



Advanced Nutrition and Human Metabolism

EIGHTH EDITION

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Dietary Reference Intakes (DRI)

The Dietary Reference Intakes (DRI) include two sets of values that serve as goals for nutrient intake—Recommended Dietary Allowances (RDA) and Adequate Intakes (AI). The RDA reflect the average daily amount of a nutrient considered adequate to meet the needs of most healthy people. If there is insufficient evidence to determine an RDA, an AI is set. AI are more tentative than RDA, but both may be used as goals for nutrient intakes. (Chapter 9 provides more details.)

In addition to the values that serve as goals for nutrient intakes (presented in the tables on these two pages), the DRI include a set of values called Tolerable Upper Intake Levels (UL). The UL represent the maximum amount of a nutrient that appears safe for most healthy people to consume on a regular basis. Turn the page for a listing of the UL for selected vitamins and minerals.

Estimated Energy Requirements (EER), Recommended Dietary Allowances (RDA), and Adequate Intakes (AI) for Water, Energy, and the Energy Nutrients

Age (yr)	Reference BMI (kg/m ²)	Reference Height cm (in)	Reference Weight kg (lb)	Water ^a AI (L/day)	Energy EER ^b (kcal/day)	Carbohydrate RDA (g/day)	Total Fiber AI (g/day)	Total Fat AI (g/day)	Linoleic Acid AI (g/day)	Linolenic Acid ^c AI (g/day)	Protein RDA (g/day) ^d	Protein RDA (g/kg/day)
Males												
0–0.5	—	62 (24)	6 (13)	0.7 ^e	570	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8 ^f	743	95	—	30	4.6	0.5	11	1.20
1–3 ^g	—	86 (34)	12 (27)	1.3	1046	130	19	—	7	0.7	13	1.05
4–8 ^g	15.3	115 (45)	20 (44)	1.7	1742	130	25	—	10	0.9	19	0.95
9–13	17.2	144 (57)	36 (79)	2.4	2279	130	31	—	12	1.2	34	0.95
14–18	20.5	174 (68)	61 (134)	3.3	3152	130	38	—	16	1.6	52	0.85
19–30	22.5	177 (70)	70 (154)	3.7	3067 ^h	130	38	—	17	1.6	56	0.80
31–50	22.5 ⁱ	177 (70) ⁱ	70 (154) ⁱ	3.7	3067 ^h	130	38	—	17	1.6	56	0.80
>50	22.5 ⁱ	177 (70) ⁱ	70 (154) ⁱ	3.7	3067 ^h	130	30	—	14	1.6	56	0.80
Females												
0–0.5	—	62 (24)	6 (13)	0.7 ^e	520	60	—	31	4.4	0.5	9.1	1.52
0.5–1	—	71 (28)	9 (20)	0.8 ^f	676	95	—	30	4.6	0.5	11	1.20
1–3 ^g	—	86 (34)	12 (27)	1.3	992	130	19	—	7	0.7	13	1.05
4–8 ^g	15.3	115 (45)	20 (44)	1.7	1642	130	25	—	10	0.9	19	0.95
9–13	17.4	144 (57)	37 (81)	2.1	2071	130	26	—	10	1.0	34	0.95
14–18	20.4	163 (64)	54 (119)	2.3	2368	130	26	—	11	1.1	46	0.85
19–30	21.5	163 (64)	57 (126)	2.7	2403 ^j	130	25	—	12	1.1	46	0.80
31–50	21.5 ⁱ	163 (64) ⁱ	57 (126) ⁱ	2.7	2403 ^j	130	25	—	12	1.1	46	0.80
>50	21.5 ⁱ	163 (64) ⁱ	57 (126) ⁱ	2.7	2403 ^j	130	21	—	11	1.1	46	0.80
Pregnancy												
1st trimester				3.0	+0	175	28	—	13	1.4	46	0.80
2nd trimester				3.0	+340	175	28	—	13	1.4	71	1.10
3rd trimester				3.0	+452	175	28	—	13	1.4	71	1.10
Lactation												
1st 6 months				3.8	+330	210	29	—	13	1.3	71	1.30
2nd 6 months				3.8	+400	210	29	—	13	1.3	71	1.30

NOTE: For all nutrients, values for infants are AI. Dashes indicate that values have not been determined.

^aThe water AI includes drinking water, water in beverages, and water in foods; in general, drinking water and other beverages contribute about 70 to 80 percent, and foods, the remainder. Conversion factors: 1 L = 33.8 fluid oz; 1 L = 1.06 qt; 1 cup = 8 fluid oz.

^bThe Estimated Energy Requirement (EER) represents the average dietary energy intake that will maintain energy balance in a healthy person of a given gender, age, weight, height, and physical activity level. The values listed are based on an “active” person at the reference height and weight and at the midpoint ages for each group

until age 19. Chapter 8 provides equations and tables to determine estimated energy requirements.

^cThe linolenic acid referred to in this table and text is the omega-3 fatty acid known as alpha-linolenic acid.

^dThe values listed are based on reference body weights.

^eAssumed to be from human milk.

^fAssumed to be from human milk and complementary foods and beverages. This includes approximately 0.6 L (~2½ cups) as total fluid including formula, juices, and drinking water.

^gFor energy, the age groups for young children are 1–2 years and 3–8 years.

^hFor males, subtract 10 calories per day for each year of age above 19.

ⁱBecause weight need not change as adults age if activity is maintained, reference weights for adults 19 through 30 years are applied to all adult age groups.

^jFor females, subtract 7 kcalories per day for each year of age above 19.

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Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Vitamins

Age (yr)	Thiamin RDA (mg/day)	Riboflavin RDA (mg/day)	Niacin RDA (mg/day) ^a	Biotin AI (µg/day)	Pantothenic acid AI (mg/day)	Vitamin B ₆ RDA (mg/day)	Folate RDA (µg/day) ^b	Vitamin B ₁₂ RDA (µg/day)	Choline AI (mg/day)	Vitamin C RDA (mg/day)	Vitamin A RDA (µg/day) ^c	Vitamin D RDA (IU/day) ^d	Vitamin E RDA (mg/day) ^e	Vitamin K AI (µg/day)
Infants														
0–0.5	0.2	0.3	2	5	1.7	0.1	65	0.4	125	40	400	400 (10 µg)	4	2.0
0.5–1	0.3	0.4	4	6	1.8	0.3	80	0.5	150	50	500	400 (10 µg)	5	2.5
Children														
1–3	0.5	0.5	6	8	2	0.5	150	0.9	200	15	300	600 (15 µg)	6	30
4–8	0.6	0.6	8	12	3	0.6	200	1.2	250	25	400	600 (15 µg)	7	55
Males														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	600 (15 µg)	11	60
14–18	1.2	1.3	16	25	5	1.3	400	2.4	550	75	900	600 (15 µg)	15	75
19–30	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	600 (15 µg)	15	120
31–50	1.2	1.3	16	30	5	1.3	400	2.4	550	90	900	600 (15 µg)	15	120
51–70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	600 (15 µg)	15	120
>70	1.2	1.3	16	30	5	1.7	400	2.4	550	90	900	800 (20 µg)	15	120
Females														
9–13	0.9	0.9	12	20	4	1.0	300	1.8	375	45	600	600 (15 µg)	11	60
14–18	1.0	1.0	14	25	5	1.2	400	2.4	400	65	700	600 (15 µg)	15	75
19–30	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	600 (15 µg)	15	90
31–50	1.1	1.1	14	30	5	1.3	400	2.4	425	75	700	600 (15 µg)	15	90
51–70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	600 (15 µg)	15	90
>70	1.1	1.1	14	30	5	1.5	400	2.4	425	75	700	800 (20 µg)	15	90
Pregnancy														
≤18	1.4	1.4	18	30	6	1.9	600	2.6	450	80	750	600 (15 µg)	15	75
19–30	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	600 (15 µg)	15	90
31–50	1.4	1.4	18	30	6	1.9	600	2.6	450	85	770	600 (15 µg)	15	90
Lactation														
≤18	1.4	1.6	17	35	7	2.0	500	2.8	550	115	1200	600 (15 µg)	19	75
19–30	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	600 (15 µg)	19	90
31–50	1.4	1.6	17	35	7	2.0	500	2.8	550	120	1300	600 (15 µg)	19	90

NOTE: For all nutrients, values for infants are AI.

^aNiacin recommendations are expressed as niacin equivalents (NE), except for recommendations for infants younger than 6 months, which are expressed as preformed niacin.

^bFolate recommendations are expressed as dietary folate equivalents (DFE).

^cVitamin A recommendations are expressed as retinol activity equivalents (RAE).

^dVitamin D recommendations are expressed as cholecalciferol and assume an absence of adequate exposure to sunlight.

^eVitamin E recommendations are expressed as α-tocopherol.

Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) for Minerals

Age (yr)	Sodium AI (mg/day)	Chloride AI (mg/day)	Potassium AI (mg/day)	Calcium RDA (mg/day)	Phosphorus RDA (mg/day)	Magnesium RDA (mg/day)	Iron RDA (mg/day)	Zinc RDA (mg/day)	Iodine RDA (µg/day)	Selenium RDA (µg/day)	Copper RDA (µg/day)	Manganese AI (mg/day)	Fluoride AI (mg/day)	Chromium AI (µg/day)	Molybdenum RDA (µg/day)
Infants															
0–0.5	120	180	400	200	100	30	0.27	2	110	15	200	0.003	0.01	0.2	2
0.5–1	370	570	700	260	275	75	11	3	130	20	220	0.6	0.5	5.5	3
Children															
1–3	1000	1500	3000	700	460	80	7	3	90	20	340	1.2	0.7	11	17
4–8	1200	1900	3800	1000	500	130	10	5	90	30	440	1.5	1.0	15	22
Males															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.9	2	25	34
14–18	1500	2300	4700	1300	1250	410	11	11	150	55	890	2.2	3	35	43
19–30	1500	2300	4700	1000	700	400	8	11	150	55	900	2.3	4	35	45
31–50	1500	2300	4700	1000	700	420	8	11	150	55	900	2.3	4	35	45
51–70	1300	2000	4700	1000	700	420	8	11	150	55	900	2.3	4	30	45
>70	1200	1800	4700	1200	700	420	8	11	150	55	900	2.3	4	30	45
Females															
9–13	1500	2300	4500	1300	1250	240	8	8	120	40	700	1.6	2	21	34
14–18	1500	2300	4700	1300	1250	360	15	9	150	55	890	1.6	3	24	43
19–30	1500	2300	4700	1000	700	310	18	8	150	55	900	1.8	3	25	45
31–50	1500	2300	4700	1000	700	320	18	8	150	55	900	1.8	3	25	45
51–70	1300	2000	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
>70	1200	1800	4700	1200	700	320	8	8	150	55	900	1.8	3	20	45
Pregnancy															
≤18	1500	2300	4700	1300	1250	400	27	12	220	60	1000	2.0	3	29	50
19–30	1500	2300	4700	1000	700	350	27	11	220	60	1000	2.0	3	30	50
31–50	1500	2300	4700	1000	700	360	27	11	220	60	1000	2.0	3	30	50
Lactation															
≤18	1500	2300	5100	1300	1250	360	10	13	290	70	1300	2.6	3	44	50
19–30	1500	2300	5100	1000	700	310	9	12	290	70	1300	2.6	3	45	50
31–50	1500	2300	5100	1000	700	320	9	12	290	70	1300	2.6	3	45	50

NOTE: For all nutrients, values for infants are AI.

Tolerable Upper Intake Levels (UL) for Vitamins

Age (yr)	Niacin (mg/day) ^a	Vitamin B ₆ (mg/day)	Folate (μg/day) ^a	Choline (mg/day)	Vitamin C (mg/day)	Vitamin A (μg/day) ^b	Vitamin D (IU/day)	Vitamin E (mg/day) ^c	
Infants									
0–0.5	—	—	—	—	—	600	1000 (25 μg)	—	
0.5–1	—	—	—	—	—	600	1500 (38 μg)	—	
Children									
1–3	10	30	300	1000	400	600	2500 (63 μg)	200	
4–8	15	40	400	1000	650	900	3000 (75 μg)	300	
9–13	20	60	600	2000	1200	1700	4000 (100 μg)	600	
Adolescents									
14–18	30	80	800	3000	1800	2800	4000 (100 μg)	800	
Adults									
19–70	35	100	1000	3500	2000	3000	4000 (100 μg)	1000	
>70	35	100	1000	3500	2000	3000	4000 (100 μg)	1000	
Pregnancy									
≤18	30	80	800	3000	1800	2800	4000 (100 μg)	800	
19–50	35	100	1000	3500	2000	3000	4000 (100 μg)	1000	
Lactation									
≤18	30	80	800	3000	1800	2800	4000 (100 μg)	800	
19–50	35	100	1000	3500	2000	3000	4000 (100 μg)	1000	

^aThe UL for niacin and folate apply to synthetic forms obtained from supplements, fortified foods, or a combination of the two.

^cThe UL for vitamin E applies to any form of supplemental α-tocopherol, fortified foods, or a combination of the two.

^bThe UL for vitamin A applies to the preformed vitamin only.

Tolerable Upper Intake Levels (UL) for Minerals

Age (yr)	Sodium (mg/day)	Chloride (mg/day)	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day) ^d	Iron (mg/day)	Zinc (mg/day)	Iodine (μg/day)	Selenium (μg/day)	Copper (μg/day)	Manganese (mg/day)	Fluoride (mg/day)	Molybdenum (μg/day)	Boron (mg/day)	Nickel (mg/day)	Vanadium (mg/day)	
Infants																	
0–0.5	—	—	1000	—	—	40	4	—	45	—	—	0.7	—	—	—	—	
0.5–1	—	—	1500	—	—	40	5	—	60	—	—	0.9	—	—	—	—	
Children																	
1–3	1500	2300	2500	3000	65	40	7	200	90	1000	2	1.3	300	3	0.2	—	
4–8	1900	2900	2500	3000	110	40	12	300	150	3000	3	2.2	600	6	0.3	—	
9–13	2200	3400	3000	4000	350	40	23	600	280	5000	6	10	1100	11	0.6	—	
Adolescents																	
14–18	2300	3600	3000	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
Adults																	
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8	
51–70	2300	3600	2000	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8	
>70	2300	3600	2000	3000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	1.8	
Pregnancy																	
≤18	2300	3600	3000	3500	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
19–50	2300	3600	2500	3500	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—	
Lactation																	
≤18	2300	3600	3000	4000	350	45	34	900	400	8000	9	10	1700	17	1.0	—	
19–50	2300	3600	2500	4000	350	45	40	1100	400	10,000	11	10	2000	20	1.0	—	

^dThe UL for magnesium applies to synthetic forms obtained from supplements or drugs only.

NOTE: An Upper Limit was not established for vitamins and minerals not listed and for those age groups listed with a dash (—) because of a lack of data, not because these nutrients are safe to consume at any level of intake. All nutrients can have adverse effects when intakes are excessive.

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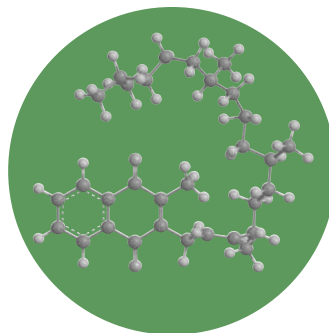
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To my children Michelle and Michael and their spouses, and to my husband, Daniel, for their ongoing encouragement, support, faith, and love and to the students who continue to impress and inspire me.

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To my wife, Carol, for her continued support, constant inspiration, and assistance in the preparation of this book.

Jack Smith

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Tim Carr



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PREFACE

Since the first edition was published in 1990, much has changed in the science of nutrition. But the purpose of the text—to provide thorough coverage of normal metabolism for upper-division undergraduate and graduate students majoring in nutrition or other health-related fields—remains the same. We continue to strive for a level of detail and scope of material that satisfy the needs of both instructors and students. With each succeeding edition, we have responded to suggestions from instructors, content reviewers, and students that have improved the text by enhancing the clarity of the material and by ensuring accuracy. In addition, we have included the latest and most pertinent nutrition science available to provide future nutrition professionals with the fundamental information vital to their careers and to provide the basis for assimilating new scientific discoveries.

Just as the body of information on nutrition science has increased, so has the team of authors working on this text. Dr. James Groff and Dr. Sara Hunt coauthored the first edition. In subsequent editions, Dr. Sareen Gropper became a coauthor as Dr. Hunt entered retirement. In the fourth edition, Dr. Jack L. Smith joined the author team now led by Dr. Gropper. In the seventh and eighth editions, Dr. Tim Carr has provided additional expertise and coauthorship on several chapters following Dr. Smith's retirement.

NEW TO THIS EDITION

All chapters of the eighth edition have been updated and feature new or enhanced tables and illustrations. The organization of the content among the chapters has remained similar to the previous editions.

Chapter 1 The Cell: A Microcosm of Life

- Expanded content in several sections including, for example, the nucleus where additional information is presented on genes and chromosomes
- Added additional information on mechanism of apoptosis
- Created new Perspective on Nutritional Genomics

Chapter 2 The Digestive System: Mechanism for Nourishing the Body

- Expanded information on the structural features of the small intestine
- Added new information on probiotics and intestinal conditions

Chapter 3 Carbohydrates

- Reorganized the chapter sections to improve flow and readability
- Revised sections on stereoisomers, ring structures, and derivatives of monosaccharides
- Added new information related to dextrans and dextrose equivalents
- Added new information on SGLTs and GLUTs
- Expanded sections on blood–tissue barriers and the electron transport chain
- Reorganized sections related to carbohydrate absorption and transport; added new discussion on membrane transport
- Revised section on metabolic regulation; added new information on enzyme translocation
- Updated and modified several figures and figure legends
- Added new Box feature on syrups
- Added new Box feature on uncoupling oxidative phosphorylation
- Updated the end-of-chapter Perspective

Chapter 4 Fiber

- Added new information on another form of resistant starch
- Provided new information on the properties of fiber important for laxation
- Added information on a new mechanism by which phytochemicals may regulate mRNA translation

Chapter 5 Lipids

- Added new section on odd-chain and branched-chain fatty acids
- Expanded discussion related to conjugated linoleic acid
- Revised information related to trans fatty acids, mono- and diacylglycerols, and the biological roles of phospholipids
- Updated information on fatty acid transport into enterocytes
- Added new section on the lipid hypothesis
- Expanded discussion on β -oxidation, including new sections related to oxidation of odd-chain and branched-chain fatty acids
- Updated and modified several figures and figure legends
- Added new Box feature on the gallbladder
- Added new Box feature on coconut oil

Chapter 6 Protein

- Added a new figure showing intestinal amino acid transport
- Expanded the discussion addressing the mechanisms of protein degradation
- Expanded the discussion on the need for protein with aging
- Expanded the section addressing plant proteins

Chapter 7 Integration and Regulation of Metabolism and the Impact of Exercise

- Revised Table 7.1
- Added new section on adiponectin; updated Table 7.3 to include adiponectin
- Revised figures and figure legends
- Added new Box feature on the metabolic similarity between type 1 diabetes and starvation

Chapter 8 Energy Expenditure, Body Composition, and Healthy Weight

- Added new information on the origin of body mass index
- Revised section related to adiponectin regulation
- Added new and updated information in the end-of-chapter Perspective on eating disorders
- Revised figures and figure legends

- Added new Box feature of how to measure what people eat

Chapter 9 Water-Soluble Vitamins

- Updated daily values
- Added photos showing the physical manifestations of several vitamin deficiencies
- Added information on another coenzyme role of thiamin tied to fatty acid oxidation
- Expanded the figures showing pantothenic acid metabolism
- Added new information on the functions of pantothenic acid linking it to folate metabolism
- Expanded the information on the non-coenzyme roles of biotin
- Added a new figure showing folate metabolism within the cytosol, nucleus, and mitochondria
- Expanded the discussion of intracellular chaperones involved in vitamin B₁₂ transport
- Added information on the Dietary Reference Intakes, including chronic disease risk reduction
- Developed a new Perspective addressing types of research study designs

Chapter 10 Fat-Soluble Vitamins

- Updated daily values
- Provided more details on the mechanisms of absorption of vitamins A, E, and K
- Added a new figure and information on the functions and metabolism of vitamin E
- Added new figures showing some manifestations of deficiencies of vitamins A and D
- Added a new figure showing phyloquinone metabolism
- Created a new table providing the phyloquinone and menaquinone contents of foods
- Expanded information on the carotenoid content of foods

Chapter 11 Major Minerals

- Expanded discussion of calcium functions
- Expanded the discussion providing an overview of bone
- Expanded discussions of calcium, phosphate, and magnesium homeostasis
- Added a new table showing factors regulating serum phosphate
- Improved figure depicting phosphate absorption

- Added information on the use of topical magnesium oils
- Updated daily values

Chapter 12 Water and Electrolytes

- Expand list of dietary sources for sodium and potassium
- Added a table with food sources of potassium
- Updated recommendations and daily values
- Added information on the chronic disease risk reduction recommendation for sodium
- Updated the Perspective on macrominerals and hypertension to reflect the latest dietary recommendations

Chapter 13 Essential Trace and Ultratrace Minerals

- Revised tables showing food sources of trace minerals
- Expanded section on iron as a pro-oxidant
- Added new information describing zinc/cancer association
- Added new information on mercury/selenium interaction
- Updated the Daily Value for each mineral
- Added photos showing deficiency symptoms of zinc, copper, selenium, and iodine
- Revised figures and figure legends
- Added new Box feature on selenium in the environment

Chapter 14 Nonessential Trace and Ultratrace Minerals

- Expanded the sections on the sources of fluoride and supplemental forms of boron
- Expanded the discussions addressing fluoride's and boron's mechanisms of action
- Updated information on recommendations for intake of boron
- Updated information on toxicity-related concerns with vanadium
- Expanded the Perspective to include information to consider when buying supplements

PRESENTATION

The presentation of the text is designed to make the book easy for the reader to use. The added color(s) draws attention to important elements in the text, tables, and figures

and helps generate reader interest. The Perspectives provide applications or expansion of the information in the chapter text.

Because this book focuses on normal human nutrition and physiological function, it is an effective resource for students majoring in either nutrition sciences or dietetics and for other health care professionals enrolled in a graduate nutrition course. Intended for a course in advanced nutrition, the text presumes a sound background in the biological sciences. At the same time, however, it provides a review of the basic sciences, particularly biochemistry and physiology, which are important to understanding the material. This text applies biochemistry to nutrient use from consumption through digestion, absorption, distribution, and cellular metabolism. Health practitioners may find that the book is a useful resource to refresh their memories with regard to metabolic and physiological interrelationships and to obtain a concise update on current concepts related to human nutrition.

We continue to present nutrition as the science that integrates life processes from the molecular to the cellular level and on through the multisystem operation of the whole organism. Our primary goal is to give a comprehensive picture of cell reactions at the tissue, organ, and system levels. Subject matter has been selected for its relevance to meeting this goal.

ORGANIZATION

Each of the 14 chapters begins with a topic outline, followed by a brief introduction to the chapter's subject matter. These features are followed in order by the chapter text, a brief summary that ties together the ideas presented in the chapter, a reference list, and a Perspective with its own reference list.

The text is divided into three sections. Section I (Chapters 1 and 2) focuses on cell structure, gastrointestinal tract anatomy, and function with respect to digestion and absorption.

Section II (Chapters 3–8) discusses metabolism of the macronutrients. This section reviews primary metabolic pathways for carbohydrates, lipids, and proteins, emphasizing those reactions particularly relevant to issues of health. Since most of the body's energy production is associated with glycolysis or the tricarboxylic acid cycle by way of the electron transport chain and oxidative phosphorylation, the carbohydrates chapter (Chapter 3) covers these aspects of energy transformation. We include a separate chapter (Chapter 4) on fiber. The metabolism of alcohol, which contributes to the caloric intake of many people, is discussed within the lipids chapter (Chapter 5). Alcohol's chemical structure more closely resembles that of carbohydrates, but its metabolism is more similar to that of lipids. Chapter 7 discusses the interrelationships among the metabolic pathways that are common to the

macronutrients. This chapter also includes a discussion of the regulation of the metabolic pathways and a description of the metabolic dynamics of the fed-fast cycle, along with a presentation of the effects of physical exertion on the body's metabolic pathways. Chapter 8 focuses on energy expenditure, energy balance, and healthy weight and also includes a brief discussion of measuring body composition and the health implications of altered body weight.

Section III (Chapters 9–14) concerns those nutrients considered regulatory in nature: the water- and fat-soluble vitamins and the minerals, including the major minerals, trace minerals, and ultratrace minerals. These chapters cover nutrient features such as digestion, absorption, transport, function, metabolism, excretion, deficiency, toxicity, and assessment of nutriture, as well as the latest Recommended Dietary Allowances or Adequate Intakes for each nutrient. Information about the major minerals has been split into two chapters: Chapter 11 addresses calcium, phosphorus, and magnesium, and Chapter 12 discusses sodium, potassium, and chloride. Chapter 12 integrates coverage of the maintenance of the body's homeostatic environment—including discussions of body fluids, electrolyte balance, and pH maintenance—with the presentation of the electrolytes.

SUPPLEMENTARY MATERIAL

MindTap for Gropper's *Advanced Nutrition and Human Metabolism*, 8th Edition, is a digital learning solution that empowers learners to go beyond memorization—enabling a deeper understanding of concepts and topics. MindTap provides engaging content and activities that help build student confidence. Accelerate progress with MindTap. Visit cengage.com/login to learn more. Additional instructor resources for this product are available online. Instructor assets include an Instructor's Manual, Educator's Guide, PowerPoint® slides, and a test bank powered by Cognero®. Sign up or sign in at www.cengage.com to search for and access this product and its online resources.

ACKNOWLEDGMENTS

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THE CELL: A MICROCOSM OF LIFE

LEARNING OBJECTIVES

- 1.1 Identify cellular components and their functions.
- 1.2 Describe the roles of cell receptors and enzymes.
- 1.3 Explain the mechanisms by which enzymatic reactions are regulated.
- 1.4 Discuss the need for and pathways involved in apoptosis.
- 1.5 Describe how energy is released and utilized in chemical reactions.

CELLS ARE THE VERY ESSENCE OF LIFE. Cells may be defined as the basic living, structural, and functional units of the human body. They vary greatly in size, chemical composition, and function, but each one is a remarkable miniaturization of human life. Cells move, grow, ingest “food,” excrete wastes, react to their environment, and reproduce. This chapter provides a brief review of the basics of a cell, including cellular components, biological energy, and an overview of a cell’s natural life span.

Cells of multicellular organisms are called **eukaryotic cells** (from the Greek *eu* meaning “true” and *karyon* meaning “nucleus”). Eukaryotic cells evolved from simpler, more primitive cells called **prokaryotic cells** (from the Greek meaning “before nucleus”). One distinguishing feature between the two cell types is that eukaryotic cells possess a defined nucleus, whereas prokaryotic cells do not. Also, eukaryotic cells are larger and much more complex structurally and functionally than their ancestors. Because this text addresses human metabolism and nutrition, all descriptions of cellular structure and function in this and subsequent chapters pertain to eukaryotic cells.

While specialization among cells is necessary for life, cells, in general, have certain basic similarities. All human cells have a **plasma membrane** and a nucleus (or have had a nucleus), and most contain an endoplasmic reticulum, Golgi apparatus, and mitochondria. For convenience of discussion, a “typical cell” is presented (Figure 1.1) to enable the identification of the various organelles and their functions, which characterize cellular life. Our discussion begins with the plasma membrane, which forms the outer boundary of the cell, and then moves inward to examine the organelles found within the cell.

1.1 COMPONENTS OF CELLS

Plasma Membrane

The plasma membrane is a sheetlike structure that encapsulates and surrounds the cell, allowing it to exist as a distinct unit. The plasma membrane, like other membranes within the cell, has distinct structural characteristics and functions.

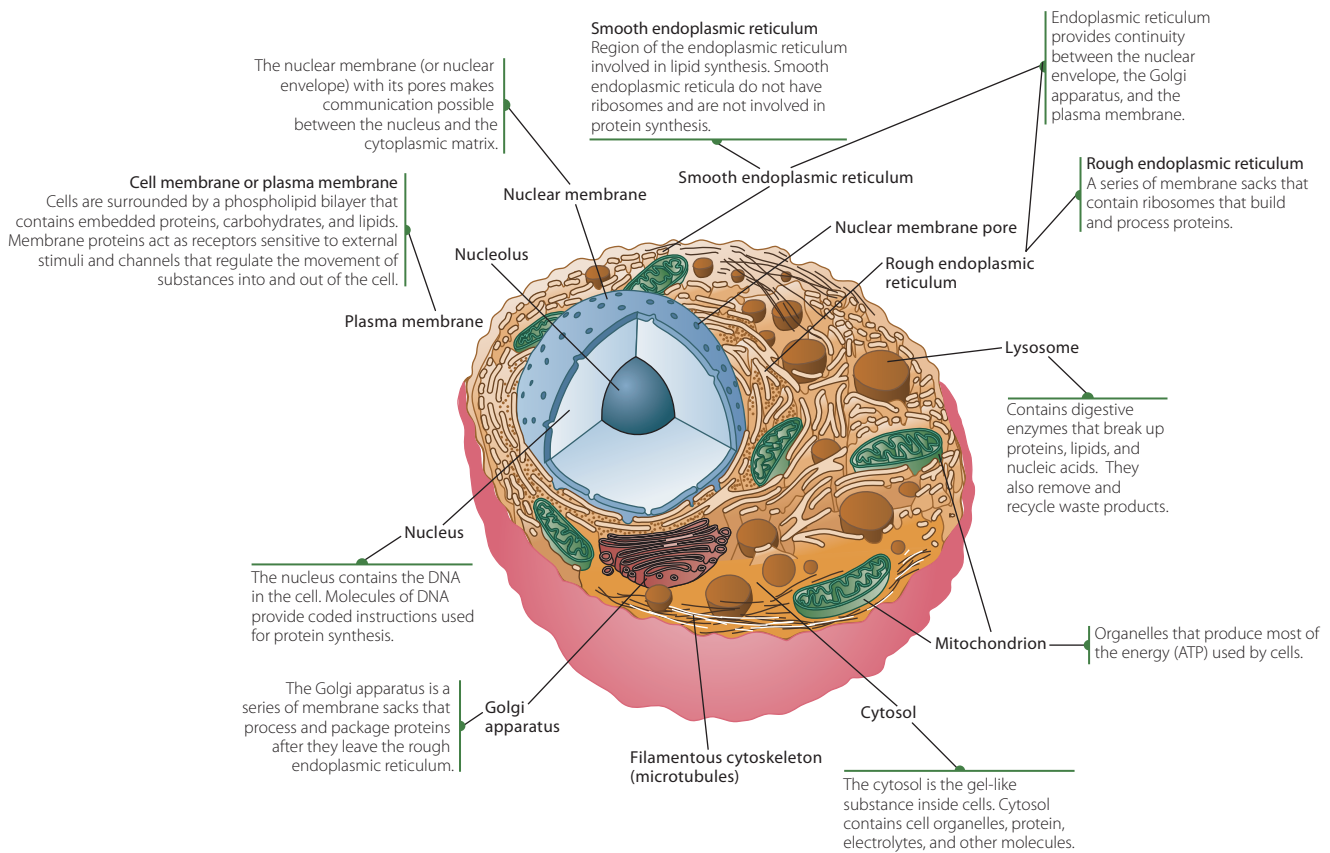


Figure 1.1 Three-dimensional depiction of a typical mammalian liver cell.

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- Plasma membranes are asymmetrical, with different inside and outside “faces.”
- Plasma membranes are not static but are fluid structures.

Plasma membranes are composed primarily of proteins, cholesterol, and phospholipids. Phospholipids, shown in Figure 1.2, provide both a **hydrophobic** and a hydrophilic moiety that allows them to spontaneously form bimolecular sheets, called lipid bilayers, in aqueous environments like the human body. It is this lipid bilayer that determines the structure of the plasma membrane. The fatty acid portion (hydrocarbon chain) of the phospholipids forms the hydrophobic (water-fearing) core of the membrane bilayer; it also inhibits many water-soluble compounds from passing into the cell and helps to retain water-soluble substances within the cell. The glycerol and phosphate-containing portions (polar head) of the phospholipid are hydrophilic (i.e., polar, water loving) and thus are oriented toward the cell’s aqueous environments found both outside the cell and in the cell cytosol.

Another important membrane lipid is cholesterol (Figure 1.3). Cholesterol influences the fluidity and thus permeability of membranes, affecting what may pass into and out of the cell; membranes with higher levels of cholesterol are less fluid. Within the membrane, cholesterol’s

hydrocarbon side chain associates with that of phospholipids, and cholesterol’s hydroxyl groups are positioned close to the phospholipid’s polar head groups. Cholesterol’s rigid planar steroid rings are positioned so as to interact with and stabilize the regions of the hydrocarbon chains closest to the polar head groups of the phospholipids. The rest of the hydrocarbon chain remains flexible and fluid.

Both integral and peripheral proteins are found interspersed with the plasma membrane’s lipid bilayer (Figure 1.3). These proteins are responsible for several membrane functions including mediating information transfer (as **receptors**), transporting ions and molecules (as channels, carriers, gates, and pumps), acting as cell adhesion molecules, and speeding up metabolic activities (as **enzymes**). Integral proteins are attached and embedded in the membrane through hydrophobic interactions; they are often transmembrane, spanning the entire structure. Peripheral proteins, in contrast, are associated with membranes through ionic interactions and are located on or near the membrane surface. Peripheral proteins may be attached to integral membrane proteins either directly or through intermediate proteins. Many of these membrane proteins have either lipid or carbohydrate attachments.

Carbohydrates are present in plasma membranes as glycolipids and glycoproteins. While some carbohydrate

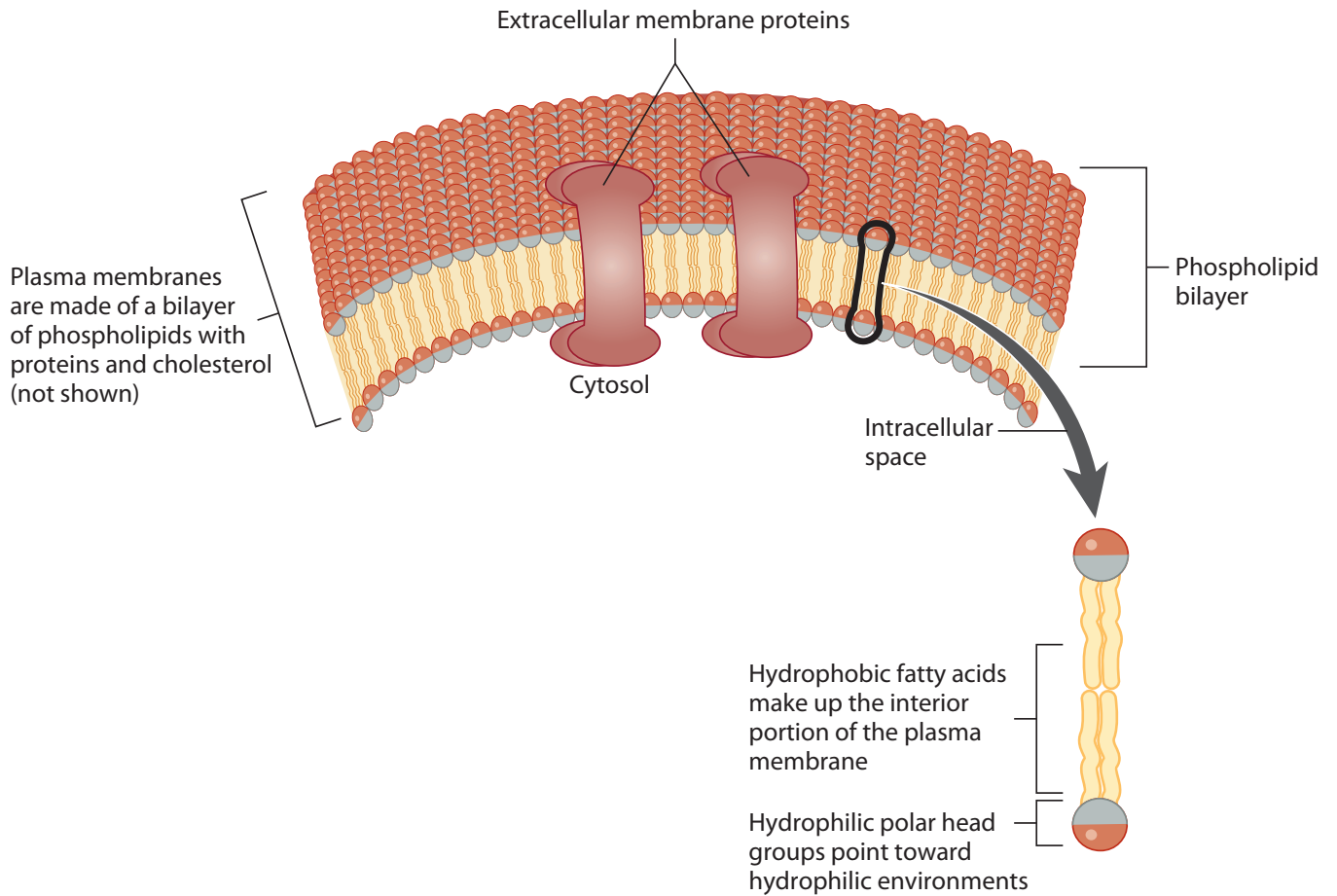


Figure 1.2 Lipid bilayer structure of biological membranes.

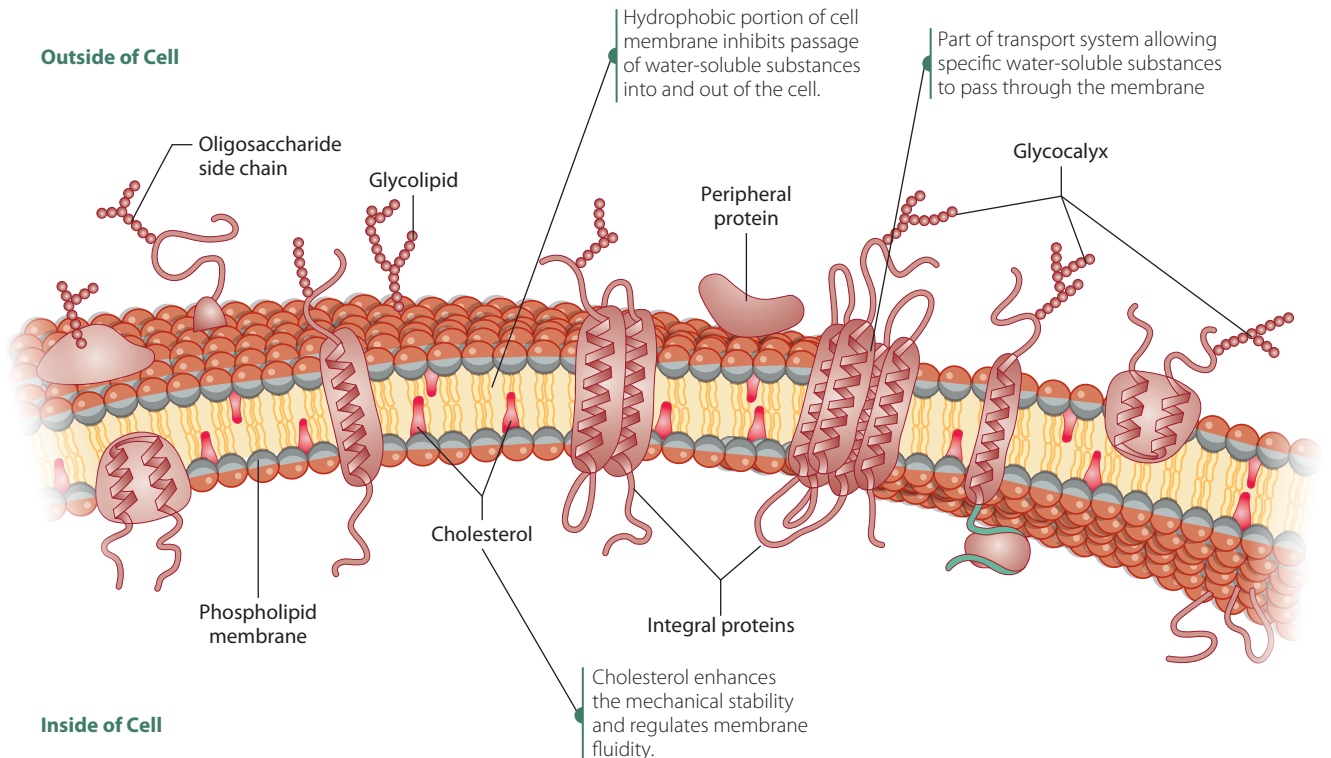


Figure 1.3 Fluid model of cell membrane. Lipids and proteins are mobile and can move laterally in the membrane.

is found in all membranes, most of the glycolipids and glycoproteins of the cell are associated with the plasma membrane. The carbohydrate moiety of the membrane glycoproteins and glycolipids provides asymmetry to the membrane because the oligosaccharide side chains are located exclusively on the membrane layer facing the cell's outer surface (and not toward the cytosol). In plasma membranes, these outer sugar residues form what is called the glycocalyx, the layer of carbohydrate on the cell's outer surface. On the membranes of the organelles within the cell, however, the oligosaccharides are directed inward. The plasma membrane glycoproteins may serve as the receptors for hormones, certain nutrients, and other substances that influence cellular function. Glycoproteins also may help regulate the intracellular communication necessary for cell growth and functions. Intracellular communication occurs through pathways that convert information from one part of a cell to another in response to external stimuli. Generally, it involves the passage of chemical messengers from organelle to organelle or within the lipid bilayers of membranes. Intracellular communication is examined more closely in the “Receptors and Intracellular Signaling” section of this chapter.

Membranes are not structurally distinct from the aqueous compartments of the cell they surround. For example, the **cytosol**—which is the aqueous, gel-like, transparent substance—fills the cell and, together with a system of filaments, connects the various membranes of the cell. This interconnection creates a structure that makes it possible for a signal generated at one part of the cell to be transmitted quickly and efficiently to other regions of the cell.

Cytosol and Cytoskeleton

The **cytoplasm**, found inside the cell's plasma membrane but outside of the nucleus, includes the cytosol (a gel-like liquid), a cytoskeletal/cytomatrix, and organelles. The cytoskeleton consists of a system of filaments or fibers (Figures 1.1 and 1.4). The cytoskeleton provides cells with:

- structural support, which defines the cell's shape and helps to maintain its function
- a framework for positioning the various organelles (such as microvilli, which are extensions of intestinal cells)
- a network to direct the movement of materials and organelles within the cells
- a means of independent locomotion for specialized cells (such as sperm, white blood cells, and fibroblasts)
- a pathway for intercellular communication among cellular components (vital for cell activation and survival)
- possible transfer of RNA and DNA.

The cytoskeleton is made up of three groups of fibers: **microtubules**, **intermediate filaments**, and **microfilaments**.

Microtubules, Intermediate Filaments, and Microfilaments

Microtubules are hollow (with about a 24 nm outer diameter), relatively rigid tubular structures (Figure 1.4). They consist of primarily two proteins— α -tubulin and β -tubulin—which form heterodimers that polymerize end-to-end. Microtubules, once formed, can be further lengthened at one end by the addition of more dimers; the other end, however, may undergo disassembly. Microtubules interact with a number of intracellular components, including proteins. They provide mechanical support, like a platform or scaffold, to influence cell shape. They also provide a structure for the intracellular movement of organelles and the assembly of cellular components (such as spindle fibers for mitosis). Flagella and cilia also rely on microtubules for movement.

Intermediate filaments, about 10 nm in diameter, are a heterogeneous group of fibers that are dynamic, undergoing constant assembly and disassembly, controlled in part by phosphorylation and dephosphorylation. Intermediate filaments (Figure 1.4) provide mechanical strength to cells that are subjected to physical stress, such as neurons, muscle cells, and epithelial cells lining body cavities.

Microfilaments, the thinnest (about 4–6 nm in diameter) of the fibers making up the cytoskeleton, are long, linear, solid fibers made up of actin. Microfilaments, like the other fibers, polymerize and unpolymerize according to the needs of the cells. Microfilaments provide scaffolding or tracks for various cell functions. Microfilaments interact with microtubules to facilitate the movement of cellular organelles and vesicles, and their interactions with intermediate filaments are thought to enable communication from extracellular stimuli to organelles within the cytosol.

Structural Arrangement

The structural arrangement within the cell influences metabolic pathways. The fluid portion of the matrix contains small molecules such as glucose, amino acids, oxygen, and carbon dioxide. This aqueous part of the cell is in contact with the cytoskeleton over a very broad surface area and enables enzymes that are associated with the polymeric lattice to be in close proximity to their substrate molecules in the aqueous portion. Furthermore, the enzymes that catalyze the reactions of many metabolic pathways are oriented sequentially so that the product of one reaction is released in close proximity to the next enzyme for which it is a substrate; this enhances the velocity of the overall metabolic pathway. Such an arrangement exists among the enzymes that participate in glycolysis. Some other metabolic pathways that occur in the cytoplasmic matrix and that might be similarly affected include the **hexose monophosphate shunt** (pentose phosphate pathway), glycogenesis, glycogenolysis, and fatty acid synthesis. The cytoplasmic matrix of eukaryotic cells contains a number of organelles,

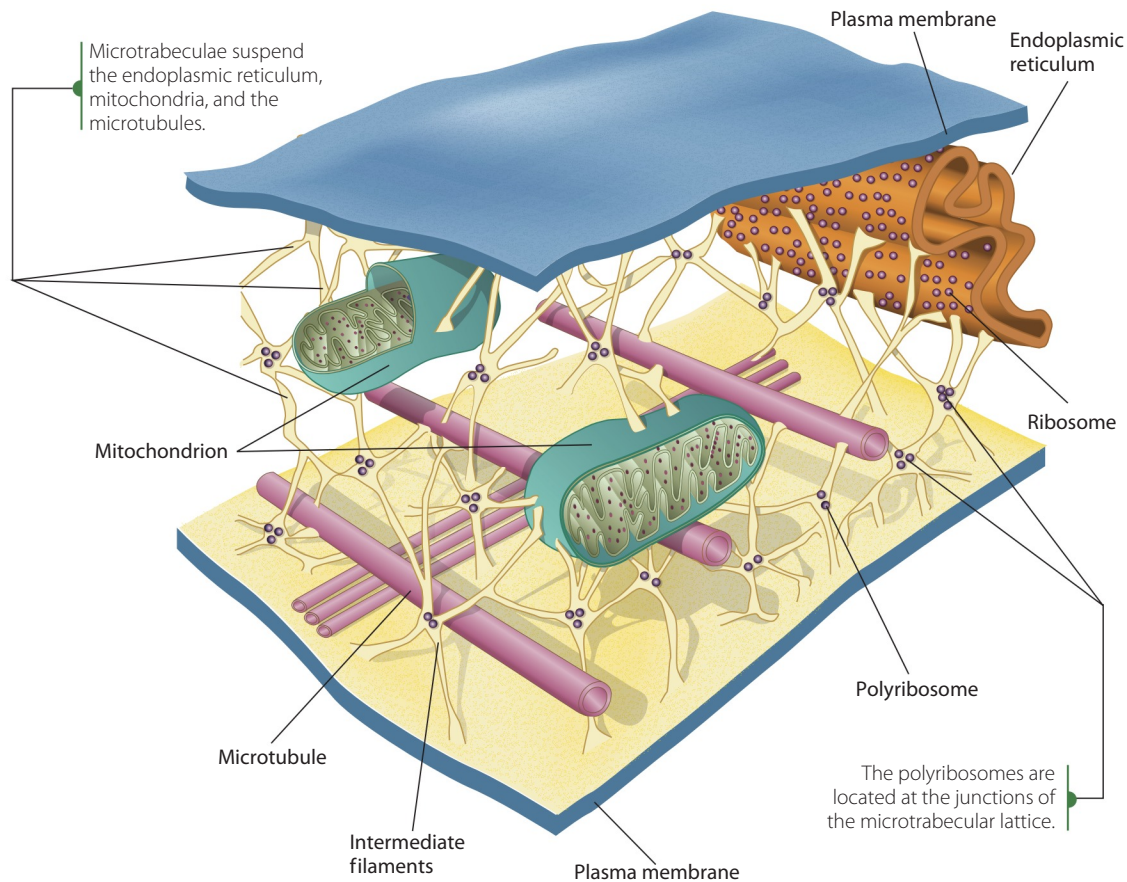


Figure 1.4 The cytoskeleton (microtrabecular lattice) provides a structure for cell organelles, microvilli (as found in intestinal mucosa cells), and large molecules. The cytosol is shown at about 300,000 times its actual size and was derived from hundreds of images of cultured cells viewed in a high-voltage electron microscope.

Source: Adapted from Porter and Tucker, "The Ground Substance of the Cell," 1981, *Scientific American*.

enclosed in bilayer membranes and described briefly in the following sections.

Mitochondrion

The **mitochondria** are the primary sites of oxygen use in cells and are responsible for most of the metabolic energy (ATP) produced in cells. All cells in the body, with the exception of the erythrocyte, possess mitochondria. The erythrocyte disposes of its mitochondria and nucleus during the maturation process and then must depend solely on energy produced through anaerobic mechanisms, primarily glycolysis. The mitochondria in different tissues vary according to the function of the tissue. In muscle, for example, the mitochondria are held tightly among the fibers of the contractile system. In the liver, however, the mitochondria have fewer restraints and move freely through the cytoplasmic matrix. Mitochondria are surrounded by two bilayer membranes.

Mitochondrial Membrane

The mitochondrion consists of a matrix or interior space surrounded by a double membrane (Figures 1.5 and 1.6). The mitochondrial outer membrane is relatively porous

(allowing for free diffusion of molecules up to about 5 kDa), whereas the inner membrane is selectively permeable (preventing free diffusion except for oxygen and carbon dioxide), serving as a barrier between the cytoplasmic matrix and the mitochondrial matrix. The inner membrane has many invaginations, called the cristae, which increase its surface area and has all the components of the electron transport chain embedded within it.

The electron transport (respiratory) chain is central to the process of **oxidative phosphorylation**, the mechanism by which most cellular ATP is produced. The components of the electron transport chain carry electrons and hydrogens during the catalytic oxidation of nutrients by enzymes in the mitochondrial matrix. The details of this process are described more fully in Chapter 3. Briefly, the mitochondria carry out the flow of electrons through the electron transport chain. This electron flow is strongly exothermic, and the energy released is used in part for ATP synthesis, an endothermic process. Molecular oxygen is ultimately, but indirectly, the oxidizing agent in these reactions. The function of the **electron transport chain** is to couple the energy released by nutrient oxidation to the formation of ATP. The chain components are precisely positioned within the inner mitochondrial membrane, an important

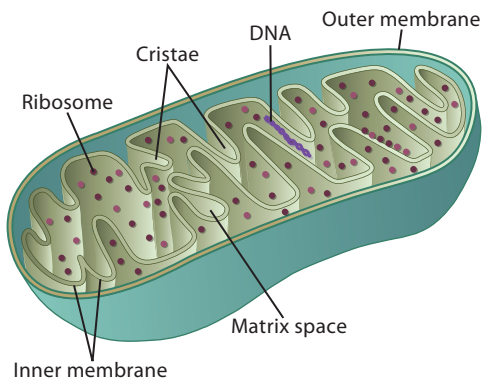


Figure 1.5 The mitochondrion.

feature of the mitochondria because it brings the products released in the matrix into close proximity with molecular oxygen. Figure 1.6 shows the flow of major reactants into and out of the mitochondrion.

Mitochondrial Matrix

Among the metabolic enzyme systems functioning in the mitochondrial matrix are those that catalyze the reactions of the tricarboxylic cycle (TCA cycle; Chapter 3) and fatty acid oxidation (Chapter 5). Other enzymes are involved in the oxidative decarboxylation and carboxylation of

pyruvate (Chapter 3) and in certain reactions of amino acid metabolism (Chapter 6).

Mitochondria are capable of both fission and fusion, depending on the needs of the cell. They reproduce by dividing in two. Although the nucleus contains most of the cell's deoxyribonucleic acid (DNA), the mitochondrial matrix contains a small amount of DNA and a few ribosomes, enabling limited synthesis of protein within the mitochondrion. Most mitochondrial enzymes are coded by nuclear DNA, synthesized on the rough endoplasmic reticulum (RER) in the cytosol, and then incorporated into existing mitochondria. The genes contained in mitochondrial DNA, unlike those in the nucleus, are inherited only from the mother and code primarily for proteins needed for normal mitochondrial function and for ATP production. Several diseases—such as cytochrome c oxidase deficiency (also called complex IV deficiency), Leigh syndrome, and Kearns-Sayre syndrome—result from mutations in mitochondrial genes.

Nucleus

The nucleus (see Figure 1.1) is the largest of the organelles within the cell. Because of its DNA content, the nucleus initiates and regulates most cellular activities. Surrounding

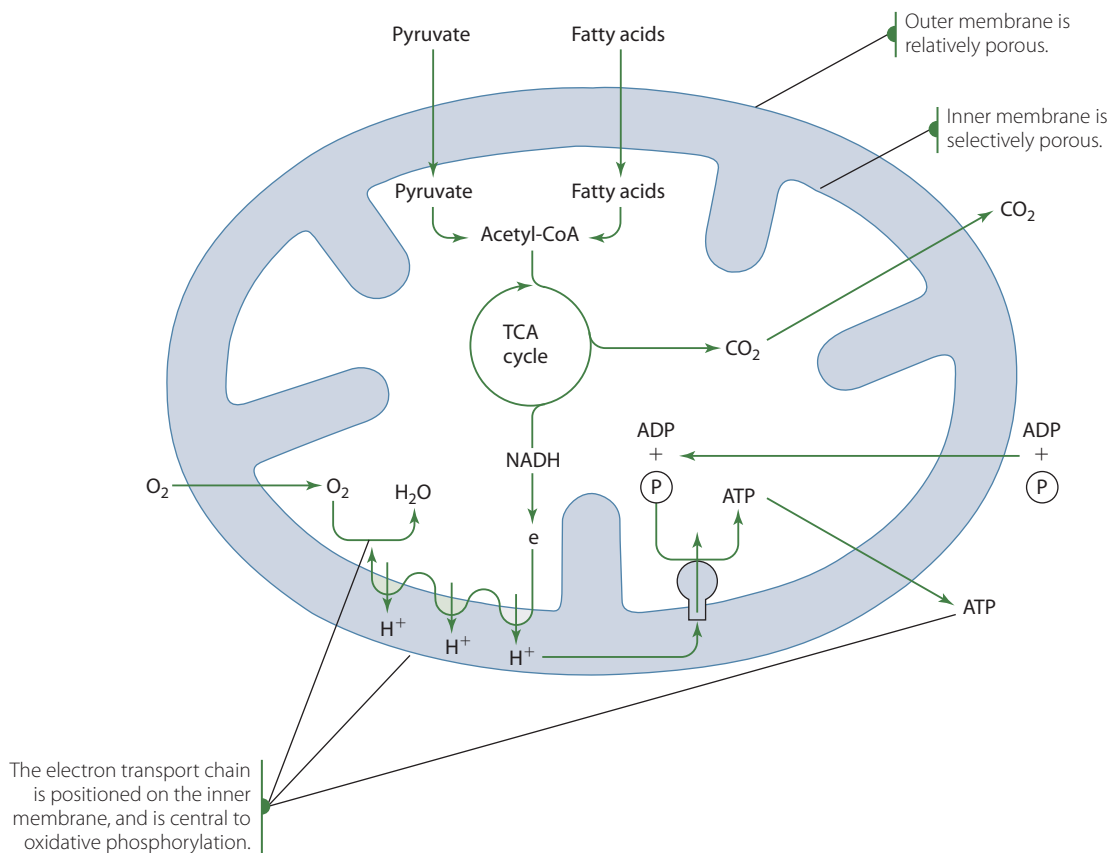


Figure 1.6 Overview of a cross section of the mitochondria.

the nucleus is the **nuclear envelope**, a dynamic structure composed of an inner and an outer membrane. The dynamic nature of these membranes makes communication possible between the nucleus and the cytoplasmic matrix and allows a continuous channel between the nucleus and the endoplasmic reticulum. At various intervals the two membranes of the nuclear envelope fuse, creating pores in the envelope. Clusters of proteins on the outer nuclear membrane serve as microtubule organization centers (MTOCs); these centers function to begin polymerizing and organizing the microtubules during mitosis. Within the nucleus, a matrix exists to facilitate nuclear functions.

The nucleus (or nuclear matrix) contains substances such as minerals needed for nuclear function and molecules of DNA. DNA encodes the cell's genetic information plus all the enzymes needed for its duplication. DNA is found wrapped around proteins called histones and organized into structures called chromatin. Long strands of DNA and histones are known as chromosomes. Also, within the nucleus is the **nucleolus**, a non-membrane-bound structure, containing ribosomal RNA (rRNA), proteins, and DNA; it is the site of rRNA transcription and processing and of ribosome assembly/synthesis.

Encoded within the nuclear DNA are thousands of **genes** that direct the synthesis of proteins. Each gene can be thought of as a nucleotide sequence that codes for an amino acid sequence representing a single specific protein. Genes are found on chromosomes. Human cells contain 23 pairs of chromosomes, which makes up the genome.

The cell **genome** is the entire set of genetic information, that is, all of the DNA within the cell. During cell division, the 23 pairs of chromosomes are duplicated to create daughter cells. Barring mutations that may arise in the DNA, daughter cells, produced from a parent cell by mitosis, possess the identical genomic makeup of the parent cell. During meiosis (cell reproduction), one from each of the original pairs of chromosomes is found in the sperm or ovum cell. Individuals receive a copy of each gene (allele) from each parent.

The process of DNA replication within cells enables the DNA to be precisely copied at the time of mitosis. After the cell receives a signal that protein synthesis is needed, protein biosynthesis occurs in phases referred to as transcription, translation, and elongation (Figure 1.7). Each phase requires DNA activity, RNA activity, or both. These phases, together with replication, are reviewed briefly in this chapter, but the scope of this subject is large; interested

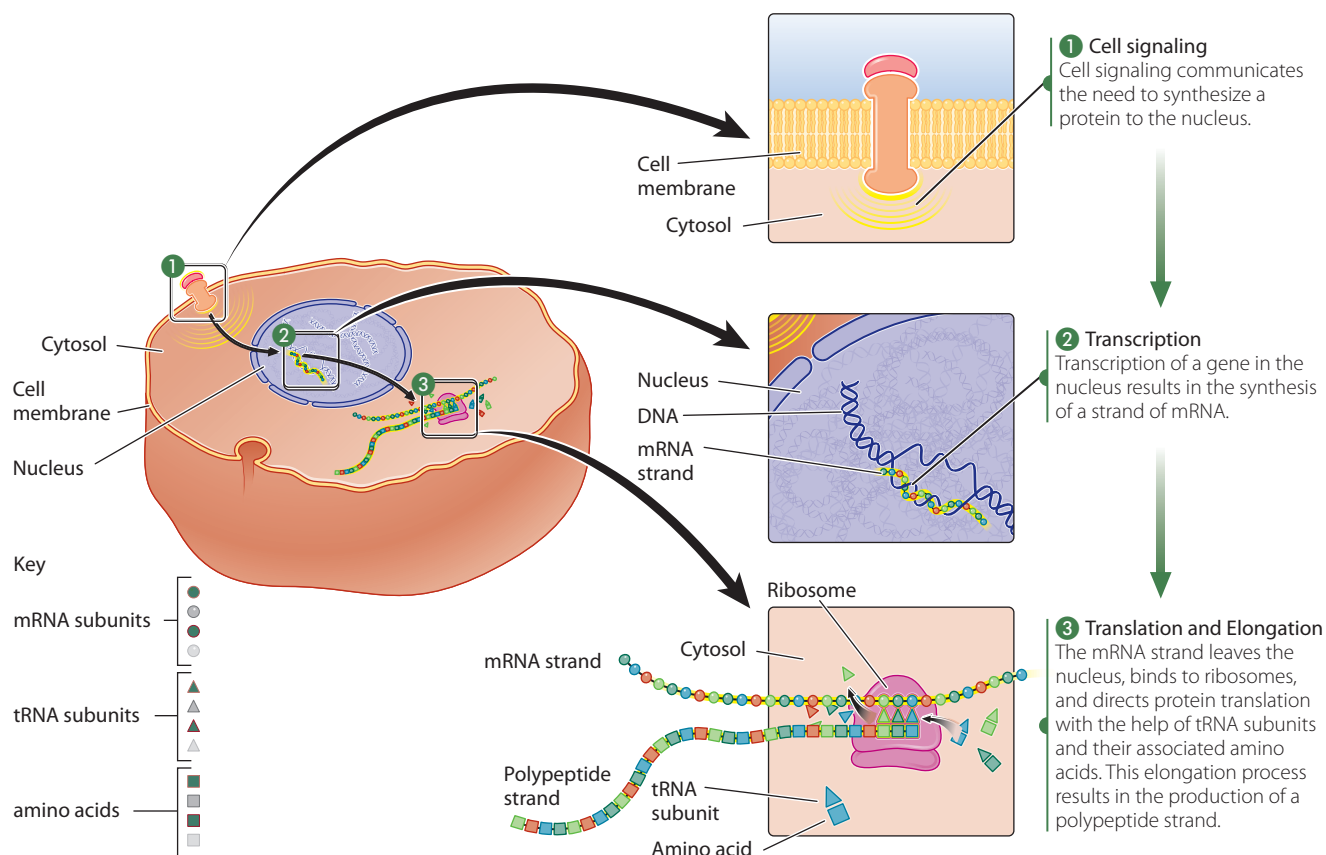


Figure 1.7 Steps of protein synthesis. (1) Signals that protein synthesis needs to occur. (2) Transcription: The DNA molecule (gene) synthesizes the corresponding mRNA. (3) Translation: The corresponding mRNA molecule binds to a ribosome and directs protein synthesis based on the codon for each amino acid and the appropriate tRNA.

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readers should consult a current cell biology text or comprehensive biochemistry text for a more thorough description of protein biosynthesis.

Nucleic Acids

Nucleic acids (DNA and RNA) are macromolecules formed from repeating units called **nucleotides**, sometimes referred to as nucleotide bases or just bases. Structurally, they consist of a nitrogenous core (either purine or pyrimidine), a pentose sugar (ribose in RNA, deoxyribose in DNA), and phosphate. Five different nucleotides are contained in the structures of nucleic acids: adenylic acid and guanylic acid are purines and cytidylic acid, uridylic acid, and thymidylic acid are pyrimidines. The nucleotides are more commonly referred to by their nitrogenous base core only—namely, adenine, guanine, cytosine, uracil, and thymine, respectively. For convenience, particularly in describing the sequence of the polymeric nucleotides in a nucleic acid, the single-letter abbreviations are most often used. Adenine (A), guanine (G), and cytosine (C) are common to both DNA and RNA, whereas uracil (U) is unique to RNA and thymine (T) is found only in DNA. When two strands of nucleic acids interact with each other—as occurs in replication, transcription, and translation—bases in one strand pair specifically with bases in the second strand: A always pairs with T or U and G pairs with C, in what is called **complementary base pairing**.

The nucleotides are connected by phosphates esterified to hydroxyl groups on the pentose—that is, deoxyribose or ribose—component of the nucleotide. The carbon atoms of the pentoses are assigned prime (') numbers for identification. The phosphate group connects the 3' carbon of one nucleotide with the 5' carbon of the next nucleotide in the sequence. The 3' carbon of the latter nucleotide in turn is connected to the 5' carbon of the next nucleotide in the sequence, and so on. Therefore, nucleotides are attached to each other by 3', 5' diester bonds. The ends of a nucleic acid chain are called either the free 3' end or the free 5' end, meaning that the hydroxyl groups at those positions are not attached by phosphate to another nucleotide.

Cell Replication

Cell replication involves the synthesis of daughter DNA molecules that are identical to the parental DNA. At cell division, the cell must copy its genome with a high degree of fidelity. Each strand of the DNA molecule acts as a template for synthesizing a new strand (Figure 1.8). The DNA molecule consists of two large strands of nucleic acid that are intertwined to form a double helix. During cell division the two unravel, with each forming a template for synthesizing a new strand through complementary base pairing. Incoming nucleotide bases first pair with their complementary bases in the template and then are connected through phosphate diester bonds by the enzyme DNA polymerase. The end result of the **replication** process is

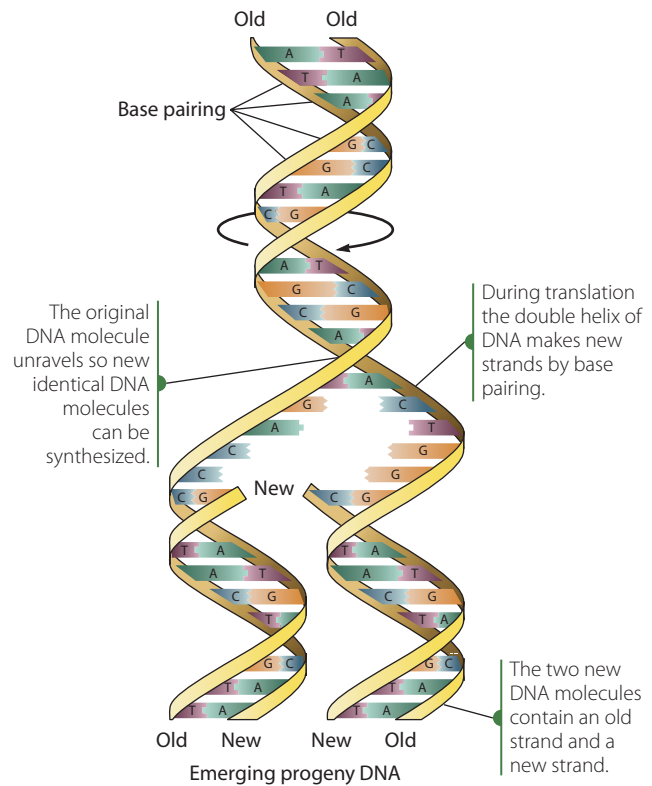


Figure 1.8 DNA replication.

two new DNA chains that join with the two chains from the parent molecule to produce two new DNA molecules. Each new DNA molecule is therefore identical in base sequence to the parent, and each new cell of a tissue consequently carries within its nucleus identical information to direct its functioning. The two strands in the DNA double helix are antiparallel, which means that the free 5' end of one strand is connected to the free 3' end of the other. With this process, a cell is able to copy or replicate its genes before it passes them on to the daughter cell. Although errors sometimes occur during replication, mechanisms exist that correct or repair mismatched or damaged DNA.

Transcription

Transcription is the process by which the genetic information (through the sequence of base pairs) in a single strand of DNA makes a specific sequence of bases in a messenger RNA (mRNA) chain (see Figure 1.7). A single strand of DNA can make many copies of the corresponding mRNA, which become multiple templates for the assembly of a specific protein. This process multiplies the information contained in the DNA to produce many corresponding protein molecules. Transcription may require **transcription factors**, discussed under the subsection “Control of Gene Expression.”

Transcription proceeds continuously throughout the entire life cycle of the cell. In the process, various sections of the DNA molecule unravel, and one strand—called

the **sense strand**—serves as the template for synthesizing mRNA. Sequences of DNA known as promoters allow genes to be turned “off” or “on” and can initiate transcription; this promoter is usually found near (upstream) of the gene. The genetic code (gene) of the DNA is transcribed into mRNA through complementary base pairing, as in DNA replication, except that the purine adenine (A) pairs with the pyrimidine uracil (U) instead of with thymine (T). Genes are composed of critically sequenced base pairs along the entire length of the DNA strand that is being transcribed. A gene, on average, is just over 1,000 base pairs in length, compared with the nearly 5 million (5×10^6) base pair length of typical chromosomal DNA chains. Although these figures provide a rough estimate of the number of genes per transcribed DNA chain, not all the base pairs of a gene are transcribed into functional mRNA.

Many genes for specific proteins are located on regions of the DNA nucleotide sequences that are not adjacent to each other. Those regions that are part of the gene but do not code for a protein product are called **introns** (intervening sequences) and have to be removed from the mRNA before it is translated into protein (see the “Translation” section of this chapter). Enzymes excise the introns from the newly formed mRNA, and the ends of the functional, active mRNA segments are spliced together in a process called post-transcriptional processing. The gene segments that get both transcribed and translated into the protein product are called **exons** (expressed sequences).

Translation

Translation is the process by which genetic information in an mRNA molecule is turned into the sequence of amino acids in the protein. After the mRNA is synthesized in the nucleus (see Figure 1.7), the mRNA is exported into the cytoplasmic matrix, where it is attached to ribosomal RNA (rRNA) of the ribosomes of the RER or to the free-standing polyribosomes (also called polysomes). On the ribosomes, the transcribed genetic code in the mRNA is used to bring amino acids into a specific sequence that produces the specified protein.

The genetic code for specifying the amino acid sequence of a protein resides in the mRNA in the form of three-base sequences called **codons**. Each codon codes for a single amino acid. Although a given amino acid may have several codons (e.g., the codons CUU, CUC, CUA, and CUG all code for the amino acid leucine), codons can code for only one amino acid. Each amino acid has one or more transfer RNAs (tRNAs), which deliver the amino acid to the mRNA for peptide synthesis. The three-base sequences of the tRNA attach to the codons by complementary base pairing.

Amino acids are first activated by ATP at their carboxyl end and then transferred to their specific tRNAs that bear the **anticodon** complementary to each amino acid's codon. For example, because codons that code for leucine are

sequenced CUU, CUC, CUA, or CUG, the only tRNAs to which an activated leucine can be attached would need to have the anticodon sequence GAA, GAG, GAU, or GAC. The tRNAs then bring the amino acids to the mRNA situated at the protein synthesis site on the ribosomes. After the amino acids are positioned according to codon–anticodon association, peptide bonds are formed between the aligned amino acids in a process called **elongation** (see Figure 1.7). Elongation extends the polypeptide chain of the protein product by translation. Each incoming amino acid is connected to the end of the growing peptide chain with a free carboxyl group (C-terminal end) by formation of further peptide bonds. New amino acids are incorporated until all the codons (corresponding to one completed protein or polypeptide chain) of the mRNA have been translated. At this point, the process stops, signaled by a “nonsense” codon that does not code for any amino acid. The completed protein dissociates from the mRNA. After translation, the newly synthesized protein may require some chemical, structural, or spatial (three-dimensional) modification to attain its active form.

Post-translational modifications of proteins may involve, for example, the covalent addition of functional groups or the cleavage of a portion of the protein. Common modifications include phosphorylation as well as glycosylation, ubiquitination, methylation, and acetylation, among others. An example of protein modifications involving proteolytic cleavage is that needed to convert zymogens, such as those involved in protein digestion, to active enzymes.

Control of Gene Expression

Each cell in the body contains a complete set of genes. Only a portion of the genes are expressed in specialized cells of a given organ. The regulation of gene expression occurs primarily at three different levels.

- Transcription-level control mechanisms determine if a particular gene can be transcribed. Transcriptional control is accomplished by large numbers of proteins (called transcriptional factors) that bind to the DNA at a site other than the one involved in serving as a template for the mRNA. These transcriptional factors can enhance, inhibit, or, in some cases, alter the frequency (number of times transcription occurs within a specified time span) of the gene's transcription. Several hormones, such as insulin, thyroid hormone, glucagon, and glucocorticoids, as well as nutrients, such as essential fatty acids and vitamins A and D, can alter the transcription of DNA by binding along with transcription-factor proteins to DNA. Expression may be activated or silenced fully or partially to meet the ever-changing needs of the cells; these actions often occur to a greater extent in metabolically active (vs. lesser active) cells such as in the liver. Further examples of such interactions

are discussed in the section on iron in Chapter 12. The effects of nutrients and bioactive dietary components on gene expression can also occur by more indirect means. The communication, for example, may result from interactions with cell surface receptors to trigger **signal transduction**, a cascade of events that can lead to the **translocation** of a transcription factor to the nucleus, where it can then bind DNA and turn gene expression on or off, as appropriate.

- Processing-level control mechanisms determine the path by which mRNA can be translated into a polypeptide. This mechanism of regulating gene expression is based on the splicing of RNA molecules, thus making it possible for one gene to code for two associated proteins.
- Translation-level control mechanisms determine whether a particular mRNA is actually translated and, if so, how often and for how long. The translation-level control mechanism can involve the localization of the mRNA in a particular part of the cell or organ. It can also operate through interactions between specific mRNAs and various small RNA strands present within the cytosol. **MicroRNAs** (abbreviated miRNA) are small noncoding RNAs, about 19–25 nucleotides in length, that silence gene expression post-translationally by binding to the 3' end of untranslated mRNA to inhibit its translation and/or promote its degradation. MicroRNAs are thought to regulate about one-third of the protein-coding genome and affect multiple cellular processes, including cell differentiation, proliferation, cell cycle progression, and apoptosis.

For more detailed information on the control of gene expression and its relationship to disease, which is vastly more complex than has been presented here, the reader is referred to a recent textbook on molecular biology and biochemistry or cell biology.

Endoplasmic Reticulum and Golgi Apparatus

The **endoplasmic reticulum (ER)** is an extensive network of membranous channels pervading throughout the cytosol and providing continuity among the nuclear envelope, the Golgi apparatus, and the plasma membrane (see Figure 1.1). This structure, therefore, is a mechanism for communication from the innermost part of the cell to its exterior. In the laboratory, however, the ER cannot be separated from the cell as an isolated entity; during mechanical homogenization, the structure is disrupted and reforms into small spherical particles called microsomes.

The ER is classified as either rough (granular) or smooth (agranular). The granularity or lack of granularity is determined by the presence or absence of ribosomes. Rough endoplasmic reticulum, so named because it is studded

with ribosomes, abounds in cells where protein synthesis is a primary function. Smooth endoplasmic reticulum (SER) is found in most cells; however, because it is the site of synthesis for a variety of lipids, it is more abundant in cells that synthesize steroid hormones (e.g., within the adrenal cortex and gonads) and in liver cells, which synthesize fat transport molecules (the lipoproteins). In skeletal muscle, the smooth endoplasmic reticulum is called **sarcoplasmic reticulum** and is the site of the calcium ion pump, a necessity for the contractile process.

Ribosomes associated with RER are composed of ribosomal RNA and structural protein. All proteins to be secreted (or excreted) from the cell or destined to be incorporated into an organelle membrane in the cell are synthesized on the RER. The clusters of ribosomes (i.e., polyribosomes or polysomes) that are freestanding in the cytosol are also the synthesis site for some proteins. All proteins synthesized in polyribosomes in the cytosol remain within the cytoplasmic matrix or are incorporated into an organelle.

Located on the RER of liver cells is a system of enzymes important in metabolizing many different drugs. This enzyme complex consists of a family of **cytochromes** called the P450 system that functions along with other enzymes. The P450 system is particularly active in oxidizing drugs, but because its action results in the simultaneous **oxidation** of other compounds as well, the system is collectively referred to as the mixed-function oxidase system. **Lipophilic** substances—such as steroid hormones and numerous drugs—can be made **hydrophilic** by oxidation, reduction, or hydrolysis to enable their excretion in the bile or urine. This system is discussed further in Chapter 5.

The **Golgi apparatus** functions closely with the ER in trafficking and sorting proteins that are synthesized in the cell; it is particularly prominent in neurons and secretory cells. It consists of four to eight membrane-enclosed, flattened cisternae that are stacked in parallel (see Figure 1.1). The Golgi cisternae are often referred to as “stacks” because of this arrangement. Tubular networks are present at either end of the Golgi stacks.

- The *cis*-Golgi network is a compartment that accepts newly synthesized proteins coming from the ER.
- The *trans*-Golgi network is the exit site of the Golgi apparatus. It sorts proteins for delivery to their next destination.

Proteins destined for the Golgi apparatus form within the RER. Once they are transferred to the Golgi apparatus, additional molecules (such as carbohydrates or lipids) can be added to them there. The Golgi apparatus is the site for membrane differentiation and the development of surface specificity. For example, the polysaccharide moieties of mucopolysaccharides and of the membrane glycoproteins are synthesized and attached to the protein

during its passage through the Golgi apparatus. Such an arrangement allows for the continual replacement of cellular membranes, including the plasma membrane.

The ER is a quality-control organelle in that it prevents proteins that have not achieved their normal tertiary or quaternary structure from reaching the cell surface. The ER can retrieve or retain proteins destined for residency within the ER, or it can target proteins for delivery to the *cis*-Golgi compartment. Retrieved or exported protein “cargo” is coated with protein complexes called coatomers, abbreviated COPs (coat proteins). Some coatomers are structurally similar to the clathrin coat of endocytic vesicles and are described later in this chapter. The choice of what is retrieved or retained by the ER and what is exported to the Golgi apparatus is mediated by signals that are inherent in the terminal amino acid sequences of the proteins in question. Certain amino acid sequences of cargo proteins are thought to interact specifically with certain coatomers.

The membrane-bound compartments of the ER and the Golgi apparatus are interconnected by transport vesicles, in which cargo proteins are moved from compartment to compartment. The vesicles leaving a compartment are formed by a budding and pinching-off of the compartment membrane, and the vesicles then fuse with the membrane of the target compartment.

Secretion of products such as proteins from the cell can be either constitutive or regulated. If secretion follows a constitutive course, the secretion rate remains relatively constant, uninfluenced by external regulation. Regulated secretion, as the name implies, is affected by regulatory factors, and therefore its rate is changeable.

Among the more interesting areas of biomolecular research has been determining how newly synthesized proteins find their way from the ribosomes to their intended destinations. While proteins synthesized on the free polyribosomes remain within the cell to perform their specific structural, digestive, regulatory, or other functions, other proteins are destined elsewhere. At the time of synthesis, signal sequences direct proteins to their appropriate target compartment. These targeting sequences, located at the N-terminus of the protein, are generally cleaved (though not always) when the protein reaches its destination. Interaction between the signal sequences and specific receptors located on the various membranes permits the protein to enter its designated membrane or become incorporated into the designated organelle. It is believed that in at least some cases, diseases result not just from the synthesis of enzymes that are inactive or deficient, but also result from the synthesis of proteins that fail to reach their correct destination.

Lysosomes and Peroxisomes

Lysosomes and **peroxisomes** are cell organelles packed with enzymes. Whereas the lysosomes (see Figure 1.1) serve as the cell’s digestive system, the peroxisomes

perform some specific oxidative catabolic reactions. Lysosomes are found in all cells, with the exception of red blood cells, but in varying numbers. Approximately 36 enzymes capable of degrading substances such as proteins, polysaccharides, nucleic acids, and phospholipids are held within the confines of a single thick lysosomal membrane. The membrane surrounding these catabolic enzymes has the capacity for selective fusion with other vesicles so that **catabolism** (or degradation) may occur as necessary. Further information on the role of lysosomes in protein and cell turnover is provided in Chapter 6.

Peroxisomes are small, intracellular, enzyme-containing organelles surrounded by a single bilayer membrane. The membrane has membrane-spanning pores (channels) through which small compounds/solutes may diffuse. Peroxisomes are believed to originate by “budding” from the smooth endoplasmic reticulum. The peroxisomes are similar to the lysosomes; however, rather than having digestive action, the peroxisomal enzymes are catabolic oxidative enzymes. Very-long-chain fatty acids and some methyl-branched fatty acids are oxidized in peroxisomes, whereas most other fatty acids are oxidized in the mitochondrial matrix. Peroxisomes are also the site for certain reactions of amino acid catabolism and for the oxidation of ethanol to acetaldehyde. Hydrogen peroxide (H_2O_2) is often produced within peroxisomes; this peroxisomal segregation from other cell parts is helpful given the reactive and destructive nature of H_2O_2 to cell components. The presence of the enzyme catalase within peroxisomes is also helpful for H_2O_2 degradation into water and molecular oxygen.

1.2 SELECTED CELLULAR PROTEINS

Two roles of cellular proteins are discussed; these roles include **receptors**, that is, proteins that modify the cell’s response to its environment, and **enzymes**, that is, proteins serving as catalysts for biochemical reactions within cells. The reader is directed to Chapter 6 for information on other roles of proteins in the body.

Receptors

Receptors are highly specific proteins located in the plasma membrane and facing the exterior of the cell. Bound to the outer surface of these specific proteins are oligosaccharide chains, which are believed to act as recognition markers. Membrane receptors act as attachment sites for specific external stimuli such as hormones, growth factors, antibodies, lipoproteins, and certain nutrients (examples are shown in Figures 1.9 and 1.10). These molecular stimuli, which bind specifically to receptors, are called **ligands**.

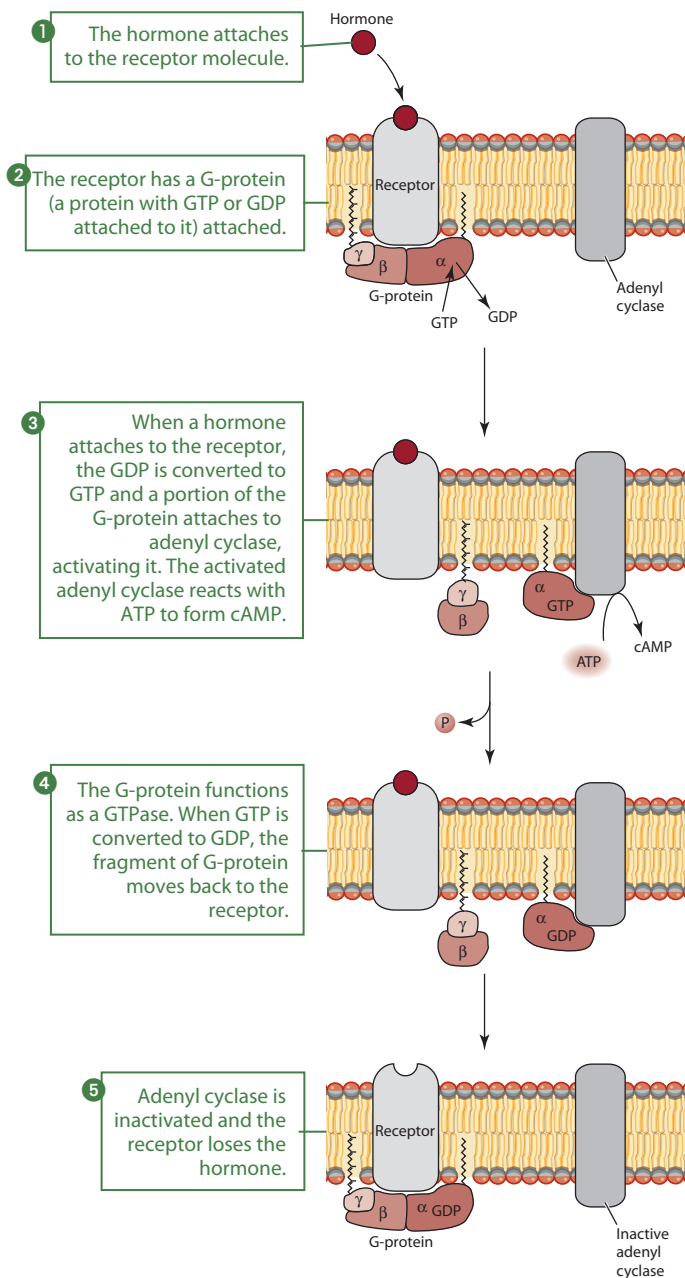
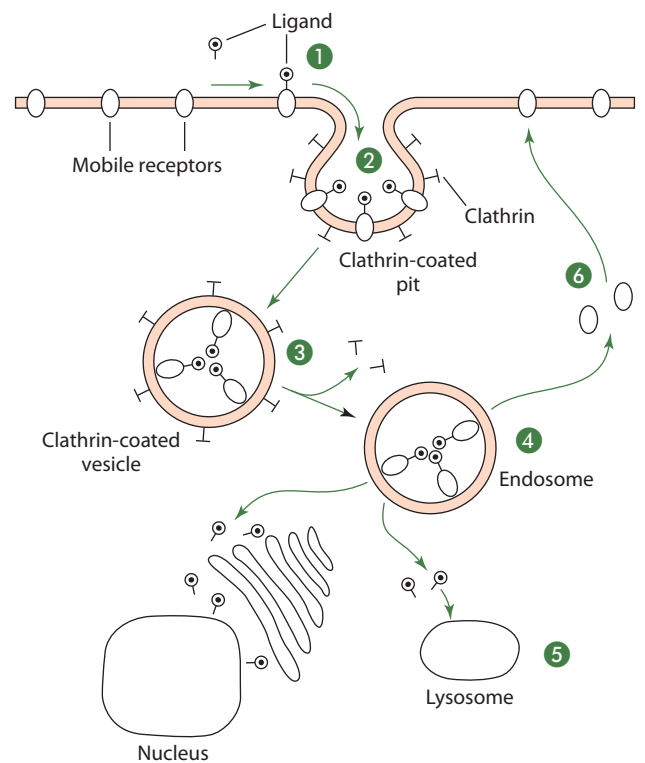


Figure 1.9 Example of an internal chemical signal by a second messenger.

Receptors are also located on the membranes of cell organelles; less is known about these receptors, but they appear to be glycoproteins necessary for correctly positioning newly synthesized cellular proteins.

Although most receptor proteins are probably integral membrane proteins, some may be peripheral. In addition, receptor proteins can vary widely in their composition and mechanism of action. Although the composition and mechanism of action of many receptors have not yet been determined, at least three distinct types of receptors are known to exist and are listed and described hereafter:

- Those that generate internal chemical signals
- Those that function as ion channels
- Those that internalize stimuli.



- 1 Ligand binds with its receptor on the cell membrane.
- 2 Ligand and receptor move into a clathrin-coated pit.
- 3 Pit closes off and forms a clathrin-coated vesicle.
- 4 The vesicle forms an endosome.
- 5 Ligand can be used by the cell or undergo lysosomal degradation.
- 6 Receptor is recycled to the surface of the cell membrane.

Figure 1.10 Internalization of a stimulus into a cell via its receptor.

Receptors That Generate Internal Chemical Signals

Upon interaction between some receptors and ligands, an internal chemical signal is generated to affect internal cellular processes. The internal chemical signal most often produced by a stimulus–receptor interaction is 3', 5'-cyclic adenosine monophosphate (cyclic adenosine monophosphate [AMP], or cAMP). It is formed from adenosine triphosphate (ATP) by the enzyme adenylyl cyclase. Cyclic AMP is frequently referred to as the second messenger in the stimulation of target cells by hormones. Figure 1.9 presents a model for the ligand-binding action of receptors, which leads to production of the internal signal cAMP. As shown in the figure, the stimulated receptor reacts with guanosine triphosphate (GTP)–binding protein (G-protein), which activates adenylyl cyclase, triggering production of cAMP from ATP. G-protein is a trimer with three subunits (designated α , β , and γ). The α -subunit binds with GDP or GTP and has GTPase activity.

Attachment of a hormone to the receptor stimulates the exchange of GDP for GTP. The GTP binding causes the trimers to disassociate and the α unit to associate with an effector protein, adenyl cyclase. A single hormone-binding site can produce many cAMP molecules.

The mechanism of action of cAMP signaling within the cell is complex, but it can be viewed briefly as follows. cAMP is an activator of protein kinases. **Protein kinases** are enzymes that phosphorylate (add phosphate groups to) other enzymes and, in doing so, generally convert the enzymes from inactive forms into active forms. Protein kinases that can be activated by cAMP contain two subunits: one catalytic and one regulatory. In the inactive form of the kinase, the two subunits are bound in such a way that the catalytic portion of the molecule is inhibited sterically by the presence of the regulatory subunit. Phosphorylation of the enzyme by cAMP causes the subunits to dissociate, thereby freeing the catalytic subunit, which regains its full catalytic capacity. As protein kinases serve to phosphorylate proteins and generally activate them, phosphatases work in opposition in order to remove phosphate groups from proteins and inactivate them. Thus, together the protein kinases and phosphatases function to turn on and off enzymes.

Many intracellular chemical messengers are known other than those cited as examples in this section. Listed here, along with cAMP, are several additional examples:

- Cyclic AMP (cAMP)
- Cyclic GMP (cGMP)
- Ca^{2+}
- Inositol triphosphate
- Diacyl glycerol
- Fructose-2,6-bisphosphate.

Receptors that Function as Ion Channels

Receptors can also act as ion channels. In some cases, the binding of the ligand to its receptor causes a voltage change, which then becomes the signal for a cellular response. Such is the case when the neurotransmitter acetylcholine is the stimulus. The receptor for acetylcholine appears to function as an ion channel in response to voltage change. Stimulation by acetylcholine signals the channels to open, allowing sodium (Na^+) ions to pass through an otherwise impermeable membrane.

Receptors That Internalize Stimuli

The internalization of a stimulus into a fibroblast by way of its receptor is illustrated in Figure 1.10. Receptors that perform in such a manner exist for a variety of biologically active molecules, including several hormones. Low-density lipoproteins (LDLs) are taken up by certain cells in much the same fashion (see Chapter 5), except that their receptors, rather than being mobile, are already clustered in coated pits. These pits, vesicles formed from the plasma membrane,

are coated with several proteins, among which clathrin is primary. A coated pit containing the receptor with its ligand soon loses the clathrin coating and forms a smooth-walled vesicle. This vesicle delivers the ligand into the cell and then is recycled, along with the receptor, into the plasma membrane. If the endocytotic process is for scavenging, the ligand (perhaps a protein) is not used by the cell but instead undergoes lysosomal degradation, as shown in Figure 1.10 and exemplified by the endocytosis of LDL.

Receptors' Role in Homeostasis

The cells of every organ in the body have specialized receptors that respond to changes in external conditions. The reaction of a fibroblast to changes in blood glucose level is a good example of cellular adjustment to the existing environment that is made possible through receptor proteins. When blood glucose levels are low, the hormone epinephrine is released by the adrenal medulla. Epinephrine attaches to and activates its receptor protein on the fibroblast, thereby causing it to stimulate G-protein and adenyl cyclase, which catalyzes the formation of cAMP from ATP. Then cAMP initiates a series of enzyme phosphorylation modifications, as described earlier in this section, which ultimately generate glucose-1-phosphate for use by the fibroblast. In contrast, when blood glucose levels are elevated, the hormone insulin is secreted by the β -cells of the pancreas and reacts with receptors on the fibroblast membrane. Insulin facilitates glucose entry by increasing the number of cell membrane glucose receptors, which transport glucose in the cell. (Glucose transporters are covered in Chapter 3.)

Catalytic Proteins (Enzymes)

Enzymes, which are found in all cellular compartments, are catalysts that take part in a reaction but are not part of the final product of that reaction. Some enzymes function externally (such as within the digestive tract); examples include some digestive enzymes, such as isomaltase, lactase, sucrase, maltase, and some peptidases, which are located on the brush border membrane of the epithelial cells lining the small intestine. Other enzymes that are components of the cellular membranes and most enzymes associated with organelle membranes are found on the inner membrane surface. For example, the enzymes of the electron transport chain are located within the inner membrane of the mitochondria.

Enzymes have an “active site” where they bind with a substrate. The functional activity of some enzymes, however, depends not only on the enzyme’s protein portion, but also on a nonprotein prosthetic group or coenzyme/cofactor. Many of the B-vitamins serve as coenzymes and several minerals—such as Mg, Zn, Cu, Mn, and Fe—serve as inorganic prosthetic groups (or cofactors) for enzymes.

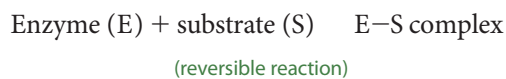
An enzyme’s active site possesses high specificity. This means that a substrate must “fit” perfectly into the

specific contours of the enzyme's active site so that the reacting parts of the substrate are in close proximity to the reacting parts of the enzyme. The most common analogy used to describe this is a lock and key. The concept of interlocking pieces of a puzzle has also been used to convey that the substrate and enzyme must fit. The enzyme's specificity can come from the reactive groups of its amino acids as part of the amino acid sequence or primary structure. The specificity may also originate from the three-dimensional or tertiary structure of the enzyme. Mutations in genes that alter a protein's amino acid composition can result in changes in enzyme structure and/or its active site and thus affect its ability to bind to its substrate(s). Such defects can lead to inborn errors (genetic disorders) of metabolism such as phenylketonuria.

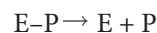
The velocity of an enzyme-catalyzed reaction (the number of molecules of substrate reacted on in a specified time) increases if all of the active sites on the enzyme are "filled" with substrate. As the concentration of the substrate increases, the number of molecules of substrate available to the enzyme increases. This increases the number of substrate molecules acted on by the enzyme-catalyzed reaction and is said to increase the rate of the reaction. However, this relationship applies only to a concentration of substrate that is less than the concentration that "saturates" the enzyme. At saturation levels of substrate, the enzyme functions at its maximum velocity ($V_{\max}$), and the occurrence of a still higher concentration of substrate cannot increase the velocity further.

The velocity of a chemical reaction is defined by an equilibrium constant. For enzyme-catalyzed reactions this equilibrium constant is known as K_m , or the Michaelis constant. K_m is a useful parameter that aids in establishing how enzymes react in the living cell. K_m represents the concentration of a substrate that is found in an occurring reaction when the reaction is at one-half its maximum velocity. If an enzyme has a high K_m value, then an abundance of substrate must be present to raise the rate of reaction to half its maximum velocity; in other words, the enzyme has a low affinity for its substrate and it takes more substrate to react with the active site of the enzyme. An example of an enzyme with a high K_m is glucokinase, found in the liver cells. Because glucose can diffuse freely into the liver, the fact that glucokinase has a high K_m is very important to blood glucose regulation. If glucokinase had a low K_m affinity for glucose, too much glucose would be removed from the blood during periods of fasting. Glucokinase (with its high K_m but low affinity) can still convert excess glucose to glucose phosphate when the glucose load is high—for example, following a high-carbohydrate meal; however, the liver glucokinase does not function at its maximum velocity when glucose levels are in the normal range. The enzyme thus protects against high cellular concentrations of glucose.

The nature of enzyme catalysis can be described by the following reactions:



The substrate activated by combining with the enzyme is converted into an enzyme-product (E-P) complex through rearrangement of the substrate's ions and atoms:



The product is released, and the enzyme is free to react with more of the substrate.

Reversibility

Most biochemical reactions are reversible, meaning that the same enzyme catalyzes a reaction in both directions. The extent to which a reaction can proceed in a reverse direction depends on several factors, the most important of which are the relative concentrations of substrate (reactant) and product and the differences in energy content between reactant and product. In instances when a large disparity in either energy content or concentration exists between reactant and product, the reaction can proceed in only one direction. Such a reaction is unidirectional rather than reversible. This topic is discussed later in this chapter. In unidirectional reactions, the same enzyme cannot catalyze in both directions. Instead, a different enzyme is required to catalyze the reverse direction of the reaction. Comparing glycolysis (the oxidation of glucose) with gluconeogenesis (the synthesis of glucose) allows us to see how unidirectional reactions may be reversed by introducing a different enzyme.

Simultaneous reactions, catalyzed by various multienzyme systems or pathways, constitute cellular metabolism. Enzymes are compartmentalized within the cell and function in sequential chains. An example of a multienzyme system is the TCA cycle located in the mitochondrial matrix. Each sequential reaction is catalyzed by a different enzyme, and some reactions are reversible, whereas others are unidirectional. Although some reactions in almost any pathway are reversible, it is important to understand that removal of one of the products (by that product reacting to produce the next compound in the pathway) drives the reaction toward forming more of that product. Removing (or using) the product, then, becomes the driving force that causes reactions to proceed primarily in the desired direction.

Regulation

An important aspect of nutritional biochemistry is the regulation of metabolic pathways. Anabolic (synthetic) and catabolic (oxidative) reactions must be kept in a balance

appropriate for life (and perhaps growth). Regulation primarily involves the adjustment of the catalytic activity of certain participating enzymes. This enzyme regulation occurs through three major mechanisms:

- Covalent modification of enzymes (also referred to as post-translational modification)
- Modulation of allosteric enzymes
- Increase in enzyme concentration by induction (synthesis of more enzyme).

Covalent Modification With the first of these mechanisms, covalent modification, the enzyme is inactive until a post-translational modification is made. This is usually achieved by the addition or hydrolytic removal of phosphate groups to or from the enzyme, as previously discussed in the subsection “Receptors That Generate Internal Chemical Signals.” One example of covalent modification of enzymes is the regulation of glycogenesis (synthesis of glycogen from glucose) and glycogenolysis (breakdown of glycogen to glucose) (see Chapter 3). Another covalent modification involves cleavage; for example, some enzymes (like those secreted into the digestive tract to digest proteins) are synthesized as inactive proenzymes (also called zymogens). To activate the proenzyme (make it a functional enzyme), a portion of it is hydrolyzed.

Allosteric Enzyme Modulation A second regulatory mechanism is that exerted by certain unique enzymes called allosteric enzymes. The term *allosteric* refers to the fact that these enzymes possess an allosteric or specific “other” site besides the catalytic site. Specific compounds, called modulators, can bind to these allosteric sites and profoundly influence the activity of these regulatory enzymes. Modulators may be positive (i.e., causing an increase in enzyme activity) or they may exert a negative effect (i.e., inhibit activity). Modulating substances are believed to alter the activity of the allosteric enzyme by changing the conformation (three-dimensional structure) of the polypeptide chain or chains of the enzyme, thereby altering the binding of its catalytic site with the intended substrate. Negative modulators are often the end products of a sequence of reactions. As an end product accumulates above a certain critical concentration, it can inhibit, through an allosteric enzyme, its own further production.

An excellent example of an allosteric enzyme is phosphofructokinase in the glycolytic pathway. Glycolysis gives rise to pyruvate, which is decarboxylated and oxidized to acetyl-CoA, which enters the mitochondrion and is further oxidized by the TCA cycle by combining with oxaloacetate to form citrate. Citrate is a negative modulator of phosphofructokinase. Therefore, an accumulation of citrate in the cell matrix causes the glycolytic pathway to be inhibited by regulating phosphofructokinase. In contrast,

an accumulation of AMP or adenosine diphosphate (ADP), which indicates that ATP is depleted, signals the need for additional energy in the cell in the form of ATP. AMP or ADP therefore modulates phosphofructokinase positively. The result is an active glycolytic pathway that ultimately leads to the formation of more ATP through the TCA cycle–electron transport chain connection.

Allosteric mechanisms of regulation are considered to be of one of two types. In one type, the K series, the K_m is affected, which alters the binding of the substrate to the enzyme. If the allosteric effect is positive, the enzyme can become “saturated” at a lower concentration. The other type of allosteric regulation, called the V series, increases the maximum velocity of the enzymatic reaction. If the allosteric effector is an inhibitor, the maximum velocity (V_m) of the reaction will be decreased.

Induction The third mechanism of enzyme regulation, *enzyme induction*, creates changes in the concentration of certain inducible enzymes by increasing enzyme synthesis. Inducible enzymes are adaptive, meaning that they are synthesized at rates dictated by cellular circumstances. In contrast, constitutive enzymes, which are synthesized at a relatively constant rate, are uninfluenced by external stimuli. Induction usually occurs through the action of certain hormones, such as the steroid hormones and the thyroid hormones, and is exerted through changes in the expression of genes encoding the enzymes. Dietary changes can elicit the induction of some enzymes necessary to cope with the changing nutrient load. This regulatory mechanism is relatively slow, however, compared to the first two mechanisms, which exert their effects in terms of seconds or minutes.

The reverse of induction is the blockage of enzyme synthesis by blocking the formation of the mRNA of specific enzymes. This regulation of translation is one of the means by which small molecules, reacting with cellular proteins, can exert their effect on enzyme concentration and the activity of metabolic pathways.

Specific examples of enzyme regulation are described in subsequent chapters addressing nutrient metabolism. It should be noted at this point, however, that *enzymes targeted for regulation essentially catalyze unidirectional reactions*. In every metabolic pathway, at least one reaction is essentially irreversible, exergonic, and enzyme limited. That is, the rate of the reaction is limited only by the activity of the enzyme catalyzing it. Such enzymes are frequently called the regulatory enzymes, capable of being stimulated or suppressed by one of the mechanisms described. Logically, an enzyme catalyzing a reaction reversibly at near equilibrium in the cell cannot be a regulatory enzyme because its up- or downregulation would affect its forward and reverse activities equally. This effect, in turn, would not accomplish the purpose of regulation,

which is to stimulate the rate of the metabolic pathway in one direction to exceed the rate of the pathway in the reverse direction.

Examples of Enzyme Types

Enzymes participating in cellular reactions are located throughout the cell in both the cytoplasmic matrix and the various organelles. The location of specific enzymes depends on the site of the metabolic pathways or metabolic reactions in which those enzymes participate. Enzyme classification, therefore, is based on the type of reaction catalyzed by the various enzymes. Enzymes fall within six general classifications:

- **Oxidoreductases** (dehydrogenases, reductases, oxidases, peroxidases, hydroxylases, and oxygenases) are enzymes that catalyze all reactions in which one compound is oxidized and another is reduced. Examples of oxidoreductases are the enzymes found in the electron transport chain located on the inner membrane of the mitochondria. Other examples are the cytochrome P450 enzymes located on the ER of liver cells.
- **Transferases** are enzymes that catalyze reactions not involving oxidation and reduction in which a functional group is transferred from one substrate to another. Included in this group of enzymes are transketolase, transaldolase, transmethylase, and the transaminases. The transaminases (α -amino transferases), which figure so prominently in protein metabolism, are located primarily in the mitochondrial matrix.
- **Hydrolases** (esterases, amidases, peptidases, phosphatases, and glycosidases) are enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by adding water. Digestive enzymes fall within this classification, as do those enzymes contained within lysosomes.
- **Lyases** (decarboxylases, aldolases, synthetases, cleavage enzymes, deaminases, nucleotide cyclases, hydrases or hydratases, and dehydratases) are enzymes that catalyze cleavage of carbon–carbon, carbon–sulfur, and certain carbon–nitrogen bonds (peptide bonds excluded) without hydrolysis or oxidation–reduction. Citrate lyase, which frees acetyl-CoA for fatty acid synthesis in the cytosol, is a good example of an enzyme belonging to this classification.
- **Isomerases** (racemases, epimerases, and mutases) are enzymes that catalyze the interconversion of optical or geometric isomers. Phosphohexose isomerase, which converts glucose-6-phosphate to fructose-6-phosphate in glycolysis (occurring in the cytosol), exemplifies this particular class of enzyme.
- **Ligases** are enzymes that catalyze the formation of bonds between carbon and a variety of other atoms, including oxygen, sulfur, and nitrogen. Forming bonds

catalyzed by ligases requires energy that usually is provided by hydrolysis of ATP. An example of a ligase is acetyl-CoA carboxylase, which initiates fatty acid synthesis in the cytosol. Through the action of acetyl-CoA carboxylase, a bicarbonate ion (HCO_3^-) is attached to acetyl-CoA to form malonyl-CoA, the initial compound formed in the synthesis of fatty acids.

Clinical Applications of Cellular Enzymes

Enzymes in the body are synthesized intracellularly, and most of them function within the cell in which they were formed. Variations in amino acid sequence are not uncommon among some enzymes that catalyze the same reaction but are found in different tissues (such as the liver, muscle, and heart); such enzymes may be referred to as isozymes (or isoenzymes or protein isomers). Once made, some enzymes are secreted in an inactive form and are rendered active in the extracellular fluids where they function. Those that function in the blood are called plasma-specific enzymes.

Diagnostic enzymology focuses on intracellular enzymes, which, because of a problem within the cell structure, escape from the cell and ultimately express their activity in the serum. By measuring the serum activity of these released enzymes, both the site and often the extent of the cellular damage may be determined. If the site of the damage is to be determined with reasonable accuracy, the enzyme being measured must exhibit a relatively high degree of organ or tissue specificity. For instance, lactate dehydrogenase (LDH) is an enzyme that is widely distributed among cells such as the heart, liver, skeletal muscle, lymph nodes, erythrocytes, and platelets. Elevated serum levels of LDH do not have diagnostic value until the enzyme is separated into its five different isozyme forms and each is measured individually. Each isozyme is organ specific. The amount of elevation of the isozyme from the heart is an indication of the extent of tissue damage following, for example, a heart attack.

Intracellular enzymes are normally retained within the cell where they are produced by the plasma membrane. The plasma membrane is metabolically active, and its integrity depends on the local environment. Any process, for example, that impairs the cell's use of nutrients can compromise the structural integrity of the plasma membrane. Membrane failure can also arise from mechanical disruption, such as would be caused by a viral attack on the cell. Damage to the plasma membrane is manifested as leakiness and eventual cell death, allowing an unimpeded passage of substances, including enzymes, from intracellular to extracellular compartments such as the blood.

Factors contributing to cellular damage and resulting in abnormal egress of cellular enzymes include, for example, hypoxia (inadequate oxygen supply), tissue necrosis and **ischemia** (impaired blood flow to a tissue or part of a tissue that in turn deprives affected cells of oxygen and

nutrients), and damage from viral attack or organic chemicals such as alcohol and organophosphorus pesticide. Increased production of enzymes and other substances can also cause a spike in its serum concentration. Cancers affecting certain tissues can cause such increases. Substances that occur in body fluids as a result of malignant disease are called tumor markers. A tumor marker may be produced by the tumor itself or by the host, in response to a tumor. In addition to enzymes and isozymes, other forms of tumor markers include hormones, oncofetal protein antigens such as carcinoembryonic antigen (CEA), and products of oncogenes. **Oncogenes** are mutated genes that encode abnormal, mitosis-signaling proteins, which, in turn, can promote unregulated cell division.

Increases in blood serum concentrations of cellular enzymes can be indicators of even minor cellular damage because the intracellular concentration of enzymes is hundreds or thousands of times greater than in blood. However, not all intracellular enzymes are valuable in diagnosing damage to the cells in which they are contained. Several conditions must be met for the enzyme to be suitably diagnostic:

- The enzyme must have a sufficiently high degree of organ or tissue specificity.
- A steep concentration gradient of enzyme activity must exist between the interior and exterior of the cells under normal conditions. This makes small increases in serum activity detectable (assuming the laboratory assay is sensitive).
- The enzyme must function in the cytosol of the cell so that it leaks out whenever the plasma membrane suffers significant damage.
- The enzyme must be stable for a reasonable time period in the vascular compartment.

1.3 APOPTOSIS

Dying is said to be a normal part of living. So, it is with the cell. Like every living thing, a cell has a well-defined lifespan, after which its structural and functional integrity diminishes and it is removed from the body. Many terms have been used to describe naturally occurring cell death. It is now most commonly referred to as *programmed cell death*, to distinguish it from pathological cell death (necrosis), which is not part of the normal physiological process and uncontrolled. The term describing programmed cell death is **apoptosis**, a word borrowed from the Greek meaning to “fall out.”

Cells are constantly turned over in the body. For instance, 10^{10} neutrophils (a type of white blood cell) die and are replaced each day. As cells die, they are replaced by new cells that are continuously being formed through

cell mitosis. However, both daughter cells formed in the mitotic process do not always enjoy the full lifespan of the parent. If they did, the number of cells, and consequently tissue mass, could increase inordinately. Therefore, one of the two cells produced by mitosis generally is programmed to die before its sister. In fact, most dying cells are already doomed at the time they are formed. Those targeted for death are usually smaller than their surviving sisters, and their degradation begins even before the mitosis generating them is complete. The processes of cell division and cell death must be carefully regulated to generate the proper number of cells during development. Once cells mature, the appropriate number of cells must be maintained.

Apoptotic cell death (and cell survival) is brought about by two general mechanisms. An intracellular (or intrinsic) pathway can be triggered by several different stimuli, stress or signals that damage has occurred. Some examples include irreparable DNA damage, hypoxia, cytokine deprivation, calcium flux, and glucose deprivation, among others. Upon stimulation, proapoptotic factors (such as Bax, Bad, Bid, Noxa, and PUMA) are released into the cytosol from the mitochondria secondary to increased outer mitochondrial membrane permeability. Activation of mitochondrial death signaling occurs via the release of cytochrome c (among other cytotoxic proteins) into the cytosol. The binding of cytochrome c to apoptotic protein activating factor (Apaf-1) with involvement from caspase-9 and ATP leads to the formation of a multiprotein complex called an apoptosome. The apoptosome facilitates the recruitment and activation of other selected **caspases** (proteases with cysteine at their active sites) including caspase-3 and caspase-7. While the exact sequence of events leading to cell death is unclear, it is thought to involve the production of reactive oxygen species among other substances that induce structural alterations to the cell and its components, resulting in its death.

The extracellular (extrinsic) pathway, also called the caspase 8/10 dependent pathway, for apoptosis is triggered when specific ligands (such as molecules that belong to the **tumor necrosis factor** [TNF] family) bind to cell surface death receptors (such as Fas/CD95 and TNFR1) and generate apoptotic signaling. The ligands are released as part of immune responses as well as under other circumstances. Immune-system actions, such as natural killer cells' release (from cytosolic granules) of granzymes and a protein called perforin (which create pores in the membranes of cells targeted for destruction), facilitate the process. Next, a series of protein–protein interactions occur that ultimately activate caspase-8 and caspase-10 to induce cell death. The death signals initiated as part of the extrinsic pathway, however, may be enhanced (especially in nonimmune cells) secondary to connections with the intrinsic pathway.

The removal of a dead cell's contents occurs without any of its contents escaping into the extracellular fluid. Thus, apoptosis does not trigger autoimmunity. However,

defects in the apoptotic process may increase susceptibility to autoimmune diseases. Studies are ongoing to determine if specific human autoimmune diseases are related to such defects.

In contrast to apoptosis, which is programmed and characterized by cell shrinkage followed by cell breakup, the cell death process of **oncosis** (from *onksos*, meaning “swelling”) results from cell injury and is characterized by cellular swelling, along with swelling of the mitochondrial, nucleus and cytosol, and cytosol vacuolization.

Because cell death can be activated by specific genes, the expression of these genes must be tightly controlled to avoid inappropriate cell death. Interestingly, many of the proteins released in the process of apoptosis are found in the mitochondria or in its outer membrane space. Most have a specific role there and only when they are released into the cytosol do they have a role in apoptosis. The BCL-2 family is one key group of proteins involved in the regulation of the mitochondrial intrinsic apoptotic pathway by promoting or inhibiting mitochondrial outer membrane permeability. Examples of some antiapoptotic factors include Bcl-xL and Bcl-2, which protect the cell against apoptotic stimuli. Heat shock proteins may also attenuate apoptosis. Nutrients including vitamins A and D also exhibit roles in cell proliferation, differentiation, and growth, and sphingolipids are involved in survival along with cell growth, adhesion, and **motility**.

The study of how cell death can be controlled has important implications since the dysregulation of apoptosis is thought to be involved in the pathophysiology of numerous diseases. If cell death is prevented, then a transformed cell can continue to grow (rather than be destroyed) and promote oncogenesis (the formation of a tumor).

1.4 BIOLOGICAL ENERGY

The previous sections of this chapter provide some descriptive insight into the makeup of a cell, how it reproduces, and how large and small molecules are synthesized within a cell or move in or out of a cell. All of these activities require energy. The cell obtains this energy from small molecules transformed (oxidized) to provide heat and chemical energy. The small molecules that are constantly required are supplied by the nutrients in food. The next section covers some basics of energy needs in the cell.

Most of the processes that sustain life involve energy. Some processes use energy, and others release it. The term *energy* conjures an image of physical “vim and vigor,” the fast runner or the weightlifter straining to lift hundreds of pounds. This notion of energy is accurate insofar as the contraction of muscle fibers associated with mechanical work is an energy-demanding process, requiring adenosine triphosphate (ATP), the major storage form of molecular energy in the cell. Beyond the ATP required for

physical exertion, the living body has other, equally important, requirements for energy, including:

- Biosynthetic (anabolic) systems by which substances can be formed from simpler precursors
- Active transport systems by which compounds or ions can be moved across membranes against a concentration gradient
- Transfer of genetic information.

This section addresses the key role of energy transformation and heat production in using nutrients and sustaining life.

Energy Release and Consumption in Chemical Reactions

Energy used by the body is ultimately derived from the energy contained in the **macronutrients**—carbohydrate, fat, and protein (and alcohol). If this energy is released, it may simply be expressed as heat, as would occur in the combustion of flammable substances, or be preserved in the form of other chemical energy. Energy cannot be created or destroyed; it can only be transformed. Burning a molecule of glucose outside the body liberates heat, along with CO₂ and H₂O as products of combustion, as shown:



The metabolism of glucose to the same CO₂ and H₂O within the cell is nearly identical to that of simple combustion. The difference is that in metabolic oxidation a significant portion of the released energy is salvaged as chemical energy in the form of new, high-energy bonds. These bonds represent a usable source of energy for driving energy-requiring processes. Such stored energy is generally contained in phosphate anhydride bonds, chiefly those of ATP (Figure 1.11). The analogy between the combustion and the metabolic oxidation of a typical nutrient (palmitic acid) is illustrated in Figure 1.12. The metabolic oxidation illustrated releases 59% of the heat produced by the combustion and conserves about 40% of the chemical energy.

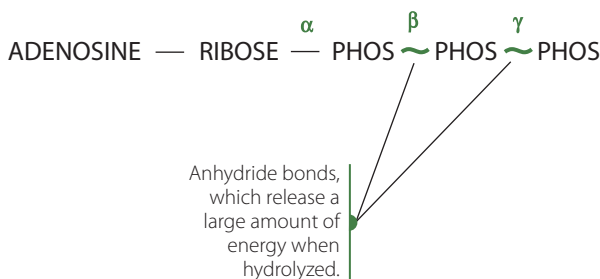


Figure 1.11 Adenosine triphosphate (ATP).

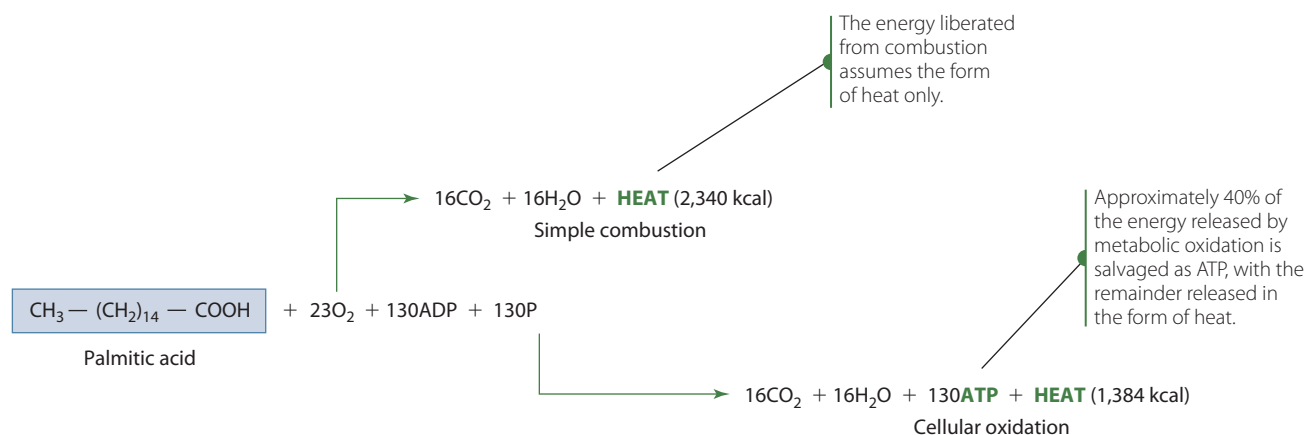


Figure 1.12 Comparison of the simple combustion and the metabolic oxidation of the fatty acid palmitate.

Units and Expressions of Energy

The unit of energy used throughout this text is the *calorie*, abbreviated *cal*. In the expression of the higher caloric values encountered in nutrition, the unit *kilocalories* (*kcal*) is often used: $1\text{kcal} = 1,000\text{ cal}$. The international scientific community and many scientific journals use another unit of energy, called the *joule* (*J*) or the *kilojoule* (*kJ*). Calories can easily be converted to joules by the factor 4.18:

$$1\text{ cal} = 4.18\text{ J, or } 1\text{ kcal} = 4.18\text{ kJ}$$

To help you become familiar with both terms, this text primarily uses calories or kilocalories, followed by the corresponding values in joules or kilojoules in parentheses; joules and kilojoules are sometimes used in scientific publications.

Free Energy

The potential energy inherent in the chemical bonds of nutrients is released if the molecules undergo oxidation either through combustion or through oxidation within the cell. This energy is defined as **free energy** if, on its release, it is capable of doing work at constant temperature and pressure—a condition that is met within the cell. In equations, *G* is used as an abbreviation for free energy and ΔG for the change in free energy.

CO_2 and H_2O are the products of the complete oxidation of organic molecules containing only carbon, hydrogen, and oxygen, and they have an inherent free energy. The energy released in the course of oxidation of the organic molecules is in the form of either heat or chemical energy. The products have less free energy than do the original reactants. Because energy is neither created nor lost during the reaction, the total energy remains constant. Thus, the difference between the free energy in the products and that in the reactants in a given chemical reaction is a useful parameter for estimating the tendency

for that reaction to occur. This difference is symbolized as follows:

$$G_{\text{products}} - G_{\text{reactants}} = \Delta G \text{ of the reaction}$$

where *G* is free energy and Δ is a symbol signifying change.

Exothermic and Endothermic Reactions

If the *G* value of the reactants is greater than the *G* value of the products, as in the case of the oxidation reaction, the reaction is said to be **exothermic**, or energy releasing, and the change in *G* (ΔG) is negative. In contrast, a positive ΔG indicates that the *G* value of the products is greater than that of the reactants, indicating that energy must be supplied to the system to convert the reactants into the higher-energy products. Such a reaction is called **endothermic**, or energy requiring.

Exothermic and endothermic reactions are sometimes referred to as downhill and uphill reactions, respectively, terms that help create an image of energy input and release. The free energy levels of reactants and products in a typical exothermic, or downhill, reaction can be likened to a boulder on a hillside that can occupy two positions, A and B, as illustrated in Figure 1.13. As the boulder descends to level B from level A, energy capable of doing work is liberated, and the change in free energy is a negative value. The reverse reaction, moving the boulder uphill to level A from level B, necessitates an input of energy, or an endothermic process, and the change is a positive value. The quantity of energy released in the downhill reaction is precisely the same as the quantity of energy required for the reverse (uphill) reaction—only the sign of ΔG changes.

Activation Energy

Although exothermic reactions are favored over endothermic reactions in that they require no external energy input, they do not occur spontaneously. If they

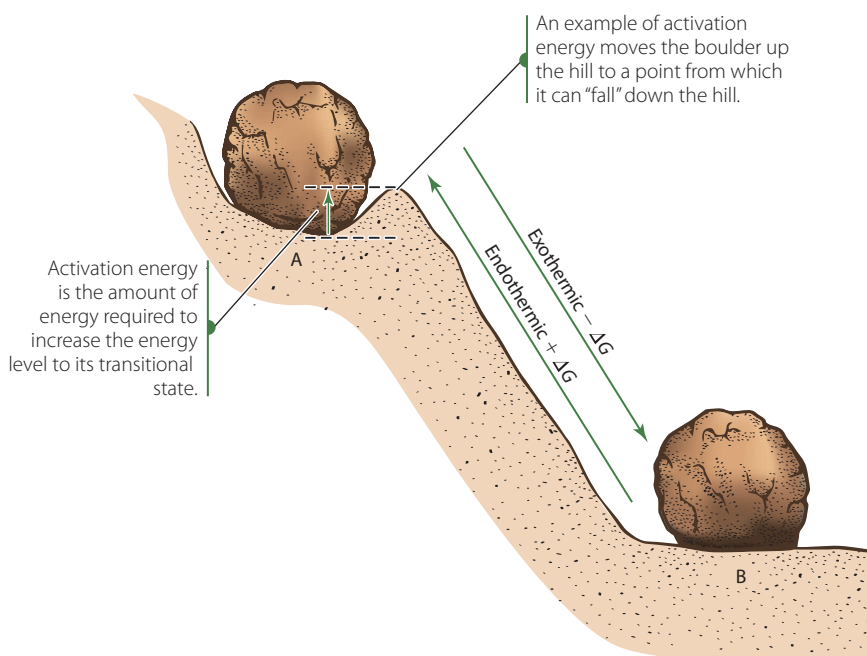


Figure 1.13 The uphill–downhill concept illustrating energy-releasing and energy-demanding processes.

did, no energy-producing nutrients or fuels would exist throughout the universe because they would all have transformed spontaneously to their lower energy level. A certain amount of energy must be introduced into reactant molecules to activate them to their **transition state**, a higher energy level or barrier at which the exothermic conversion to products can indeed take place. The energy that must be imposed on the system to raise the reactants to their transition state is called the **activation energy**. Refer again to the boulder-and-hillside analogy in Figure 1.13. The boulder does not spontaneously descend until the required activation energy can dislodge it from its resting place to the brink of the slope.

Cellular Energy

The cell derives its energy from a series of chemical reactions, each of which exhibits a free energy change. The reactions occur sequentially as nutrients are systematically oxidized ultimately to CO_2 and H_2O . Nearly all the reactions in the cell are catalyzed by enzymes. Within a given catabolic pathway—for example, the oxidation of glucose to CO_2 and H_2O —some reactions may be energy consuming (have a $+\Delta G$ for the reaction). However, energy-releasing (those with a $-\Delta G$) reactions are favored, so the net energy transformation for the entire pathway has a $-\Delta G$ and is exothermic.

Reversibility of Chemical Reactions

Most cellular reactions are reversible, meaning that an enzyme (E) that can catalyze the conversion of hypothetical

substance A into substance B can also catalyze the reverse reaction, as shown:



Using the A, B interconversion as an example, let us review the concept of reversibility of a chemical reaction. In the presence of the specific enzyme E, substance A is converted to substance B. Initially, the reaction is unidirectional because only A is present. However, because the enzyme is also capable of converting substance B to substance A, the reverse reaction becomes significant as the concentration of B increases. From the moment the reaction is initiated, the amount of A decreases, while the amount of B increases to the point at which the rate of the two reactions becomes equal. At that point, the concentrations of A and B no longer change, and the system is said to be in equilibrium. Enzymes are only catalysts and do not change the equilibrium of the reaction. This concept is discussed more fully later. Whether the $A \rightarrow B$ reaction or the $B \rightarrow A$ reaction is energetically favored is indicated by the relative concentrations of A and B at equilibrium.

The equilibrium between reactants and products can be defined in mathematical terms and is called the equilibrium constant (K_{eq}). K_{eq} is simply the ratio of the equilibrium concentration of product B to that of reactant A: $K_{eq} = [B]/[A]$. The [] signify the concentration. If the denominator ([A]) is very small, dividing it into a much larger number results in K_{eq} being large. [A] will be small if most of A (the reactant) is converted to the product B. In other words, K_{eq} increases in value when the concentration

of A decreases and that of B increases. If K_{eq} has a value greater than 1, substance B is formed from substance A, whereas a value of K_{eq} less than 1 indicates that at equilibrium A will be formed from B. An equilibrium constant equal to 1 indicates that no bias exists for either reaction. The K_{eq} of a reaction can be used to calculate the standard free energy change of the reaction.

Standard Free Energy Change

To compare the energy released or consumed in different reactions, it is convenient to define the free energy at standard conditions. Standard conditions are defined precisely: a temperature of 25°C (298 K); a pressure of 1.0 atm (atmosphere); and the presence of both the reactants and the products at their standard concentrations, namely 1.0 mol/L. The standard free energy change ΔG^0 (the superscript zero designates standard conditions) for a chemical reaction is a constant for that particular reaction. The ΔG^0 is defined as the difference between the free energy content of the reactants and the free energy content of the products under standard conditions. Under such conditions, ΔG^0 is mathematically related to K_{eq} by the equation

$$\Delta G^0 = -2.3 RT \log K_{eq}$$

where R is the gas constant (1.987 cal/mol) and T is the absolute temperature, 298 K in this case. The factors 2.3, R, and T are constants, and their product is equal to $-2.3(1.987)(298)$, or $-1,362$ cal/mol. The equation therefore simplifies to

$$\Delta G^0 = -1,362 \log K_{eq}$$

This topic is important in understanding the energetics of metabolic pathways, but you should refer to a biochemistry textbook for additional information on this subject.

Equilibrium Constant and Standard Free Energy Change

The equilibrium constant of a reaction determines the sign and magnitude of the standard free energy change. For example, referring once again to the $A \rightarrow B$ reaction, the logarithm of a K_{eq} value greater than 1.0 will be positive, and because it is multiplied by a negative number, the

sign of ΔG^0 will be negative. We have established that the reaction $A \rightarrow B$ is energetically favored if ΔG^0 is negative. Conversely, the log of a K_{eq} value less than 1.0 would be negative, and when multiplied by a negative number the sign of ΔG^0 would be positive. The ΔG^0 in this case indicates that the formation of A from B ($B \rightarrow A$) is favored in the equilibrium.

Standard pH

For most compartments in the body, the pH is near neutral; for biochemical reactions, a standard pH value of 7 is adopted by convention. For human nutrition, the standard free energy change of reactions is designated $\Delta G^{0'}$. This book uses this notation.

Nonstandard Physiological Conditions

Physiologically standard conditions do not often exist. The difference between standard conditions and nonstandard conditions can explain why a reaction having a positive $\Delta G^{0'}$ can proceed exothermically ($-\Delta G^0$) in the cell. For example, consider the reaction catalyzed by the enzyme triosephosphate isomerase (TPI) shown in Figure 1.14. This particular reaction occurs in the glycolytic pathway through which glucose is converted to pyruvate. (The chemical structures and the pathway are discussed in detail in Chapter 3.) In the glycolytic pathway, the enzyme aldolase produces 1 mol each of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G-3-P) from 1 mol of fructose-1,6-bisphosphate. Let us focus on the reaction that TPI catalyzes, which is an isomerization between the two products of the aldolase reaction. As explained in Chapter 3, only the G-3-P is further degraded in the subsequent reactions of glycolysis. This circumstance results in a substantially lower concentration of the G-3-P metabolite than of DHAP.

For this reaction, two important conditions within the cell deviate from “standard conditions”: namely, the temperature is the temperature of the body, $\sim 37^\circ\text{C}$ (310 K), and neither the G-3-P nor DHAP are at 1.0 mol/L concentrations. The value of $\Delta G^{0'}$ for the reaction DHAP (reactant) $\rightarrow$ G-3-P (product) is $+1,830$ cal/mol ($+7,657$ J/mol), indicating that under standard conditions the formation

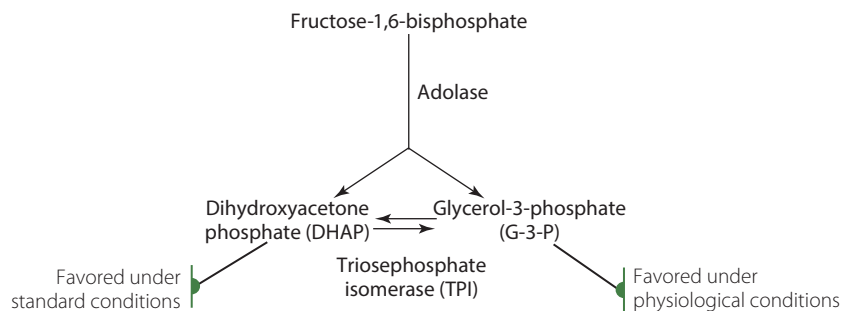


Figure 1.14 Example of a shift in the equilibrium by changing from standard conditions to physiological conditions.

of DHAP is preferred over the formation of G-3-P. If we assume that the cellular concentration of DHAP is 50 times that of G-3-P because G-3-P is further metabolized, ΔG^0 for the reaction is calculated to be equal to -577 cal/mol ($-2,414 \text{ J/mol}$). The negative ΔG^0 shows that the reaction to form G-3-P is favored, as shown, despite the positive ΔG^0 for this reaction.

The Role of High-Energy Phosphate in Energy Storage

The preceding section addressed the fundamental principle of free energy changes in chemical reactions and the fact that the cell obtains this chemical free energy through the catabolism of nutrient molecules. It also stated that this energy must somehow be used to drive the various energy-requiring processes and anabolic reactions so important in normal cell function. This section explains how ATP can be used as a universal source of energy to drive reactions. Examples of very-high-energy phosphate compounds are shown in Figure 1.15. Phosphoenolpyruvate and 1,3-bisphosphoglycerate are components of the oxidative pathway of glucose (Chapter 3), and creatine phosphate (also called phosphocreatine) is a storage form of high-energy phosphate available to replenish ATP in muscle. The hydrolysis of the phosphate anhydride bonds of ATP can liberate the stored chemical energy when needed. ATP thus can be thought of as an energy reservoir, serving as the major linking intermediate between energy-releasing and energy-demanding chemical reactions in the cell. In nearly all cases, the energy stored in ATP is released by the enzymatic hydrolysis of the anhydride bond connecting the β - and γ -phosphates in the molecule (see Figure 1.11).

The products of this hydrolysis are adenosine diphosphate (ADP) and inorganic phosphate (P_i). In certain instances, the free phosphate group is transferred to various acceptors, a reaction that activates the acceptors to higher energy levels. The involvement of ATP as a link between the energy-releasing and energy-requiring cellular reactions and processes is summarized in Figure 1.16.

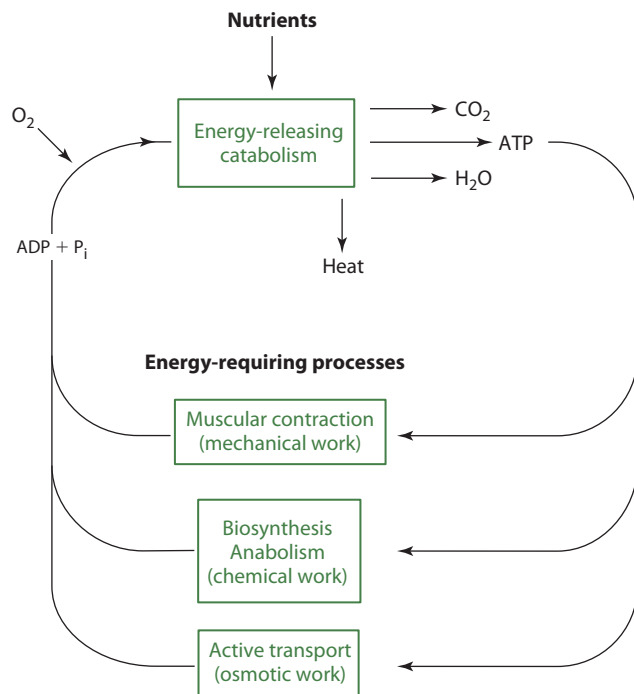


Figure 1.16 Illustration of how ATP is generated from the coupling of ADP and phosphate through the oxidative catabolism of nutrients and how it in turn is used for energy-requiring processes.

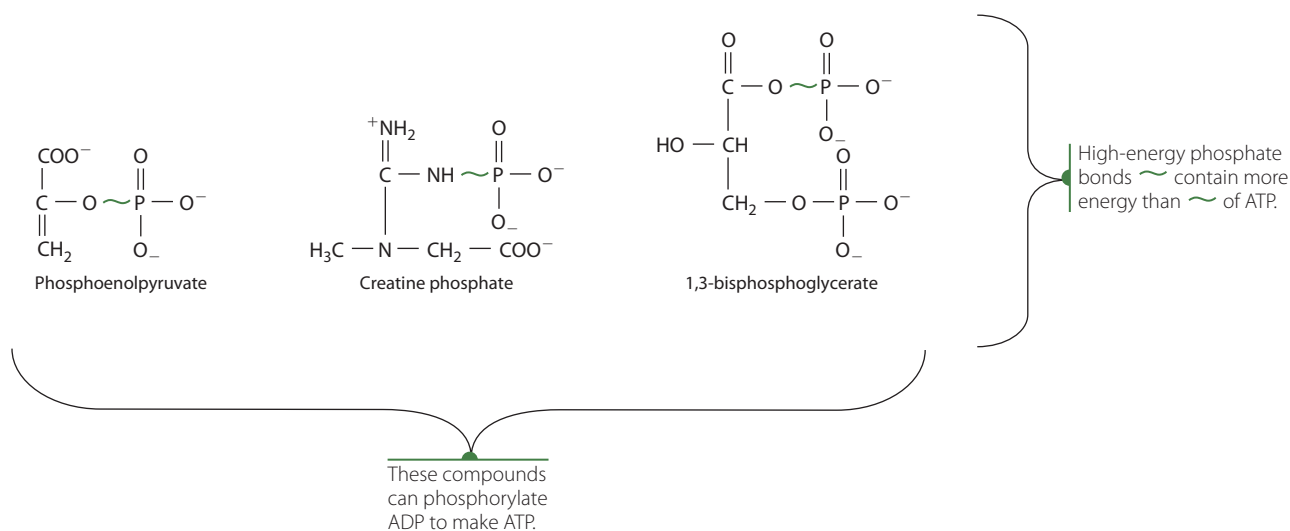


Figure 1.15 Examples of very-high-energy phosphate compounds.

Coupled Reactions in the Transfer of Energy

Some reactions require energy, and others yield energy. The coupling of these reactions makes it possible for a pathway to continue. The oxidation of glucose in the glycolysis pathway demonstrates the importance of coupled reactions in metabolism. An understanding of how chemical energy is transformed from macronutrients (the carbohydrate, protein, fat, and alcohol in food) to storage forms (such as ATP) and how the stored energy is used to synthesize needed compounds for the body is fundamental to the study of human nutrition. These topics are covered in this section as well as throughout this book. The $\Delta G^{0'}$ value for the phosphate bond hydrolysis of ATP is intermediate between those of certain high-energy phosphate compounds and compounds that possess relatively low-energy phosphate esters. ATP's central position on the energy scale lets it serve as an intermediate carrier of phosphate groups. ADP can accept the phosphate groups from high-energy phosphate donor molecules and then, as ATP, transfer them to lower-energy receptor molecules. Two examples of this transfer are shown in Figure 1.17. By receiving the phosphate groups, the acceptor molecules become activated to a higher energy level, from which they can undergo subsequent reactions such as entering the glycolysis pathway. The end result is the transfer of chemical energy from donor molecules through ATP to receptor molecules. The second example is the release of a P_i group from creatine phosphate; this P_i joins with ADP, forming ATP. Creatine phosphate serves as a ready reservoir to renew ATP levels quickly, particularly in muscle.

If a given quantity of energy is released in an exothermic reaction, the same amount of energy must be added to the system for that reaction to be driven in the reverse direction. For example, hydrolysis of the phosphate-ester bond of glucose-6-phosphate liberates 3,300 cal/mol (13.8 kJ/mol) of energy, and the reverse reaction, in which

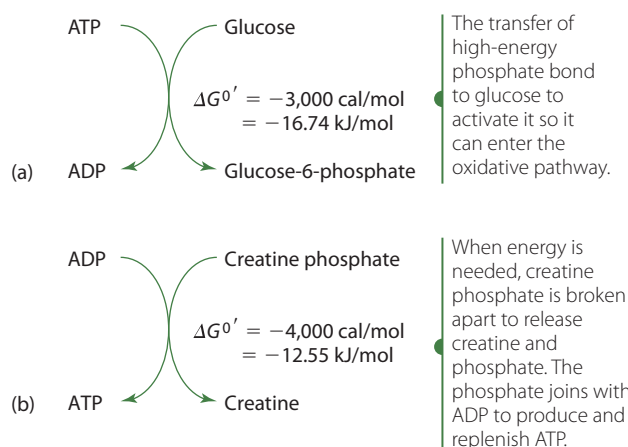


Figure 1.17 Examples of high-energy phosphate bonds being transferred.

the phosphate is added to glucose to form glucose-6-phosphate, necessitates the input of 3,300 cal/mol (13.8 kJ/mol). These reactions can be expressed in terms of their standard free energy changes, as shown in Figure 1.18. To phosphorylate glucose, the reaction must be coupled with the hydrolysis of ATP, which provides the necessary energy. The additional energy from the reaction is dissipated as heat.

The addition of phosphate to a molecule is called a phosphorylation reaction. It is generally accomplished by the enzymatic transfer of the terminal phosphate group of ATP to the molecule, rather than by the addition of free phosphate, as suggested in Figure 1.18. The reverse reaction is hypothetical, designed only to illustrate the energy requirement for phosphorylation of the glucose molecule. In fact, the enzymatic phosphorylation of glucose by ATP is the first reaction glucose undergoes upon entering the cell. This reaction promotes glucose to a higher energy level, from which it may be indirectly incorporated into glycogen as stored carbohydrate or systematically oxidized for energy. Phosphorylation therefore can be viewed as occurring in two reaction steps: (1) hydrolysis of ATP to ADP and phosphate and (2) addition of the phosphate to the substrate (glucose) molecule. A net energy change for the two reactions coupled together is shown in Figure 1.18. The net $\Delta G^{0'}$ for the coupled reaction is $-4,000 \text{ cal/mol}$ (16.7 kJ/mol).

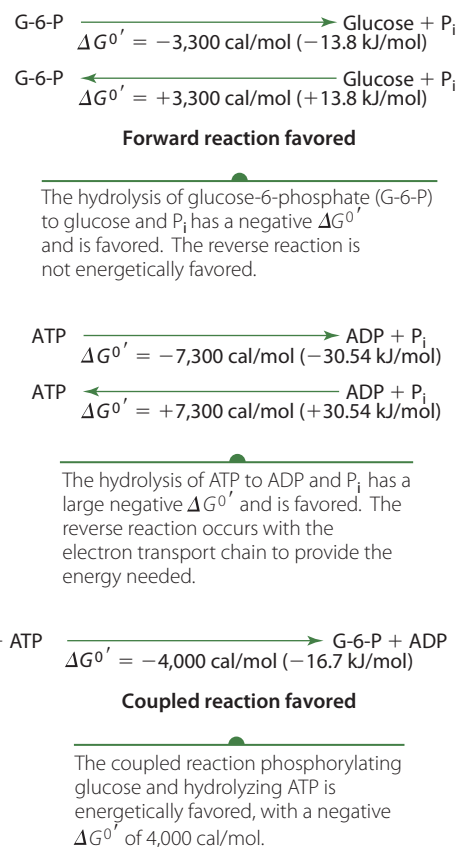


Figure 1.18 Exothermic reactions.

The significance of these coupled reactions cannot be overstated. They show that even though energy is consumed in the endothermic formation of glucose-6-phosphate from glucose and phosphate, the energy released by the ATP hydrolysis is sufficient to force (or drive) the endothermic reaction that “costs” only 3,300 cal/mol. The coupled reactions result in 4,000 cal/mol (16.7 kJ/mol) left over. The reaction is catalyzed by the enzyme hexokinase or glucokinase, both of which hydrolyze the ATP and transfer the phosphate group to glucose. The enzyme brings the ATP and the glucose into close proximity, reducing the activation energy of the reactants and facilitating the phosphate group transfer. The overall reaction, which results in activating glucose at the expense of ATP, is energetically favorable, as evidenced by its high, negative standard free energy change.

Reduction Potentials

As we will see when we discuss the formation of ATP in Chapter 3, ATP is formed in the electron transport chain after the macronutrients are oxidized. To better understand these oxidations and reductions, you need to understand reduction potentials. The energy to synthesize ATP becomes available following a sequence of individual reduction-oxidation (redox) reactions along the electron transport chain, with each component having a characteristic ability to donate and accept electrons. The released energy is used in part to synthesize ATP from ADP and phosphate. The tendency of a compound to donate and to receive electrons is expressed in terms of its **standard reduction potential**, E_0' . The more negative the values of E_0' are, the greater the ability of the compound to donate electrons, whereas increasingly positive values signify an increasing tendency to accept electrons. The reducing capacity of a compound (its tendency to donate H^+ and electrons) can be expressed by the E_0' value of its half-reaction, also called the compound's electromotive potential.



Free energy changes accompany the transfer of electrons between electron donor-acceptor pairs of compounds and are related to the measurable electromotive force of the electron flow. Remember: *In electron transfer, an electron donor reduces the acceptor, and in the process the electron donor becomes oxidized. Consequently, the*

acceptor, as it is reduced, oxidizes the donor. The quantity of energy released is directly proportional to the difference in the standard reduction potentials, $\Delta E_0'$, between the partners of the redox pair. The free energy of a redox reaction and the $\Delta E_0'$ of the interacting compounds are related by the expression

$$\Delta G^{0'} = -nF\Delta E_0'$$

where ΔG_0 is the standard free energy change in calories, n is the number of electrons transferred, and F is a constant called the Faraday (23,062 cal absolute volt equivalent).

An example of a reduction-oxidation reaction that occurs within the electron transport system is the transfer of **hydrogen atoms** and electrons from NADH through the flavin mononucleotide (FMN)-linked enzyme NADH dehydrogenase to oxidized coenzyme Q (CoQ). The half-reactions and E_0' values for each of these reactions follow:

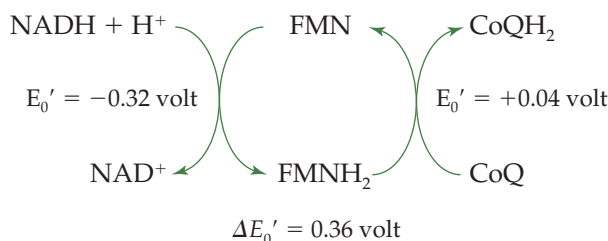


$$E_0' = -0.32 \text{ volt}$$



$$E_0' = +0.04 \text{ volt}$$

Because the NAD^+ system has a relatively more negative E_0' value than the CoQ system, NAD^+ has a greater reducing potential than the CoQ system because electrons tend to flow toward the system with the more positive E_0' . The reduction of CoQ by NADH is therefore predictable, and the coupled reaction, linked by the FMN of NADH dehydrogenase, can be written as follows:



Inserting this value for $\Delta E_0'$ into the energy equation gives

$$\Delta G^{0'} = -2(23,062)(0.36) = -16,604 \text{ cal/mol}$$

The amount of energy liberated from this single reduction-oxidation reaction within the electron transport chain is therefore more than enough to phosphorylate ADP to ATP, which, as you will recall, requires about 7,300 cal/mol (35.7 kJ).

SUMMARY

This brief journey through the cell—beginning with its outer surface, the plasma membrane, and moving into its innermost part, where the nucleus is located—provides a view of how this living entity functions. Characteristics of the cell that seem particularly notable are as follows:

- The flexibility of the plasma membrane in adjusting or reacting to its environment while protecting the cell as it monitors what may pass into or out of the cell. Prominent in the membrane's reaction to its environment are the receptor proteins, which are synthesized on the rough endoplasmic reticulum and moved through the Golgi apparatus to their intended site on the plasma membrane.
- The communication among the various components of the cell made possible through the cytosol, with its microtrabecular network, and also through the endoplasmic reticulum and Golgi apparatus. The networking is such that communications flow not only among components within the cell but also between the nucleus and the plasma membrane.
- The efficient division of labor among the cell components (organelles). Each component has its own specific functions to perform, with little overlap. Furthermore, much evidence is accumulating to support the concept of an “assembly line” not only in oxidative phosphorylation on the inner membrane of the mitochondrion but also in other metabolic pathways, wherever they occur.
- The superb management exercised by the nucleus to ensure that all the needed proteins are synthesized. The proteins needed as recognition markers, receptors, transport vehicles, and enzymes are available and located in the appropriate place in the cell as needed.

- The fact that, like all living things, cells must die a natural death. This programmed process is called apoptosis, a particularly attractive focus of current research.

Despite the efficiency of the cell, it is still not a totally self-sufficient unit. Its continued operation is contingent on receiving appropriate and sufficient nutrients. Nutrients needed include not only those that can be used to produce energy, ATP, but also those stored as chemical energy.

- Most of the stored chemical energy is needed to maintain normal body temperature (released as heat energy). About 40% of the stored energy is conserved in the form of high-energy phosphate bonds, principally ATP. The ATP can, in turn, activate various substrates by phosphorylation to higher energy levels from which they can undergo metabolism by specific enzymes. The exothermic hydrolysis of the ATP phosphate is sufficient to drive the endothermic phosphorylation, thereby completing the energy transfer from nutrient to metabolite.
- The oxidative pathways for the macronutrients (carbohydrate, fat, and protein) and alcohol provide a continuous flow of energy for maintaining heat and replenishing ATP.
- The cell also needs nutrients required as building blocks for structural macromolecules. In addition, the cell must have an adequate supply of the so-called regulatory nutrients (i.e., vitamins, minerals, and water).

With a view of the structure of the “typical cell,” the division of labor among cellular component parts, and the location within the cell where many of the key metabolic reactions necessary to continue life take place, we can now consider in subsequent chapters how the cell receives its nourishment and how the nutrients are metabolized.

Perspective

NUTRITIONAL GENOMICS

Nutritional genomics (or nutrigenomics) addresses interactions between nutrients (and bioactive dietary components) and the human genome, that is, the cell's entire set of genetic information. Nutrigenomics includes mutual interactions between nutrients and genes whereby nutrition impacts gene expression and genetics affects nutrient metabolism. Within the study of nutritional genomics are the subareas of *nutrigenetics*, which focuses on nutrient (and bioactive dietary component) interactions with genes, including gene variants, and of *epigenetics*, which encompasses alterations in gene expression (i.e., the turning “on” and “off” of genes) that are not the result of changes in DNA's nucleotide sequence.

Genetic variations are often linked to disease or disease risk. Some disorders resulting from single gene variants can alter nutrient utilization and affect dietary and nutrient needs. However, the development of other diseases, such as heart disease, some cancers, and type 2 diabetes, are influenced by multiple genes, as well as by environmental/lifestyle factors. The Human Genome Project and genome-wide association studies (GWAS) have provided tremendous knowledge about genes and their products, including a more thorough identification and understanding of genetic polymorphisms and their contribution to disease or risk of disease. The findings help to explain, for example, why not all people with medical conditions such as hypercholesterolemia or hypertension (among others) respond equally to dietary interventions. Moreover, the research provides the potential foundation for “personalized nutrition,” that is, a diet tailored for an individual based on their own gene variations with the ultimate goals of promoting health and reducing disease risk.

While this chapter's “Nucleus” section provided information on nucleic acids, cell replication, transcription, translation, and

some aspects of controls of gene expression, this next section briefly expands on this information to address inheritance. The Perspective also provides discussions of nutrigenetics and epigenetics with some examples illustrating how nutrient needs can be affected by gene variants and how nutrients affect gene expression.

SOME BASICS OF INHERITANCE

Genes represent segments of DNA (which consists of nucleotides), code for specific proteins, and determine particular traits. Individuals inherit genes from each parent; these inherited genes are a person's genotype. The copy of the particular gene inherited from each parent is referred to as an allele. Alleles occur in pairs (one from each parent). Two alleles when the same are termed *homozygous*, and two alleles that differ are termed *heterozygous*. Alleles control the expression of genes and bring variations to the trait. The inheritance of certain genes is categorized as autosomal dominant or recessive. (Note: Some are also classified based on linkage to the X or Y chromosome). With a heterozygous allele pair, the expressed allele (i.e., the displayed phenotypic trait) is termed *dominant*, and the other allele is termed *recessive*. Using the example of eye color, a person who inherits a gene for blue eyes from one parent and a gene for brown eyes from the other parent will exhibit brown eyes (the phenotype) since brown is **autosomal dominant**. In some cases, however, both alleles may be expressed (i.e., codominant); an example of codominance occurs in those with blood type AB, with both one “A” allele and one “B” allele expressed.

NUTRIGENETICS

Gene variants result from changes in the nucleotide subunits of DNA. Single-nucleotide polymorphisms, abbreviated SNPs (pronounced “snips”), represent variants that affect one nucleotide base (such

as cytosine replacing thymine) in a particular region of the DNA. SNPs, which represent one type of **mutation** that can affect DNA, contribute to the uniqueness of each individual and are thought to represent the overwhelming majority of all polymorphisms in DNA in humans. Some result in differences in observable traits. Some may have no observable effects on proteins (including their production or function). Others, however, may influence metabolic processes critical to the workings of the body's trillions of cells. Thus, outcomes from a SNP in the DNA vary from negligible to extensive.

Multiple SNPs have been identified that directly result in medical conditions/diseases. For example, inheritance from both parents of certain SNPs in the gene coding for the enzyme phenylalanine hydroxylase result in the autosomal recessive inherited disorder phenylketonuria (PKU). The SNP occurs due to a one base pair substitution (point mutation) in a nucleotide of the gene for phenylalanine hydroxylase. While the enzyme is still produced, because of the mutation and resulting amino acid substitution, the enzyme's activity is significantly reduced, and thus the cell's ability to metabolize the amino acid phenylalanine is negligible. Changes to the diet, including restriction of phenylalanine (and thus protein-rich foods) along with other dietary modifications (such as added tyrosine), are required to ensure normal development and growth. (PKU as well as other inborn errors of metabolism affecting amino acids are discussed further in Chapter 6.) Additional examples of SNPs and their effects of nutrient utilization and/or disease risk are provided throughout the book. Chapter 9, for example, provides a discussion on SNPs in the gene for methyltetrahydrofolate reductase and its ramifications on disease risk. A discussion of apolipoprotein E alleles and its effects on blood lipid concentrations and risks for heart disease and Alzheimer disease is given in Chapter 5.

Other polymorphisms, besides SNPs, also contribute to genetic variants. Extra copies (repeats), insertions, and deletions of nucleotides (or parts of chromosomes) are relatively common causes of genetic disorders. Frameshift mutations whereby one nucleotide (or more) may be inserted or deleted from genes can profoundly alter the amino acid sequence and thus the protein end product. Depending on the number of amino acids affected by the frameshift and their position within the protein, the resulting protein may be nonfunctional and may contribute to (or increase) risk of disease. If, however, the changes occur in noncoding regions of the genes, the results may be less significant.

While the aforementioned examples have focused on the impact of gene variants on nutrient utilization, nutrients also influence gene transcription, as discussed in this chapter. Further information is provided in Chapter 10 on the roles of vitamins A and D and their interactions with transcription factors to influence gene expression. Chapter 13 discusses iron's interactions with DNA and mRNA and the subsequent effects of transcription and translation.

EPIGENETICS

Changes in gene expression can also result from factors that have no effect on DNA's nucleotide sequence, but instead modify DNA structure. Histones (proteins), which are found wrapped around DNA, control gene expression through their ability to condense (close or wind up) and decondense (open or unwind) the DNA. DNA that is tightly compacted/condensed is not available to be transcribed whereas DNA that is decondensed is more available for transcription. Epigenetic regulation of gene expression involves, for example, modifications to histones which in turn close or open the DNA. Examples of some ways in which histones can be modified include acetylation, biotinylation, methylation, and phosphorylation, to name a few. In addition, DNA can be directly modified

by methylation. Nutrients from the diet provide the “resources” needed to modify the histones and DNA as well as to affect other processes, such as meiosis, mitosis, DNA repair, and apoptosis. Many enzymes responsible for altering the histones, for example, require vitamins as coenzymes. Some deacetylases, which function to remove acetyl groups, are niacin dependent; deacetylation of histones limits DNA accessibility and thus inhibits gene transcription. Another B-vitamin biotin provides for biotinylation of histones, which may also decrease gene expression. In contrast, acetylation of histones, which may require pantothenic acid, increases accessibility of the DNA and enhances transcription. Moreover, bioactive food components also have been shown to affect the activities of enzymes responsible for altering histones and thus exert effects on gene expression.

Direct methylation of DNA (vs. histones) influences gene expression, especially among different tissues. For example, while all nucleated cells contain the same DNA in the nucleus, all of the genes on the DNA are not expressed in all body cells. It is the pattern of the methylation of the DNA in the cells that influences gene expression within given tissues. The patterns of DNA methylation are inherited. Methyl groups are derived from the diet, and methylation reactions require the B-vitamins folate and vitamin B₁₂ (along with methionine, choline, or betaine). More specifically, folate and vitamin B₁₂ contribute to the epigenetic regulation of gene expression through their roles in transferring methyl groups to form S-adenosylmethionine (SAM). SAM provides the methyl groups (via the actions of DNA methyltransferase) with selected cytosine-containing nucleotides (usually located 5' to a guanosine; i.e., at a CpG site) within a gene's promoter region. Up to about 5% of cytosines in DNA nucleotides are thought to be methylated. Methylation of DNA represses gene expression (transcription) by condensing the chromatin structure (chromatin can be thought of as DNA plus the histones) and by inhibiting transcription

factor binding to DNA. Hypomethylation of DNA has been associated with, for example, increased cancer risk.

Gene expression can also be modified through effects on micro (mi) RNA, an area of study referred to as transcriptomics. Bioactive dietary components (found naturally in foods, especially fruits, vegetables, tea, and other plants, although many are now also included in supplements) are thought to promote positive changes to health through interactions with DNA and other required “machinery” needed for gene expression. Resveratrol, a bioactive compound found in berries and grapes, for example, binds to and modulates miRNA expression, resulting in reduced translation and thus protein production. Many other phytochemicals are also thought to exert their effects in the body through interactions with miRNA. Epigenetics is thought to be particularly important during early development, including in utero stages of life, but its effects extend to advanced age and the epigenetic patterns may be passed onto offspring.

SUMMARY

The field of nutritional genomics continues to identify associations among genes, diseases, and dietary nutrients and bioactive components. Such findings should provide for more personalized diet recommendations for individuals and thus more effective therapeutic approaches to diet-related diseases. We will one day know that if you have alleles *x* and *y* for gene *ABZ*, the most effective approaches to address the manifestations associated with the genetic variant are to consume a diet that has *x* amount of nutrient *F*, *x* amount of nutrient *T*, *x* amount of nutrient *W*, and so forth. At present, however, there are far more questions than answers to the precise nutrient and dietary modifications needed both to promote optimal health and to treat or reduce risk of diseases, especially those affected by multiple genes. Answers to these questions are coming, however, with time and ongoing research efforts.

THE DIGESTIVE SYSTEM: MECHANISM FOR NOURISHING THE BODY

LEARNING OBJECTIVES

- 2.1** Identify the organs of the digestive tract and their roles/functions in nutrient digestion and absorption.
- 2.2** Describe the secretions released by the digestive tract organs, including accessory organs, and factors influencing the release of these secretions.
- 2.3** Describe the factors influencing digestive tract motility.
- 2.4** Describe the structural features of the small intestine that facilitate nutrient absorption.
- 2.5** Identify the beneficial effects of the gut microflora.
- 2.6** Describe the roles of the nervous system and regulatory peptides in regulation of the digestive process.

NUTRITION INCLUDES THE SCIENCE OF NOURISHMENT. Ingestion of foods and beverages provides the body with at least one, if not more, of the nutrients needed to nourish the body. The body needs six classes of nutrients: carbohydrate, lipid, protein, vitamins, minerals, and water. For the body to use the carbohydrate, lipid, protein, and some vitamins and minerals found in foods, the food must first be digested—in other words, the food first must be broken down mechanically and chemically. This process of digestion occurs in the digestive tract and, once complete, yields nutrients ready for absorption and use by the body.

2.1 THE STRUCTURES OF THE DIGESTIVE TRACT AND THE DIGESTIVE AND ABSORPTIVE PROCESSES

The digestive tract, approximately 16 feet in length, includes organs that comprise the gastrointestinal (GI) tract (also called the alimentary canal or gut) as well as three accessory organs. The main structures of the digestive tract include the oral cavity, esophagus, and stomach (collectively referred to as the upper digestive tract) and the small and large intestines (called the lower digestive tract). The accessory organs include the pancreas, liver, and gallbladder. The accessory organs provide or store secretions that ultimately are delivered to the lumen (interior passageway) of the digestive tract and aid in the digestive and absorptive processes. Figure 2.1 illustrates the digestive tract and accessory organs. Figure 2.2 provides a cross-sectional view of the gastrointestinal tract

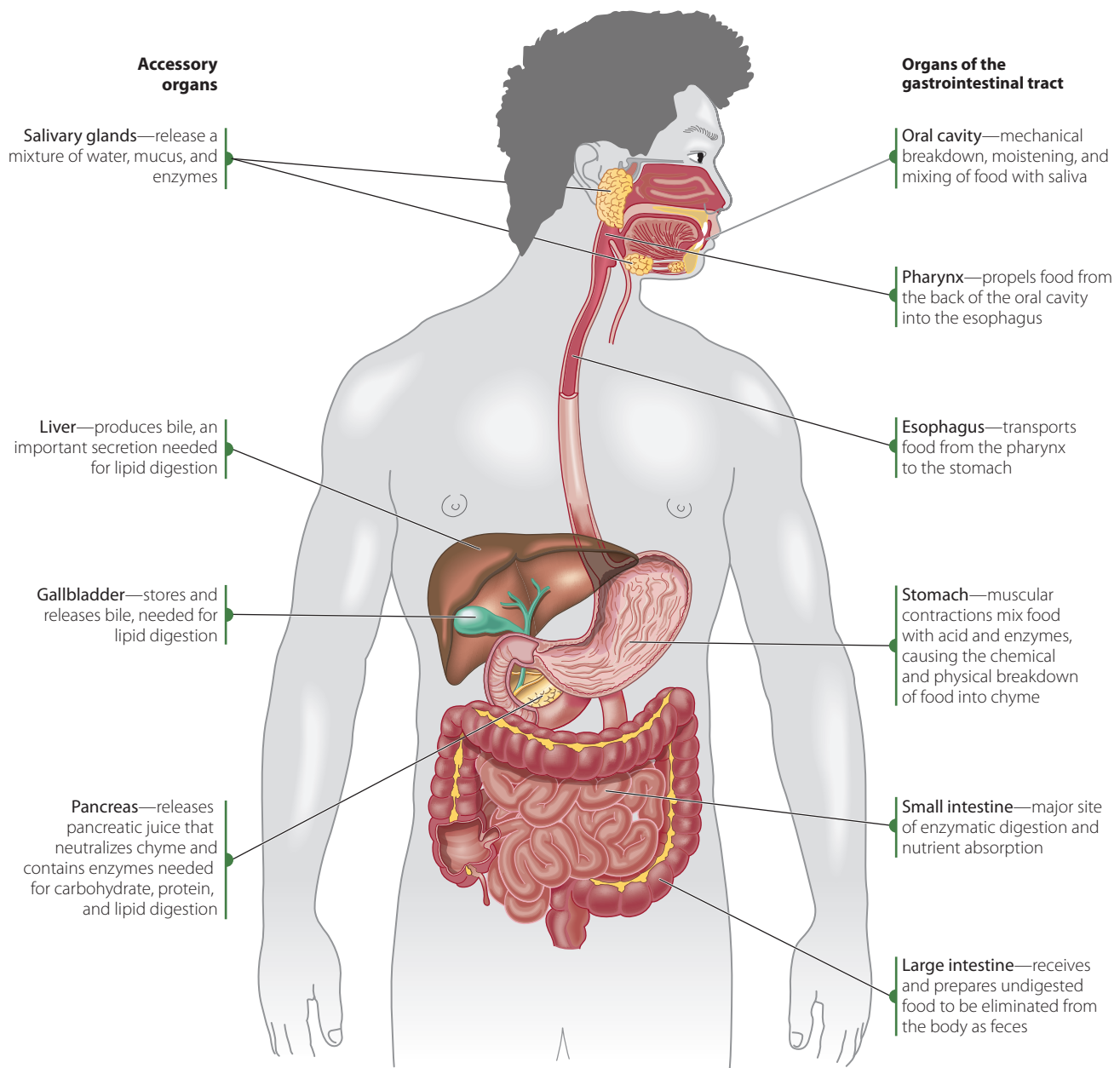


Figure 2.1 The digestive tract and its accessory organs.

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that shows the lumen and the four main tunics, or layers, of the gastrointestinal tract:

- The mucosa
- The submucosa
- The muscularis externa
- The serosa.

This first layer, the mucosa, is the innermost layer and is made of three sublayers: the mucosal membrane, the lamina propria, and the muscularis mucosa. The mucosa acts as a membrane, consists of epithelial cells that line the lumen of the gastrointestinal tract, and is the inner surface

layer that is in contact with the food (and its nutrients) that we eat. In the small intestine, this layer is arranged differently than in other sections of the digestive tract (as discussed under “Structural Aspects, Secretions, and the Digestive Processes of the Small Intestine”). Both exocrine and **endocrine** cells are found among the epithelial cells of the mucosa. The exocrine cells secrete a variety of enzymes and juices into the lumen of the gastrointestinal tract, and the endocrine (also called enteroendocrine) cells secrete various hormones into the blood. The lamina propria, another sublayer, lies adjacent to the epithelium and consists of primarily connective tissue and lymphoid tissue. This lymphoid tissue contains a number of cells,

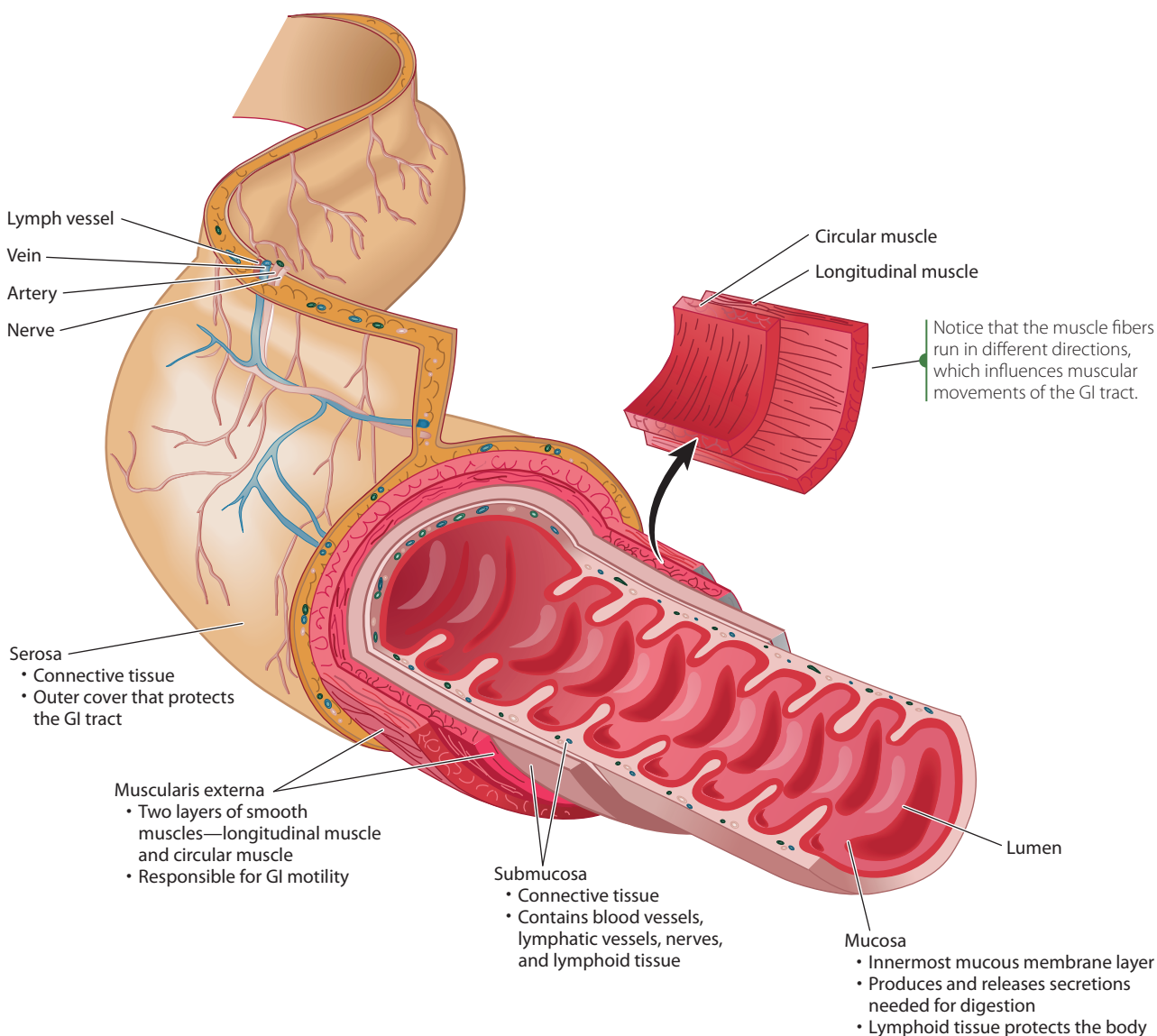


Figure 2.2 Sublayers of the small intestine.

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especially macrophages and lymphocytes, which provide protection against microorganisms. The third sublayer of the mucosa, the muscularis mucosa, is made up of a thin layer of smooth muscle.

Next to the mucosa is the submucosa. The submucosa, the second tunic or layer, is made up of connective tissue, blood and lymphatic vessels, more lymphoid tissue, and a network of nerves called the submucosal plexus, or plexus of Meissner. This plexus (or network) controls, in part, gastrointestinal secretions and local blood flow. The lymphoid tissue in the submucosa is similar to that found in the mucosa and protects the body against ingested foreign substances. The submucosa connects the first mucosal layer of the gastrointestinal tract to the muscularis externa, or third layer of the gastrointestinal tract.

The muscularis externa contains inner circular and outer longitudinal smooth muscles that surround (lie on top of) the submucosa and facilitate motility. This layer also includes the myenteric plexus, or plexus of Auerbach, which lies between the circular and the longitudinal muscles. This plexus controls the frequency and strength of contractions of the muscularis to regulate gastrointestinal motility.

The outermost layer, the serosa (sometimes called the adventitia) consists of relatively flat mesothelial cells that produce small amounts of lubricating fluids. For many areas of the digestive tract, this layer is continuous with the peritoneum. The peritoneum is a membrane with two layers within the abdominal cavity. In the abdominal cavity, the visceral peritoneum surrounds the stomach and

intestine, and the parietal peritoneum lines the pelvic cavity walls. These membranes are somewhat permeable and highly vascularized. Between the two membranes is the peritoneal cavity. The selective permeability and the rich blood supply of peritoneal membranes allow the peritoneal cavity to be used in dialysis, an ultra-filtration process used to treat kidney failure.

Immune system protection is located throughout the gastrointestinal tract (called gut-associated lymphoid tissue or GALT), especially the mucosa and submucosa layers of the small intestine (sometimes called mucosa-associated lymphoid tissue or MALT). Atrophy of these mucosa and submucosa layers can result in bacterial translocation from the intestine into the blood, leading to sepsis (infection). Within these layers of the digestive tract, immunoprotection is provided by leukocytes, especially T- and B-lymphocytes; plasma cells; natural killer (NK) cells; macrophages; microfold (M) cells; and dendritic cells, among others. Many of these cells are found in Peyer's patches, which are aggregates of lymphoid tissue, usually present in a single layer, in the mucosa and submucosa.

- The plasma cells produce secretory IgA, which binds antigens ingested with foods, inhibits the growth of pathogenic bacteria, and inhibits bacterial translocation.
- Tissue macrophages secrete cytokines, which exhibit a variety of immunoprotective effects to defend against foreign substances.

- The M-cells are antigen-presenting cells; these M-cells pass or transport foreign antigens to the Peyer's patches or lymphocytes, which in turn mount an immune response. After processing the foreign antigens, some of these lymphocytes are released from the Peyer's patches and enter circulation to augment the immune response.
- Dendritic cells, a type of macrophage, are also found in the gastrointestinal tract. Dendritic cells destroy foreign substances and then serve as antigen-presenting cells to stimulate lymphocyte activity and proliferation. The processing and presentation of antigens by antigen-presenting cells further triggers recognition of antigens by other parts of the immune system as "safe" or "harmful."

The digestive process begins in the oral cavity and proceeds sequentially through the esophagus, stomach, small intestine, and finally into the colon (large intestine). The next subsections of this chapter describe the structures and digestive processes that occur in each of these parts of the digestive tract. Other sections include information on the structures and roles of the pancreas, liver, and gallbladder and the roles of a variety of enzymes. Table 2.1 provides an overview of some of the enzymes and **zymogens** (also referred to as proenzymes or inactive enzymes, which must be altered to function as an enzyme) that participate in digesting the nutrients in foods.

Table 2.1 Digestive Enzymes and Their Actions

Enzyme or Zymogen/Enzyme	Site of Secretion	Preferred Substrate(s)	Primary Site of Action
Salivary α -amylase	Mouth	α (1-4) bonds in starch, dextrins	Mouth, stomach
Lingual lipase	Mouth	Triacylglycerol	Mouth, stomach
Pepsinogen/pepsin	Stomach	Carboxyl end of phe, tyr, trp, met, leu, glu, asp*	Stomach
Gastric lipase	Stomach	Triacylglycerol (mostly medium chain)	Stomach
Trypsinogen/trypsin	Pancreas	Carboxyl end of lys, arg*	Small intestine
Chymotrypsinogen/chymotrypsin	Pancreas	Carboxyl end of phe, tyr, trp, met, asn, his*	Small intestine
Procarboxypeptidase/carboxypeptidase A	Pancreas	C-terminal neutral amino acids	Small intestine
Carboxypeptidase B	Pancreas	C-terminal basic amino acids	Small intestine
Proelastase/elastase	Pancreas	Fibrous connective tissue proteins—elastin	Small intestine
Collagenase	Pancreas	Collagen	Small intestine
Ribonuclease	Pancreas	Ribonucleic acids	Small intestine
Deoxyribonuclease	Pancreas	Deoxyribonucleic acids	Small intestine
Pancreatic α -amylase	Pancreas	α (1-4) bonds, in starch, maltotriose	Small intestine
Pancreatic lipase and colipase	Pancreas	Triacylglycerol	Small intestine
Phospholipase	Pancreas	Lecithin and other phospholipids	Small intestine
Cholesterol esterase	Pancreas	Cholesterol esters	Small intestine
Retinyl ester hydrolase	Pancreas	Retinyl esters	Small intestine
Amino peptidases	Small intestine	N-terminal amino acids	Small intestine

(Continued)

Table 2.1 Digestive Enzymes and Their Actions (*Continued*)

Enzyme or Zymogen/Enzyme	Site of Secretion	Preferred Substrate(s)	Primary Site of Action
Dipeptidases	Small intestine	Dipeptides	Small intestine
Nucleotidase	Small intestine	Nucleotides	Small intestine
Nucleosidase	Small intestine	Nucleosides	Small intestine
Alkaline phosphatase	Small intestine	Organic phosphates	Small intestine
Monoglyceride lipase	Small intestine	Monoglycerides	Small intestine
Alpha dextrinase or isomaltase	Small intestine	α (1-6) bonds in dextrins, oligosaccharides	Small intestine
Glucoamylase, glucosidase, and sucrase	Small intestine	α (1-4) bonds in maltose, maltotriose	Small intestine
Trehalase	Small intestine	Trehalose	Small intestine
Disaccharidases	Small intestine		Small intestine
Sucrase		Sucrose	
α -glucosidase		Maltose	
Lactase		Lactose	

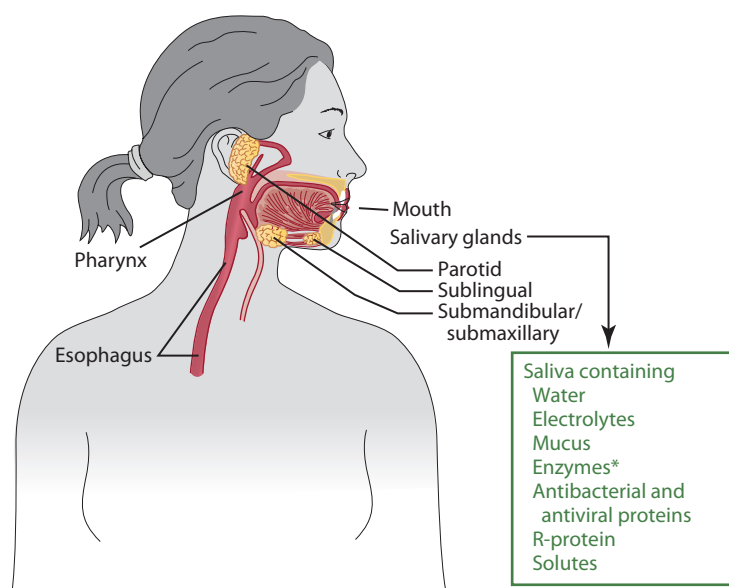
*Amino acid abbreviations: phe, phenylalanine; tyr, tyrosine; trp, tryptophan; met, methionine; leu, leucine; glu, glutamic acid; asp, aspartic acid; lys, lysine; arg, arginine; asn, asparagine; and his, histidine.

The Oral Cavity

The mouth and pharynx (or throat) constitute the oral cavity and provide the entryway to the digestive tract. On entering the mouth, food is chewed by the actions of the teeth and jaw muscles and is made ready for swallowing by mixing with secretions (saliva) released from the salivary glands. Three pairs of small, bilateral saliva-secreting salivary glands—the parotid, the submandibular, and the sublingual—are distributed throughout the lining of the oral cavity, along the jaw from the base of the ear to

the chin (Figure 2.3). Secretions (about 1–2 L/day) from these glands constitute saliva, which is made up of mostly water (99.5%) along with proteins (enzymes, mucus, antiviral/antibacterial proteins), electrolytes (sodium, potassium, chloride), and some solutes (urea, phosphates, bicarbonate).

- The water in saliva helps dissolve foods.
- The principal enzyme of saliva is salivary α -amylase (also called ptyalin; see Table 2.1). This enzyme hydrolyzes internal α (1-4) bonds within starch.



*Main enzyme in saliva is salivary amylase, which hydrolyzes α (1-4) bonds in starch.

Figure 2.3 Secretions of the oral cavity.

- A second digestive enzyme, **lingual lipase**, is produced by lingual serous glands on the tongue and in the back of the mouth. This enzyme hydrolyzes dietary triacylglycerols (triglycerides) primarily after food has been swallowed and is in the stomach. The enzyme's activity diminishes with age and is limited by the coalescing of the fats within the stomach. Lingual lipase activity is most helpful in infants, enhancing the digestion of triacylglycerols in milk.
- Mucus in the saliva lubricates food and coats and protects the oral mucosa.
- Some of the antibacterial and antiviral proteins in saliva include the antibody IgA (immunoglobulin A) and the enzyme lysozyme, which lyses (destroys) the cell walls of some bacteria.
- An R-protein in saliva functions in the stomach to enhance the absorption of vitamin B₁₂.
- Bicarbonate in saliva assists in neutralizing acids in consumed foods and acids produced by bacteria inhabiting the oral cavity. The pH of saliva is about 7.

Saliva is released into the oral cavity 24 hours a day. Basal, or resting, secretion rates (when we are not eating) are about 0.3–0.5 mL/minute, and with food consumption, saliva secretion rates usually increase to about 2 mL/minute. Insufficient saliva production results in xerostomia (dry mouth), and may occur with the use of some medications, cancer-associated radiation and chemotherapies, and disorders such as Parkinson's disease and Sjögren's syndrome, among others. Insufficient saliva production not only causes the mouth and throat to become dry but also impairs swallowing and diminishes the cleansing of our teeth and gums from food residue, acids, and old epithelial cells that have been shed from the oral mucosa. Dental caries and gum disease result if preventative care is not taken. Saliva substitutes and stimulants to increase saliva production can be helpful for some with xerostomia.

The Esophagus

From the mouth, food, now mixed with saliva and called a bolus, is passed through the pharynx into the esophagus. The esophagus is about 10 inches long and close to an inch (2 cm) in diameter (see Figure 2.1). The passage of the bolus of food from the oral cavity into the esophagus constitutes swallowing. Swallowing, which can be divided into several stages (voluntary, pharyngeal, and esophageal), is a **reflex** response initiated by a voluntary action and regulated by the swallowing center in the medulla of the brain. To swallow food, the esophageal sphincter relaxes, allowing the esophagus to open. Food then passes into the esophagus. Simultaneously, the larynx (part of the respiratory tract) moves upward, inducing the epiglottis to shift over the glottis. The closure of the glottis is important

in keeping food from entering the trachea, which leads to the lungs. Once food is in the esophagus, the larynx shifts downward to allow the glottis to reopen.

When the bolus of food moves into and down the esophagus, both the striated (voluntary) muscle of the upper portion of the esophagus and the smooth (involuntary) muscle of the distal portion are stretched and stimulated by the **nervous system**. The result is peristalsis, a progressive wavelike motion that moves the bolus through the esophagus into the stomach in usually less than 10 seconds. While the swallowing of food triggers the primary peristaltic wave, secondary waves (through the activation of stretch receptors in the esophagus) may also be initiated if, for example, food gets lodged in the esophagus. Peristalsis occurs throughout the digestive tract from the esophagus to the colon and propels the contents in the lumen distally.

At the lower (distal) end of the esophagus, just above the juncture with the stomach, lies the gastroesophageal sphincter, also called the lower esophageal sphincter (Figure 2.4). Calling it a sphincter may be a misnomer because no consensus exists about whether this particular muscle area is sufficiently hypertrophied to constitute a true sphincter. Several sphincters or valves, which are circular muscles, are located throughout the digestive tract; these sphincters allow food to pass from one section of the gastrointestinal tract to another. On swallowing, the gastroesophageal sphincter pressure drops. This drop in gastroesophageal sphincter pressure relaxes (opens) the sphincter so that food may pass from the esophagus into the stomach.

Multiple mechanisms, including neural and hormonal, regulate gastroesophageal sphincter pressure. The musculature of the gastroesophageal sphincter has a tonic pressure that is normally higher than the intragastric pressure (the pressure within the stomach). This high tonic pressure keeps the sphincter closed. Keeping this sphincter closed is important because it prevents gastroesophageal reflux (the movement of substances from the stomach back into the esophagus).

Selected Disorders of the Esophagus

A person experiencing gastroesophageal reflux feels a burning sensation (known as heartburn or pyrosis) in the midchest. The burning usually occurs after eating and may last for several hours. Repeated episodes may be diagnosed as gastroesophageal reflux disease (GERD), also called acid reflux disease. Because of the low (acidic) pH of gastric (stomach) juices and because the esophageal mucosa does not have the same protective layers as does the gastric mucosa, significant damage to the esophagus may occur with chronic acid reflux including edema (swelling); tissue erosion and ulceration; blood vessel (usually capillary) damage; spasms; and fibrotic tissue formation, which can cause a narrowing (stricture) within the esophagus.

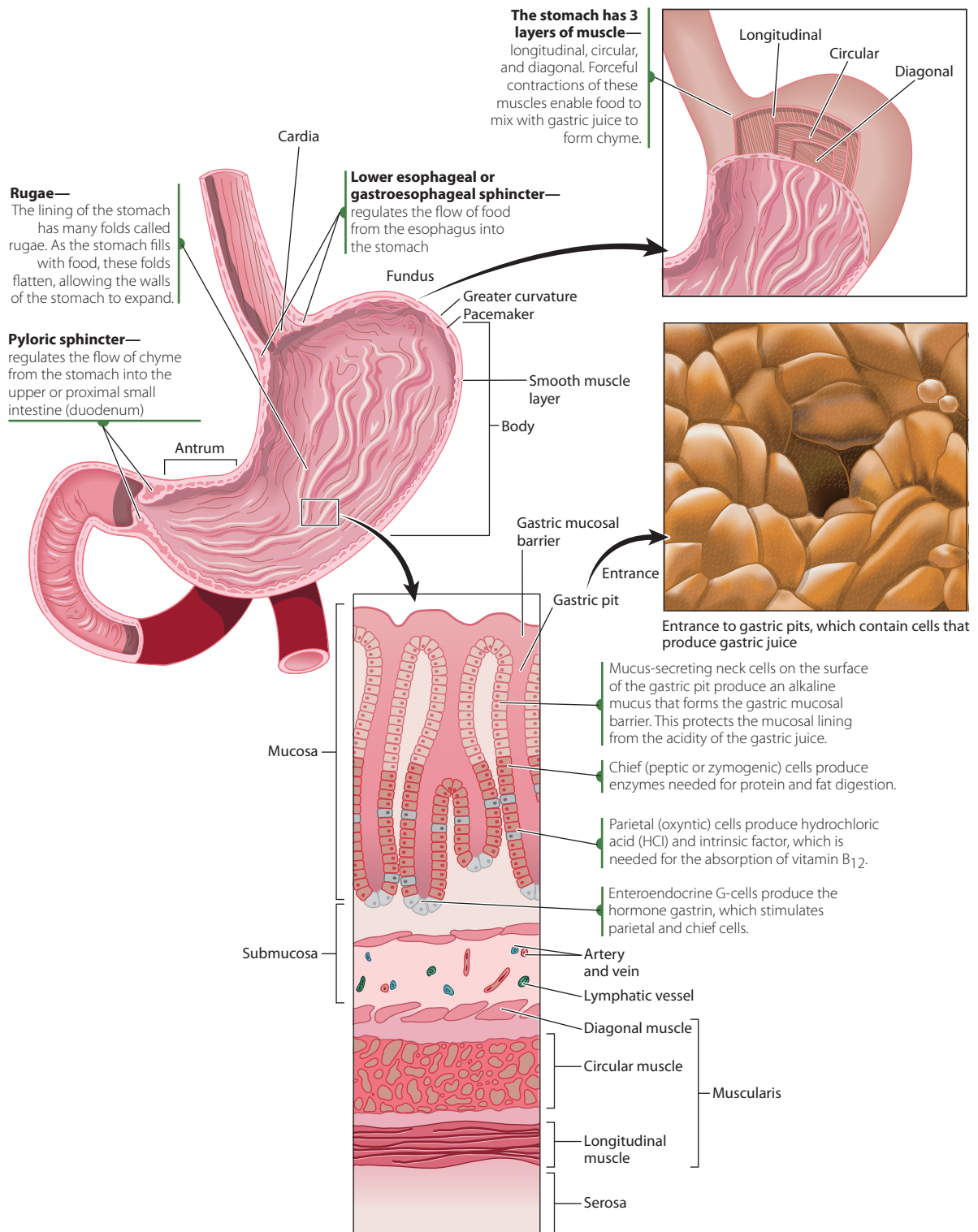


Figure 2.4 Structure of the stomach including a gastric gland and its secretions.

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Additional symptoms may include a chronic cough, excessive belching, and/or a sour taste in the mouth.

While medications to neutralize the acid and/or to reduce acid production are important to promote healing, some dietary changes can also help.

- To minimize reductions in sphincter pressure, high-fat foods as well as chocolate, nicotine, alcohol, and carminatives (volatile oil extracts of plants, most often oils of spearmint and peppermint) should be avoided.

- Substances that increase gastric acid production (such as alcohol, excessive calcium, and decaffeinated and caffeinated coffee and tea) should also be avoided.
- Because citrus products and other acidic foods or beverages, as well as spices such as red and black pepper, nutmeg, cloves, and chili powder, can directly irritate inflamed tissues, avoidance of these substances is also encouraged.

Additional suggestions include (1) eating smaller (vs. larger) meals and drinking fluids between meals (vs. with meals), since large gastric volume may promote reflux; (2) losing weight (if overweight or obese) and avoiding tight-fitting clothes, since these may directly increase gastric pressure; and (3) avoiding lying down, lifting, or bending for at least 2 hours after eating, since such actions place gastric contents nearer to the sphincter and may promote reflux. A discussion of some of the medications used in the management of gastroesophageal reflux disease as well as ulcers is presented in the section “Selected Disorders of the Stomach.” Surgical treatment of chronic acid reflux that has not responded to medications and dietary changes usually involves fundoplication, a procedure in which a portion of the stomach (the fundus) is wrapped around the sphincter (and thus tightens it).

The Stomach

Once the bolus of food has passed through the gastroesophageal sphincter, it enters the stomach, a J-shaped saclike organ located on the left side of the abdomen under the diaphragm. The stomach extends from the gastroesophageal sphincter to the duodenum, the upper or proximal section of the small intestine. The stomach contains four main regions (shown in Figure 2.4):

- The cardia region lies below the gastroesophageal sphincter and receives the swallowed food (bolus) from the esophagus.
- The fundus lies adjacent or lateral to and above the cardia.
- The body, the large central region, serves primarily as the reservoir for swallowed food and is the main production site for gastric juice.
- The antrum or pyloric portion, the lower (distal) one-third of the stomach, provides strong peristalsis to grind and mix food with the gastric juices (which forms **chyme**, a thick, semiliquid mass of partially digested food) and to empty the chyme into the duodenum.

The stomach’s circular, longitudinal, and oblique smooth muscles enable the mixing of the food with gastric juices, including its acid and enzymes. The volume of the stomach when empty (resting) is about 50 mL (~2 oz),

but on being filled it can expand to accommodate from 1 L to approximately 1.5 L (~37–52 oz). When the stomach is empty, folds (called rugae) present in all but the antrum section are visible; however, as we eat and the stomach fills, the rugae disappear. Receptive relaxation allows gastric expansion with food intake with minimal impact on intragastric pressure unless food intake exceeds the stomach’s volume capacity.

Gastric juices, which are produced in significant quantities by glands found within the gastric mucosa and submucosa, facilitate the digestion of nutrients within the chyme. These glands include:

- The cardiac glands, found in a narrow rim at the juncture of the esophagus and the stomach
- The oxyntic glands, found in the fundus and body
- The pyloric glands, located primarily in the antrum.

Several cell types, which secrete different substances, are found within gastric glands, as shown in Figure 2.4. Some of the cells and their secretions that are found in a gastric oxyntic gland include:

- Neck (mucous) cells, which secrete mucus
- Parietal (oxyntic) cells, which secrete hydrochloric acid and intrinsic factor
- Chief (peptic) cells, which secrete pepsinogen and gastric lipase
- Enteroendocrine cells, which secrete a variety of hormones.

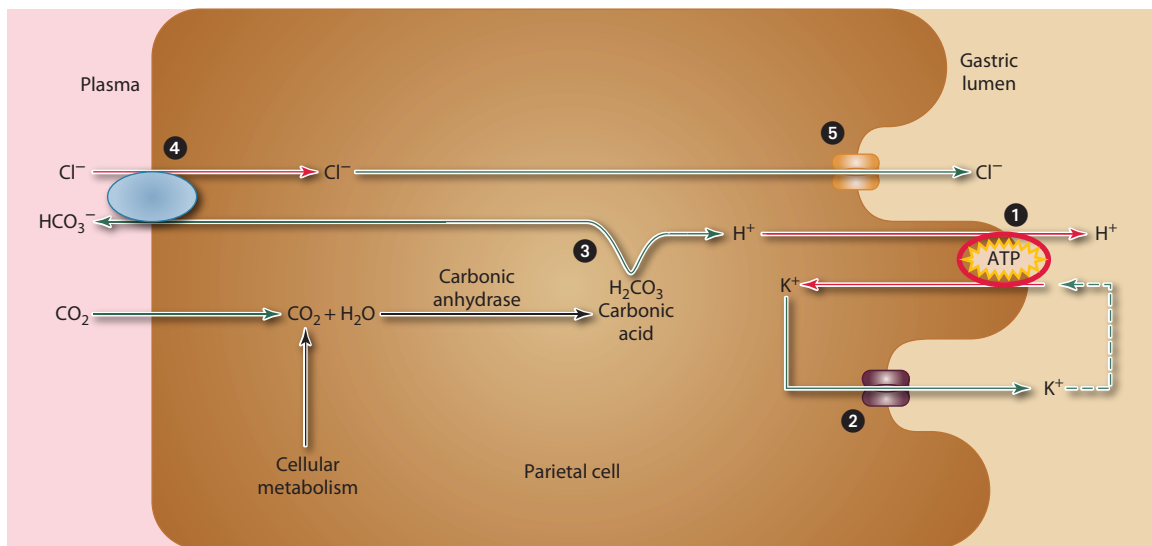
Unlike the oxyntic glands, the cardiac glands contain no parietal cells and the pyloric glands contain no chief cells.

The main constituents of gastric juice produced by the different cells of these gastric glands include water, electrolytes, hydrochloric acid, enzymes, mucus, and intrinsic factor. About 2 L (usual range 1–3 L) of this juice are secreted each day. The next section describes some of these constituents: hydrochloric acid, enzymes, and mucus. A discussion of intrinsic factor, which is found in gastric juice and needed for vitamin B₁₂ absorption, is provided in Chapter 9.

Gastric Juice

Gastric juice contains an abundance of hydrochloric acid, which is secreted as separate hydrogen ions (H⁺) and chloride ions (Cl[−]) from parietal cells into the lumen of the stomach. The mechanism by which hydrochloric acid is secreted is shown and described in Figure 2.5.

The high concentration of hydrochloric acid in the gastric juice is responsible for its low pH, about 2. The pH value is the negative logarithm of the hydrogen ion concentration. The lower the pH, the more acidic the solution. Figure 2.6 shows the approximate pH values of body fluids and, for comparison, some other compounds and



- 1 Parietal cells actively secrete hydrogen (H^+) and chloride (Cl^-) by two different transport systems. A hydrogen (proton) potassium ATPase exchange system (H^+ , K^+ -ATPase), also referred to as a proton pump, secretes hydrogens (protons) into the lumen in exchange for potassium ions (K^+) with each ATP molecule hydrolyzed.
- 2 Following the active exchange, the potassium ions typically diffuse out of the parietal cells and back into the lumen.
- 3 The hydrogen arises, along with bicarbonate, from the dissociation of carbonic acid (H_2CO_3). The carbonic acid is generated within the parietal cell from carbonic anhydrase, an enzyme found in high concentrations within parietal cells, using water and carbon dioxide. The water and carbon dioxide are produced within the cell from normal metabolism; the carbon dioxide also may arise in the cell following diffusion from the plasma.
- 4 The chloride ions needed to form hydrochloric acid arise initially from the plasma from which they are transported by a secondary active transport system in exchange for bicarbonate into the parietal cells. This antiporter carries simultaneously the bicarbonate down its concentration gradient into the plasma and the chloride against its concentration gradient into the parietal cell.
- 5 From the parietal cells, the chloride ions then diffuse out via a chloride channel into the gastric lumen joining the hydrogen ions to generate hydrochloric acid.

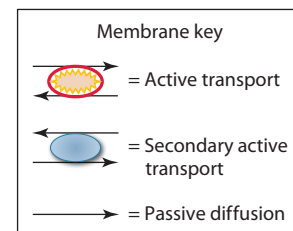


Figure 2.5 Mechanism of HCl secretion.

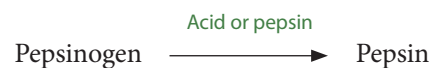
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beverages. Notice that the pH of orange juice (and typically of all fruit juices) is higher than that of gastric juice. Thus, drinking such juices does not lower the gastric pH.

In addition to creating an acidic environment, hydrochloric acid has several other functions in gastric juice, including:

- Converting or activating the zymogen pepsinogen to form pepsin (needed for protein digestion)
- Denaturing proteins (i.e., destructing or “uncoiling” the tertiary and secondary protein structures to expose the protein’s interior peptide bonds so pepsin can perform its enzymatic functions)
- Releasing various nutrients such as minerals from organic complexes so absorption can occur
- Acting as a bactericide agent (needed to kill bacteria ingested along with food).

Three enzymes (see Table 2.1) are found in gastric juice. The enzyme pepsin is secreted into gastric juice initially as a zymogen called pepsinogen. Specifically, pepsinogen is secreted in granules into the gastric lumen by chief cells when they are stimulated by acetylcholine and/or acid. Pepsinogen is then converted (activated) to pepsin, an active enzyme, by hydrochloric acid or the presence of previously formed pepsin in the gastric lumen.



Pepsin functions as a protease, an enzyme that hydrolyzes proteins. Specifically, pepsin is an endopeptidase, meaning that it hydrolyzes interior peptide bonds within proteins. Optimal pepsin activity occurs at about pH 3.5. Another enzyme made by gastric chief cells is gastric lipase. Gastric lipase hydrolyzes fatty acids from glycerol’s

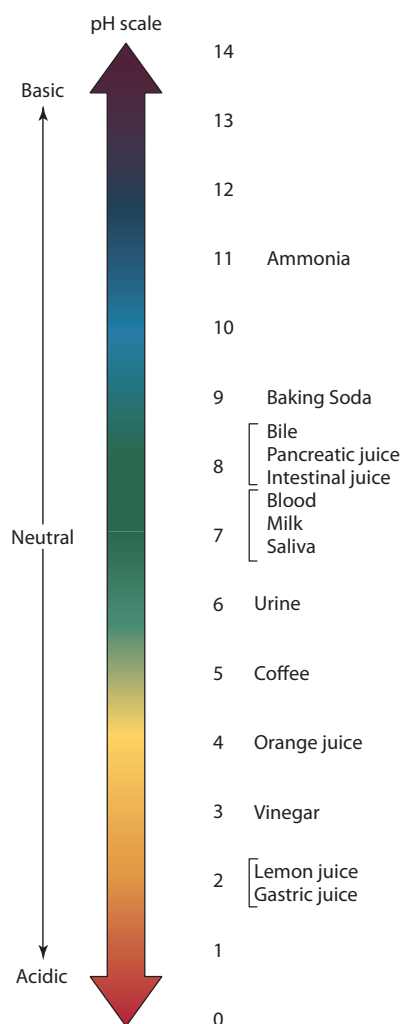


Figure 2.6 Approximate pHs of selected body fluids, compounds, and beverages.

third carbon in triacylglycerols. This enzyme is thought to be responsible for up to about 20% of lipid digestion. The salivary α -amylase found in gastric juice originates from the salivary glands of the mouth. This enzyme, which hydrolyzes starch, retains some activity in the stomach until it is inactivated by the low pH of gastric juice. Additional information about pepsin and salivary α -amylase can be found in Chapters 6 and 3, respectively. Gastric lipase is discussed further in Chapter 5.

Gastric juice also contains mucus, which is secreted both by neck (mucous) cells in gastric glands and by mucosal epithelial cells; these epithelial cells also release bicarbonate (HCO_3^-). Mucus composition varies depending on its location in the digestive tract, but it generally consists of a network of different glycoproteins called **mucins**. Most mucins bind water and are gel-forming and thus provide lubrication and protection. In the stomach, mucus both coats the gastric contents as well as forms a layer about 2 mm thick on the gastric mucosal membrane to coat and protect it. Embedded within this gastric mucus layer is

bicarbonate, creating a local pH of about 6–7 versus the very acidic pH of about 2 in the gastric lumen. Production and release of mucus within the stomach is enhanced by **prostaglandins**, vagal nerve stimulation, acetylcholine, and various hormones. Substances that inhibit or diminish mucus secretion increase the risk for ulcer formation.

Regulation of Gastric Secretions

The regulation of gastric secretions can be divided into three phases based on events occurring before food reaches the stomach, once food is in the stomach, and after food has left the stomach. Multiple mechanisms, both neural and chemical, influence each of the three phases; some of the many hormones and peptides that are involved are shown in Figure 2.7 and are presented later in the chapter in Table 2.2.

In the cephalic (first) phase, eating or tasting food, as well as thinking about, seeing, and/or smelling food, stimulates gastric secretions. Vagal stimulation of primarily the submucosal plexus promotes the secretion of the neurotransmitter acetylcholine and enhances the release of the hormone gastrin from G cells. Acetylcholine and gastrin both trigger the release of the paracrine histamine by mast cells and enterochromaffin-like cells in the gastric glands. Each stimulates hydrochloric acid secretion by parietal cells—histamine binds to H_2 receptors, gastrin binds to gastrin receptors, and acetylcholine acts on muscarinic receptors on the parietal cells. Additionally, acetylcholine stimulates the chief cells, promoting enzyme release.

The second, or gastric, phase occurs when ingested food reaches the stomach. Distension of the stomach (identified by stretch receptors in the stomach layers) along with the presence of protein and some other consumed substances, especially caffeine and alcohol, in the stomach enhance gastric secretions in this phase. The ability of proteins, primarily those that have been digested into small peptides and/or amino acids, to enhance gastric secretions occurs through multiple pathways including, for example, stimulating chemoreceptors that initiate submucosal plexus nerve activity; promoting gastrin release; and activating the parasympathetic nervous system, which further enhances vagal activity to the stomach.

The third, or intestinal, phase of gastric secretions occurs after food has left the stomach and has entered the duodenum. In this phase, a reduction in chyme volume in the stomach and a reduction in the pH of gastric juice (to < 2) trigger the release of somatostatin by D cells in the pancreas, antrum, and duodenum. Somatostatin, which acts in a paracrine fashion by entering gastric juice, diminishes parietal cell, G cell, and enterochromaffin-like cell secretions. Additionally, some of the factors that inhibit gastric emptying (as discussed in the next section) also inhibit the release of gastric secretions. These factors include the presence of hyperosmolar chyme and acidic chyme, as well as fat-containing chyme in the duodenum.

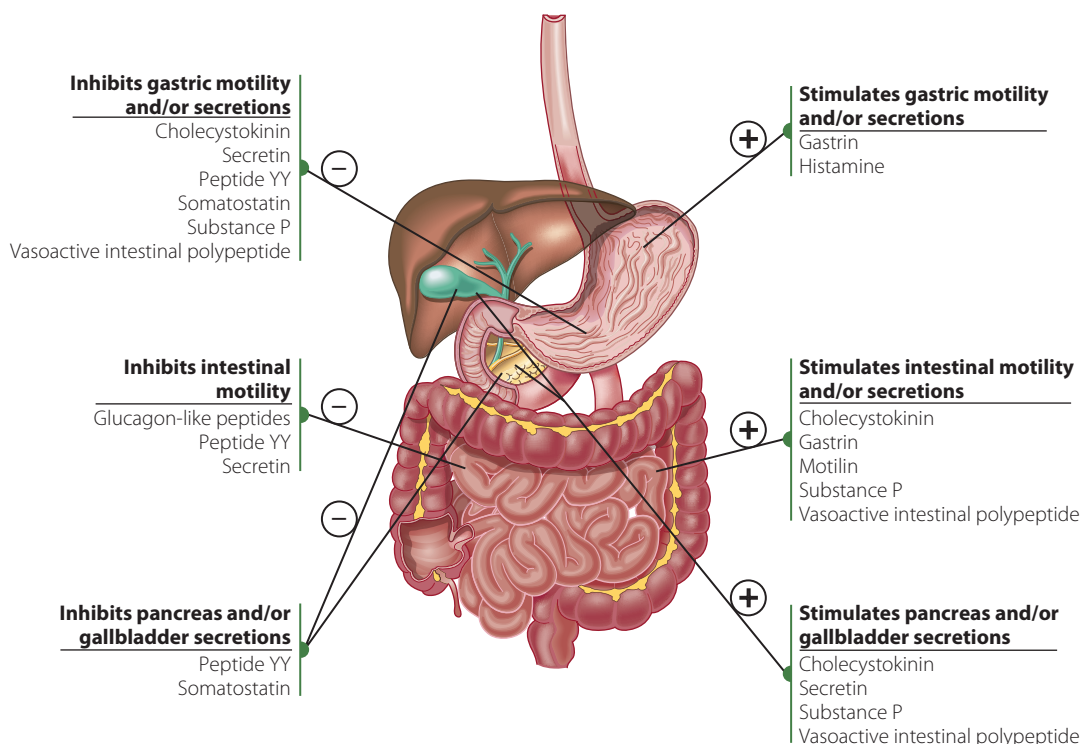


Figure 2.7 Effects of selected gastrointestinal hormones/peptides on gastrointestinal tract secretions and motility.

Furthermore, chyme's presence in the duodenum both causes distension (eliciting responses from the submucosal plexus and myenteric plexus) and triggers the release of secretin and cholecystikinin. These actions reduce gastric secretions, reduce peristalsis in the antrum, and slow gastric emptying to “finish up” digestive actions in the stomach, but simultaneously these hormones also promote digestive processes within the small intestine. Other hormones that play lesser roles in diminishing gastric acid production include glucose-dependent insulintrophic peptide and peptide YY, the paracrine glucagon-like peptides, and the neurocrine vasoactive intestinal polypeptide.

Regulation of Gastric Motility and Gastric Emptying

Peristalsis occurring in the stomach is strongest in the lower body and **antral** sections. The peristaltic waves propel the digestive contents through the stomach as well as through most of the other portions of the digestive tract. Additionally, in the antrum, retropulsion pushes the chyme back and forth between peristaltic contractions to help grind and liquify food particles. Another means of motility present in the stomach is a basic electrical rhythm that is initiated by the interstitial cells of Cajal (also referred to as pacemaker cells), found in the outer circular muscles (muscularis externa) near the myenteric plexus in the body of the stomach at the greater curvature. The pacemaker cells in the stomach generate wave-like signals (or slow-wave potentials) at a rate of about

three per minute that move from the fundus toward the pyloric sphincter and help to coordinate peristalsis and other motor activity.

Gastric emptying is affected by factors both in the stomach and duodenum. In the antrum of the stomach, the strength of the peristaltic contractions and gastric motility are affected by the volume and fluidity of the chyme. Increased gastric volume promotes gastric distension. The distension is detected by not only the nerves innervating the stomach (vagus and intrinsic plexuses), but also the smooth muscle within the stomach. These factors along with increased gastrin release and fluidity of the gastric contents in turn increase gastric emptying and motility. In the duodenal bulb (the first few centimeters of the proximal duodenum), receptors are sensitive to distension/volume, as well as the **osmolarity**, nutrient content, and acidity of the chyme.

- Distension from an excessive volume of chyme in the duodenum reduces gastric emptying.
- The presence in the duodenum of hypertonic/hyperosmolar (very concentrated) chyme, which occurs, for example, with increased gastric emptying and/or delayed nutrient (especially amino acid and/or glucose) absorption, slows gastric emptying.
- Dietary fat intake also has an inhibitory effect on gastric emptying, versus carbohydrate-rich and protein-rich foods; in fact, a high-fat meal may take up to 6 hours to digest versus typically less than 4 hours for a meal

consisting of mostly carbohydrates and protein. The delay in gastric emptying is mediated primarily by the hormone cholecystokinin whose release is triggered by the presence of fat in the duodenum. Cholecystokinin primarily promotes **bile** secretion into the duodenum, enabling fat emulsification and digestion, but also inhibits gastric emptying.

- The presence of unneutralized acidic chyme in the duodenum stimulates the release of secretin that both slows gastric emptying of the acidic chyme into the duodenum and stimulates the release of pancreatic juice, which functions in part to neutralize the acid.
- In addition to cholecystokinin and secretin, the paracrine somatostatin and, to a lesser extent, the hormones pancreatic polypeptide and peptide YY and the paracrine glucagon-like peptides, diminish gastric emptying.

Some other factors affecting gastric motility result from neural reflexes and involvement of the autonomic nervous system. Distension in the duodenum inhibits gastric emptying, as previously discussed; additionally, distension in the distal small intestine also impacts gastrointestinal tract motility. The nerve reflex known as the ileogastric reflex is elicited by distension in the ileum and results in diminished gastric emptying. This action allows more time for the contents of the ileum to be emptied before more chyme is released from the stomach into the duodenum. Finally, emotions such as fear, anger, and sadness, among others, inhibit or excite the digestive system's smooth muscles via the autonomic nervous system to affect gastric emptying and intestinal motility.

The secretions and contractions within the stomach promote physical disintegration of solid foods into liquid form and continue the digestive processes that began in the oral cavity. However, most nutrients from these digestive actions in the upper gastrointestinal tract are not yet ready to be absorbed into the body; the stomach absorbs only alcohol and small quantities of water and a few minerals including iodide and fluoride. Before most nutrient absorption can occur, additional digestive actions are needed within the small intestine. Complete liquefaction of chyme is not necessary for the stomach contents to empty into the duodenum (which occurs via the pyloric sphincter, found at the junction of the antrum and the duodenum). Particles as large as 3 mm in diameter (~1/8 inch) can be emptied from the stomach through the sphincter, but solid particles are usually emptied with fluids when they have been degraded to a diameter of about 2 mm or less. Approximately 1–5 mL (~up to 1 tsp) of chyme enters the duodenum about twice per minute. Gastric emptying following a meal usually takes between 1 and 4 hours; however, in those who are critically ill, gastric emptying may be delayed and can result in larger gastric residual volumes. These gastric residuals need to be monitored closely in hospitalized patients being fed into their stomach via a tube. Should the rate of tube feeding be greater than the

rate of gastric emptying, vomiting (emesis) and aspiration may occur. Problems from delayed gastric emptying (called gastroparesis) can also occur in those who are not critically ill and/or being tube-fed. Gastroparesis can occur with damage to the vagus nerve from diabetes and some neurological conditions. If untreated, gastroparesis may cause malnutrition and, in those with diabetes, difficulty controlling blood glucose concentrations.

Selected Disorders of the Stomach

Peptic ulcer disease (PUD), commonly referred to as ulcers, is characterized by the presence of ulcerations or erosions usually in the mucosa and submucosa layers of the stomach (antrum area), duodenum (first few centimeters), and/or lower esophagus. Perforations, however, can also occur, affecting all four layers of the digestive tract. Multiple factors promote the formation of ulcers. Zollinger-Ellison syndrome, from the presence of a gastrin-producing tumor, is a rare condition characterized by extremely copious secretion of gastrin into the blood. The hypergastrinemia (higher than normal blood gastrin concentrations) promotes excessive hydrochloric acid release into the stomach and the formation of numerous ulcers in the stomach and duodenum, and sometimes even the jejunum. A more common cause of ulcers is from the bacterium *Helicobacter (H.) pylori*, but any factor that disrupts the integrity of the mucosa (including normal defense and repair systems) can increase the likelihood of ulcer formation. Chronic ingestion of alcohol as well as the excessive use of aspirin and nonsteroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, for example, both disrupt the normally tight junctions between gastric mucosal cells (that prevent acid penetration) and diminish the production of the bicarbonate and mucus (which form a protective barrier on the mucosal membrane of the gastrointestinal tract). The dietary recommendations and medications used to treat peptic ulcers are similar to those described for gastroesophageal reflux disease. (Some of the dietary changes have been previously addressed; see “Selected Disorders of the Esophagus.”) A brief discussion of the mechanisms of action for two groups of frequently used medications in the treatment of ulcers and gastroesophageal reflux disease and their effects on nutrient utilization follows.

One group of medications used to treat these conditions is called H_2 receptor blockers—including Tagamet (cimetidine), Pepcid (famotidine), and Axid (nizatidine). These medications function by binding to the H_2 receptors on the parietal cells. Consequently, when histamine is released, it cannot bind to these H_2 receptors (the drug blocks histamine's ability to bind), and acid release from the parietal cell is diminished. Another group of drugs, referred to as proton pump inhibitors—including Prilosec (omeprazole), Nexium (esomeprazole), Protonix (pantoprazole), Aciphex (rabeprazole), and Dexilant (dexlansoprazole)—works by binding to the ATPase/proton pump (see Figure 2.5) at

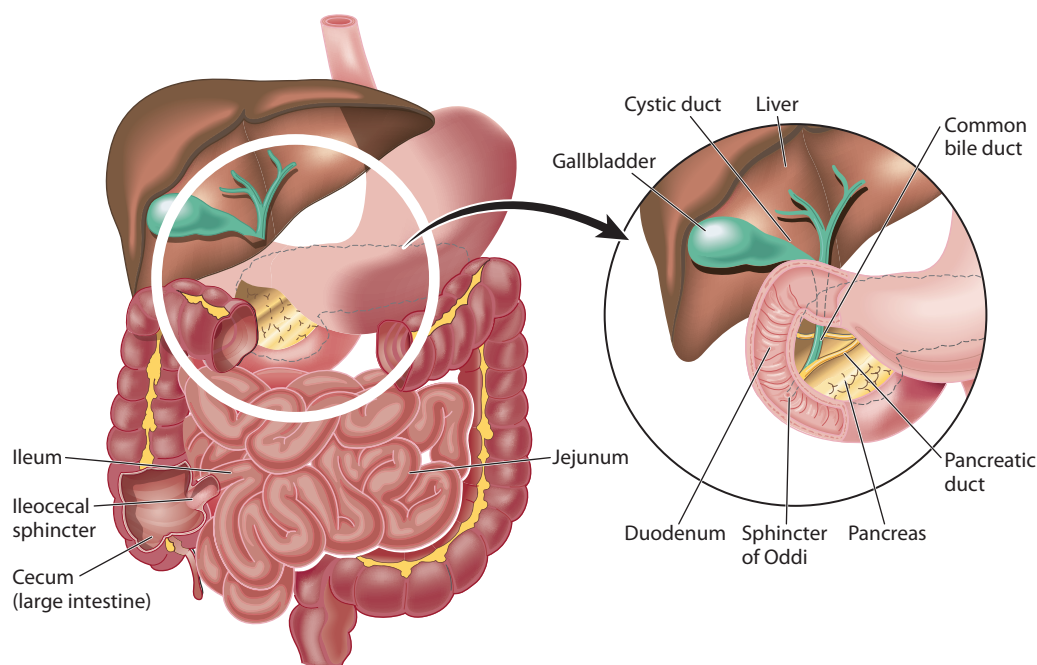
the secretory surface of the parietal cell and thus directly inhibits hydrogen release. In comparison with other medications, the proton pump inhibitors are the most effective at inhibiting acid production. However, long-term use can cause bacterial overgrowth and can negatively impact the absorption of vitamin B₁₂ and several minerals that tend to benefit from an acidic environment.

Recurrent ulcers that are not responsive to medications and diet changes as well as other conditions affecting the stomach, such as cancer, may necessitate the surgical removal (resection) of a portion of the stomach. Gastric restriction and resection procedures are also used for the treatment of obesity and are discussed further in the Perspective for this chapter. Removing a portion of the stomach negatively impacts normal digestive tract functions. One such complication is a condition called dumping syndrome. Dumping syndrome occurs after eating (from about 30 minutes to 3 hours) and results initially from hyperosmolar (concentrated) chyme getting released “too rapidly” into the duodenum. This “dumping” occurs because the size of the stomach, which is now considerably reduced, can no longer serve as a storage reservoir, produce its usual volume of digestive juices, or mix the ingested food with gastric juices to create a diluted, partially digested chyme mixture. The hyperosmolar (concentrated) chyme in the duodenum in turn causes fluid from the blood to be “pulled or drawn” quickly into the lumen of the duodenum to dilute its contents and create

a more isotonic chyme. Such actions promote some of the symptoms of dumping syndrome, which include dizziness, weakness, tachycardia (rapid heartbeat), and hypotension (associated with the reduction in vascular fluid), as well as nausea, abdominal distension, and pain. Other symptoms may include gas, diarrhea, and abdominal pain from the fermentation of the undigested nutrients by bacteria in the intestines, and weakness, palpitations, and hypoglycemia (low blood glucose). The hypoglycemia results when there is excessive **insulin** secretion occurring secondary to the consumption of foods usually rich in simple sugars (monosaccharides and disaccharides), which get absorbed too quickly from the duodenum and into the blood. To help alleviate some of the nutritional complications of gastric resection, some of the several dietary modifications include eating foods slowly, limiting the intake of foods high in simple sugars, and limiting the consumption of fluids with meals (to lessen the gastric volume, which promotes rapid emptying). Medications that delay gastric emptying and reduce gut motility may also ameliorate some of the symptoms.

The Small Intestine

Once through the pyloric sphincter, chyme enters the small intestine. The small intestine (Figure 2.8), which represents the main site for both nutrient digestion and absorption, is composed of the duodenum (slightly less than 1 foot long



The small intestine is divided into three regions: the duodenum, jejunum, and ileum. The ileocecal sphincter regulates the flow of material from the ileum, the last segment of the small intestine, into the cecum, the first portion of the large intestine.

The duodenum receives secretions from the gallbladder via the common bile duct. The pancreas releases its secretions into the pancreatic duct, which eventually joins the common bile duct. The sphincter of Oddi regulates the flow of these secretions into the duodenum.

Figure 2.8 The small intestine.

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with about a 2-inch diameter), the jejunum (slightly over 8 feet long), and the ileum (about 11½ feet long). Microscopy is generally needed to identify where one of these sections of the small intestine ends and the other begins. However, the Treitz ligament, a suspensory ligament, is found at about the site where the duodenum and jejunum meet. Furthermore, there is a slight size difference, with the lumen of the jejunum (about 1¼–1½ inches) being generally slightly larger than that of the ileum (about 1–1¼ inches).

Structural Aspects, Secretions, and Digestive Processes of the Small Intestine

Although the structure of the small intestine consists of the same layers identified in Figure 2.2, the small intestine is structured with an enhanced surface area to absorb nutrients. The small intestine has a surface area of approximately 300 m², an area about equal to a 3-foot-wide sidewalk more than three football fields in length. Several structures, shown in Figure 2.9, that contribute to this enormous surface area include:

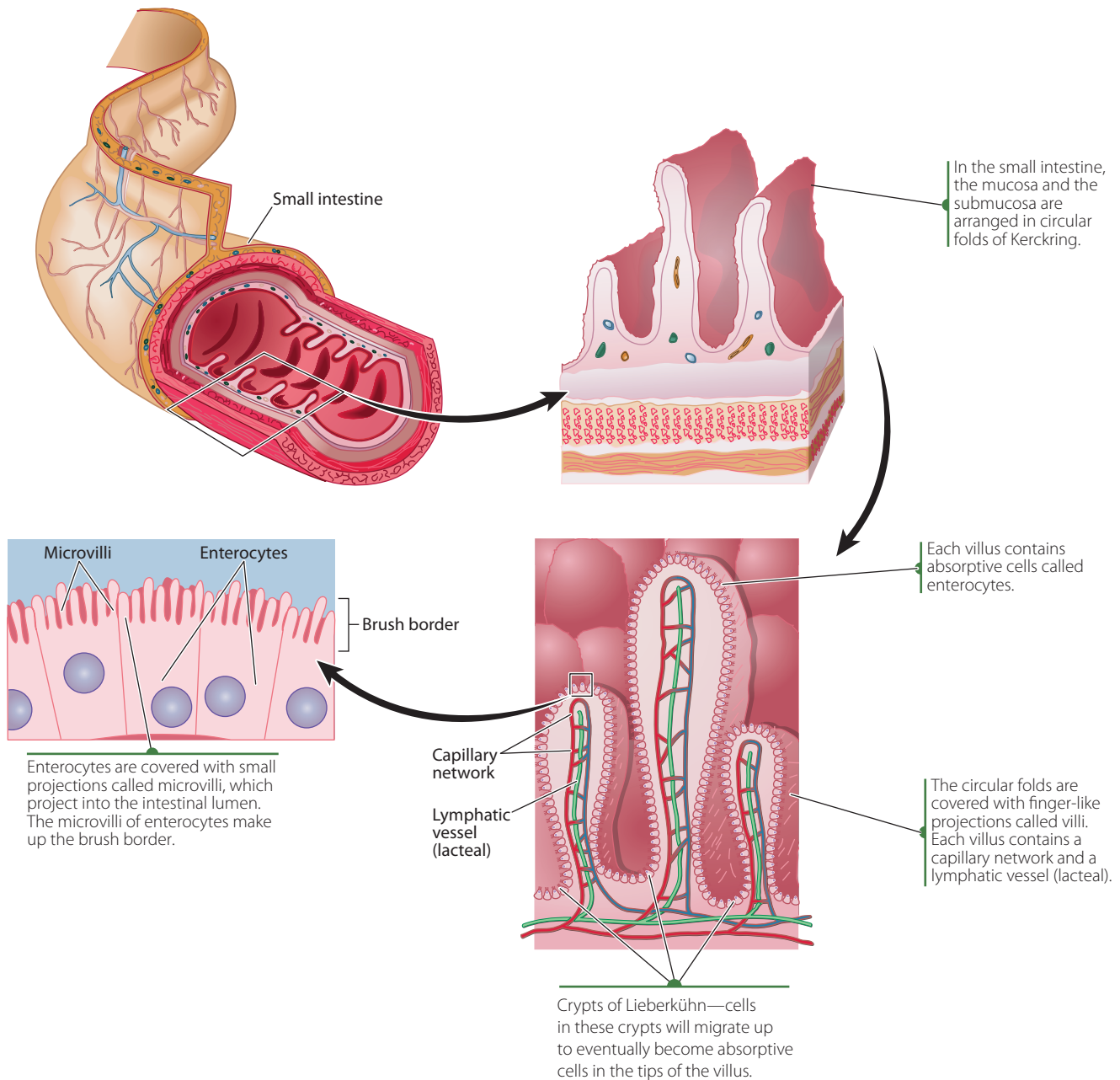


Figure 2.9 Structure of the small intestine.

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- Large circular folds of the mucosa, called the folds of Kerckring, that protrude into the lumen of the small intestine
- Finger-like projections, called villi, that project out into the lumen of the intestine and consist of hundreds of intestinal cells called **enterocytes** (these cells are also referred to as absorptive, epithelial, and/or mucosal cells) along with blood capillaries and a lacteal (lymphatic vessel) for transport of nutrients out of the enterocytes
- **Microvilli**, hairlike extensions of the plasma membrane of the enterocytes that make up the villi. A square millimeter of cell surface is believed to have as many as 2×10^5 microvilli projections. The microvilli (Figure 2.10) possess a surface coat, or glycocalyx, consisting of numerous fine filaments that extend almost perpendicular from the membrane to which it is attached out into the lumen.

The enterocyte membrane bordering the lumen is referred to as the enterocyte's brush border (also called **apical**) membrane. It consists of membranes from a single layer of enterocytes (shown in Figures 2.9 and 2.10) and forms a continuous membrane border as the result of precise alignments of the cells and tight intercellular junctions. The role of tight junctions is discussed further under the section addressing "The Absorptive Process."

Many of the digestive enzymes produced by the enterocytes are structurally glycoproteins, and the carbohydrate (glyco) portion of these glycoprotein enzymes

make up part of the **glycocalyx**. These enzymes hydrolyze already partially digested nutrients, especially carbohydrates and protein. Some nutrients that are not completely digested on the brush border, however, may be further digested within the cytosol of the enterocytes.

Covering the brush border membrane is an area called the unstirred water (fluid) layer. That is, the unstirred water layer lies between the enterocyte's brush border membrane and the intestinal lumen. Its presence can affect lipid and fat-soluble vitamin absorption. More detailed information on carbohydrate, fat, and protein digestion and absorption is provided in Chapters 3, 5, and 6, respectively.

Between the villi of the small intestine are small pits or pockets called the crypts of Lieberkühn (Figure 2.9). Stem cells in these crypts continuously undergo mitosis. The new cells migrate upward and out of the crypts toward the tips of the villi, and as they migrate, they differentiate into other cell types. Billions of old enterocytes, which die by apoptosis and are sloughed off daily into the intestinal lumen for excretion in the feces, are replaced by new enterocytes about every 3–5 days. Some of the other cells found in the crypts include Paneth cells that secrete both antimicrobial peptides (called defensins), lysozymes that can destroy bacterial cell walls, and goblet cells that secrete both small cysteine-rich proteins with antifungal activity and mucus, which adheres to the mucosa and acts as a protective barrier. Cells and glands in the crypts of Lieberkühn also secrete large volumes of intestinal juices into the lumen of the small intestine to facilitate nutrient digestion. Much of this fluid is reabsorbed.

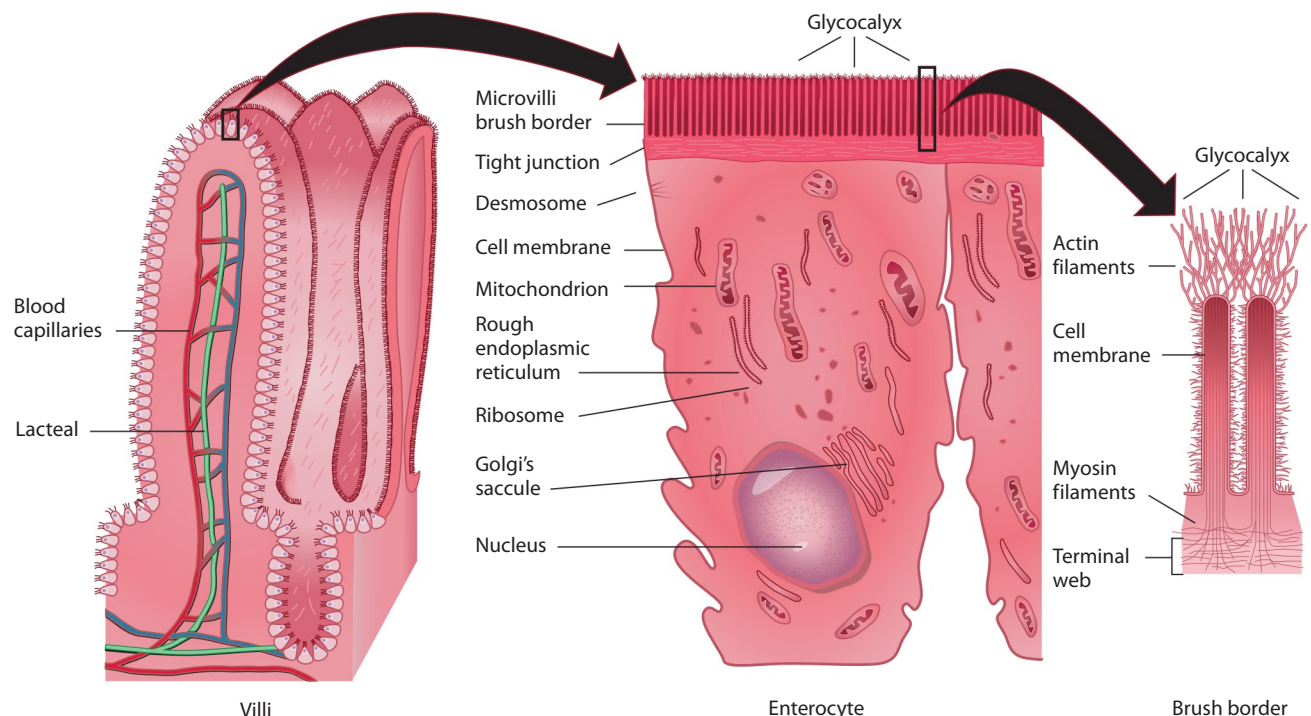


Figure 2.10 Structure of the absorptive cell of the small intestine.

Brunner's glands, located in the mucosa and submucosa of the first few centimeters of the duodenum (duodenal bulb), as well as exocrine cells of the pancreas, also release secretions into the small intestine. The secretions of the Brunner's glands are rich in mucus to coat (protect) the intestinal mucosa cells. The secretions of the pancreas are rich in bicarbonate to neutralize the acidic chyme (released by the stomach) and create a more alkaline environment, with a pH of approximately 8.2–9.3. This higher pH is also important for optimal enzyme activity within the intestine.

Regulation of Intestinal Motility and Secretions

Chyme is propelled through the small intestine by contractions (Figure 2.11) that are influenced by the nervous system as well as various hormones and peptides. For example, the neuropeptide vasoactive intestinal polypeptide promotes intestinal motility and secretions, while the paracrine glucagon-like peptides diminish intestinal motility.

Peristaltic waves, or progressive contractions (like those that are in the esophagus and stomach), direct the chyme

distally from the duodenum toward the ileocecal sphincter. Segmentation contractions, standing contractions of intestinal circular smooth muscles, also occur as nutrients from food are being digested. The segmentation contractions are especially important for promoting a bidirectional flow of the chyme in the small intestine, thus prolonging contact between the intestinal cells and the digested nutrients within the chyme for absorption to occur. The basal electrical rhythm generated from the interstitial cells of Cajal located throughout the muscularis externa layer of the small intestine induces the contractions, which occur at a frequency of about 11 or 12 contractions per minute in the duodenum and about 7 or 8 contractions per minute in the ileum.

Neural reflexes also affect motility during digestion. These reflexes, discussed in more detail in the section “Neural Regulation,” generally help to coordinate motility and secretions between one section of the digestive tract and another. These actions, for example, may slow processes in one organ to allow actions in another organ to “catch up”; for example, slowing gastric secretions and/

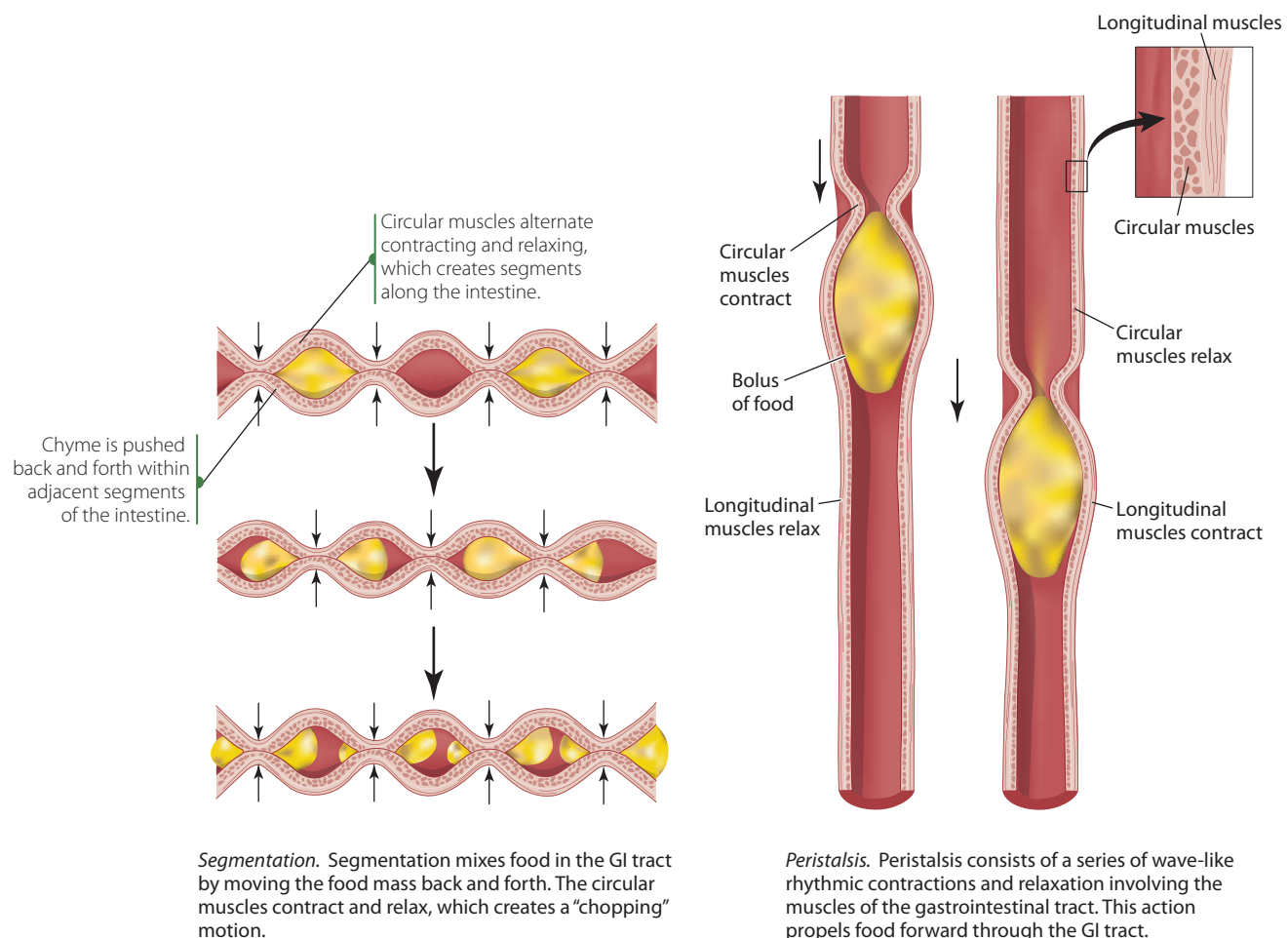


Figure 2.11 Movement of chyme in the gastrointestinal tract.

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or gastric emptying if large volumes of chyme requiring digestion were present in the small intestine.

While the aforementioned processes regulate intestinal motility during meals (i.e., digestive period), another type of motor activity occurs largely in the small intestine between meals. The migrating motility or myoelectric complex (MMC), a series of weak contractions that occur in several phases, moves distally down the intestine at regular intervals between periods of digestion (i.e., between meals). The MMC helps to empty or “sweep out” the intestines as well as to prevent bacterial overgrowth. The hormone motilin, secreted by M cells of the stomach, small intestine, and colon during fasting (i.e., between meals), primarily stimulates the activity of this complex. Transit time within the small intestine ranges from about 3 to 5 hours.

The Accessory Organs

Three organs—the pancreas, liver, and gallbladder—facilitate the digestive and absorptive processes in the small intestine. The next section of this chapter describes each of these organs and its role in nutrient digestion, absorption, or both.

The Pancreas

The pancreas is a slender, elongated organ that ranges in length from about 6 to 9 inches. The pancreas is found behind the greater curvature of the stomach, lying between the stomach and the duodenum (Figures 2.1 and 2.12). The organ contains both endocrine and exocrine cells (Figure 2.12b).

The endocrine cells are found among the 1–2 million cells that make up the islets of Langerhans, located primarily in the tail region of the pancreas. While these cells comprise less than 5% of the gland’s volume, they are responsible for the secretion of several important hormones. The A or α cells secrete glucagon, the B or β cells secrete insulin, and the D or δ cells secrete somatostatin. Yet, while these hormones exert enormous regulatory control, it is the exocrine cells of the pancreas that are more involved in the digestive processes with the production of pancreatic juice and enzymes.

The exocrine portion of the pancreas contains acinar secretory cells, which are arranged in a circular pattern and are attached to small ducts. Cells in the ducts produce the pancreas’s alkaline-rich juice, while the acinar secretory cells produce and package into granules the digestive enzymes that get released by **exocytosis** into the lumens of the small ducts. The small ducts within the pancreas coalesce to form the pancreatic duct of Wirsung, which runs the length of the pancreas and connects with the common bile duct at the ampulla of Vater to form a common channel (bile pancreatic duct). The bile pancreatic duct empties through the sphincter of Oddi (Figure 2.12a) into the duodenum. The enzyme-rich and alkaline-rich secretions from the pancreas are needed for the digestive processes within the small intestine.

Pancreatic Juice and Digestive Enzymes The pancreas releases up to about 2 L of its secretions daily into the duodenum. The juice contains mostly water, electrolytes (the cations sodium, potassium, and calcium and the

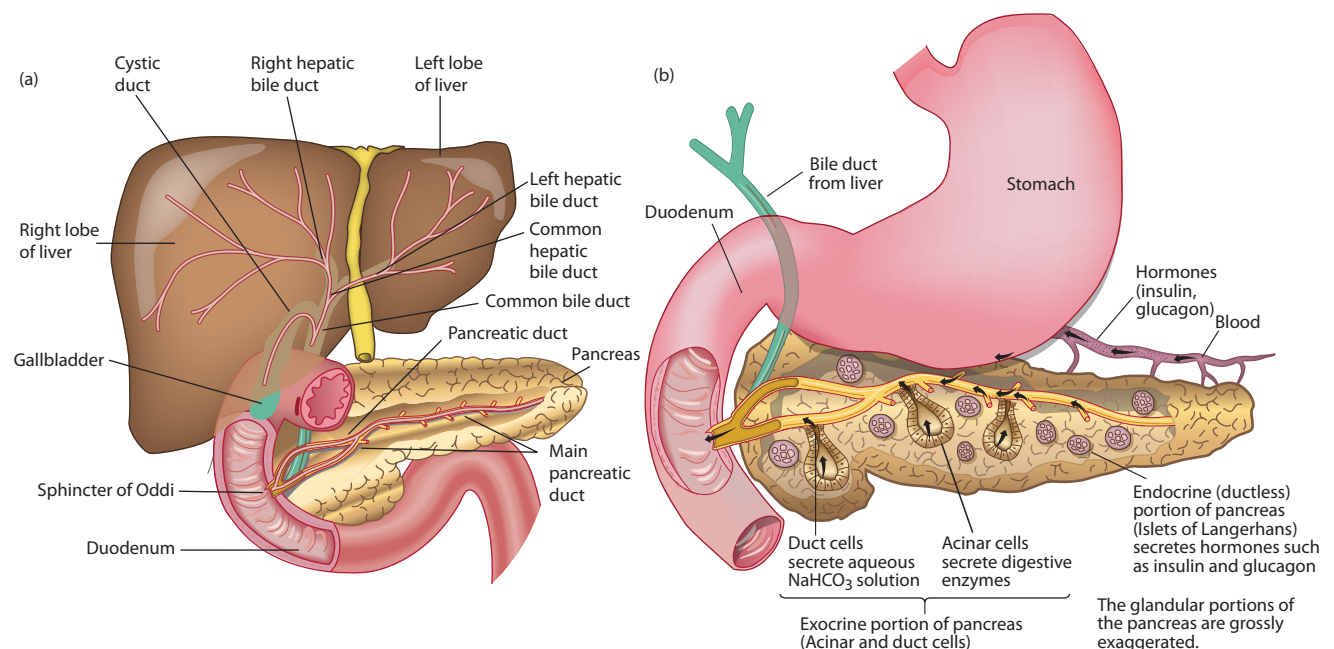


Figure 2.12 (a) The ducts of the gallbladder, liver, and pancreas. (b) Schematic representation of the exocrine and endocrine portions of the pancreas.

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anion chloride), and bicarbonate (as NaHCO_3). The bicarbonate neutralizes the acidic chyme released from the stomach into the duodenum and creates a more alkaline pH needed for enzyme activity within the intestinal lumen. The enzymes released by the acinar secretory cells, listed in Table 2.1, digest approximately half (50%) of all ingested carbohydrates, half (50%) of all proteins, and almost all (80–90%) of ingested fat. The **proteases**—enzymes that digest proteins—are typically released as zymogens and include trypsinogen, chymotrypsinogen, procarboxypeptidases, proelastase, and collagenase. Release in this inactive state is important because, if secreted in an active form, they could digest the proteins within the pancreatic cells in which they were formed. The zymogen trypsinogen is of particular significance in that once it has become activated in the duodenum, it then functions to activate several other zymogens (chymotrypsinogen, procarboxypeptidase, proelastase) and the enzyme phospholipase A_2 needed for fat digestion. A protein called trypsin inhibitor, also synthesized by the pancreas, protects the pancreas by binding to trypsin should it have been accidentally activated within the pancreas. By binding to trypsin, the inhibitor prevents trypsin from activating other zymogens within the pancreas and causing pancreatitis (inflammation of the pancreas).

As a group, proteases hydrolyze peptide bonds within proteins, resulting in the production of smaller polypeptides or proteins that are shorter in length than the original polypeptide or protein (see Chapter 6 for additional information on protein digestion). Enzymes that are released also participate in the digestion of starch (pancreatic α -amylase) and fats (pancreatic lipase, phospholipase A_2 , and colipase), as discussed further in Chapters 3 and 5, respectively.

Regulation of Pancreatic Secretions The primary stimuli for the release of pancreatic juice and enzymes are the hormones secretin and cholecystokinin and to a lesser extent the neurotransmitter acetylcholine.

- Secretin, produced by S cells in the proximal small intestine, is secreted into the blood primarily in response to the presence of unneutralized acidic chyme in the duodenum. Secretin stimulates pancreatic duct cells to secrete into the duodenum its bicarbonate-rich juices, which in turn neutralize the acidic chyme.
- Cholecystokinin is secreted by I cells of the proximal small intestine and enteric nerves in response to the presence of fat and partially digested proteins in the duodenum. Cholecystokinin acts on the acinar secretory cells to stimulate digestive enzyme release into the duodenum.
- Acetylcholine also functions to enhance enzyme release by the acinar cells.

In contrast, the hormone pancreatic polypeptide and the paracrine somatostatin act in reverse, that is, inhibiting pancreatic exocrine secretions.

Selected Disorders of the Pancreas Pancreatitis (the suffix *-itis* meaning “inflammation”) occurs when zymogens become activated within the pancreas and digest pancreatic tissue and sometimes associated tissues including blood vessels and fat. The condition can occur with excessive alcohol consumption, hypertriglyceridemia (**serum** triglycerides in excess of usually about 1,000 mg/dL), blockage of the pancreatic duct (e.g., from gallstones), viral infections, and pancreatic injury, among others. Abdominal pain, usually in the upper left quadrant and that worsens with food intake, is a major symptom. A number of biochemical blood indices become altered with pancreatitis. Most notably, the enzymes pancreatic amylase and lipase, which are normally released into the duodenum, leak out of the damaged pancreas and become elevated in the blood (where they are not normally found). The nutritional management of individuals with especially **acute**, severe pancreatitis is quite involved (and beyond the scope of this book); however, a few nutritional implications of pancreatitis related to digestive functions are provided here. First, because the damaged pancreas cannot produce enzymes in sufficient quantities, the patient often requires the provision of nutrients that are already partially hydrolyzed (rather than intact) or supplements of pancreatic enzymes, especially lipase, to replace those not being released by the malfunctioning pancreas. In addition, because bicarbonate secretion from the pancreas is frequently diminished with pancreatitis, and because this bicarbonate is needed to neutralize acid from the stomach and increase the pH of intestinal juices for enzyme function, medications such as antacids are sometimes provided. Depending on the severity, the individual may also need to be fed through a tube placed into the jejunum and may require suctioning of his or her gastric contents to minimize stimulation of the pancreas.

The Liver

Another accessory organ to the gastrointestinal tract is the liver, pictured in Figures 2.1, 2.12, and 2.13. The liver, the largest single internal organ of the body, is made up of two lobes, the right lobe and the left lobe. These lobes in turn contain functional units called lobules. The lobules are made up of plates or sheets of hepatocytes (liver cells) (Figure 2.13). The plates of cells are arranged so that they radiate out from central veins. Thus, the liver has multiple plates of cells radiating from multiple central veins. The central veins direct blood from the liver into general circulation through the hepatic veins and then ultimately into the inferior vena cava. Blood passes between the plates of liver cells by way of sinusoids, which function like a

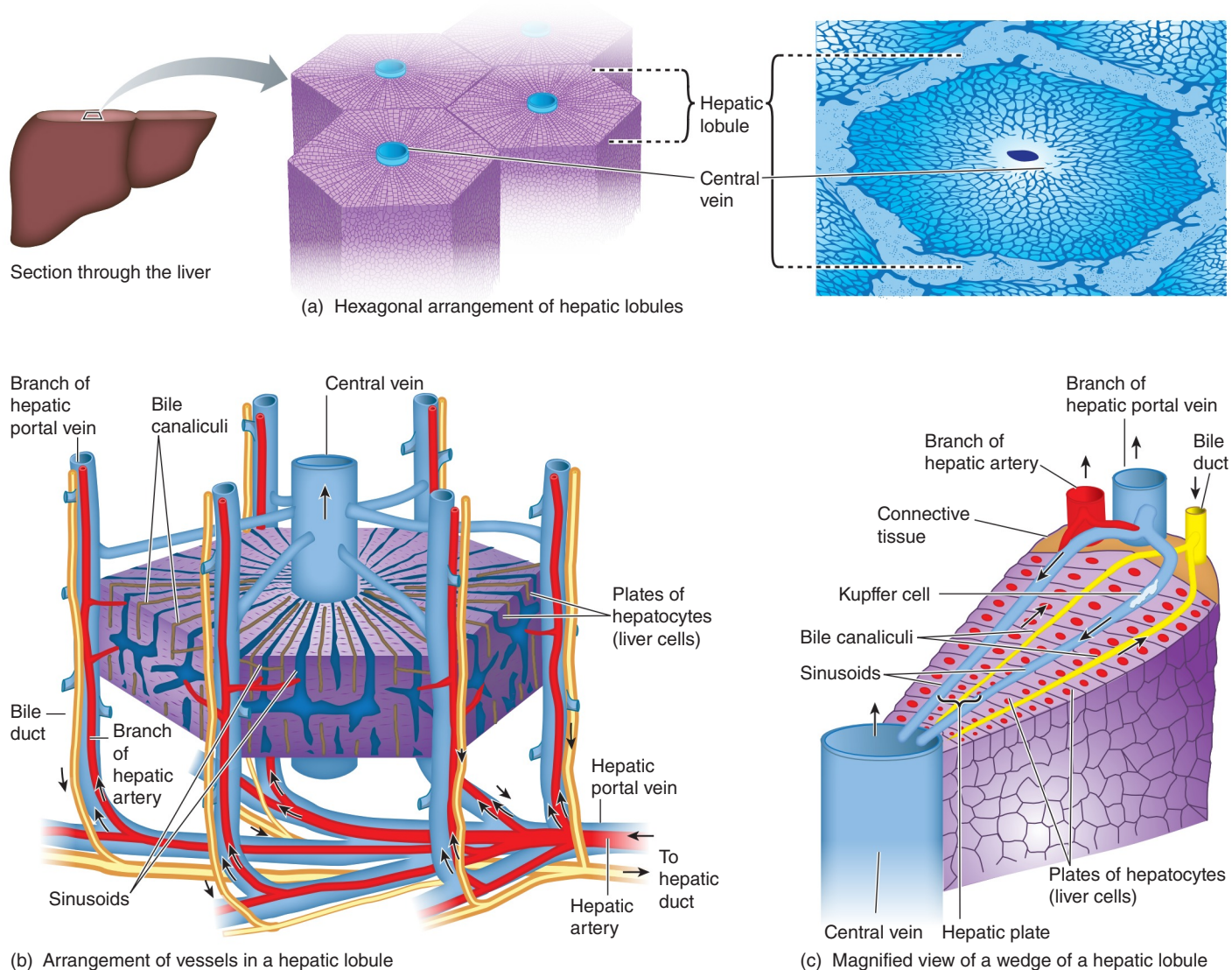


Figure 2.13 Anatomy of the liver.

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channel and arise from branches of the hepatic artery and from the portal vein. The portal vein brings blood rich in nutrients from the digestive tract to the liver. Sinusoids allow blood from these two blood vessels (the portal vein and the hepatic artery) to mix and also enable uptake of nutrients through the endothelial cells that line the sinusoids. Sinusoids also contain macrophages called Kupffer's cells, which phagocytize bacteria and other foreign substances and thus serve to protect the body. Bile canaliculi lie between the hepatocytes in the hepatic plates. Following hepatocyte production of bile, the bile is secreted into the canaliculi, which then carry it to a duct at the periphery of the lobules. The hepatic ducts from the different lobules unite and join with the cystic duct from the gallbladder to form the common bile duct.

Bile Synthesis and Function The liver produces bile, a greenish-yellow fluid composed mainly of bile acids and salts but also cholesterol, phospholipids, and bile pigments dissolved in an alkaline solution. The bile acids are synthesized in the hepatocytes from cholesterol, which in a series of reactions is oxidized to generate chenodeoxycholic acid and cholic acid, the two principal or primary bile acids. These bile acids combine primarily with sodium, but also with potassium and calcium, to form bile salts. Once formed, these bile salts conjugate primarily (~75%) with the amino acid *glycine*, forming *glycocholic acid* and *glycochenodeoxycholic acid*, and to a lesser extent (25%) with the amino acid *taurine*, forming *taurocholic acid* and *taurochenodeoxycholic acid*. Conjugation of the bile with these amino acids improves their ability to form micelles.

In addition to bile salts, small amounts of cholesterol and phospholipids, especially lecithin, are found in bile, and make up what is referred to as the bile acid-dependent fraction of bile. In addition, bile contains water, bicarbonate, and bile pigments, mainly bilirubin and/or biliverdin (waste end products of hemoglobin degradation) that are conjugated with glucuronic acid. It is these bile pigments that give bile much of its color. This fraction of the bile is referred to as bile acid independent.

Bile acts like a detergent to emulsify fat, that is, to break down large fat globules into small (about 1 mm diameter) fat droplets. More specifically, the bile helps to absorb lipids by forming small (<10 nm) spherical, cylindrical, or disklike complexes called micelles. Micelles, which can contain as many as 40 bile salt molecules, allow pancreatic lipase to better access and hydrolyze bonds within the

triacylglycerols in the micelles. More thorough coverage of the function of bile is found in Chapter 5.

The Gallbladder

The gallbladder, a small organ with a capacity of approximately 40–50 mL (1.4–1.8 oz), is located on the surface of the liver (Figure 2.14). Bile that is made in the liver is concentrated in the gallbladder whose mucosa absorbs large quantities of water along with some electrolytes that were originally present in the bile. This concentration of the bile facilitates its storage given the small volume capacity of the gallbladder.

Cholecystokinin, secreted into the blood by I cells of the proximal small intestine in response to the presence of fat-laden chyme in the duodenum, stimulates the gallbladder to contract and the sphincter of Oddi to relax.

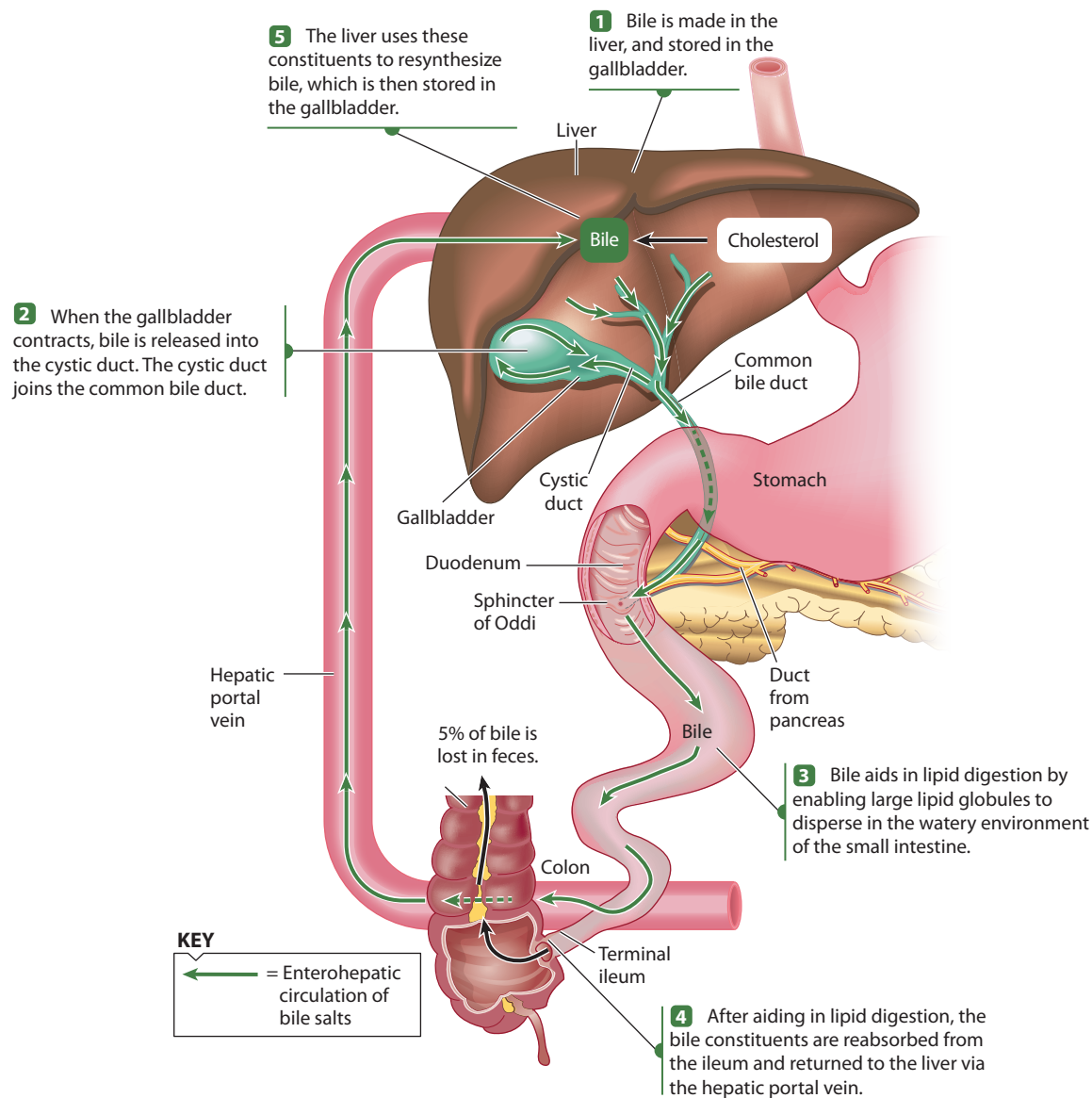


Figure 2.14 Enterohepatic circulation of bile.

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These actions allow the release of bile into the duodenum where it functions to emulsify fat. The closing of the sphincter of Oddi directs the bile back into the gallbladder.

Selected Disorders of the Gallbladder The presence of gallstones (cholelithiasis) in the gallbladder is fairly common, especially among older adults. Most stones are cholesterol based, although some consist primarily of pigments, usually bilirubin. Stones can form when bile remains sequestered within the gallbladder and is infrequently released (gallbladder hypomotility occurring, for example, with the use of parenteral nutrition versus oral intake). More commonly, gallstones form when the bile becomes supersaturated with cholesterol. The presence in the bile of large amounts of cholesterol, which is not very soluble, causes the cholesterol to precipitate out of the solution and provides a crystalline-like structure or nuclei around or within which calcium, bilirubin, phospholipids, and other compounds deposit, to ultimately form a “stone.” Other unknown factors may also promote nucleation, or the formation of a crystalline-like structure, upon which gallstones form. Gallstones, once produced, may reside silently in the gallbladder; irritate the organ, causing cholecystitis (inflammation of the gallbladder); or lodge in the common bile duct, blocking the flow of bile (choledocholithiasis) or the flow of pancreatic juice, causing pancreatitis. When gallstones block any of these ducts, surgical removal of the gallbladder (cholecystectomy) is often needed. However, in those without a gallbladder, the common bile duct generally remains intact, allowing bile release from the liver directly (without any storage in the gallbladder) into the duodenum. Individuals who have had a cholecystectomy often need to eat low-fat

foods, as high-fat meals can promote abdominal pain and **steatorrhea**.

The Recirculation and Excretion of Bile The human body has a total bile pool of about 2.5–5.0 g. Greater than 90% of the bile salts secreted into the duodenum are reabsorbed primarily by active transport in the distal ileum. Small amounts of the bile may also be passively reabsorbed in the colon. Absorbed bile enters the portal vein and is transported, attached to the plasma protein albumin in the blood, back to the liver. Once in the liver, the reabsorbed bile is reconstituted to amino acids, if necessary, and secreted along with the newly synthesized bile into the duodenum during digestion. New bile acids are typically synthesized in amounts about equal to those lost in the feces. The circulation of bile, termed **enterohepatic circulation**, is pictured in Figure 2.14. The pool of bile is thought to recycle at least twice per meal.

Some of the bile acids that are not reabsorbed in the small intestine may be deconjugated by bacteria (via bacterial bile salt hydrolase) to form secondary bile acids (Figure 2.15). Cholic acid is converted to the secondary bile acid deoxycholic acid. Chenodeoxycholic acid is converted to the secondary bile acid lithocholic acid, which, unlike deoxycholic acid, is typically excreted in the feces. It has been suggested that this bacterial enzymatic activity on bile salts may regulate in part both cholesterol metabolism and energy balance in the host. About 0.5 g of bile salts are lost daily in the feces.

Bile Circulation and Hypercholesterolemia Knowing how bile is recirculated and excreted helps in understanding the mechanisms by which various drug therapies and

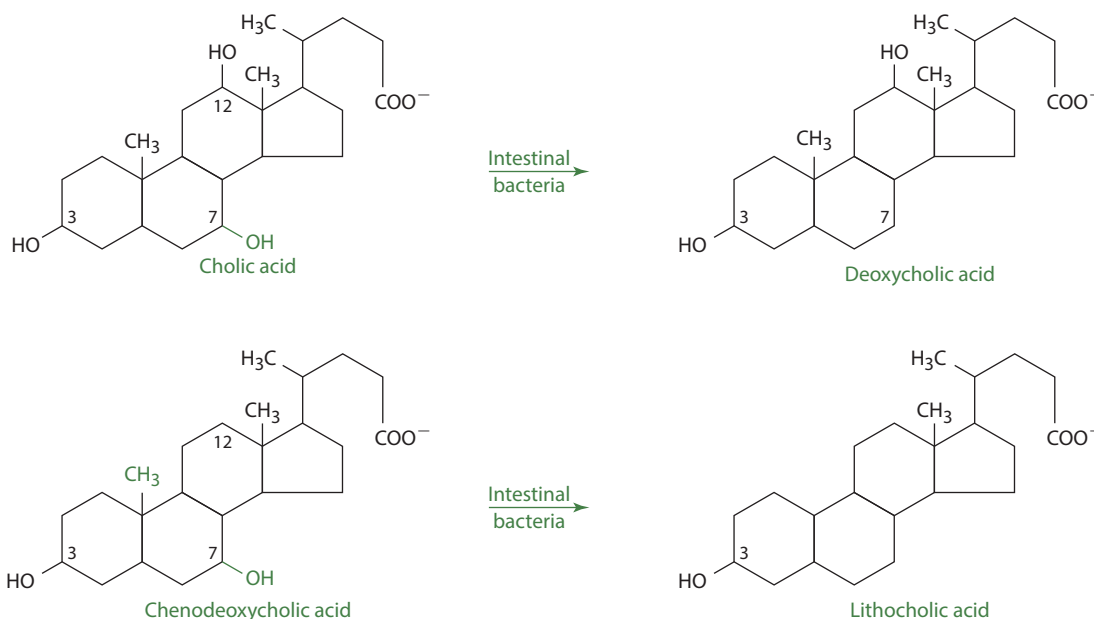


Figure 2.15 Synthesis of secondary bile acids by intestinal bacteria.

functional foods help in the treatment of high blood cholesterol concentrations (hypercholesterolemia). Medications—specifically, **resins** such as cholestyramine (Questran)—that bind bile in the gastrointestinal tract and enhance its fecal excretion from the body are used to treat hypercholesterolemia in some individuals. Additionally, plant (phyto-) stanols and sterols are added to some foods such as margarines, orange juice, and granola bars. These phytosterols and phytosterols (as well as some dietary fibers) bind bile and dietary and endogenous cholesterol in the gastrointestinal tract and enhance their fecal excretion from the body. An increased fecal excretion of the bile, a decreased recirculation of the bile, and a decreased absorption of cholesterol require the body to use cholesterol to synthesize new bile acids. The increased use of cholesterol to make more bile diminishes the body's cholesterol concentrations. Thus, the use of such medications and functional foods can lead to lower blood cholesterol concentrations and reduced risk of cardiovascular disease. Health claims on the labels

of some of the products containing phytosterols state that “Plant sterols, eaten twice a day with food for a total of 1.3 g daily total, may reduce heart disease risk in a diet low in saturated fat and cholesterol.” Daily consumption of plant sterols has been shown to decrease total and low-density lipoprotein (LDL) **plasma** cholesterol concentrations in people with normal or high blood lipid concentrations.

The Absorptive Process

Following all the actions of the various secretions and enzymes from the mouth, stomach, pancreas, and small intestine and with the help of bile from the liver and gallbladder, the now digested nutrients are ready to be absorbed, that is, to enter the cells of the gastrointestinal tract. The absorption of most nutrients begins in the duodenum and continues throughout the jejunum and ileum, as shown in Figure 2.16. Generally, most absorption occurs in the proximal (upper) portion of the small intestine, but some nutrients are absorbed primarily in the distal

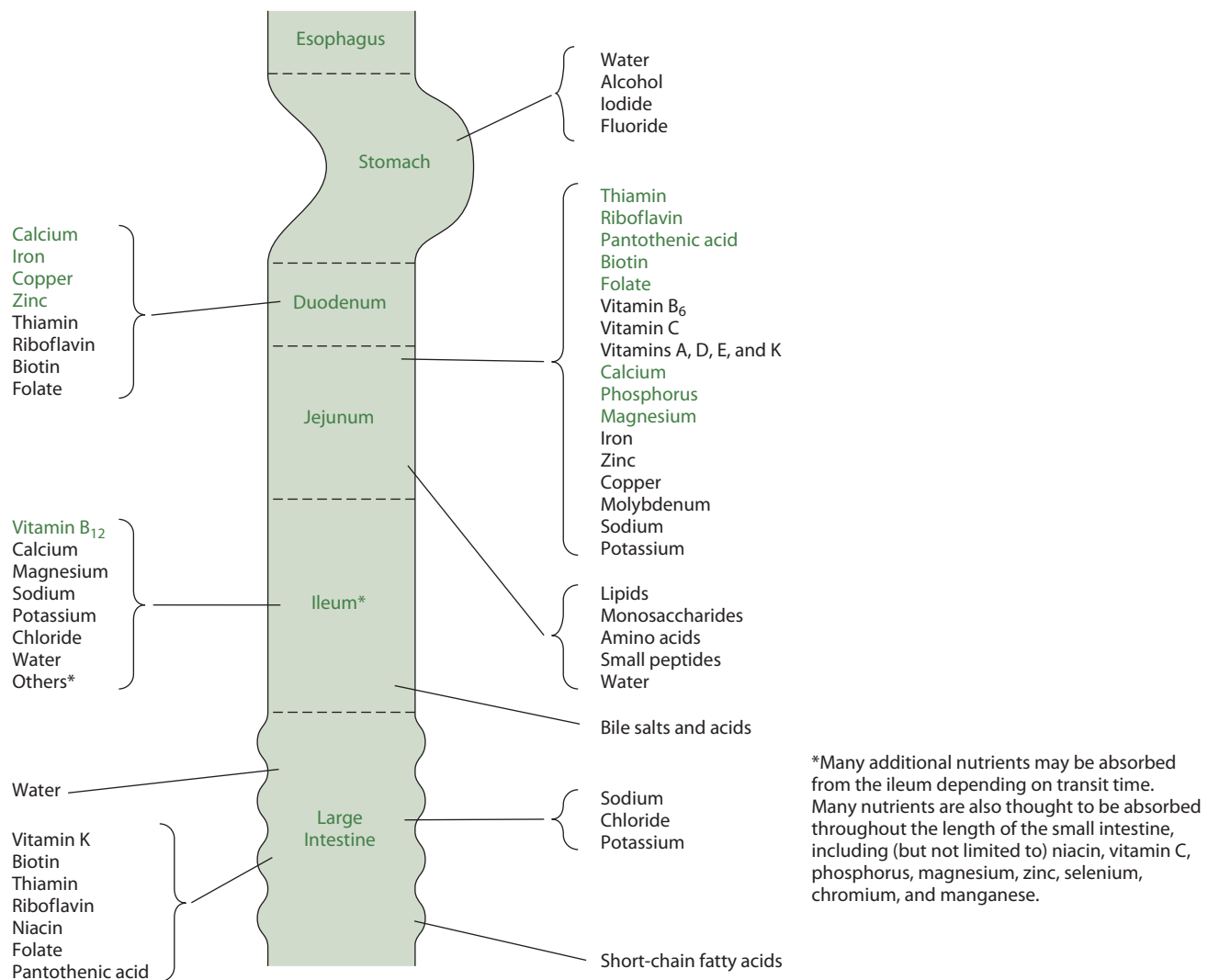


Figure 2.16 Primary sites of nutrient absorption in the gastrointestinal tract.

ileum, making all regions of the small intestine valuable to adequately nourishing the body.

The digestion and absorption of nutrients within the small intestine are fairly rapid, with most of the carbohydrate, protein, and fat being absorbed within a few hours after chyme has reached the small intestine. The presence of unabsorbed food in the ileum may increase the amount of time material remains in the small intestine and therefore may increase nutrient absorption. Transit time of unabsorbed substances in the small intestine is about 3–6 hours.

Nutrients are absorbed across the brush border membrane into enterocytes primarily by diffusion,

facilitated diffusion, active transport, and/or, occasionally, **pinocytosis** or **endocytosis** (Figure 2.17). To be available for use in the body, transport across the enterocyte's basolateral (serosal) membrane is also needed. A few nutrients, however, gain entry into the body via a paracellular (between cells) route. Tight junctions are found throughout the digestive tract to help regulate “what gets in” and “what does not get in” to the body via this paracellular route. Tight junctions consist of multiple protein complexes including several membrane-spanning proteins and scaffolding proteins which function together to alter these junctions and thus to regulate permeability. Examples of membrane-spanning proteins include

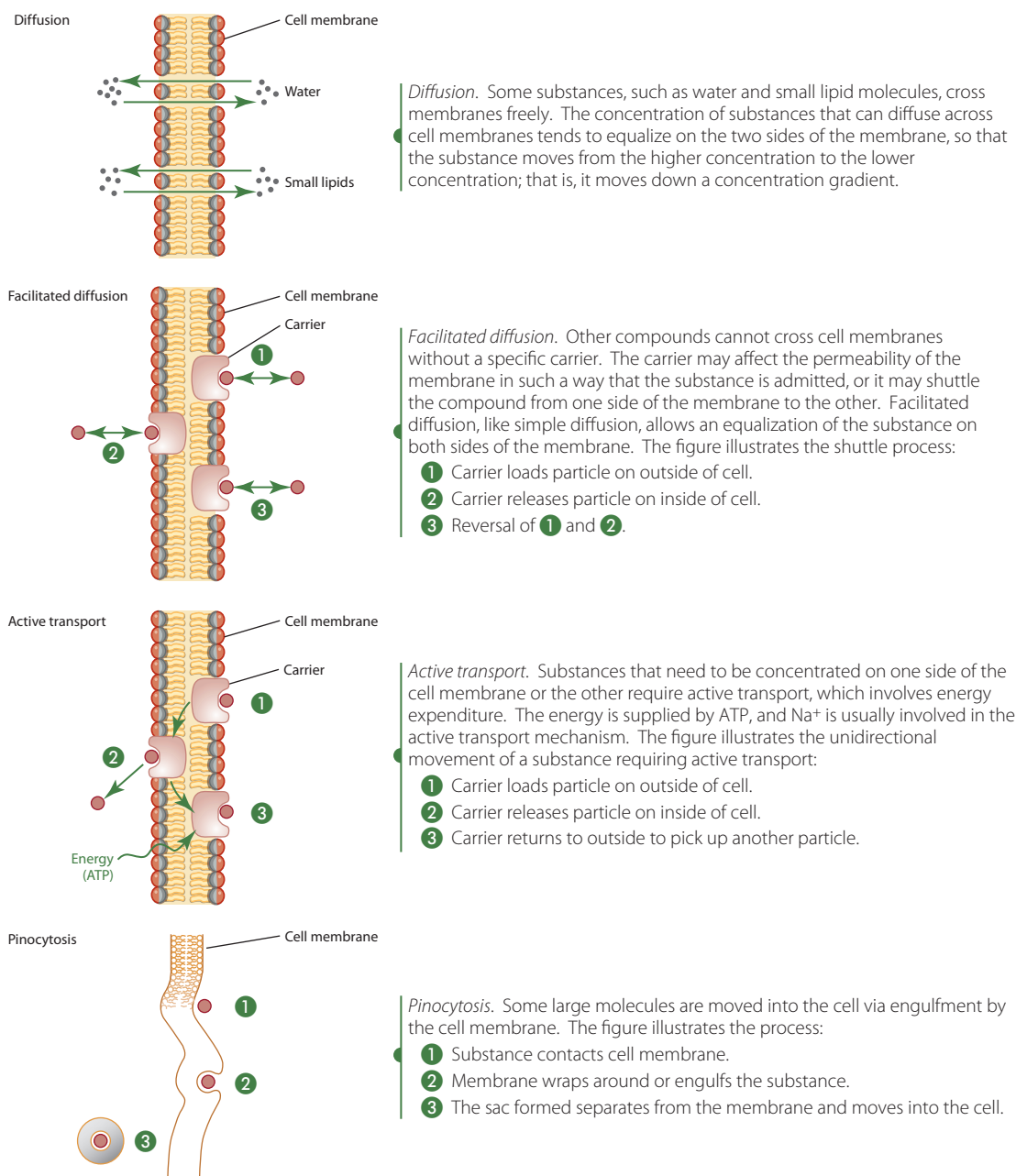


Figure 2.17 Primary mechanisms for nutrient absorption.

occludin, claudins, and junctional adhesion molecule. The intracellular regions of these membrane-spanning proteins interact with intracellular scaffolding proteins such as zonula occludens proteins. The scaffolding proteins in turn link membrane-spanning proteins to the cell's cytoskeleton. Membrane-spanning proteins also traverse the paracellular space and function to seal up or tighten the paracellular space and thus minimize entry into the body. Other membrane-spanning proteins, such as claudin-2, form ion-selective pores that open up the space to facilitate paracellular absorption for nutrients like calcium and magnesium. Changes in tight junctions (as occur with mutations in specific claudins) that increase intestinal permeability (and allow substances to enter the body that would not normally “get in”) have been associated with intestinal inflammation and disease.

The mechanism of absorption for a nutrient depends on several factors: solubility (fat vs. water) of the nutrient, the concentration or electrical gradient, and the size of the molecule to be absorbed. The absorption and transport of amino acids, peptides, monosaccharides, fatty acids, monoacylglycerols, and glycerol—that is, the end products of **macronutrient** digestion—are considered in depth in Chapters 3, 5, and 6. The digestion and mechanisms of absorption for each of the vitamins and minerals are described in detail in Chapters 9–14; the general sites of absorption are shown in Figure 2.16.

Unabsorbed intestinal contents are passed from the ileum (the terminal or most distal section of the small intestine) through the ileocecal sphincter into the colon. Some of these unabsorbed materials, however, serve as substrates for bacteria that inhabit the small intestine and colon. The ileocecal sphincter, in addition to controlling the passage of contents from the small intestine into the large intestine, helps to prevent the migration of bacteria from the large intestine back into the small intestine. Bacterial overgrowth in the small intestine may result in nutritional deficiencies not only from bacteria-induced mucosal cell destruction, but also from direct bacterial use of nutrients, bacterial destruction of the nutrients, and/or bacterial destruction of substances (such as bile) needed for nutrient absorption. Criterion for bacterial overgrowth is typically the presence of fecal microorganisms in the small intestine at a density of $> 10^5$ microbes/mL. Bacterial overgrowth in the small intestine induces deficiencies of nutrients, such as vitamin B₁₂ and iron, which the bacteria use for their own growth. Additionally, the bacteria may induce deficiencies of thiamin and fat-soluble vitamins. Fat-soluble vitamin deficiencies occur with bacterial deconjugation of bile that is needed for fat and fat-soluble vitamin absorption. Thiamin can be destroyed in the small intestine from thiaminases released by the bacteria.

The Colon (Large Intestine)

Once through the ileocecal sphincter, materials move into the cecum, the right side of the colon, and then move sequentially through the ascending, transverse, descending, and sigmoid sections (Figure 2.18). The colon in its entirety is almost 5 feet long and is larger in diameter (about 3 inches) than the small intestine (about 1½ inches), thus explaining the terminology distinction (large vs. small) between the two intestines.

Rather than being a part of the entire wall of the digestive tract, as it is in the upper digestive tract, the longitudinal muscle in the colon is gathered into three muscular bands or strips called teniae (also spelled taenia or teneae) coli that extend throughout most of the colon. The length of the teniae coli is smaller than that of the underlying circular muscles and mucosa, which causes the underlying layers to form pouches called haustra.

On initially entering the colon, the contents are still quite fluid. Contraction of the musculature of the large intestine is coordinated so as to mix the intestinal contents and to keep material in the proximal (ascending) colon a sufficient length of time for absorption of nutrients to occur. The proximal colonic mucosal cells typically absorb sodium, chloride, and water. About 90–95% of the water and sodium entering the colon each day is absorbed. Colonic absorption of sodium, which occurs by active transport and which enhances water absorption, is influenced by a number of factors, including hormones. Antidiuretic hormone (also called vasopressin) secreted from the pituitary gland, for example, decreases sodium absorption, whereas glucocorticoids like cortisol secreted from the adrenal gland and mineralocorticoids such as aldosterone secreted from the adrenal gland increase sodium absorption in the colon. Further information on water and electrolyte absorption is found in Chapter 12.

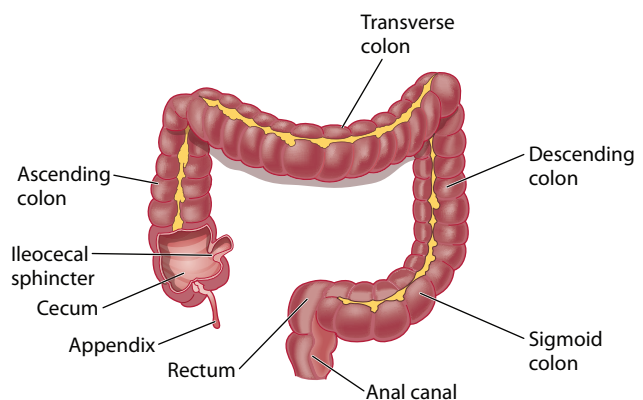


Figure 2.18 The colon.

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Colonic Secretions and Motility and Their Regulation

Secretions into the lumen of the colon are few, but present. Goblet cells secrete mucus. Mucus acts as a lubricant for fecal matter and protects the colonic mucosal cells. The mucus, present in a double layer, lies between the colonic mucosal cells and the bacteria that reside in the colon and thus help reduce the likelihood of bacterial translocation. Bicarbonate is also secreted into the lumen in exchange for chloride, which is absorbed. Bicarbonate provides an alkaline environment that helps neutralize acids produced by colonic anaerobic bacteria.

Haustral contractions, characterized as oscillating contractions of the circular muscles, provide one form of motility within the large intestine. These contractions are regulated in part by the basic electrical rhythm of the colon's smooth muscle layer and occur at a rate of about two to six contractions per minute. Peristalsis provides minimal motility in the colon. Instead, more vigorous mass-action peristaltic-like contractions (i.e., contractions of large sections of smooth muscle within the colon) promote movement of material from one section of the colon to the next toward the rectum. Neural reflexes also affect motility. For example, the gastrocolic reflex, which occurs in response to gastrin and enteric nervous system activity, promotes contractions within the distal colon and rectum to promote defecation.

The end result of the passage of material through the colon, which usually takes about 12–72 hours, is that the unabsorbed materials are progressively dehydrated. Typically, the approximately 500 mL to 1 L of materials that enter the large intestine each day is reduced to about 150–200 g of defecated material. This fecal material is about 75% water and 25% solids. Fecal solids usually include sloughed gastrointestinal cells, digestive juice constituents, fiber, small amounts of unabsorbed fat and bile, and bacteria. The bacteria may account for about 30% of dry fecal weight. Frequency of bowel movements among adults in the United States ranges from 3 to 21 per week.

Colonic Bacteria

The trillions of microorganisms (which can weigh up to 5 lbs) that are living in the gastrointestinal tract make up our gut microbiota (or **microflora**). These microorganisms include both gram-negative and gram-positive bacterial strains, representing over 1,000 species. Although intestinal bacterial counts in the large intestine have been reported to be as high as 10^{12} per gram of gastrointestinal tract contents, bacteria are found throughout the gastrointestinal tract.

- The mouth contains mostly anaerobic bacteria.
 - The stomach contains few bacteria because of its low pH, but some more acid-resistant bacteria that are present include lactobacilli and streptococci.
 - The proximal small intestine contains both aerobes and facultative anaerobes.
 - Most bacteria found in the ileum and large intestine are anaerobes, including bacteroides, lactobacilli, and clostridia. Other examples of bacteria that inhabit the large intestine are bifidobacteria, methanogens, eubacteria, and streptococci. Anaerobic species are thought to outnumber aerobic species by at least 10-fold, but the exact composition of the microflora is affected by a variety of factors such as substrate availability, pH, medications, and diet, among others.
- Bacteria gain nutrients for their own growth from undigested and/or unabsorbed food residues in the intestines. Enzymes synthesized by the bacteria but lacking in humans allow for the digestion of many nutrients to generate substrates for bacterial energy production and to attain, for example, carbon atoms necessary for bacterial maintenance and/or growth. Starch that has not undergone hydrolysis by pancreatic amylase, for example, may be used by gram-negative bacteroides and by gram-positive bifidobacteria or eubacteria. Mucins found in mucus secretions of the gastrointestinal tract may be broken down and used by bacteria such as bacteroides, bifidobacteria, and clostridia. Digestive enzymes themselves may even serve as substrates for bacteria such as bacteroides and clostridia. In addition, sugar alcohols such as sorbitol and xylitol, disaccharides such as lactose, and some fibers may be degraded by selected bacteria in the colon.
- Many products are generated from the bacterial use of undigested and unabsorbed materials in the colon. Several B-vitamins as well as vitamin K are produced by bacteria in the colon and may be absorbed to varying degrees. Some particularly beneficial acids that are produced during carbohydrate **fermentation** (an anaerobic process by which bacteria break down substances, primarily carbohydrate and protein) by specific strains of bacteria include lactic acid and three **short-chain fatty acids**—acetic acid, butyric acid, and propionic acid. These short-chain fatty acids provide many benefits to the host, as shown in part in Figure 2.19, and more specifically listed hereafter.
- **Acidify the luminal environment.** The presence of short-chain fatty acids in the colon decreases the pH within the lumen of the colon. This more acidic environment has several positive effects.
 - With the more acidic pH, free bile acids become less soluble and the activity of bacterial 7 α dehydroxylase diminishes (optimal pH ~ 6–6.5), resulting in decreased conversion of primary bile acids to secondary (more harmful) bile acids.

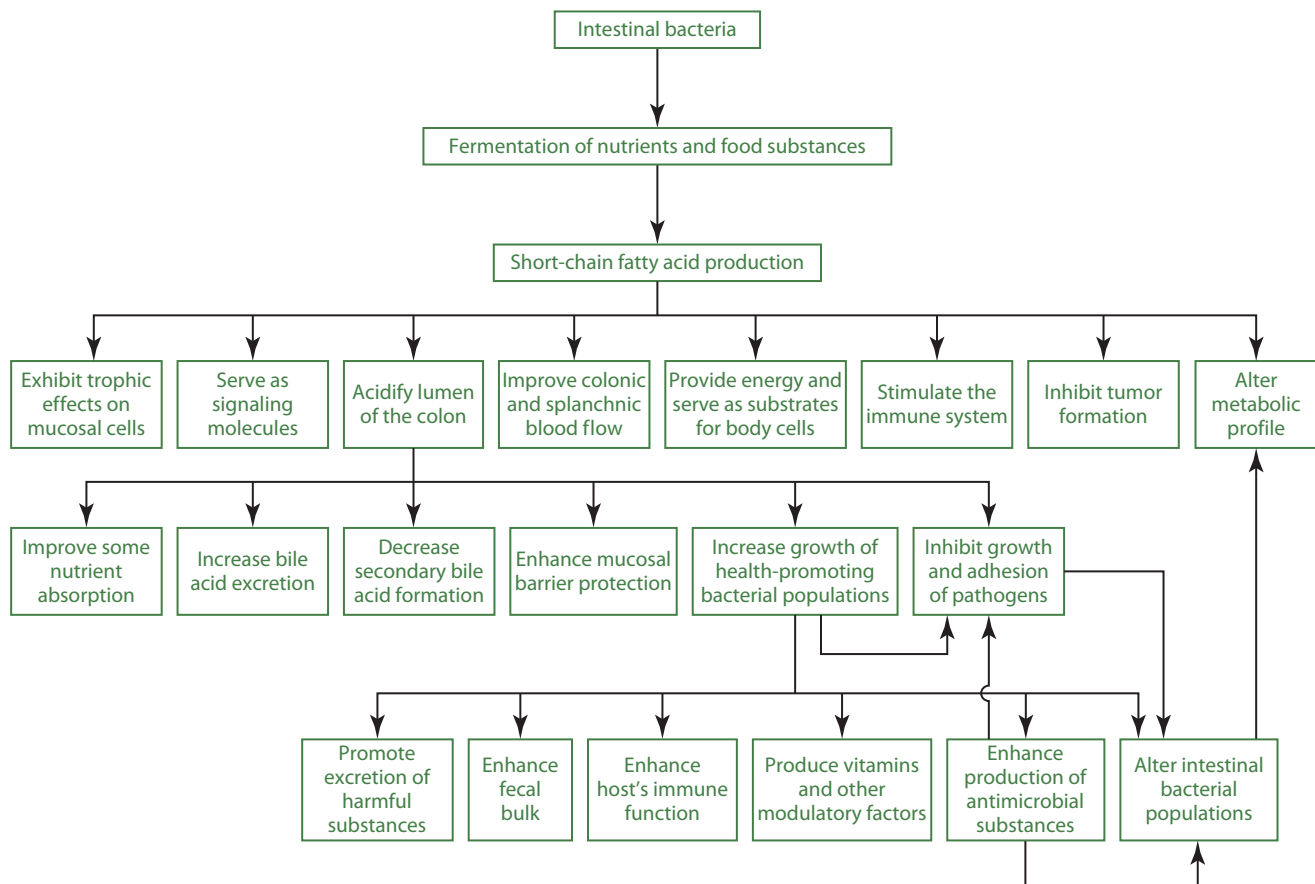


Figure 2.19 Some benefits from the presence of bacteria in the large intestine.

- With the lower pH, calcium, released with fiber degradation, binds to and promotes the excretion of bile acids (and thus prevents their conversion to secondary bile acids).
- The lower pH favors the growth of beneficial lactobacilli and bifidobacteria and inhibits the growth of pH-sensitive pathogenic bacteria.
- The acidic environment enhances the production of mucin, which forms part of the physical barrier overlying intestinal cells. This increased mucin content provides a greater physical barrier and decreases the likelihood of pathogenic bacterial colonization as well as bacterial translocation.
- The low pH may improve the absorption of minerals released during fermentation.
- **Serve as signaling molecules** by interacting with receptors on enteroendocrine cells that mediate the synthesis of hormones and peptides and by effects on histone acetylation involved in gene expression.
- **Exhibit trophic effects**, specifically stimulating proliferation and growth and maintaining the integrity (preventing atrophy) of the colonic mucosal cells.
- **Improve colonic and splanchnic blood flow.** Short-chain fatty acids are thought to directly affect smooth muscle as well as to interact with the enteric nervous system. This improved blood flow enhances both the delivery of nutrients to the colon and the transport of nutrients from the colon to the liver.
- **Increase water and sodium absorption in the colon.** The absorption of the short-chain fatty acids in turn stimulates water and sodium absorption into the mucosal cells of the colon.
- **Provide energy and serve as substrates for use within cells.** Over 95% of the short-chain fatty acids are absorbed and utilized by the body.
 - Butyric acid serves as a major energy source for colonic mucosal cells. In fact, butyric acid is thought to supply colonic cells with over two-thirds of their energy needs.
 - Absorbed propionic acid and acetic acid are transported via the portal vein to the liver. In the liver, propionic acid is largely metabolized along with small amounts of acetic acid. Much of the propionic acid is converted to succinyl-CoA, which may

be used by the liver for glucose or energy production. Propionic acid may also alter cholesterol metabolism.

- Most of the acetic acid passes through the liver and is used by other tissues, including skeletal and cardiac muscle and the kidneys and brain. Acetic acid may be used for the synthesis of cholesterol and fatty acids.
- Short-chain fatty acids may also impact glycogenolysis and play a role in insulin release and/or sensitivity.
- **May inhibit tumors.** *In vitro* studies suggest short-chain fatty acids promote apoptosis and promote the arrest of growth and differentiation in tumor cell lines.
- **Stimulate the immune system** by enhancing the production of macrophages, T-helper lymphocytes, leukocytes, antibodies, and cytokines and improving antibody response.

As can be gleaned from this list, the short-chain fatty acids generated by bacteria in the gastrointestinal tract play several important roles. The bacteria themselves also provide direct benefits and augment some of the benefits attained from the short-chain fatty acids. Some examples of healthful actions of bacteria include the ability to:

- **Enhance the host's immune defense system** by increasing secretory IgA production, tightening the mucosal barrier, enhancing cytokine responses, enhancing phagocytic activity, and producing antimicrobial substances such as bacteriocin.
- **Displace, exclude, or antagonize pathogenic bacteria from colonizing**, for example, by competing for attachment sites on the intestinal mucosa, by strengthening the mucosal barrier to normalize intestinal permeability and to prevent pathogenic bacterial translocation, and by producing substances like biosurfactants that reduce adhesion of pathogens to the mucosa.
- **Scavenge, sequester, transform, and/or promote the excretion of harmful/carcinogenic substances** such as bile acids, nitrosamines, heterocyclic amines, and mutagenic compounds. Moreover, some bacteria, such as *Lactobacillus acidophilus*, may be able to inhibit the production of carcinogenic compounds.
- **Enhance fecal bulk and dilute fecal contents** to minimize exposure with colonic mucosal cells.

Another possible role of the microbiota is in energy metabolism and thus regulation of body weight. Data are limited, but some studies suggest products generated by colonic microbes may exert signals that influence brain activity, including effects on appetite regulation and energy metabolism.

A less desirable result of the presence of colonic bacteria is gas production, although swallowed air also contributes

to this problem. Several different gases are generated by these bacteria, including methane (CH_4), hydrogen (H_2), hydrogen sulfide (H_2S), and carbon dioxide (CO_2). One estimate suggests that colonic bacterial fermentation of about 10 g of carbohydrate can generate several liters of hydrogen gas. While much of the hydrogen and other gases that are generated can be used by other bacteria in the colon, gases that are not used are excreted.

Measurement of hydrogen gas produced by bacteria is used as a basis to diagnose lactose intolerance, a condition in which the enzyme lactase is not made in sufficient quantities to digest the disaccharide lactose. Lactose intolerance is fairly common among adults, especially those of African American, Native American, and Asian heritage. When a person with lactose intolerance ingests the carbohydrate lactose (e.g., by drinking milk), the undigested lactose enters the colon and is **fermented** by colonic bacteria. These colonic bacteria, upon fermenting the lactose, produce more hydrogen gas than usual. Much of this hydrogen gas made by the bacteria is absorbed by the body and then exhaled in the breath. In fact, to diagnose lactose intolerance, a person may be asked to consume about 50 g of lactose and have their breath analyzed for hydrogen gas for the next several hours. Generally, if the person is lactose intolerant, hydrogen gas excretion in the breath increases for about 1–1½ hours after lactose is consumed. An absence of an increase in breath hydrogen gas concentrations suggests adequate lactose digestion. Symptoms of lactose intolerance include bloating, gas, and abdominal pain.

Other products are made as bacteria degrade amino acids in the colon. For example, bacterial degradation of the branched-chain amino acids generates the branched-chain fatty acids isobutyric acid and isovaleric acid. Deamination (removal of the amino group) of aromatic amino acids yields phenolic compounds. Amines such as histamine result from bacterial decarboxylation of amino acids such as histidine. Ammonia is generated by bacterial deamination of amino acids as well as by bacterial urease action on urea that has been secreted into the gastrointestinal tract from the blood. The ammonia can be absorbed by the colon and circulated to the liver, where it can be reused to synthesize urea or amino acids. About 25%, or 8 g, of the body's urea may be handled in this fashion. This process must be controlled in people with liver disease (cirrhosis), as high amounts of ammonia in the blood are thought to contribute to the development of hepatic encephalopathy (coma). Uric acid and creatinine may also be released into the digestive tract and metabolized by colonic bacteria.

Intestinal Conditions and Probiotics Imbalances in the number and composition of gut microbiota have been linked to a number of conditions like inflammatory bowel diseases (Crohn's disease and ulcerative colitis), colon

cancer, rheumatoid arthritis, and diabetes, among others, and have prompted increased therapeutic use of **probiotics** (*pro* means “life” in Greek) and **prebiotics**. Probiotics are live microorganisms (i.e., active cultures of specific strains of bacteria) that when administered in adequate amounts confer health benefits to its hosts. Prebiotics (discussed in more detail in Chapter 4) are substances that are not digested by human digestive enzymes but confer health benefits to the host by acting as substrates for the growth and/or activity of one or more species of healthful bacteria in the colon.

The most common probiotic bacteria are lactic acid bacteria, usually strains of *Lactobacillus* and *Bifidobacterium* genera. To be considered a probiotic, the product must contain 100 million live active bacteria per gram. At present, probiotics are mostly consumed as yogurt with live cultures and as fermented or cultured milk and milk products (such as buttermilk and kefir). In the United States, yogurt is often fermented by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and milk is usually fermented by *Lactobacillus acidophilus* and *Lactobacillus casei*. Other bacteria used to manufacture dairy products include *Leuconostoc mesenteroides* and *Lactococcus lactis*. Other food sources of probiotics include miso, tempeh, and some soy beverages/products.

Consumption of probiotics has been shown to improve symptoms of irritable bowel syndrome and inflammatory bowel diseases as well as several types of diarrhea. To be effective, probiotics usually need to contain 1–10 billion colony-forming units (CFUs) per dose, with doses given once or twice daily or sometimes a few times per week. Tolerance is typically satisfactory; however, bacterial sepsis (infection) is possible, especially in those with impaired immune function (immunosuppression), intestinal tract dysfunction (characterized by increased gastrointestinal permeability or a defective barrier), or other chronic health conditions such as diabetes mellitus, cancer, abscesses, and organ transplant.

2.2 COORDINATION AND REGULATION OF THE DIGESTIVE PROCESS

The central nervous system, which comprises the brain and spinal cord, affects the body via efferent neurons. Efferent neurons to skeletal muscles make up the somatic division, and efferent neurons to the internal organs represent the autonomic division of the nervous system. The autonomic division can be divided into the sympathetic and the parasympathetic nervous systems.

Neural Regulation

The autonomic division communicates with the digestive organs directly, but it can also communicate with the digestive tract’s own (local) nervous system. Generally,

the sympathetic system decreases or slows down digestive tract motility and secretions, whereas the parasympathetic nervous system stimulates the digestive tract, promoting motility (such as peristalsis), gastrointestinal reflexes, and the secretion of hormones and enzymes. The parasympathetic system interacts with the digestive tract primarily through the vagus nerve.

The digestive system’s local nervous system is known as the enteric nervous system or the intrinsic nerve plexuses and includes about 100 million neurons and their processes embedded in the layers of the gastrointestinal tract beginning in the esophagus and extending to the anus. The enteric nervous system consists of two neuronal networks or plexuses: the myenteric or Auerbach plexus and the submucosal or Meissner plexus.

- The myenteric plexus, which lies between the circular and longitudinal smooth muscles of the digestive tract, generally controls motility, and when this plexus is stimulated, gastrointestinal activity generally increases.
- The submucosal plexus typically controls the release of secretions and affects local blood flow.

Sensory information is received by the enteric nervous system in part from different receptors within the gastrointestinal tract layers; these receptors monitor “local” conditions within the digestive organs. Mechanoreceptors detect distension or pressure in the gastrointestinal tract walls. Chemoreceptors monitor changes in chemical composition, and osmoreceptors detect changes in the osmolarity, such as that of chyme. Receipt of this sensory information by the enteric nervous system results in changes in the digestive tract’s smooth muscle functions (affecting motility) and/or changes to specific cells and glands (affecting the release of enzymes and hormones). Neural reflexes may also result from the stimulation of these receptors, as discussed in the next paragraph. Some of the many neurotransmitters released by the enteric nervous system are acetylcholine, 5-hydroxytryptamine (serotonin), norepinephrine, gamma aminobutyric acid (GABA), vasoactive intestinal polypeptide, and nitric oxide.

Neural reflexes also occur within the digestive tract to effect changes in secretions, blood flow, and/or motility. For example, with the ileogastric reflex, gastric motility is inhibited when the ileum becomes distended. This action allows more time for the contents of the lower small intestine, the ileum, to be emptied before more chyme is released from the stomach into the upper small intestine. With the gastroileal reflex, ileal motility is stimulated when gastric motility and secretions increase. This neural reflex promotes overall motility within the stomach and small intestine. Other reflexes also affect the intestines. For example, with the colonoileal reflex, stimulation of receptors within the colon in turn inhibits the emptying of the contents from the ileum into the colon. Such actions slow down overall motility in these organs. Similar actions

occur with the intestinointestinal reflex, which diminishes intestinal motility when a segment of the intestine is overdistended.

Regulatory Peptides

Factors influencing digestion and absorption are coordinated, in part, by a group of gastrointestinal tract molecules called regulatory peptides or, more specifically, gastrointestinal hormones and neuropeptides. More than 100 regulatory peptides are thought to affect gastrointestinal functions. These peptides are released by endocrine cells within the digestive tract or its accessory organs, by enteric nerves, or both. These enteroendocrine cells, which are often identified by letters (e.g., G cells, S cells, I cells, etc.), are found throughout the digestive system. Most of the regulatory peptides released by these cells work in an endocrine manner, being released into the blood in response to specific stimuli and traveling to region(s) of the digestive tract and/or its accessory organs to evoke changes. A few regulatory peptides, however, work in a paracrine manner, being released into the local area where they diffuse through extracellular spaces to evoke changes in target tissues.

Regulatory peptides affect a variety of digestive functions, such as gastrointestinal tract motility, cell growth, and the secretion of digestive enzymes, electrolytes, and water. Most, but not all, have multiple actions; some are strictly inhibitory or stimulatory, whereas some mediate both types of responses. Many of the functions of regulatory peptides have been addressed to varying degrees in

the sections on regulation of gastric and intestinal secretions and motility. Table 2.2 summarizes some of the functions of a few of these peptides, while more detailed information is provided hereafter.

- **Gastrin**, secreted into the blood primarily by G cells in the antrum of the stomach and proximal small intestine, acts mainly in the stomach to stimulate the release of hydrochloric acid and pepsin, and to a lesser extent to stimulate gastric motility and emptying. Gastrin also stimulates the release of histamine, which further induces gastric acid release, and has trophic actions (stimulates cell growth) on gastric and intestinal mucosa. Gastrin release is stimulated mainly by gastric distension and the presence of protein digestion products in the stomach, as well as by the release of gastrin-releasing polypeptide by the vagus nerve. Gastrin secretion is inhibited by the presence of acid in the antrum and by the release of somatostatin.
- **Cholecystokinin (CCK)**, secreted into the blood by I cells of the proximal small intestine and by enteric nerves in the distal ileum and colon, principally stimulates pancreatic acinar secretory cells to release digestive enzymes into the duodenum. It also has trophic actions on the pancreas and stimulates gallbladder contraction and the relaxation of the sphincter of Oddi to facilitate the release of bile into the duodenum. Lesser roles of cholecystokinin include decreasing gastric emptying and gastric acid secretion. Cholecystokinin release is stimulated by the presence of protein digestion products

Table 2.2 Selected Regulatory Hormones/Peptides of the Gastrointestinal Tract, Their Main Production Site(s), and Selected Digestive Tract Functions

Hormone/Peptide	Main Production Sites	Selected Function(s)
Gastrin	Stomach and small intestine	Stimulates gastric acid secretion Stimulates pepsinogen secretion
Cholecystokinin	Small intestine and enteric nerves	Stimulates gallbladder contraction Stimulates sphincter of Oddi relaxation Stimulates pancreatic enzyme secretion
Secretin	Small intestine	Stimulates pancreas juice secretion Diminishes gastric emptying Diminishes gastric acid secretion
Motilin	Stomach and intestines	Stimulates gastric and intestinal motility between meals
Glucose-dependent insulintropic peptide	Small intestine	Stimulates insulin secretion May diminish gastric acid secretion
Peptide YY	Small and large intestines	Diminishes gastric acid secretion Diminishes gastric emptying
Somatostatin	Pancreas, stomach, and small intestine	Diminishes gastric acid secretion Diminishes gastric emptying Diminishes pancreatic enzyme secretions Inhibits gallbladder contraction
Glucagon-like peptides	Small and large intestines	Stimulates insulin secretion Reduces digestive tract motility Reduces gastric secretions
Pancreatic polypeptide	Pancreas	Decreases gastric emptying Reduces pancreatic exocrine secretions

and fat in the duodenum, which is logical given the hormone's actions on the pancreas, but its release diminishes as nutrients are absorbed or moved into more distal sections of the digestive tract. In neurons in the brain, cholecystokinin is thought to influence the perception of appetite, among other processes.

- Secretin is secreted into the blood by S cells in the proximal small intestine in response to the presence of unneutralized acidic chyme and the products of protein digestion in the duodenum. Secretin acts primarily on pancreatic duct cells, stimulating the release of pancreatic juice rich in bicarbonate. The presence of this bicarbonate in the duodenum in turn neutralizes the acidic chyme and serves as feedback control. Secretin also exhibits trophic action on the pancreas and decreases gastric acid secretion and gastric emptying.
- Peptide YY (PYY), secreted into the blood by L cells of the ileum and colon, decreases appetite as well as decreases gastric acid secretion and gastric emptying. Its release is stimulated by the presence of fat in the small intestine.
- Motilin, secreted by M cells in the stomach, small intestine, and colon, controls the MMC, promoting gastric emptying and stimulating motility in the intestines between meals. Its release is stimulated by acetylcholine and serotonin. Acetylcholine is released by nerves. Serotonin is released both from nerves and from enterochromaffin-like cells within the gastrointestinal tract.

Four paracrine factors affecting the digestive tract are somatostatin, histamine, glucagon-like peptides, and insulin-like growth factor-1.

- Somatostatin, synthesized by pancreatic δ (D) cells as well as cells in the antrum and duodenum, inhibits gastrin release, and thus inhibits gastric acid secretion, through effects on parietal and enterochromaffin-like cells. Somatostatin also suppresses the actions of gastrin, glucose-dependent insulinotropic peptide, secretin, vasoactive intestinal polypeptide, and motilin. Further actions include inhibition of gastric emptying, pancreatic exocrine secretions, and gallbladder contraction. Release of the somatostatin is promoted by a drop, below about 2, in the pH of gastric juice.
- Histamine, secreted by mast cells and enterochromaffin-like cells in the stomach, stimulates parietal cells to secrete hydrochloric acid. Histamine release is stimulated by both gastrin and acetylcholine.
- Glucagon-like peptides, secreted by L cells of the distal small intestine and colon and by the nervous system, primarily stimulate the pancreas to release insulin and inhibit glucagon secretion. The peptides may also decrease appetite and diminish gastric emptying, gastric secretions, and intestinal motility. Release of the peptides occurs with the presence of nutrients in the lumen of the small intestine.

- Insulin-like growth factor-1, also secreted by endocrine cells of the gastrointestinal tract, increases proliferation of the gastrointestinal tract. Its release is stimulated by the presence of nutrients in the digestive tract.

Of the following neurocrine peptides involved with digestive tract functions, vasoactive intestinal polypeptide (VIP) has one of the larger roles. VIP is present in gastrointestinal tract nerves and the central nervous system and may also be present in the blood. The peptide is thought to stimulate intestinal and pancreatic secretions, relax intestinal smooth muscle including most gastrointestinal sphincters, and inhibit gastric acid secretion. Another neuropeptide called neurotensin is produced by both neurons and N cells of the small intestine (especially the ileum), but its exact physiological role in the digestive process at normal circulating concentrations is unclear. The peptide, however, is known to have multiple actions in the brain.

Two hormones exhibiting lesser direct effects on the digestive tract but impacting nutrient utilization include glucose-dependent insulinotropic peptide (GIP; previously called gastric inhibitory peptide) and amylin. GIP, a peptide produced by K cells of the duodenum and jejunum, primarily functions to stimulate insulin release by the pancreatic beta cells. The hormone may also inhibit gastric acid secretion. Amylin, a hormone that is cosecreted with the insulin by pancreatic beta cells, functions to inhibit glucagon secretion as well as gastric emptying. Insulin's role in promoting glucose uptake, along with the role of another pancreatic hormone called glucagon, is discussed in detail in Chapter 3.

In addition to direct effects on the digestive tract and effects on nutrient utilization, other hormones affect appetite. While a discussion of appetite regulation is beyond the scope of this chapter, information of a few appetite-regulating hormones is presented here as well as in Chapter 8. **Ghrelin**, a peptide secreted primarily from endocrine cells of the stomach, acts on the hypothalamus to stimulate food intake. Plasma concentrations of ghrelin typically rise before eating (e.g., a fasting situation) and decrease immediately after eating, especially carbohydrates. Two other appetite-enhancing peptides include neuropeptide Y (NPY) and agouti-related protein (AGRP). **Leptin**, secreted mainly by white adipose tissue in proportion to fat stores, suppresses food intake. Leptin's activity occurs at least in part in conjunction with α -melanocyte-stimulating hormone (α -MSH), which stimulates MC₄ receptors, primarily in the hypothalamus. Another hormone suppressing food intake in conjunction with leptin is corticotropin-releasing hormone (CRH).

A review of these regulatory peptides clearly shows that these various mediators of the digestive processes work in concert to stimulate and inhibit food intake as needed and to coordinate the movement of digestive tract contents and the breakdown of the nutrients within the digestive tract.

SUMMARY

Examining the various mechanisms in the gastrointestinal tract that allow food to be ingested, digested, and absorbed, and its residue to then be excreted reveals the complexity of the digestion and absorption processes. Normal digestion and absorption of nutrients depend not only on a healthy digestive tract but also on integration of the digestive system with the nervous, endocrine, and circulatory systems.

The many factors that influence digestion and absorption—including dispersion and mixing of ingested food, quantity and composition of gastrointestinal secretions, enterocyte integrity, the expanse of intestinal absorptive area, and the transit time of intestinal contents—must be coordinated so that the body can be nourished without disrupting the homeostasis of body fluids. Much of the coordination required is done by regulatory peptides, some of which are provided by the nervous system as well as by the endocrine cells of the gastrointestinal tract.

Although the basic structure of the digestive tract—which consists of the mucosa, submucosa, muscularis

externa, and serosa—remains the same throughout, structural modifications enable various segments of the gastrointestinal tract to perform more specific functions. Gastric glands that underlie the gastric mucosa secrete fluids and compounds necessary for the stomach's digestive functions. Other particularly noteworthy features are the villi and the microvilli, which dramatically increase the surface area exposed to the contents of the intestinal lumen. This enlarged surface area helps maximize absorption not only of ingested nutrients but also of endogenous secretions released into the gastrointestinal tract.

Study of the digestive system makes abundantly clear the fact that a person's adequate nourishment, and therefore his or her health, depends in large measure on a normally functioning gastrointestinal tract. Particularly crucial to nourishment and health is a normally functioning small intestine because that is where the greatest amount of digestion and absorption occurs. Later chapters of this book expand on digestion and absorption of individual nutrients.

Perspective

THE NUTRITIONAL IMPACT OF ROUX-EN-Y GASTRIC BYPASS, A SURGICAL APPROACH FOR THE TREATMENT OF OBESITY

Obesity is a national epidemic in the United States and one of the most prevalent health conditions worldwide, with close to 2 billion people classified as either overweight or obese. While changes in lifestyle, including diet and physical activity, and, if needed, pharmacological intervention, are the preferred treatment approaches for obesity, obese individuals who meet selected criteria and who are at increased risk of obesity-related mortality may be candidates for bariatric surgery.

Bariatric surgical options can be classified as restrictive, malabsorptive, or both. Restrictive procedures, such as gastric banding and sleeve gastrectomy, reduce the size of the stomach by up to 85%, which thus limits gastric volume and food intake. The digestive tract, however, remains intact with these restrictive procedures. Malabsorptive procedures reduce nutrient absorption. Bariatric surgical procedures, such as biliopancreatic diversion with or without duodenal switch and Roux-en-Y gastric bypass (RYGB), are both restrictive and malabsorptive, reducing the size of the stomach as well as altering intestinal tract continuity. Specifically, with RYGB, the proximal and distal portions of the stomach are surgically separated and a small gastric pouch is created. A loop of the jejunum (referred to as the Roux limb) is attached to the gastric pouch with shorter Roux limbs resulting in greater postoperative malabsorption. A biliopancreatic limb is attached to the Roux limb at a site distal to the anastomosis (attachment) of the stomach pouch and jejunum. A large section of the stomach and the duodenum are surgically stapled and bypassed (Figure 1).

RYGB is the most common bariatric procedure performed in the United States. Yet, it is not without complications, both medical and nutritional. Macronutrient and micronutrient deficiencies occur following RYGB. Some result from poor compliance to postsurgical nutritional treatment

plans, while many others occur due to RYGB-induced modifications to the digestive tract. Some of the surgically induced alterations most impacting digestion and absorption include reducing the size of the stomach, shortening the length of the small intestine in contact with nutrients, and disrupting the normal continuity of the digestive system and its accessory organs (affecting bile release and pancreatic secretions). Additionally, bacterial overgrowth in the “bypassed section” of the small intestine can promote deficiencies of some nutrients. This perspective focuses on some of the most prevalent nutritional consequences associated with RYGB.

Of the macronutrients, protein deficiency occurs rather frequently. It typically results from inadequate protein intake, reduced gastric acid secretion (which normally facilitates protein denaturation and pepsinogen activation in the stomach to facilitate protein digestion), insufficient amino acid absorption (reduced absorptive surface), and extreme weight loss. For several weeks post operation, only very small amounts of

foods, usually in liquid form, are permitted; such restrictions make ingestion of recommended amounts of nutrients, especially protein, challenging. Protein intakes of 1.1–1.5 g/kg per ideal body weight or in total amounts ranging from about 60 to 120 g daily are recommended for bariatric surgical patients. Additionally, supplemental leucine (which has been shown to promote protein synthesis) for protein-malnourished bariatric patients has also been recommended. Postsurgical monitoring should include regularly scheduled measurements of muscle strength and muscle mass, which are often negatively impacted with poor protein status. Some physical symptoms suggesting protein deficiency may include brittle hair and alopecia (hair loss), generalized edema (swelling), and asthenia (weakness).

Several vitamin deficiencies occur among bariatric surgical patients. Of the water-soluble vitamins, deficiencies of thiamin, vitamin B₁₂, and folate are common. Thiamin deficiency occurs with excessive or recurrent vomiting (emesis), which is often present, as well as from reductions

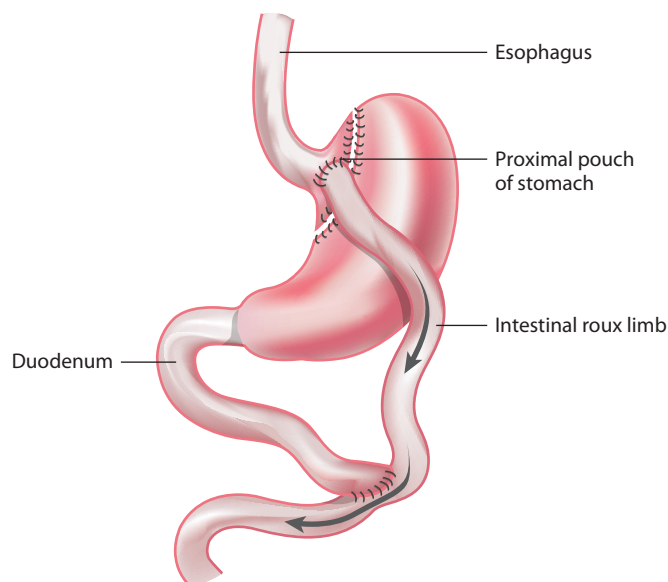


Figure 1 Anatomy of a Roux-en-Y gastric bypass.

in thiamin intake and absorption (normally from the proximal small intestine). Treatment of thiamin deficiency characterized by neurologic symptoms may require parenteral administration of the vitamin. In the absence of neurologic symptoms, oral thiamin supplementation in doses of about 50–100 mg/day is usually recommended to attain or maintain thiamin status.

Vitamin B₁₂ deficiency results from several surgery-induced problems, especially insufficient intrinsic factor. Intrinsic factor is made by parietal cells in the stomach and binds to the vitamin in the duodenum so it can be absorbed in the ileum. However, with RYGB, secretions from gastric parietal cells are reduced, so little intrinsic factor is released. Additionally, hydrochloric acid in the stomach is needed to help release the vitamin from foods, but as with intrinsic factor, the amount of acid produced after RYGB is often not sufficient to facilitate this release. A third factor contributing to deficiency is inadequate intake. Finally, should bacterial overgrowth occur, the bacteria use the vitamin for their own growth needs and thus limit the vitamin's availability. Because most individuals have large stores of vitamin B₁₂, deficiency symptoms (i.e., neurological problems, cognitive dysfunction, and macrocytic anemia, among others) may not appear for some time. Treatment of a vitamin B₁₂ deficiency generally requires injections of the vitamin, but because about 1–3% of vitamin B₁₂ may be absorbed without intrinsic factor, oral ingestion of high doses of the vitamin (about 1,000–2,000 µg/day) or vitamin B₁₂ nasal sprays can sometimes correct the deficiency.

Folate deficiency may result from inadequate dietary intake and/or from insufficient absorption of folate due to surgery-induced changes in the intestinal continuity. Oral, supplemental folate in amounts of 800–1,000 µg per day for several months is usually needed to treat the deficiency.

Fat malabsorption occurs in RYGP primarily if the common channel below the biliopancreatic and Roux limb anastomosis is too short (i.e., less than about 100 cm). Fat malabsorption in turn leads to malabsorption and deficiencies of the fat-soluble vitamins. Insufficient bile (which is no longer directed into the duodenum through the sphincter of Oddi) and the bypassing of much of the jejunum, where most

fat-soluble vitamins are absorbed, also contribute to the malabsorption. Vitamin D problems also occur with obesity because the greater amounts of subcutaneous fat that are present store more of the vitamin and don't release (mobilize) the vitamin into the blood as quickly when intake is insufficient. Of the fat-soluble vitamins, deficiencies of vitamins D and A are common, although physical signs of a vitamin D deficiency are not usually present. Low serum 25-hydroxyvitamin D concentrations, especially if coupled with high serum parathyroid hormone concentrations, suggest impaired vitamin D status. Treatment requires oral supplements of the vitamin in amounts ranging from about 75 to 250 µg (but sometimes higher doses) per day for several months or until serum 25-hydroxyvitamin D concentrations exceed 30 ng/mL. Vitamin A deficiency is typically characterized by low serum retinol and vision/ophthalmological problems. Symptomatic vitamin A deficiency is usually treated with oral supplements, providing 1,500–7,500 µg vitamin A per day, and may be needed for 6–12 months to correct the deficit. Short-term treatment with larger doses has also been used with severe vitamin A deficiency.

Of the minerals, calcium, iron, zinc, and copper deficiencies are commonly reported. Calcium is best absorbed from a slightly acidic environment in the proximal small intestine and requires adequate vitamin D status; however, these conditions do not exist following RYGB. General practice guidelines suggest up to 2 g of elemental calcium along with vitamin D supplements daily for those who have had RYGB.

Iron deficiency is one of the most well-studied and documented deficiencies in those who have had RYGB. Aforementioned reductions in acid production and rerouting of the proximal intestine represent surgical-induced changes contributing to the deficiency. Inflammation, which may be present with obesity, also diminishes intestinal iron absorption. Finally, iron intake is often poor because meat (a good source of iron) is frequently not tolerated. Deficiency is usually detected by evaluation of biochemical indices such as low serum ferritin, increased serum soluble transferrin receptors, low transferrin saturation, elevated total iron-binding capacity, low serum iron, and low mean cell volume (MCV). MCV, however, may be normal with the copresence of vitamin B₁₂ and folate

deficiencies, and ferritin concentrations may be elevated in the presence of inflammation. While treatment of deficiency often requires intravenously administered iron, oral doses (in amounts up to 300 mg) may be tried initially. Typically, lower doses of iron taken orally a couple of times per day are better tolerated (less side effects) than higher doses taken less frequently. Ingestion of foods rich in vitamin C along with the iron supplements is normally recommended to facilitate iron absorption.

Zinc and copper deficiencies have also been documented in bariatric surgery patients. Poor dietary intake of foods rich in these trace minerals and reductions in gastric acid contribute to the deficiencies. Additionally, both nutrients, like calcium and iron, are better absorbed from a slightly acidic environment in the proximal small intestine. Classic symptoms of zinc deficiency include skin lesions, poor wound healing, and hair loss (alopecia). Plasma or blood cell zinc concentrations also decrease with deficiency, along with 24-hour urinary zinc excretion. Practice guidelines suggest oral supplementation providing about 10–40 mg elemental zinc per day to treat deficiency; prolonged intakes in amounts higher than 40 mg per day can induce copper deficiency or impair copper status. Providing 1–2 mg of elemental copper with such zinc supplementation is suggested to minimize this interaction. However, dosages of 2–5 mg (sometimes higher) of elemental copper per day (given in divided doses) for up to 3 months may be needed to correct copper deficiency and replenish stores. In some cases, intravenous infusion of copper may be initially needed prior to oral supplementation. Serum copper and ceruloplasmin concentrations, which are reduced with deficiency, can be used to assess copper. Copper deficiency is also characterized by neutropenia, thrombocytopenia, hypochromic anemia, decreased erythropoiesis, and neurologic dysfunction.

Bariatric surgery is an effective treatment for obesity and many of its comorbidities. Yet, as can be gleaned from this perspective, the RYGB procedure is not without nutritional consequences. This perspective has reviewed some of the more prevalent nutritional complications associated with RYGB. The articles at the end of this Perspective provide additional information on the complications associated with bariatric surgeries.

Suggested Readings

Mangan A, Le Roux CW, Miller NG, Docherty NG. Iron and vitamin D/calcium deficiency after gastric bypass: mechanisms involved and strategies to improve oral supplement disposition. *Curr Drug Metab.* 2019; 20:244–52.

Patel JJ, Mundi MS, Hurt RT, Wolfe B, Martindale RG. Micronutrient deficiencies after bariatric surgery: an emphasis on vitamins and trace minerals. *Nutr Clin Pract.* 2017; 32:471–80.

Via MA, Mechanick JL. Nutritional and micronutrient care of bariatric surgery patients: current evidence update. *Curr Obes Rep.* 2017; 6:286–96.

CARBOHYDRATES

LEARNING OBJECTIVES

- 3.1** Describe the structural and functional features of the main carbohydrate classes.
- 3.2** Explain how dietary carbohydrates are digested, absorbed, transported and stored in the body.
- 3.3** Define glycemic index and its application in maintaining health.
- 3.4** Explain how blood glucose is metabolically controlled and the role of insulin.
- 3.5** Describe carbohydrate utilization in energy metabolism and the role of ATP.
- 3.6** Describe the relationships among glycolysis, the TCA cycle, the electronic transport chain, and oxidative phosphorylation.
- 3.7** Explain the importance of glucose synthesis from noncarbohydrate sources.

CARBOHYDRATES ARE THE MOST ABUNDANT ORGANIC MOLECULES ON EARTH. They are the main structural component of plants and they provide food energy in the form of starch and sugars. In fact, carbohydrates provide half or more of the food energy consumed by humans worldwide. Carbohydrates also act as metabolic intermediates, as constituents of RNA and DNA, as structural elements of cells and tissues, and as energy storage molecules in the body. The functional diversity of carbohydrates is due to their structural diversity.

Carbohydrates are constructed from carbon, oxygen, and hydrogen atoms that occur in a proportion that approximates that of a “hydrate of carbon,” $(C-H_2O)_n$, accounting for the term *carbohydrate*. More precisely, carbohydrates are aldehydes or ketones that have multiple hydroxyl groups. Carbohydrates are usually categorized into *simple* carbohydrates and *complex* carbohydrates. Simple carbohydrates include monosaccharides and disaccharides. Complex carbohydrates include oligosaccharides containing 3–10 saccharide units and polysaccharides containing more than 10 units (Figure 3.1).

3.1 SIMPLE CARBOHYDRATES

Monosaccharides

Monosaccharides are structurally the simplest carbohydrates and cannot be hydrolyzed into smaller units by digestive enzymes. Monosaccharides are commonly called sugars and are sometimes referred to as monosaccharide units or residues. The suffix *-ose* is used to name monosaccharides and many other carbohydrates. Monosaccharides may contain from three to seven carbon atoms and accordingly are termed *trioses*, *tetroses*, *pentoses*, *hexoses*, and *heptoses*. In addition to having hydroxyl groups, monosaccharides possess a functional carbonyl group, $C=O$, that is either an aldehyde or a ketone. Hence, they are further designated as *aldoses*, sugars having an aldehyde group, and *ketoses*, sugars possessing a ketone group. Combining the functional group name with the number of carbon atoms can describe a particular monosaccharide. For example, a five-carbon sugar having a ketone group is a *ketopentose*; a six-carbon aldehyde-possessing sugar is an *aldohexose*.

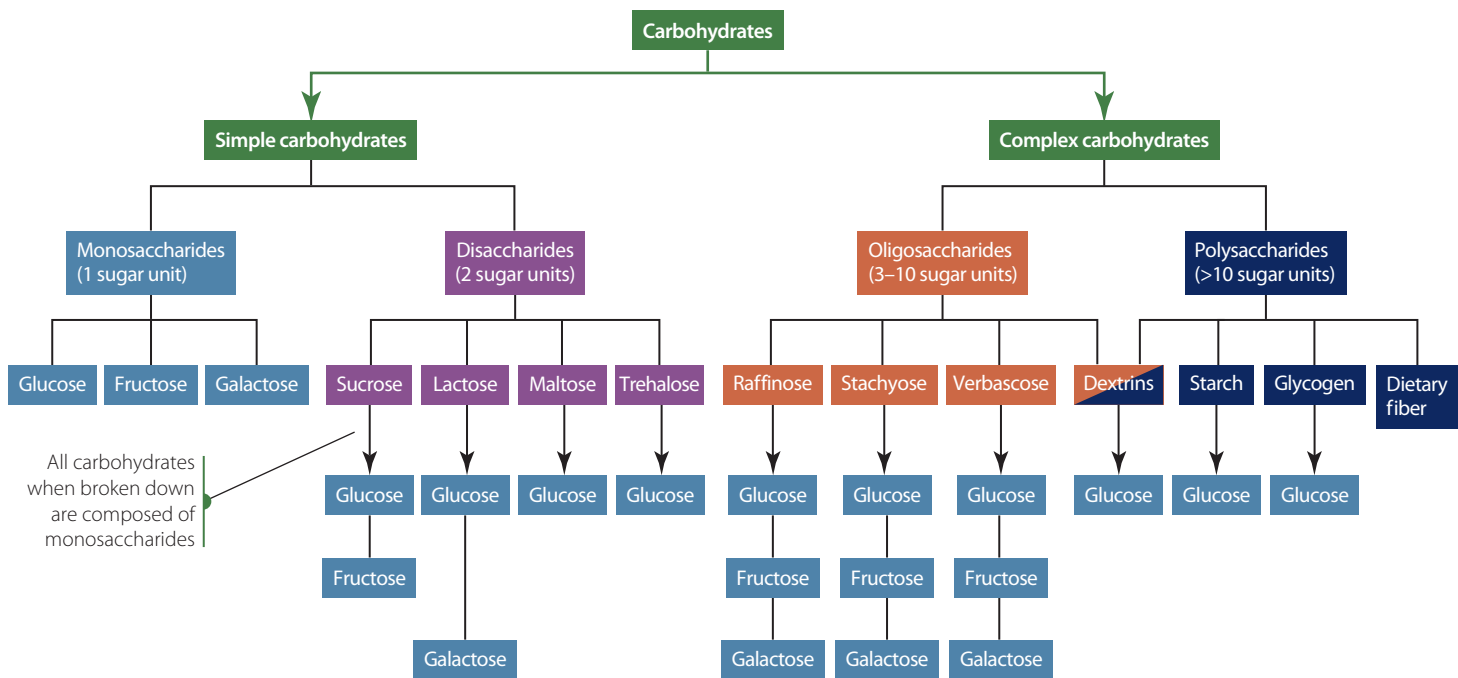


Figure 3.1 Classification of carbohydrates, showing the monosaccharide composition upon hydrolysis.

Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

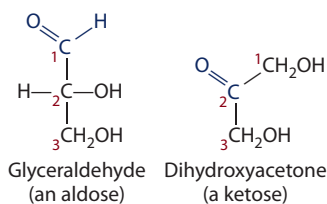


Figure 3.2 Three-carbon monosaccharides. Shown here are an aldose, with the carbonyl group at C-1, and a ketose, with the carbonyl group at C-2.

Figure 3.2 illustrates the chemical structure of the smallest monosaccharides, glyceraldehyde and dihydroxyacetone. Note that the carbonyl group of aldoses is located at carbon number one (C-1), whereas the carbonyl group of ketoses is located at C-2.

Stereoisomers

A brief discussion of stereoisomerism as it relates to monosaccharides is provided here to emphasize the importance of stereospecificity in biological systems. To review, isomers are compounds with identical molecular formulas but have

different structures. **Stereoisomers** are spatial isomers, meaning that two or more compounds have the same molecular composition and the same bonds, but the bonds differ in their three-dimensional orientation in space.

The existence of stereoisomers is due to the presence of **asymmetric carbon atoms**. Recall that an asymmetric (chiral) carbon atom has four different atoms or groups covalently attached to each of its four bonds. In the case of glyceraldehyde (Figure 3.3), C-2 is an asymmetric carbon; therefore, the hydroxyl group at C-2 can exist in two different spatial configurations. When drawn as Fischer projections, placing the hydroxyl group on the left side of the carbon atom designates the L stereoisomer, whereas the hydroxyl group on the right indicates the D stereoisomer.

Monosaccharides with four or more carbons have multiple asymmetric carbons. In the case of glucose (Figure 3.3), the asymmetric carbons are C-2, C-3, C-4, and C-5. By convention, the asymmetric carbon atom farthest from the aldehyde or keto group designates D or L configuration. For glucose,

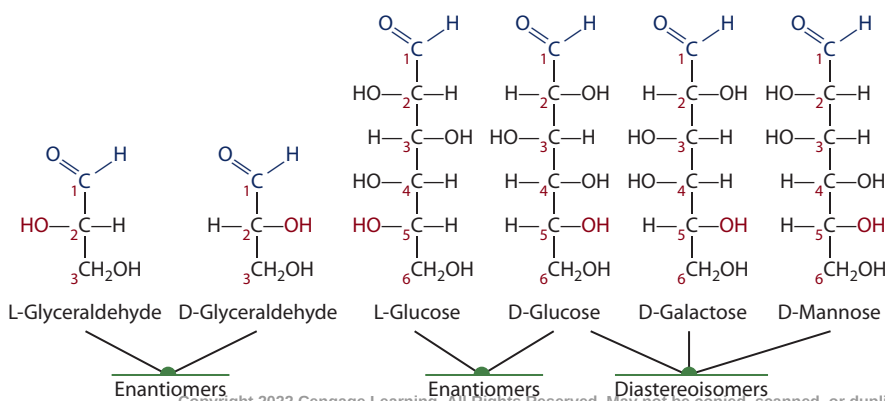


Figure 3.3 Stereoisomers of monosaccharides. When drawn as Fischer projections, the asymmetric carbon atom farthest from the carbonyl group indicates the D- or L-stereoisomer, with the hydroxyl group on the left side for the L configuration. Stereoisomers that are not mirror images and not superimposable are called diastereoisomers.

C-5 is farthest from the aldehyde group, so D-glucose is identified by the C-5 hydroxyl group on the right side.

Stereoisomers that exist as D and L configurations are mirror images of each other (and not superimposable), much like a person's left and right hands. These types of stereoisomers are called *enantiomers*. D- and L-glyceraldehyde are enantiomers, as are D- and L-glucose. In contrast, close inspection of D-glucose and D-galactose show nearly identical structures, except for the hydroxyl group at C-4. Both monosaccharides are stereoisomers, but they are not mirror images and not superimposable. These types of stereoisomers are called *diastereoisomers*.

Both D and L monosaccharides are present in nature, although the vast majority are D isomers. Consequently, digestive and cellular enzymes tend to be specific for D monosaccharides, as discussed later in the chapter.

Ring Structures

In solution, monosaccharides do not exist in an open-chain form, even though straight-line Fischer projections are frequently used for illustrative purposes. Instead, the molecules form a cyclic ring structure through a reaction between the carbonyl group and a single hydroxyl group. The rings form spontaneously in solution and are energetically more stable than the open chain. If the cyclized sugar is formed with an aldehyde, it is called a *hemiacetal*; if the cyclized sugar is formed with a keto group, it is called a *hemiketal*.

Table 3.1 illustrates the cyclization of monosaccharides using the examples of D-glucose, D-galactose, D-fructose, and D-ribose. The rings are best illustrated using Haworth projections because the three-dimensional structure can be inferred more easily. Hydroxyl groups pointing upward

Table 3.1 Structural Representation of D-Monosaccharides

Hexose	Fischer Projection	Cyclized Fischer Projection	Haworth Projection
α -D-glucose (an aldohexose)			
β -D-galactose (an aldohexose)			
β -D-fructose (a ketohexose)			
β -D-fructose (a aldopentose)			

in Haworth projections are above the plane of the ring (they point left in the cyclized Fischer projections).

Formation of the ring structure creates a new and unique asymmetric carbon atom from the carbonyl group called an **anomeric carbon**. The anomeric carbon, indicated by an asterisk in Table 3.1, is C-1 for the hemiacetals (glucose, galactose, and ribose) and C-2 for the hemiketal (fructose). The hydroxyl group attached to the anomeric carbon can exist on either side of the ring structure, thus creating another type of stereoisomer—*anomers*—designated α and β . On Haworth projections, the β isomer is drawn with the $-\text{OH}$ group above the plane of the ring structure. To be more precise, the $-\text{OH}$ group of the β isomer resides on the same side of the ring as the $-\text{CH}_2\text{OH}$ next to the carbon atom that determines D or L configuration. In aqueous environments, an equilibrium occurs with approximately two times more of the β configuration.

Stereoisomerism among the monosaccharides, and also among other nutrients such as amino acids and lipids, has important metabolic implications because of the stereospecificity of certain metabolic enzymes. An important example of stereospecificity is the action of the digestive enzyme α -amylase, which hydrolyzes the bond between glucose units in the polysaccharide starch. The α -amylase enzyme recognizes only the α -linkage between α -D-glucose molecules found in starch, but does not recognize the β -linkage between β -D-glucose molecules found in cellulose.

Reducing Sugars

Monosaccharides that are cyclized into hemiacetals or hemiketals are sometimes called reducing sugars because they are capable of reducing other substances, such as the copper ion (from Cu^{2+} to Cu^{1+}). This property is useful in identifying which end of a polysaccharide chain has the monosaccharide unit that can open and close; in other words, which end has the anomeric carbon unattached to another sugar unit. This role of reducing sugars is discussed in more detail in the “Polysaccharides” section.

Pentoses

Compared to the hexoses, pentose sugars furnish little dietary energy because relatively few are available in the diet. However, they are readily synthesized in the cell from hexose precursors and are incorporated into metabolically important compounds. The aldopentose ribose, for example, is a constituent of key nucleotides such as the adenosine phosphates: adenosine triphosphate (ATP), adenosine diphosphate (ADP), adenosine monophosphate (AMP), and cyclic adenosine monophosphate (cAMP). Ribose is a constituent of the nicotinamide adenine dinucleotides (NAD^+ , NADP^+). Ribose and its deoxygenated form, deoxyribose, are part of the structures of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA), respectively. Ribitol, a reduction product of ribose, is a constituent of

the vitamin riboflavin and of the flavin coenzymes: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN).

Derivatives of Monosaccharides

Monosaccharides are routinely modified in cells to serve specialized functions. As mentioned above, ribose can be modified by deoxygenation or reduction, resulting in deoxyribose and ribitol (Figure 3.4). Substitution of the C-6 hydroxyl group of glucose with a carboxyl group results in glucuronic acid. Substitution of the C-2 hydroxyl group of galactose results in galactosamine; acetylation of the amino group results in acetylgalactosamine.

The monosaccharide derivatives in Figure 3.4 represent only a few examples that occur in the body. Such modifications are important to the formation of larger molecules in which monosaccharides are joined—conjugated—to noncarbohydrate components such as proteins and lipids. For example, certain proteoglycans (protein + sugar) containing glucuronic acid and acetylgalactosamine are found in abundance in connective tissue, including bone and cartilage. Dietary supplements containing glucosamine are marketed to support joint health, despite little evidence of its effectiveness.

Disaccharides

Disaccharides contain two monosaccharide units joined through a glycosidic bond. The attachment is formed between a hydroxyl group of one monosaccharide unit and a hydroxyl group of a second monosaccharide, while forming one molecule of water. Glycosidic bonds generally involve the hydroxyl group on the anomeric carbon of one monosaccharide and the hydroxyl group on a

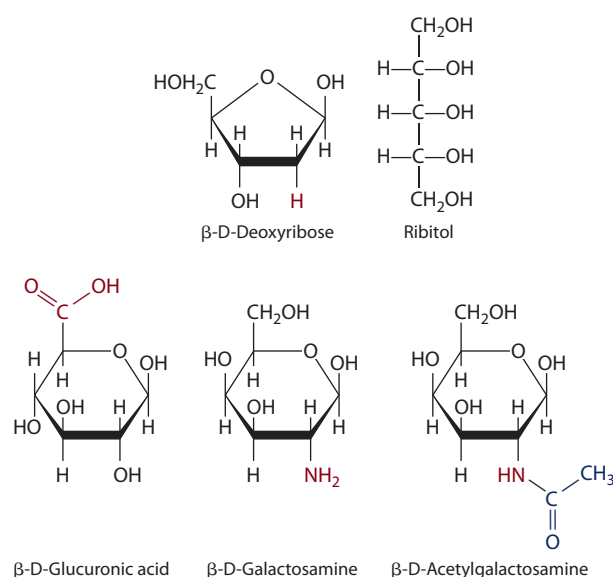


Figure 3.4 Common derivatives of monosaccharides.

nonanomeric carbon of the second monosaccharide. Furthermore, the glycosidic bond is termed α or β in reference to the anomeric carbon's hydroxyl group orientation before the glycosidic bond was formed. Specific glycosidic bonds therefore may be designated $\alpha(1-4)$, $\beta(1-4)$, $\alpha(1-6)$, and so on. Disaccharides are important energy-supplying nutrients in the diet. The most common disaccharides in the diet are maltose, lactose, and sucrose (Figure 3.5).

Maltose

Maltose is formed primarily from the partial hydrolysis of starch and therefore is found naturally in malt beverages such as beer and malt liquors. Maltose is also added as an ingredient in a variety of foods. It consists of two glucose units linked through an $\alpha(1-4)$ glycosidic bond (Figure 3.5). The α designation refers to the left-side glucose unit, in which the C-1 anomeric carbon is attached to the glycosidic bond below the plane of the ring structure. The enzyme that hydrolyzes this bond recognizes only the α orientation. Maltose is a reducing sugar because the C-1 anomeric carbon of the glucose unit on the right is available to react as a free hemiacetal.

Lactose

Lactose is found naturally only in milk and milk products. It is composed of galactose linked by a $\beta(1-4)$ glycosidic bond to glucose (Figure 3.5). The C-1 anomeric carbon of galactose on the left is attached to the glycosidic bond in the β position. Lactose is a reducing sugar.

Sucrose

Sucrose (cane sugar, beet sugar) is the most widely consumed disaccharide and is the most commonly used natural sweetener. It is composed of glucose and fructose and is structurally noteworthy because its glycosidic bond involves the anomeric carbons of both monosaccharides. The linkage is α with respect to the glucose residue and β with respect to the fructose residue (Figure 3.5). The glycosidic bond is written as $\alpha(1-2)$, acknowledging the glucose unit first. Because it has no free hemiacetal or hemiketal function, sucrose is not a reducing sugar.

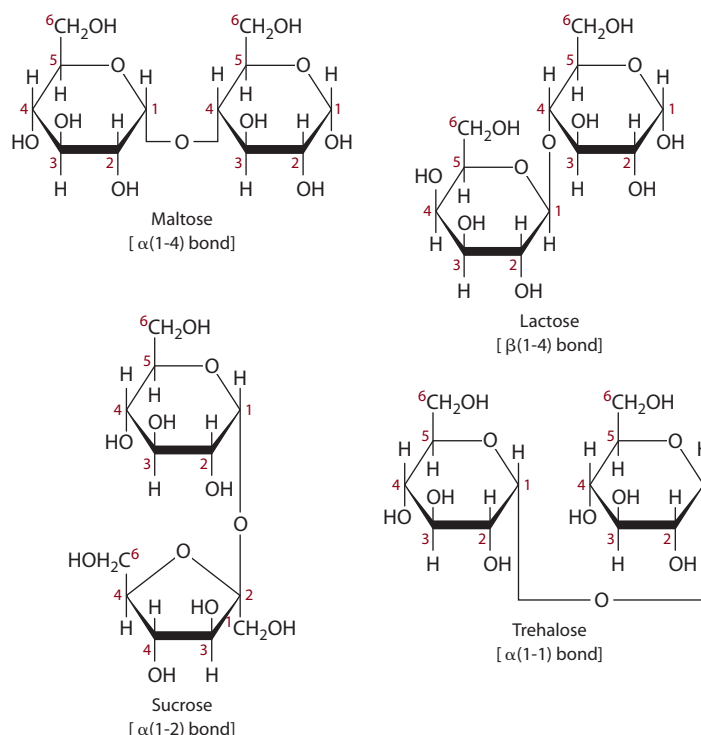


Figure 3.5 Disaccharides. The structures are shown as Haworth projections, indicating the glycosidic bonds.

Trehalose

Trehalose is found naturally in bacteria, yeast, fungi (mushrooms), shrimp, and plants, although its native abundance in the human diet is minimal. Trehalose possesses different physical and chemical properties from other sugars. Consequently, it is used as an additive in processed foods in Japan and other countries because it acts as a stabilizer and protects against moisture loss. When consumed, trehalose is digested slowly and elicits a low glycemic response, thus raising interest in trehalose as a treatment for metabolic disease [1]. It has been granted Generally Recognized as Safe (GRAS) status by the U.S. Food and Drug Administration [2]. Trehalose has an $\alpha(1-1)$ glycosidic bond between two glucose molecules. It is a nonreducing sugar (Figure 3.5).

SYRUPS – LIQUID SUGAR

A syrup is a viscous solution of sugars dissolved in water. The concentration of sugars is high enough to create the viscosity, but not so high as to cause the sugars to crystallize. Syrups can be found in their native state (such as honey), can be made by reducing the water content

of natural juices (such as maple sap), or made commercially using enzymatic processes (such as corn syrup). The specific type and proportion of sugars in syrups vary widely. Depending on its source and purpose, a syrup may contain ingredients other than sugars that contribute to its

unique flavor, appearance, and functional properties.

Honey

Honey is a natural syrup made by bees. The bees collect sugars from the floral nectar of plants, giving honey varieties their

(Continued)

distinctive flavor and color. Honey can be consumed directly from the beehive.

Molasses

A by-product of “table sugar” refining, molasses is the viscous liquid that remains after most of the sucrose crystals have been removed from the sugar cane or sugar beets. Although molasses contains residual sugars, it can also taste bitter because of the high mineral content.

Maple Syrup

Start by tapping a sugar maple tree to collect the sap. The sap is mostly water, so boiling the sap is necessary to evaporate the water and concentrate the sugar.

It takes 40 liters of sap to make 1 liter of maple syrup.

Agave Syrup

The juice of the agave plant is rich in polysaccharides called fructans. The juice is treated with dilute acid, enzymes or heat to break down the fructans into fructose. After the juice is concentrated, the resulting syrup is sometimes marketed as agave nectar.

Corn Syrup

A favorite of candy makers, corn syrup is also known as glucose syrup. It is made by dissolving corn starch in water, then adding enzymes to hydrolyze the glycosidic

bonds. Because only glucose units comprise starch, the resulting syrup contains only glucose. Confectioners prefer glucose syrup to table sugar because glucose resists crystallizing.

High-Fructose Corn Syrup

During corn syrup production, a second step is added to enzymatically convert some glucose molecules to fructose. The conversion rate is controlled, so only 42% or 55% is converted to fructose, resulting in syrups called HFCS-42 and HFCS-55. HFCS-42 is primarily used in the food industry for making cereals and baked goods. HFCS-55 is used primarily in soft drinks. So is HFCS really “high” in fructose?

	Fructose (g/100 g)	Glucose (g/100 g)	Sucrose (g/100 g)	Water (g/100 g)
Honey	40.9	35.8	0.9	17.1
Sugar cane molasses	12.8	11.9	29.4	21.9
Maple syrup	0.5	1.6	58.3	32.4
Agave syrup	55.6	12.4	0	22.9
Corn syrup, light	0	74.4	0	22.8
High-fructose corn syrup-55	41.6	34.0	0	24.0

Source: U.S. Department of Agriculture, FoodData Central (<https://fdc.nal.usda.gov/index.html>)

3.2 COMPLEX CARBOHYDRATES

Complex carbohydrates are polymers of saccharide units linked together by glycosidic bonds. By convention, oligosaccharides contain 3–10 saccharide units and polysaccharides contain more than 10 units, usually thousands of units. The type of saccharide present in complex carbohydrates can vary, although glucose is the most abundant. Complex carbohydrates are a major component of the human diet. In the body, oligosaccharides are usually conjugated to proteins and lipids associated with cell membranes. When present on the cell surface, the conjugated oligosaccharides act as important modulators of cell function (see Figure 1.3).

Oligosaccharides

Raffinose, stachyose, and verbascose are common food oligosaccharides consisting of three, four, and five saccharides, respectively. Each is composed of glucose, galactose, and fructose and are found in dried beans, peas, lentils, bran, and whole grains. Human digestive enzymes do not hydrolyze their glycosidic bonds, but the bacteria within the intestine can digest them. As a result, these oligosaccharides can cause intestinal discomfort and flatulence.

Dextrins are a category of oligosaccharides composed entirely of glucose units. They are not naturally present in food; instead, they are produced commercially and used as an additive in foods, pharmaceuticals, and nutritional supplements. Dextrins are made from starch, which is hydrolyzed under controlled conditions to produce glucose chains of desired lengths. The shorter-chain dextrins (3–20 glucose units) are used most frequently for food and drug applications. Note that dextrins can be categorized as either oligo- or polysaccharides, depending on chain length. Dextrins are listed on product labels as maltodextrin, corn syrup solids, or hydrolyzed corn starch. Some of the desirable properties and applications of dextrin products include:

- Thickening agent
- Inhibition of sugar crystallization in confections
- Fat replacer
- Crisping agent in food batters and coatings
- Energy source in enteral nutrition (tube feeding) formulas, infant formulas, and sports drinks.

When consumed, most dextrins are easily digested. An exception is wheat dextrin used as a dietary supplement, which contains nondigestible $\beta(1-2)$ and $\beta(1-3)$

glycosidic linkages. Because of the nondigestible bonds, wheat dextrin is considered a soluble fiber.

Polysaccharides

The glycosidic bonding of monosaccharide units may be repeated many times to form high-molecular-weight polymers called polysaccharides. If the structure is composed of a single type of sugar, it is called a *homopolysaccharide*. If two or more different types of sugars make up its structure, it is called a *heteropolysaccharide*. Both types exist in nature; however, homopolysaccharides are of far greater importance in nutrition because of their abundance in many natural foods. Starch and glycogen, for example, are made entirely of glucose units. Starch and glycogen are the major storage forms of carbohydrate in plant and animal tissues, respectively. These polysaccharides range in molecular weight from a few thousand to 500,000.

The reducing property of a saccharide is useful in describing polysaccharide structure by enabling one end of a linear polysaccharide to be distinguished from the other. In a polyglucose chain, for example, the glucose unit at one end of the chain has an available hemiacetal group because its anomeric carbon atom is not involved in a glycosidic bond. This glucose unit has reducing capacity. The glucose unit at the other end, however, has its anomeric carbon involved in a glycosidic bond and cannot act as a reducing sugar.

Understanding the reducing property of saccharides is important in determining how digestive enzymes work together when hydrolyzing dietary starch. Some enzymes (e.g., α -amylase) hydrolyze glycosidic bonds in the interior of the polyglucose chain, whereas other enzymes (e.g., glucoamylase) hydrolyze the glycosidic bond at the terminal nonreducing end.

In the food industry, hydrolysis of starch to produce dextrans and smaller saccharides can be easily monitored by the rate of appearance of reducing sugars. Each time a glycosidic bond is hydrolyzed, a hemiacetal at the anomeric carbon is created. A simple test, called *dextrose equivalents (DE)*, is used to measure the extent of hydrolysis. A starch solution with a DE value of 0 means no hydrolysis has occurred. A starch solution with a DE value of 100 means all glycosidic bonds have been hydrolyzed. The DE of food dextrans is typically 3–20.

Starch

The most common digestible polysaccharide in plants is starch. Its two forms, amylose and amylopectin, are both polymers of α -D-glucose. The amylose molecule is a linear, unbranched chain in which the glucose units are attached solely through $\alpha(1-4)$ glycosidic bonds. In water, amylose chains adopt a helical conformation, as shown in Figure 3.6a. Amylopectin, on the other hand, is a branched-chain polymer, with branch points occurring through $\alpha(1-6)$ bonds, as illustrated in Figure 3.6b. Both amylose and amylopectin occur in cereal grains, potatoes,

legumes, and other vegetables. Amylose contributes about 15–20%, and amylopectin 80–85%, of the total starch content of these foods.

Glycogen

The structure of glycogen is similar to amylopectin but is more highly branched (Figure 3.6c). Glycogen is the major form of stored carbohydrate in animal tissues, localized primarily in liver and skeletal muscle. The glucose units within glycogen serve as a readily available source of glucose. When dictated by the body's energy demands, glucose units are sequentially removed by enzymatic hydrolysis from the nonreducing ends of the glycogen chains. The liberated glucose molecule then enters energy-releasing pathways of metabolism. This process, called **glycogenolysis**, is discussed later in this chapter. The high degree of branching in glycogen and amylopectin offers a distinct metabolic advantage because it presents a large number of nonreducing ends from which glucose units can be cleaved.

Essentially no glycogen is consumed in meat products, despite muscle being a primary location for glycogen storage. During meat animal processing, the glycogen in muscle is quickly hydrolyzed to glucose, which in turn is converted to lactic acid.

Cellulose

Cellulose is the major component of cell walls in plants and, like starch, is a homopolysaccharide of glucose. It differs from starch because the glycosidic bonds connecting the glucose units are $\beta(1-4)$, rendering the molecule resistant to the digestive enzyme α -amylase, which is stereospecific to favor $\alpha(1-4)$ linkages. Because cellulose is not digestible by mammalian digestive enzymes, it is defined as a dietary fiber and is not considered an energy source. However, colonic bacteria can digest it, resulting in several digestion products including short-chain fatty acids that provide energy to the body and play important roles in the gastrointestinal tract. A more extensive discussion of fiber and short-chain fatty acids is presented in Chapter 4.

3.3 DIGESTION

Polysaccharides are the most abundant carbohydrates in the food supply. Disaccharides, mainly sucrose and lactose, are also abundant in food. Before these dietary carbohydrates can be used by the body's cells, they must first be hydrolyzed into their constituent monosaccharides within the gastrointestinal (GI) tract. Only monosaccharides can be absorbed into intestinal mucosal cells (enterocytes). The hydrolytic enzymes involved in digestion of complex carbohydrates and disaccharides are collectively called **glycosidases** or, alternatively, **carbohydrases**. Glucose and fructose, when present in food as monosaccharides, require no digestion prior to being absorbed into intestinal cells.

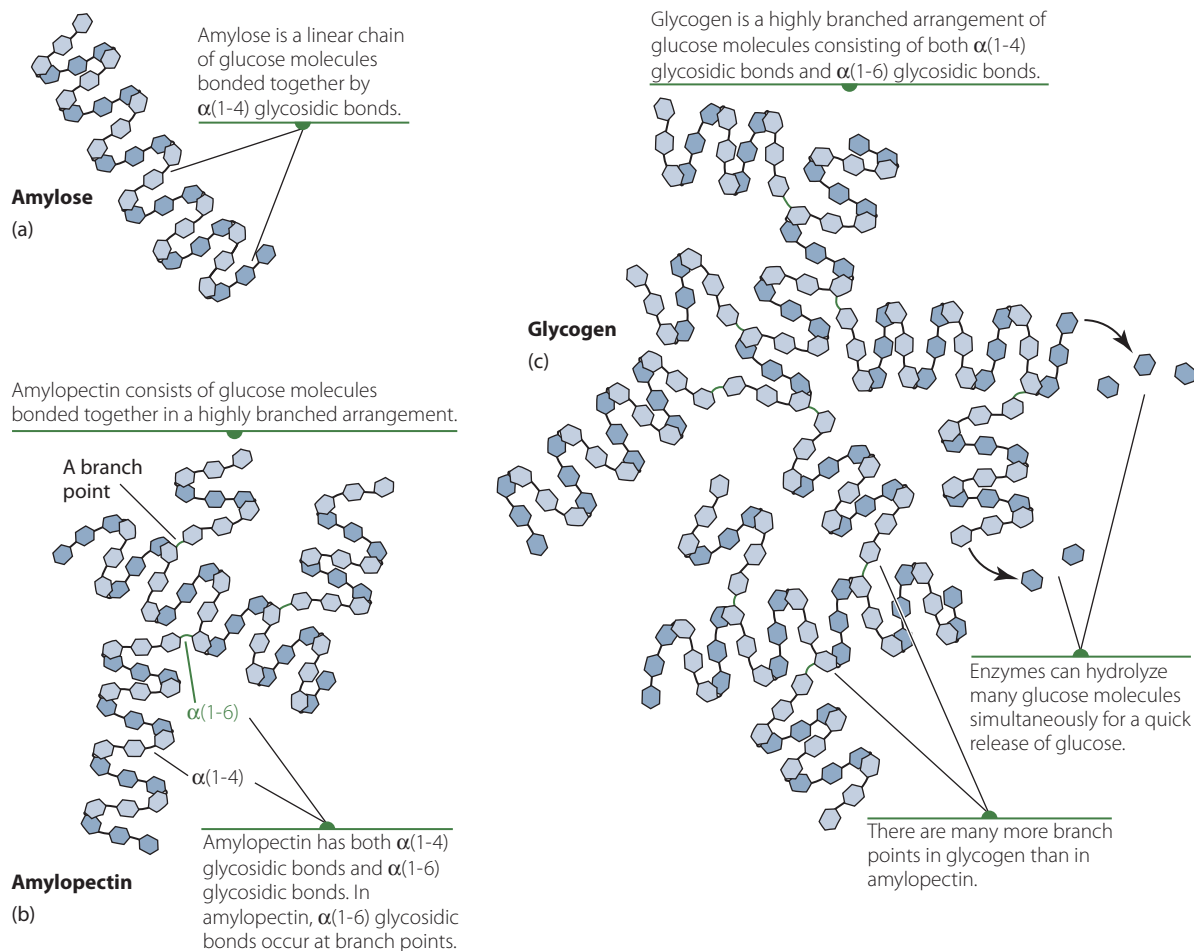


Figure 3.6 Structure of starches and glycogen.

Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Digestion of Polysaccharides

Digestion of the starches, amylose and amylopectin, starts in the mouth. The key enzyme is salivary α -amylase, a glycosidase that specifically hydrolyzes $\alpha(1-4)$ glycosidic linkages. α -Amylase is unable to hydrolyze the $\beta(1-4)$ bonds of cellulose, the $\beta(1-4)$ bonds of lactose, the $\alpha(1-2)$ bonds of sucrose, and the $\alpha(1-6)$ linkages that form branch points in amylopectin. Given the short period of time that food is in the mouth before being swallowed, this phase of digestion produces mostly oligosaccharides (dextrins), but few mono- or disaccharides. The salivary α -amylase action continues in the stomach until the gastric acid penetrates the food bolus and lowers the pH sufficiently to inactivate the enzyme.

The dextrins move into the duodenum and jejunum, where they are acted upon by pancreatic α -amylase. The presence of pancreatic bicarbonate in the duodenum elevates the pH to a level favorable for enzymatic function. Pancreatic α -amylase continues to hydrolyze $\alpha(1-4)$ glycosidic bonds to produce maltose, maltotriose, and limit dextrins (Figure 3.7). Maltotriose contains three glucose units with $\alpha(1-4)$ linkages. Limit dextrins are branched remnants of amylopectin containing the $\alpha(1-6)$ linkage that α -amylase is unable to hydrolyze.

The maltose, maltotriose, and limit dextrins are further digested by specific enzymes in the enterocyte brush border. Maltose and maltotriose are hydrolyzed by α -glucosidase (also called maltase). Limit dextrins are acted on by α -limit dextrinase (also called isomaltase). α -Dextrinase is the only intestinal enzyme that will hydrolyze $\alpha(1-6)$ glycosidic bonds. Glucose is the final digestion product of the combined action of α -amylase and the brush border enzymes (Figure 3.7).

A portion of the starch of beans and certain vegetables and other resistant starches are not fully digested (see also Chapter 4). This is partially due to the inaccessibility of the food to the enzyme and to naturally occurring inhibitors of α -amylase and α -glucosidase in some foods. The latter observation has led to the use of enzyme inhibitors to slow starch digestion for controlling glycaemic response [3].

Digestion of Disaccharides

No significant digestion of disaccharides or small oligosaccharides occurs in the mouth, stomach, or lumen of the small intestine. Digestion of disaccharides takes place almost entirely within the brush border of the upper small

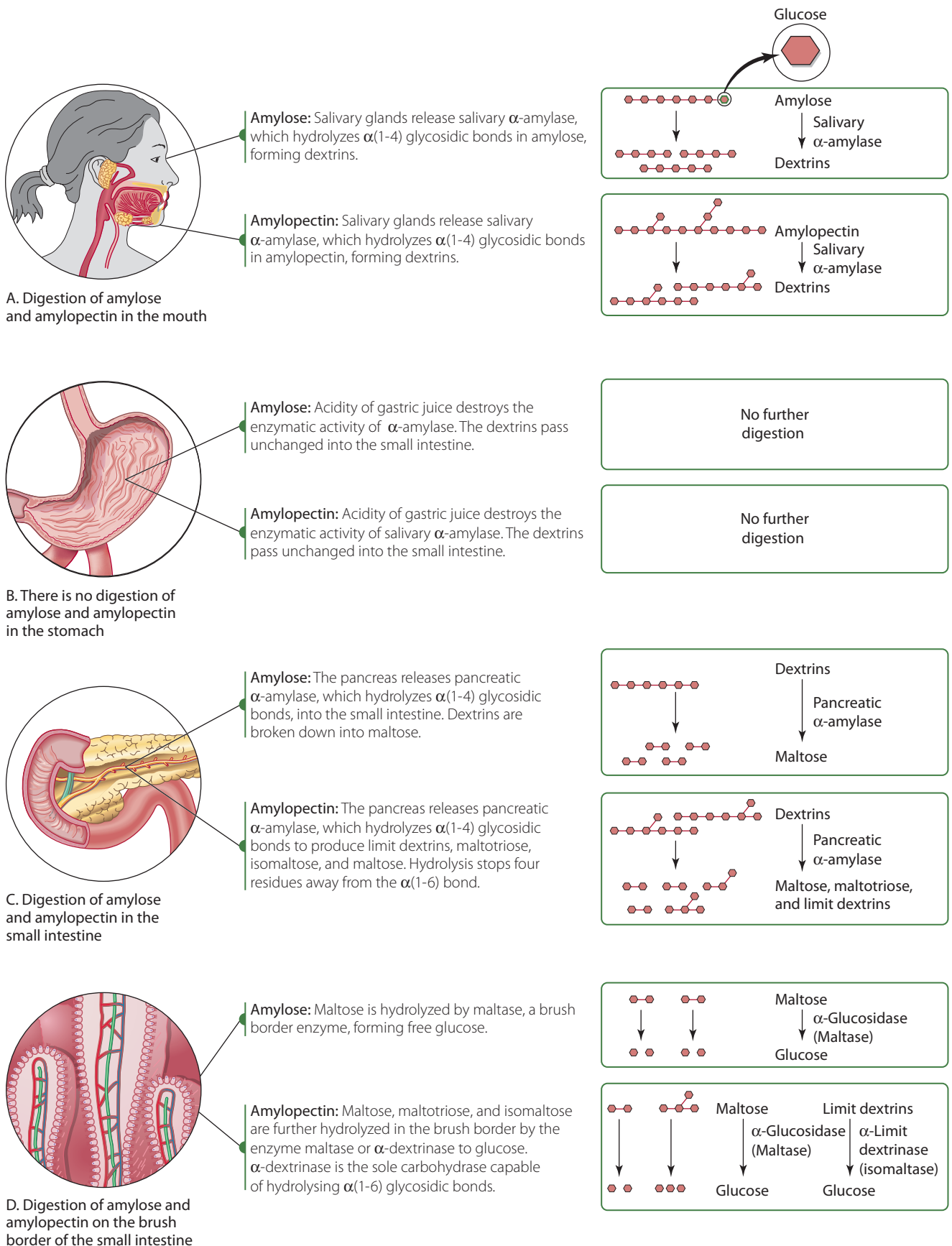


Figure 3.7 Starch digestion.

Source: Cengage Learning Inc. Reproduced by permission. www.cengage.com/permissions

intestine via disaccharidase activity. The resulting monosaccharides immediately enter the enterocytes with the aid of specific transporters (see Figure 2.17).

Among the enzymes located on the enterocytes are lactase, sucrase, α -glucosidase (maltase), and trehalase. Lactase catalyzes the cleavage of lactose to equimolar amounts of galactose and glucose. As was pointed out earlier, lactose has a $\beta(1-4)$ glycosidic bond, and lactase is stereospecific for this β linkage.

Lactase activity is high in infants, but in most mammals, including humans, it decreases a few years after weaning. This diminishing activity can lead to lactose malabsorption and intolerance. The frequency of lactose intolerance in human populations varies widely depending on geography, race, and ethnicity. The highest frequency is seen in Native Americans and in people of Asian, African, and Middle Eastern descent. The lowest frequency is seen in white individuals originating from northern European countries (Figure 3.8). Many lactose-free products are available for individuals with lactose intolerance. Additionally, lactase can be added directly to regular milk products to hydrolyze the lactose.

Sucrase hydrolyzes sucrose to yield one glucose and one fructose unit. α -Glucosidase (maltase) hydrolyzes maltose to yield two glucose units. Trehalase hydrolyzes the $\alpha(1-1)$ glycosidic bond of trehalose to yield two molecules of glucose. The final products of carbohydrate digestion, monosaccharides, can now be absorbed by the intestinal mucosal cells.

3.4 ABSORPTION AND TRANSPORT

For dietary monosaccharides to be absorbed into the bloodstream, they must twice cross the plasma membrane of enterocytes (see Figure 2.10 in Chapter 2). The monosaccharides first enter the cell on the brush border (apical) side, then exit on the basolateral side that faces a network of capillaries connected to the hepatic portal vein. In this way, the newly absorbed sugars are delivered directly to

the liver where they will be metabolized according to the body's needs, which are discussed later in the chapter.

The movement of molecules across cell membranes, including those of enterocytes, is a highly regulated process. To better understand intestinal absorption and transport of monosaccharides, a general discussion of membrane transport is presented here.

Membrane Transport

Cellular membranes are basically impermeable to molecules, yet normal cell function depends on the ability of molecules to cross these membranes. In the case of monosaccharides, crossing membranes is mediated by specialized transport proteins integrated in the cell membrane. Two major families of monosaccharide transporters have been identified in humans: the energy-dependent *sodium-glucose cotransporters* (SGLTs) and the facilitated diffusion *glucose transporters* (GLUTs). The distribution of SGLTs and GLUTs throughout the body is tissue-specific, each having different regulatory properties and substrate specificity [4,5].

SGLTs

Of the seven isoforms identified so far, SGLT1 and SGLT2 are known to play prominent roles in monosaccharide transport (Table 3.2). The function of SGLTs is coupled with sodium cotransport and ATP hydrolysis. Their activity is therefore dependent on cellular energy and exemplify active transport.

- SGLT1 is expressed mainly in the brush border membrane of enterocytes where its primary role is the absorption of dietary glucose and galactose. It is also expressed in kidney and other tissues, but its significance in other tissues is unclear. The importance of intestinal SGLT1 is evident in the genetic abnormality called *glucose-galactose malabsorption*, the result of mutations in the *SGLT1* gene. The condition is immediately recognized in newborn infants. Patients with the mutation experience severe diarrhea unless food

Worldwide prevalence of lactose intolerance in recent populations (schematic)

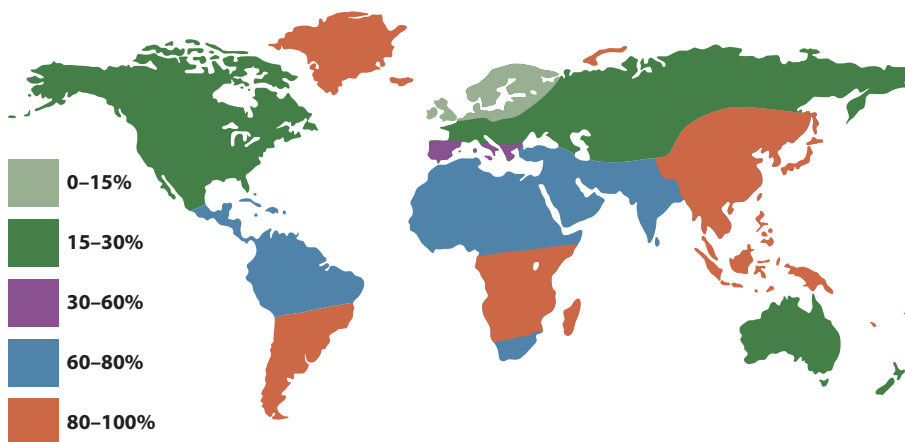


Figure 3.8 Worldwide prevalence of lactose intolerance.

Table 3.2 Membrane Transporters of Monosaccharides

Transporter Protein	Major Substrates	Major Sites of Expression
Sodium-Glucose Cotransporter Family		
SGLT1	Glucose, galactose	Small intestine, heart, kidney
SGLT2	Glucose	Kidney
SGLT3	No transport activity; acts as a glucose sensor	Enteric nervous system
SGLT4	Mannose	Small intestine, kidney
SGLT5	Mannose, fructose	Kidney
SGLT6	Inositol	Brain, kidney
SMIT1	Inositol, glucose	Wide tissue distribution
Glucose Transporter Family		
Class I		
GLUT1	Glucose, galactose, mannose, glucosamine	Erythrocytes, central nervous system, blood–brain barrier, placenta, fetal tissues in general
GLUT2	Glucose, galactose, fructose, mannose, glucosamine	Liver, β -cells of pancreas, kidney, small intestine
GLUT3	Glucose, galactose, mannose, xylose, dehydroascorbic acid	Brain (neurons), white blood cells, testis, placenta, preimplantation embryos
GLUT4 (insulin dependent)	Glucose, glucosamine	Skeletal muscle, heart, brown and white adipocytes
GLUT14	Glucose, dehydroascorbic acid	Testis
Class II		
GLUT5	Fructose, but not glucose	Small intestine, kidney, brain, skeletal muscle, adipocytes
GLUT7	Glucose, fructose	Small intestine, testis, prostate
GLUT9	Uric acid, glucose, fructose	Liver, kidney, small intestine
GLUT11	Glucose, fructose	Kidney, placenta, skeletal muscle, pancreas
Class III		
GLUT6	Glucose	Spleen, brain, white blood cells
GLUT8	Glucose, galactose, fructose	Testis, brain (neurons), adipocytes
GLUT10	Glucose, galactose, dehydroascorbic acid	Liver, pancreas, smooth muscle (aorta)
GLUT12	Glucose	Small intestine, skeletal muscle, adipocytes
GLUT13	Myo-inositol	Brain

sources of glucose and galactose, but not fructose, are removed from the diet [6].

- SGLT2 is highly expressed in the proximal tubule of the kidney where it is responsible for reabsorbing glucose from the glomerular filtrate. In type 2 diabetes mellitus, SGLT2 may be upregulated, which can exacerbate **hyperglycemia**. Alternatively, a class of anti-hyperglycemic drugs called SGLT2 inhibitors are being used to promote the urinary excretion of glucose by blocking the glucose reabsorption in the kidney proximal tubule [7].

GLUTs

Fourteen members of the GLUT family have been identified in humans. GLUTs are distributed throughout the body and function to transport glucose and other molecules by facilitated diffusion. Transport may be bidirectional depending on the substrate concentration gradient.

All of the GLUTs share a common structure and have similar sequences in the genes that code for them. The GLUT proteins are composed of about 500 amino acid residues. Each GLUT is an integral protein, penetrating and

spanning the lipid bilayer of the plasma membrane. Twelve transmembrane α -helix segments are present in each of the transporters. Figure 3.9 shows a typical GLUT protein, which is oriented so that hydrophilic regions of the protein chain protrude into the extracellular and cytosolic media, while the hydrophobic regions traverse the membrane. When glucose or other substrate attaches to the protein's binding site, it causes a conformational change in the protein, allowing the substrate to translocate to the other side of the membrane. After the substrate is released, the conformational change is reversed and the GLUT protein can repeat the process.

The GLUT family is generally divided into three classes based on their sequence similarities (Table 3.2). All cells express at least one GLUT isoform on their plasma membrane and on intracellular membranes of organelles. Redundancy of GLUTs throughout the body helps to ensure the uptake and use of glucose as a critical fuel source under a variety of physiological conditions. GLUTs 1–5 are the most studied and have well-established roles in monosaccharide transport:

- GLUT1 was the first GLUT identified and is the most ubiquitously expressed glucose transporter. It allows glucose

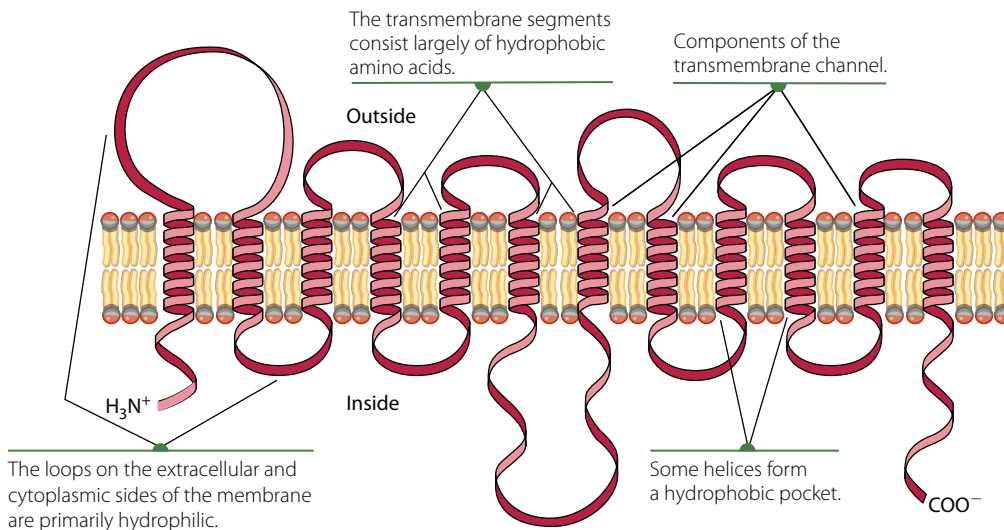


Figure 3.9 A model for the structural orientation of a glucose transporter.

to cross the blood–brain barrier and supplies glucose to the developing central nervous system during embryogenesis. GLUT1 is responsible for the supply of glucose to erythrocytes, endothelial cells of the brain, and most fetal tissue. Its importance is evident in *GLUT1 deficiency syndrome* in which patients experience seizures beginning in early infancy due to insufficient glucose supply to the brain. Treatment includes strict adherence to a ketogenic diet that raises levels of ketone bodies in the blood to be used as fuel for the brain and other tissues [8].

- GLUT2 is a low-affinity, high-capacity transporter with predominant expression in the β -cells of the pancreas, liver, small intestine, and kidney. GLUT2 is involved in the transport of most monosaccharides from enterocytes into the portal blood via the basolateral membrane. And when the concentration of glucose in the intestinal lumen is high, it can transport glucose and fructose into the enterocyte through the brush border. The rate of transport is highly dependent upon the blood glucose concentration. In the pancreas, GLUT2 appears to be the sensitive indicator of blood glucose levels and is involved in the release of insulin from the β -cells. High insulin levels cause GLUT2 to leave the plasma membrane of the enterocyte and return to storage vesicles.
- GLUT3 is a high-affinity glucose transporter with predominant expression in tissues such as the brain and neurons that are highly dependent on glucose as a fuel. It is also expressed in cells and tissues that have a high requirement for glucose such as spermatozoa, the placenta, and preimplantation embryos. Some data suggest that a possible dysregulation of GLUT3 might lead to glucose deficits in the brain and thus to dyslexia in children.
- GLUT4 is the primary means by which insulin regulates the cellular uptake of blood glucose in muscle and adipose tissue. Other cells and tissues such as the liver, kidneys, erythrocytes, and brain do not express GLUT4 and therefore are not dependent upon insulin for glucose

uptake. One of the actions of insulin is to cause the translocation of GLUT4 from intracellular storage vesicles to the plasma membrane (discussed in the next section).

- GLUT5 is highly specific for fructose and does not recognize glucose. It is expressed primarily in the small intestine and to a lesser degree in kidney, brain, skeletal muscle, and adipose tissue. Its main function is to transport dietary fructose across the brush border membrane of enterocytes.

Figure 3.10 illustrates the physiological role of representative SGLTs and GLUTs in the enterocyte and epithelial cell of the kidney proximal tubule. Both cell types are distinctive because of their brush border membranes. The increased surface area created by the brush border allows for maximum absorption of monosaccharides and other nutrients from the small intestine and reabsorption of glucose from the glomerular filtrate (discussed in Chapter 12). Both active transport (SGLTs) and facilitated diffusion

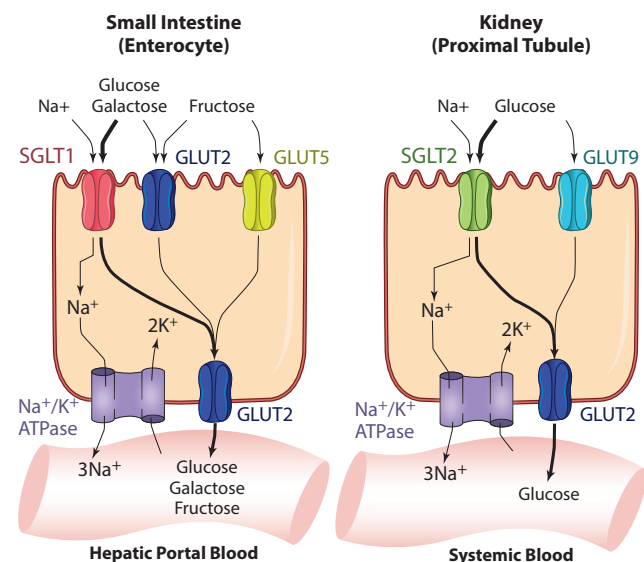


Figure 3.10 Membrane transport of monosaccharides. The SGLTs represent active transport. The GLUTs represent facilitated diffusion.

(GLUTs) are present in these cells to ensure maximum absorption. Transporters in the enterocyte encounter the greatest variety of monosaccharides derived from dietary sources. In contrast, the kidney filters and reabsorbs primarily glucose because glucose is practically the only monosaccharide in the systemic circulation. The fact that blood glucose concentration is precisely controlled under every metabolic condition emphasizes the importance of SGLTs and GLUTs in maintaining glucose homeostasis.

Intestinal Absorption of Glucose and Galactose

The villi and microvilli of the intestinal brush border present an enormous surface area for nutrient absorption to occur. The absorptive capacity of the human intestine has been estimated to be about 5,400 g/day for glucose and 4,800 g/day for fructose—a capacity that would never be reached in a normal diet. Digestion and absorption of carbohydrates are so efficient that, under normal conditions, nearly all monosaccharides are absorbed by the end of the jejunum.

After carbohydrate digestion, glucose and galactose are transported into the enterocyte by the same mechanisms involving both active transport (SGLTs) and facilitated diffusion (GLUTs). The relative contribution of active transport versus facilitated diffusion depends on the amount of carbohydrate consumed; facilitated diffusion participates to a greater extent following a large carbohydrate meal.

Active Transport

The active transport mechanism for glucose and galactose absorption into enterocytes requires cellular energy as ATP and the involvement of SGLT1 (Figure 3.10). The SGLT1 is positioned on the brush border membrane and simultaneously transports one molecule of glucose (or galactose) and two molecules of Na^+ in the same direction and is thus a *symporter*. The SGLT1 protein has two binding sites: one binds Na^+ and the other binds glucose. The glucose binding site is not available unless the transport protein has already bound Na^+ . The attachment of Na^+ to the carrier increases the transport protein's affinity for glucose. Sodium is moving down a concentration gradient because the intracellular concentration of Na^+ is normally quite low. After Na^+ and glucose are transported into the enterocyte, they are released from SGLT1. As the intracellular glucose concentration increases, it binds to GLUT2 in the basolateral membrane. GLUT2 is a low-affinity, high-capacity transporter that facilitates the exit of glucose and other monosaccharides from the enterocyte into the underlying capillaries for delivery into the hepatic portal vein.

Na^+ that has entered the cell is “pumped” back out by the energy-requiring Na^+/K^+ -ATPase (also called the *sodium-potassium pump*) located in the basolateral membrane. Na^+/K^+ -ATPase works by first combining with ATP in the presence of Na^+ on the inner surface of the

cell membrane. The enzyme then is phosphorylated by the breakdown of ATP to adenosine diphosphate (ADP) and consequently is able to move three Na^+ out of the enterocyte. On the outer surface of the cell membrane, the ATPase becomes dephosphorylated by hydrolysis in the presence of K^+ and then is able to return two K^+ into the cell. The term *pump* is used because the Na and K ions are both transported across the membrane against their concentration gradients.

Note that the activity of Na^+/K^+ -ATPase occurs in a different membrane location opposite of SGLT1, and that SGLT1 itself is not phosphorylated by ATP. Yet the overall process of glucose (and galactose) transport involving SGLT1 is referred to as active transport. This illustrates how active transport can occur in a variety of cells with many types of membrane transporters, as long as they cotransport Na^+ . In fact, the activity of the Na^+/K^+ -ATPase is responsible for most of the active transport in cells and is the major energy demand of the body at rest.

Facilitated Transport

Some glucose (and galactose) can be absorbed into the enterocyte independent of SGLT1 and without the input of energy. When glucose concentration in the intestinal lumen is high, such as after the ingestion of a large carbohydrate-containing meal, glucose is transported into the enterocyte by GLUT2 in the brush border membrane. When large amounts of glucose enter the enterocyte, intracellular GLUT2 is translocated to the brush border membrane by the movement of the cytoskeleton and the contraction of myosin. After high-carbohydrate meals, more glucose is transported into the enterocyte by facilitated transport than by active transport via SGLT1.

Rising levels of blood glucose following a meal triggers insulin secretion, causing GLUT2 to be translocated from the brush border membrane back to intracellular vesicles. While GLUT2 is not directly dependent on insulin for facilitated transport, this indirect effect of insulin results in reduced intestinal glucose absorption when blood glucose levels are high. In insulin-resistant individuals or those with type 2 diabetes, GLUT2 is resistant to the effect of insulin, and the GLUT2 remains in the brush border membrane. The result is that glucose continues to be absorbed at a higher rate [9]. The role of insulin in metabolic regulation is discussed in detail later in this chapter and in Chapters 7 and 8.

Intestinal Absorption of Fructose

Absorption of dietary fructose occurs by facilitated diffusion and is mediated primarily by GLUT5 (Figure 3.10). GLUT5 has a high affinity for fructose and is not influenced by the presence of glucose. Fructose is not absorbed by SGLT1 and therefore its absorption does not require energy. The rate of fructose absorption is much slower than that of both glucose and galactose but is increased when GLUT2

is present in the brush border membrane of the enterocyte, as discussed previously. When the intracellular concentration increases, fructose is transported out of the enterocyte into the hepatic portal vein by GLUT2 in the basolateral membrane, the same transporter that moves glucose and galactose out of the cell. The facilitated transport process can proceed only down a concentration gradient.

Because fructose is absorbed entirely by facilitated diffusion, its overall absorption rate is slower than glucose or galactose, but faster than sugar alcohols such as sorbitol and xylitol, which are absorbed purely by passive diffusion.

At typical dietary intakes, fructose is efficiently absorbed and there is no fructose in the systemic circulation due to removal by the liver, where it is phosphorylated and trapped in the hepatocytes. Many individuals (about 60%) cannot completely absorb fructose when consumed in large amounts, ranging from 20 to 50 g [10]. Those with limited absorption who ingest large amounts of fructose experience intestinal pain, gas, and diarrhea, symptomatic of malabsorption. This level of intake is readily achievable for individuals who consume 25–30 ounces of a carbonated beverage sweetened with either sugar or high-fructose corn syrup. The simultaneous ingestion of glucose can improve fructose absorption and prevent symptoms of malabsorption, possibly due to the increased presence of GLUT2 in the brush border membrane [11]. Examples include ingestion of sucrose and high-fructose corn syrup, which contain equivalent amounts of glucose and fructose. The Perspective at the end of this chapter discusses the trends in carbohydrate intake over the past several decades and the major food sources that deliver the glucose and fructose to the enterocyte for absorption.

Hepatic Metabolism of Dietary Monosaccharides

Following the intestinal absorption of glucose, galactose, and fructose, they enter the hepatic portal vein, where they are carried directly to the liver. Essentially all of the galactose and fructose is taken up by the liver through specific GLUTs and metabolized. In contrast, only 30–40% of glucose is taken up by the liver, with the majority passing through into the systemic circulation. This explains why glucose, but not galactose or fructose, is found in the peripheral blood and why the latter sugars are not directly subject to the strict hormonal regulation that is such an important part of glucose homeostasis. Galactose is largely converted to glucose derivatives and stored as liver glycogen through pathways described later in this chapter. The majority of fructose enters an alternative pathway and is catabolized for energy according to the liver's energy demand. If an occasional meal is high in fructose, and the liver's energy needs have been met, excess fructose is converted to triacylglycerol and transported out of the liver for distribution to muscle and adipose tissue. However, diets

chronically high in fructose can cause **hyperlipidemia** and triacylglycerol accumulation in the liver.

Glucose is nutritionally the most abundant monosaccharide because it is the exclusive constituent of starch and also occurs in each of three major disaccharides (Figure 3.1). The portion of dietary glucose taken up by the liver can be used for energy, stored as glycogen, or returned to the blood during nonfed periods by pathways described in Chapter 7. The portion of dietary glucose that passes into the systemic blood supply is thus available to all the organs of the body. Glucose enters the cells in these organs by facilitated transport (GLUTs). In the case of skeletal muscle and adipose tissue, the uptake of glucose is mediated by a unique glucose transporter, GLUT4, that is dependent on insulin, whereas the liver, kidneys, brain, erythrocytes (red blood cells), and other tissues are insulin independent.

3.5 MAINTENANCE OF BLOOD GLUCOSE CONCENTRATION

Maintaining normal blood glucose concentration is an important homeostatic function, requiring the coordinated effort of the small intestine, liver, kidneys, skeletal muscle, and adipose tissue. Regulation is the net effect of the organs' metabolic processes that remove glucose from or return glucose to the blood. These pathways, which are examined in detail in the section “Integrated Metabolism in Tissues,” are hormonally influenced, primarily by the antagonistic pancreatic hormones insulin and **glucagon** and to a lesser extent by the glucocorticoid hormones of the adrenal cortex.

The rise in blood glucose following the ingestion of carbohydrate, for example, triggers the release of insulin while reducing the secretion of glucagon. Insulin is the main hormone that lowers blood glucose levels and is the primary anabolic hormone. Insulin stimulates the cellular uptake of glucose, amino acids, and lipid, leading to their conversion to storage forms in muscle and adipose tissue. The storage form of glucose, glycogen, is synthesized through the process called **glycogenesis**.

Glucagon, the primary catabolic hormone having opposite effects on insulin, increases the breakdown of liver glycogen by a process called glycogenolysis. Additional mechanisms to increase blood glucose levels include an increase in the secretion of glucocorticoid hormones, primarily cortisol. Glucocorticoids cause increased activity of hepatic **gluconeogenesis**, a process of glucose synthesis described in detail in a later section of this chapter.

Role of Insulin

Insulin and GLUT4 play extremely important roles in the uptake of glucose in muscle and adipose tissue, especially following a carbohydrate-rich meal. The sequence of events involving insulin and GLUT4 are critical to normalizing

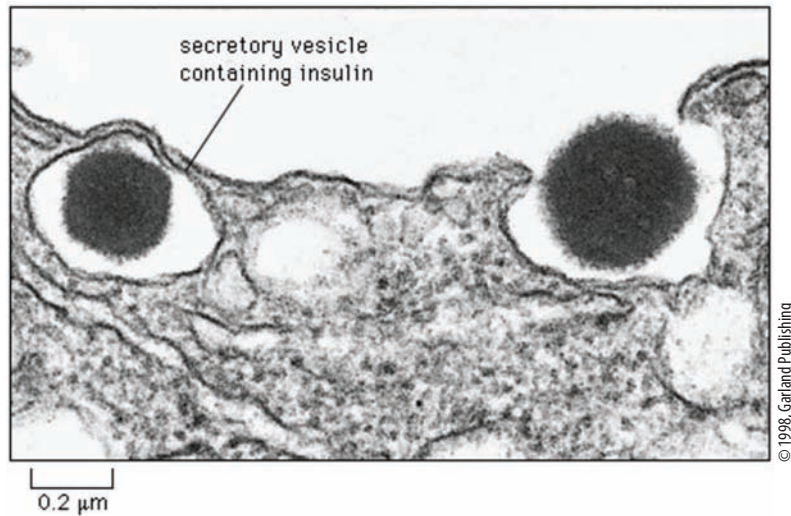


Figure 3.11 Secretion of insulin into the systemic circulation by β -cells of the pancreas. The image shows insulin clusters being delivered to the cell surface by secretory vesicles.

blood glucose and thus preventing hyperglycemia. When blood glucose levels raise after eating, insulin is released by the β -cells of the pancreas into the bloodstream by a process of exocytosis (Figure 3.11). The circulating insulin binds with specific insulin receptors on cell membranes of muscle and adipose tissue. Insulin binding causes GLUT4 to translocate to the cell surface, where it can remove glucose from the blood. Insulin binding also results in other important cellular responses, as depicted in Figure 3.12.

GLUT4 is an insulin-responsive transporter that is synthesized on the ribosomes of the rough endoplasmic reticulum and then transferred to the Golgi apparatus, where it is packaged into GLUT4 storage vesicles (GSVs). Binding of insulin to its receptor causes the GSV to translocate to the cell membrane. Key to the ability of insulin to bind to the receptor site on the cell membranes of skeletal muscle, cardiac muscle, or adipose tissue cells are the activation of phosphatidylinositol-3-kinase and the cascading

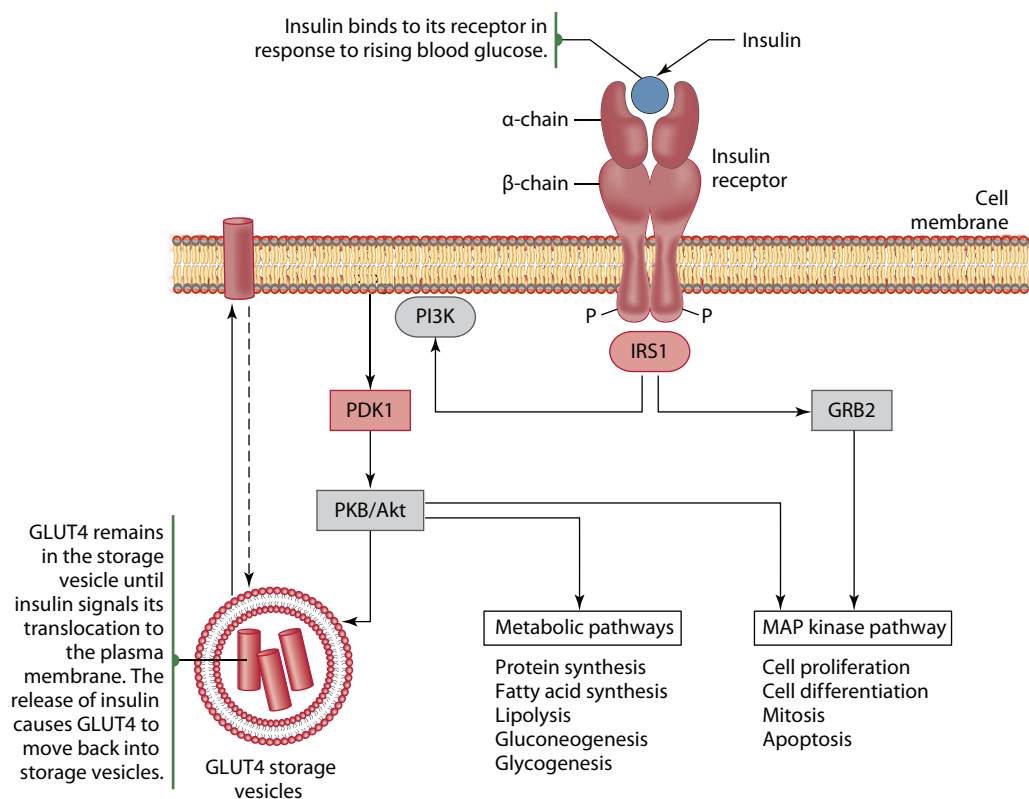


Figure 3.12 Insulin signaling pathways and the translocation of GLUT4. Abbreviations: GRB2, growth factor receptor binding protein-2; IRS1, insulin receptor substrate 1; PI3K, phosphatidylinositol-3-kinase; PIP₃, phosphatidylinositol-3,4,5-trisphosphate; PDK1, PIP₃-dependent kinase 1; PKB, protein kinase B (also called Akt).

Source: Adapted from Augustin R., *Life*, 2010; 62:315–33, Figure 3B.

reactions that follow. This activity is discussed more fully in Chapter 7. The net result of insulin's effects on the cell membrane is to cause translocation of GLUT4 to the cell membrane; this process can be described simply as follows:

- 1 The biosynthesis of GLUT4 and its storage in GSVs are stimulated.
- 2 The GSVs are transported to the cell membrane by elements of the cytoskeleton including the microtubules and actin.
- 3 An interaction between GSVs and the plasma membrane occurs, mediated by a tethering complex; this step is called *tethering*.
- 4 The GSVs dock with the plasma membrane in preparation for fusion.
- 5 The lipid bilayers of the GSVs and plasma membrane fuse.
- 6 Endocytosis—the GLUT4 becomes part of the plasma membrane and is available for transporting glucose into the cell.

In the presence of insulin, GLUT4 cycles continuously through the endosomal system. In insulin-resistant states or at low insulin levels, the GLUT4 stays in the GSVs and its presence in the cell membrane is reduced. Interestingly, exercise causes similar translocation of GLUT4 from the GSVs to the cell membrane, as well as increased GLUT4 expression [12].

Blood–Tissue Barriers

The primary function of *blood–tissue barriers* is to protect vital organs from harmful substances in the bloodstream. This is achieved by selective permeability of endothelial cells that line the inside of blood vessels. While some molecules can freely diffuse through the endothelial cell membrane, most molecules require specific transporters, thus ensuring a high level of protection to the recipient tissue. Some tissues possess an additional layer of epithelial cells as reinforcement to the blood–tissue barrier. The endothelial and epithelial cells also form tight junctions that prevent paracellular transport (the movement of molecules in the interstitial space between cells). Common examples are the blood–brain, blood–retina, blood–placenta, and blood–testes barriers.

The continuous supply of blood glucose is critical to normal cellular function, particularly in the brain and other organs with blood–tissue barriers. The endothelial and epithelial cells comprising blood–tissue barriers are replete with transport proteins, primarily GLUTs, to ensure the delivery of glucose. GLUT1 appears to be the primary isoform for cross-barrier glucose transport, though other GLUTs may be involved (see Table 3.2).

In tissues lacking a blood–tissue barrier, the endothelial cells of blood vessels are freely permeable to glucose. This property allows glucose to move between blood capillaries and the interstitial fluid without the need of a membrane transporter. The concentration of glucose in interstitial fluid is driven by a concentration gradient that reaches equilibrium with the blood plasma. One such example is the skin. In patients with diabetes mellitus, in whom blood glucose must be monitored, the skin interstitial glucose concentration provides an adequate surrogate for blood glucose concentration. This phenomenon is the basis for electronic devices that continuously measure interstitial glucose. The device has a tiny probe that penetrates the skin's surface and transmits the information to a monitor. The device can be worn on the skin for several days, minimizing the need for constant blood sampling by fingerstick.

Glycemic Response to Carbohydrates

Glycemic response refers to the change in blood glucose after eating a carbohydrate-containing food. Some foods cause blood glucose to increase slowly, whereas other foods cause a more rapid and prolonged increase. The glycemic response is an important parameter in controlling blood glucose homeostasis, insulin release, and obesity. Persistently elevated blood glucose and insulin levels are linked with obesity and the development of chronic diseases. The role of these factors in the development of insulin resistance and type 2 diabetes is covered in Chapters 7 and 8.

A widely accepted quantitative measure of glycemic response is the **glycemic index** (GI). It provides a numerical value for the glycemic effect of a particular food and was initially developed as a tool for people with diabetes in selecting foods. The GI is defined as the increase in blood glucose level above the baseline fasting level during a 2-hour period following the ingestion of a defined amount of carbohydrate, usually 50 g, compared with the same amount of carbohydrate in a reference food.

Some studies of GI have used glucose as the reference food, while others used white bread (Table 3.3). The reference food is assigned a score of 100. In practice, the GI is measured by determining the elevation of blood glucose for 2 hours following ingestion and plotting the values against time. The area-under-the-curve for the test food is divided by the area-under-the-curve for the reference food, then multiplied by 100 (Figure 3.13). If glucose is used as the reference food and assigned a GI of 100, white bread has a GI of about 71. When white bread is used as the reference, some foods will have a GI of greater than 100.

High GI foods are quickly digested and absorbed, causing a rapid rise in blood glucose levels. A rapid rise in insulin occurs in parallel, so glucose is quickly removed from the blood that leads to a rapid fall below the fasting level.

Table 3.3 Glycemic Index of Common Foods with White Bread and Glucose Used as the Reference Food

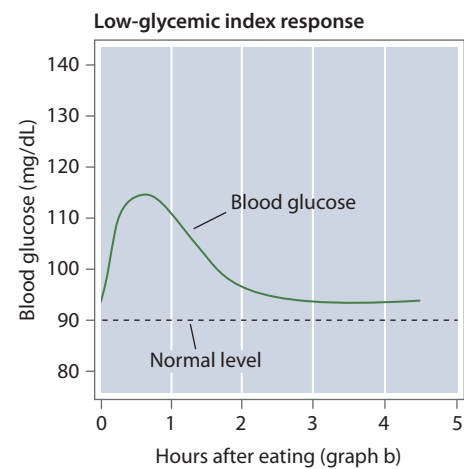
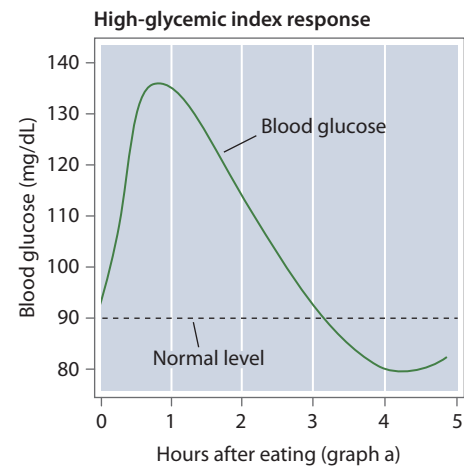
Food Tested	Glycemic Index	
	White Bread = 100	Glucose = 100
White bread ¹	100	71
Baked russet potato ¹	107.7	76.5
Instant mashed potatoes ¹	123.5	87.7
Boiled red potato (hot) ¹	125.9	89.4
Boiled red potato (cold) ¹	79.2	56.2
Bran muffin ²	85	60
Coca Cola ²	90	63
Apple juice, unsweetened ²	57	40
Tomato juice ²	54	38
Bagel ²	103	72
Whole-meal rye bread ²	89	62
Rye-kernel bread ² (pumpernickel)	58	41
Whole-wheat bread ²	74	52
All-Bran cereal ²	54	38
Cheerios ²	106	74
Corn Flakes ²	116	81
Raisin Bran ²	87	61
Sweet corn ²	86	60
Couscous ²	81	61
Rice ²	73	51
Brown rice ²	72	50
Ice cream ²	89	62
Soy milk ²	63	44
Raw apple ²	57	40
Banana ²	73	51
Orange ²	69	48
Raw pineapple ²	94	66
Baked beans ²	57	40
Dried beans ²	52	36
Kidney beans ²	33	23
Lentils ²	40	28
Spaghetti, durum wheat (boiled) ²	91	64
Spaghetti, whole meal (boiled) ²	32	46
Sucrose ²	83	58

¹Source for data: Fernandes G, Velangi A, Wolever TM. Glycemic index of potatoes commonly consumed in North America. *J Am Diet Assoc.* 2005; 105:557–62.

²Source for data: Foster-Powell K, Holt SH, Brand-Miller JC. International table of glycemic index and glycemic load values: 2002. *Am J Clin Nutr.* 2002; 76:5–56.

In contrast, low GI foods cause a slower rise in blood glucose and insulin, with a more gradual fall in blood glucose (Figure 3.13).

A related quantitative measure, the **glycemic load** (GL), considers portion size as a contributing factor in a food's glycemic response. The GL is calculated by dividing the GI by 100, then multiplying by the grams of carbohydrate.



Calculation of Glycemic Index

- 1 The elevation in blood glucose level above the baseline following consumption of a high-glycemic index food or 50 g of glucose in a reference food (glucose or white bread). The glycemic index of the reference food is by definition equal to 100 (graph a).
- 2 The elevation of blood glucose levels above the baseline following the intake of 50 g of glucose in a low-glycemic index food (graph b).
- 3 The glycemic index is calculated by dividing the area under the curve for the test food by the area under the curve for the reference food and multiplying the result by 100.

Figure 3.13 Blood glucose changes following carbohydrate intake (glycemic index).

Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

A food's GI and GL can be quite different; for example, the carbohydrate in carrots has a high GI score, but the GL for carrots is low because a half-cup serving of carrots contains only 6.13 g of carbohydrate. The higher the GL, the greater the expected elevation in blood glucose and the insulinogenic effect of the food.

Many published tables provide the GI for different foods. The most complete is an international table [13]. Remember that food products differ in different regions of the world. The GI values listed in Table 3.3 are intended to illustrate trends, not to prepare diets.

There are many potential criticisms of the use of GI and GL for food labeling purposes, foremost the wide variation of GI values for apparently similar foods and between laboratories. Factors that may cause this variation include the amount of carbohydrate in the meal, composition of the meal (particularly fiber, protein, and fat), previous meal composition, physical activity level of the subjects, choice of the reference food, rate and extent of digestion, and glucose tolerance of the subjects [14,15]. The variations observed could also reflect real differences among samples of the same food due to factors such as its food form, ripeness, location of growth, and variety. For example, the GI for a baked russet potato is 76.5 and for an instant mashed potato is 87.7 (using glucose as the reference food) [16]. Even the temperature of the food can make a difference: A boiled red potato eaten hot (with the starch gelatinized) has a GI of 89.4, but the same potato eaten cooler (with the starch back to a crystalline structure) has a GI of 56.2 (Table 3.3).

Despite its limitations, the GI/GL concept has been widely touted as a tool for identifying foods associated with chronic diseases and obesity. The literature suggests that the longer and higher the elevation of blood glucose and insulin, the greater the risk of developing chronic diseases and obesity. Some studies suggest a useful application of GI/GL for managing body weight, diabetes, cardiovascular disease, and certain cancers [17]. Conversely, other studies show no relationship between GI/GL and physiological measures of disease risk, suggesting that other food components such as fiber or whole grains are better predictors of health outcomes [18].

3.6 INTEGRATED METABOLISM IN TISSUES

The metabolic fate of the monosaccharides, especially glucose, depends to a great extent on the body's energy needs. This section covers the individual pathways of carbohydrate metabolism. The following section addresses the ways metabolism is regulated, including covalent modifications, allosteric mechanisms, substrate-level regulation, induction, post-translational modification, and translocation. Several terms used in carbohydrate metabolism sound and appear to be similar but are in fact quite different. The metabolic pathways of carbohydrate metabolism are listed below.

- **Glycogenesis:** The synthesis of glycogen
- **Glycogenolysis:** The breakdown of glycogen
- **Glycolysis:** The oxidation of glucose to pyruvate

- **Gluconeogenesis:** The synthesis of glucose from non-carbohydrate sources
- **Pentose phosphate pathway (hexose monophosphate shunt):** The production of five-carbon monosaccharides (pentoses) and nicotinamide adenine dinucleotide phosphate (NADPH)
- **Tricarboxylic acid (TCA) cycle:** The oxidation of acetyl-CoA to yield CO_2 and high-energy electrons.

An integrated overview of these pathways is given in Figure 3.14. The metabolism of glycogen is covered first to emphasize the body's ability to store energy from the diet (glucose) to be used at a later time. Then we cover the pathways (glycolysis and the TCA cycle) that transfer the glucose energy to ATP so the energy can be used in a multitude of reactions in the body. Finally, we cover gluconeogenesis, which emphasizes the body's need to make glucose when dietary sources are insufficient. The detailed pathways with the names of the molecules and their structures are shown in the later figures. These are followed with a discussion of the individual reactions and additional comments that are particularly significant from a nutritional standpoint. It is important to recognize that under physiological conditions, many of these molecules exist as conjugated bases and are named accordingly (e.g., *pyruvate* instead of *pyruvic acid*, *lactate* instead of *lactic acid*). Because of the central role of glucose in carbohydrate nutrition, its metabolic fate is featured here. The entry of fructose and galactose into the metabolic pathways is introduced later in the discussion.

Glycogenesis

The term *glycogenesis* refers to the pathway by which glucose is converted into its storage form glycogen—a process vital to ensuring a reserve of quick energy. The major sites of glycogen synthesis and storage are the liver and skeletal muscle, while a small amount of glycogen is found in the kidneys and heart, among other tissues.

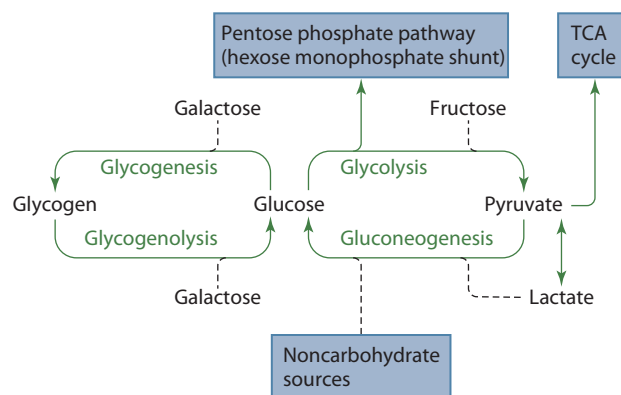


Figure 3.14 Overview of carbohydrate metabolic pathways.

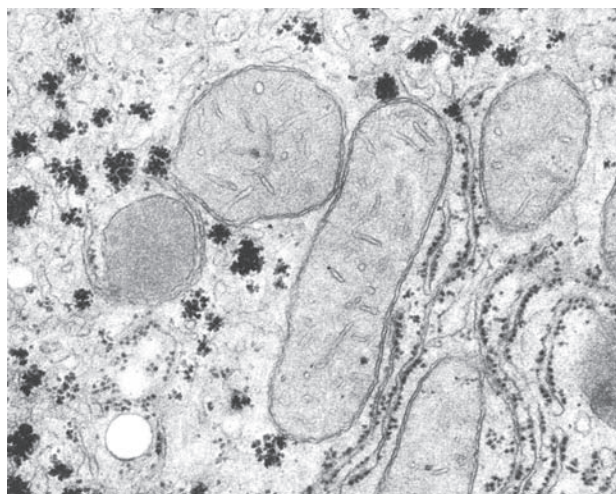


Figure 3.15 Glycogen storage in liver cells. Glycogen granules are the large dark clusters within the cytosol.

Glycogen accounts for as much as 7% of the weight of the liver, particularly following a high-carbohydrate meal. Liver glycogen can be broken down to glucose and reenter

the bloodstream. Therefore, it plays an important role in maintaining blood glucose homeostasis. The other major site of glycogen storage is skeletal muscle. In human skeletal muscle, glycogen generally accounts for a little less than 1% of the weight of the tissue. Although the concentration of glycogen in the liver is greater, muscle stores account for most of the body's glycogen because the muscle makes up a much greater portion of the body's weight. The liver can store approximately 100 g of glycogen, whereas muscle can store about 500 g (Figure 3.15). The glycogen stores in muscle are an energy source within that muscle fiber and cannot directly contribute to blood glucose levels. Muscle lacks the enzyme that converts the phosphorylated glucose back to free glucose.

The initial part of the glycogenic pathway is illustrated in Figure 3.16. Glucose is first phosphorylated upon entering the cell, producing glucose-6-phosphate. In muscle and other nonhepatic cells, the enzyme catalyzing this phosphate transfer from ATP is *hexokinase*, a mixture of hexokinase isozymes type 1 and 2. The properties of this enzyme are shown in Table 3.4. Muscle hexokinase

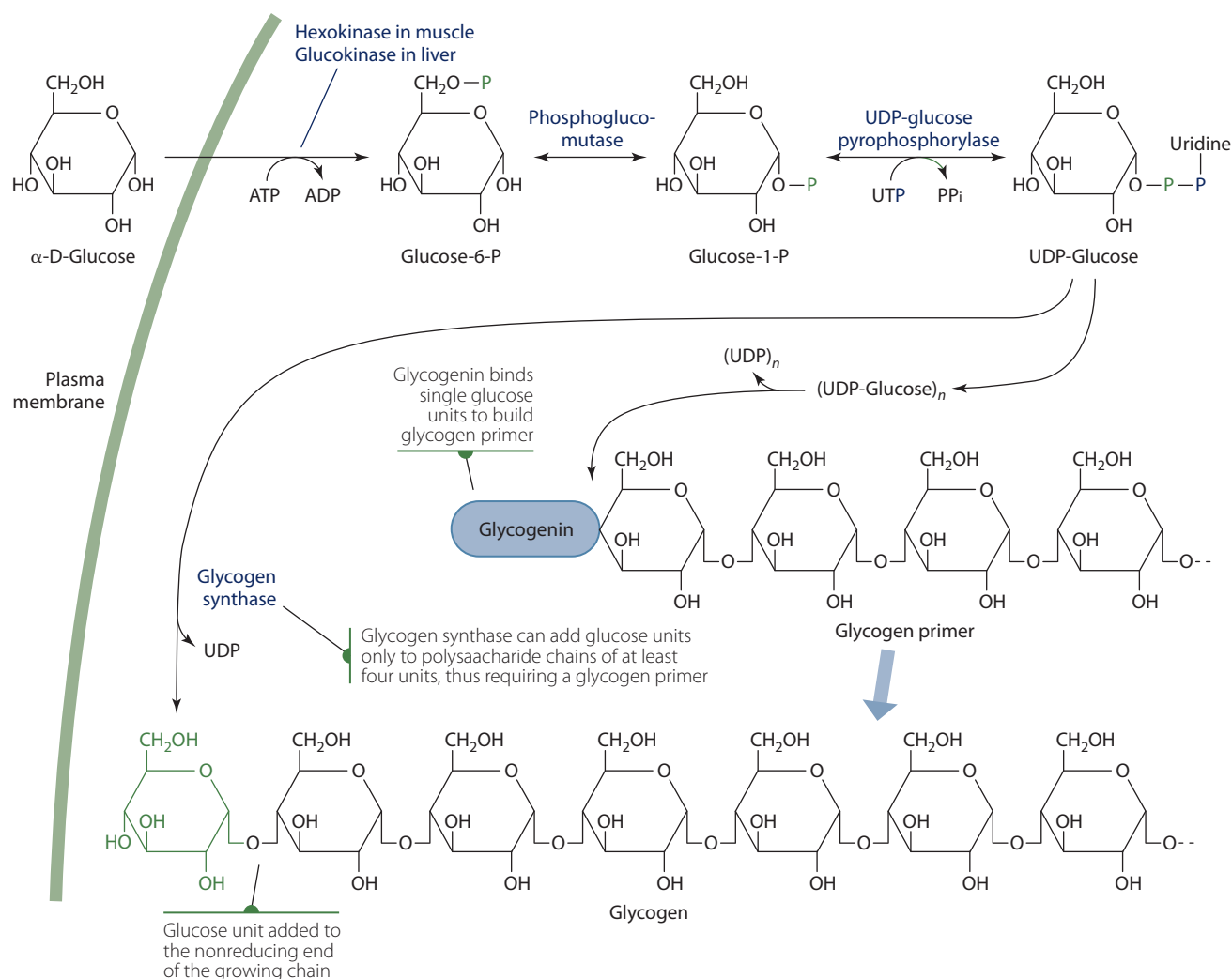


Figure 3.16 Glycogenesis. The synthesis of glycogen is initiated by the protein glycogenin, which assembles single glucose units (as UDP-glucose) into a short chain called a "glycogen primer." Glycogen synthase continues adding glucose units.

Table 3.4 Properties of Hexokinase and Glucokinase

Hexokinase (Types 1 and 2)	Glucokinase (Hexokinase Type 4)
Located in muscle, brain, and adipose tissue	Located in liver and pancreas
Allosterically inhibited by glucose-6-phosphate (its product)	Not inhibited by glucose-6-phosphate
Low K_m ; function at maximum velocity at fasting blood glucose concentrations	High K_m ; functions at maximum velocity only when glucose levels are high (such as following a high-carbohydrate meal)
Not induced by insulin in normal individuals	Induced by insulin in normal individuals
Not induced by insulin in insulin-resistant individuals	Not induced by insulin in insulin-resistant individuals

is an allosteric enzyme that is negatively modulated by the product of the reaction, glucose-6-phosphate. This means that when the muscle cell has adequate glucose-6-phosphate, the entry of additional glucose into the cell is slowed. Muscle hexokinase has a low K_m , which means it can function at maximum velocity when blood glucose levels are at normal (fasting) levels.

Glucose **phosphorylation** in the liver is catalyzed primarily by a hexokinase isozyme called *glucokinase* (sometimes called hexokinase 4). Although the reaction product, glucose-6-phosphate, is the same as in other tissues, interesting differences distinguish glucokinase from hexokinase (Table 3.4). For example, muscle hexokinase is allosterically inhibited by glucose-6-phosphate, whereas liver glucokinase is not. This characteristic allows excess glucose entering the liver cell to be phosphorylated quickly and encourages glucose entry when blood glucose levels are elevated. Also, glucokinase has a much higher K_m than hexokinase, meaning that it can convert glucose to its phosphorylated form at a higher velocity should the blood concentration of glucose rise significantly, particularly after a carbohydrate-rich meal.

Phosphorylation of glucose effectively decreases the free glucose concentration in the cell, which enhances more blood glucose into the liver cell due to the concentration gradient that is created. The main glucose transporter in the liver, GLUT2, has a high capacity and is not dependent on insulin. Therefore, the liver has the capacity to reduce blood glucose concentration as long as the cellular free glucose concentration remains lower than the blood.

Unlike GLUT2, glucokinase is inducible by insulin. Glucokinase activity is below normal in people with type 1 diabetes mellitus because they have very low insulin levels, and the glucokinase is therefore not induced. In type 2 diabetes, the glucokinase becomes resistant to the effects of insulin. In either case, the low glucokinase activity contributes to the liver cell's inability to rapidly take up and metabolize glucose.

After glucose-6-phosphate is produced, the next step in glycogenesis is to move the phosphate group from C-6 of the glucose molecule to C-1 in a reaction catalyzed by the enzyme *phosphoglucomutase* (Figure 3.16). Nucleoside triphosphates other than ATP sometimes function as activating substances in intermediary metabolism.

In the next reaction of glycogenesis, energy derived from the hydrolysis of the α - β -phosphate anhydride bond of uridine triphosphate (UTP to UMP) allows the resulting uridine monophosphate to be coupled to the glucose-1-phosphate to form uridine diphosphate-glucose (UDP-glucose). The reaction is catalyzed by *UDP-glucose pyrophosphorylase*. Attachment of glucose, as UDP-glucose, to the growing glycogen molecule is catalyzed by *glycogen synthase*.

Glycogen synthase can add UDP-glucose only to polysaccharide chains containing at least four glucose units. This requires some short glycogen “primer” molecules of only a few glucose units. The initial glycogen primer is formed by a protein called *glycogenin* that binds a single UDP-glucose unit to a tyrosine residue in its binding site. Glycogenin then stimulates autoglucosylation of the growing chain using more UDP-glucose as the glucose donor [19]. Glycogen synthase takes over once the glucose chain reaches a sufficient length. Glycogenin in muscle remains in the core of the glycogen molecule, but in the liver more glycogen molecules than glycogenin molecules are present, suggesting the glycogen must separate from the protein.

Glycogen synthase exists in an active (dephosphorylated) form and a less active (phosphorylated) form. Insulin facilitates glycogen synthesis by stimulating the dephosphorylation of glycogen synthase. The glycogen synthase reaction is the primary target of insulin's stimulatory effect on glycogenesis.

When six or seven glucose molecules are added to the glycogen chain, the *branching enzyme* transfers them to a hydroxyl group at C-6 (Figure 3.17). Glycogen synthase cannot form the $\alpha(1-6)$ bonds of the branch points. This action is left to the branching enzyme, also called amylo(1-4 $\rightarrow$ 1-6)-transglycosylase, which transfers a seven-residue oligosaccharide segment from the end of the main glycogen chain to a C-6 hydroxyl group. Branching within the glycogen molecule is important because it increases the molecule's solubility and compactness. Branching also makes available many nonreducing ends of chains from which glucose residues can be cleaved rapidly and used for energy, in the process known as glycogenolysis and described in the following section. The overall pathway of glycogenesis, like most synthetic

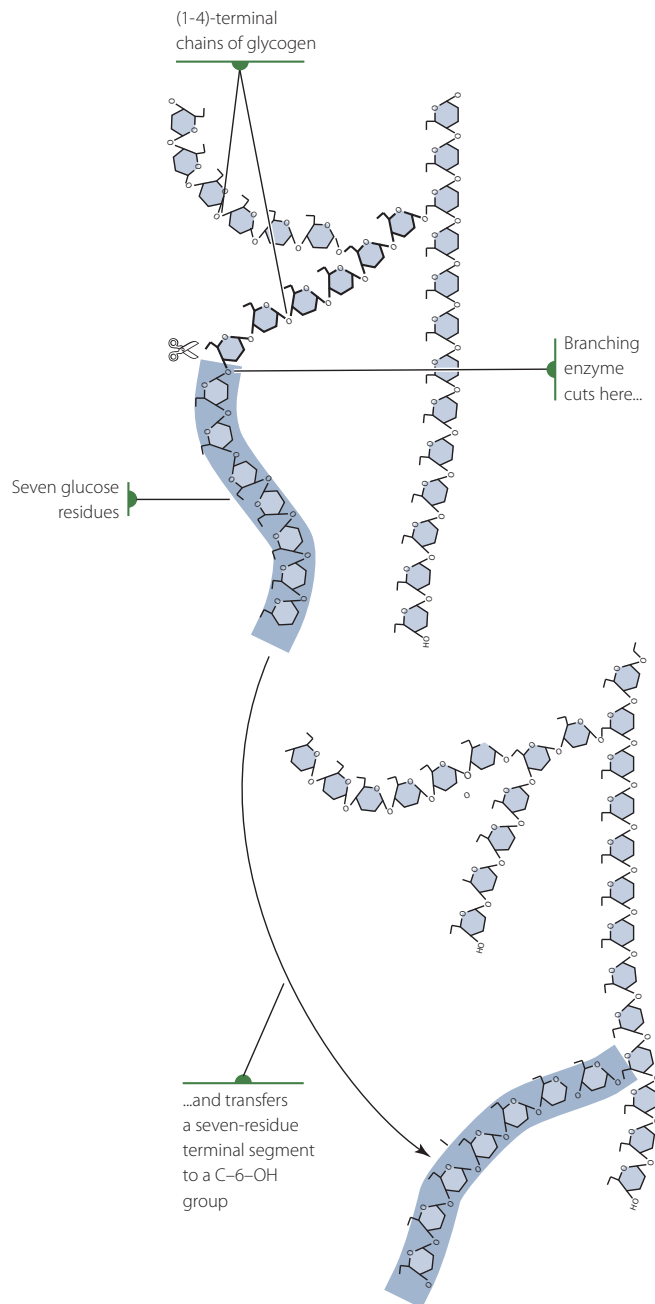


Figure 3.17 Formation of glycogen branches by the branching enzyme.

pathways, consumes energy because an ATP and a UTP are consumed for each molecule of glucose introduced.

Dietary carbohydrate is not the only source of glucose used in glycogen synthesis. Newly synthesized glucose via gluconeogenesis provides another source of glucose-6-phosphate that can be used for glycogen synthesis in the liver, even when there is an abundance of glucose following a carbohydrate-rich meal. As discussed in detail later in this chapter, gluconeogenesis produces glucose-6-phosphate from noncarbohydrate sources including lactate, a by-product of glycolysis in red blood cells and muscle. While it may seem paradoxical for both

gluconeogenesis and glycogenesis to function simultaneously, gluconeogenesis provides about one-third of the glucose-6-phosphate used for glycogen synthesis in the liver.

Glycogenolysis

The potential energy of glycogen is contained within the glucose residues that make up its structure. In accordance with the body's energy demands, the residues can be systematically cleaved one at a time from the nonreducing ends of the glycogen branches and routed through energy-releasing pathways. The breakdown of glycogen into individual glucose units, in the form of glucose-1-phosphate, is called *glycogenolysis* and is catalyzed by the enzyme *glycogen phosphorylase*. The steps involved in glycogenolysis are shown in Figure 3.18.

Although glycogen phosphorylase cleaves $\alpha(1-4)$ glycosidic bonds, it cannot hydrolyze $\alpha(1-6)$ bonds. Phosphorylase acts repetitively along linear portions of the glycogen molecule until it reaches a point four glucose residues away from an $\alpha(1-6)$ branch point. Here the degradation process stops, resuming only after another enzyme, called the *debranching enzyme*, cleaves the $\alpha(1-6)$ bond at the branch point.

At times of heightened glycogenolytic activity, the formation of increased amounts of glucose-1-phosphate shifts the phosphoglucomutase reaction toward production of the 6-phosphate isomer. In the liver (and, to some extent, the kidneys), glucose-6-phosphate can become free glucose or enter into the oxidative pathway for glucose (glycolysis). The conversion of glucose-6-phosphate to free glucose requires the action of *glucose-6-phosphatase*. This enzyme is not expressed in muscle cells or adipocytes. Therefore, free glucose can be formed only from liver or kidney glycogen and transported through the bloodstream to other tissues for oxidation.

Like its counterpart glycogenesis, glycogenolysis is highly regulated. Its catalyzing enzyme, glycogen phosphorylase, is regulated by both covalent and allosteric mechanisms. The regulation is different for the phosphorylation isozymes in muscle than in liver. The muscle and liver isozymes fulfill different physiological purposes: In muscle, the glucose is released from glycogen to provide glucose for energy within the cell, whereas in the liver the glucose is released to provide blood glucose. As phosphorylase is activated for glycogen phosphorylation, glycogen synthase is inhibited.

Glycogenolysis Regulation

Covalent Regulation Covalent regulation of phosphorylase is enhanced by glucagon and the catecholamines epinephrine and norepinephrine. These hormones cause a covalent modification of phosphorylase by converting it to an active form through the second messenger cAMP, which regulates the phosphorylation site of the enzymes

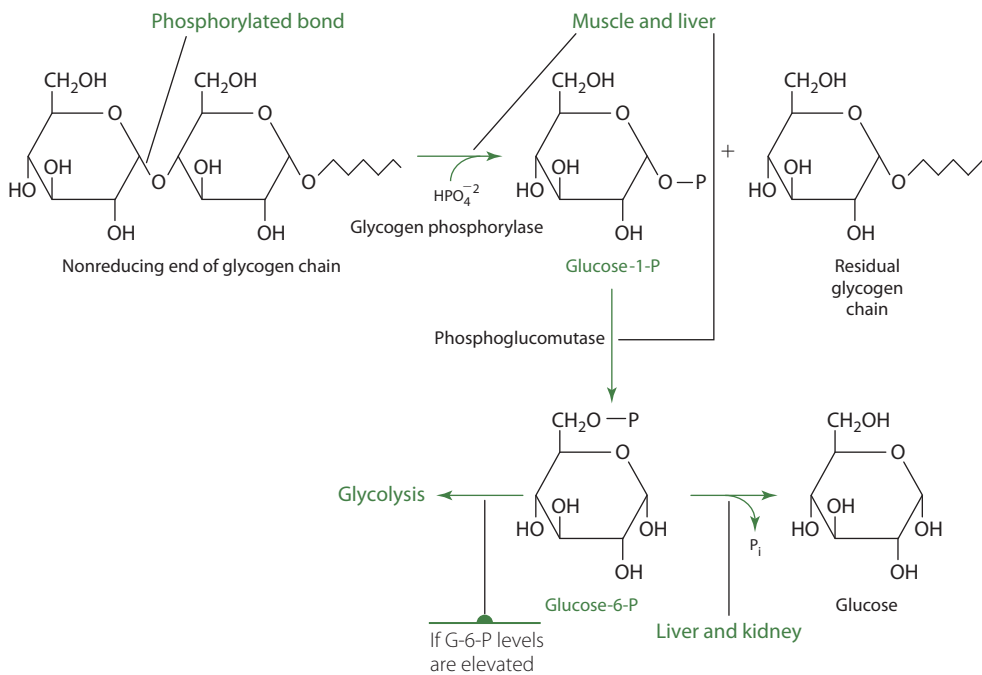


Figure 3.18 Glycogenolysis. Glucose residues are sequentially removed from the nonreducing ends of glycogen branches.

involved, as discussed in Chapter 1. These hormones bind to a receptor on the cell membrane that causes adenylyl cyclase to be activated to produce cAMP. The cAMP causes inactive phosphorylase kinase to become active by phosphorylating it. The active phosphorylase kinase plus ATP converts inactive (nonphosphorylated) phosphorylase *b* to active (phosphorylated) phosphorylase *a*. The phosphorylated phosphorylase *a* is less sensitive to the allosteric activation discussed later in this chapter. Phosphorylase *a* can be converted back to the inactive form, phosphorylase *b*, by phosphoprotein phosphatase 1. A Nobel Prize was awarded for elucidating this pathway (Figure 3.19).

Allosteric Activation The allosteric activation of phosphorylase *b* is carried out by AMP to convert it to the active phosphorylase *a*. When energy levels are low, cellular

ATP has been hydrolyzed to AMP, more energy is needed, and the phosphorylase *a* releases glucose-1-phosphate. The AMP binds to an allosteric site on phosphorylase *b*, which increases the binding of the glycogen. This allosteric site can also bind ATP, which is an allosteric inhibitor of the enzyme. Glucose-6-phosphate and caffeine are also allosteric inhibitors of the enzyme.

Muscle Phosphorylase The muscle and liver phosphorylase are isozymes. The muscle enzyme releases glucose-1-phosphate, which can be converted to glucose-6-phosphate that enters into the glycolysis pathway to provide energy for the cell. Muscle phosphorylase is more sensitive to intracellular ligands such as AMP for activation. The muscle enzyme is inhibited by metabolites, ATP, glucose-6-phosphate, and glucose. During times of stress, the hormones epinephrine and norepinephrine stimulate cAMP

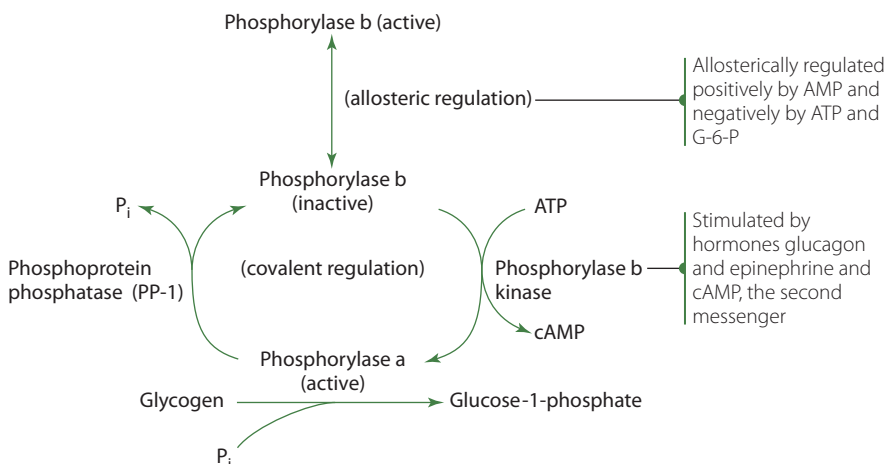


Figure 3.19 Regulation of glycogen phosphorylase. The enzyme is positively regulated covalently by cAMP and positively regulated allosterically by AMP. It is negatively regulated by ATP and glucose-6-phosphate, which cause shifts in the equilibrium between the inactive and active forms.

synthesis and, along with phosphoprotein phosphatase 1, covalently modify phosphorylase to the active form. Nervous stimulation and Ca^{2+} ions have the same effect.

Liver Phosphorylase Liver phosphorylase is less sensitive to intracellular ligands. It shows a weak increase in activity in the presence of AMP (10–20%) and is insensitive to inhibition by ATP or glucose-6-phosphate. Liver phosphorylase is regulated by hormones, including glucagon.

Glycolysis

Glycolysis is the pathway by which glucose is catabolized to pyruvate in the initial steps of harvesting energy from the glucose molecule. The energy is captured by transferring it to ATP. The reactions in glycolysis convert one molecule of glucose into two molecules of pyruvate, with a net yield of two molecules of ATP. The conversion of glucose to pyruvate is not very efficient; in other words, there is still a lot of energy remaining in the pyruvate molecule. Glycolysis is anaerobic and therefore does not require oxygen to produce pyruvate, so glycolysis will function under either aerobic or anaerobic conditions. From pyruvate, the metabolic course depends largely on the availability of oxygen and reducing units within the cell. When oxygen is lacking, pyruvate is converted to lactate. When sufficient oxygen is present, glucose is catabolized more efficiently in mitochondria to carbon dioxide and water.

Under anaerobic conditions, or in a situation without sufficient reducing equivalents due to the lack of oxygen or high cellular metabolism, pyruvate is converted to lactate. Under otherwise normal conditions, the conversion to lactate occurs mainly in times of strenuous exercise when the demand for oxygen by the working muscles exceeds that which is available. Lactate produced under anaerobic conditions can also diffuse from the muscle to the bloodstream and be carried to the liver for conversion back to glucose. Under these anaerobic conditions, glycolysis releases a small amount of usable energy that can help sustain the muscles even in a state of oxygen insufficiency. Providing this energy is the major function of the anaerobic pathway of glucose to lactate. Anaerobic glycolysis is the sole source of energy for erythrocytes because these cells do not contain mitochondria, the site of aerobic metabolism. The brain and gastrointestinal tract also produce much of their ATP energy from anaerobic glycolysis.

Under aerobic conditions, pyruvate can be transported into the mitochondria and participate in the TCA cycle, in which it becomes completely oxidized to CO_2 and H_2O . Complete oxidation is accompanied by the release of relatively large amounts of energy, most of which is captured in ATP molecules by the mechanism of oxidative phosphorylation. The enzymes in glycolysis function within the cytosol of the cell, but the enzymes catalyzing the TCA

cycle reactions are located within the mitochondrion. Therefore, pyruvate must enter the mitochondrion for complete oxidation.

The complete aerobic catabolism of glucose demands an ample supply of oxygen, a condition that generally is met in normal, resting mammalian cells. Under such conditions, only a small amount of lactate is formed. The primary importance of glycolysis in energy metabolism, therefore, is in providing the initial sequence of reactions (glucose $\rightarrow$ pyruvate) necessary for the complete oxidation of glucose by the TCA cycle, which supplies relatively large quantities of ATP.

Nearly every cell conducts glycolysis to meet the constant need for cellular energy. Following a meal, most of the energy derived from dietary carbohydrates is stored or utilized by the liver, muscle, and adipose tissue, which together constitute a major portion of total body mass. The brain is an extravagant consumer of carbohydrate energy, but lacks the ability to store it. In cells that lack mitochondria, such as erythrocytes, the pathway of glycolysis is the sole provider of ATP by the mechanism of substrate-level phosphorylation of ADP, discussed later in this chapter.

The pathway of glycolysis is summarized in Figure 3.20. The figure illustrates how the common dietary monosaccharides (glucose, galactose, and fructose) enter the glycolytic pathway, as is typical of the liver following a meal. Other cells of the body will normally encounter only glucose because the liver removes essentially all dietary galactose and fructose. Following are comments on the numbered reactions in Figure 3.20; reactions numbered 1–10 represent glycolysis.

- 1 After glucose enters the cell, it is phosphorylated at C-6 by either hexokinase or glucokinase, depending on the tissue. Phosphorylating glucose serves to “prime” the glycolytic pathway by trapping glucose in the cell and energizing the molecule for subsequent reactions. The properties of these enzymes were covered in Table 3.4. Glucokinase is present in the liver and the β -cells of the pancreas. Hexokinase is located in muscle, adipose tissue, brain, and virtually all other tissues. As discussed earlier, the hexokinase in muscle has a low K_m , which means it can function at maximum velocity at normal blood glucose levels. Hexokinase is inhibited by the accumulation of its product, glucose-6-phosphate. Liver glucokinase, in contrast, has a high K_m , which means it requires a high concentration of glucose in blood to function at maximum velocity. Liver glucokinase functions uninhibited and will remove large quantities of glucose from blood when blood glucose is elevated. Liver glucokinase is induced by insulin. The hexokinase/glucokinase reaction consumes 1 mol ATP/mol glucose.
- 2 Phosphoglucose isomerase (also called glucose phosphate isomerase) catalyzes movement of the carbonyl group of glucose from C-1 to C-2, thus converting the

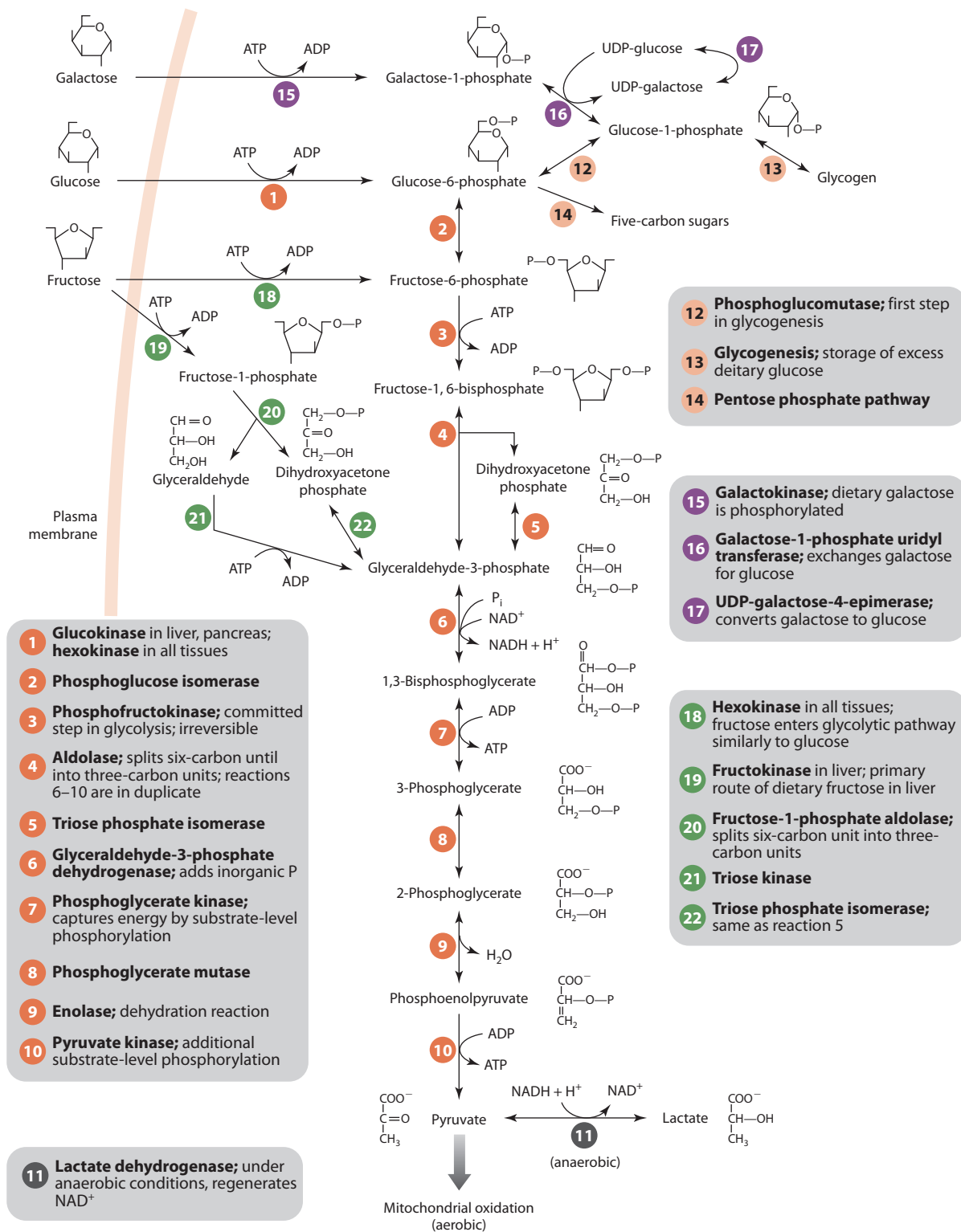


Figure 3.20 Glycolysis and related pathways. Reactions 1–10 represent glycolysis; reaction 11 represents anaerobic metabolism; reactions 12–14 represent alternative glucose pathways; reactions 15–17 represent galactose entry into glycolysis; reactions 18–22 represent fructose entry into glycolysis.

aldose into a ketose (fructose). This is an interconversion of isomers—glucose-6-phosphate to fructose-6-phosphate—and is reversible.

- 3 Phosphofructokinase catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphate using an ATP. The term *bis* means that the two phosphates are on different carbons. The phosphofructokinase reaction is an important regulatory step. This irreversible reaction commits the cell to metabolize glucose rather than converting it to another sugar or storing it as glycogen. Phosphofructokinase is an allosteric enzyme that is negatively modulated by ATP and citrate (a product of the TCA cycle and an indication that energy needs are met). The inhibition by ATP is reversed by AMP, an indication that the cell needs more energy. There is a relationship among the levels of ATP, ADP, and AMP. They are interconverted by the reaction:



This reaction is catalyzed by adenylate kinase. When the reaction reaches equilibrium, the quantity of ADP is about 10% of that of ATP, and AMP levels are less than 1% of those of ATP. Small changes in ATP are amplified in changes in AMP. In this way, the regulation of phosphofructokinase reaction is modulated by the relative amounts of ATP and AMP.

Phosphofructokinase is also regulated by fructose-2,6-bisphosphate, which is a potent allosteric activator that increases the affinity of the enzyme for its substrate, fructose-6-phosphate. Levels of fructose-2,6-bisphosphate are controlled by the enzyme phosphofructokinase-2. This enzyme is induced by the hormone glucagon and is different from the phosphofructokinase in the glycolytic pathway. Other activities of this enzyme are discussed in the “Gluconeogenesis” section of this chapter.

- 4 Fructose bisphosphate aldolase (or simply aldolase) cleaves fructose-1,6-bisphosphate, a hexose, into two trioses, glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. The remaining steps in glycolysis involve three-carbon units rather than six-carbon units.
- 5 Glyceraldehyde-3-phosphate is on the direct pathway of glycolysis, but dihydroxyacetone phosphate is not. Dihydroxyacetone phosphate must be converted to glyceraldehyde-3-phosphate by the enzyme triose phosphate isomerase. The reaction is reversible, but driven in the direction of glyceraldehyde-3-phosphate because the product is continuously “removed” by the next reaction in glycolysis. Note that the combination of reactions 4 and 5 produce two molecules of glyceraldehyde-3-phosphate for every glucose molecule entering the glycolytic pathway. Consequently, each of the following reactions occurs in duplicate.

- 6 In this reaction, glyceraldehyde-3-phosphate is oxidized to a carboxylic acid, 1,3-bisphosphoglycerate, while inorganic phosphate is incorporated as a carboxylic phosphoric anhydride bond (a high-energy compound). The enzyme is glyceraldehyde-3-phosphate dehydrogenase, which uses NAD^+ as its hydrogen-accepting cosubstrate. Under aerobic conditions, the NADH formed is reoxidized to NAD^+ by O_2 through the electron transport chain in the mitochondria, as explained in the next section. The reason why O_2 is not necessary to sustain the reaction of converting glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate is that under anaerobic conditions, the NAD^+ consumed is restored by a subsequent reaction converting pyruvate to lactate (see reaction 11).
- 7 Up to this point, glycolysis has required the input of energy supplied by ATP. This reaction is the first to capture energy by generating ATP. The reaction is catalyzed by phosphoglycerate kinase and exemplifies substrate-level phosphorylation of ADP. A more detailed review of substrate-level phosphorylation, by which ATP is formed from ADP by the transfer of a phosphate from a high-energy donor molecule, is covered in the “Substrate-Level Phosphorylation” section. Remember that the reaction is in duplicate, so two moles of ATP are synthesized from one mole of glucose. This reaction replaces the two ATPs used to prime glycolysis. Under conditions of high ATP and low ADP, the reaction can be reversed.
- 8 Phosphoglycerate mutase catalyzes the transfer of the phosphate group of 3-phosphoglycerate from the number 3 carbon to the number 2 carbon.
- 9 Dehydration of 2-phosphoglycerate by the enzyme enolase introduces a double bond that imparts high energy to the phosphate bond of the product, phosphoenolpyruvate.
- 10 Phosphoenolpyruvate donates its phosphate group to ADP in a reaction catalyzed by pyruvate kinase to yield pyruvate. This is the second site of substrate-level phosphorylation of ADP in the glycolytic pathway. Because the reaction occurs in duplicate, it makes two ATPs. In summary, two ATPs were produced in reaction 7 and two were produced in this reaction. Two ATPs were used to prime glycolysis, for a net gain of two ATPs to this point. Pyruvate kinase is a highly regulated enzyme. It is activated allosterically by AMP and fructose-1,6-bisphosphate and inhibited by ATP, acetyl-CoA, and alanine. In the liver, pyruvate kinase is regulated covalently by glucagon through the cAMP mechanism discussed earlier, which transfers a phosphoryl group from ATP. The phosphorylated enzyme is more sensitive to inhibition by ATP.

- 11 Under anaerobic conditions, the lactate dehydrogenase reaction reduces pyruvate to lactate while oxidizing NADH to NAD⁺. The NAD⁺ formed in the reaction can replace the NAD⁺ consumed earlier in reaction 6. Lactate dehydrogenase is most active in situations of oxygen insufficiency, as occurs in prolonged muscular activity. Under aerobic conditions, pyruvate enters the mitochondrion for complete oxidation via the TCA cycle. A third important option available to pyruvate is its conversion to the amino acid alanine by amino transferase, a reaction by which pyruvate acquires an amino group from the amino acid glutamate (Chapter 6). The alternate pathways for pyruvate, together with the fact that pyruvate is also the product of the catabolism of various amino acids, makes pyruvate an important link between protein (amino acid) and carbohydrate metabolism.
- 12–13 When glucose is abundant following a meal, the liver and skeletal muscle can store large amounts of glucose as glycogen. Other tissues, including the kidneys, can also convert glucose to glycogen, but to a lesser extent. Accumulation of glucose-6-phosphate drives the reaction toward production of glucose-1-phosphate, catalyzed by phosphoglucomutase. Glucose-1-phosphate then enters the glycogenesis pathway. Conversely, when ATP is in high demand, reactions 12 and 13 are reversed and glycogen is broken down (glycogenolysis). In skeletal muscle, the liberated glucose-1-phosphate enters glycolysis for energy utilization. In the liver, glucose-1-phosphate can enter glycolysis or it can be converted to free glucose for release into the system circulation.
- 14 Some glucose-6-phosphate is diverted into another pathway called the pentose phosphate pathway (also called the hexose monophosphate shunt), which is discussed later in this chapter. This pathway generates important metabolic intermediates not produced in other pathways.
- 15 Dietary galactose, like glucose and fructose, is immediately phosphorylated upon entering the cell. The reaction occurs primarily in the liver when galactose is first absorbed from the gastrointestinal tract. The reaction is catalyzed by galactokinase and produces galactose-1-phosphate.
- 16–17 These two reactions are collectively called the *galactose-glucose interconversion pathway* in which galactose-1-phosphate is converted to glucose-1-phosphate. The fate of glucose-1-phosphate from galactose depends on the energy status of the cell. Following a meal when dietary galactose is accompanied by comparatively large amounts of glucose, the glucose-1-phosphate from galactose is driven mostly toward glycogenesis as the flow of glucose-1-phosphate from glucose pushes the reaction toward glycogenesis.
- 18 This reaction is catalyzed by hexokinase, the same enzyme present in all tissues that phosphorylates glucose. In contrast to glucose, which circulates throughout the body, fructose is metabolized primarily by the liver when first absorbed from the gastrointestinal tract. Because the liver also has fructokinase (see reaction 19), phosphorylation of fructose by liver hexokinase is a relatively unimportant reaction. The hexokinase reaction is slow and occurs only in the presence of high levels of blood fructose, a situation that is rarely encountered in humans.
- 19 Fructokinase is abundant in the liver and catalyzes the conversion of dietary fructose to fructose-1-phosphate. Following a carbohydrate-rich meal, the majority of fructose is committed to glycolysis rather than being converted to glucose and stored as glycogen. Notice that fructose-1-phosphate enters glycolysis at the point of glyceraldehyde-3-phosphate. Also note that reaction 3 is irreversible and stimulated by insulin following a meal. Consequently, fructose in the liver follows a one-way trip to becoming pyruvate (and possibly lactate).
- 20 The six-carbon fructose-1-phosphate molecule is split into three-carbon molecules by fructose-1-phosphate aldolase. The products are glyceraldehyde and dihydroxyacetone phosphate.
- 21 Triose kinase phosphorylates glyceraldehyde at C-3 using an ATP. The product can now enter glycolysis as glyceraldehyde-3-phosphate.
- 22 Dihydroxyacetone phosphate is isomerized to glyceraldehyde-3-phosphate by triose phosphate isomerase, the same enzyme that catalyzes reaction 5.

The Tricarboxylic Acid Cycle

The tricarboxylic acid (TCA) cycle, also called the **Krebs cycle** or the citric acid cycle, is central to energy metabolism in the body. Under aerobic conditions, the TCA cycle is the final pathway by which fuel molecules—carbohydrates, fatty acids, and amino acids—are completely oxidized to CO₂ so that energy is released and transferred to ATP molecules. Greater than 90% of food energy captured and used in the human body involves the TCA cycle in conjunction with oxidative phosphorylation (see Figure 1.6).

The enzymes of the TCA cycle are located in the mitochondrial matrix and work in concert with “energy carriers” that shuttle high-energy electrons released by the TCA cycle to the electron transport chain located in the inner mitochondrial membrane. These so-called energy carriers, NADH and FADH₂, are formed by reduction (accept electrons) when fuel molecules are oxidized (lose electrons). In this way, the main function of the TCA cycle is to release high-energy electrons that power the synthesis of ATP via oxidative phosphorylation. The TCA cycle is shown in Figure 3.21.

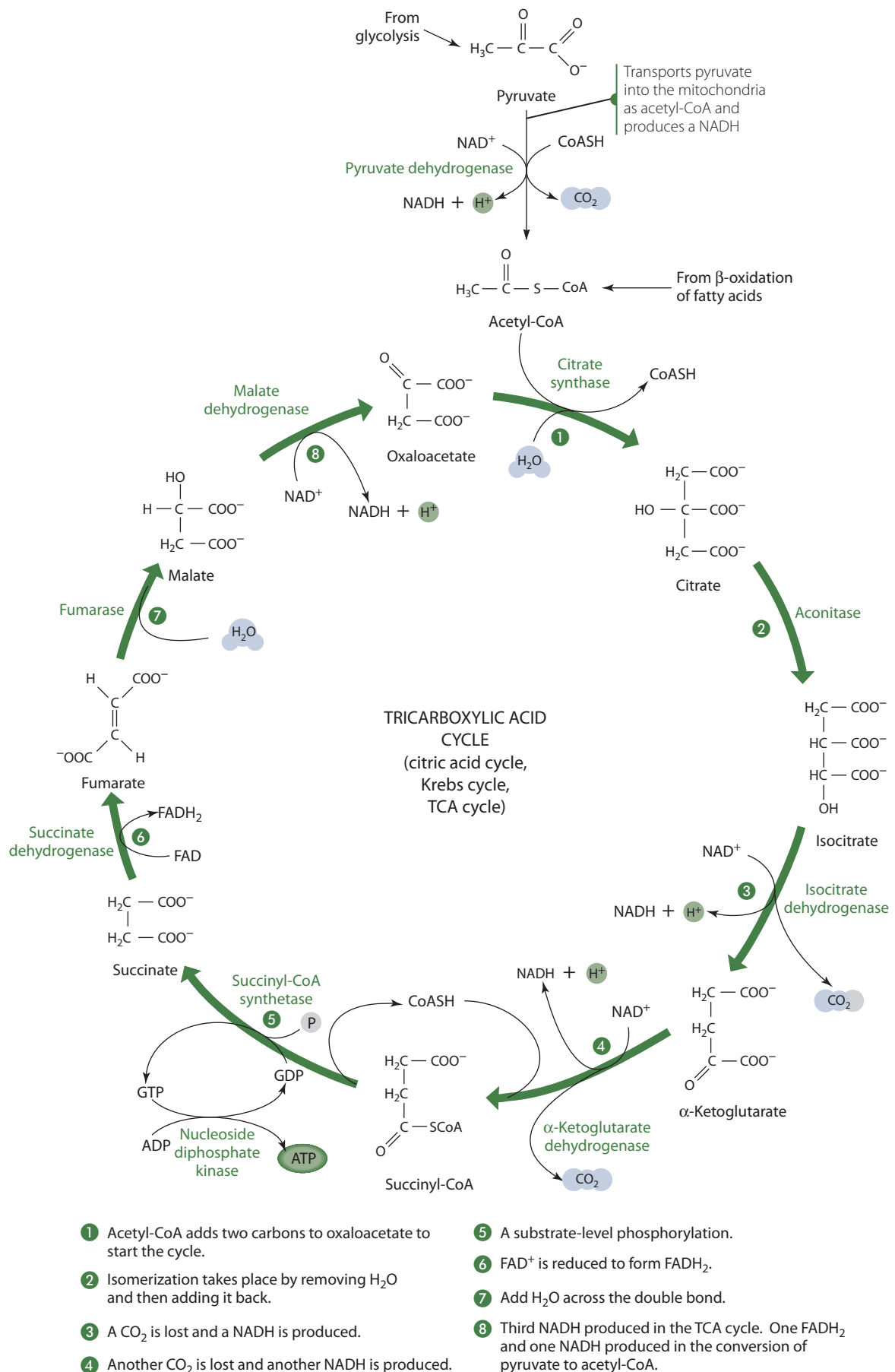


Figure 3.21 The tricarboxylic acid (TCA) cycle.

The primary molecule entering the TCA cycle is acetyl-CoA. Therefore, fuel molecules must first be transported into mitochondria by specific carrier proteins and converted to acetyl-CoA for complete oxidation. In the case of carbohydrate, glycolysis in the cytosol produces pyruvate, which is transported into mitochondria and converted to acetyl-CoA (discussed in the next section). Fatty acids and amino acids are also transported into mitochondria and converted to acetyl-CoA (discussed in later chapters).

Conversion of Pyruvate to Acetyl-CoA

Conversion of pyruvate to acetyl-CoA is irreversible and represents a committed step in energy metabolism. The reaction is accomplished in the mitochondrial matrix by a multienzyme complex called the *pyruvate dehydrogenase complex* (PDC). This multienzyme system is made up of three enzymes: pyruvate dehydrogenase, dihydrolipoamide acetyltransferase, and dihydrolipoamide dehydrogenase. Several cofactors are required for the reaction, including coenzyme A (CoA), thiamin pyrophosphate, Mg^{2+} , NAD^+ , FAD, and lipoic acid. Four vitamins, therefore, are necessary for the activity of the complex: pantothenic acid (a component of CoA), thiamin, niacin, and riboflavin. The role of these vitamins and others as precursors of coenzymes is discussed in Chapter 9.

The net effect of the PDC is decarboxylation, producing CO_2 . In the process, pyruvate is dehydrogenated, with NAD^+ serving as the terminal acceptor of a hydride ion (one proton and two electrons). The active sites of the three enzymes are packed closely together, which allows the passing of the product of one reaction to the next enzyme. This reaction yields energy because the oxidation of NADH produces ATP by oxidative phosphorylation. The reaction is regulated allosterically: negatively by ATP, acetyl-CoA, and NADH, and positively by NAD^+ and ADP. The PDC is also regulated covalently: a Mg^{2+} -dependent enzyme, pyruvate dehydrogenase kinase, phosphorylates the complex when NADH and acetyl-CoA levels rise. Reactivation of the PDC occurs by the enzyme pyruvate dehydrogenase phosphatase, which removes the phosphate. Insulin and Ca^{2+} ions activate the kinase to activate the PDC.

Release of High-Energy Electrons

The condensation of acetyl-CoA with oxaloacetate initiates the TCA cycle reactions. Note that the TCA cycle itself does not directly generate much ATP. Rather, it generates high-energy electrons that are transferred to NAD^+ and FAD, thus yielding NADH and $FADH_2$, respectively. Because the reactions are cyclic, oxaloacetate is regenerated after one trip through the cycle, so a relatively small number of oxaloacetate molecules can generate large amounts of NADH and $FADH_2$ as acetyl-CoA continually

feeds into the cycle. Following are comments on the individual reactions in Figure 3.21:

- 1 The formation of citrate from oxaloacetate and acetyl-CoA is catalyzed by the enzyme citrate synthase. The reaction is regulated negatively by NADH and succinyl-CoA.
- 2 The isomerization of citrate to isocitrate results in the repositioning of the $-OH$ group onto an adjacent carbon, catalyzed by aconitase. The reaction occurs in two steps involving dehydration followed by hydration, with *cis* aconitate as an intermediate.
- 3 This is the first of four dehydrogenation reactions within the TCA cycle and is catalyzed by the enzyme isocitrate dehydrogenase. The main products are NADH, CO_2 , and α -ketoglutarate. The reaction is positively modulated by ADP and negatively modulated by ATP and NADH.
- 4 Decarboxylation and dehydrogenation of α -ketoglutarate is catalyzed by a multienzyme, multicofactor system called the α -ketoglutarate dehydrogenase complex. It involves the coordination of three enzymes that are homologous to the pyruvate dehydrogenase complex. The main products are NADH, CO_2 , and succinyl-CoA.
- 5 Succinyl-CoA contains a high-energy thioester bond that is hydrolyzed by succinyl-CoA synthetase (also called succinyl thiokinase). The reaction releases sufficient energy to drive the phosphorylation of guanosine diphosphate (GDP) by inorganic phosphate. The resulting guanosine triphosphate (GTP) can transfer its phosphate to ADP to make ATP in a reaction catalyzed by the enzyme nucleoside diphosphate kinase. This reaction is another example of ATP production through substrate-level phosphorylation.
- 6 The succinate dehydrogenase reaction yields fumarate and uses FAD instead of NAD^+ as a proton and electron acceptor. Succinate dehydrogenase is bound in the inner membrane of the mitochondria because it is part of enzyme Complex II in the electron transport chain. Other TCA cycle enzymes are found in the mitochondrial matrix.
- 7 Fumarase catalyzes a hydration reaction that incorporates the elements of H_2O across the double bond of fumarate to form malate.
- 8 The conversion of malate to oxaloacetate completes the cycle. NAD^+ acts as the proton and electron acceptor in this dehydrogenation reaction, catalyzed by malate dehydrogenase.

Replenishing Oxaloacetate

To keep the TCA cycle functioning, oxaloacetate—and other TCA cycle intermediates that can lead to oxaloacetate—must be replenished in the cycle. Oxaloacetate, fumarate, succinyl-CoA, and α -ketoglutarate can all be formed from certain amino acids, but the single most important mechanism for ensuring an ample supply of oxaloacetate is the reaction that forms oxaloacetate (four carbons) directly from pyruvate (three carbons) by the addition of CO_2 . This reaction, shown in Figure 3.22, is catalyzed by pyruvate carboxylase. The “uphill” incorporation of CO_2 is accomplished at the expense of ATP, and the reaction requires the participation of biotin (see Chapter 9). The conversion of pyruvate to oxaloacetate is called an **anaplerotic** (replenishing) process because of its role in restoring oxaloacetate in the cycle. Increasing levels of acetyl-CoA stimulates pyruvate carboxylase, thus ensuring oxaloacetate formation.

NADH from Glycolysis: The Shuttle Systems

NADH produced in the cytosol during glycolysis via the glyceraldehyde-3-phosphate dehydrogenase reaction is unable to participate directly in oxidative phosphorylation because the inner mitochondrial membrane is impermeable to NADH. Under anaerobic conditions, NADH in the cytosol is used in the lactate dehydrogenase reduction of pyruvate to lactate, thereby becoming reoxidized to NAD^+ without involving oxygen. In this manner, NAD^+ is restored to sustain the glyceraldehyde-3-phosphate dehydrogenase reaction, allowing the production of lactate to continue in the absence of oxygen.

When the supply of oxygen is adequate to allow complete oxidation of incoming glucose, the production of pyruvate and NADH from glycolysis is accelerated and lactate is not formed. In this situation, the reducing equivalents of NADH (the protons and electrons) are transported from the cytosol to the mitochondrial matrix by two separate shuttle systems. These shuttle systems are specific to certain tissues. The glycerol-3-phosphate shuttle functions in the brain and skeletal muscle, whereas the more active malate–aspartate shuttle functions in the liver, kidney, and heart.

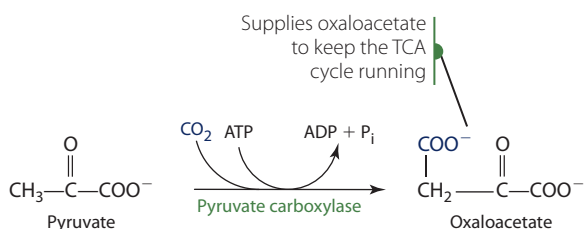


Figure 3.22 Formation of oxaloacetate from pyruvate and CO_2 .

Glycerol-3-Phosphate Shuttle System NADH in the cytosol transfers its reducing equivalents to dihydroxyacetone phosphate, forming glycerol-3-phosphate that freely diffuses across the outer mitochondrial membrane. The reaction is catalyzed by the cytosolic isoform of glycerol-3-phosphate dehydrogenase. The reducing equivalents of glycerol-3-phosphate are then transferred to FAD that is associated with a membrane-bound isoform of glycerol-3-phosphate dehydrogenase located on the outer face of the inner mitochondrial membrane. Finally, the resulting FADH_2 transfers its electrons directly to the electron transport chain, producing 1.5 moles of ATP per mole of NADH (Figure 3.23). This shuttle is not reversible.

Malate–Aspartate Shuttle System The most active shuttle compound, malate, is freely permeable to the inner mitochondrial membrane. Oxaloacetate from the cytosol is reduced by the NADH to form malate and NAD^+ . The malate is oxidized by the enzyme malate dehydrogenase to oxaloacetate in the matrix of mitochondria, producing NADH that enters the electron transport chain and generates 2.5 moles of ATP per mole of NADH. The oxaloacetate undergoes transamination by aspartate amino transferase to form aspartate, which is freely permeable to the inner membrane and can move back out into the cytosol. The effect is that reducing equivalents of NADH are transferred into mitochondria, even though the inner mitochondrial membrane is impermeable to NADH itself (Figure 3.24). This shuttle is reversible.

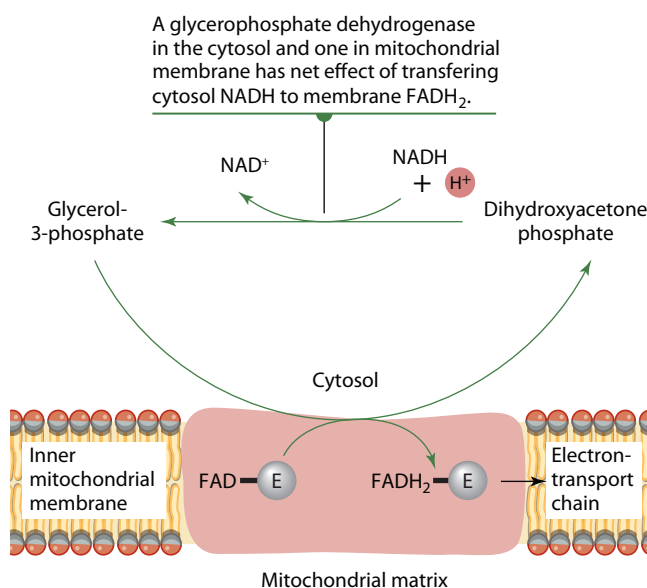


Figure 3.23 Glycerol-3-phosphate shuttle.

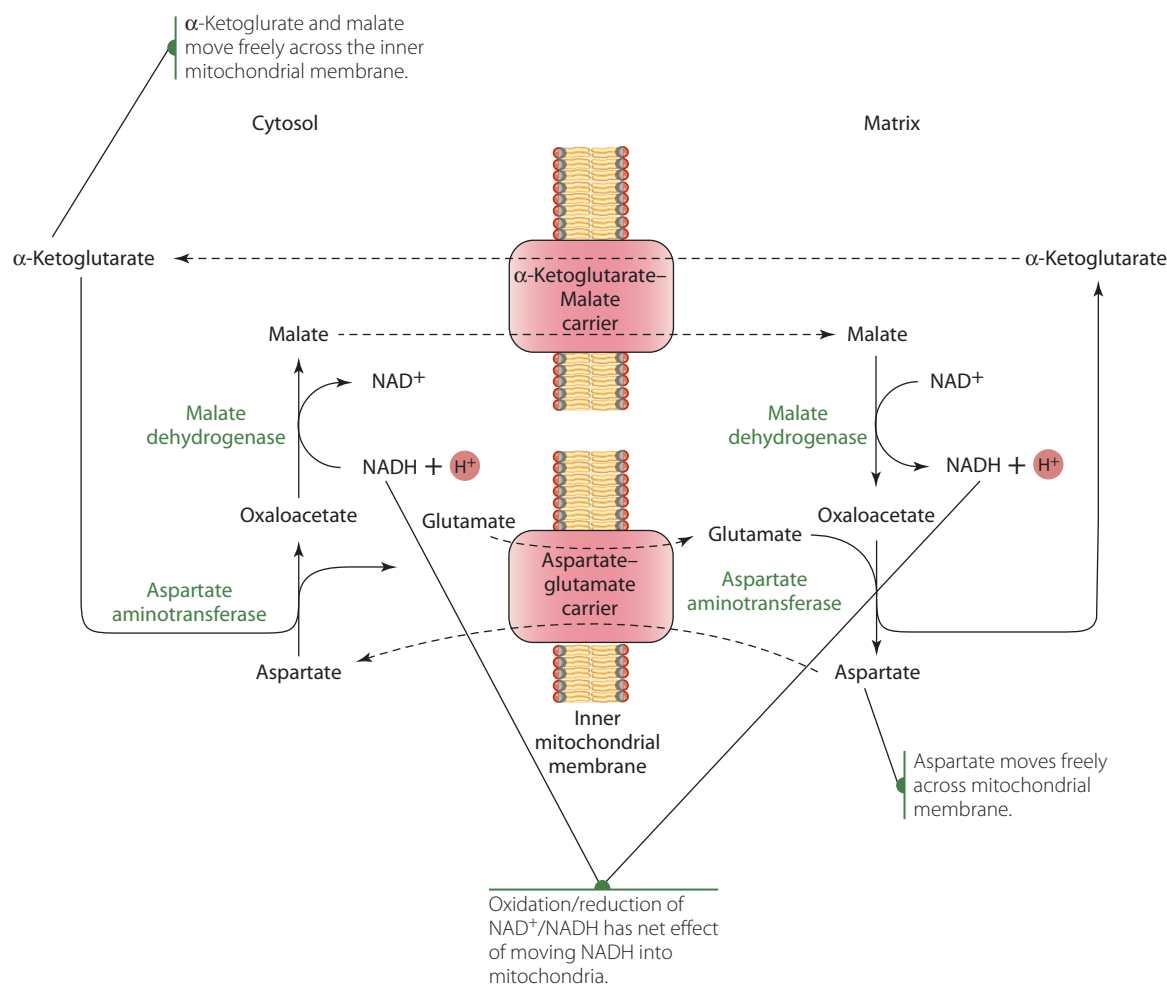


Figure 3.24 Malate–aspartate shuttle.

Formation of ATP

The majority of energy-requiring reactions in the body depend on ATP as a cosubstrate to furnish the energy that drives the reaction. Thus, ATP acts as the main energy currency and must be continually synthesized from the energy provided by macronutrients, primarily carbohydrates. A small proportion of ATP is produced in the body's cells by substrate-level phosphorylation, but the majority of ATP is synthesized in mitochondria by oxidative phosphorylation.

Substrate-Level Phosphorylation

Some ATP are synthesized by direct phosphorylation involving high-energy phosphate donors, referred to as substrate-level phosphorylation. Two reactions in glycolysis and one reaction in the TCA cycle produce ATP by substrate-level phosphorylation. Phosphorylation of ADP to form ATP is accomplished by phosphate donors having more energy than the amount needed ($\Delta G^\circ = +7,300 \text{ cal/mol}$ or $+35.7 \text{ kJ/mol}$) for the reaction.

Table 3.5 lists the standard free energy of hydrolysis of selected phosphate-containing compounds in both cal and

Table 3.5 Free Energy of Hydrolysis (Phosphate Group Transfer Potential) of Some Phosphorylated Compounds

Compound	$\Delta G^\circ(\text{cal})$	$\Delta G^\circ(\text{kJ})$
Phosphoenolpyruvate	−14,800	−62.2
1,3-Bisphosphoglycerate	−11,800	−49.6
Phosphocreatine	−10,300	−43.3
ATP	−7,300	−35.7
Glucose-1-phosphate	−5,000	−21.0
Adenosine monophosphate (AMP)	−3,400	−14.2
Glucose-6-phosphate	−3,300	−13.9

kJ. The ΔG° of hydrolysis of these compounds is called the *phosphate group transfer potential* and is a measure of a compound's capacity to donate phosphate groups to other substances. Phosphorylated molecules have a wide range of free energies of hydrolysis of their phosphate groups. Many of them release less energy than ATP, but some release more. Phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine have more free energy than ATP and are capable of phosphorylating ADP. The more negative the transfer potential, the more potent the phosphate-donating

power. Therefore, a compound that releases more energy on hydrolysis of its phosphate can transfer that phosphate to an acceptor molecule having a less negative transfer potential. For this transfer to actually occur, however, there must be a specific enzyme to catalyze the transfer. For example, a phosphate group can be enzymatically transferred from ATP to glucose, a transfer that can be predicted from Table 3.5. It can also be predicted from Table 3.5 that compounds with a more negative phosphate group transfer potential than ATP can transfer phosphate to ADP, forming ATP. This kind of reaction does, in fact, occur in the hexokinase/glucokinase reactions. The phosphorylation of ADP by phosphocreatine represents an important mode for ATP formation in muscle, and the reaction exemplifies a substrate-level phosphorylation (Figure 3.25).

Macronutrient Oxidation and Electron Transfer

The production of ATP in mitochondria by oxidative phosphorylation begins with the oxidation of fuel molecules by the TCA cycle and the release of electrons and protons. The electrons and protons are captured by NADH and FADH₂ and delivered to the inner mitochondrial membrane. Here, the electrons are passed through a series of oxidation-reduction reactions and ultimately to molecular oxygen, which becomes reduced to H₂O in the process. The compounds that participate in electron transfer within the inner mitochondrial membrane comprise the *electron transport chain*, also known as the respiratory chain because the electron transfer is linked to the availability of O₂ through tissue respiration (see Figure 1.6). The energy provided by the electron flow allows the protons to be translocated from the mitochondrial matrix to the space between the inner and outer membranes, which creates an energy gradient that powers the phosphorylation of ADP to form ATP. The driving

force in oxidative phosphorylation is the electron transfer potential in NADH and FADH₂ relative to O₂. The term *oxidative phosphorylation* is a descriptive blend of simultaneous processes involving electron transport, translocation of protons, oxidation of a metabolite by oxygen, and the phosphorylation of ADP to make ATP.

Cellular oxidation of a compound can occur by several different reactions: the addition of oxygen, the removal of electrons, or the removal of protons and electrons together (as hydrogen atoms or hydride ions). All of these reactions are catalyzed by enzymes collectively termed *oxidoreductases*. Among these, the **dehydrogenases** remove protons and electrons from nutrient metabolites and are particularly important in energy transformation. The protons and electrons removed from metabolites by dehydrogenases generally produce NADH or FADH₂, which are either already in or shuttled into the mitochondria and move along the electron transport chain. Many dehydrogenases catalyze reactions of the TCA cycle.

After oxidation of substrate molecules by a dehydrogenase enzyme, the protons and electrons are transferred to a cosubstrate. In the TCA cycle, these cosubstrates are the vitamin-derived nicotinamide adenine dinucleotide (NAD⁺) or flavin adenine dinucleotide (FAD). The structures of both the oxidized and reduced forms of these cosubstrates are shown in Figures 3.26 and 3.27. After accepting protons and electrons from reactions of the TCA cycle, NADH and FADH₂ move to the inner mitochondrial membrane to initiate the electron transport chain. The sequence of reactions in the electron transport chain is shown in Figure 3.28.

The Electron Transport Chain

The flow of electrons from NADH and FADH₂ to molecular oxygen takes place in four protein complexes within the mitochondrial inner membrane. The outer membrane is permeable to most molecules smaller than 10 kilodaltons, but the inner membrane has very limited permeability. Each complex is a cluster of enzymes, peptides, and other molecules that transfer electrons along the chain. The main constituents of the complexes are:

Complex I

- Flavin mononucleotide (FMN)
- Iron-sulfur center

Complex II

- Succinate dehydrogenase
- Iron-sulfur center

Complex III

- Cytochrome *b*
- Cytochrome *c*₁
- Heme groups
- Iron-sulfur center

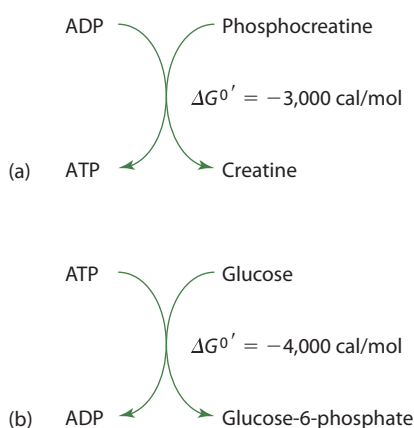


Figure 3.25 (a) Example of high-energy phosphate bond being transferred from high-energy compound phosphocreatine to form ATP. (b) The transfer of the high-energy phosphate bond to a compound that becomes activated, allowing it to enter into the glycolytic pathway.

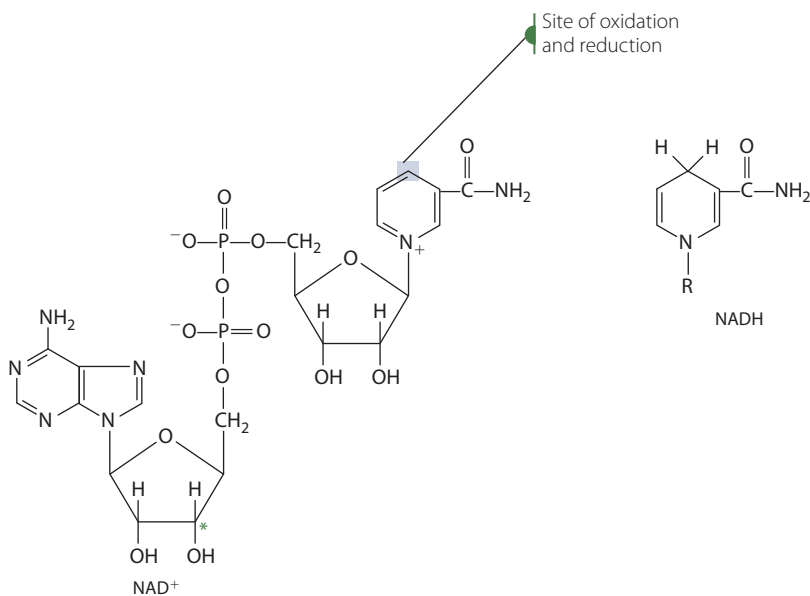


Figure 3.26 Nicotinamide adenine dinucleotide (NAD⁺) and its reduced form (NADH).

Complex IV

- Cytochrome *a*
- Cytochrome *a*₃
- Heme groups
- Iron-sulfur center

The strategic location of the electron transport chain within the inner membrane allows for the simultaneous translocation of protons (H⁺) from within the matrix to the intermembrane space (the space between the cristae and outer membrane). The translocation of protons provides much of the energy that drives the final step of phosphorylating ADP to make ATP.

The electron transport chain starts with NADH or FADH₂ produced within the mitochondria or shuttled in from the cytosol. Glycolysis produces cytosolic NADH and FADH₂, and their shuttling into the mitochondria has already been discussed (see Figures 3.23 and 3.24). As noted in Figure 3.28, NADH and FADH₂ donate their electrons in different places along the electron transport chain. The main flow of electrons from NADH to O₂ occurs through Complex I, III, and IV, whereas the electrons in FADH₂ flow through Complex II, III, and IV. In both cases, the flow of electrons requires the help of two electron carriers, coenzyme Q (CoQ), also called *ubiquinone*, and cytochrome *c*. These carriers are mobile and are able to ferry the electrons from one complex to the next. Also note that each complex contains protein-associated iron-sulfur (Fe-S) centers that readily accept electrons by reducing Fe³⁺ to Fe²⁺. In contrast to FMN and CoQ, the Fe-S centers can undergo oxidation-reduction cycles without binding or releasing protons.

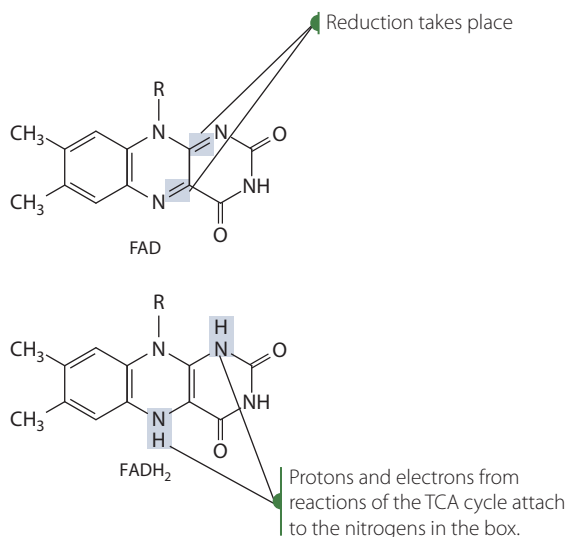


Figure 3.27 Flavin adenine dinucleotide (FAD) and its reduced form (FADH₂).
R = ribitol phosphate + AMP.

Complex I Also called *NADH-coenzyme Q oxidoreductase*, Complex I transfers a pair of electrons from NADH to CoQ by a series of oxidation-reduction reactions. The reactions also promote the transfer of protons from the matrix side of the inner mitochondrial membrane to the intermembrane space. The importance of the buildup of protons in the intermembrane space is discussed in the following sections. Complex I is made of many polypeptide chains, a molecule of FMN, and several Fe-S centers, along with additional iron molecules. The iron molecules bind with the sulfur-containing amino acid cysteine. The iron transfers one electron at a time, cycling between Fe²⁺ and Fe³⁺.

The transfer of electrons through Complex I reactions are ultimately accepted by CoQ (ubiquinone). CoQ is a

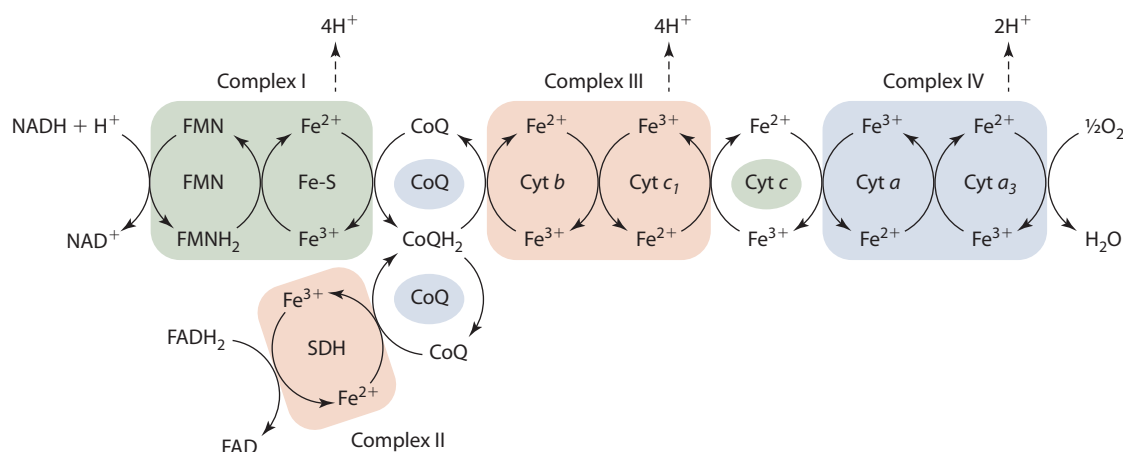


Figure 3.28 Electron transfer sequence from NADH and FADH₂ to O₂ in the electron transport chain. Coenzyme Q (CoQ) and cytochrome *c* (Cyt *c*) act as carriers of electrons between complexes. FMN, flavin mononucleotide; FeS, iron-sulfur center; SDH, succinate dehydrogenase; Cyt *b*, cytochrome *b*; Cyt *c*₁, cytochrome *c*₁; Cyt *a*, cytochrome *a*; Cyt *a*₃, cytochrome *a*₃.

highly hydrophobic compound and it diffuses freely in the hydrophobic core of the inner membrane. The transfer of electrons to CoQ occurs one electron at a time. Transfer of the first electron creates an unstable intermediate ion radical (semiquinone), followed quickly by the second electron, resulting in fully reduced CoQH₂ (ubiquinol). The structures of the oxidized and reduced forms of CoQ are shown in Figure 3.29. The overall oxidation of NADH through the electron transport chain results in the synthesis of approximately 2.5 ATP molecules.

Complex II The main component of Complex II (also called *succinate-coenzyme Q reductase*) is the succinate dehydrogenase enzyme, the same enzyme that produces FADH₂ in the TCA cycle. Succinate dehydrogenase is the only TCA cycle enzyme associated with the inner

mitochondrial membrane, which allows the oxidation of succinate to fumarate (in the matrix) to occur simultaneously with the reduction of CoQ to CoQH₂ within the membrane. Unlike the other complexes, Complex II does not pump protons into the intermembrane space.

Besides succinate dehydrogenase, Complex II contains a FAD protein and Fe-S centers. When succinate is converted to fumarate in the TCA cycle, FAD is reduced to FADH₂. The FADH₂ is oxidized by electron transfer through the Fe-S centers to reduce CoQ to CoQH₂. The oxidation of FADH₂ through the electron transport chain results in the formation of approximately 1.5 molecules of ATP.

Complex III The electrons derived from both NADH and FADH₂ are passed from CoQH₂ to cytochrome *c* through the reactions of Complex III, also called *coenzyme Q-cytochrome c oxidoreductase*. Complex III contains two different cytochromes (*b* and *c*₁) and a Fe-S protein. The cytochromes contain heme molecules with an iron molecule in the center. The iron in the center of the cytochromes is oxidized and reduced as electrons flow through, releasing four protons to the intermembrane space.

During the oxidation-reduction reactions of Complex III, two electrons and two protons are donated by CoQH₂, but the final acceptor molecule, cytochrome *c*, can accept only one electron. To optimize electron transfer and proton translocation, a unique mechanism exists called the *Q cycle*. It starts with two CoQH₂ molecules that bind to the complex, releasing a total of four electrons and four protons. All four protons are translocated to the intermembrane space. In the first half of the cycle, one electron flows to cytochrome *c*₁ and is passed on to cytochrome *c*. A second electron flows to cytochrome *b* where it transfers to a CoQ molecule, creating an unstable semiquinone ion. In the second half of the cycle, a third electron flows

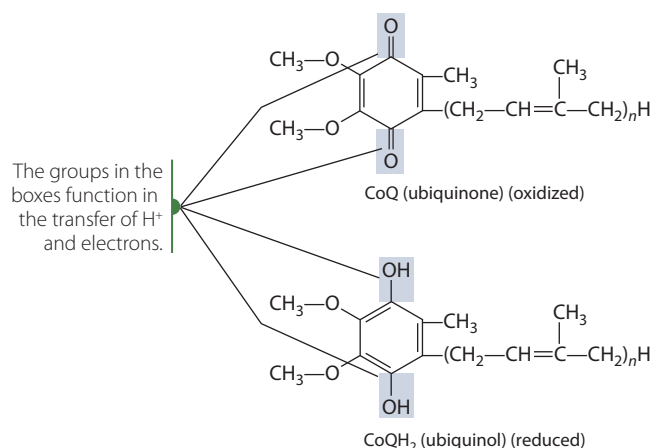


Figure 3.29 Oxidized and reduced forms of coenzyme Q, or ubiquinone. The subscript *n* indicates the number of isoprenoid units in the side chain (most commonly 10). A one-electron transfer results in the formation of a semiquinone with only one of the quinone groups reduced.

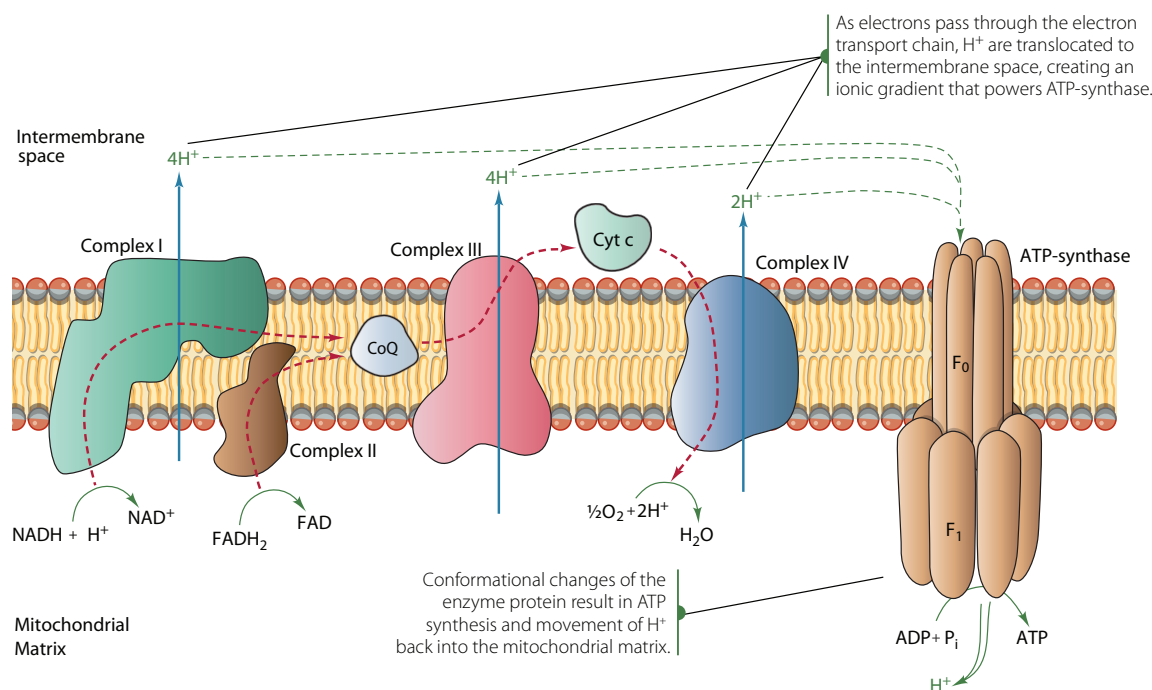


Figure 3.30 Oxidative phosphorylation. Electrons from NADH and FADH_2 travel along the electron transport chain (dashed red arrows) releasing energy that pumps protons (H^+) from the matrix into the intermembrane space (blue arrows). The increased H^+ concentration gradient causes H^+ to move back into the matrix through channels in ATP synthase, thus providing the energy to phosphorylate ADP to form ATP.

to cytochrome c_1 and on to cytochrome c , just as the first electron. The fourth electron flows to cytochrome b where it joins the semiquinone ion and two protons from the matrix to form CoQH_2 . This means that one full turn of the Q cycle results in the oxidation of 2CoQH_2 , the release of four protons in the intermembrane space, the reduction of one CoQ to CoQH_2 , and the release of one CoQ back to the “CoQ pool” to continue the cycle.

Cytochrome c accepts electrons emerging from Complex III and transfers them to Complex IV. Cytochrome c is highly water soluble and, unlike CoQ, can ferry electrons outside of the membrane within the intermembrane space. This characteristic allows cytochrome c to migrate along the membrane, a feature that is particularly important for transferring electrons between two distinct proteins such as those present in Complex III and IV.

Complex IV Complex IV is also called *cytochrome c oxidase*. It accepts electrons from cytochrome c and catalyzes a four-electron reduction of oxygen to form water. This reaction is the final step in the electron transport chain that releases energy from nutrients (carbohydrate, fat, protein, and alcohol) to produce usable chemical energy in the form of ATP.

The structure of cytochrome c oxidase is known; it is made up of multiple subunits. Some of the subunits are encoded from nuclear DNA and some from mitochondrial DNA. These latter proteins contain iron and copper. The metal ions cycle between their oxidized (Fe^{3+} , Cu^{2+}) and reduced (Fe^{2+} , Cu^{1+}) states. Cytochrome c oxidase also contains two cytochromes, cytochrome a and cytochrome

a_3 , which contain different heme moieties. The reactions of Complex IV result in the transport of protons to the intermembrane space.

Electron transport can carry on without phosphorylation, but the phosphorylation of ADP to form ATP (discussed in the next section) is dependent upon electron transport that terminates as molecular oxygen is reduced to H_2O . The relationship between electron transport and oxidative phosphorylation within the inner mitochondrial membrane is shown in Figure 3.30. The free energy change at various sites within the electron transport chain is shown in Table 3.6.

Phosphorylation of ADP to Form ATP

The intimate association of energy release with oxidation is exemplified by the oxidation of glucose to CO_2 plus water and energy, discussed earlier in this chapter. Glycolysis occurs in the cytosol; the TCA cycle, electron transport,

Table 3.6 Free Energy Changes at Various Sites within the Electron Transport Chain Showing Phosphorylation Sites

Reaction	ΔG° (cal/mol)	ADP Phosphorylation Site?
$\text{NAD}^+ \rightarrow \text{FMN}$	−922	No
$\text{FMN} \rightarrow \text{CoQ}$	−15,682	Yes
$\text{CoQ} \rightarrow \text{cyt } b$	−1,380	No
$\text{Cyt } b \rightarrow \text{cyt } c_1$	−7,380	Yes
$\text{Cyt } c_1 \rightarrow \text{cyt } c$	−922	No
$\text{Cyt } c \rightarrow \text{cyt } a$	−1,845	No
$\text{Cyt } a \rightarrow \frac{1}{2}\text{O}_2$	−24,450	Yes

proton translocation, and oxidative phosphorylation occur in the mitochondria. It has already been established that the complete oxidation of 1 mol of glucose yields either 30 or 32 ATPs. The complete biological oxidation of 1 mol of glucose yields approximately 700 kcal (or 2,937 kJ). The standard free energy for the hydrolysis of ATP that has been used throughout this chapter is 7.3 kcal (30.5 kJ). However, standard conditions are at a concentration of 1 mol/L, whereas the concentration of ATP within the cell is closer to 1–5 mmol/L. The free energy of hydrolysis at this concentration is closer to 12 kcal (50 kJ). The free energies of other compounds with high phosphate transport potential such as phosphoenolpyruvate, 1,3-bisphosphoglycerate, and phosphocreatine are also increased proportionally. It is more straightforward to use standard free energy in talking about these reactions. However, to determine the energy efficiency of the biological oxidation of glucose, the free energy of ATP under biological conditions must be considered. In living cells, 32 mol of ATP capture 384 kcal (32×12). The efficiency is therefore $384/700 \times 100$, or about 54% [20]. The remaining energy is released as heat. This is an efficient process as engines go.

The previous discussion on electron transport focused on the translocation of protons from the matrix to the intermembrane space. This translocation is vital to the phosphorylation of ADP to form ATP. The translocation of protons requires energy from the electron transport chain but in return creates a pool of potential energy. The generally accepted mechanism for the synthesis of ATP was first proposed by Peter Mitchell in 1961. He proposed that the energy stored in the difference in concentration of protons between the mitochondrial matrix and the intermembrane space was the driving force for coupled ATP formation. This proposal was called the *chemiosmotic hypothesis*. We examine its main points to support our understanding of the coupling of phosphorylation with the electron transport chain.

Translocation of Protons To determine if the pH gradient and electrical charge difference are sufficient to provide the energy for ATP synthesis, we must examine the number of protons translocated at each complex. Direct measurements have been difficult and disagreement exists among experts, but the consensus is that for every two electrons that pass through Complex I and Complex III, four H^+ are translocated by each complex, for a total of eight H^+ . For Complex IV, an additional two H^+ are translocated by each pair of electrons passing through the complex. No protons are translocated in Complex II. This means that for every mol of NADH oxidized to water, a total of 10 protons are translocated from the matrix to the intermembrane space. The electrical charge across the inner membrane changes because of the positively charged protons in the intermembrane space, a difference estimated to be approximately 0.18 volts. It is also assumed that the pH difference between the mitochondrial matrix and the inner membrane is one unit. Using these assumptions, the

free energy available is -94.49 kcal/mol (-23.3 kJ/mol). This is the potential free energy available to move protons back into the matrix of the mitochondria through the ATP synthase enzyme, thus coupling electron transport with the phosphorylation of ADP to form ATP. Paul Boyer and John Walker shared the 1997 Nobel Prize for chemistry for their work on ATP synthase. A review of Paul Boyer's research on ATP synthase sums up several decades of work [21].

ATP Synthase Figure 3.30 illustrates electron transport, proton translocation, and oxidative phosphorylation. The disparity in both the proton concentration and electrical charge on either side of the inner membrane of mitochondria has already been discussed. It is this proton gradient that provides the energy for ATP synthesis that occurs with the aid of ATP synthase. (ATP synthase is sometimes called Complex V, even though it does not participate in the electron transport chain.)

ATP synthase is made up of two main components, F_0 and F_1 , each with multiple subunits. F_0 is anchored in the membrane and F_1 sticks out of the membrane into the mitochondrial matrix. Respiratory stalks extend from the cristae. If these stalks are removed, electron transport continues, but phosphorylation of ADP does not occur. Some of the subunits of F_1 are capable of rotating and have sites that bind ATP, ADP, and P_i . They also contain channels that allow proton movement through the membrane. For each pair of electrons traversing Complex IV, the rotating subunits of F_1 can complete one rotation and produce three ATPs. At the same time, protons from the intermembrane space are moved back into the matrix from the intermembrane space. The number of protons moved back depends on the number of subunits in the rotating stalk (this can vary between 10 and 15), resulting in three to five protons per ATP formed moving back into the matrix. The return flow of protons furnishes the energy necessary for the synthesis of ATP from ADP and P_i .

ATP is synthesized in mitochondria but must be moved to the cytosol to supply energy for the cell. The enzyme *ATP-ADP translocase* shuttles ATP out of the mitochondria and ADP in. The transport is reversible and exchanges ATP and ADP in a 1:1 ratio. At the same time, another carrier transports inorganic phosphate and one proton into the mitochondrial matrix. Because of the large amount of ATP produced each day (the equivalent of a person's body weight), the ATP-ADP translocase comprises 10–15% of the total protein found in the inner mitochondrial membrane.

ATPs Produced by Complete Glucose Oxidation

The complete oxidation of glucose to CO_2 and H_2O can be shown by this equation:



Complete oxidation is achieved by the combined reaction sequences of the glycolytic and TCA cycle pathways. The energy-conserving steps yield a net of two ATPs by

substrate-level reactions in the glycolytic pathway and two ATPs (or one ATP and one GTP) by substrate-level reactions in the TCA cycle. In addition, there are three NADH and one FADH₂ produced from each acetyl-CoA that goes through the TCA cycle. Two acetyl-CoAs are produced from each molecule of glucose, which releases two molecules of CO₂ and two NADH. In summary, one molecule of glucose produces:

- Six molecules of CO₂ (released)
- Four ATP
- Ten NADH
- Two FADH₂.

The NADH and FADH₂ are in the matrix of the mitochondria and are oxidized by the electron transport chain and coupled with oxidative phosphorylation to ultimately produce ATP. By convention, it is assumed that three ATPs are formed by oxidative phosphorylation from NADH and two ATPs are formed from FADH₂. As previously discussed, the actual number of ATPs formed from NADH is closer to 2.5; for FADH₂, it is 1.5. If the integers (3/2) are used for the number of ATPs produced from NADH/FADH₂, a total of 38 mol of ATP are formed. If we accept the 2.5/1.5 ratio, 32 mol of ATP are produced from each mol of glucose. Oxidative phosphorylation is only active under aerobic conditions. Under anaerobic conditions, only two ATPs are produced from each glucose at substrate level.

The actual number of ATPs formed aerobically from glucose varies because of the two different shuttle mechanisms that transport the electrons from NADH produced by the glycolytic pathway into the mitochondria. One mechanism, the glycerol-3-phosphate shuttle system, transfers the electrons to FADH₂ and therefore yields only 1.5 ATPs. The other shuttle system, the malate-aspartate shuttle, transfers the electrons to NADH inside the mitochondria and yields 2.5 ATPs.

The conversion of the chemical energy of carbohydrates to form ATP is an integral part of carbohydrate metabolism. Historical reviews of electron transport, proton translocation, and oxidative phosphorylation are available to the interested reader [22,23]. The next sections cover other aspects of carbohydrate metabolism.

The Pentose Phosphate Pathway (Hexose Monophosphate Shunt)

The pentose phosphate pathway (also called the hexose monophosphate shunt) is one of the pathways that is available to glucose in the cytosol and is shown in Figure 3.31. It generates important intermediates not produced in other pathways. The pentose phosphate pathway has two important products:

- Pentose phosphates, necessary for the synthesis of the nucleic acids found in DNA and RNA and for other nucleotides

UNCOUPLING ELECTRON TRANSPORT AND ATP SYNTHESIS

Translocation of protons into the intermembrane space is the biological event that links electron transport with ATP synthesis. The energy potential created by the proton gradient provides the power for ATP synthase to phosphorylate ADP, forming ATP. In doing so, ATP synthase acts as a *proton channel* so the protons can relocate to the mitochondrial matrix. When oxidative phosphorylation is operating at peak efficiency, the maximum number of ATP are synthesized per molecule of glucose.

But what happens when the proton gradient in the intermembrane space is uncoupled from the synthesis of ATP? There are, in fact, *uncoupling proteins* that reside in the inner mitochondrial membrane. They act as proton channels to redirect protons back into the matrix and away from ATP synthase. By diffusing the

proton gradient, the potential energy is no longer available for ATP production but, instead, is dissipated as heat. The degree of uncoupling reflects the *metabolic efficiency* in converting nutrient fuels into ATP. In this way, uncoupling proteins and ATP synthase work together to maintain body temperature while meeting the cellular demand for ATP.

During electron transport, some electrons escape and lead to the production of reactive oxygen species (ROS), a phenomenon called electron leak. It is hypothesized that uncoupling proteins may protect mitochondria by decreasing the production of ROS. On the other hand, overexpression of uncoupling proteins can lead to cell death due to inadequate ATP production. The latter observation could be used to our advantage by intentionally

stimulating uncoupling in cancer cells and other human diseases. Several chemical uncouplers have been studied, with varying results.

An important concern in nutrition is the activity of uncoupling proteins in the mitochondria of pancreatic β -cells. Recall that insulin is secreted by β -cells in response to increased blood glucose from the diet. When glucose is taken into the β -cells, it is metabolized to produce ATP, which triggers a cascade of signals resulting in insulin secretion. However, prolonged exposure of pancreatic β -cells to high glucose increases ROS production and stimulates expression of uncoupling proteins. This leads to decreased ATP production and decreased insulin secretion. A hallmark of type 2 diabetes mellitus is diminished insulin production by the pancreas.

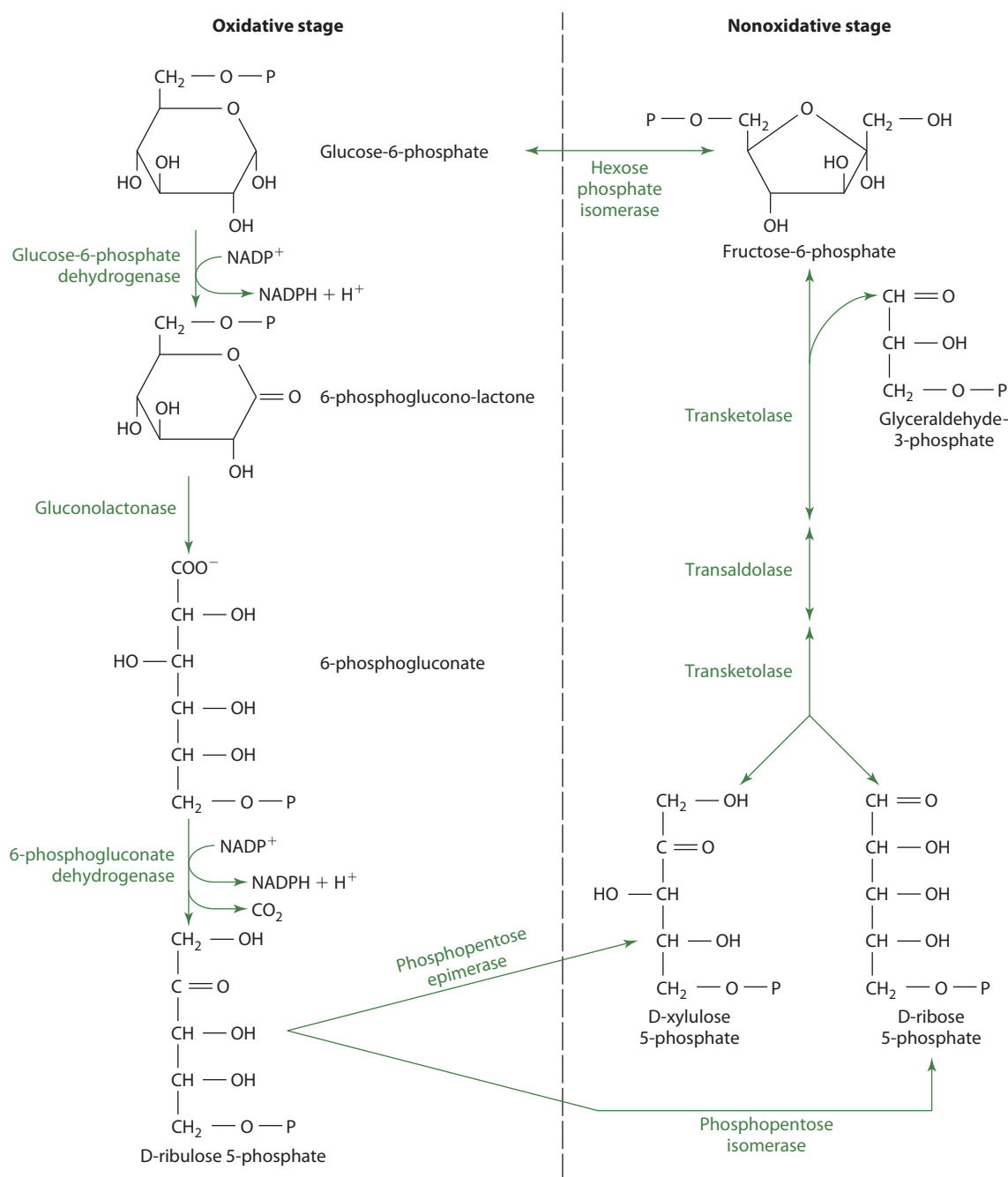


Figure 3.31 The pentose phosphate pathway (hexose monophosphate shunt), showing the oxidative stage and the nonoxidative stage

- Reduced cosubstrate NADPH, used for important metabolic functions, including the biosynthesis of fatty acids (Chapter 5), the maintenance of reducing substrates in red blood cells necessary to ensure the functional integrity of the cells, and drug metabolism in the liver.

The cells of some tissues have a high demand for NADPH, particularly those that are active in the synthesis of fatty acids, such as cells of the mammary gland, adipose tissue, adrenal cortex, and liver. These tissues predictably engage the entire pentose phosphate pathway, recycling pentose phosphates back to glucose-6-phosphate to repeat the cycle and ensure an ample supply of NADPH.

The pathway reactions that include the dehydrogenase reactions and therefore the formation of NADPH from NADP^+ are called the oxidative reactions of the pathway. This segment of the pathway is illustrated on the left in Figure 3.31. The pentose phosphate pathway also synthesizes three-, four-, five-, six-, and seven-carbon sugars.

This pathway begins by oxidizing glucose-6-phosphate in two consecutive reactions catalyzed by *glucose-6-phosphate dehydrogenase* and *6-phosphogluconate dehydrogenase*. Both reactions require NADP^+ as cosubstrate, accounting for the formation of NADPH as a reduction product. The first dehydrogenase reaction is irreversible

and highly regulated. It is strongly inhibited by the cosubstrate NADPH and fatty acid CoAs. Pentose phosphate formation is achieved by the decarboxylation of 6-phosphogluconate to form the pentose phosphate, ribulose-5-phosphate. This product can take two pathways: isomerization to ribose-5-phosphate or epimerization to xylulose-5-phosphate.

Pentose phosphates can subsequently be “recycled” back to hexose phosphates through the transketolase and transaldolase reactions illustrated in Figure 3.31. This recycling of pentose phosphates to hexose phosphates does not produce pentoses, but it does ensure generous production of NADPH as the cycle repeats. It also links the pentose phosphate pathway to glycolysis.

The re-formation of glucose-6-phosphate from the pentose phosphates, through reactions catalyzed by transketolase, transaldolase, and hexose phosphate isomerase, is called the nonoxidative reactions of the pathway and is shown on the right in Figure 3.31. Transketolase and transaldolase enzymes catalyze complex reactions in which three-, four-, five-, six-, and seven-carbon phosphate sugars are interconverted. These reactions are detailed in most comprehensive biochemistry texts.

The reversibility of the transketolase and transaldolase reactions allows hexose phosphates to be converted directly into pentose phosphates, bypassing the oxidative reactions. Therefore, cells that undergo a more rapid rate of replication and that consequently have a greater need for pentose phosphates for nucleic acid synthesis can produce these products in this manner.

The pathway's activity is low in skeletal muscle because of the limited demand for NADPH (fatty acid synthesis) in this tissue and also because of muscle's reliance on glucose and fatty acids for energy metabolism. Glucose-6-phosphate can be used for either glycolysis or for the pentose phosphate pathway. The choice is made based on the cell's needs for energy (by assessing the ATP/ADP ratio) or for biosynthesis (by assessing the NADP⁺/NADPH ratio). The level of NADPH is generally much higher than that of NADP⁺.

Gluconeogenesis

Glucose is an essential nutrient for most cells. The brain and other tissues of the central nervous system (CNS) and red blood cells are particularly dependent upon glucose as a nutrient. When dietary intake of carbohydrate is decreased and blood glucose concentration declines, hormones including glucagon trigger accelerated glucose synthesis from noncarbohydrate sources in a process called *gluconeogenesis*. Lactate, glycerol (a product of triacylglycerol hydrolysis), and certain amino acids represent important noncarbohydrate sources. The liver is the major site of this activity, although under certain circumstances, such as prolonged starvation, the kidneys become increasingly

important in gluconeogenesis. Most of the glucose formed by the liver and the kidneys is released into the blood to maintain blood glucose levels.

Many steps in gluconeogenesis are the reverse of glycolysis. Gluconeogenesis synthesizes glucose and consumes ATP and NAD⁺ rather than producing ATP and NADH. Most of the cytosolic enzymes involved in glycolysis, which is the conversion of glucose to pyruvate, catalyze their reactions reversibly and therefore provide the means for also converting pyruvate to glucose. But when the cell is oxidizing glucose for energy, it does not need to make glucose from gluconeogenesis.

Both glycolysis and gluconeogenesis must be regulated, and it is the nonreversible reactions that are regulated. Three reactions in the glycolytic sequence are highly exergonic, highly regulated, and *not* reversible: those catalyzed by the enzymes glucokinase (hexokinase), phosphofructokinase, and pyruvate kinase (reactions 1, 3, and 10 in Figure 3.20). These three steps involve ATP and are unidirectional by virtue of the high, negative free energy change of the reactions. Therefore, the process of gluconeogenesis requires that the reverse of these steps be either bypassed or circumvented by other enzyme systems. The presence or absence of specific enzymes determines whether a certain organ or tissue is capable of conducting gluconeogenesis. As shown in Figure 3.32, the glucokinase and phosphofructokinase reactions can be bypassed by specific phosphatases (glucose-6-phosphatase and fructose-1,6-bisphosphatase, respectively) that remove phosphate groups by hydrolysis.

The bypass of the pyruvate kinase reaction involves the formation of oxaloacetate as an intermediate. Mitochondrial pyruvate can be converted to oxaloacetate by pyruvate carboxylase, a reaction that was discussed earlier as an *anaplerotic* process. Oxaloacetate, in turn, can be decarboxylated and phosphorylated to phosphoenolpyruvate by phosphoenolpyruvate carboxykinase, thereby completing the bypass of the pyruvate kinase reaction. However, the phosphoenolpyruvate carboxykinase reaction is a cytosolic reaction and therefore oxaloacetate must leave the mitochondrion to be acted upon by the enzyme. Because the mitochondrial membrane is impermeable to oxaloacetate, it must first be converted to either malate (by malate dehydrogenase) or aspartate (by transamination with glutamate; see Chapter 6), both of which freely traverse the mitochondrial membrane. This mechanism is similar to the malate–aspartate shuttle previously discussed. In the cytosol, the malate or aspartate can be converted back to oxaloacetate by malate dehydrogenase or aspartate aminotransferase (glutamate oxaloacetate transaminase), respectively.

Noncarbohydrate Sources

Amino Acid Utilization The conversion of pyruvate to oxaloacetate in the initial steps of gluconeogenesis allows for the carbon skeletons of various amino acids

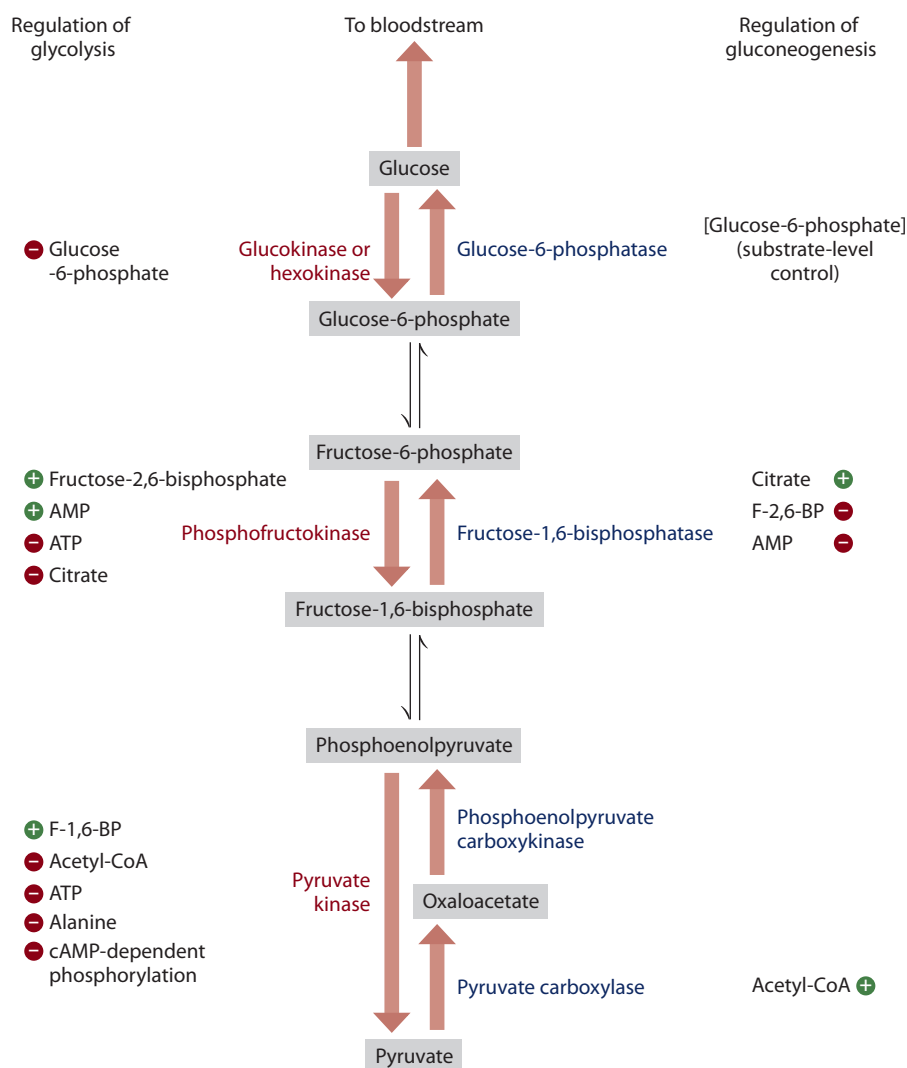


Figure 3.32 Reciprocal regulation of glycolysis and gluconeogenesis. Nonreversible reactions of glycolysis and gluconeogenesis are regulated steps. Inhibitors are indicated by minus signs and activators by plus signs.

Source: Garrett & Grisham, *Biochemistry*, 4/e. © Cengage Learning.

to enter the gluconeogenic pathway. Such amino acids accordingly are called glucogenic. Glucogenic amino acids can be catabolized to pyruvate or oxaloacetate when metabolic conditions favor glucose synthesis. Furthermore, since some amino acids can convert to various TCA cycle intermediates—to replenish the intermediates that have exited the mitochondrion in the form of malate or aspartate—utilization of TCA cycle intermediates represents another way that amino acids can be converted to glucose. Reactions showing the entry of noncarbohydrate substances into the gluconeogenic system are shown in Figure 3.33.

Lactate Utilization Lactate is produced by red blood cells continuously and by skeletal muscle during strenuous physical exertion. The majority of lactate produced is released into the blood, where it travels to the liver for conversion to glucose via gluconeogenesis. The newly

made glucose can, in turn, be released into the blood. Recall that muscle cells lack glucose-6-phosphatase and cannot produce free glucose from noncarbohydrate sources. Thus, the liver is able to prevent the accumulation of lactate while replenishing blood glucose. This is an important relationship between muscle and liver, especially during strenuous (anaerobic) physical activity when blood glucose is being used, at least in part, to fuel muscle by glycolysis that produces lactate. The ability of the liver to convert muscle-derived lactate to glucose, and for muscle to take up that glucose and use it in glycolysis, constitutes the **Cori cycle**.

Glycerol Utilization The hydrolysis of triacylglycerols stored in adipose tissue produces fatty acids and glycerol (discussed further in Chapter 5). Fatty acids are a rich source of energy that provides fuel for muscle and other tissues by being catabolized to acetyl-CoA for entry into

body synthesis during carbohydrate deficit (see Chapter 5), the by-product acetone can be used to make pyruvate, which can enter the gluconeogenic pathway. While theoretically possible, additional metabolic research is needed to confirm whether this pathway is a quantitatively important source of glucose.

3.7 REGULATION OF METABOLISM

As discussed throughout this chapter, glucose is a central player in energy metabolism. It is the most abundant fuel molecule in food and it is a primary energy metabolite in the body. Glucose breakdown (glycolysis) initiates the release of its energy for use in the body's cells. In contrast, glucose synthesis (gluconeogenesis) occurs when energy needs to be distributed—as blood glucose—to tissues in need. This section focuses on the regulation of these metabolic pathways. The principles described here apply as well to the metabolic regulation of other monosaccharides, fatty acids and certain amino acids that enter the energy pathways.

Glycolysis and gluconeogenesis exemplify pathways that are highly regulated by changes in the nutritional and biochemical demands of the body. The purpose of regulation of glycolysis and gluconeogenesis is to maintain homeostasis. An excellent example of metabolic regulation is the reciprocal regulation of the glycolysis (catabolic) pathways and the gluconeogenic (anabolic) pathways. The glycolytic conversion of glucose to pyruvate liberates energy, whereas the reversal of the process from pyruvate to glucose consumes energy. The pyruvate kinase bypass in itself is energetically expensive, considering that one mol of ATP and one mol of GTP must be expended in converting intramitochondrial pyruvate to extramitochondrial phosphoenolpyruvate (Figure 3.33). It follows that among the factors that regulate the glycolysis/gluconeogenesis activity ratio is the body's need for energy.

Nearly all biological reactions in the body are catalyzed by enzymes, including the reactions of metabolic pathways. In a broad sense, regulation is achieved by four mechanisms:

- Negative or positive modulation of allosteric enzymes by effector compounds
- Hormonal activation by covalent modification or induction of specific enzymes
- Directional shifts in reversible reactions by changes in reactant or product concentrations
- Translocation of enzymes within the cell.

The concept of enzyme regulation was covered in Chapter 1, but a brief discussion of the principles is included here, with an emphasis on the regulation of carbohydrate metabolism.

Allosteric Enzyme Modulation

Allosteric mechanisms can stimulate or suppress the enzymatic activity of a pathway. An allosteric, or regulatory, enzyme is said to be positively or negatively modulated. Modulators, which are usually compounds within the pathway, generally act by altering the conformational structure of the allosteric enzyme. Allosteric enzymes catalyze unidirectional, or nonreversible, reactions. The modulators of the enzymes of the unidirectional reactions must either stimulate or suppress a reaction in one direction only. General examples of allosteric modulators are presented in the following sections.

AMP, ADP, and ATP as Allosteric Modulators

An indication of the energy status of a cell and an important regulatory factor in energy metabolism is the ratio of the cellular concentrations of ADP (or AMP) to ATP. The usual breakdown product of ATP is ADP, but as ADP increases in concentration, some of it becomes enzymatically converted to AMP as a phosphate is transferred to produce an ATP. Therefore, ADP and/or AMP accumulation can signify an excessive use of ATP and its depletion.

AMP, ADP, and ATP all act as modulators of certain allosteric enzymes, but the effect of AMP or ADP opposes that of ATP. For example, if ATP is abundant and ADP is scarce, additional energy is not needed. Energy-releasing (ATP-producing) pathways are negatively modulated, reducing the production of additional ATP. The reverse is also true; an increase in AMP (or ADP) concentration conversely signifies a depletion of ATP and the need to produce more of this energy source. In such a case, AMP or ADP can positively modulate allosteric enzymes of the energy-releasing pathways.

Two examples of positive modulation by AMP are its ability to cause a shift from the inactive form of phosphorylase *b* to an active form in glycogenolysis and the activation of phosphofructokinase in the glycolytic pathway, discussed in the next paragraph. Increased levels of AMP are accompanied by an enhanced activity of either of these reactions that encourages glucose catabolism. The resulting shift in metabolic direction, as signaled by the AMP buildup, causes the release of energy as glucose is metabolized and helps restore depleted ATP stores.

Phosphofructokinase is modulated positively by AMP and ADP and negatively by ATP. As the store of ATP increases, slowing of the glycolytic pathway is called for. Phosphofructokinase is an extremely important rate-controlling allosteric enzyme and is modulated by a variety of substances. Its regulatory function has already been described in Chapter 1.

Other regulatory enzymes in carbohydrate metabolism that are modulated by ATP—all negatively—are pyruvate dehydrogenase complex, citrate synthase, and isocitrate dehydrogenase. Pyruvate dehydrogenase complex is

positively modulated by AMP, and citrate synthase and isocitrate dehydrogenase are positively modulated by ADP.

Regulatory Effect of NADH/NAD⁺ and NADPH/NADP⁺

Another example of allosteric mechanisms is the ratio of NADH to NAD⁺. NADH and NAD⁺ can regulate their own formation through negative modulation. NADH is a product of glycolysis. Its buildup would indicate the pathway is not needed to produce additional ATP. If NAD⁺ accumulates, the oxidative step in glycolysis would be favored. In the fasted state, the liver typically has a high NAD⁺/NADH ratio (about 700, meaning that the level of NADH is low) and it produces more glucose than it needs through gluconeogenesis, releasing the glucose into the blood. In contrast, muscle will be actively catabolizing glucose, and its NAD⁺/NADH ratio will be lower and will favor lactate production. Dehydrogenase reactions, which involve the interconversion of the reduced and oxidized forms of the cosubstrate, are reversible. If metabolic conditions cause either NADH or NAD⁺ to accumulate, the equilibrium is shifted to return the ratio to normal. Pyruvate dehydrogenase complex is positively modulated by NAD⁺, whereas pyruvate kinase, citrate synthase, and α -ketoglutarate dehydrogenase are negatively modulated by NADH.

The pentose phosphate pathway, which makes pentoses or NADPH under different conditions, is dependent upon the level of NADPH and NADP⁺. Glucose-6-phosphate dehydrogenase is inhibited by high levels of NADPH and acetyl-CoA, indicating that demands for lipid biosynthesis are met. If the NADPH levels drop, the pathway can produce ribose. If the cell has more ribose than needed, the pathway follows the nonoxidative reactions on the right side of Figure 3.31 and makes more glucose and more NADPH.

Covalent Regulation

Covalent modification is another mechanism of enzyme and metabolic pathway regulation. This involves the binding or unbinding of a group by a covalent bond and is one of the mechanisms by which hormones can exert their action. Examples include the covalent regulation of glycogen synthase and glycogen phosphorylase, enzymes discussed in the sections on glycogenesis and glycogenolysis, respectively.

Phosphorylation inactivates glycogen synthase, whereas dephosphorylation activates it. In contrast, phosphorylation activates glycogen phosphorylase, and dephosphorylation inactivates it. These actions can be controlled by the actions of glucagon and epinephrine. Both hormones function by the phosphorylation of pathway enzymes through the second messenger cAMP.

Directional Shifts in Reversible Reactions

Another control mechanism for pathways is based on enzyme kinetics, the concentration of the reactants and products in the cell. Most enzymes catalyze reactions reversibly, and the preferred direction in which a reversible reaction is proceeding at a particular moment is largely dependent upon the relative concentration of each reactant and product. An increasing concentration of one of the reactants drives or forces the reaction toward forming the other.

This concept is exemplified by the phosphoglucomutase reaction, which interconverts glucose-6-phosphate and glucose-1-phosphate and which functions in the pathways of glycogenesis and glycogenolysis (see Figures 3.16 and 3.18). At times of heightened glycogenolytic activity (rapid breakdown of glycogen), glucose-1-phosphate concentration rises sharply, driving the reaction toward the formation of glucose-6-phosphate. With the body at rest, gluconeogenesis and glycogenesis are accelerated, increasing the concentration of glucose-6-phosphate. This increase in turn shifts the phosphoglucomutase reaction toward the formation of glucose-1-phosphate and ultimately glycogen.

Enzyme Translocation

The movement and position of an enzyme within a cell influences its catalytic activity. An example is hexokinase in skeletal muscle. Recall that hexokinase catalyzes the phosphorylation of glucose entering the muscle cell (Figure 3.20). The product, glucose-6-phosphate, can enter glycolysis for ATP production or enter glycogenesis for storage, depending on the energy needs of the cell. Hexokinase functions in the cytosol, but can physically bind to the outer membrane of mitochondria. This creates a biological advantage of having preferential access to ATP generated by mitochondria so that glucose is immediately phosphorylated upon entry into the cell.

When glucose and insulin are abundant following a meal, hexokinase translocates to the mitochondrial surface in response to insulin, thus ensuring rapid phosphorylation of glucose entering the cell. In the absence of insulin, hexokinase dissociates from the mitochondria, slowing its activity to basal levels.

In resting muscle, the need to store glucose as glycogen is high and the enzyme glycogen synthase is physically associated with the growing glycogen molecule. However, glycogen breakdown during exercise causes glycogen synthase to translocate away from glycogen to the cytoskeleton within the cell, thus slowing its activity [25].

Genetic Regulation

Another important example of enzyme regulation is through genetic control. The abundance of an enzyme can be either induced or suppressed. Such a change might arise through a prolonged shift in the dietary intake of certain nutrients. Induction stimulates transcription of new messenger RNA, programmed to produce the enzyme.

Specific hormones can influence (induce or suppress) the expression of a gene. One of the actions of certain hormones such as cortisol is to stimulate protein breakdown and decrease protein synthesis in skeletal muscle. In the liver, cortisol stimulates glycogen synthesis and gluconeogenesis by increasing the expression of several genes that encode for enzymes of the gluconeogenic pathway.

Metabolic Control of Glycolysis and Gluconeogenesis

Most enzymatic reactions are reversible, depending on their free energy. Yet, at any given moment, the pathways in a cell are going in only one direction depending on the cell's metabolic status. The previous sections reviewed the different methods the body uses for controlling metabolic pathways. Glycolysis and gluconeogenesis provide examples of these control mechanisms in action. Figure 3.32 shows the reactions in both pathways that are under metabolic control by the mechanisms discussed, with the regulation of glycolysis on the left and that of gluconeogenesis on the right. The modulators that are activators are indicated by a plus sign, and those that are inhibitors by a minus sign.

The end result of gluconeogenesis is the formation of glucose, the molecule with which glycolysis begins. It is also true that the end product of glycolysis is pyruvate, and pyruvate is the first reactant of gluconeogenesis. But as was pointed out earlier, gluconeogenesis is not simply the reversal of glycolysis. These two pathways are controlled reciprocally. Which of the two pathways is active at a given time depends on the energy status of the cell. In glycolysis there are three regulated enzymes, all of which catalyze exergonic reactions: hexokinase (glucokinase), phosphofructokinase, and pyruvate kinase. These three reactions are replaced in the gluconeogenic pathway with those catalyzed by glucose-6-phosphatase; fructose-1,6-bisphosphatase; and the pyruvate carboxylase–phosphoenolpyruvate carboxylase pair. The control of these reactions is considered for each pathway. The fate of pyruvate is strongly dependent upon acetyl-CoA levels. Acetyl-CoA inhibits the glycolytic enzyme pyruvate kinase allosterically and activates pyruvate carboxylase. This latter enzyme is found only in the mitochondria and is part of the gluconeogenic pathway that transfers mitochondrial pyruvate to phosphoenolpyruvate. If the TCA cycle is not active (adequate

cellular ATP), the pyruvate is converted to glucose via gluconeogenesis.

In gluconeogenesis, glucose-6-phosphatase is controlled by the level of substrate. Because the K_m for this enzyme is much higher than the level of glucose-6-phosphate that is normally present, the reaction proceeds very slowly unless a high concentration of this substrate accumulates. A buildup of glucose-6-phosphate is needed to activate the gluconeogenesis pathway.

Another control point for gluconeogenesis is the enzyme fructose-1,6-bisphosphatase, which is allosterically inhibited by AMP and activated by citrate. The effects of AMP and citrate on this enzyme are the opposite in glycolysis. When AMP levels are low (which means ATP is adequate), the gluconeogenesis pathway is active and glycolysis is decreased. Another allosteric regulator of fructose-1,6-bisphosphatase is fructose-2,6-bisphosphate. The levels of fructose-2,6-bisphosphate are controlled by the enzyme phosphofructokinase-2. This enzyme is different than the phosphofructokinase of the glycolytic pathway. Fructose-6-phosphate (the substrate of phosphofructokinase of glycolysis) activates phosphofructokinase-2, which would inhibit gluconeogenesis.

Another means of control for these two pathways is the level of enzymes. In glycolysis, glucokinase, phosphofructokinase, and pyruvate kinase are inducible enzymes, meaning that their concentrations can rise and fall in response to molecular signals, particularly sustained change in the concentration of a certain metabolite. In the gluconeogenic pathway glucose-6-phosphatase, fructose bisphosphatase, phosphoenolpyruvate carboxykinase, and pyruvate carboxylase are inducible. The other enzymes of both pathways are constitutive, meaning that their rate of synthesis is constant. Glucagon and glucocorticoid hormones are known to stimulate gluconeogenesis by inducing the key gluconeogenic enzymes to form, and insulin may stimulate glycolysis by inducing increased synthesis of key glycolytic enzymes.

The interrelationship among pathways of carbohydrate metabolism is exemplified by the regulation of blood glucose concentration. The integration of the pathways, a topic of Chapter 7, is best understood after metabolism of lipids and amino acids has been discussed (Chapters 5 and 6). Largely through the opposing effects of insulin and glucagon, the fasting serum glucose level is normally maintained within the approximate range of 80–100 mg/dL (4.5–5.5 mmol/L). Whenever blood glucose levels are excessive or sustained at high levels because insulin is insufficient, other insulin-independent pathways of carbohydrate metabolism for lowering blood glucose become increasingly active. Such insulin-independent pathways are indicated in Figure 3.34. The overactivity of these pathways in certain tissues is believed to be partly responsible for the clinical manifestations of type 1 diabetes mellitus.

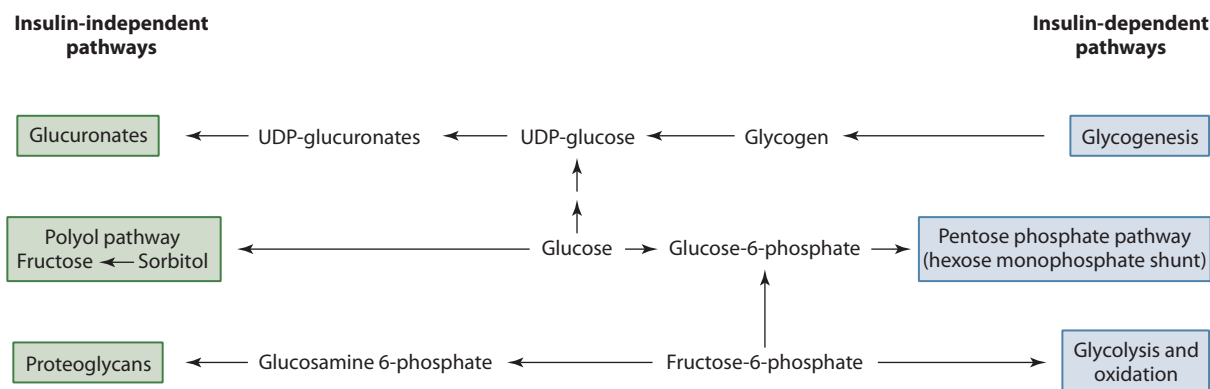


Figure 3.34 Insulin-independent and -dependent pathways of glucose metabolism.

SUMMARY

This chapter deals with a subject of vital importance in nutrition: the transfer of energy from nutrient molecules to ATP energy usable by the body. Important food sources of that energy are carbohydrates.

- The major sources of dietary carbohydrate are the starches and the disaccharides (sugars). During digestion, these are hydrolyzed by specific glycosidases to their component monosaccharides, primarily glucose, fructose and galactose.
- The monosaccharides are absorbed into the intestine cell by active and facilitated transport.
- Practically all dietary fructose and galactose is transported into the liver to be metabolized. Some glucose is transported into the liver, while the majority of glucose is transported in the blood to various tissues.
- Transport from the blood across the cell membrane occurs by facilitated transport, mediated by GLUT proteins.
- Different tissues use different GLUTs that are part of the family of glucose transporters. The GLUT4 that transports glucose into muscle and adipose tissue is stimulated by insulin. Insulin translocates the pre-formed GLUT4 from intracellular vesicles to the cell membrane.
- In the cells, monosaccharides are immediately phosphorylated at the expense of ATP, and then the monosaccharides can follow any of several integrated pathways of metabolism.
- In muscle, brain, and adipose tissue, glucose is phosphorylated by hexokinase (types 1 and 2). In the liver, glucose is phosphorylated by an isoenzyme of hexokinase called glucokinase; fructose is phosphorylated mainly by fructokinase; and galactose is phosphorylated by galactokinase.

During times of energy excess, cellular glucose and certain metabolites can be converted to glycogen, primarily in liver and skeletal muscle. Liver glycogen is mostly made from dietary and circulating glucose, while about one-third of the glucose-6-phosphate converted to glycogen is derived from gluconeogenesis (lactate, pyruvate, and TCA cycle intermediates).

When energy is needed, cellular glucose can be routed through the energy-releasing pathways of glycolysis and the TCA cycle for ATP production.

- Glycolytic reactions convert glucose from the blood or from glycogen stores to pyruvate.
 - Under anaerobic conditions, pyruvate is converted to lactate.
 - Under aerobic conditions, pyruvate is completely oxidized in the TCA cycle, releasing CO_2 and energy in the form of electrons.
 - The electrons (and protons) are captured as reduced coenzymes (NADH and FADH_2) that are delivered to the mitochondrial electron transport chain. The energy released by electron transfer drives the phosphorylation of ADP to form ATP.
 - On complete oxidation, approximately 40% of this energy is retained in the high-energy phosphate bonds of ATP. The remaining energy supplies heat to the body.
- Noncarbohydrate substances derived from the other major nutrients can be converted to glucose or glycogen by the pathways of gluconeogenesis.
- Noncarbohydrate sources include lactate from red blood cells and muscle, glycerol from triacylglycerols, and certain amino acids.
 - The basic carbon skeleton of fatty acids (metabolized to acetyl-CoA units) cannot be converted to a net

synthesis of glucose, but some of the carbons from fatty acids find their way into the carbohydrate molecule due to small amounts of TCA cycle intermediates being used in gluconeogenesis.

- In gluconeogenesis, the reactions are basically the reversible reactions of glycolysis, shifted toward glucose synthesis in accordance with reduced energy demand by the body. Three kinase reactions occurring in glycolysis are not reversible, requiring the involvement of different enzymes and pathways to circumvent those reactions in the process of gluconeogenesis.
- Muscle glycogen provides a source of glucose for energy only for muscle fibers in which it is stored because muscle lacks glucose-6-phosphatase, the enzyme that produces free glucose from glucose-6-phosphate.
- Glucose-6-phosphatase is active in the liver, however, which means that the liver can release free glucose from its glycogen stores into the circulation for maintaining blood glucose and for use by other tissues. The Cori cycle describes the liver's uptake and gluconeogenic conversion of muscle-produced lactate to glucose.

In Chapters 5 and 6, we see that fatty acids and the carbon skeleton of various amino acids are ultimately oxidized through the TCA cycle.

- The amino acids that become TCA cycle intermediates, however, may not be completely oxidized to CO_2 and H_2O , but instead may leave the cycle to be converted to glucose or glycogen (by gluconeogenesis) should dietary intake of carbohydrate be low.
- The glycerol portion of triacylglycerols enters the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point it can be oxidized for energy or used to synthesize glucose or glycogen. The fatty acids from triacylglycerols enter the TCA cycle as acetyl-CoA, which is oxidized to CO_2 and H_2O but cannot contribute carbon for the net synthesis of glucose.

- Entrance of noncarbohydrate substances into the energy pathways is an important reminder that these pathways are not exclusively committed to carbohydrate metabolism. Rather, they represent common ground for the interconversion and oxidation of fats and proteins as well as carbohydrate.

Much of the energy needs of the body are met by the production and utilization of ATP, the main distributor of energy for metabolic reactions.

- ATP can be generated by “substrate-level phosphorylation” that involves the direct transfer of a phosphate group from compounds with a very-high-energy phosphate transfer potential to ADP.
- ATP is also generated by the TCA cycle and oxidative phosphorylation. This process involves the passage of high-energy electrons derived from food molecules through the electron transport chain in mitochondria, creating an energy gradient used to phosphorylate ADP to form ATP.
- Oxidative phosphorylation is the major route for ATP production. Electron flow in the electron transport chain is from reduced cosubstrates to molecular oxygen. Molecular oxygen becomes the ultimate oxidizing agent and becomes H_2O in the process. The downhill flow of electrons and proton translocation generate sufficient energy to affect oxidative phosphorylation at multiple sites along the chain. The energy from this process that is not conserved as chemical energy (ATP) is given off as heat. About 60% of the energy assumes the form of heat.

The pentose phosphate pathway generates important intermediates not produced in other pathways of the body, including pentose phosphates for RNA and DNA synthesis and NADPH, which serves as an electron (and H^+) donor in the synthesis of fatty acids.

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Perspective

WHAT CARBOHYDRATES DO AMERICANS EAT?

Although a seemingly simple question, measuring the food and nutrients we eat is a difficult task. Several methods, based primarily on self-reported data, have been used to directly assess the amount of food consumed by individuals [1]. The accuracy of such methods depends entirely on the ability of subjects to know the foods they are eating; to know portion size and record the amount of each food; to record every food and beverage consumed; and to be truthful. In view of these requirements, it is easy to see why direct methods frequently result in underreporting of intake, particularly by subjects with elevated body mass index [2–5]. The National Health and Nutrition Examination Surveys (NHANES), funded and managed by the Centers for Disease Control and Prevention, have been ongoing since the 1960s and represent the most widely used dataset for estimating food intake using direct assessment [6].

A different approach to estimating food intake is to measure the amount of food available for human consumption in the United States. The total amount available of each food category is then divided by the total population for each year and expressed on a per capita basis. The U.S. Department of Agriculture (USDA) has been reporting such data since 1909, which is useful for determining food consumption trends because they are a proxy for actual food intake. Food availability data—sometimes called food “disappearance” because the data reflect available food that “disappears” into the food marketing system—may overestimate actual intake by individuals due to inclusion of nonedible food portions and food lost through waste and spoilage in the home and marketing system. Consequently, the USDA provides loss-adjusted food availability data to more closely reflect actual intake [7].

Documenting food intake by direct or indirect methods is just the first step in learning what nutrients we consume. Converting food intake into nutrient intake requires knowledge about the chemical

composition of every food consumed. The Agricultural Research Service of the USDA maintains the most comprehensive system for collecting and disseminating food composition data. Information compiled at *FoodData Central* provides the basis for nearly all public and commercial nutrient databases and food composition tables used in the United States and several foreign countries [8]. The Nutrient Database contains information for approximately 150 components of food, including essential and nonessential nutrients, for thousands of individual foods. The database is constantly being updated and expanded as new information becomes available. The data comes from academic research, the food industry, government laboratories, and independent food-testing laboratories. Values in the database may also be based on calculations using appropriate algorithms, factors, or recipes.

Valuable information regarding food and nutrient intake can be obtained by combining the food availability and nutrient composition data. Each database is freely accessible and can be downloaded for combining, although the USDA has already done much of the work for us. Spreadsheets containing the combined data through 2010 are available for downloading; combined data later than 2010 must be calculated by the user [8]. With this arsenal of data, one can choose to examine the type and amount of food consumed, their nutrient composition, or the amount of nutrients consumed by major food groups.

CARBOHYDRATES IN THE FOOD SUPPLY

Examining the USDA data reveals many things. First, carbohydrates are the most abundant macronutrient (by weight) in the food supply and contribute most of the total energy in the American diet (as shown in Figure 1). Second, carbohydrate availability since 1970 has increased about 10% [9]. This period of time is significant because obesity prevalence in children and adults

increased in parallel [10]. It is tempting to blame the increase in carbohydrate intake for the increase in obesity prevalence, but one should be cautious in assuming a direct causal relationship on the basis of correlations alone without further research.

A third observation gleaned from the USDA data for the year 2010 is that most of the carbohydrate was provided by grain products (42%) and sugar and sweeteners (35%). The remaining contributors of carbohydrate were comparatively minor and included vegetables (7%), fruits (6%), and dairy products (6%). Moreover, during the four-decade period between 1970 and 2010 the carbohydrate contributed by grain products increased 24%, whereas the contribution from sugar and sweeteners as a group increased only about 1% [8]. Many consumers may be confused by this outcome because the facts contradict the avalanche of misinformation in the lay press and on social media claiming that intake of sugar and sweeteners has skyrocketed—a conclusion that is clearly not supported by data.

Confusion may also stem from the widespread misunderstanding of sugar and sweeteners in the food supply. As illustrated in Figure 2, sugar (sucrose) was the primary sweetening agent used in 1970. High-fructose corn syrups (HFCS) were introduced after 1970 as a sugar alternative because of lower cost and desirable functional properties. Two major types of HFCS are used by food manufacturers, HFCS-42 and HFCS-55. The saccharide composition of HFCS-42 is 42% fructose, 52% glucose, and 6% other saccharides, whereas the saccharide composition of HFCS-55 is 55% fructose, 41% glucose, and 4% other saccharides. Food manufacturers generally use HFCS-42 as a sweetening agent in dry products such as cereals and baked goods. HFCS-55 is used mainly in beverages such as fruit juices and soft drinks. When present together in the U.S. food supply, the two HFCS contribute about equal proportions of fructose and glucose, which is identical to the saccharide composition of sugar (50% fructose,

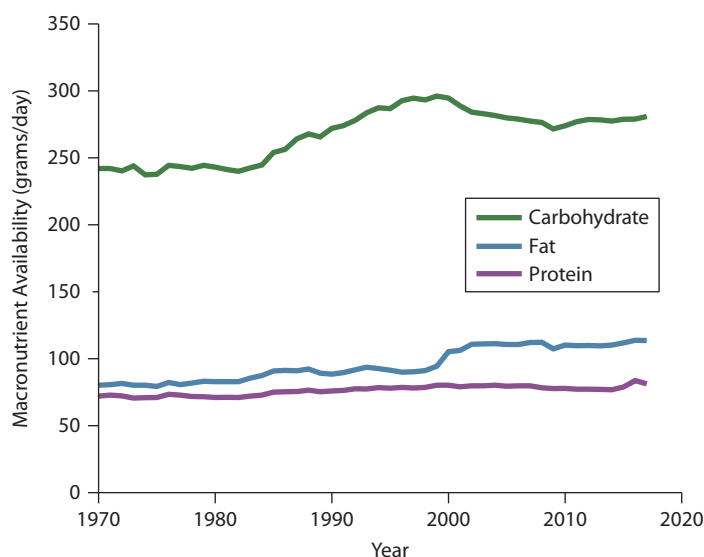


Figure 1 Per capita availability of macronutrients from all food sources in the U.S. food supply.

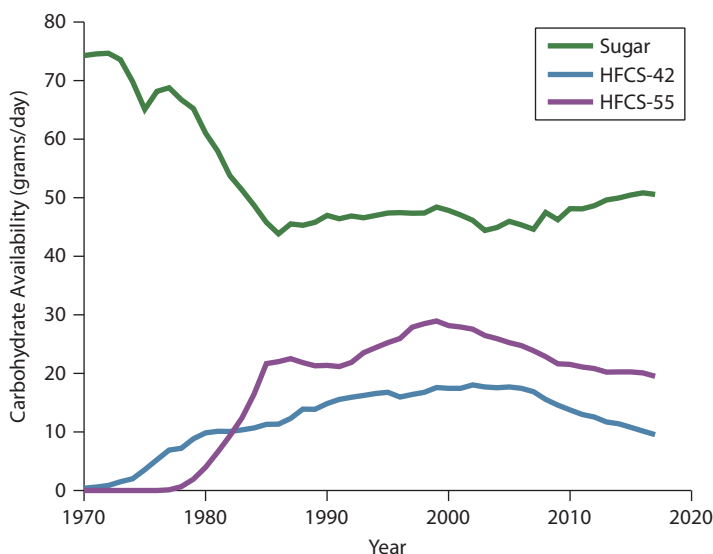


Figure 2 Per capita availability of carbohydrates from sugar and high-fructose corn syrups (HFCS) in the U.S. food supply.

50% glucose). So while it is true HFCS have significantly increased in the food supply since 1970, the availability of sugar and sweeteners combined has changed very little, due to the replacement of sugar with HFCS. Consumers can be easily misled when they hear only the HFCS story.

GLUCOSE VERSUS FRUCTOSE

All digestible carbohydrates in the food supply, irrespective of the food source, must be broken down to their monosaccharide units for absorption into the body. Nature provides foods that, when digested, yield mostly glucose and fructose (and, to a lesser extent, galactose if dairy products are

consumed). Starch yields exclusively glucose; sugar and HFCS yield equal amounts of glucose and fructose; dairy products containing lactose yield glucose and galactose; and some foods provide glucose and fructose as monosaccharides. In view of the heightened awareness of fructose as a potential contributor to obesity-related diseases [11a], it is useful to express the USDA food availability data in terms of the component monosaccharides resulting from carbohydrate digestion. Making such a calculation, as shown in Figure 3, allows us to examine the amounts of glucose, fructose, and galactose available for absorption from all digestible carbohydrates consumed.

Figure 3 can be interpreted as the “carbohydrate” line from Figure 1, broken down into its monosaccharide units. Viewing the data this way clearly shows glucose, not fructose, is the most abundant saccharide provided by food carbohydrates. Recall that the major food source of carbohydrates is grain products that contribute only glucose when starch is digested. The second most abundant food source of carbohydrates, sugars and sweeteners, contributes equal amounts of glucose and fructose, while fruits and vegetables contribute smaller amounts of both glucose and fructose. Thus, every food category that contains carbohydrate contributes glucose, resulting

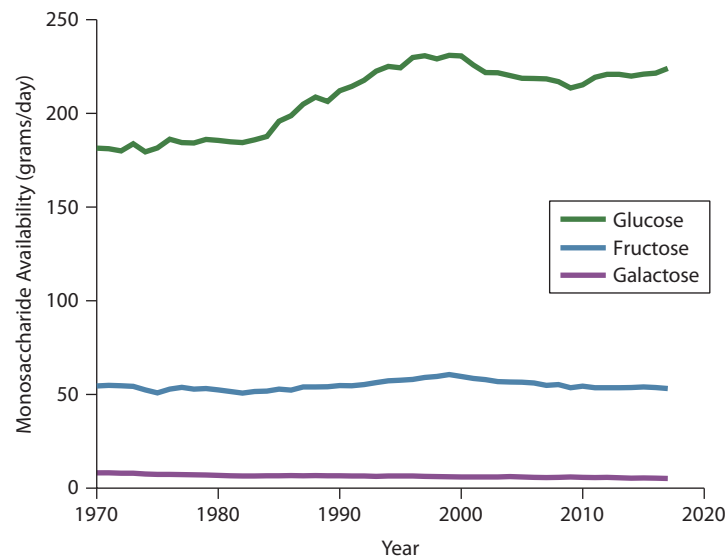


Figure 3 Per capita availability of monosaccharides from digestible carbohydrates in the U.S. food supply.

in four times more glucose than fructose in the food supply.

Another important observation from Figure 3 is the change that occurred in monosaccharide availability between 1970 and 2017. The overall trend in glucose availability increased 13%, whereas the overall trend in fructose availability has not changed during this 48-year period [9]. Once again, these facts contradict information found in the lay press and on social media that incorrectly emphasize an increase in fructose when the spotlight should be focused on the significant increase in glucose.

Conclusion

Measuring food and nutrient intake of Americans can be accomplished by indirect methods using the USDA food availability and nutrient composition databases. This approach offers the advantage of examining trends over time (decades) and it avoids the difficulties of surveying individuals directly. Using the loss-adjusted USDA data also allows us to determine the food sources of all major nutrients and the amounts available on a per capita basis. In this Perspective, examination of the food sources and amounts of carbohydrate in the U.S. food supply reveals the following:

- Carbohydrates are the most abundant macronutrient in the food supply and provide the majority of dietary energy.
- Grain products are the primary source of dietary carbohydrate, followed by sugar and sweeteners.

- Between 1970 and 2010, the availability of carbohydrate from all food sources increased 10%.
- Between 1970 and 2010, the availability of carbohydrate from grain products increased 24%.
- Between 1970 and 2010, the availability of carbohydrate from sugar and sweeteners increased 1%.
- The sugar and sweeteners category has not significantly increased because HFCS have merely replaced sugar.
- Upon digestion, food carbohydrates yield four times more glucose than fructose.
- Between 1970 and 2010, the availability of glucose from all food sources increased 13%.
- Between 1970 and 2010, the availability of fructose from all food sources did not change.

Conclusions from these findings are best made when the entire picture is considered. Misinterpretations can easily be made when only a portion of the findings is used. For example, the use of HFCS has significantly increased since 1970, leading some to conclude that HFCS (and the fructose they contribute) are the cause of obesity and metabolic diseases. However, when one considers the entire picture, the increased use of HFCS has mirrored the decline in sugar usage, resulting in virtually no change in the amount of saccharides contributed by the combined sugar

and sweeteners group. Also, the glucose-to-fructose ratio in sugar and the HFCS together is approximately the same and has not changed since 1970, so fructose availability has remained relatively unchanged.

Perhaps the most important conclusion from the USDA data should focus on glucose as the major saccharide contributed by carbohydrates in the U.S. food supply. Significantly more glucose compared to fructose is available for intestinal absorption as a result of eating a typical American diet. Furthermore, the overall trend in glucose availability has increased since 1970, due mainly to increased availability of grain products. When addressing the dietary factors that contribute to obesity, it is logical to focus attention on all carbohydrates, the main energy source from food, to control total energy intake. The USDA data indicate that glucose from starch in grain products is the major carbohydrate that contributes to total energy intake.

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FIBER

LEARNING OBJECTIVES

- 4.1 Identify the different types of fiber.
- 4.2 Describe the properties of fiber and their physiological impact.
- 4.3 Explain how high-fiber diets may reduce risk for some diseases.
- 4.4 Describe recommendations for fiber intake.
- 4.5 Identify food sources of fiber.

FIBER NOT ONLY ENHANCES THE HEALTH OF THE GASTROINTESTINAL TRACT, BUT FIBER-RICH FOODS PLAY KEY ROLES IN THE PREVENTION AND MANAGEMENT OF SEVERAL DISEASES. The varied health benefits of fiber are related to the fact that fiber is not a single entity or even a group of chemically related compounds, but instead consists of multiple different components with distinctive characteristics. This chapter addresses definitions, chemistries, properties, sources, health benefits, allowed health claims, food labels, and recommended intake of fiber.

4.1 DEFINITIONS

With the publication of the 2002 Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein, and Amino Acids by the National Academy of Sciences Food and Nutrition Board, definitions for dietary, functional, and total fiber were established.

- **Dietary fiber** refers to nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants [1]. Dietary fibers include cellulose, hemicellulose, pectins, lignin, gums, β -glucans, fructans, and resistant starches [1].
- **Functional fiber** consists of isolated, extracted, or manufactured nondigestible carbohydrates that have been shown to have beneficial physiological effects in humans [1]; they are usually added to foods as well as found in supplements.
- **Total fiber** refers to dietary fiber present within the food plus functional fiber that has been added to the food.

All dietary fibers and the mucilage psyllium are functional fibers with the exceptions of hemicellulose, fructans, and lignin (the Food and Nutrition Board stated that fructans and lignin require additional studies showing beneficial physiological effects in humans to be classified as functional fibers) [1]. Chitin and chitosan and polydextrose and polyols also require additional studies showing positive physiological effects in humans to be considered functional fibers [1].

A branch of the World Health Organization adopted another definition of dietary fiber.

- Dietary fiber includes carbohydrate polymers with 10 or more monomeric units (i.e., monosaccharides), which are not hydrolyzed by human digestive enzymes or absorbed and
 - are in foods (intrinsic and intact), or
 - have been extracted from food and have physiological benefits to health, or
 - are synthetic or modified and have physiological benefits to health [2].

Not included under this definition are oligosaccharides with degrees of polymerization between 3 and 9 (i.e., oligosaccharides containing chains of three to nine monosaccharides) such as some fructooligosaccharides and galactooligosaccharides [2]. The term *fiber* that appears on food labels reflects dietary fiber but includes oligosaccharides.

4.2 FIBER AND PLANTS

Fiber is found in plant foods. Figure 4.1 shows the anatomy of a wheat plant. The endosperm of the plant contains mostly starch along with small quantities of fiber (mainly cellulose, hemicellulose, and resistant starch). The germ layer is rich primarily in some vitamins, minerals, and essential fatty acids, but also contains small amounts of fiber (mainly cellulose, lignin, and fructans). It is the bran component of cereals that contains the most fiber (over 95%). The outer bran layer of cereals consists of primary and secondary cell walls. These walls are fiber-rich, containing strands of cellulose arranged within a matrix of other fibers, especially hemicellulose and pectins, but also lesser amounts of fructans, resistant starch, and β -glucans. Other substances such as suberin (consisting of various phenolic compounds, long-chain alcohols, and polymeric

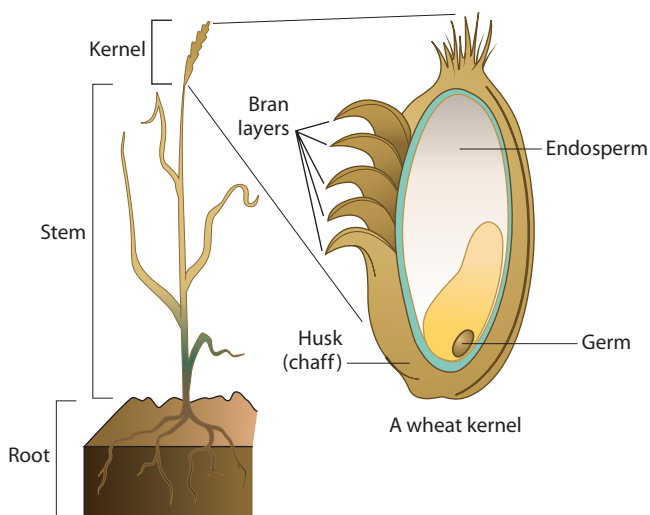


Figure 4.1 The partial anatomy of a wheat plant.

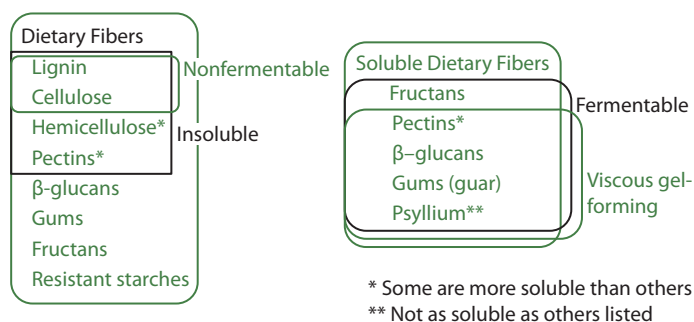


Figure 4.2 Dietary fibers and some of their selected properties.

esters of fatty acids), cutin (also made of polymeric esters of fatty acids that is secreted onto the plant surface), and waxes (complex hydrophobic, hydrocarbon compounds that coat the plant's external surfaces) are also components of the cell wall but do not contribute to the fiber content. Additional fibers may also be found within plants, but these vary with the plant species, the part of the plant (leaf, root, or stem), and the plant's maturity.

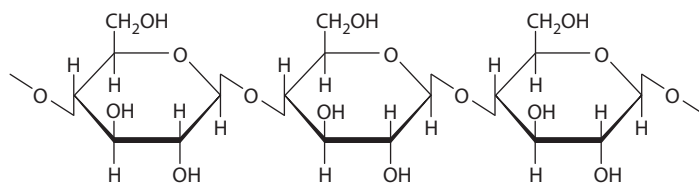
Whole-grain cereals and grain products provide cellulose, hemicellulose, lignin, some gums, β -glucans, some galactooligosaccharides (mainly raffinose and stachyose), and some fructans. Of the cereals, rye and barley typically contain more fiber than other grains. Fruits and vegetables provide almost equal quantities (~30%) of cellulose and pectin as well as some hemicellulose and, in selected fruits, some fructans and lesser amounts of other fibers. Legumes are also fiber-rich, containing cellulose, hemicellulose, pectins, gums, galactooligosaccharides, and resistant starches, among others. This next section reviews the chemistry and characteristics of fibers. Figure 4.2 shows these fibers and selected characteristics of the fibers; these characteristics and their impact on physiological processes and health are discussed in later sections of the chapter.

4.3 CHEMISTRY AND CHARACTERISTICS OF FIBER

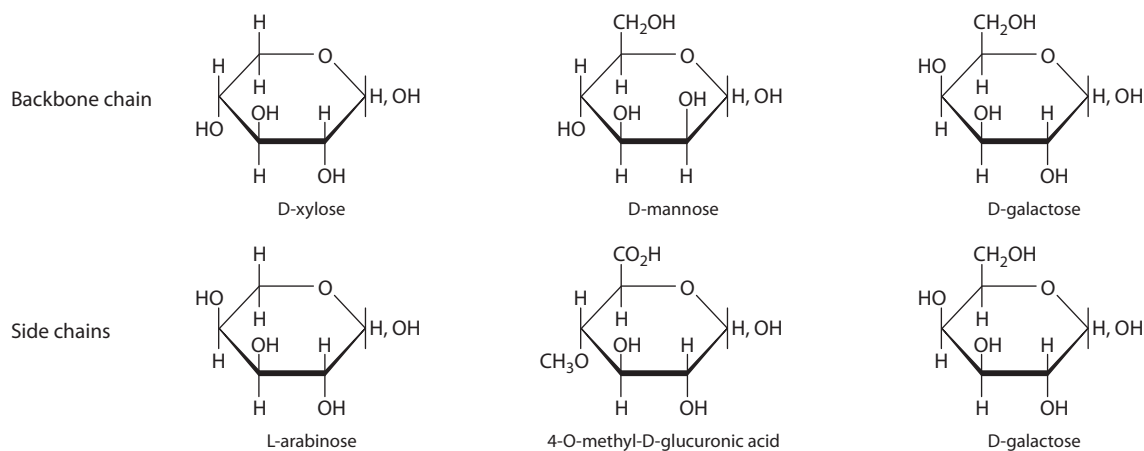
Cellulose

Cellulose (Figure 4.3a), a dietary fiber and functional fiber, is a long, linear **polymer** (a high-molecular-weight substance made up of a repeating chain) of up to 10,000 β (1-4)-linked glucose units. Hydrogen bonding between sugar residues in adjacent, parallel-running cellulose chains imparts a three-dimensional structure to cellulose. Being a large, linear, neutrally charged molecule, cellulose is water insoluble, although it can be modified chemically (e.g., carboxymethyl cellulose, methylcellulose, and hydroxypropyl methylcellulose) for use as a food additive and this modified form may be more water soluble and a

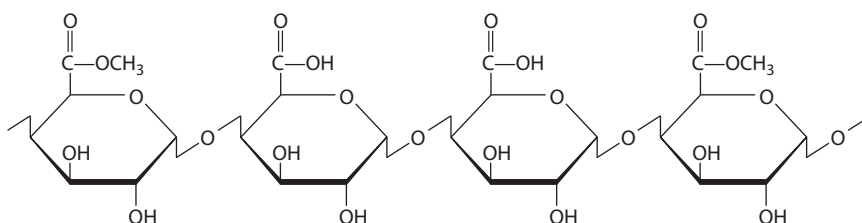
(a) Cellulose



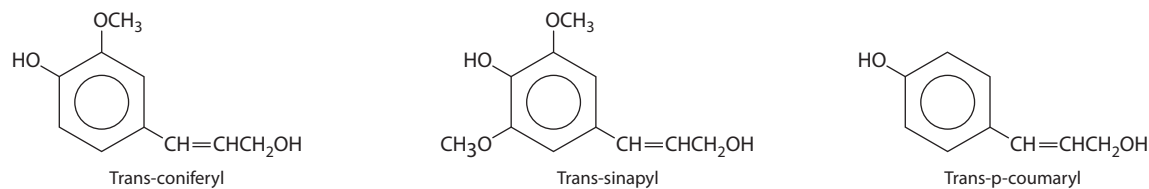
(b) Hemicellulose (major component sugars)



(c) Pectin



(d) Phenols in lignin



(e) Gum arabic

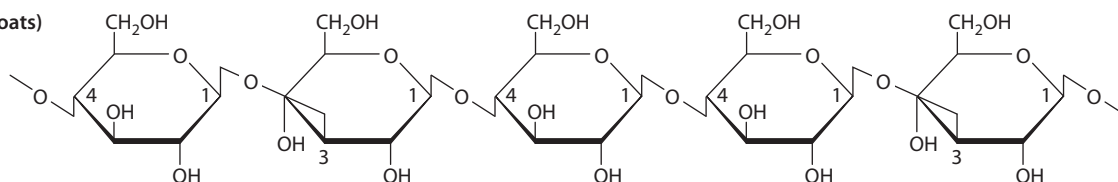
(f) β -glucan (from oats)

Figure 4.3 Chemical structures of dietary fibers and some functional fibers.

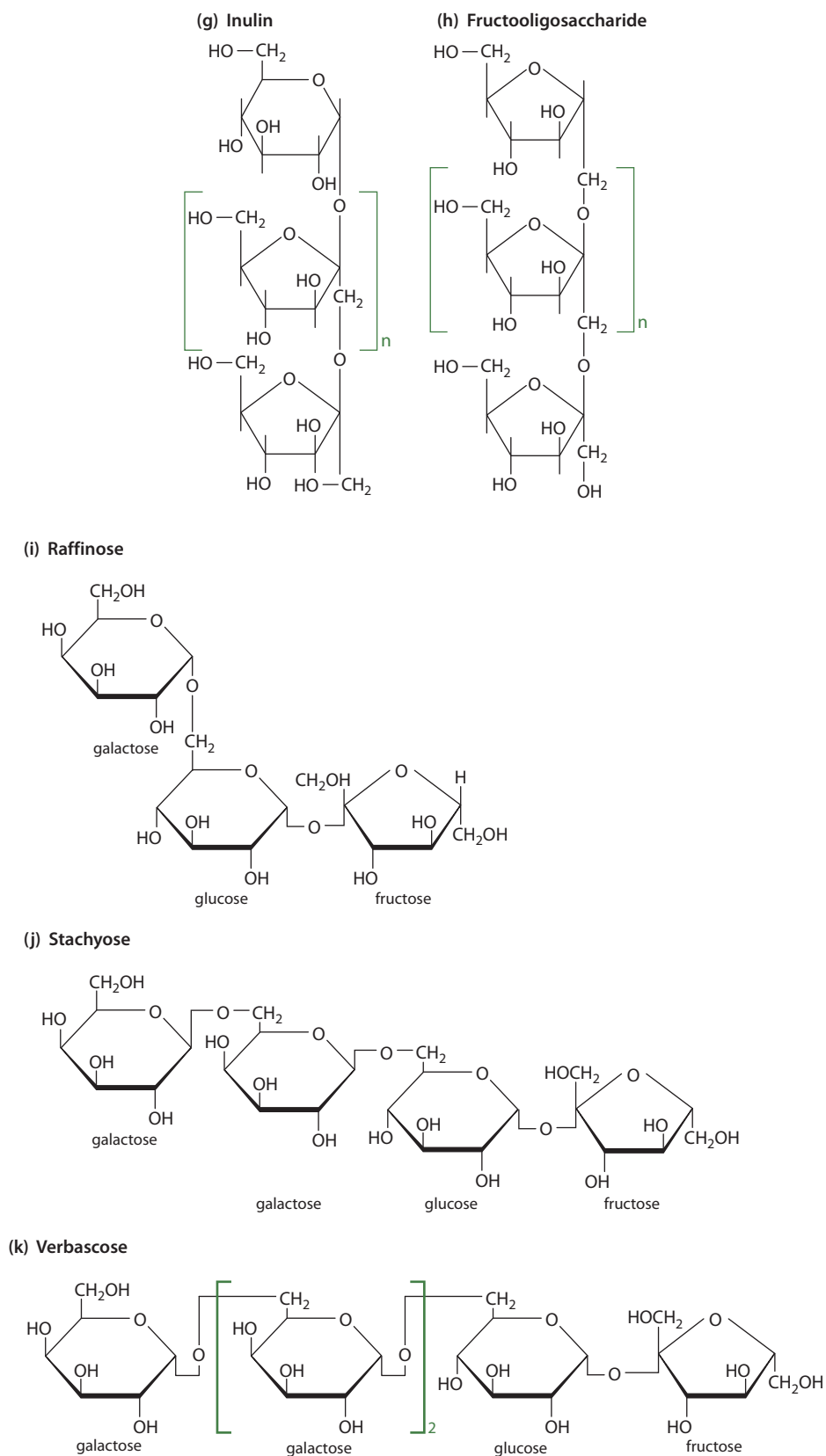


Figure 4.3 (Continued)

little more fermentable by colonic bacteria than naturally occurring cellulose. Cellulose that is found naturally in foods is not typically degraded by colonic bacteria. Examples of some cellulose-rich foods include whole grains, bran, legumes, peas, nuts, root vegetables, vegetables of the cabbage family, seeds (mainly the outer covering), and apples. Purified, powdered cellulose (usually isolated from wood) and modified cellulose are added to foods, for example, as a thickening or texturing agent or to prevent caking or syneresis (leakage of liquid). Some examples of foods to which cellulose or a modified form of cellulose is added include breads, cake mixes, sauces, sandwich spreads, dips, frozen meat products (e.g., chicken nuggets), and fruit juice mixes.

Hemicellulose

Hemicellulose, another dietary fiber, consists of a heterogeneous group of polysaccharides. These polysaccharides vary among plants and within a plant depending on location. One example of a hemicellulose structure is β (1-4)-linked D-xylopyranose units with branches of 4-O-methyl D-glucopyranose uronic acids linked by α (1-2) bonds or with branches of L-arabinofuranosyl units linked by α (1-3) bonds. Hemicelluloses contain both hexoses and pentoses in their backbone and branched side chains. The β (1-4)-linked sugars in the backbone, which form a basis for hemicellulose classification, usually include the pentose xylose and hexoses such as mannose and galactose, while sugars such as arabinose, glucuronic acid, and galactose, among others, are found in the side chains. Some of these sugars are shown in Figure 4.3b. The sugars in the side chains confer important characteristics on the hemicellulose. For example, hemicelluloses that contain acids in their side chains are slightly charged and more water soluble, whereas other hemicelluloses are water insoluble. Similarly, fermentability of the hemicelluloses by intestinal bacteria is also influenced by these sugars and their positions. For example, hexose and uronic acid components of hemicellulose are more accessible to bacterial enzymes and thus more fermentable than are the other hemicellulose sugars. Foods that are relatively high in hemicellulose include whole grains as well as nuts, legumes, and some vegetables and fruits.

Pectins

Pectins, a dietary and functional fiber, represent another family of heterogeneous polysaccharides found in plant cell walls, intercellular regions of plants, and in the outer skin and rind of some fruits and vegetables. Galacturonic acid is a primary constituent of pectin's backbone and is found as an unbranched chain of α (1-4)-linked D-galacturonic acid units, as shown in Figure 4.3c. Chains of pentoses (xylose and arabinose) and hexoses (galactose,

rhamnose, and fucose) are attached to pectin's backbone. Rich sources of pectins include many fruits—apples, berries, apricots, cherries, grapes, and citrus fruits—as well as legumes, nuts, and some vegetables. In some fruits, pectin is broken down as the fruit ripens and becomes softer. Commercially, pectins are usually extracted from citrus peel or apples and may be added to products, such as fruit strips, fruit juices, and icing, among others. In jellies and jams, pectin is used to promote gelling. Pectin is added to some enteral nutrition products used for tube feeding to provide a source of fiber in the diet. Pectins are highly water soluble and have a high ion-binding potential. In the digestive tract, pectins form gels (but to a lesser extent than some other fibers) and are almost completely fermented by bacteria in the colon.

Lignin

Lignin is a highly branched polymer of phenol units (versus sugars) with strong intramolecular bonding. The primary phenols that compose lignin include trans-coniferyl, trans-sinapyl, and trans-p-coumaryl, shown in Figure 4.3d. Lignin provides structural support in plant cell walls. It is found in the bran layer of cereals and in the stems and seeds of fruits and vegetables. Lignin is insoluble in water, has hydrophobic binding capacity, and is generally not fermented by colonic bacteria. Lignin is a dietary fiber and may serve as a functional fiber. Foods high in lignin include wheat, rye, mature root vegetables such as carrots, flaxseed, and fruits with edible seeds such as many berries.

Gums

Gums, also called *hydrocolloids*, are secreted at the site of plant injury by specialized secretory cells and can be exuded from plants (i.e., forced out of plant tissues). Gums that originate as tree **exudates** include gum arabic, gum karaya, and gum ghatti; gum tragacanth is a shrub exudate. Gums are often highly branched and are composed of a variety of sugars and sugar derivatives. Gum arabic, shown in Figure 4.3e, for example, contains a main galactose backbone joined by β (1-3) linkages and β (1-6) linkages with side chains of galactose, arabinose, rhamnose, glucuronic acid, or methylglucuronic acid. The nonreducing ends terminate with a rhamnopyrosyl unit. Of the tree exudates, gum arabic is most commonly used as a food additive to promote gelling, thickening, and stabilizing. It is found in candies such as caramels, gumdrops, and toffees, as well as in other assorted products.

Guar gum and locust bean gum (also called carob gum) are made from the ground endosperm of guar seeds and locust bean seeds, respectively. These water-soluble gums consist mostly of galactomannans, which contain a mannose backbone in 1-4 linkages and in a 2:1 or 4:1 ratio with

galactose present in the side chains. Guar galactomannans have more branches than locust bean galactomannans. Both guar gum and locust bean gum are added as a thickening agent and water-binding agent (among other roles) to products such as bakery goods, sauces, dairy products, ice creams, dips, and salad dressings. Gums are also found naturally in foods such as oatmeal, barley, and legumes. Gums are dietary and functional fibers. They are water soluble and some (like guar gum) form viscous gels. Gums are fermentable by colonic bacteria, especially if the gum has been partially hydrolyzed before being added to a food.

β-Glucans

β-glucans (Figure 4.3f) are homopolymers of glucose units, but are smaller in size and contain different linkages than cellulose. Oat β-glucan consists of a chain of glucoses joined mostly in β (1-4) linkages but also some β (1-3) linkages. β-glucans are highly water soluble, highly fermentable by colonic bacteria, and form viscous gels within the digestive tract. β-glucans are found in relatively high amounts in two grains: oats (oat bran, rolled oats, and whole oat flour) and barley (whole grain and dry milled). β-glucans extracted from cereals are used commercially as a functional fiber because of their effectiveness in reducing serum cholesterol and moderating blood glucose concentrations. The U.S. Food and Drug Administration (FDA) permits a health claim for β-glucans describing reductions in serum LDL cholesterol resulting from the daily consumption of ≥ 3 g of β-glucans from oats [3].

Fructans

Fructans, sometimes called *polyfructose*, include inulin, oligofructose, and fructooligosaccharides. Fructans are chemically composed of fructose units in chains of varying lengths. Inulin consists of a β (2-1)-linked fructose chain that contains from 2 to about 60 units (usually at least 10), with a glucose molecule at the end of the fructose chain linked by an α (1-2) bond (Figure 4.3g). Oligofructose is similar in structure to inulin but generally contains less than 10 fructose units. Inulin and oligofructose are dietary fibers. Fructooligosaccharides are a functional fiber formed from the partial hydrolysis of inulin or synthesized from sucrose by adding fructose; they typically contain about two to four or five fructose units and may or may not contain an end glucose molecule (Figure 4.3h). Fructans, especially fructooligosaccharides and oligofructose, are water soluble and highly fermentable by colonic bacteria, but do not form viscous gels in the digestive tract. Fructooligosaccharides and inulin also function as prebiotics, promoting the growth of healthful bifidobacteria.

Fructans (mainly inulin) are found naturally in some plants. The most common food sources of inulin include

chicory, asparagus, leeks, onions, garlic, Jerusalem artichoke, tomatoes, and bananas. The highest amounts are found in chicory with about 15–20 g per 100 g. Slightly lower amounts are found in artichoke and asparagus. Less than about 6 g of inulin are provided by 100 g of minced dried onion flakes. Wheat, barley, and rye also contain some fructans. Fructans are also added to some foods. Oligofructose is commonly used, for example, in cereals, fruit preparations for yogurt, dairy products, and frozen desserts. Inulin is used to replace fat in fillings, table spreads, dairy products, dressings, and frozen desserts, to name a few examples. Both inulin and fructooligosaccharides are found in supplements (such as fiber gummy supplements), and fructooligosaccharides are also added to foods. Americans are thought to consume up to about 4 g of fructans each day from foods.

Galactans

Galactans, also called galactooligosaccharides, are made up of about 2–10 molecules of galactose and one glucose molecule and include sugars such as raffinose, stachyose, and verbascose, among others. Raffinose is a trisaccharide of fructose, glucose, and galactose (Figure 4.3i). Stachyose is a tetrasaccharide of fructose, glucose, and galactose to which another galactose is linked (Figure 4.3j). Verbas-cose is an oligosaccharide containing fructose, glucose, and three galactose molecules (Figure 4.3k). Galactooligosaccharides are found naturally in human milk and in peas (field peas, chickpeas, and green peas), lentils, and beans (such as soy, mung, lima, snap, northern, and navy, among others). Galactooligosaccharides, like fructooligosaccharides, are not digestible by human digestive enzymes but are highly soluble and fermentable by colonic bacteria.

Resistant Starch

Resistant starch (RS) is starch that cannot be or is not easily enzymatically digested. There are five main types (numbered 1 to 5) of resistant starch; these types are based on the characteristics and nature of the resistant starch granule.

- RS₁ represents starch granules that are physically inaccessible to digestion due to their location within a section of the plant's structure (typically the cell wall or matrix). Food sources of RS₁ include whole or partially milled grains, cereals, seeds, and legumes.
- RS₂ represents starch that resists digestion because it is tightly packaged inside of granules within foods. The tight packaging is associated with the linear structure of amylose (a component of starch along with amylopectin) and is especially prevalent in some “raw or uncooked” plant foods such as unripe (green) bananas,

raw potatoes, some legumes and high-amylose maize. The heating of foods with these starches, however, gelatinizes the starch and increases its ability to be digested.

- RS_3 is called retrograde starch or amylose. It is formed with moist-heat cooking and then cooling of starch that has gelatinized. This cooking and then cooling alters the starch to make it more crystal-like and resistant to digestion. Examples of foods rich in RS_3 include cooked and cooled potatoes, rice, pasta, bread, and some corn.
- RS_4 results from chemical modifications of starch (usually isolated from corn) that is not naturally occurring in food. Examples of modifications include the formation of starch esters or cross-linked starches, which retard the ability of the starch to swell during cooking and thus keep it in a more granular form that resists digestion. This type of resistant starch is found in some corn-based products.
- RS_5 is formed when amylose in the starch granule binds to lipids; this interaction impairs the expansion of the starch granule, which is needed for digestion by enzymes. Resistant dextrins, also called *resistant maltodextrins*, are considered RS_5 . The resistant dextrin wheat dextrin is added to foods as well as found as a dietary supplement. Wheat dextrin is water soluble and fermentable by colonic bacteria; it has also been shown to enhance the growth of healthful bacteria in the colon.

Both RS_1 and RS_2 are dietary fibers. RS_3 and RS_4 are also sometimes added to foods and are considered functional fibers. Both RS_3 and RS_4 also may be partially fermented by colonic bacteria. RS_3 may also stimulate the growth of healthful bacteria in the colon and may improve the glycemic response following carbohydrate ingestion. Americans are thought to consume up to about 10 g of resistant starch daily. Consumption of up to 20 g of resistant starch has been recommended to obtain health benefits. See reference [4] for the resistant starch content of some foods commonly consumed in the United States.

Mucilages (Psyllium)

Mucilages are plant polysaccharides with a structure similar to gums. Mucilages are found in the seeds of a variety of plants, including flax and psyllium, among others. Psyllium, from the husk of psyllium seeds (also called plantago or fleas seed), contains several polysaccharides, including arabinoxylan, which has a xylose backbone and arabinose side chains. Psyllium is not fermentable, but fairly soluble in water, containing about 70–80% water-soluble polysaccharides and 20–30% water-insoluble polysaccharides. Products to which psyllium has been added have high water-binding capacities and form viscous gels in the digestive tract. Psyllium is added to Metamucil® for its laxative properties as well as other products to promote

reductions in serum lipids. The FDA permits a health claim for psyllium, with consumption of 10.2 g (providing 7 g of viscous fiber) resulting in significant reductions in serum LDL cholesterol [3]. Foods containing psyllium that bear a health claim are required to state on the label that the food should be eaten with at least a full glass of liquid, otherwise choking may result [3]. In addition, the label should state that the food should not be eaten if a person has difficulty swallowing [3].

Polydextrose and Polyols

Polydextrose is a polysaccharide consisting of glucose and sorbitol units that have been polymerized at high temperatures and under a partial vacuum. Polydextrose, available commercially, is added to foods as a bulking agent or as a sugar substitute. Polyols are hydrogenated carbohydrates or sugar alcohols and are used commercially to replace sugars in some foods; they do not, however, raise blood glucose concentrations to the same extent as sucrose and some other naturally occurring sugars. Examples of polyols include polyglycitol, sorbitol, xylitol, maltitol, mannitol, and isomalt. Polyglycitol and maltitol, for example, are found in syrups; others are found in mints and gums. Polyols are also found naturally in some fruits like apples, watermelon, plums, peaches, and pears, to name a few. Polyols absorb water in the colon. Both polyols and polydextrose can be partially fermented by colonic bacteria and may enhance the growth of healthful bacteria. The Food and Nutrition Board of the National Academy of Sciences, with the 2002 publication of Dietary Reference Intakes for fiber, designated polydextrose and polyols as functional fibers pending the results of additional studies showing physiological effects in humans [1].

Chitin and Chitosan

Chitin is a straight-chain polymer containing β (1-4)-linked glucose units, similar in structure to cellulose, but with an N-acetyl amino group substituted for the hydroxyl group at carbon 2 of glucose. Chitin is a component of the exoskeleton of insects and is found in the shells of crabs, shrimp, and lobsters.

Chitosan is a deacetylated form of chitin. Both chitin and chitosan have high molecular weights, are insoluble in water, and can adsorb (interact or complex with) dietary lipids, primarily unesterified cholesterol and phospholipids, and promote their excretion in the feces. Modified forms of chitin and chitosan have been designed for nutraceutical and functional food applications. The Food and Nutrition Board of the National Academy of Sciences designated chitin and chitosan as functional fibers pending the results of additional studies showing physiological effects in humans. Table 4.1 lists some food sources of fiber.

Table 4.1 Food Sources of Fiber

Type of Fiber	Examples of Food Sources
Cellulose	All plant foods, especially wheat bran, legumes, nuts, peas, root vegetables (such as carrots), vegetables of the cabbage family, celery, broccoli, coverings of seeds, and apples
Hemicellulose	Whole grains, especially bran, nuts, and legumes
Lignin	Whole grains, especially wheat bran, mature root vegetables (such as carrots), fruits with edible seeds (such as strawberries), and broccoli (especially the stalk)
Pectins	Citrus fruits, strawberries, apples, raspberries, legumes, nuts, some vegetables (such as carrots), and oat products
Gums	Oatmeal, barley, and legumes
β -glucans	Oat products and barley
Resistant starches	RS ₁ : partially milled grains and seeds; RS ₂ : unripe (green) bananas, legumes, raw potato, and high-amylose corn; RS ₃ : rice, pasta, cold cooked potatoes, and high-amylose corn
Fructans	Chicory, asparagus, onion, garlic, artichoke, tomatoes, bananas, rye, and barley
Chitosan, chitin	Shells of crab, shrimp, and lobster

4.4 SELECTED PROPERTIES OF FIBER AND THEIR PHYSIOLOGICAL IMPACT

The physiological effects and ultimately the health benefits of fiber vary based on certain characteristics of fiber, most notably viscosity and fermentability, but also to a lesser extent based on solubility and chain length (longer vs. shorter chain). Shorter-chain fibers are highly fermentable and water soluble. In contrast, longer-chain fibers vary in degree of solubility and fermentability, and thus are sometimes subdivided into four groups: (1) soluble and highly fermentable, (2) intermediately soluble and fermentable, (3) insoluble and slowly fermentable, and (4) insoluble and nonfermentable. This section discusses the solubility, viscosity, and fermentability of fibers. However, as you read about these characteristics and their effects on the physiological processes, remember that we eat foods containing a mixture of dietary fiber, not foods with just cellulose, hemicellulose, pectins, gums, and so forth. Thus, the described effects on the body processes are more variable and are not as straightforward as presented in this chapter.

Solubility in Water

One approach to classifying fiber that has been used for decades is based on fiber's solubility or insolubility. Water-soluble fibers are those that dissolve in hot water, whereas insoluble fibers do not dissolve in hot water. Shorter-chain water-soluble fibers include both fructooligosaccharides and galactooligosaccharides. Longer-chain water-soluble fibers include pectins, gums (mainly guar), inulin, and resistant starches, as well as the resistant dextrin wheat dextrin. Fibers that are intermediately soluble include

psyllium, β -glucans, and some hemicelluloses and pectins. Foods typically rich in water-soluble fibers include legumes, oats, barley, rye, chia, flaxseeds, most fruits (especially berries, bananas, apples, pears, plums, and prunes), some vegetables (carrots, broccoli, artichokes, and onions), and cooked and cooled pasta, rice, and potatoes.

Insoluble fibers include mainly cellulose, lignin, and some hemicelluloses, and to a lesser extent some pectins, some resistant starches, chitosan, and chitin. Examples of foods rich in insoluble fiber include whole-grain products, bran, legumes, nuts, seeds, some vegetables (such as cauliflower, zucchini, celery, and green beans), and some fruits. Generally, vegetables and most grain products contain more insoluble fibers than soluble fibers. Fruits tend to be higher in soluble fibers, which are found in the fruit's pulp and skin; the skin of fruit, however, also provides some insoluble fibers.

This solubility/insolubility approach to classifying fibers, which has been used as a basis for some observed biomarkers and health outcomes, is now considered, because of inconsistent findings, to be of less significance. For example, soluble fibers were generally accepted to delay gastric emptying, increase intestinal transit time (slower movement/takes a longer time to move through), and decrease nutrient absorption. These effects in turn positively impact blood glucose and lipid concentrations. In contrast, insoluble fibers were generally accepted to decrease (speed up/take less time to move through) intestinal transit time and increase fecal weight to positively impact laxation. However, it is now known that not all soluble fibers alter nutrient absorption and that insoluble fibers have varied effects on fecal weight. With these observations, the focus has shifted away from classifications based on solubility/insolubility and more toward viscosity and gel formation.

Viscosity and Gel Formation

Viscosity is related to fiber's ability both to bind or hold water (think of fiber as a dry sponge that hydrates or soaks up water and digestive juices as it moves through the digestive tract) and to form a gel (think of freshly made Jello® as it is starting to “set”) within the digestive tract. The water-holding capacity of fibers is influenced by chemical structure, particle size, processing, and pH, among other factors. Similarly, the ability of fibers to form viscous gels when interacting with fluids within the digestive tract is also affected by various factors, such as chemical structure and processing. It is this viscosity property of fiber, as well as another property, fermentability, that is most associated with health benefits, as discussed in the next sections of this chapter.

Viscous gel-forming fibers include mainly β -glucans, mucilages (e.g., psyllium), and gums (especially unprocessed guar gum). These fibers, upon absorbing in some cases up to several times their weight in water, produce a viscous, gelatinous mass within the digestive tract. Ingestion of these fibers may reduce serum lipids (total and LDL cholesterol). The fibers function by

- interacting with bile within the digestive tract,
- reducing micelle formation, which occurs as the viscous gel traps bile (needed for micelle formation), and
- reducing the reabsorption of bile in the ileum.

Because this bile is excreted in the feces, the bile pool in the liver becomes reduced. To accommodate the loss, the liver cells increase LDL cholesterol uptake via an upregulation of LDL receptors. The hepatocytes then use the cholesterol from increased LDL cholesterol uptake to synthesize new bile. Such actions effectively decrease serum cholesterol. The more viscous the fiber, typically the greater the fiber's ability to reduce bile reabsorption.

Ingestion of viscous gel-forming soluble fibers also improves glycemic control (lower fasting blood glucose, insulin, and hemoglobin A1c values). These actions are mediated by the fiber's ability to increase the viscosity of the contents of the digestive tract (e.g., the chyme) and thus

- reduce nutrient digestion, which occurs as the viscous gel traps nutrients and interferes with their ability to interact with the digestive enzymes;
- decrease nutrient diffusion rates, which now must occur through a thickened, unstirred water layer that has become viscous and more “resistant” to nutrient movement (needed for absorption); and
- decrease convective movement of nutrients (especially amino acid and fatty acids) within the intestinal lumen.

Convective currents, induced by peristaltic movements, bring nutrients from the lumen to the intestinal cell's brush border membrane for absorption.

The slowed carbohydrate digestion and glucose absorption reduce the peak rise in blood glucose. In addition, alterations in the release of gastrointestinal tract hormones/peptides, such as glucagon-like peptide 1, associated with nutrient delivery to more distal areas of the small intestine may also impact glycemic control through effects on insulin release. Similar to what has been observed with serum cholesterol, the more viscous the fiber is upon hydration within the digestive tract, the greater the fiber's ability to improve glycemic control.

Additional effects associated with the ingestion of gel-forming fibers include

- increased gastric distension,
- delayed gastric emptying, and
- longer intestinal transit time.

These actions slow down the digestive process and may increase satiety (feelings of fullness).

Fermentability

Fiber reaches the colon undigested by human digestive enzymes. Colonic bacteria then ferment (degrade to varying degrees) this undigested mass. Fibers that are not typically fermented include principally the water-insoluble fibers—cellulose and lignin, along with some hemicelluloses and some resistant starches like RS₁. Wheat bran found in some breakfast cereals contains primarily insoluble fibers and is poorly fermented. In addition, psyllium, a viscous, gel-forming fiber, is also not fermented.

The fermentation of fibers occurs mainly in the proximal (upper) colon—that is, by the cecum and in the ascending region of the colon—and diminishes as the undigested mass moves through the transverse and descending sections of the colon. Fermentable fibers do not remain intact as they move through the large intestine. Moreover, although the fibers may initially have had gel-forming capabilities in the proximal gastrointestinal tract, as they are fermented within the large intestine, they can lose their water-holding capacity and gel consistency. Fermentable fibers do not contribute substantially to fecal bulk, but do increase fecal bacteria mass. Information on the fermentability of some fibers is listed hereafter.

- Shorter-chain fibers, fructooligosaccharides and galactooligosaccharides, are rapidly and almost completely fermented.
- Longer-chain fermentable fibers include pectins, inulin, some resistant starch, and guar gums.

- Fermentable viscous, gel-forming fibers include β -glucans and partially hydrolyzed guar gum.
- Slowly fermentable, insoluble fibers include some lignin and some hemicelluloses.
- Wheat dextrin, a resistant starch, and polydextrose are also fermentable.

Fermentation of fiber by colonic bacteria provides energy and substances for microbial growth as well as products such as short-chain fatty acids (discussed in Chapter 2) that may be used by the human host. *Prebiotics* is the term used for substrates that are selectively utilized by host microorganisms and that confer a health benefit [5]. Fructans and galactooligosaccharides along with lactulose (at sub laxative doses) serve as prebiotics. Some other fibers (such as wheat dextrin, aracia gum, and polydextrose) have also been shown to stimulate the growth of various species of healthful bacteria or have been shown to provide health benefits through the production of short-chain fatty acids. The amount of energy realized to the host from fiber fermentation depends mostly on the amount and type of fiber that is ingested and the short-chain fatty acids that are produced, but is usually estimated at about 1.5–2.5 kcal/g.

The amounts of the various prebiotic fibers that need to be ingested to promote desirable effects vary. Similarly, the side effects from prebiotic use (which may include excessive gas, abdominal bloating, cramping, and osmotic

diarrhea) also vary with the amount and type of fiber consumed. Generally, shorter-chain fibers produce side effects at lower intakes than longer-chain fibers. This next section of the chapter addresses some of the health benefits and proposed mechanisms of action of fiber. Figure 4.4 reviews some of the physiological effects on the digestive tract from the consumption of fiber.

4.5 HEALTH BENEFITS OF FIBER

Several systematic reviews and meta-analyses have been conducted examining relationships between fiber intake and/or the intake of foods rich in fiber (most commonly whole grains, fruits, and vegetables) and specific diseases. Positive outcomes are reported, especially for cardiovascular disease but also for health, with inverse relationships between dietary fiber intake and overall mortality shown in both men and women. The roles of fiber in four areas—cardiovascular disease; diabetes; appetite, satiety, and weight control; and selected gastrointestinal disorders—are reviewed briefly hereafter.

Cardiovascular Disease

Studies examining fiber intake consistently report that ingestion of diets high in fiber is associated with a reduced risk of death from cardiovascular disease. Consistent

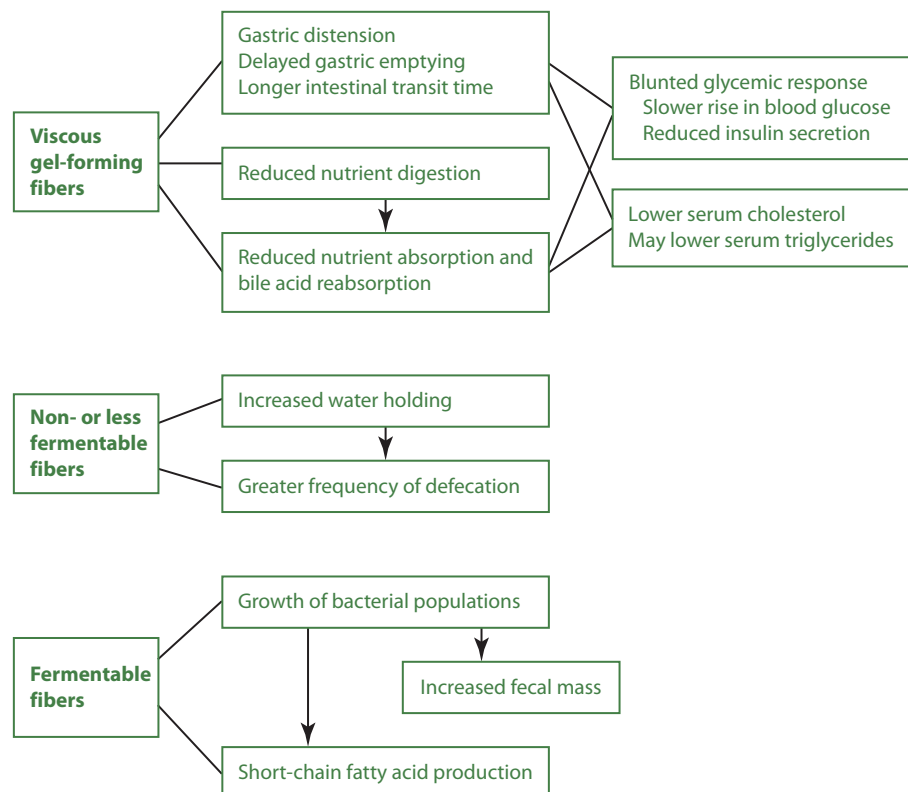


Figure 4.4 Selected gastrointestinal responses to fiber ingestion.

evidence has also been reported for inverse relationships between intake of fruits and vegetables (primarily greater than five servings per day) and heart attack and stroke and between intake of whole grains and heart disease. Diets rich in high-fiber foods and the consumption of functional fibers like psyllium have also been associated with both lower systolic and diastolic blood pressure readings and with reductions in blood pressure among those with hypertension, another risk factor for heart disease.

Studies focusing on the effects of fiber or diets rich in fiber on heart disease risk factors, usually serum cholesterol concentrations, have also typically been favorable. Lower serum total and LDL cholesterol concentrations have been demonstrated with ingestion of primarily viscous gel-forming fibers, especially β -glucans, psyllium, and guar gum. The most well-studied cholesterol-lowering high-fiber foods/fibers are β -glucan from barley and oats and psyllium. In fact, each of these has been studied sufficiently to have health claims.

Quantities of fiber needed to lower serum lipid concentrations vary. Effective LDL-cholesterol lowering quantities for psyllium range from about 7 to 15 g, and for β -glucan about 5–6 g [6,7]. Similar amounts (5–6 g) of β -glucan and psyllium, as well as about 7 g guar gum, have been shown to improve glycemic control [6,7]. In addition, phytosterols and phytosterols, in amounts ranging from about 1.6 to 3 g/day, have been shown to decrease total and LDL serum cholesterol concentrations. Modes of action thought to promote fiber's hypercholesterolemic effects were discussed under the section "Viscosity and Gel Formation." In addition, cholesterol synthesis may be inhibited by increases in propionic acid generated by bacteria and absorbed (the mechanisms are not known) and by fiber-induced shifts in bile acid production. Specifically, shifts from cholic acid toward chenodeoxycholic acid. These changes are thought to contribute to reduced serum cholesterol concentrations.

Diabetes Mellitus

Inverse associations between dietary fiber intake (as well as high intakes of fruits, vegetables, and complex carbohydrates) and risk of developing type 2 diabetes have been demonstrated in several studies. Consumption of diets high in fiber has also been generally associated with improved glycemic control (also referred to as blunting the glycemic response) in individuals with diabetes and prediabetes. Specifically, the ingestion of fiber supplements or foods rich in viscous gel-forming fibers improves glycemic control largely through reduced rates of glucose absorption and insulin secretion. Reductions in insulin secretion are thought to result at least in part both from slower glucose absorption into the blood as well as from altered secretion of gastrointestinal tract regulatory peptides such as glucagon-like peptides and glucose-dependent insulinotropic peptide. Changes in glycogen catabolism and

the resulting release of glucose into the blood also may be influenced by short-chain fatty acids that are produced with fiber fermentation in the colon. Improvements in glycemic control are usually observed with fiber intakes of at least 30 g per day, although fiber supplementation in doses similar to those used to improve hypercholesterolemia appear to be beneficial.

Appetite and/or Satiety and Weight Control

Fiber-rich foods, versus low-fiber foods, tend to have a lower energy density and a higher volume, which can promote satiety. Satiety may also result from ingestion of foods containing viscous gel-forming fibers due to fiber-induced delays in gastric emptying and/or alterations in the release of digestive tract hormones known to modulate appetite such as ghrelin, glucagon-like peptide-1, peptide YY, and cholecystokinin. Additionally, however, the consumption of nonviscous fibers such as galactooligosaccharides has been shown to reduce appetite; such actions have been attributed to changes in metabolism, gut microbiota, and gastrointestinal tract peptides. Gut bacteria are known to generate neurotransmitters as well as other metabolites that can impact brain function through the microbiome–gut–brain axis. As expected from the diversity of polysaccharides, fiber's effects on satiety and appetite vary with the type, amount, and form (supplement or food) of fiber consumed, among other factors. And, while some studies have reported reduced energy intakes and weight loss or reduced weight gain over time on high-fiber diets, others have not. Similarly, while some studies have demonstrated inverse correlations between dietary fiber intake and weight gain, others have not.

Gastrointestinal Disorders

Fiber intake has been linked with several gastrointestinal conditions, especially constipation and diverticular disease. The consumption of specific fibers has also been linked with irritable bowel syndrome. Each are discussed here.

Constipation is characterized by long transit time, difficult stool expulsion, low stool output, and incomplete rectal emptying. Increasing consumption of fiber through supplements or fiber-rich foods can improve constipation. The properties of the fiber needed to improve constipation/promote regular bowel function include:

- the ability to directly interact with the mucosa of the large intestine,
- a high water-holding capacity, and
- nonfermentability.

Fibers with larger, coarser particle sizes enable physical interaction with the mucosa; smaller, fine, or smoother

particles do not generate the same interactions. Such interactions between the fibers and mucosa promote the secretion of water and mucus, and the formation of bulky, soft stools that are easily excreted [6]. Nonfermentable fibers, such as those found in insoluble forms of wheat bran, are highly effective in laxation. They absorb several times their weight of water as they migrate through the large intestine, resist fermentation, and “interact” with the colonic mucosa, leading to a larger fecal volume, softer stool, and a greater frequency of defecation. Several fiber-rich products designed to help individuals with constipation are available in the marketplace. Psyllium-containing products, such as Fiberall® and Metamucil®, and those containing other nonfermentable, insoluble fibers, such as chemically modified (methyl) cellulose found in MiraFiber® and Citrucel®, can be of benefit [6,7].

Another gastrointestinal tract disorder that has been linked to diets low in fiber is diverticular disease, which is characterized by the presence of diverticula in the colon. Diverticula, protruding or bulging pouches of the wall of the colon, are thought to form when the colon’s wall weakens. This weakening is theorized to result in chronic constipation associated with low fecal bulk and straining to pass hard fecal matter. (The straining increases the pressure inside the colon and weakens its walls.) When fecal matter becomes trapped in the diverticula, the pouches become inflamed (called diverticulitis) and the person experiences pain and sometimes fever, diarrhea, gastrointestinal bleeding, and infection. Diets high in fibers that increase stool weight, as discussed in the preceding section on constipation, reduce straining and the likelihood of fecal matter becoming trapped in the diverticula. However, whether a high-fiber diet reduces the likelihood of formation of new diverticula once the condition has developed is unclear. Recommendations for fiber intake for those with diverticular disease, as well as with constipation, are the same as those recommended for all Americans, about 20–35 g per day.

Whereas the aforementioned health conditions have been linked with inadequate fiber intake, symptoms of irritable bowel syndrome, a functional digestive disorder, often occur after eating and have been attributed to gut microbial dysbiosis and consumption of certain foods (some of which are high in fiber). The classic symptoms of this syndrome are bloating, gas (flatulence), abdominal cramping, and diarrhea or constipation or a mixed bowel pattern. Some of the causative agents triggering these symptoms in susceptible individuals include fructooligosaccharides and galactooligosaccharides (short-chain, highly fermentable fibers) as well as polyols. In addition to these fibers, the monosaccharide fructose and the disaccharide lactose (along with wheat bran) may also trigger symptoms. These substances have been coined FODMAP—fermentable, oligo-, di-, monosaccharides, and polyols—and restriction of foods rich in these carbohydrates is purported to help alleviate symptoms of irritable bowel syndrome for some individuals.

The low-FODMAP diet consists of an extensive list of “foods to avoid.” For example, to minimize fructose consumption, one must limit foods with added high-fructose corn syrup, which include many beverages along with sauces and condiments (barbecue sauce, ketchup, syrups, etc.), along with foods naturally rich in fructose such as agave, honey, and many fruits. Fructans (which must be limited) are found in many vegetables. To avoid galactooligosaccharides, one must minimize intakes of most legumes and peas. Lactose is found primarily in dairy products. Polyols are found in chewing gums and mints as well as some fruits. Variable benefits from these dietary restrictions on observed symptoms have been reported, but long-term efficacy data for the low-FODMAP diet are needed. Similarly, the effectiveness of fiber in treating irritable bowel syndrome symptoms has been examined in systematic reviews with generally mixed results. Of the various fibers, psyllium appears to be relatively helpful in improving some symptoms in some individuals [6].

4.6 FOOD LABELS AND HEALTH CLAIMS

Nutrient recommendations for fiber, as well as for other nutrients, are found on the Nutrition Facts panel on food labels. The recommendation for fiber provided on food panel labels is 25 g of dietary fiber for a 2,000-kcal diet. Some food labels also provide information on quantities of soluble and insoluble fibers in the product. For example, the label on a box of cereal might show that a serving (1 cup) provides 7 g of dietary fiber, with 6 g listed as insoluble and 1 g listed as soluble.

Based on the total amount of dietary fiber provided by a serving of the food, food labels may state that the food is an “excellent” or “good” source of fiber. Foods claiming to be an “excellent source of fiber” by the manufacturer must provide at least 20% of recommendations in a serving—that is, 0.20×25 g, or 5 g of fiber. Foods may be considered a “good source of fiber” if they provide 10% of recommendations or 2.5 g of fiber/serving.

The FDA has approved several fiber-related health claims [3]. The claims typically focus on consumption of fiber-rich foods such as fruits, vegetables, and whole grains coupled with consumption of a low-fat diet, as shown below.

- Diets low in fat and rich in high-fiber foods (or rich in fruits and vegetables) may reduce the risk of certain cancers.
- Diets low in saturated fat (or low in fat) and rich in soluble fiber (or rich in whole oats and psyllium seed husk) may reduce the risk of heart disease.
- Diets low in total fat, saturated fat, and cholesterol and rich in whole grains and other plant foods may help reduce the risk of heart disease.

4.7 RECOMMENDED FIBER INTAKE

Dietary Reference Intakes, specifically Adequate Intakes, for fiber are shown in Table 4.2. They were established based on the amounts of fiber shown to protect against heart disease [1]. Unfortunately, most Americans fail to meet recommendations, with intakes commonly less than 15 g of fiber per day [1].

Table 4.3 shows the dietary fiber content of selected foods. General estimates of fiber intake can be calculated

Table 4.2 Recommended Fiber Intakes [1]

Population Group	Age (years)	Total Fiber (g)
Men	19–50	38
	≥ 51	31
Women	19–50	25
	≥ 51	21
Children	1–3	19
	4–8	25
Girls	9–18	26
Boys	9–13	31
	14–18	38

Table 4.3 Dietary Fiber Content of Selected Foods* [8]

Food Group	Soluble Fiber (g / 100 g)	Insoluble Fiber (g / 100 g)	Total
Fruits (raw)			
Apple with skin	0.70	2.00	2.70
Banana	0.58	1.21	1.79
Grapes	0.24	0.36	0.60
Mango	0.69	1.08	1.76
Orange	1.37	0.99	2.35
Peach with skin	1.31	1.54	2.85
Pear with skin	0.92	2.25	3.16
Pineapple	0.04	1.42	1.46
Plum with skin	1.12	1.76	2.88
Strawberries	0.60	1.70	2.30
Watermelon	0.13	0.27	0.40
Legumes/Beans (cooked)			
Black			8.7
Kidney	1.36	5.77	7.13
Lima	1.02	4.21	5.23
Navy			10.5
Pinto	0.99	5.66	6.65
Nuts			
Almonds			12.3
Cashews			3.2
Pecans			9.6
Peanuts			8.1
Walnuts			6.7

assuming each serving of fruits, vegetables, and whole grains provides 2 g of dietary fiber and each serving of legumes contributing 5 g of dietary fiber. To complete the estimation, fiber from any consumed fiber supplements and from the ingestion of any high-fiber cereals or other products should be added to the total.

No Tolerable Upper Intake Level for dietary fiber or functional fiber has been established [1]. Tolerance to fiber intake varies from person to person, and problems associated with the use of supplements vary with the type and dose of fiber ingested. Generally, supplements containing fibers that are very rapidly fermented are associated with more undesirable side effects than those that are more slowly or not fermented. The most common complaints with fiber “over” consumption include abdominal discomfort, bloating, gas, and altered stool output; however, gastrointestinal tract tolerance generally improves over time.

Reduced absorption of some minerals has also been purported as an adverse effect of ingesting too much dietary fiber. And, while this may be a problem in individuals consuming fiber in quantities well in excess of recommended amounts, it is not likely that healthy adults consuming recommended amounts of fiber will develop mineral deficiencies. The proposed “problem”

Food Group	Soluble Fiber (g / 100 g)	Insoluble Fiber (g / 100 g)	Total
Vegetables (cooked)			
Asparagus			2.0
Broccoli	1.85	2.81	4.66
Carrots	1.58	2.29	3.87
Cauliflower	0.70	3.50	4.20
Corn			2.0
Lettuce (raw)			1.3
Mushrooms			2.4
Potato baked			
With skin	0.61	1.70	2.31
Boiled, no skin	0.99	1.06	2.05
Grain and Grain Products			
Rice			
White			0.3
Brown			1.8
Couscous			2.8
Bread			
White			2.4
Whole grain			6.8
Crackers (wheat)			10.6
Cereals (cold)			
All Bran®			29.3
Raisin Bran®			11.1
Cheerios®			10

*Soluble and insoluble fiber contents provided when available.

is thought to occur because of the adsorption of some divalent minerals (including calcium, magnesium, zinc, and iron) to some fibers (like those containing uronic acid, such as hemicellulose, pectins, and gums, as well as with lignin, which has both carboxyl and hydroxyl groups). Yet, countering these possible effects are studies that show that fermentation of these fibers and the resulting acidic environment enhance the release of minerals from fiber and promote mineral absorption from

the colon. Maillard products are also mentioned in the scientific literature as having mineral binding potential. These products contain enzyme-resistant linkages between the amino group of amino acids, especially lysine, and the carbonyl group of reducing sugars, which have formed during cooking, particularly in baking and frying foods. Yet, as with fiber ingestion, mineral deficiencies are not thought to be likely from the ingestion of Maillard products.

SUMMARY

The physiological effects of fiber in the gastrointestinal tract are as varied as the number of fiber components and their physiochemical properties.

- Two important characteristics related to health are viscosity/gel formation and fermentability. These characteristics not only impact digestive tract function and health but also affect risk factors for disease, especially heart disease and diabetes.

To obtain fiber through the diet, food sources of fiber need to be varied, ideally within and across all plant-based food groups including whole-grain cereals and cereal products, legumes, nuts, seeds, fruits, and vegetables.

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Perspective

THE FLAVONOIDS: ROLES IN HEALTH AND DISEASE PREVENTION

Chapter 4 described fiber and some of its characteristics that make it important in the diet. However, other substances in plant foods are also of significance. These substances are known as phytochemicals, a group of compounds that are biologically active in the body. Of the thousands of phytochemicals, polyphenolic phytochemicals (also referred to as polyphenols, meaning they contain more than one phenol unit), make up the largest group. The polyphenols include more than 8,000 compounds and can be divided into a variety of classes. One of the largest of these classes is the flavonoids, which include a group of over 4,000 plant metabolites. This Perspective reviews some of the more ubiquitous flavonoids in foods and their potential roles in maintaining health and preventing disease.

FLAVONOIDS

The flavonoids are organic, bioactive, polyphenolic secondary metabolites that occur in small quantities in a wide variety of plants (especially fruits, vegetables, nuts, seeds, herbs, spices, and tea). The flavonoids of dietary significance can be divided, based on functional groups attached to the common flavone backbone, into six subclasses—flavonols, flavanols, flavones, flavanones, anthocyanins, and isoflavones. The

flavone and flavonols are subclasses, however; they are sometimes grouped together and referred to as 4-oxoflavonoids. Table 1 provides a list of these flavonoid subclasses along with major food sources.

Flavonols

The flavonol subclass includes two main compounds—quercetin and kaempferol, but also myricetin, isorhamnetin, and rutin. These flavonols are widely found in foods (Table 1). Quercetin is among the more well studied of the flavonoids. Quercetin, along with the other flavonols, exhibits several biological actions helpful in the prevention of cardiovascular disease and some of its risk factors like hypertension.

Flavanols

Flavanols, also called flavan-3-ols, are another subclass of flavonoids and can be further categorized based on chemical structure. Monomer forms are called catechins, and condensed or polymerized forms are called proanthocyanidins or tannins. Some of the food sources containing these flavanols are listed in Table 1. Catechins may help reduce the risk of hypertension and cardiovascular disease. Of the proanthocyanidins in foods, procyanidin is one of the most common. Studies suggest flavanols

may be beneficial in reducing risk factors associated with cardiovascular disease and diabetes.

Flavones and Flavanones

Another category of flavonoids are the flavones, which include luteolin and apigenin. Only a few foods, listed in Table 1, have been identified as good sources of flavones. In comparison with the other flavonoids, not as much research has been conducted on these phytochemicals.

The flavanones also consist of just a few compounds, primarily narigenin, hesperetin, and eriodictyol, and are found mostly in citrus fruits and their juices. Both hesperetin and its glycoside (meaning attached to a sugar) form hesperidin are found in relatively high amounts in oranges. The flavanones exhibit several biological properties that are thought to aid in the prevention of both cardiovascular disease and cancer.

Anthocyanins

Anthocyanins are pigments found mostly in the skin of plants, and thus provide color (usually red, blue, or purple) to many fruits and vegetables. Major food sources include blueberries, strawberries, raspberries, red grapes, and blackberries, among others listed in Table 1.

Table 1 Flavonoid Subclasses, Common Phytochemicals, and Their Sources

Flavonoid Subclass	Common Phytochemicals	Main Sources
Flavonols	Quercetin, kaempferol, myricetin, isorhamnetin, and rutin	Onions, tea, olives, kale, leafy lettuce, cranberries, tomatoes, cherries, apples, applesauce, turnip greens, endive, ginkgo biloba, chili peppers, chives, celery, tea (black and green), wine (red and white), and dark chocolate
Flavanols	Catechins, epicatechin, and epigallo-catechin-3-gallate	Green tea, pears, grapes, wine, berries, apples, applesauce, apple juice, and cocoa and cocoa products
Derived tannins	Theaflavins, theorubigins, and theabrownins	Fermented teas (black and oolong)
Condensed tannins/proanthocyanidins	Procyanidins, prodelphinidins, and propelargonidin	Cocoa and cocoa products, stone fruits, grapes, grape seed, wine, strawberries, cranberries, black currant, legumes, cinnamon, beer, tea (black and green), and barley

(Continued)

Table 1 Flavonoid Subclasses, Common Phytochemicals, and Their Sources (Continued)

Flavonoid Subclass	Common Phytochemicals	Main Sources
Flavones	Apigenin and luteolin	Parsley, thyme, celery, celery seed, oregano, and peppers (hot and sweet)
Flavanones	Hesperetin, naringenin, and eriodictyol	Citrus fruits and juices, and tomatoes and tomato-derived products
Anthocyanins	Cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin	Berries, cherries, bananas, plums, oranges, grapes, pomegranate, and red wine
Isoflavones	Genistein, daidzein, equol, and Glycitein	Legumes, especially soybeans and soy foods—soy nuts, soy milk, tofu, miso, soy sauce, and edamame

Anthocyanins are found free (unattached) as well as attached to sugars (anthocyanidin glycosides) or acyl groups in foods. Of the dozens of anthocyanidins, the six most commonly found include cyanidin, delphinidin, petunidin, peonidin, pelargonidin, and malvidin. Consumption of anthocyanins and/or foods rich in these flavonoids has been suggested to benefit the heart, eyes (vision), and nerves and may also be protective against cancer and diabetes.

Isoflavones

A final category of flavonoids is the isoflavones; the two main isoflavones are genistein and daidzein. They are found mostly in soybeans and soy products, as presented in Table 1. Isoflavones, along with lignans (found in seeds, whole grains, nuts, and some fruits and vegetables) and coumestans (found in broccoli and sprouts) are phytoestrogens; they are structurally similar to estrogen in that the phenol ring can bind to estrogen receptors on some body

tissues. Soy products have been marketed for use by women during perimenopause to help alleviate some of the side effects of diminished natural estrogen in the body.

Other Phytochemicals

These flavonoids are among the thousands of phytochemicals found in foods. Some additional classes and examples of phytochemicals within each class, along with some food sources, are listed in Table 2. Within each of these classes are phytochemicals with a wide range of biological actions that are also thought to help protect against disease and maintain health.

Although Tables 1 and 2 provide examples of foods containing different phytochemicals, note that most plant foods contain multiple phytochemicals. Tomatoes, for example, may contain as many as 10,000 different phytochemicals; tea is also rich in multiple phytochemicals, including several flavonoids, flavonols, flavanols, and proanthocyanidins, among others. Phytochemical contents further vary based

on the plant species, its stage of ripeness, and the methods used for storing and processing the plant as well as with the climate or environmental conditions in which the plant was grown.

An Overview of Flavonoid Digestion, Absorption, and Metabolism

Most phytochemicals are found in foods in a variety of forms, and these forms influence the digestion and the rate and extent of absorption of the phytochemical. Polyphenols in foods may exist free (unattached) or in some cases as a glycoside conjugate (also called a glycone). The names of the conjugated and unconjugated forms differ slightly; for example, the flavanone hesperidin is conjugated to sugar, and its free/unconjugated form is known as hesperetin.

In some cases, the glycoside forms of the flavonoids must be digested to soluble forms before being absorbed. Other phytochemicals do not require extensive digestion and may be more directly absorbed from the small intestine (and to a small

Table 2 Phytochemicals and Their Sources

Phytochemical Class	Common Phytochemicals	Sources
Carotenoids	β -carotene, α -carotene, lutein, and lycopene	Tomatoes, pumpkins, squash, carrots, watermelon, papayas, and guavas
Terpenes	Limonene and carvone	Citrus fruits, cherries, and ginkgo biloba
Organosulphides	Diallyl sulphide, allyl methyl sulphide, and S-allylcysteine	Garlic, onions, leeks, and cruciferous vegetables (broccoli, cabbage, Brussels sprouts, mustard, watercress)
Phenolic acids	Hydroxycinnamic acids: caffeic acid, ferulic acid, chlorogenic acid, and neochlorogenic curcumin	Coffee, blueberries, cherries, pears, apples, oranges, grapefruit, tomatoes, kiwi, plums, and white potatoes
Phenolic acids	Hydroxybenzoic acids: ellagic and gallic acids	Grapes, grape juice, red wine, tea, raspberries, strawberries, and nuts
Lignans	Secoisolariciresinol, matairesinol, and pinoresinol	Berries, flaxseeds, sesame seeds, legumes, nuts, broccoli, cabbage, kale, and whole-grain cereals
Saponins	Panaxadiol and panaxatriol	Alfalfa sprouts, potatoes, tomatoes, and ginseng
Phytosterols	β -sitosterol, campesterol, and stigmasterol	Vegetable oils (soy, rapeseed, corn, and sunflower)
Glucosinolates	Glucobrassicin, gluconapin, sinigrin, and glucoiberin	Cruciferous vegetables (see organosulphides)
Isothiocyanates	Allylisothiocyanates and indoles	Cruciferous vegetables (see organosulphides)
Stilbene	Resveratrol	Grapes, red wine, and berries

extent the stomach). Glycosylated quercetin, for example, may be absorbed directly or hydrolyzed first by β -glycosidase. Many other digestive enzymes in the small intestine also assist in the cleavage of sugars (and other functional groups) bound to the flavonoids to enable absorption. The method of absorption of most flavonoids is thought to involve carriers; however, the absorptive processes have not been clearly elucidated.

Some flavonoids are neither digested nor absorbed in the upper digestive tract, but instead undergo degradation by colonic microflora. The bacteria hydrolyze the glycosides (as well as other attached functional groups such as glucuronides, sulfates, amides, lactones, etc.), generating metabolites that may be absorbed or that exert effects on the body from within the colon. Lignans, for example, are metabolized by colonic bacteria to the metabolites enterodiol and enterolactone, which are then absorbed. These enterolignans exhibit weak estrogenic activity and/or antiestrogenic effects upon binding to estrogen receptors on various body tissues. Bacteria in the colon also utilize anthocyanin glycosides, deglycosylating them to aglycones, which are then further degraded. The extent of absorption of the products generated from the actions of the bacteria is not well established.

Once absorbed, most flavonoid metabolites are conjugated in the cells of the small intestine and then enter portal blood for transport to the liver. Some metabolites, however, efflux from the enterocyte back into the lumen of the small intestine via adenosine-binding cassette (ABC) transporters. Those flavonoid metabolites that enter portal blood are taken up largely by the liver where they undergo further metabolism, especially conjugation with methyl or sulfate groups, or glucuronic acid. These conjugated metabolites are then released into systemic circulation bound to plasma proteins like albumin. The amount of the metabolites present in the plasma varies considerably with the type of flavonoid consumed, the food source, and the amount ingested; little is known about the metabolism of all the different polyphenols in the body, and thus about what metabolites are present in the plasma after consumption of a specific polyphenol.

FLAVONOIDS AND HEALTH AND DISEASE PREVENTION

Diets rich in plant foods (whole grains, legumes, nuts, seeds, vegetables, and fruits) are typically associated with reductions in the risk of various diseases or conditions, especially cardiovascular disease and some cancers, but also to a lesser extent neurodegenerative conditions and osteoporotic fractures, among others. Diets rich in plant foods, as we now know, are also rich in flavonoids and other phytochemicals.

Flavonoids exhibit a broad spectrum of biological activities that affect a variety of metabolic processes that may be related to the development of diseases. Several flavonoids provide cardioprotective effects with antioxidant and anti-inflammatory functions, vasodilatory effects (blood vessel relaxation), antiplatelet adhesion, and anticoagulant effects. More specifically, for example in nervous system glial cells, flavonoids exert influences through inhibiting cytokine (including IL-1 β and tumor necrosis factor α) release, down-regulating proinflammatory transcription factor activity such as of NF- κ B, reducing nitric oxide production in response to glial activation, and reducing reactive oxygen species generation. Quercetin, a well-studied flavonol, for example, exhibits direct antioxidant functions (scavenging free radicals), activates signaling pathways, inhibits inflammation, and promotes vascular relaxation. Kaempferol also has antihypertensive actions via enhancing endothelium vasorelaxation and protecting against endothelial damage. Myricetin, another flavonol, also demonstrates antiplatelet, antihypertensive, and antiatherosclerotic properties. Catechins (monomeric flavanols) are also anti-inflammatory, and some isoflavones exhibit cholesterol-lowering effects that may be protective against heart disease.

It is a variety of actions of several flavonoids that are also thought to help in the prevention of some cancers. Some of these actions include antioxidant and anti-inflammatory functions, antiangiogenesis actions, and antiproliferative and apoptotic effects on tumor cells. The catechins (flavanols), for example, target signaling pathways to inhibit the growth of some cancers and promote apoptosis. The flavanols quercetin and myricetin exhibit direct antioxidant functions (scavenging free radicals), have apoptotic effects, and activate

signaling pathways, which may be beneficial in the prevention of some cancers. The antioxidant actions of some phytochemicals can also prevent oxidative damage to vitamins with antioxidant functions, such as vitamin E, to protect the body. Additionally, the isoflavone genestein, lignans, glucosinolates, isothiocyanates, terpenes, and some phenolic acids such as hydroxycinnamic acid have been shown to inhibit tumor formation and/or proliferation.

Phytochemicals can also elicit health-promoting effects through interactions with microRNAs (miRNAs). MiRNAs are small and noncoding RNAs; they function through interactions with mRNA. The miRNA-mRNA interaction results in either mRNA degradation or translation repression to disrupt protein synthesis. Aberrant miRNA expression is thought to contribute to the development of many diseases/conditions including heart disease and cancer. Phytochemicals, however, can directly interact with and alter the expression of miRNAs. The list of phytochemicals eliciting such interactions with miRNAs is growing but some examples with anticancer effects on miRNA expression include resveratrol, quercetin, genistein, curcumin, and cinnamic acid derivatives, among others. Many other miRNAs have roles in the regulation of proteins involved in cardiovascular-related pathways where phytochemicals may also play roles.

Inflammation is thought to contribute to the development of some neurodegenerative conditions. Flavonoids are thought to suppress neuroinflammation, as well as target signaling pathways and enhance cerebrovascular blood flow to improve cognitive function. Effects of flavonoids may also be mediated through changes in selected neurotrophic factors such as brain-derived neurotrophic factor. This protein, for example, plays roles in the maintenance, growth, and differentiation (maturation) of neurons, including new neurons, and nerve synapses. Metabolism of phytochemicals by gut bacteria may promote or inhibit the growth of other bacteria as well as generate neurotransmitters or metabolites that impact brain function through the microbiome-gut-brain axis.

Many of the demonstrated actions of flavonoids have been studied *in vitro*, in cultured cells, or in isolated tissues using specific glycosides or aglycone forms of

the various phytochemicals. The forms of the polyphenolic phytochemicals used in the studies, however, have not been consistently the same as the forms of polyphenolic phytochemicals found naturally in the body. Moreover, the amounts or concentrations of the phytochemicals used in the studies have often been much higher than the amounts found in the body. Differences in the metabolism of the thousands of phytochemicals in the body also complicate the interpretation of research studies and the ability to make recommendations. Study findings, especially when examining diet, continue to be mixed. Some support the consumption of diets rich in flavonoids in reducing the risk of cardiovascular diseases and/or its risk factors, some cancers, and all-cause mortality, whereas other studies do not. For example, one investigation reported all-cause, cardiovascular- and cancer-related mortality were inversely associated with flavonoid intake (plateauing at 500 mg/day) [1]. Such quantities of flavonoids can be found with consumption of about 100 g of berries, which provides up to about 500 mg of anthocyanins along with other phytochemicals. Yet, another study found no relationship between mortality and intakes of flavonols, flavanones, and flavones, but did show that higher intakes of certain foods (red wine, tea, peppers, blueberries, and strawberries) were associated with reduced risk of total and cause-specific mortality [2]. The possibilities of additive

or synergistic interactions among bioactive compounds as well as neutralizing or opposing interactions among food components represent yet another consideration when examining diet, phytochemicals, and disease [3]. See *Suggested Readings* for more information on phytochemicals, including specific mechanisms by which the various flavonoids and other phytochemicals are thought to function.

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Suggested Website

The U.S. Department of Agriculture maintains nutrient databases providing information on the flavonoid content of foods. See <https://www.nal.usda.gov/fnic/phytonutrients>.

LIPIDS

LEARNING OBJECTIVES

- 5.1** Describe the structural and functional features of the main lipid classes.
- 5.2** Describe the major food sources of lipids.
- 5.3** Explain how dietary lipids are digested, absorbed, transported and stored in the body.
- 5.4** Describe the structure and function of lipoproteins.
- 5.5** Explain how fatty acids are degraded by oxidation.
- 5.6** Define ketone bodies.
- 5.7** Explain how fatty acids and triacylglycerols are synthesized.
- 5.8** Describe atherogenesis and the role of lipids.

THE PROPERTY THAT SETS LIPIDS APART FROM OTHER MAJOR NUTRIENTS IS THEIR SOLUBILITY IN ORGANIC SOLVENTS SUCH AS ETHER, CHLOROFORM, AND ACETONE. If lipids are defined according to this property, as is generally the case, many diverse molecules fit the criteria and are thus considered lipids. Unlike carbohydrates and proteins, classifying lipids on the basis of solubility covers a broad range of molecules with diverse structural and functional properties. Such biological diversity is a benefit to plants and animals due to the many roles lipids play. As body fat, lipids serve as a depot of stored energy, provide protection to internal organs, and insulate against heat loss. Lipids also form the basis of cellular membranes, steroid hormones, bile acids, **eicosanoids**, and other signaling molecules. The roles of the fat-soluble vitamins are discussed in Chapter 10.

The diversity of lipids poses a challenge in creating a classification system beyond their solubility property. A traditional way of classifying lipids is based on how many products result from hydrolysis: “simple” lipids are those yielding two types of products on hydrolysis, whereas “complex” lipids yield three or more products. An alternative way of classifying lipids is based on the products of synthesis. In this system, lipids are defined as molecules arising from two distinct pathways that produce fatty acids (and their derivatives) or **sterols** (and their derivatives). Neither system is entirely adequate for the study of nutrition in which emphasis is placed on the structure and function of lipids. Consequently, the lipids discussed in this chapter are limited to those most relevant to human nutrition and are organized by their structural and functional similarities:

- Fatty acids
- Triacylglycerols, diacylglycerols, and monoacylglycerols
- **Phospholipids**
- **Sphingolipids**
- Sterols (cholesterol, bile acids, and phytosterols).

This chapter also describes lipoproteins—complexes of lipids and proteins—that allow lipids to be transported in the aqueous environment of the blood. Finally, this chapter discusses the metabolism of ethyl alcohol. Although not a lipid, ethyl alcohol is a common dietary component and is catabolized similarly to lipids.

5.1 STRUCTURE AND BIOLOGICAL IMPORTANCE

The structure of each lipid class is strongly related to its biological function. Of the five major lipid classes discussed in this chapter, fatty acids are structurally the simplest and are a component of the other lipid classes.

Fatty Acids

Fatty acids are composed of a hydrocarbon chain with a methyl group at one end and a carboxylic acid group at the other. Therefore, fatty acids have a polar, hydrophilic end and a nonpolar, hydrophobic end that is insoluble in water (Figure 5.1). Fatty acids exist alone or as components of the more complex lipids, discussed in later sections. They are of vital importance as an energy nutrient, furnishing most of the calories derived from dietary fat.

The lengths of the hydrocarbon chains of fatty acids found in foods and body tissues vary from 4 to about

24 carbon atoms, although the most common fatty acids in nature are 18 carbons. The fatty acids may be saturated (SFA), monounsaturated (MUFA; possessing one carbon-carbon double bond), or polyunsaturated (PUFA; having two or more carbon-carbon double bonds). Nutritionally important PUFA may have as many as six double bonds.

Where a carbon-carbon double bond exists, there is an opportunity for either *cis* or *trans* geometric isomerism that significantly affects the molecular configuration and functionality of the molecule. The *cis* isomer results in folding and bending of the molecule into a U-like orientation, whereas the *trans* form has the effect of extending the molecule into a linear shape similar to that of saturated fatty acids. The more carbon-carbon *cis* double bonds occurring within a chain, the more pronounced is the bending effect. The degree of bending plays an important role in the structure and function of cell membranes. The structures in Figure 5.1 illustrate saturation and unsaturation in an 18-carbon fatty acid and show how *cis* or *trans* isomerization affects the molecular configuration.

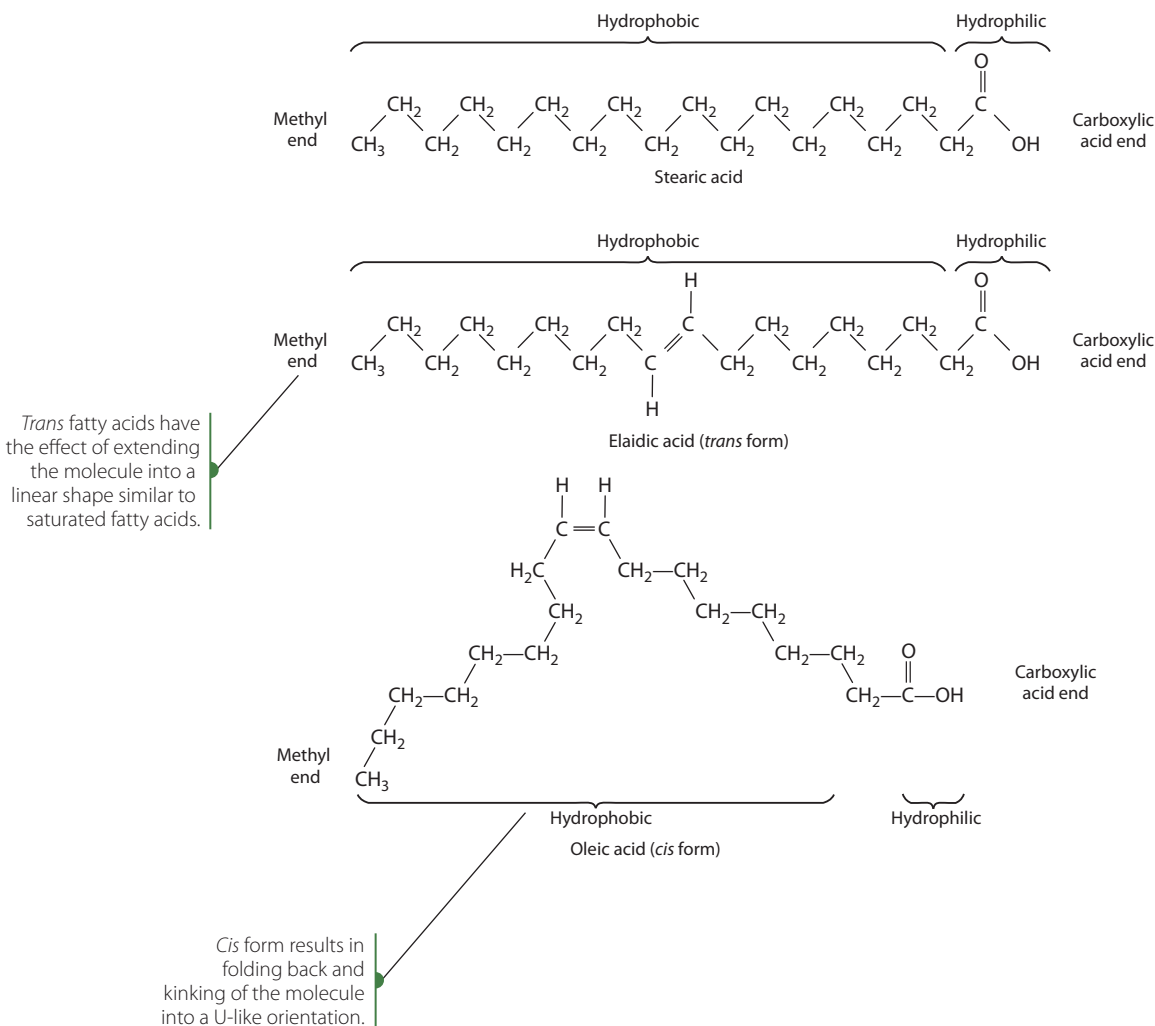


Figure 5.1 Structures of selected fatty acids.

Most naturally occurring unsaturated fatty acids are of the *cis* configuration, although the *trans* form does appear in some natural plant oils, in dairy products, and lamb and beef fat as a result of biohydrogenation by ruminant bacteria. *Trans* fatty acids can also be commercially produced by a process called hydrogenation. *Trans* fatty acids resulting from biohydrogenation contain the *trans* double bond mostly at the Δ^{11} carbon, whereas commercial hydrogenation produces a normal distribution of *trans* isomers with the peak around the Δ^{10} carbon. Partial hydrogenation, a process historically used in making frying oils and commercial food products, was designed to solidify vegetable oils at room temperature. The chemical and physical properties of partially hydrogenated oils provided many advantages to food manufacturers and consumers. However, mounting evidence linking partially hydrogenated oils to cardiovascular disease risk prompted the U.S. Food and Drug Administration (FDA) in 2015 to remove them from the list of foods “generally recognized as safe.” Furthermore, the FDA banned the use of partially hydrogenated oils in processed foods manufactured after June 18, 2018 [1]. A small amount of *trans* fatty acids are still found naturally in meat and dairy products from ruminant animals.

Fatty Acid Nomenclature

Two systems of notation have been developed to provide a shorthand way to indicate the chemical structure of a fatty acid. Both systems are used regularly and are used interchangeably in the text for different purposes.

The delta (Δ) system of notation has been established to denote the chain length of the fatty acids and the number and position of any double bonds that may be present. For example, the notation 18:2 $\Delta^{9,12}$ describes linoleic acid. The first number, 18 in this case, represents the number of carbon atoms; the number following the colon refers to the total number of double bonds present; and the superscript numbers following the delta symbol designate the carbon atoms at which the double bonds begin, counting from the carboxyl end of the fatty acid.

A second commonly used system of notation locates the position of double bonds on carbon atoms counted from the methyl, or omega (ω), end of the hydrocarbon chain. For instance, the notation for linoleic acid would be 18:2 ω -6. Substitution of the omega symbol with the letter *n* has been popularized. Using this designation, the notation for linoleic acid would be expressed as 18:2 *n*-6. In this system, the total number of carbon atoms in the chain is given by the first number, the number of double bonds is given by the number following the colon, and the location (carbon atom number) of the first double bond counting from the methyl end is given by the number following ω - or *n*-. This system of notation takes into account the fact that double bonds in a fatty acid are usually positioned so that they are separated by three carbons. Thus, if you know the total number of double bonds and the location of the first relative to either the methyl or carboxylic end, you can determine the locations of the remaining double bonds.

Figure 5.2 demonstrates the designation of linoleic acid using each of the two systems: 18:2 $\Delta^{9,12}$ (delta) or 18:2 ω -6 or 18:2 *n*-6 (omega). The fatty acid α -linolenic acid, which contains three double bonds, is identified as 18:3 $\Delta^{9,12,15}$ or 18:3 ω -3 or 18:3 *n*-3.

Table 5.1 lists some naturally occurring fatty acids and their common dietary sources. For unsaturated fatty acids, the table shows the Δ and ω system designations and their commonly used abbreviations. The list includes only those fatty acids with chain lengths of 14 or more carbon atoms because these fatty acids are most important both nutritionally and functionally. For example, palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), and linoleic acid (18:2) together account for about 90% of the fatty acids in the average U.S. diet. However, shorter-chain fatty acids do occur in nature and are present in the food supply. Butyric acid (4:0) and lauric acid (12:0), for instance, are abundant in milk fat and coconut oil, respectively.

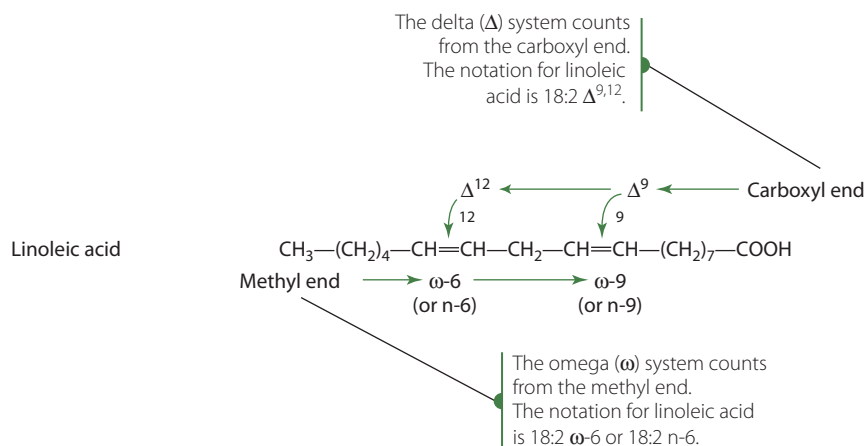


Figure 5.2 The structure of linoleic acid, showing the two systems for nomenclature.

Table 5.1 Some Naturally Occurring Fatty Acids

Notation	Common Name	Formula	Source*
Saturated Fatty Acids			
14:0	Myristic acid	$\text{CH}_3-(\text{CH}_2)_{12}-\text{COOH}$	Coconut and palm kernel oil, fish oils
16:0	Palmitic acid	$\text{CH}_3-(\text{CH}_2)_{14}-\text{COOH}$	All animal and plant fats, notably palm oil
18:0	Stearic acid	$\text{CH}_3-(\text{CH}_2)_{16}-\text{COOH}$	All animal and plant fats, notably cocoa butter
20:0	Arachidic acid	$\text{CH}_3-(\text{CH}_2)_{18}-\text{COOH}$	Peanut oil, wild-caught salmon oil
24:0	Lignoceric acid	$\text{CH}_3-(\text{CH}_2)_{22}-\text{COOH}$	Peanut oil
Unsaturated Fatty Acids			
16:1 Δ^9 (n-7)	Palmitoleic acid	$\text{CH}_3-(\text{CH}_2)_5-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	Fish oils, poultry fat
18:1 Δ^9 (n-9)	Oleic acid	$\text{CH}_3-(\text{CH}_2)_7-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	All animal and plant fats
18:2 $\Delta^{9,12}$ (n-6)	Linoleic acid	$\text{CH}_3-(\text{CH}_2)_4-\text{CH}=\text{CH}-\text{CH}_2-\text{CH}=\text{CH}-(\text{CH}_2)_7-\text{COOH}$	Most plant oils, poultry fat
18:3 $\Delta^{9,12,15}$ (n-3)	α -Linolenic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_3-(\text{CH}_2)_2-\text{COOH}$	Linseed (flax), soybean, and canola oils
20:4 $\Delta^{5,8,11,14}$ (n-6)	Arachidonic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_5-(\text{CH}_2)_3-\text{COOH}$	Fish oils
20:5 $\Delta^{5,8,11,14,17}$ (n-3)	Eicosapentaenoic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_5-(\text{CH}_2)_3-\text{COOH}$	Marine algae and fish that consume the algae
22:6 $\Delta^{4,7,10,13,16,19}$ (n-3)	Docosahexaenoic acid	$\text{CH}_3-(\text{CH}_2-\text{CH}=\text{CH})_6-(\text{CH}_2)_2-\text{COOH}$	Marine algae and fish that consume the algae

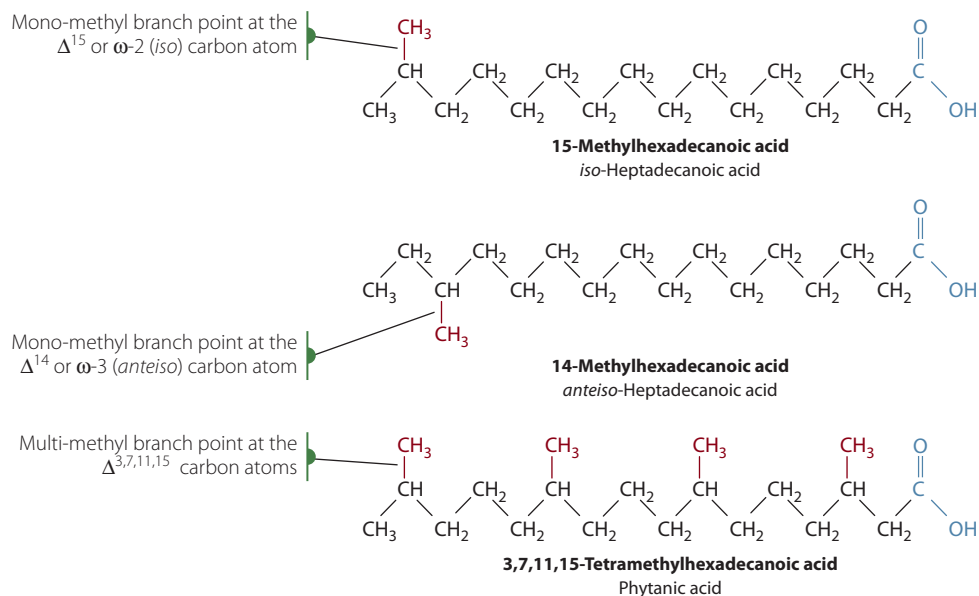
*Fats and oils in the food supply contain many types of fatty acids of varying proportions. The sources listed here indicate foods that are comparatively enriched in the specific fatty acid.

Odd-Chain and Branched-Chain Fatty Acids

Most fatty acids have an even number of carbon atoms. The reason for this will be evident in the sections related to fatty acid oxidation and synthesis. Fatty acids with an odd number of carbon atoms, although less abundant, are found in certain foods and in human tissues. Meat and dairy products from ruminant animals and some fatty fish are foods that contain detectable amounts of odd-chain fatty acids, mainly pentadecanoic acid (15:0) and heptadecanoic acid (17:0). In ruminant animals, these fatty acids are made by rumen bacteria and are incorporated into meat and milk fat. Fish can make odd-chain fatty

acids in oil-producing glands in addition to eating aquatic organisms that produce them. Recent studies also indicate that peroxisomes of human cells can make 17:0 from 18:0 in a process called α -oxidation that removes a single carbon atom [2].

Branched-chain fatty acids are normally saturated with one or more methyl groups attached along the hydrocarbon chain. Each methyl group represents a branch point. The majority of branched-chain fatty acids in human diets have 14 or 16 carbon atoms in the backbone chain with a single methyl group attached to the second (ω -2) or third (ω -3) carbon from the end (Figure 5.3). Food sources

**Figure 5.3** Branched-chain fatty acids.

of branched-chain fatty acids are mainly meat and dairy products from ruminant animals and fatty fish. The average intake in human diets is about 500 mg/day [3].

Naming branched-chain fatty acids can be confusing because of different nomenclature in the scientific literature. One method begins by naming the backbone chain, followed by identification of methyl branch points and their location. For example, the 16-carbon saturated fatty acid in Figure 5.3 is hexadecanoic acid (commonly called palmitic acid). The three variations in Figure 5.3 illustrate the different positions of methyl branch points using the delta system, which are reflected in the fatty acid name—for example, 15-methylhexadecanoic acid.

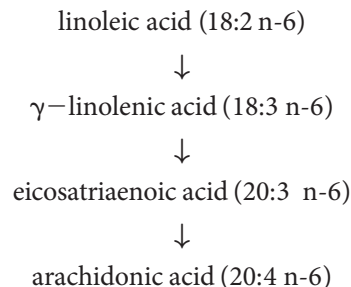
Another method simply counts the total number of carbon atoms present in the fatty acid, including all methyl groups. In this method, 15-methylhexadecanoic acid is also called *iso*-heptadecanoic acid (or *iso*-17:0). The term *iso* refers to the position of the mono-methyl branch point when located at the second carbon from the omega end. The term *anteiso* identifies the position of the mono-methyl branch point at the third carbon from the omega end. This method is not useful for naming branched-chain fatty acids having multiple methyl groups.

A unique branched-chain fatty acid, phytanic acid, contains multiple methyl branch points, as shown in Figure 5.3. Phytanic acid is a breakdown product of chlorophyll found in green plants. Humans lack digestive enzymes that break down chlorophyll, so the presence of phytanic acid in human tissues is due to dietary intake. Like other branched-chain fatty acids, meat and dairy products from ruminant animals and certain fish are food sources of phytanic acid. Humans consume about 50–100 mg/day [4].

Essential Fatty Acids

If fat is entirely excluded from the diet of humans, a condition develops that is characterized by retarded growth, dermatitis, kidney lesions, and early death. Studies have shown that eating certain unsaturated fatty acids is effective in curing the conditions related to the lack of these fatty acids. Two unsaturated fatty acids cannot be synthesized in the body and must be acquired in the diet from plant foods. The two essential fatty acids are linoleic acid (18:2 n-6 or 18:2 $\Delta^{9,12}$) and α -linolenic acid (18:3 n-3 or 18:3 $\Delta^{9,12,15}$). They are essential because humans lack enzymes called Δ^9 and Δ^{15} desaturases, which incorporate double bonds at these positions. These enzymes are found only in plants. Humans are incapable of forming double bonds beyond the Δ^9 carbon in the chain. If a $\Delta^{9,12}$ fatty acid is obtained from the diet, however, additional double bonds can be incorporated at Δ^6 (**desaturation**). Fatty acid chains can also be elongated by the enzymatic addition of two carbon atoms at the carboxylic acid end of the chain. These reactions are discussed further in the “Synthesis of Fatty Acids” section of this chapter.

In mammalian cells, linoleic acid can be converted to arachidonic acid (20:4 n-6) via the so-called “omega-6 pathway.” The intermediates in the desaturation and **elongation** pathway are:



In a similar manner, α -linolenic acid can be converted to eicosapentaenoic acid (20:5 n-3) via the “omega-3 pathway.” Both arachidonic and eicosapentaenoic acid are metabolically significant because they are precursors of eicosanoids, important signaling molecules discussed later in this chapter. Linseed (flax) oil is particularly rich in α -linolenic acid, whereas fish oils are good sources of eicosapentaenoic acid and docosahexaenoic acid (22:6 n-3). The fatty acid composition of common fats and oils is given in Table 5.2. It is interesting to note that fatty acid composition in wild-caught salmon is different than farm-raised salmon, due to differences in the diets that these fish consume. Farm-raised salmon are often fed plant sources of protein and fat (corn or soybean meal) that influences their fatty acid composition.

n-6 versus n-3 Fatty Acids

It is estimated that our human ancestors consumed foods that provided equal amounts of n-6 and n-3 fatty acids. Today, the intake of n-3 fatty acids is quite low and overwhelmed by n-6 fatty acids in the diet, with linoleic acid providing 80–90% of all PUFA. This is due to the widespread use of plant oils, such as soybean oil, in the production of manufactured food products and in foodservice frying oils, coupled with the relatively low intake of fish and other n-3 fatty acid sources in Western diets. Assessing the metabolic impact of dietary n-6 and n-3 fatty acids is important in the field of nutrition. The disproportionate amounts of n-6 and n-3 fatty acids can have metabolic consequences that are discussed further in the “Synthesis of Fatty Acids” section of this chapter.

Triacylglycerols (Triglycerides)

Most adipose tissue is composed of triacylglycerols, which represent a highly concentrated form of stored energy. (*Triacylglycerols* is the currently accepted name that has replaced the older name *triglycerides*.) When triacylglycerols in adipose tissue are used for energy, the fatty acids are cleaved from glycerol by lipases and released from the

Table 5.2. Fatty Acid Composition of Fats and Oils

	SFA										MUFA					PUFA				
	4:0	6:0	8:0	10:0	12:0	14:0	16:0	18:0	20:0	16:1 n-9	18:1 n-9	20:1 n-9	18:2 n-6	20:4 n-6	18:3 n-3	20:5 n-3	22:5 n-3	22:6 n-3		
Plant Oils																				
	Butyric	Caproic	Caprylic	Capric	Lauric	Myristic	Palmitic	Stearic	Arachidic	Palmitoleic	Oleic	Gadoleic	Linoleic	Arachadonic	α-Linolenic	Eicosapentaenoic	Docosapentaenoic	Docosahexaenoic		
Canola oil							4.3	2.1	0.7	0.2	61.7		18.6		9.1					
Cocoa butter						0.1	25.4	33.2		0.2	32.6		2.8		0.1					
Coconut oil		0.5	6.8	5.4	41.8	16.7	8.6	2.5	0.1		6.3		1.7							
Corn oil						0.1	10.6	1.8	0.4	0.1	27.3	0.1	53.2		1.2					
Cottonseed oil						0.8	22.7	2.3	0.1	0.8	17.0		51.5		0.2					
Flax (linseed) oil						0.1	5.1	3.4		0.1	18.3		14.2		53.4					
Olive oil							11.3	2.0	0.4	1.3	71.3	0.3	9.8		0.8					
Palm oil					0.1	1.0	43.5	4.3		0.3	36.6	0.1	9.1		0.2					
Palm kernel oil		0.2	3.3	3.7	47.0	16.4	8.1	2.8			11.4		1.6							
Peanut oil						0.1	9.5	2.2	1.4	0.1	44.8	1.3	32.0							
Safflower oil						0.1	4.3	2.3	0.3	0.1	12.0	0.1	77.7		0.4					
Soybean oil						0.1	10.6	4.0	0.3	0.1	23.2		53.7		7.6					
Sunflower oil						0.1	7.0	4.5	0.4	0.1	18.7	0.1	67.5		0.8					
Animal Fats																				
Beef tallow					0.9	3.7	24.9	18.9	0.1	4.2	36.0	0.3	3.1		0.6					
Chicken fat					0.1	0.9	21.6	6.0	0.1	5.7	37.3	1.1	19.5		1.0					
Lard (pork fat)					0.1	0.2	1.3	23.8	13.5	0.1	2.7	41.2	1.0	10.2		0.1				
Milk (butter) fat	3.2	1.9	1.1	2.5	2.8	10.0	26.2	12.1	0.1	2.2	25.0		2.2		1.5					
Fish Oils																				
Herring oil					0.2	7.2	11.7	0.8		9.6	12.0	13.6	1.1	0.3	0.8	6.3	0.6	4.2		
Mackerel oil						4.9	15.3	3.0		5.2	16.4	7.5	1.6	1.3	1.1	6.5	1.5	10.1		
Menhaden oil						8.0	15.1	3.8		10.5	14.5	1.3	2.2	1.2	1.5	13.2	4.9	8.6		
Salmon oil (wild)						2.1	10.0	3.3		4.0	21.3	3.5	2.7	4.2	4.7	5.1	4.5	17.6		
Salmon oil (farmed)						4.1	14.0	3.7	0.2	5.9	20.2	2.0	6.7	0.7	1.1	6.4	2.9	8.2		

Source: U.S. Department of Agriculture, Agricultural Research Service, FoodData Central, 2019. <https://fdc.nal.usda.gov> Accessed 3/12/2020.

cell in free (nonesterified) form. The free fatty acids bind to serum albumin and are transported to various tissues for oxidation via the TCA cycle.

Triacylglycerols also account for nearly 95% of dietary fat. Structurally, they are composed of a trihydroxyalcohol, glycerol, to which three fatty acids are attached by ester bonds, as shown in Figure 5.4; the formation of each of these ester bonds liberates a water molecule. The fatty acids may be all the same (a simple triacylglycerol) or different (a mixed triacylglycerol). The fatty acids in triacylglycerols can be all saturated, all monounsaturated, all polyunsaturated, or any combination.

Triacylglycerols exist as fats (solid) or oils (liquid) at room temperature, depending on the nature of the component fatty acids. In general, short-chain fatty acids, medium-chain fatty acids, and unsaturated long-chain fatty acids have relatively low melting points, so triacylglycerols with these fatty acids tend to be liquid oils at room temperature. Saturated long-chain fatty acids have higher melting points, so triacylglycerols with a high proportion of long-chain fatty acids exist as solid fats at room temperature.

The specific glycerol hydroxyl group to which a certain fatty acid is attached is indicated by a numbering system for the three glycerol carbon atoms. When different fatty acids are attached to the first and third carbons of glycerol, the second carbon becomes asymmetric. In this case, the glycerol molecule may exist in either the D or the L form. A system of nomenclature called stereospecific numbering (*sn*) has been adopted, as shown in Figure 5.4, in which the

sn-2 hydroxyl group is oriented to the left (L). Enzymes of the body are able to distinguish between the three carbons of glycerol and are generally quite specific. This specificity is important in digesting and synthesizing triacylglycerols, as is discussed later in this chapter.

Mono- and diacylglycerols contain one or two fatty acids, respectively. The single fatty acid of monoacylglycerols can be attached to any of the three carbons of glycerol. When the fatty acid is attached to *sn*-1, the molecule is called *1-monoacylglycerol*; when attached to *sn*-2, it is called *2-monoacylglycerol*. A single fatty acid attached to *sn*-1 or *sn*-3 are indistinguishable, so either case is recognized only as *1-monoacylglycerol*. Diacylglycerols exist as either *1,2-diacylglycerol* or *1,3-diacylglycerol*. Though present in the body only in small amounts, the mono- and diacylglycerols are important intermediates in some metabolic reactions and may form the basis of other lipid classes. They are also used in processed foods, where they function as emulsifying agents.

Phospholipids

Phospholipids, as the name implies, are phosphate-containing lipids that form the structural basis of all cell membranes, including the membranes of organelles within the cell (see Figures 1.2 and 1.3). Because of their **amphipathic** properties, phospholipids are also critical components of plasma lipoproteins in which phospholipids, triacylglycerols, and other lipids form stable complexes that allow them to be transported in the blood.

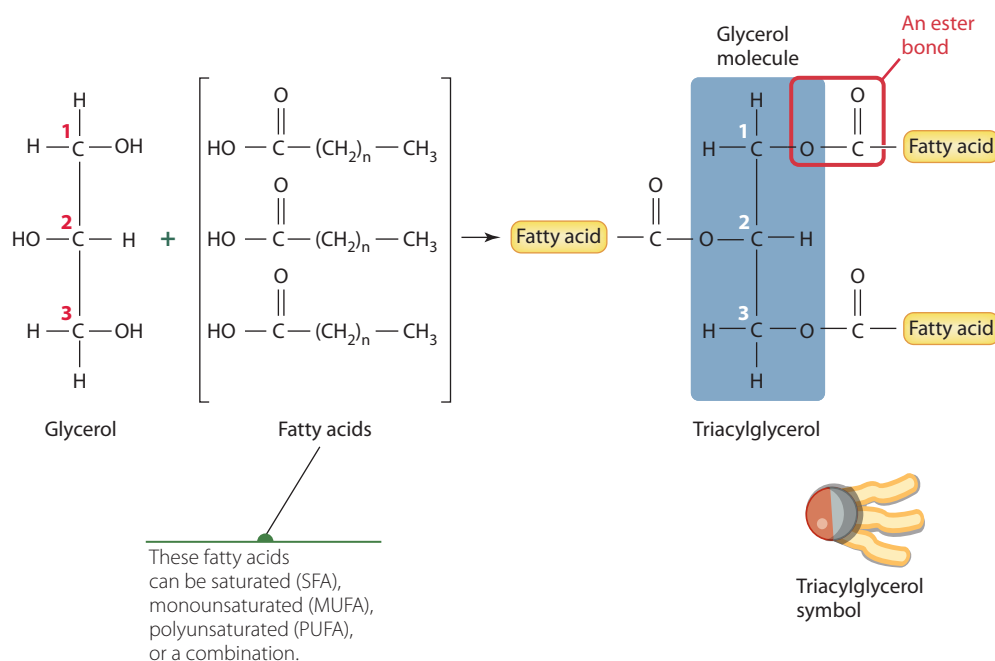


Figure 5.4 Linkage of fatty acids to glycerol to form a triacylglycerol. Fatty acids are attached to glycerol by an ester bond.

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Glycerol forms the structural backbone of phospholipids. Fatty acids are esterified to the hydroxyl groups at the *sn*-1 and *sn*-2 positions of glycerol. A phosphate group is esterified at the *sn*-3 position and, in turn, a polar “head group” is esterified to the phosphate. A phospholipid molecule lacking the head group is known as phosphatidic acid (Figure 5.5). The conventional numbering of the glycerol carbon atoms is the same as that for triacylglycerols, provided the glycerol is written in the L configuration so that the *sn*-2 fatty acid constituent is directed to the left, as shown in Figure 5.5. The fatty acid portion of the molecule is hydrophobic, whereas the phosphate and the polar head group are hydrophilic, thus giving phospholipids their amphipathic property.

Phospholipids generally have a saturated fatty acid at *sn*-1 and an unsaturated fatty acid at *sn*-2, although many fatty acid combinations are possible, resulting in a broad range of distinct phospholipids. Despite this fact, phospholipids are named according to the specific head group rather than their fatty acids. Common head group

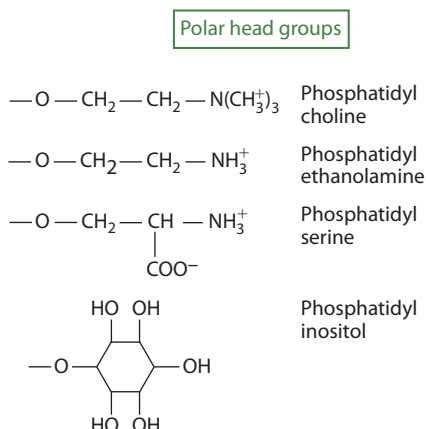
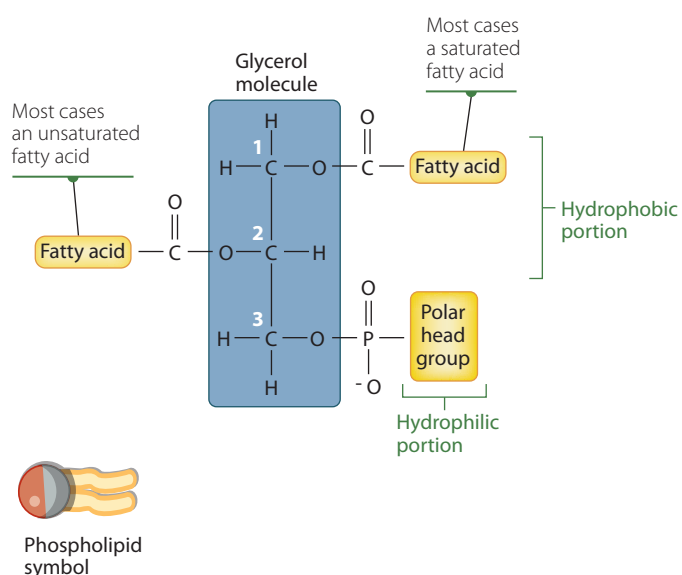


Figure 5.5 Typical structure of phospholipids.

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molecules are choline, ethanolamine, serine, and inositol, each possessing a hydroxyl group through which esterification to the phosphate takes place (Figure 5.5). The compounds are named as the phosphatidyl derivatives of the alcohols, as indicated in the figure. The most common phospholipid in mammal tissues is phosphatidylcholine, making up about half of the phospholipids in cell membranes, followed by phosphatidylethanolamine in terms of abundance. Food grade phosphatidylcholine (called *lecithin*) is produced commercially from egg yolks and soybeans for use as an emulsifier in the production of foods that contain both fat and water, such as margarine and chocolate. Phosphatidylserine and phosphatidylinositol are also found in cell membranes, but they serve important functions beyond membrane structure. Phosphatidylserine participates in apoptosis by attracting phagocytes during cellular degradation. Phosphatidylinositol participates in several cell functions, as described in the next section.

Diphosphatidylglycerol is another phospholipid found in several tissues of the body. It is also called cardiolipin and was originally identified within heart muscle (Figure 5.6). The structure of cardiolipin can be viewed as two phospholipid molecules that share a common head group of glycerol. The overall structure therefore contains three glycerol molecules and four fatty acids. Cardiolipin is located exclusively in the inner membrane of mitochondria and attaches cytochrome *c* to the membrane.

The main structure of phospholipids described thus far involves ester bonds between the glycerol backbone and the fatty acids at the *sn*-1 and *sn*-2 positions. In some cases that linkage is an ether bond (—C—O—C—) or vinyl ether bond (—C—O—C=C—), resulting in the so-called “ether phospholipids.” Platelet-activating factor is perhaps the most studied ether phospholipid, having a fatty acid ether bond at *sn*-1, an acetate ester bond at *sn*-2, and phosphocholine as the head group at *sn*-3. Platelet-activating factor is an important signaling molecule that participates

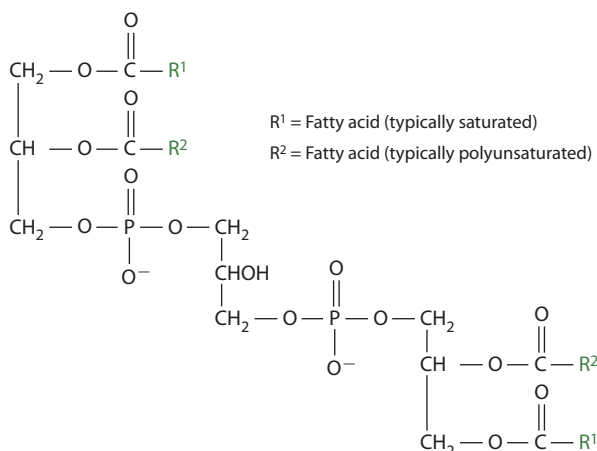


Figure 5.6 Structure of diphosphatidylglycerol (cardiolipin).

in several metabolic events including inflammation, platelet aggregation, and neural functions.

Another well-known ether phospholipid is plasmalogen, which has a fatty acid vinyl ether bond at *sn*-1, a fatty acid ester bond at *sn*-2, and phosphocholine, ethanolamine, or serine as the head group at *sn*-3. Plasmalogens are found in many tissues, most notably the heart and brain. Choline plasmalogen constitutes up to 40% of all phospholipids in the heart, while ethanolamine plasmalogen makes up about 20% of phospholipids in the brain and is concentrated mostly in the myelin sheath.

Biological Roles of Phospholipids

Phospholipids play several important roles in the body. Because of their amphipathic nature, phospholipids form the foundational structure of lipid bilayers that comprise cell membranes that serve as a selective barrier for the passage of water-soluble and fat-soluble materials across the membrane. Phospholipids also form a monolayer on the surface of bloodborne lipoprotein particles, thereby stabilizing the particles in the aqueous medium.

In addition to their structural role, phospholipids are physiologically active compounds. In particular, phosphatidylinositol plays a significant role in cell signaling and membrane dynamics. Phosphatidylinositol resides mainly on the cytosolic side of cell membranes where the inositol hydroxyl groups at C-3, C-4, and C-5 can be phosphorylated, resulting in *phosphoinositides*. Mammalian cells synthesize seven distinct phosphoinositides containing one, two, or three additional phosphate groups (Figure 5.7). The reversible phosphorylation and dephosphorylation reactions are catalyzed by phosphoinositide kinases and phosphatases, respectively, in response to extracellular stimuli [5].

Phosphoinositides exert their physiological role by attracting regulatory proteins to the membrane. These “effector” proteins regulate many activities of cell

membranes, such as endocytosis, membrane fusion, and ion channels. For example, phosphatidylinositol 4,5-bisphosphate (PIP₂) can bind protein kinase C, phospholipase C, and integral membrane proteins. In the case of phospholipase C, the enzyme hydrolyzes PIP₂, yielding inositol-1,4,5-trisphosphate and diacylglycerol. Both of these products function as second messengers in cell signaling.

Another example is PIP₃ and its role in insulin signaling pathways, as previously discussed in Chapter 3 (Figure 3.12). The binding of insulin to its receptor triggers phosphatidylinositol-3-kinase to synthesize PIP₃ (by phosphorylating PIP₂), which then recruits other protein kinases to the cell membrane, including protein kinase B. In turn, protein kinase B phosphorylates many enzymes throughout the body that regulate carbohydrate, protein, and lipid metabolism.

Sphingolipids

Sphingolipids are found in the plasma membrane of all cells, although their concentration is highest in cells of the central nervous system. Unlike the lipid classes discussed thus far, sphingolipids are built on the amino alcohol sphingosine rather than glycerol as the structural backbone (Figure 5.8). All sphingolipids have a fatty acid attached to the amino group (R¹ in the figure). The simplest sphingolipid is **ceramide**, in which the terminal hydroxyl has no other group attached. The other sphingolipids build on ceramide, with substituent molecules attached to the terminal hydroxyl group (R² in the figure). **Sphingomyelin** is formed when phosphocholine is added to ceramide. (Due to the presence of phosphate, sphingomyelin can also be considered a phospholipid, although it makes more sense to classify it primarily as a sphingolipid because of its structural similarity to other sphingolipids.) Sphingomyelin is

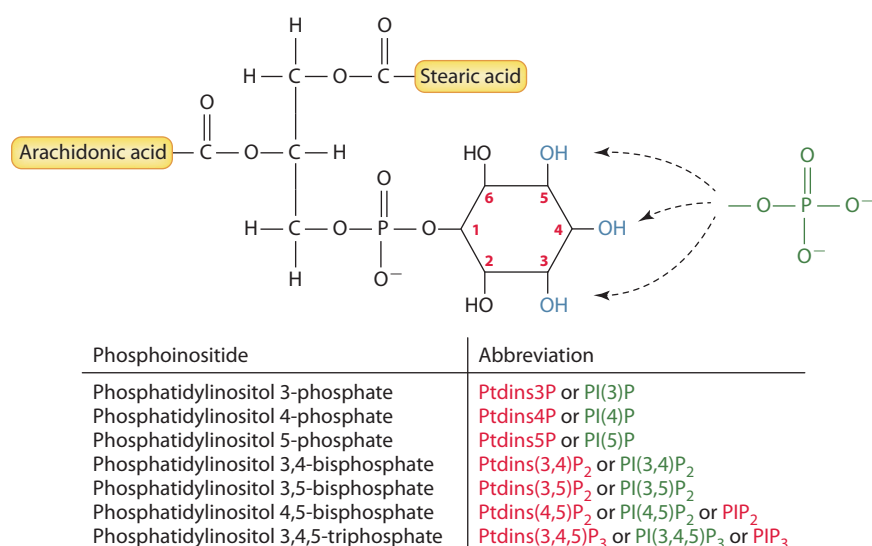


Figure 5.7 Phosphoinositide synthesis from phosphoinositol. Kinases catalyze the phosphorylation of phosphoinositol at C-3, C-4, or C-5 hydroxyl groups, producing phosphoinositides having one, two, or three additional phosphates. The most frequent fatty acids in phosphoinositides are stearic acid and arachidonic acid in the *sn*-1 and *sn*-2 positions, respectively. Various abbreviations are found in the scientific literature.

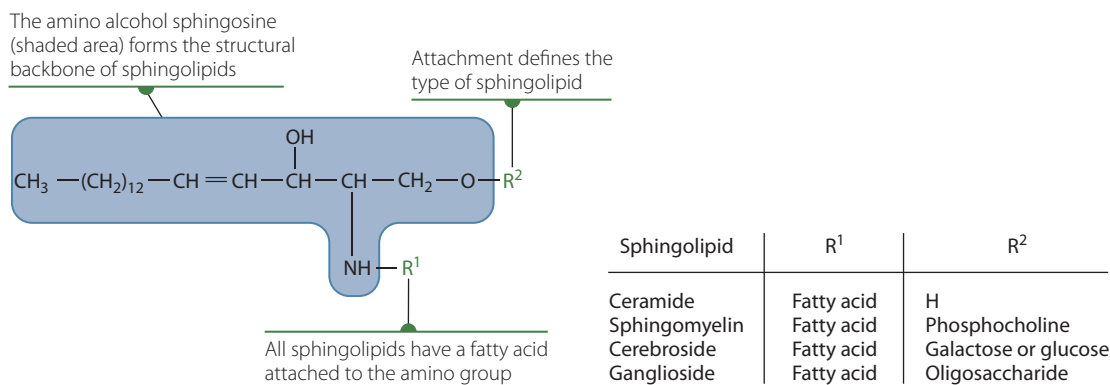


Figure 5.8 Structure of sphingolipids.

particularly abundant in the myelin sheath of nerve tissues and thus important for nervous system function.

Cerebrosides are formed when a single sugar molecule, either galactose or glucose, attaches to the terminal hydroxyl group of ceramide. Galactocerebrosides are abundant in the myelin sheath of nerves and in brain tissue, particularly the white matter, whereas glucocerebrosides are found mainly in spleen and red blood cells. Cerebrosides are located on the plasma membranes where they serve a protective role, acting as an insulator and facilitator in the proper conduction of nervous impulses. **Gangliosides** resemble cerebroside, except they have multiple sugar units linked to the terminal hydroxyl group of ceramide. In addition, gangliosides have a negatively charged sialic acid molecule attached to the oligosaccharide chain. Gangliosides are located on the outer surface of plasma membranes mainly in nerve tissue where they function as markers in cellular recognition and as receptors for certain hormones and toxins, including the cholera toxin.

Sterols

Sterols are structurally quite different than the other lipid classes. They are characterized by having a four-ring steroid nucleus and at least one hydroxyl group, hence the name sterol (steroid alcohol). This section describes three categories of sterols important to human nutrition: cholesterol, bile acids and salts, and phytosterols.

Cholesterol

Cholesterol is the most common sterol in humans. It can exist in free form or the hydroxyl group at C-3 can be esterified with a fatty acid. The structure of cholesterol is shown in Figure 5.9, along with the numbering system for the carbons in the steroid nucleus and the side chain. Cholesterol is an important constituent of plasma membranes along with phospholipids due to its amphipathic nature. In free form, the hydroxyl group of cholesterol interacts with

the phospholipid head group so that the hydrophobic side chain of cholesterol is oriented in parallel with the fatty acids of phospholipids (see Figure 1.3). Cholesterol constitutes nearly 25% of the lipids in plasma membranes of some nerve cells but may be absent in intracellular membranes. Cells can regulate the amount of cholesterol in membranes by esterifying “excess” cholesterol with a fatty acid and storing the cholesterol esters in vesicles within the cytosol. When unesterified (free) cholesterol is needed, the cholesterol esters are hydrolyzed and free cholesterol is transported back to the membrane.

Cholesterol serves as the precursor for many important steroids in the body, including the bile acids; steroid sex hormones such as estrogens, androgens, and progesterone; the adrenocortical hormones; and vitamin D (cholecalciferol). The major derivatives of cholesterol are shown in Figure 5.10. These steroids differ structurally from one another in the arrangement of double bonds in the ring system, the presence of carbonyl or hydroxyl groups, and the nature of the side chain at C-17. All of these structural modifications are mediated by enzymes that function as dehydrogenases, isomerases, hydroxylases, or desmolases. Desmolases remove or shorten the length of side chains on the steroid nucleus.

Bile Acids and Bile Salts

As discussed in Chapter 2, bile acids and bile salts are critical components of bile that act as detergents in the small intestine to emulsify dietary lipids for digestion and absorption. The liver synthesizes two bile acids, cholic acid and chenodeoxycholic acid, each of which is conjugated with either glycine or taurine, resulting in four different primary bile salts (Figure 5.11). After the newly formed bile salts enter the small intestine via bile secretion, they are subject to dehydroxylation by bacteria, thus producing secondary bile salts. All of the bile salts can be reabsorbed into the enterohepatic circulation and returned to the liver. In this way, secondary bile salts, while not directly synthesized by the liver, are present in gallbladder bile.

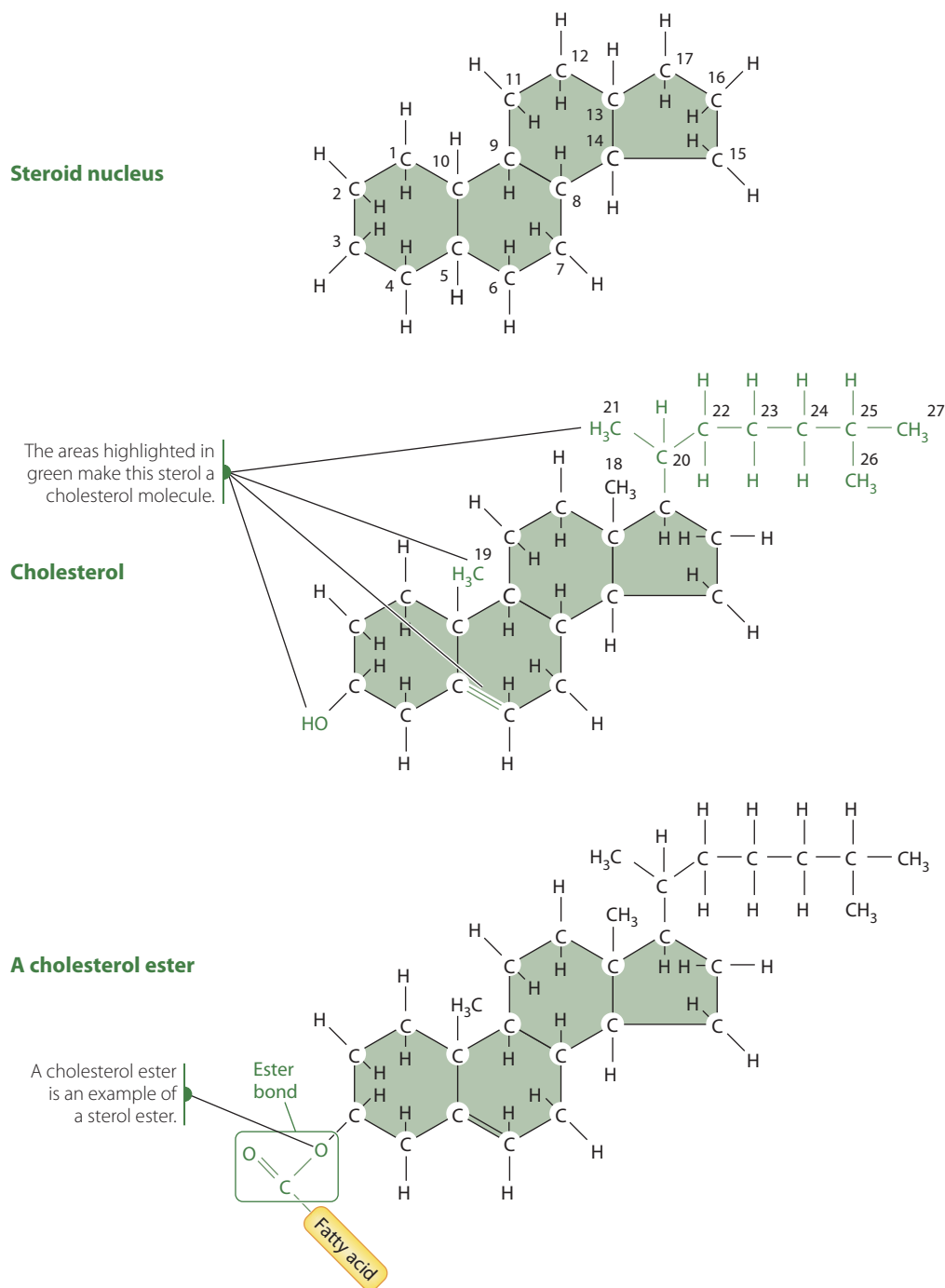


Figure 5.9 Structure of a sterol, cholesterol, and a cholesterol ester.

Source: Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Phytosterols

Plant cell membranes contain structural sterols in a manner similar to cholesterol in animal cells. These phytosterols are structurally similar to cholesterol, with only slight differences in the side chain (refer to Figure 5.9). Some phytosterols are actually stanols—meaning, the double bond between carbons 5 and 6 is eliminated by saturating the molecule with hydrogen atoms. Strictly speaking,

stanols are chemically different than sterols, but they are often counted together under the heading of phytosterols. Stanols constitute about 5–10% of total phytosterols present in nature. The hydroxyl group at C-3 of phytosterols can be esterified with a fatty acid.

Phytosterols are found throughout the food supply. Plant oils, legumes, nuts, and seeds have relatively high concentrations of phytosterols, whereas fruits and vegetables

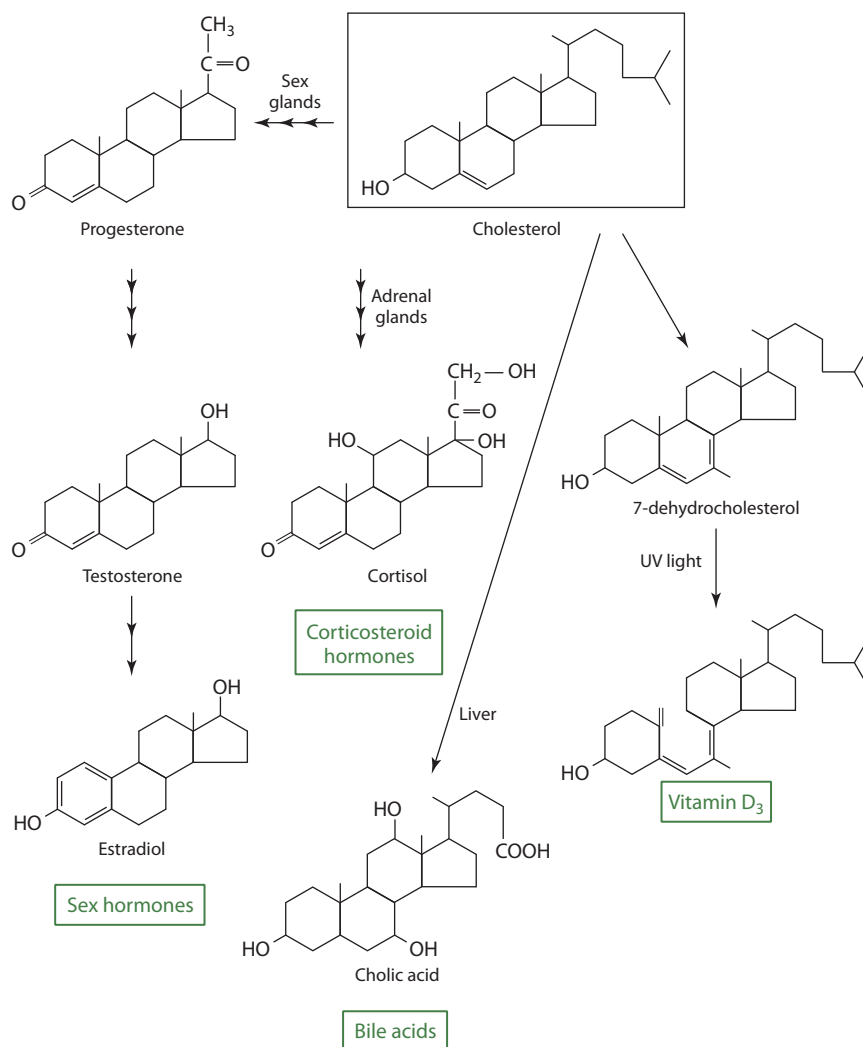


Figure 5.10 The formation of physiologically important steroids from cholesterol. Only representative compounds from each category of steroid are shown.

have low concentrations. Although cereal grains have only modest concentrations, humans consume large amounts of grain products, making them a quantitatively important source of phytosterols. Intake of total phytosterols from natural food sources is about 200–300 mg/day, with Asian and vegetarian diets providing significantly more [6]. The manufacture of foods and supplements enriched with phytosterols has increased in recent years because of their cholesterol-lowering properties. Phytosterol intake of 2 g/day results in blood cholesterol reductions of 10% or more. Approval to make health claims about phytosterols on food and supplement products has been granted by the FDA, the European Foods Safety Authority, and Health Canada. Because of their similarity to cholesterol, phytosterols have the ability to displace cholesterol from micelles that form during digestion, reducing the amount of cholesterol available for intestinal absorption.

5.2 DIETARY SOURCES

Triacylglycerols—fats and oils—are ubiquitous in the food supply. They are found naturally in both plant and animal foods. Foods prepared in restaurants often contain high-fat ingredients and are cooked in oil; grocery stores are abundant with prepared and packaged foods containing fat; and many consumers use fats and oils when cooking at home. In order to track the amount and type of fat consumed in the United States, the Food and Nutrition Service of the U.S. Department of Agriculture maintains a database of the major food groups that contribute fat to the food supply [7]. As indicated in Figure 5.12, the primary source of fat is the “Cooking Oils” category, which represents all uses of edible oils (mostly plant derived) in the United States, including those used in the food industry for the manufacture of food products; cooking and frying

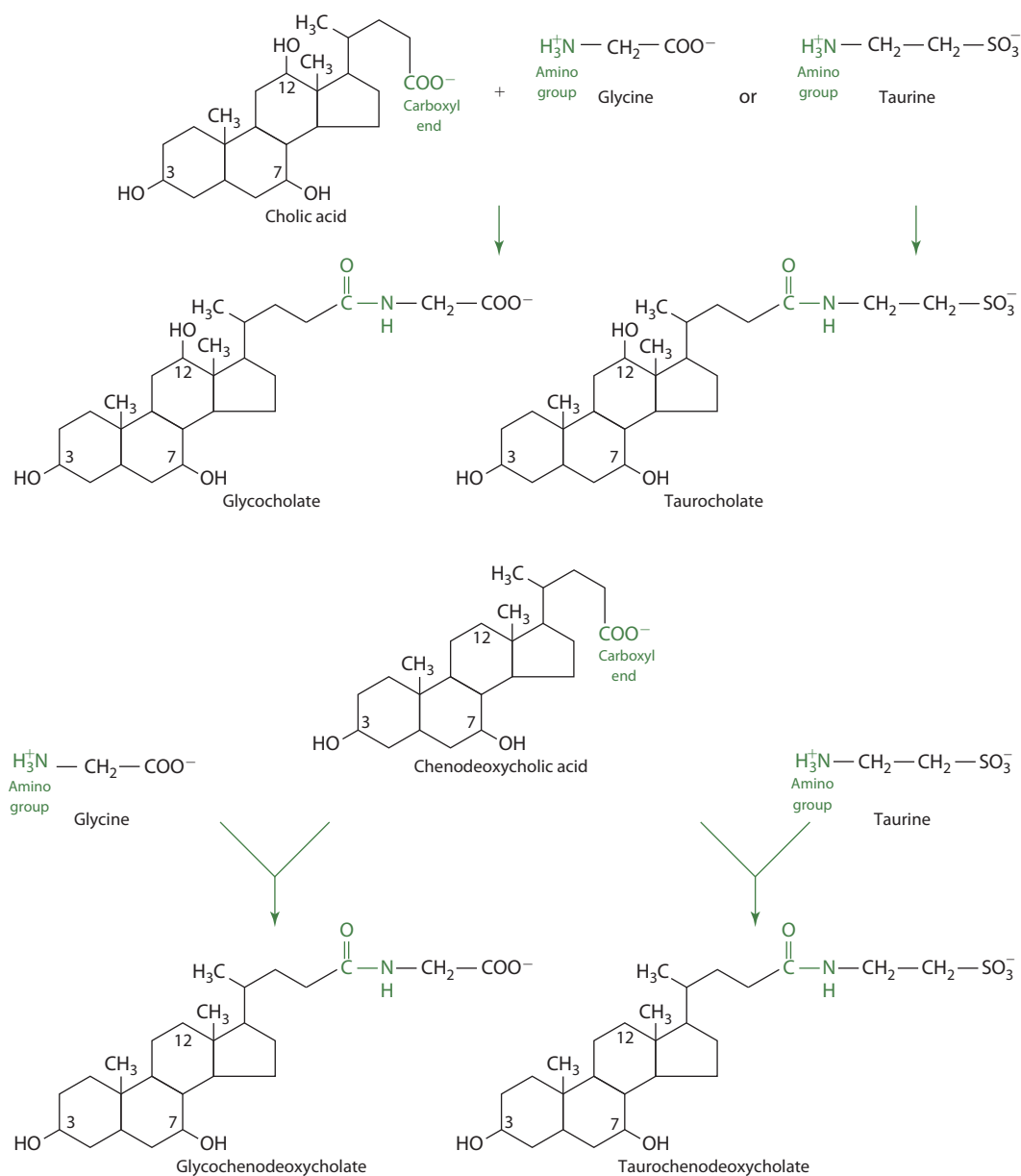


Figure 5.11 The formation of glycocholate, taurocholate, glycochenodeoxycholate, and taurochenodeoxycholate conjugated bile acids.

oils used by restaurants and other foodservice institutions; and salad and cooking oils used directly by consumers. The “Shortening” category represents solid fats (mostly plant derived) that are used for similar purposes as “Cooking Oils.” Figure 5.12 further shows that the food categories of animal origin contribute significant amounts of fat in the food supply. Note that “Butter” has been separated from “Dairy Products” to emphasize its individual contribution to overall fat intake. The “Other” category includes fat contributed by fruits, vegetables, fish, and specialty oils.

The proportion of SFA, MUFA, and PUFA comprising the fat of each food group is also illustrated in Figure 5.12.

The majority of SFA is contributed by cooking oils, red meats, and dairy products. Although the relative amount of SFA in cooking oils is lower than red meats and dairy products, the widespread use of cooking oils means they provide about 20% of all SFA in the food supply. Cooking oils (mostly plant derived) supply nearly all of the PUFA consumed in the United States, of which 80–90% is linoleic acid. Cooking oils, shortening, and red meats provide most of the MUFA, of which > 90% is oleic acid. Knowing the fatty acid composition of common fats and oils (Table 5.2) can help guide health care professionals and consumers in making well-informed decisions about dietary fats.

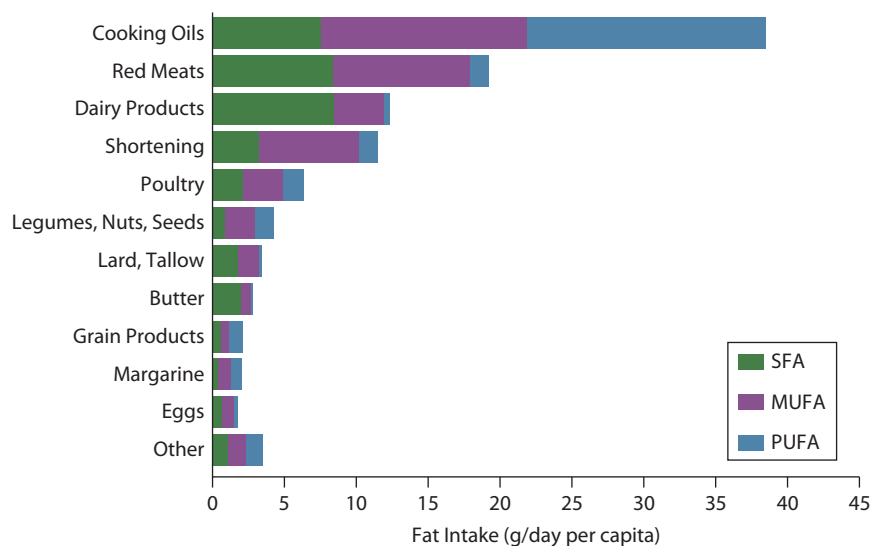


Figure 5.12 Dietary fat contribution from major food groups.

Source: U.S. Department of Agriculture, Nutrient Content of the U.S. Food Supply, 2010.

Although the information in Figure 5.12 shows only the major food groups, it is helpful to know the main contributors of fat when seeking to modify one's fat intake. For example, if reduction in total fat intake is desired, focusing on all foods made with or cooked in "cooking oils" would be a good starting point. Reduction in red meats, dairy products, and foods made with shortening would also be advisable. If reducing SFA is the goal, the obvious targets are red meats and dairy products because of their relatively high proportion of SFA. However, products containing cooking oils and shortening should not be overlooked, particularly plant-derived solid fats that have relatively high proportions of SFA, such as palm kernel, palm, and coconut oils. Table 5.3 provides the fat content of foods commonly consumed in the United States. In updating the 2015 *Dietary Guidelines for Americans*, the Advisory Committee emphasized that "strong and consistent evidence from [randomized controlled trials] shows that replacing SFA with unsaturated fats, especially PUFA, significantly reduces total and LDL cholesterol . . . and reduces the risk of [cardiovascular disease] events and coronary mortality" [8]. These relationships are discussed later in the chapter.

The intake of *trans* fatty acid is relatively minor, but their impact on cardiovascular health is a major concern. As mentioned earlier in the chapter, the use of partially hydrogenated oils has been banned by the FDA, so the presence of *trans* fatty acids is due to meat and dairy products from ruminant animals. Before the ban was enacted, *trans* fatty acid intake was estimated to be 1.3 g/day [9], with much lower intake expected after food products currently available are allowed to cycle out of the market.

A unique *trans* fatty acid found in meat and dairy products from ruminant animals is conjugated linoleic acid. Recall that the double bonds in most polyunsaturated fatty acids are separated by two single bonds between the carbon atoms in the backbone chain. In contrast, the double

Table 5.3 Fat Content of Common Foods

		SFA	MUFA	n-6 PUFA	n-3 PUFA
		grams			
Bean burrito	1 each	4.3	2.3	3.5	0.6
Beef, top sirloin, broiled	3 oz	3.1	3.4	0.3	0.0
Beef, ribeye, broiled	3 oz	4.9	5.1	0.4	< 0.1
Butter	1 Tbsp	7.3	3.0	0.4	< 0.1
Cheddar cheese	1 oz	5.3	2.6	0.3	< 0.1
Chicken breast, with skin, fried	1 med	4.9	7.6	4.0	0.2
Chicken breast, without skin, baked	1 med	0.9	1.1	0.6	< 0.1
Coconut "milk," regular	1 cup	42.7	2.1	0.5	0.0
Corn dog	1 each	2.7	3.7	2.4	0.2
Dressing, olive oil and vinegar	2 Tbsp	1.9	9.9	1.3	< 0.1
Dressing, ranch	2 Tbsp	2.1	2.8	6.7	1.1
Eggs, hard-boiled	2 large	1.6	2.0	0.6	< 0.1
Fish, Atlantic mackerel, baked	3 oz	3.5	6.0	0.2	3.4
Fish, salmon, baked	3 oz	1.4	2.3	0.3	1.8
Fish, whitefish, breaded, fried	3 oz	3.1	2.7	6.2	0.4
Ham, sliced	3 oz	2.5	3.7	0.6	< 0.1
M&Ms candies, 1.7 oz	1 pkg	6.3	2.5	0.4	< 0.1
Nuts, almonds	1 oz	1.2	9.4	3.7	< 0.1
Nuts, peanuts	1 oz	2.2	7.4	2.8	< 0.1
Nuts, walnuts	1 oz	1.7	2.5	10.8	2.6
Popcorn, microwave, regular	1 bag	12.7	0.3	3.6	0.0
Popcorn, microwave, light	1 bag	1.2	3.6	2.9	0.2

Source: U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov> Accessed 3/12/2020.

bonds in conjugated linoleic acid are separated by one single bond between carbon atoms. The most common isomer found in food is 18:2 $\Delta^{cis9,trans11}$ (rumenic acid), with smaller amounts of 18:2 $\Delta^{trans10,cis12}$ also found in food. Conjugated linoleic acid isomers have drawn attention from researchers and health professionals due to their efficacy against cancer, obesity, and cardiovascular disease [10].

Recommended Intakes

Recommendations regarding dietary fat and fatty acids have historically come from several governmental and nongovernmental (nonprofit) organizations, including the American Heart Association, the Institute of Medicine (IOM), the U.S. Department of Agriculture, and the U.S. Department of Health and Human Services. These organizations work together in order to provide a cohesive message when making recommendations to the public.

The Food and Nutrition Board of the IOM has not established a Recommended Dietary Allowance (RDA) value for total fat intake. Adequate Intake (AI) levels have been established for infants, but not for adults or children over the age of 12 months (see inside front cover of the book). Rather than focusing on total fat, current recommendations and guidelines focus on specific fatty acids due to their individual effects associated with the prevention or promotion of disease.

AIs are established for the essential fatty acids, linoleic (18:2 n-6) and α -linolenic acid (18:3 n-3), at levels that prevent deficiency symptoms. The AI for α -linolenic acid is also set at levels believed to provide overall health benefits associated with the consumption of n-3 fatty acids (discussed later in the chapter). Table 5.2 shows flax (linseed) oil as having the highest percentage of α -linolenic acid among the common plant oils, followed by canola and soybean oils. α -Linolenic acid serves as a precursor for the highly unsaturated n-3 fatty acids (EPA and DHA), although the conversion efficiency of α -linolenic to EPA and DHA acid is very low in humans. Therefore, consumption of EPA and DHA (present in fatty fish) can avert deficiencies associated with low α -linolenic acid.

Trans fatty acids appear to provide no specific health benefits beyond providing energy. Therefore, no RDA or AI has been set. Since the FDA has banned the use of partially hydrogenated oils in manufactured food products, the only dietary *trans* fatty acids are found naturally in meat and dairy products from ruminant animals. Some older food products containing partially hydrogenated oils manufactured before the ban may still be available, so consumers are advised to check the ingredient list.

The 2015–2020 *Dietary Guidelines for Americans* recommends limiting SFA intake to 10% of total calories and that unsaturated fatty acids should be the primary source of dietary fat. Whether SFA are replaced by MUFA or PUFA depends on the dietary strategy employed, but in

either case will likely result in health benefits. When SFA are replaced with PUFA, it is recommended that n-3 PUFA be selected to minimize the metabolic effects of “too much” n-6 linoleic acid. With MUFA, much attention has focused on the so-called Mediterranean diet. Defining this diet has been challenging, but its general characteristics include high levels of MUFA intake (largely from olive oil) and significantly lower PUFA intake compared to the United States [11]. The Mediterranean diet also includes relatively high amounts of fiber and protein.

5.3 DIGESTION

Dietary lipids are hydrophobic and therefore pose a special problem to digestive enzymes. Like all proteins, digestive enzymes are hydrophilic and normally function in an aqueous environment. The dietary lipid targeted for digestion is emulsified by an efficient process, mediated mainly by bile salts. This emulsification greatly increases the surface area of the dietary lipid, consequently increasing the accessibility of the fat to digestive enzymes.

Triacylglycerols, phospholipids, cholesterol, and phytosterols provide the lipid component of the typical Western diet. Of these, triacylglycerols are by far the major contributor. The National Health and Nutrition Examination Survey (NHANES) for the years 2015–2016 found that males 20 years and over consume an average of 96 g/day and females 20 years and over consume an average of 73 g/day [12]. The intake of cholesterol is significantly less, estimated to be 348 and 256 mg/day for the same groups, respectively. Phytosterol intake is not tracked by NHANES but, as mentioned earlier, it is about 200–300 mg/day, similar to cholesterol intake. Also not tracked by NHANES is phospholipid intake, although it is estimated to be about 2–3 g/day.

Digestive enzymes involved in breaking down dietary lipids in the gastrointestinal (GI) tract are esterases that cleave the fatty acid ester bonds within triacylglycerols (lipases), phospholipids (phospholipases), cholesterol esters (cholesterol esterase), and phytosterol esters (also cholesterol esterase).

Triacylglycerol Digestion

Most dietary triacylglycerol digestion is completed in the lumen of the small intestine, although the process actually begins in the mouth and stomach with lingual lipase released by the serous gland, which lies beneath the tongue, and gastric lipase produced by the chief cells of the stomach. Basal secretion of these lipases apparently occurs continuously but can be stimulated by neural sympathetic agonists, high dietary fat, and sucking and swallowing. These lipases account for limited triacylglycerol digestion (10–30%) that occurs in the stomach. The lipase

activity is made possible by the enzymes' particularly high stability at the low pH of the gastric juices. Gastric lipase readily penetrates milk fat globules without substrate stabilization by bile salts, a feature that makes it particularly important for fat digestion in the suckling infant, whose pancreatic function may not be fully developed. Both lingual and gastric lipases act preferentially on triacylglycerols containing medium- and short-chain fatty acids. They preferentially hydrolyze fatty acids at the *sn*-3 position, releasing a fatty acid and 1,2-diacylglycerols as products. This specificity is advantageous for the suckling infant because short- and medium-chain fatty acids in milk triacylglycerols are usually esterified at the *sn*-3 position [13]. Short- and medium-chain fatty acids are metabolized more directly than are long-chain fatty acids—that is, they can be absorbed into the blood and transported directly to the liver via the hepatic portal vein, as discussed later in this chapter. Commercially available high-energy formulas for preterm infants, which are rich in triacylglycerols containing short- and medium-chain fatty acids esterified at the *sn*-3 position, are designed to take advantage of the lipases' specificity. These products supply ample energy to the premature infant in a small volume [13].

For dietary triacylglycerols to be hydrolyzed by lingual and gastric lipases in the stomach, some degree of

emulsification must occur to expose a sufficient surface area of the substrate. Muscle contractions of the stomach and the squirting of the fat through a partially opened pyloric sphincter produce shear forces sufficient for emulsification. Also, potential emulsifiers in the acid milieu of the stomach include complex polysaccharides, phospholipids, and peptic digests of dietary proteins. The presence of undigested lipid in the stomach delays the rate at which the stomach contents empty, presumably by way of hormones of the enterogastrone family such as secretin, which inhibits gastric motility. Dietary fats therefore have a “high satiety value.”

The partially hydrolyzed lipid emulsion leaves the stomach and enters the duodenum as small lipid droplets. Further emulsification takes place because as mechanical shearing continues, it is complemented by bile salts that are released from the gallbladder as a result of stimulation by the hormone cholecystokinin (CCK). The small intestine has the capacity to digest a large quantity of triacylglycerols with 95% efficiency. Significant hydrolysis and absorption, especially of the long-chain fatty acids, require less acidity, appropriate lipases, more effective emulsifying agents (bile salts), and specialized absorptive cells. These conditions are provided in the lumen of the upper small intestine.

THE GALLBLADDER

Did you know that many animal species have no gallbladder? Most large mammals lack a gallbladder, including elephants, whales, rhinoceroses, horses, zebras, camels, and giraffes. Certain small animals also lack a gallbladder, including rats, some birds, and some fish. So why do humans have a gallbladder?

Evolutionary evidence suggests that species with high fat diets and those that eat sporadically need large amounts of bile when a big meal is available. The presence of a gallbladder allows for large amounts of bile to be stored and ready to help digest a big meal. Species like canines and felines need lots of bile when prey is eaten in large amounts. Humans have also evolved as “meal eaters” and benefit from having a gallbladder.

Gallstones

There are four main components of gallbladder bile: cholesterol, bile salts,

phospholipids, and pigments such as bilirubin. The proportions of these components must stay in balance so cholesterol remains dissolved in the bile milieu. Gallstones are mostly cholesterol crystals that form when an imbalance occurs among the biliary components. Diets low in fiber and high in sugar and sweet foods are among several factors that may contribute to gallstone formation [1].

Gallbladder “Cleanse”

A gallbladder cleanse is a home remedy believed to clear gallstones from the gallbladder. The practice involves fasting for several hours (or limiting intake to fruit juice), then “challenging” the gallbladder by ingesting large amounts of olive oil. In theory, the intake of olive oil will cause the gallbladder to contract, ridding itself of gallstones. There is no scientific evidence that shows a gallbladder cleanse will prevent gallstone formation. The practice can

cause abdominal pain, nausea and vomiting, and diarrhea.

Cholecystectomy

Surgical removal of the gallbladder—cholecystectomy—is sometimes necessary when gallstones block the flow of bile into the small intestine. Gallstones can also block the flow of pancreatic juices, causing pancreatitis. After removal of the gallbladder, bile is no longer stored, so bile will flow directly from the liver to the small intestine. This is precisely the situation that exists in animals that lack a gallbladder. Following a cholecystectomy, some patients need to eat a low fat diet to allow time for the liver and small intestine to adjust.

1. Di Ciaula A, Wang DQH, Portincasa P. An update on the pathogenesis of cholesterol gallstone disease. *Curr Opin Gastroenterol*. 2018; 34:71–80.

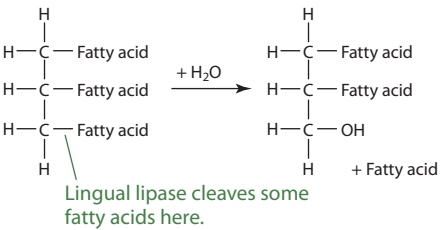
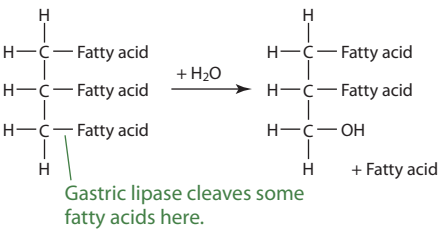
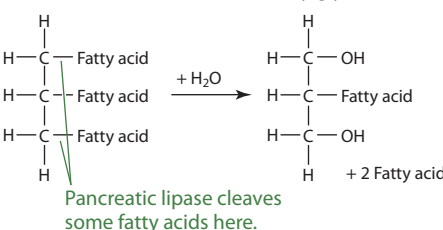
The pancreas simultaneously releases pancreatic lipase and bicarbonate, elevating the pH to a level suitable for pancreatic lipase activity. In combination with bile salts, the triacylglycerol breakdown products (free fatty acids and mono- and diacylglycerols) are themselves excellent emulsifying agents due to their amphipathic properties. Such molecules tend to arrange themselves on the surface of small fat particles, with their hydrophobic regions pointed inward and their hydrophilic regions turned outward toward the water phase. This chemical action, together with the help of peristaltic agitation, converts the fat into small droplets with a greatly increased surface area. The small droplets can then be readily acted upon by pancreatic lipase.

The action of pancreatic lipase on ingested triacylglycerols results in a complex mixture of diacylglycerols, monoacylglycerols, and free fatty acids. Its specificity is primarily toward *sn*-1-linked fatty acids and secondarily to *sn*-3 bonds. Therefore, the digestive action of pancreatic

lipase progresses from triacylglycerols → 2,3-diacylglycerols and 1,2-diacylglycerols → 2-monoacylglycerols. Only a small percentage of the triacylglycerols is hydrolyzed totally to free glycerol. The complete hydrolysis of triacylglycerols that does occur probably follows the isomerization of the 2-monoacylglycerol to 1-monoacylglycerol, which is then hydrolyzed. Thus, the action of pancreatic lipase produces mostly 2-monoacylglycerols and free fatty acids that gradually shrink the size of the small fat droplet, finally resulting in bile salt-stabilized micelles. An overview of triacylglycerol digestion is summarized in Table 5.4.

An inhibitor of gastric and pancreatic lipase, orlistat, has been developed to reduce the absorption of dietary triacylglycerols. It is marketed both as Xenical, a prescription-only product, and Alli, an over-the-counter product. The rationale for use is that when the hydrolysis of triacylglycerols is restricted, less dietary fat will be absorbed, resulting in decreased caloric intake. Xenical inhibits the absorption about 30%, equivalent to a reduction of about

Table 5.4 Overview of Triacylglycerol Digestion

Location	Major Events	Required Enzyme or Secretion	Details
Mouth	Triacylglycerol ↓ <i>Minor amount of digestion</i> Triacylglycerols, diacylglycerols, and fatty acids	Lingual lipase produced in the salivary glands	Diacylglycerol  Lingual lipase cleaves some fatty acids here.
Stomach	Triacylglycerol, diacylglycerol, and fatty acids ↓ <i>Additional digestion</i> Triacylglycerol, diacylglycerol, and fatty acids	Gastric lipase produced in the stomach	Diacylglycerol  Gastric lipase cleaves some fatty acids here.
Small intestine	↓ <i>Phase I: Emulsification</i> Emulsified triacylglycerols, diacylglycerols, and fatty acid micelles ↓ <i>Phase II: Enzymatic digestion</i> Monoacylglycerols and fatty acids	Bile; no lipase Pancreatic lipase produced in the pancreas	Monoacylglycerol  Pancreatic lipase cleaves some fatty acids here.

200 kcal from fat per day. As one might expect, the most frequently reported side effects of orlistat are gastrointestinal discomfort, fecal incontinence, and steatorrhea (presence of fat in feces).

The Role of Colipase

Pancreatic lipase activation is complex, requiring the participation of the protein colipase, calcium ions, and bile salts. Colipase is formed by the hydrolytic activation by trypsin of procolipase, also of pancreatic origin. It contains approximately 100 amino acid residues and possesses distinctly hydrophobic regions that are believed to act as lipid-binding sites. Colipase has been shown to associate strongly with pancreatic lipase and therefore may act as an anchor, or linking point, for attachment of the enzyme to the bile salt–stabilized fat droplet.

Phospholipid Digestion

Phospholipids are hydrolyzed by a specific esterase, phospholipase A₂, made and secreted by the pancreas. Recall from Chapter 2 that, in addition to dietary sources of phospholipid, the bile releases significant amounts of phospholipid (specifically phosphatidylcholine) into the small intestine, perhaps five times more than the diet provides. Both dietary and biliary phospholipid is subject to hydrolysis by phospholipase A₂, which targets the fatty acid at the *sn*-2 position of glycerol. The products of hydrolysis are lysophospholipid and a free fatty acid. These products, together with the products of triacylglycerol digestion and bile salts, incorporate into the resulting micelles for transport to the intestinal cell. Micelles that contain hydrolyzed lipids are negatively charged and have a much smaller diameter (~5 nm) than the unhydrolyzed precursor particles, allowing them access to the intramicrovillus spaces (50–100 nm) of the intestinal membrane.

Cholesterol Ester Digestion

Some of the cholesterol present in food is esterified with a fatty acid. About 10% of the cholesterol in egg yolks is esterified, whereas about 50% in meat and poultry is esterified. Cholesterol esters cannot be absorbed and therefore must be hydrolyzed to free cholesterol and free fatty acid to be incorporated into micelles for delivery to intestinal cells. Hydrolysis is achieved by cholesterol esterase, made and secreted by the pancreas. Free cholesterol from the diet (and from bile) requires no digestion and can directly incorporate in micelles. Cholesterol esterase also hydrolyzes phytosterol esters consumed in the diet. As previously mentioned, free phytosterols can displace cholesterol from the micelle, resulting in less cholesterol being available for absorption. A summary of the digestion of lipids is shown in Figure 5.13.

5.4 ABSORPTION

Micelles contain the final digestion products from lipid hydrolysis, including free long-chain fatty acids, 2-monoacylglycerols, lysophospholipids, free cholesterol, and phytosterols, as well as fat-soluble vitamins. Stabilized by the polar bile salts, the micellar particles are sufficiently water soluble to penetrate the unstirred water layer that bathes the enterocytes of the small intestine. Micelles are small enough to interact with the microvilli at the brush border, whereupon their lipid contents move into the enterocytes. As stable aggregates of lipid molecules, micelles do not cross the plasma membrane intact; they exist only as temporary structures to deliver digested lipids to the enterocyte. The term *absorption* refers to an overall process that includes the transport of digested lipids from the intestinal lumen across the brush border membrane; the reassembly of those lipids by esterification; and, finally, the release of the lipids into the circulation.

Fatty Acid, Monoacylglycerol, and Lysophospholipid Absorption

The mechanism for moving fatty acids, monoacylglycerols, and lysophospholipids across the brush border membrane is not fully understood, although two general mechanisms have been suggested involving a protein-independent diffusion model and a protein-dependent transport model.

Diffusion across the brush border membrane occurs when the concentration in the intestinal lumen exceeds that of the cell. Diffusion is made possible because of the similar amphipathic nature of both digestion products and membrane lipids, which allows the fatty acids, monoacylglycerols, and lysophospholipids to associate with the membrane lipids as they pass into the cell interior. It is thought that some remodeling of the membrane phospholipids—the shifting of fatty acids between the *sn*-1 and *sn*-2 positions—is necessary to facilitate diffusion of these dietary lipids [14].

Protein-dependent transport appears to involve transporters that are lipid specific. An important protein transporter of fatty acids located on the brush border of enterocytes is CD36 (also called SR-B2). CD36 is expressed in a variety of cells throughout the body, where it serves as the predominant membrane transporter of fatty acids [14]. CD36 also transports monoacylglycerols into the cell, but whether it transports lysophospholipids is unknown. Well-defined transport proteins for lysophospholipids are known to exist in yeast, although the presence of similar proteins in mammalian cells, while assumed to exist, have not been reported in the scientific literature.

After transport into the enterocyte, the products of lipid digestion (free fatty acids, monoacylglycerols, and

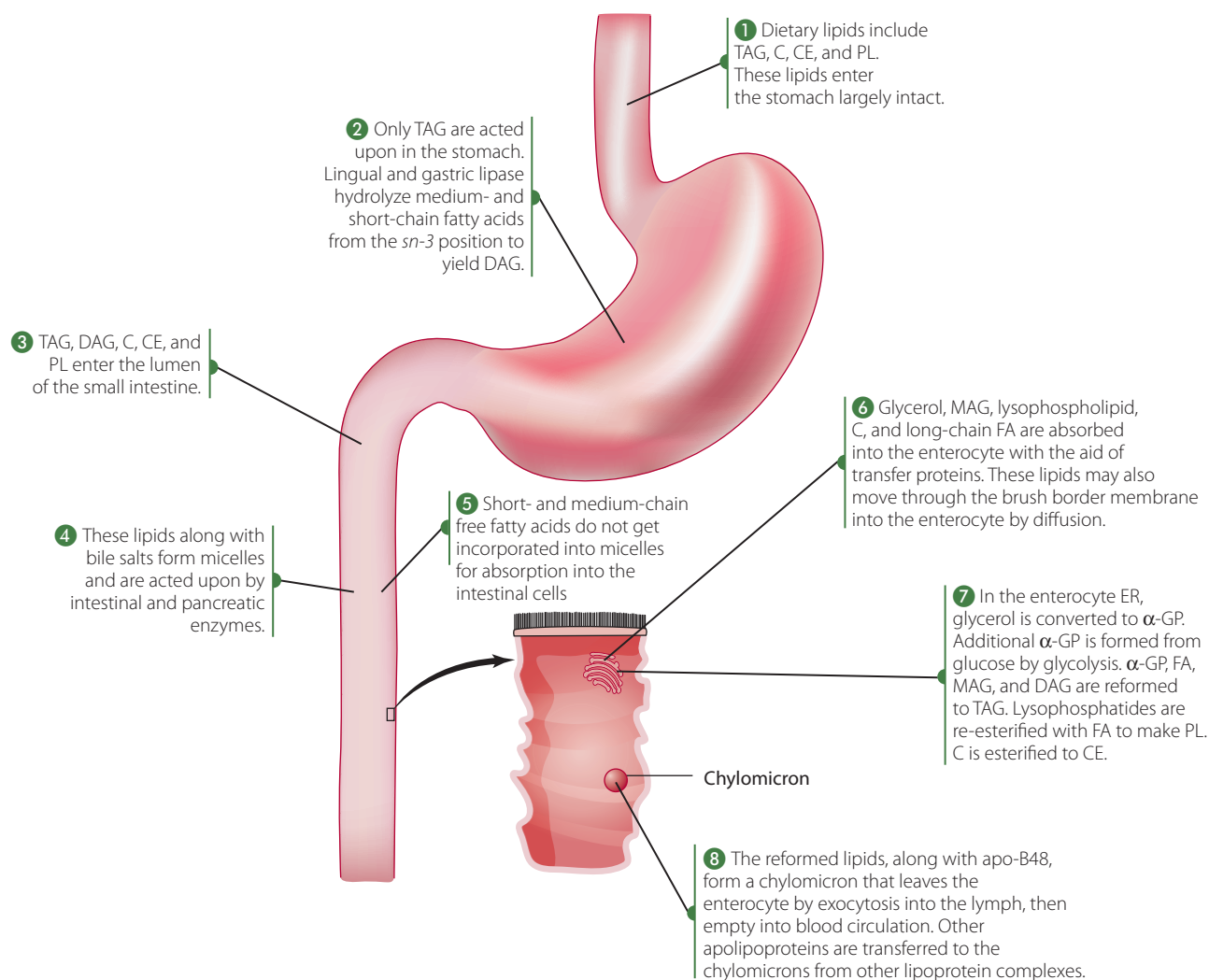


Figure 5.13 Summary of digestion and absorption of dietary lipids. Abbreviations: TAG, triacylglycerol; C, cholesterol; CE, cholesterol ester; PL, phospholipid; DAG, diacylglycerol; MAG, monoacylglycerol; FA, fatty acid; and α -GP, α -glycerolphosphate.

lysophospholipids) move to the endoplasmic reticulum where they are re-esterified. Specific transport proteins, called fatty acid binding proteins (FABP), carry the lipids in the aqueous cytosol. FABP were first discovered to carry fatty acids (hence the name) but have since been reported to transport lysophospholipids and monoacylglycerols [14]. Once in the endoplasmic reticulum, acyltransferases transfer the fatty acid–CoA molecules onto the monoacylglycerol and lysophospholipid to produce triacylglycerol and phospholipid, respectively. Note that triacylglycerols can also be synthesized from α -glycerolphosphate in the enterocytes. This metabolite can be formed either from the phosphorylation of free glycerol or from reduction of dihydroxyacetone phosphate, an intermediate in the pathway of glycolysis (see Figure 3.20).

Other proteins implicated in fatty acid uptake into enterocytes are a family of proteins called the fatty acid

transport proteins (FATP), particularly FATP4. Unlike CD36, which clearly functions as a membrane transporter, FATP4 facilitates the attachment of coenzyme A to fatty acids already in the cell, thus acting as an enzyme rather than a transporter. By priming the fatty acids for synthesis of triacylglycerols, phospholipids, and cholesterol esters, the reassembled lipids can now be incorporated into **chylomicrons** for delivery to the body's tissues. In this way, FATP4 promotes the absorption of fatty acids by facilitating the flow of lipids through the enterocyte.

Cholesterol Absorption

Cholesterol that enters the small intestine comes from two sources: the diet and bile. As previously mentioned, dietary intake of cholesterol is about 300 mg/day, whereas the bile contributes 800–1,400 mg/day. Because the majority of

cholesterol available for absorption is of hepatic origin, the efficiency of absorption can affect how much cholesterol is retained in the body. Cholesterol not absorbed is excreted in the feces. Given that no oxidative pathway for cholesterol exists in humans, fecal excretion represents the primary catabolic route in which whole-body cholesterol homeostasis is maintained. Therefore, the efficiency of cholesterol absorption is a critical point of regulation and the target of drug and dietary therapies that block absorption and promote the removal of cholesterol from the body.

Cholesterol in the intestine must incorporate into micelles for delivery to the enterocyte. Uptake by the cell is mediated by a brush border protein called Niemann-Pick C1 like 1 (NPC1L1). Once inside the cell, cholesterol is carried through the cytosol by sterol carrier proteins. Cholesterol may incorporate into enterocyte membranes, although the majority is esterified in preparation for transport out of the cell as a component of chylomicrons. Cholesterol esterification is catalyzed by acyl-CoA:cholesterol acyltransferase 2 (ACAT2), which is required for chylomicron formation to occur.

Phytosterols are also transported into the intestinal cell by NPC1L1. Despite the ability of NPC1L1 to transport both cholesterol and phytosterols, essentially no phytosterols incorporate into chylomicrons or enter the circulation. This is due to the presence of two additional proteins—members of the ATP-binding cassette (ABC) transporter family called ABCG5 and ABCG8—that reside adjacent to NPC1L1 in the brush border membrane. The role of ABCG5 and ABCG8 is to redirect phytosterols back into the intestinal lumen immediately after being taken into the cell. ABCG5 and ABCG8 also redirect some cholesterol back into the intestinal lumen, so that the overall efficiency of cholesterol absorption is about 50–60% [15]. A rare autosomal recessive disorder called sitosterolemia can occur as a result of mutations in either ABCG5 or ABCG8, causing hyperabsorption of cholesterol and phytosterols.

Strategies to block cholesterol absorption date back to the 1950s when patients with elevated blood cholesterol were given a commercial preparation of phytosterols suspended in fruit-flavored syrup (marketed as *Cytellin*). Phytosterols are known to displace cholesterol from micelles and compete for binding to NPC1L1. The product had limited success and was largely replaced by powerful prescription drugs, including ezetimibe, which directly inhibits NPC1L1, resulting in significant reductions (about 18%) in blood cholesterol levels. For patients who cannot tolerate prescription drugs, foods and supplements enriched with phytosterols are increasingly available and effective at reducing blood cholesterol concentration by 10% or more [6].

Lipid Release into Circulation

For dietary lipids to be fully absorbed into the circulation, they must first be packaged in a form that allows for transport in the aqueous bloodstream. Lipids that are

re-esterified in the endoplasmic reticulum of the enterocytes are assembled into large lipid-protein aggregate structures called chylomicrons. The formation of chylomicrons occurs in direct response to eating a fat-containing meal; therefore, the proportions of the various lipids in chylomicrons reflect that of the diet. Chylomicrons are spherical particles containing mostly triacylglycerols and some cholesterol esters in the core (due to their hydrophobicity), with amphipathic phospholipids, free cholesterol, and protein on the surface.

The main protein added to the chylomicron surface is called apolipoprotein B-48 (apoB-48) that helps stabilize the triacylglycerol-rich chylomicron. Fully formed chylomicrons are delivered to the intercellular space between enterocytes where they are released by exocytosis into the lymphatic system (Figure 5.14). The chylomicrons travel a few inches via the thoracic duct to the left subclavian vein, at which point they enter the systemic blood circulation. The metabolic advantage of first entering the lymphatic system is to bypass the liver, an organ that would have catabolized the chylomicrons if they had entered the hepatic portal vein. This short detour around the liver allows chylomicrons to deliver their triacylglycerol cargo to other tissues such as muscle and adipose tissue (discussed in detail in the next section).

Medium-chain fatty acids (those containing 6–12 carbon atoms), if present in the diet, have the ability to pass from the

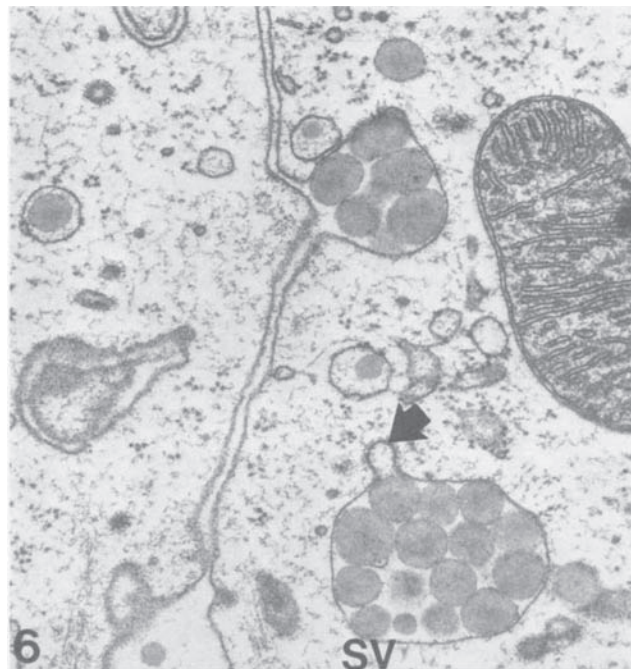


Figure 5.14 Chylomicron assembly in enterocytes. The fully formed chylomicrons (dark spheres) are transported in secretory vesicles (SV) that fuse with the lateral cell membrane and release into the intercellular space (ICS) between enterocytes. A mitochondrion is seen on the right.

Source: Sabesin SM, Frase S. Electron microscopic studies of the assembly, intracellular transport, and secretion of chylomicrons by rat intestine. *J Lipid Res.* 1977; 18:496–511.

enterocyte directly into the portal blood, where they bind to albumin and are transported directly to the liver. Most of the medium-chain fatty acids escape esterification in the enterocyte and enter the portal blood as free fatty acids. The different fates of the long- and medium-chain fatty acids result from the specificity of the acyl-CoA synthetase enzymes for long-chain fatty acids. Triacylglycerols containing medium-chain fatty acids are used clinically to treat patients with intestinal disorders because the medium-chain fatty acids can be absorbed directly to the portal blood without the need for chylomicron formation. Key features of intestinal digestion and absorption of lipids are depicted in Figure 5.13.

5.5 TRANSPORT AND STORAGE

Lipids are transported in the blood as components of highly organized lipid–protein complexes (or particles) called **lipoproteins**. Chylomicrons, as mentioned in the previous section, are a class of lipoproteins. The other classes are very-low-density lipoproteins (VLDL), intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). Each lipoprotein class participates in transport systems that can be defined as *exogenous (dietary) lipid transport*, *endogenous lipid transport*, and *reverse cholesterol transport*. In this section, the structure of lipoproteins is first described, followed by a discussion on the lipid transport systems and the central role of the liver.

Lipoprotein Structure

All lipoproteins share similar structural features in which hydrophobic, nonpolar “neutral” lipids (triacylglycerols and cholesterol esters) reside in the spherical core,

surrounded by a monolayer of amphipathic phospholipids and free cholesterol that partitions the neutral lipid from the aqueous environment. Added to the surface are proteins (called apolipoproteins or apoproteins) that impart structural stability and functionality by serving as enzyme activators or ligands for cell receptors.

The arrangement of the lipid and protein components of a typical lipoprotein is represented in Figure 5.15. The illustration depicts the apoproteins as being either peripheral (residing mostly on the external surface of the lipoprotein) or integral (having multiple regions that span the phospholipid monolayer). The apoproteins are abbreviated “apo” and are identified using letters and numbers. Each lipoprotein class will have a complement of apoproteins that is characteristic of that class; for example, chylomicrons are defined by having apoB-48, apoA-1, apoC-2, apoE, and so on. Table 5.5 shows a listing of the apoproteins, their molecular weight, the lipoprotein class with which they are associated, and their postulated physiological function.

In addition to the apoprotein composition, each lipoprotein class has its own characteristic lipid composition, physical properties, and metabolic function. Initially, lipoproteins were separated from serum by electrophoresis and therefore were named based on their movement in an electrical gradient. Later, they were separated by centrifugation and were named based on their density. These names persist even though other methods are often used for their separation. Lipoproteins with higher proportions of lipid have a lower density. The largest and least dense lipoproteins are the chylomicrons, having a high lipid:protein ratio. The smallest and most dense are HDL, having a low lipid:protein ratio. The relative percentage of lipids and protein in each lipoprotein class is shown in Figure 5.16.

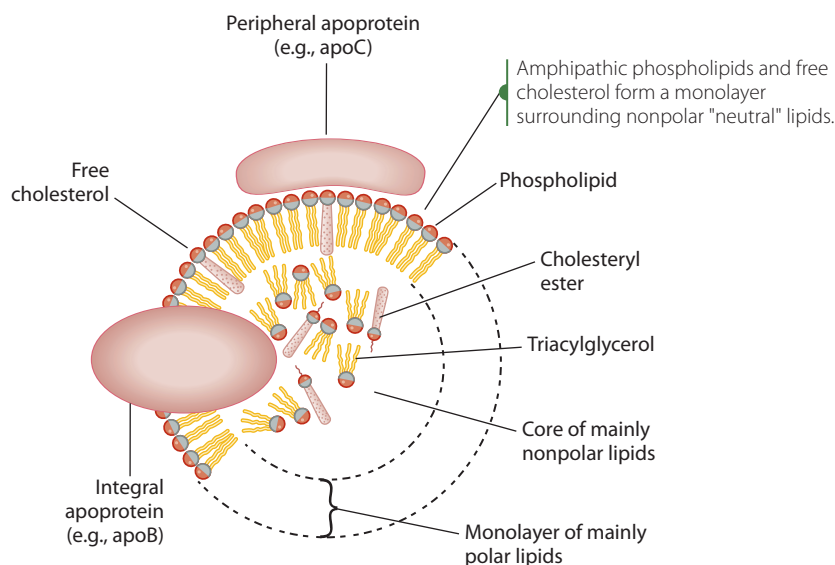
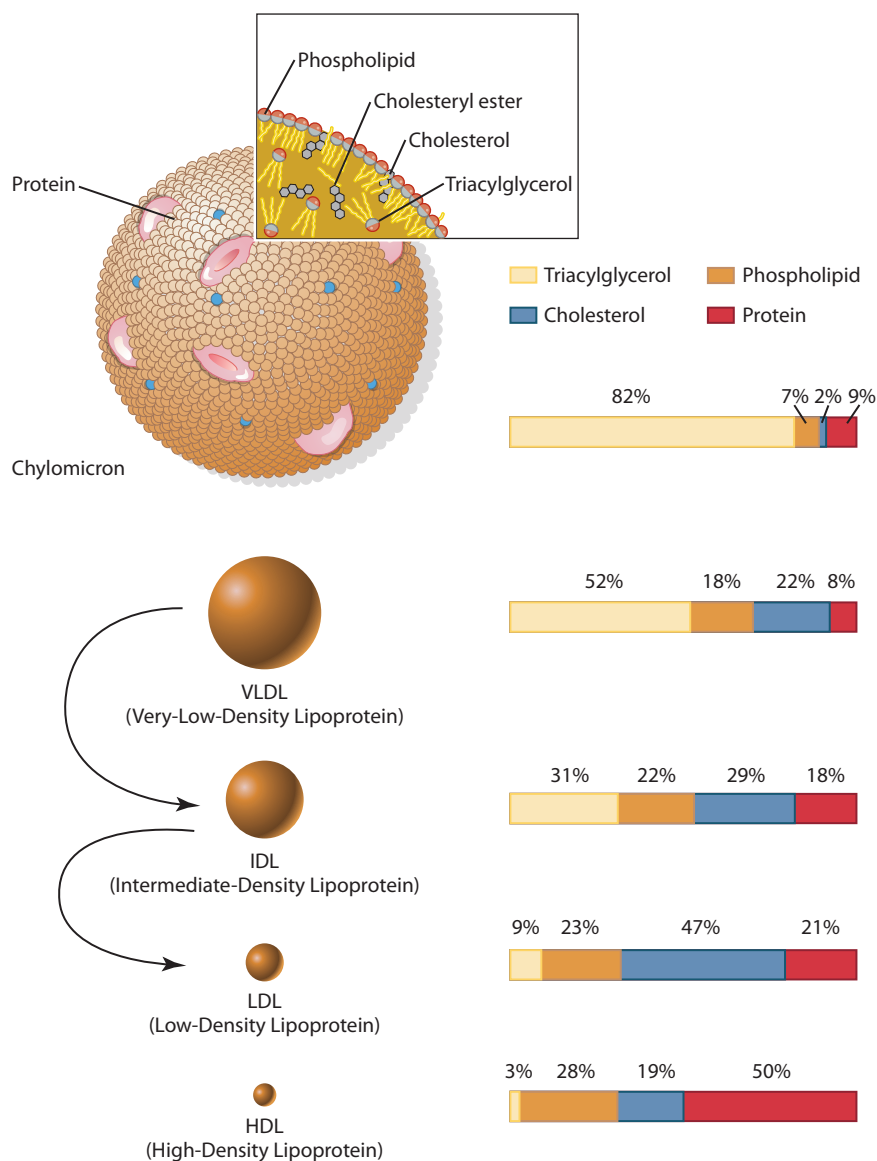


Figure 5.15 Generalized structure of a plasma lipoprotein.

Table 5.5 Apolipoproteins of Human Plasma Lipoproteins

Apolipoprotein	Lipoprotein(s)	Molecular Mass (Da)	Additional Remarks
apoA-1	HDL, chylomicrons	28,000	Activator of lecithin: cholesterol acyltransferase (LCAT); ligand for HDL receptor
apoA-2	HDL, chylomicrons	17,000	Structure is two identical monomers joined by a disulfide bridge
apoA-4	Secreted with chylomicrons but transfers to HDL	46,000	Associated with the formation of triacylglycerol-rich lipoproteins; function unknown
apoB-100	LDL, VLDL, IDL	550,000	Synthesized in liver; ligand for LDL receptor
apoB-48	Chylomicrons, chylomicron remnants	260,000	Synthesized in intestine
apoC-1	VLDL, HDL, chylomicrons	7,600	Possible activator of LCAT
apoC-2	VLDL, HDL, chylomicrons	8,916	Activator of extrahepatic lipoprotein lipase
apoC-3	VLDL, HDL, chylomicrons	8,750	Several polymorphic forms depending on content of sialic acids
apoD	Subfraction of HDL	20,000	Possible antioxidant
apoE	VLDL, HDL, chylomicrons, chylomicron remnants	34,000	Ligand for chylomicron remnant receptor

**Figure 5.16** Lipid and protein composition of lipoprotein classes.Source: Cengage Learning Inc. Reproduced by permission. www.cengage.com/permissions

Lipoprotein Metabolism

The main function of lipoproteins is to transport lipids in the blood. Each lipoprotein class is specialized with regard to the lipids they transport, where the lipids are delivered, and the lipoprotein's metabolic fate after the job is completed. The *exogenous lipid transport* system involves chylomicrons and refers to the transport of dietary lipids, primarily triacylglycerols, from the intestine to peripheral tissues for storage or energy utilization. This system operates only after a fat-containing meal. Chylomicrons disappear after all of the dietary triacylglycerols are delivered to target tissues. The *endogenous lipid transport* system involves VLDL, IDL, and LDL and refers to the transport of triacylglycerols from the liver to peripheral tissues for storage or energy utilization. This system operates continuously to maintain proper balance of fatty acids and triacylglycerols that accumulate in the liver during normal metabolism. *Reverse cholesterol transport* involves HDL and refers to the ability of HDL to pick up excess cholesterol from peripheral tissues and deliver it to the liver for conversion to other important molecules or for excretion from the body via the bile.

Exogenous Lipid Transport

Immediately after a fat-containing meal, the exogenous (dietary) lipids are packaged into chylomicrons within

the enterocyte and distributed to peripheral tissues, mainly muscle and adipose tissue. When chylomicrons are released from the enterocyte, they contain mostly triacylglycerols, reflecting the abundance of triacylglycerols in the diet. They also contain apoB-48 and apoA-1. The apoB-48 protein made by the intestine is related to apoB-100 (made by the liver), in that both arise from the same gene, although the intestinal cell contains a stop codon that results in a truncated protein that is 48% of the sequence of apoB-100. After chylomicrons enter the blood, they acquire more apoproteins (mainly apoE and apoC-2) from HDL as the lipoproteins interact in the circulation. The exogenous lipid transport system and chylomicron metabolism are illustrated in Figure 5.17.

Chylomicrons enter the bloodstream at a relatively slow rate, which prevents excessive increases in blood triacylglycerol levels. Entry of chylomicrons into the blood can continue for up to 14 hours after consumption of a large meal rich in fat. Blood triacylglycerol concentration usually peaks 30 minutes to 3 hours after a meal and returns to near normal within 5–6 hours. These times can vary, however, depending on the stomach emptying time, which in turn depends on the size and composition of the meal. The presence of triacylglycerol-rich chylomicrons accounts for the turbidity (milky appearance) of postprandial plasma and can interfere with clinical readings when “fasting

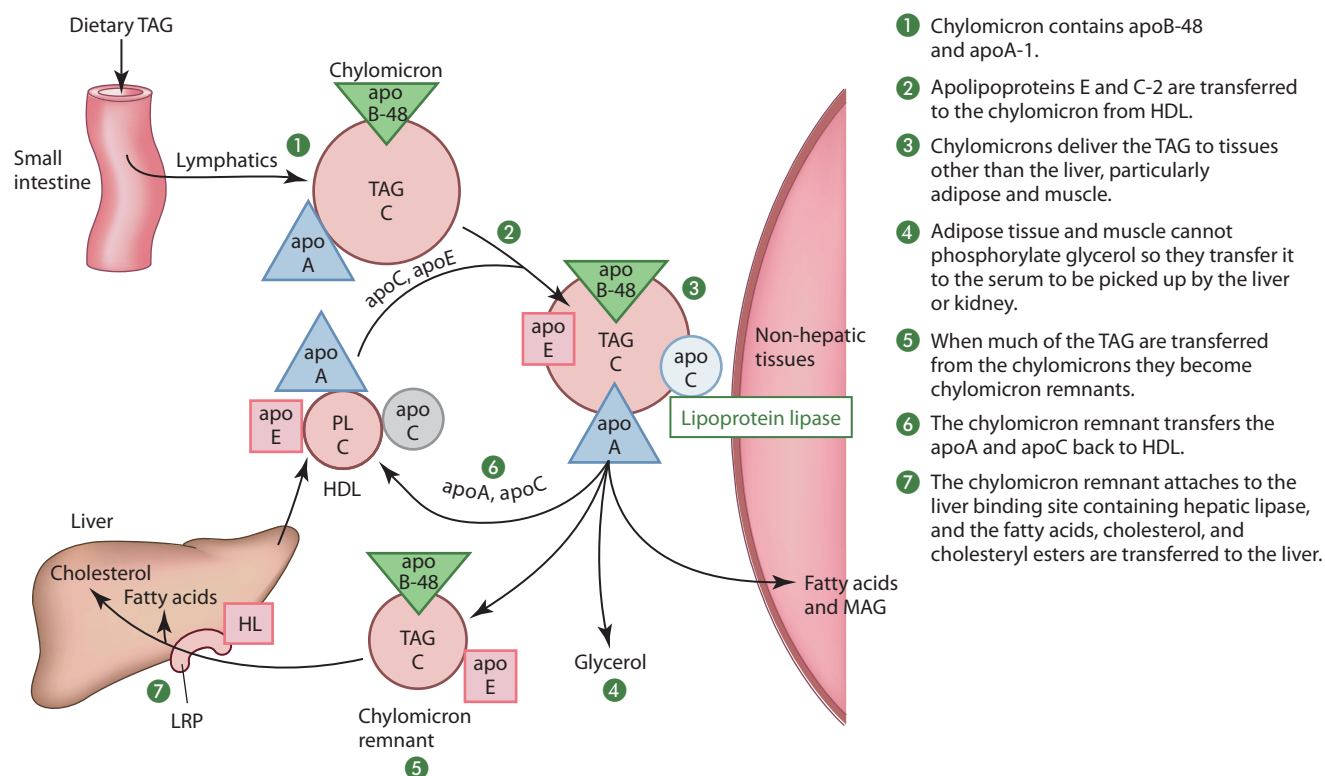


Figure 5.17 Exogenous lipid transport. Abbreviations: TAG, triacylglycerol; MAG, monoacylglycerols; PL, phospholipid; HL, hepatic lipase; LRP, LDL receptor-related protein; C, cholesterol and cholesterol esters.

Source: Modified from Murray RK, Rodwell DK, Victor W. *Harpers Illustrated Biochemistry*, 27th ed. New York: Lange Medical Books/McGraw-Hill. 2006. Figure 25-3, p. 221.

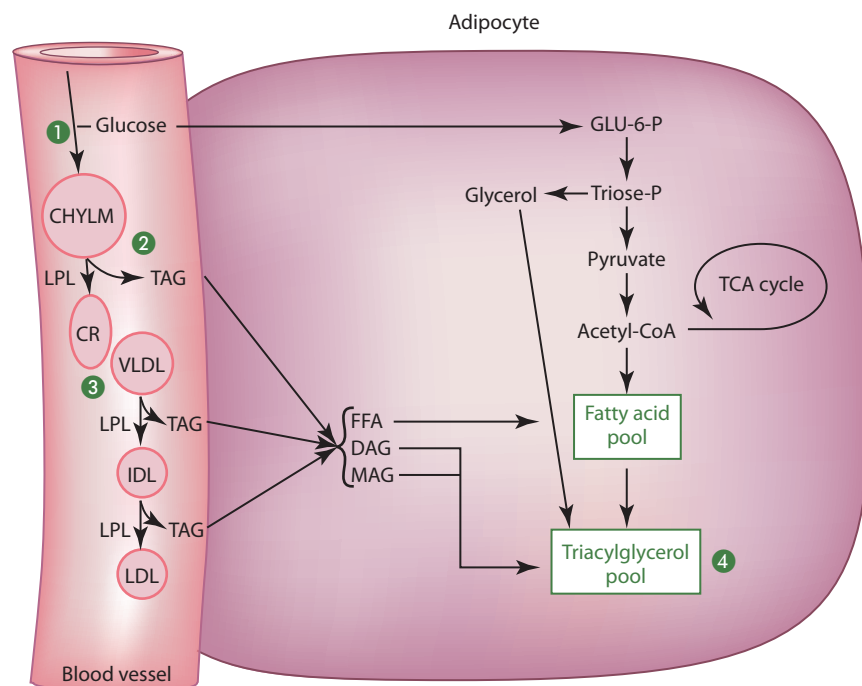
triglyceride” values are desired. Twelve hours of fasting is usually required to obtain true readings that are devoid of chylomicrons.

Circulating chylomicrons interact with tissues that express the enzyme lipoprotein lipase, primarily skeletal muscle, heart muscle, and adipose tissue (but not liver). This interaction occurs due to the presence of apoC-2, which activates the enzyme. Chylomicrons dock on the cell surface where lipoprotein lipase hydrolyzes the triacylglycerols, producing free fatty acids and 2-monoacylglycerols that are quickly taken up into the cells. As the chylomicron becomes depleted of its core triacylglycerols, the lipoprotein structure shrinks in size, yet retains the other lipids. About 80% of the chylomicron triacylglycerols are delivered to target tissues in this manner.

Once depleted of its triacylglycerols, the chylomicron “remnant” particles separate from the cell surface and reenter the circulation. The **chylomicron remnants** may donate some of its apoproteins to HDL. The chylomicron remnants travel to the liver where a specific receptor recognizes their apoE component, enabling the uptake of the entire particle into hepatocytes. The receptor is called LDL receptor-related protein 1 (LRP1). In addition, lipoproteins that have apoE, such as chylomicron remnants, can bind to the LDL receptor (discussed in the next section) and be cleared from the circulation. Hepatic lipase is a key

enzyme that hydrolyzes the remaining triacylglycerols and phospholipids of the chylomicron remnants as they enter the hepatocyte.

The interaction of chylomicrons with adipocytes and subsequent lipid metabolism in the fed state is presented in Figure 5.18. Adipocytes are the major storage site for triacylglycerol and the most likely target of chylomicrons following a fat-containing meal. Usually the amount of fat consumed by an individual in a single meal exceeds the immediate energy demands of tissues. Therefore, most dietary triacylglycerol must be stored, at least temporarily, until needed when energy demand exceeds energy intake. Triacylglycerol is in a continuous state of turnover in adipocytes; that is, constant lipolysis (hydrolysis during energy needs) is countered by constant re-esterification to form triacylglycerols (storage during energy excess). These two processes are not simply forward and reverse directions of the same reactions but are different pathways involving different enzymes and substrates. In the fed state, metabolic pathways in adipocytes favor triacylglycerol synthesis, a process strongly influenced by insulin. Insulin increases the uptake of free fatty acids and monoacylglycerols in adipocytes by stimulating lipoprotein lipase. Insulin also accelerates the entry of glucose into adipocytes and its conversion to fatty acids. Glycolysis in adipocytes provides a source of glycerol-3-phosphate



- 1 Glucose is metabolized to make acetyl-CoA, which can be converted to fatty acids.
- 2 Lipoprotein lipase acts on TAG in chylomicrons (CHYLM) causing free fatty acids (FFA) and MAG to enter the adipocyte.
- 3 Lipoprotein lipase acts on VLDL so FFA and MAG enter the cell.
- 4 The pathways favor energy storage as TAG. Insulin stimulates lipogenesis by promoting entry of glucose into the cell and by inhibiting the hormone-sensitive lipase that hydrolyzes the stored TAG to FFA and glycerol.

Figure 5.18 Lipid metabolism in the adipose cell following a meal. Abbreviations: CHYLM, chylomicron; DAG, diacylglycerol; MAG, monoacylglycerol; TAG, triacylglycerol; and FFA, free fatty acid.

for re-esterification with the fatty acids to form triacylglycerols. Absorbed monoacylglycerols also furnish the glycerol backbone for re-esterification. Insulin further exerts its lipogenic action on adipose by strongly inhibiting hormone-sensitive lipase, which hydrolyzes stored triacylglycerols, thus favoring triacylglycerol synthesis.

Endogenous Lipid Transport

The endogenous lipid transport system begins and ends with the liver. In brief, hepatic triacylglycerols are packaged in VLDL and delivered to peripheral tissues in a manner similar to chylomicrons. After delivery, the left-over particles, referred to as LDL, are depleted of triacylglycerol but relatively enriched in cholesterol. As remnant particles, LDL are removed from the circulation for catabolism by specific receptors on the plasma membrane of cells, primarily hepatocytes. If the LDL receptors are in short supply, LDL can accumulate in the blood, causing the concentration of LDL-associated cholesterol to rise. The

health implications of elevated LDL cholesterol concentration are discussed later in this chapter.

Endogenous lipid transport begins with hepatic VLDL production. The liver has a limited capacity to store triacylglycerols and must continually move them out for transport to peripheral tissues where they can be stored or used for energy. The liver's ability to synthesize and secrete triacylglycerols in VLDL helps to maintain the balance of energy-containing nutrients throughout the body. The liver is capable of synthesizing new fatty acids and triacylglycerols from nonlipid precursors such as glucose, fructose, and amino acids. It can also utilize “preformed” lipids delivered to it as chylomicron remnants, LDL, and HDL. A third source of lipid for VLDL synthesis comes from free fatty acids bound to serum albumin that are taken up by the liver. The free fatty acids may be of dietary origin (absorbed directly into the portal blood) or from adipose tissue (released into the systemic circulation during lipolysis). Figure 5.19 depicts the interrelationships among the

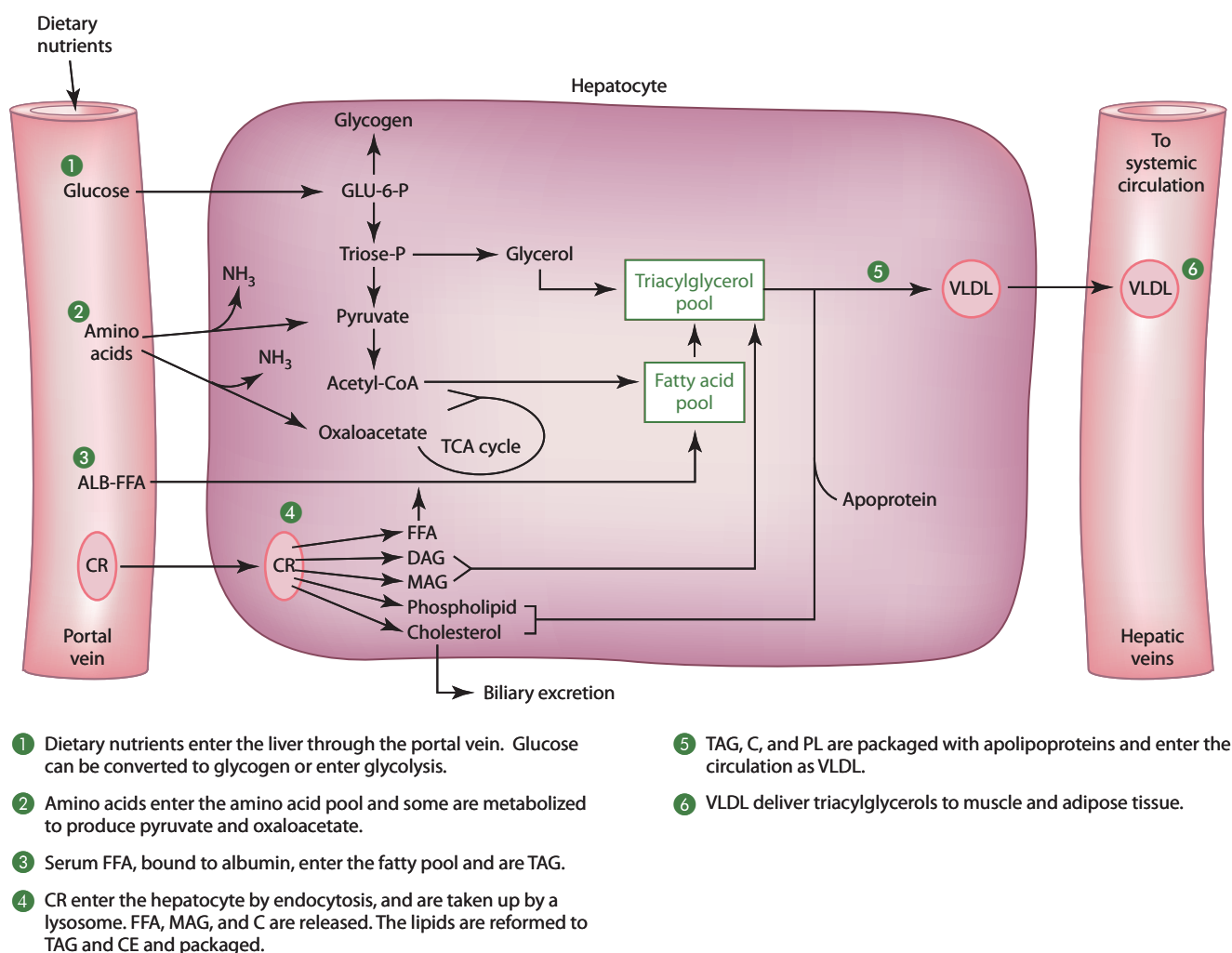


Figure 5.19 Metabolism in the liver following a fatty meal. Abbreviations: CR, chylomicron remnant; ALB, albumin; FFA, free fatty acid; MAG, monoacylglycerol; DAG, diacylglycerol; C, cholesterol; CE, cholesterol ester; TAG, triacylglycerol.

pathways of lipid, carbohydrate, and protein metabolism in the liver, illustrating how lipids from remnant particle uptake, albumin-bound free fatty acids, and nonlipid precursors can be converted to triacylglycerols and secreted as VLDL into the systemic circulation.

Glucose, fructose, and amino acids that enter the liver from the hepatic portal vein can be converted to fatty acids and incorporated into VLDL if in excess and other demands for these molecules are met. Excess glucose and fructose not used for energy via the TCA cycle results in an accumulation of acetyl-CoA, which can be used to synthesize fatty acids (see Chapter 3). The glycerol needed for triacylglycerols is made from triose phosphates such as glycerol-3-phosphate. Amino acids can serve as precursors for fatty acids because they can be metabolically converted to acetyl-CoA or pyruvate. The synthesis of fatty acids, triacylglycerols, and phospholipids is described in detail later in this chapter.

In addition to triacylglycerols, the liver processes phospholipids, cholesterol, and cholesterol esters. Phospholipids from chylomicron remnants can incorporate into cell membranes or be used in the assembly of VLDL. Cholesterol and cholesterol esters from chylomicron remnants may be used in several ways:

- Converted to bile salts and secreted in the bile
- Secreted directly into the bile as free cholesterol

- Incorporated into cellular membranes as free cholesterol
- Incorporated into VLDL and released into the blood.

VLDL are assembled in the liver from endogenous triacylglycerols in much the same way as chylomicrons are assembled in the enterocytes from dietary triacylglycerols. The lipids are carried to the endoplasmic reticulum, assembled into VLDL with its complement of apoproteins, and secreted from the cell by exocytosis. The main structural apoprotein on VLDL is apoB-100; one molecule of apoB-100 is associated with each VLDL particle. Because of its large size, the apoB-100 protein encircles the VLDL particle with several regions that anchor within the phospholipid monolayer. Newly secreted VLDL also contain apoC-1 and apoE. Circulating VLDL acquire apoC-2 and additional apoE from HDL. The main features of the endogenous lipid transport system are depicted in Figure 5.20.

By virtue of apoC-2 on its surface, VLDL bind to and interact with lipoprotein lipase on adipose and muscle cells in a manner similar to the binding and hydrolysis of triacylglycerols in chylomicrons. Within the muscle cell, the free fatty acids and monoacylglycerols from VLDL are primarily oxidized for energy, with only limited amounts resynthesized for storage as triacylglycerols. Endurance-trained muscle, however, does contain some triacylglycerol

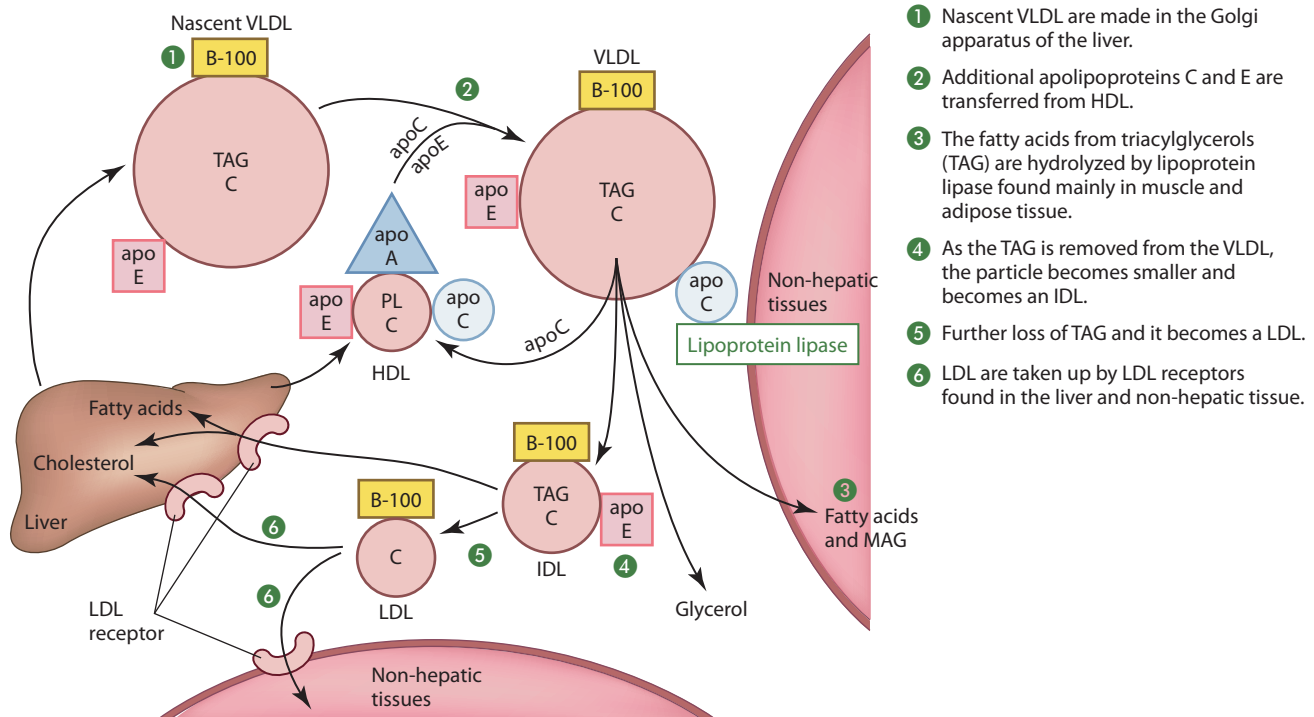


Figure 5.20 Endogenous lipid transport. Abbreviations: B-100, apolipoprotein B-100; E, apolipoprotein E; TAG, triacylglycerol; C, cholesterol and cholesterol esters; and PL, phospholipid.

Source: Modified from Murray RK, Rodwell, DK, Victor, W. *Harpers Illustrated Biochemistry*, 27th ed. New York: Lange Medical Books/McGraw-Hill. 2006. Figure 25-4, p. 222.

deposits. In adipose tissue, in contrast, the absorbed fatty acids are largely used to resynthesize triacylglycerols for storage.

As the triacylglycerols are removed from VLDL, a smaller transient IDL particle is formed. A few IDL particles may separate from the cell and return to the circulation; however, most remain attached and the removal of triacylglycerols continues until a triacylglycerol-depleted LDL particle remains. As LDL particles shrink in size, they lose all of their apoproteins except apoB-100. Several events can determine the size of LDL, including the degree of interaction with lipoprotein lipase and with other lipoproteins in the intravascular space where exchange of lipids can occur. Clinical studies have indicated that small dense LDL are more atherogenic than larger LDL, which emphasizes the importance of having a more thorough analysis conducted on LDL subfractions in individuals who are at risk for cardiovascular disease.

When LDL particles separate from lipoprotein lipase, they enter the circulation with a significantly different lipid profile compared to VLDL. Whereas VLDL are rich in triacylglycerols, LDL are composed of the remaining lipids that were initially secreted by the liver in VLDL. The relative percentage of phospholipids, free cholesterol, and cholesterol esters in LDL are greater than VLDL (see Figure 5.16), making LDL the primary carrier of cholesterol in the bloodstream of most people. Furthermore, apoB-100 is the only remaining apoprotein on LDL (one molecule per LDL particle). It is imperative that LDL, as the major carrier of cholesterol, be removed from the blood to prevent the accumulation of LDL cholesterol.

Clearance of LDL from blood is accomplished by a cell surface receptor—the LDL receptor—that recognizes apoB-100 and binds LDL particles for uptake into the cell [16]. ApoE also binds to the LDL receptor, so lipoproteins expressing apoE also have the potential to be cleared from the circulation via the LDL receptor. LDL binds to the LDL receptors on cell membranes with high affinity and specificity. The LDL receptors located on hepatocytes are particularly important, as they remove 70–80% of LDL from the circulation. Membrane-bound LDL is then internalized by endocytosis. The interaction between the receptors and apoB-100 is the key to the cell's internalization of the LDL. Figure 5.21 depicts the fate of the LDL particle following its binding to the membrane receptor. The internalized LDL particle is carried to lysosomes, and the receptor is released and returns to the surface of the cell. In the lysosome, the apoprotein and cholesterol ester components are hydrolyzed by lysosomal enzymes into amino acids, free fatty acids, and free cholesterol. The influx of free cholesterol exerts the following regulatory functions:

- The rate-limiting enzyme in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), is suppressed through decreased transcription of the reductase gene and the concomitant increased degradation of the enzyme.
- The enzyme-regulating cholesterol esterification, acyl-CoA:cholesterol acyltransferase (ACAT), is activated, thus promoting cholesterol ester storage.
- The synthesis of LDL receptors is suppressed through decreased transcription of the receptor gene, thereby preventing further entry of LDL into the cell.

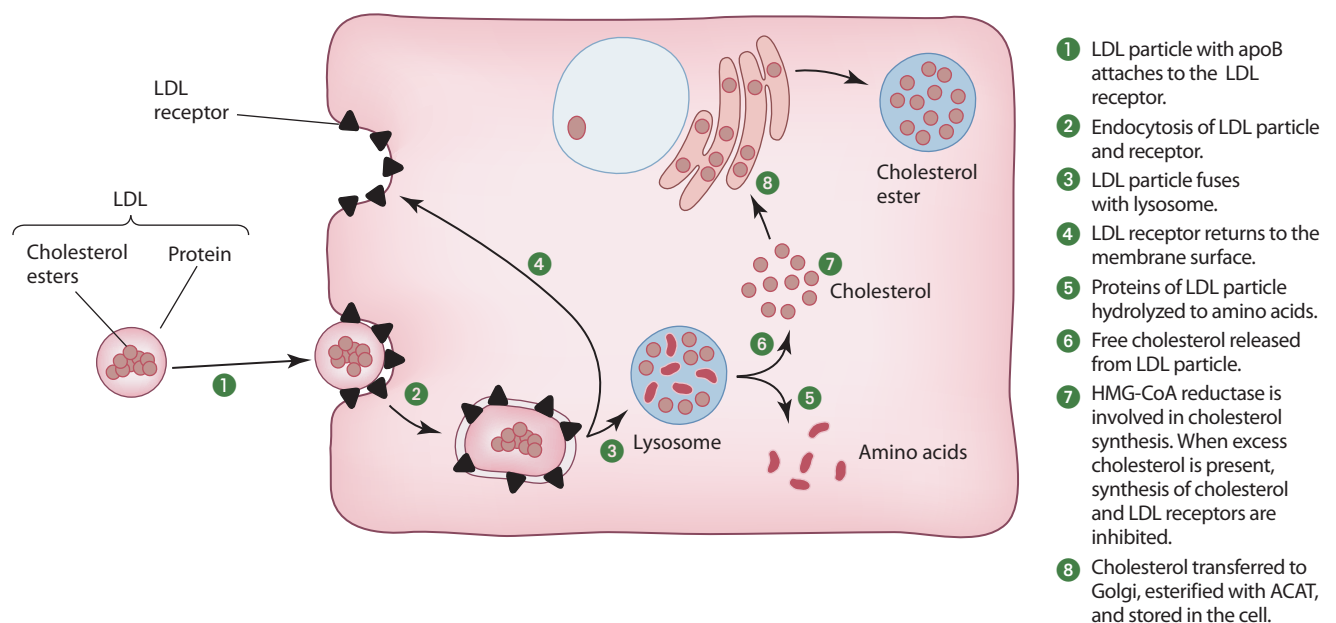


Figure 5.21 Sequential steps in endocytosis of LDL leading to synthesis and storage of cholesterol ester.

Source: M. Brown, J. Goldstein, "Receptor mediated endocytosis: insights from the lipoprotein receptor system."

The LDL receptor has been extensively studied because elevated serum LDL cholesterol concentration is a known risk factor for atherosclerotic cardiovascular disease (ASCVD). Factors that influence the number of receptors on the cell surface impact LDL cholesterol concentration. Dietary components are known to strongly influence the number of LDL receptors. Saturated and *trans* fatty acids decrease receptors, whereas soluble fiber and phytosterols increase receptors. In addition, obesity reduces the number of LDL receptors; therefore, obese individuals are less responsive to dietary interventions that normally improve serum cholesterol profiles. Genetic studies have also identified naturally occurring mutations that result in abnormal LDL receptors that can cause dramatically elevated cholesterol levels, termed *familial hypercholesterolemia*. More recently, an enzyme called PCSK9 was discovered that binds to LDL receptors and disrupts the recycling mechanism that returns the receptors to the cell surface after internalization. Interestingly, people with a mutation in PCSK9 that disables its function have low LDL cholesterol concentration and have lower risk of developing ASCVD. Injections with monoclonal antibodies that inhibit PCSK9 are highly effective at lowering LDL cholesterol concentration [17].

Reverse Cholesterol Transport

Reverse cholesterol transport refers to the ability of circulating HDL to pick up excess cholesterol from peripheral tissues and deliver it to the liver for excretion from the body via bile, as either free cholesterol or bile acids. While every cell in the body can synthesize cholesterol, mammals lack the oxidative enzymes necessary to degrade cholesterol. Therefore, the transport of cholesterol from peripheral cells to the liver for excretion is a critically important pathway for maintaining cholesterol homeostasis. Conversion of cholesterol to regulatory molecules (hormones) also occurs in the liver and other tissues, but this is quantitatively small compared to the amount excreted through the bile. The role of HDL in reverse cholesterol transport is shown in Figure 5.22.

The process by which HDL collects cholesterol from peripheral tissues and transports it to the liver involves multiple cell surface receptors, intravascular enzymes, and transfer of lipids among circulating lipoproteins. One constant throughout the entire process is HDL's main apoprotein, apoA-1. Unlike chylomicrons and VLDL, which are assembled into complete lipoproteins within the cell, HDL arise entirely within the intravascular space starting with lipid-free apoA-1. Molecules of apoA-1 are produced and secreted into the circulation by the liver and small intestine; apoA-1 released from chylomicrons and VLDL during triacylglycerol hydrolysis may also be used to create HDL.

Nascent HDL are made when the lipid-free apoA-1 binds to the liver ABCA1 receptor and acquires phospholipids

and free cholesterol from the hepatocyte. Nascent HDL are discoidal in shape due to the ability of the amphipathic lipids to form a bilayer. Additional phospholipids and cholesterol are acquired when nascent HDL interact with ABCA1 and another receptor, SR-B1, located in peripheral tissues such as muscle, adipose, and macrophages within coronary arteries. The ability of nascent HDL to accept cholesterol from macrophages benefits the cardiovascular system by reducing the amount of deposited cholesterol in the vascular endothelium, thus decreasing the risk of ASCVD (discussed in detail in the next section).

As nascent HDL acquire phospholipids and cholesterol, they also acquire an intravascular enzyme called lecithin:cholesterol acyltransferase (LCAT). This enzyme forms cholesterol esters by catalyzing the transfer of fatty acids (usually polyunsaturated) from the *sn*-2 position of phosphatidylcholine to free cholesterol within the HDL particle. Because the resulting cholesterol esters are non-polar, they migrate to the core of the particle, forming mature HDL. The small spherical HDL can further interact with peripheral tissues as the apoA-1 binds to SR-B1 and yet another receptor, ABCG1. (Mature HDL bind to ABCG1, but not ABCA1. Both nascent and mature HDL bind to SR-B1.) Further binding to cell receptors and the continued action of LCAT causes HDL to grow in size. The accumulated cholesterol esters in HDL can be transferred to other lipoproteins through the action of cholesterol ester transfer protein (CETP). By distributing cholesterol esters to VLDL and LDL, cholesterol ester transfer protein helps to reduce the size of HDL so that interaction with cell surface receptors is optimized, thus increasing HDL's ability to accept more cholesterol.

The final step in reverse cholesterol transport is the binding of HDL to SR-B1 receptors on the surface of hepatocytes. Two actions are possible: the cholesterol esters may be selectively deposited in the liver cells and the depleted HDL returned to the circulation, or the entire HDL particle may be internalized and degraded. Intracellular degradation of HDL occurs in lysosomes in a manner similar to the degradation of LDL (see Figure 5.20). The cholesterol esters are hydrolyzed by cholesterol ester hydrolase, and the free cholesterol can be secreted directly into bile or converted to bile salts and secreted (see Figure 5.11). This process is the major route by which cholesterol is eliminated from the body. The efficiency with which HDL accept and transport cholesterol is reflected in the distribution of HDL particle sizes that exist in the circulation. As large HDL represent the final stage just prior to delivery to the liver, a high proportion of large HDL are thought to be an indicator of lower ASCVD risk. A preponderance of small HDL reflects inefficiencies in the ability of HDL to gather cholesterol esters for delivery to the liver.

There are additional functions of HDL that are beyond their ability to transport cholesterol. These include a role

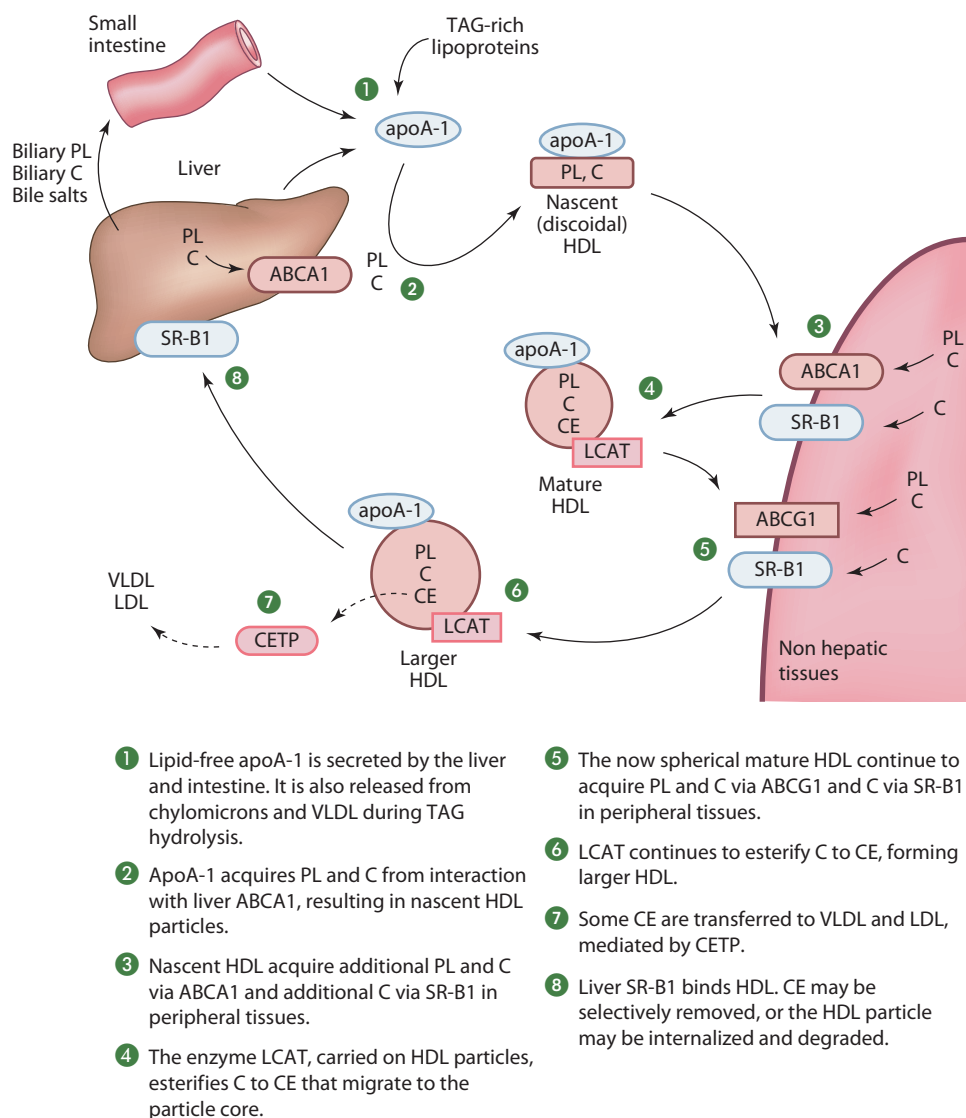


Figure 5.22 Reverse cholesterol transport.

as an anti-inflammatory regulator through interactions with the vascular endothelium and circulating inflammatory cells. Some evidence supports the idea that HDL is an integral component of innate immunity. HDL has also been shown to have antiapoptotic functions for a number of cell types, including vascular endothelial and smooth muscle cells, some leukocytes, pancreatic β cells, cardiomyocytes, and bone-forming cells. Further research will reveal its biological importance in these areas [18].

5.6 LIPIDS, LIPOPROTEINS, AND CARDIOVASCULAR DISEASE RISK

Atherosclerosis is a degenerative disease of the vascular endothelium. The principal players in the atherogenic process are cells of the immune system, which cause a pro-inflammatory environment, and lipids, primarily

cholesterol and cholesterol esters. An early response to arterial endothelial cell injury is an increased adherence of monocytes and T lymphocytes to the area of the injury. Cytokines, protein products of the monocytes and lymphocytes, mediate the atherogenic process by chemotactically attracting phagocytic cells to the area.

Additional exposure to a high level of circulating LDL and the deposition and oxidative modification of cholesterol esters further promote the inflammatory process. The process is marked by the uptake of LDL by phagocytic cells that become engorged with lipid, called foam cells. Phagocytic uptake is accelerated if the apoB-100 component of the LDL has been oxidized. The lipid-filled foam cells may then infiltrate the endothelium and develop into a fatty plaque. As lipid continues to accumulate within the plaque, the lumen of the blood vessel is progressively occluded (Figure 5.23). Atherosclerosis was once considered a disease caused exclusively by dyslipidemia; however, atherosclerosis is now considered a disease of both

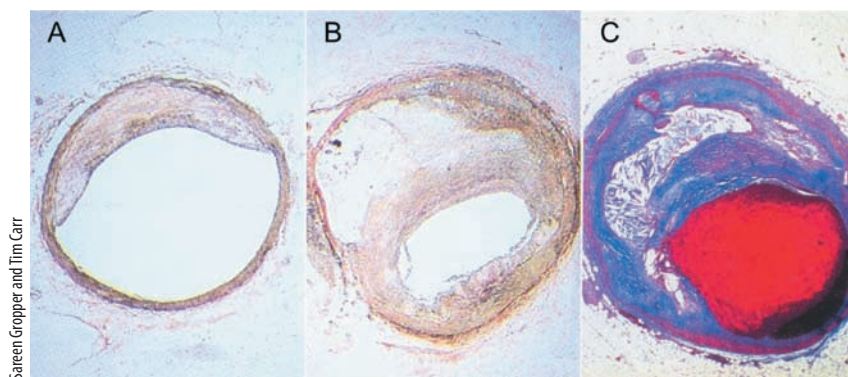


Figure 5.23 Atherosclerosis. Cross sections of coronary arteries showing occlusion: (A) 25% blockage, (B) 90% blockage, and (C) 100% blockage due to formation of a blood clot.

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dyslipidemia and immune system–induced inflammation. The Perspective at the end of this chapter discusses in detail the role of lipoproteins and inflammation in atherosclerosis development.

The Lipid Hypothesis

There is little doubt that atherosclerotic plaques contain cholesterol derived from circulating LDL. It is reasonable to presume, therefore, that lowering serum LDL cholesterol concentration will prevent ASCVD. This direct causal relationship is known as the *lipid hypothesis*. Decades of research supports the lipid hypothesis, which provides the foundation for ASCVD prevention guidelines to lower LDL cholesterol concentration [19]. However, not all of the evidence supports the lipid hypothesis. Other factors have emerged as better indicators of ASCVD risk, as discussed in the following sections.

Acceptance of the lipid hypothesis has encouraged the use of laboratory tests to measure serum lipids and other constituents. Primary clinical measurements usually include the serum concentration of LDL cholesterol, HDL cholesterol, and triacylglycerols (triglycerides). Advances in methodology has allowed LDL and HDL to be categorized into subfractions, based on size and composition. Small dense LDL subfractions are the most atherogenic and their serum concentration is a stronger indicator of ASCVD risk than the overall LDL cholesterol concentration. In contrast, a clear picture of the relationships between HDL subfractions and ASCVD has yet to emerge [20].

Other measurements focus on the apolipoproteins associated with lipoprotein classes. ApoB-100 in fasted serum can be used as a measure of VLDL and LDL particles. Recall that the total moles of serum apoB-100 indicate the number of potentially atherogenic particles. The concentration of serum apoB-100 is a stronger indicator of ASCVD risk than LDL cholesterol [19]. Serum from fasting individuals is required to avoid “contamination” from apoB-48 that would be present in chylomicrons.

ApoA-1 is the major apolipoprotein in the HDL particles that are part of the reverse cholesterol transport system. HDL particles are considered antiatherogenic. They also have anti-inflammatory and antioxidant properties. However, the concentration of serum apoA-1 does not correlate with ASCVD risk.

Lipoprotein(a)

Lipoprotein(a), abbreviated Lp(a), is composed of an LDL particle in which the apoB-100 molecule is covalently linked to a glycoprotein called apolipoprotein(a). The physiological function of the Lp(a) particle has not been identified, although it is associated with increased risk of ASCVD. Unlike other lipoprotein classes, the serum concentration of Lp(a) is genetically determined. Lp(a) exhibits a very broad and skewed distribution in the population and is not influenced by dietary or other environmental factors. The structure of apolipoprotein(a) has a strong homology—similar amino acid sequence—with plasminogen. Plasminogen is the inactive precursor of the enzyme plasmin, which dissolves blood clots by its hydrolytic action on fibrin. Apolipoprotein(a) has several genetic isoforms that vary in size. The smaller-molecular-weight isoforms appear to be more pathogenic [21].

Apolipoprotein E

Among the apolipoproteins, apoE deserves special mention because of its multiple roles in lipid metabolism, neurobiology, and cellular function. There are three isoforms of apoE in humans: apoE2, apoE3, and apoE4. One of the isoforms, apoE4, has been associated with ASCVD and Alzheimer’s disease.

A single individual inherits one apoE allele from each parent, thus various homozygous and heterozygous combinations are possible. Allele frequencies show nonrandom global distribution, with the frequency of apoE4 increasing as one moves north from the equator. The apoE4 frequency

is higher in people of northern European descent (about 25%) and lower in Mediterranean and Asian populations (< 10%). It has been known for many years that individuals of northern European origin have an increased risk for ASCVD, but only part of this increased risk is considered to be due to the serum LDL cholesterol concentration.

The isoforms of apoE display preferences for specific lipoprotein classes, with apoE4 having a preference for larger triacylglycerol-rich chylomicrons and VLDL. Consequently, apoE4 can remain associated with LDL particles during VLDL catabolism. The presence of apoE4 on LDL can increase LDL's affinity for receptors on macrophages present in atherosclerotic plaque. ApoE itself has been shown to increase oxidative stress and inflammation. While apoE is made mostly by the liver, it is also made by the brain, kidney, spleen, adipose tissue, and macrophages. Macrophage-derived apoE is abundant in atherosclerotic plaques, where it influences platelet aggregation, macrophage cholesterol efflux, expression of adhesion molecules, and inhibition of smooth muscle proliferation and migration. See the Perspective at the end of this chapter for more about the role of inflammation in atherosclerosis.

There are also several neurological consequences of apoE4. For example, it has been shown to be a risk factor in early onset of Alzheimer's disease, poorer outcomes following traumatic brain injury, and postoperative cognitive dysfunction. The mechanism for its association with these diseases is not fully understood, but it may be related to the increased oxidative stress and proinflammatory properties of apoE4 compared to the other isoforms of apoE. It is interesting that ASCVD risk is greater in smokers with apoE4 than nonsmokers. Some of the association of apoE4 with diseases is still controversial and must await additional research for confirmation [22].

Dietary Cholesterol

The impact of dietary cholesterol on serum cholesterol levels has been a controversial topic for many years. While there is definitive evidence that elevated serum LDL cholesterol increases ASCVD risk, a link between dietary cholesterol and serum cholesterol has never been firmly established. In fact, other components of the diet, particularly fats and oils, have a much greater impact on serum cholesterol levels.

The controversy surrounding dietary cholesterol started in 1968 when the American Heart Association announced a recommendation to limit cholesterol intake to less than 300 mg/day, focusing specifically on eggs (no more than three egg yolks per week) because of their high cholesterol content. Despite having weak evidence for making such a recommendation, and having no clear rationale for choosing 300 mg/day as the benchmark, the recommendation created a fear of

dietary cholesterol that has persisted for decades [23]. The preponderance of research, however, clearly indicates that dietary cholesterol has little or no impact on serum cholesterol. This is because compensatory mechanisms are engaged when cholesterol is consumed, such as increased biliary cholesterol excretion and the down-regulation of cholesterol synthesis (discussed in the "Synthesis of Cholesterol" section later in this chapter). The American Heart Association and the 2015–2020 *Dietary Guidelines for Americans* no longer recommend a restriction on cholesterol intake.

Saturated and Unsaturated Fatty Acids

Extensive research has examined the effects of ingestion of dietary fats containing primarily SFA, MUFA, PUFA, or *trans* fatty acids on serum cholesterol, particularly LDL cholesterol. The findings from older research studies generally led to the conclusions that SFA are hypercholesterolemic, PUFA are hypocholesterolemic, and MUFA are neutral (neither increasing nor lowering serum cholesterol). A comprehensive review of the scientific literature linking diet and chronic disease was published in 1989 by the National Research Council and provides an excellent historical perspective [24].

It is still thought to be important to reduce SFA intake, but it also matters what is used to replace SFA in the diet. When 1% of energy from SFA is replaced with PUFA, the LDL cholesterol is reduced and is likely to produce a 2–3% reduction in the incidence of coronary heart disease [25]. Insufficient evidence exists to judge the effects of replacing SFA with MUFA. Furthermore, replacing SFA with carbohydrate produces no benefits and may even be associated with moderately higher risk of coronary heart disease.

The potential risk of ASCVD is actually more complicated than what is implied by correlations to total serum cholesterol or LDL cholesterol. It involves a combination of genetics, dietary factors, level of obesity, exercise, and other lifestyle determinants. The cholesterol response to individual fatty acids, even those within a single fatty acid class, is heterogeneous. This heterogeneity is particularly noticeable among the long-chain SFA. Strong evidence indicates that lauric (12:0), myristic (14:0), and palmitic (16:0) acids are all hypercholesterolemic, specifically raising LDL cholesterol. On the other hand, stearic acid (18:0) reduces levels of total cholesterol and LDL cholesterol when compared to other long-chain SFA and appears more neutral in its effect. Therefore, stearic acid should not be grouped with other SFA with respect to LDL cholesterol effects. Oleic acid (18:1) and linoleic acid (18:2 n-6) are hypocholesterolemic compared to 12:0, 14:0, and 16:0 fatty acids, with linoleic acid being the more potent of the two, independently lowering total and LDL cholesterol.

COCONUT OIL: HERO OR VILLAIN?

Coconut oil is often called a superfood and natural curative. Eating coconut oil is believed to slow or prevent Alzheimer's disease, obesity, hyperglycemia, high blood pressure, arthritis, and cardiovascular disease. On the other hand, health experts warn that coconut oil is uniquely high in saturated fatty acids, well known for promoting cardiovascular disease. With such divergence of opinion, a closer look at the composition and metabolism of coconut oil is warranted.

Types of Coconut Oil

Two basic types of coconut oil are available to consumers: refined and virgin coconut oil. Refined coconut oil is processed to remove nontriacylglycerol components that impart unwanted flavors and colors. Refined oils generally have a neutral taste and a longer shelf life. Virgin coconut oil is minimally processed to retain the additional components, giving the final product a nutty flavor and off-white color. Virgin coconut oil is more susceptible to oxidation and shorter shelf life. The fatty acid profiles of refined and virgin coconut oil are the same.

Unique Fatty Acid Profile

Coconut oil is very high in saturated fatty acids (SFA), containing more SFA than practically any other oil in nature. Coconut oil has greater than 85% SFA, including both medium-chain (6:0, 8:0, 10:0, and 12:0) and long-chain (14:0, 16:0, 18:0, and 20:0) saturated fatty acids. Coconut oil is particularly rich in medium-chain SFA, making it slightly soluble in water. Triacylglycerols containing only medium-chain SFA can be

separated from coconut oil and are marketed as "MCT oils."

Other Components of Coconut Oil

Besides triacylglycerols, most common plant oils (soybean, canola, corn) contain significant amounts of vitamin E, vitamin K, phytosterols, and phenolic compounds. Coconut oil contains very low amounts of vitamins and phytosterols, but relatively high amounts of phenolic compounds. Virgin coconut oil, compared to refined coconut oil, retains more phenolic compounds due to less processing.

Metabolism of Coconut Oil

When consumed, medium-chain fatty acids can be absorbed and transported in the hepatic portal vein for direct delivery to the liver. Long-chain fatty acids are primarily packaged in chylomicrons that bypass the liver. In this way, most medium-chain fatty acids are metabolized by the liver and avoid being deposited in adipose tissue, at least initially. If overconsumption of coconut oil occurs, the excess medium-chain fatty acids in the liver will be packaged in VLDL for transport and storage in adipose tissue.

The effectiveness of coconut oil in preventing human diseases, including cardiovascular disease, has not been reported in the scientific literature. Dietary SFA from any source, including coconut oil, raises serum LDL cholesterol, a known risk factor for cardiovascular disease. Coconut oil intake also raises HDL cholesterol, but the clinical benefit in humans has not been established.

The phenolic compounds in coconut oil act as antioxidants that protect the body's

cells. As a point of reference, 100 g of virgin coconut oil contains about 30 mg of phenolic compounds (and 833 kcal). One hundred grams of blueberries contains about 800 mg of phenolic compounds (and 57 kcal). Most fruits and vegetables provide significantly more phenolic compounds than coconut oil, and with far fewer calories. Fruits and vegetables are the more obvious choice for weight loss strategies.

Using Coconut Oil

Proponents of coconut oil tout its benefits as a skin moisturizer, a UV protectant, an insect repellent, a hair treatment, and in the practice of "oil pulling" (swishing oil in the mouth to prevent dental caries). When consumed as food, coconut oil is neither a *hero* nor a *villain*. Coconut oil is an energy-dense food containing high amounts of SFA. It lacks many micronutrients and bioactive compounds found in fruits, vegetables, grains, and other high-fiber foods. In the absence of human studies showing clear metabolic benefits (beyond providing energy), consumers should be cautious about consuming too much coconut oil. As food fads come and go, history has shown that no single food can make a person healthy or cure disease.

1. Wallace TC. Health effects of coconut oil—a narrative review of current evidence. *J Am Coll Nutr.* 2019; 38:97–107.
2. Santos HO, Howell S, Earnest CP, Teixeira FJ. Coconut oil intake and its effects on the cardiometabolic profile: A structured literature review. *Prog Cardiovasc Dis.* 2019; 62:436–43.
3. U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov>

Trans Fatty Acids

The reason for the concern about dietary *trans* fatty acids is primarily because of their effects on serum lipids. Dietary *trans* fatty acids may be more unfavorable than SFA because not only do *trans* fatty acids raise LDL cholesterol, but they lower HDL cholesterol. *Trans* fatty acids also appear to correlate more strongly with ASCVD mortality than SFA [26]. However, some caution is needed

when interpreting such data because in nearly all clinical studies, the metabolic effects of *trans* fatty acids have been compared to other dietary fatty acids on a gram-per-gram basis, which misrepresents their actual proportions in the food supply. According to the U.S. Department of Agriculture food availability database, the per capita intake of SFA, MUFA, and PUFA is approximately 35, 45, and 26 g/day, respectively [7]. The per capita intake of *trans* fatty acids is significantly less at 1.3 g/day [9]. When expressed as

a percentage of total energy consumed, the contribution of *trans* fatty acids is only 0.5% of total energy, whereas SFA, MUFA, and PUFA contribute 13%, 17%, and 10% of energy, respectively. Therefore, studies that examine the isocaloric substitution of fatty acids (often at levels of 2% of energy or more) overestimate the impact of *trans* fatty acids, given their low abundance in the food supply. Interpretation of results is also complicated by the uncertainty of knowing whether experimental outcomes were due to inclusion of *trans* fatty acids or the removal of displaced fatty acids. Despite these experimental shortcomings, the American Heart Association and the *Dietary Guidelines for Americans* recommend that *trans* fatty acid intake should be avoided (zero intake, though advisable, is not considered practical because of the small amount of natural *trans* fat in the food supply).

5.7 INTEGRATED METABOLISM IN TISSUES

Triacylglycerols stored in adipose tissue represent a major energy reserve. During times of energy need such as engaging in exercise, consuming low-calorie diets, or simply sleeping through the night, the stored triacylglycerols are mobilized by lipase-catalyzed hydrolysis and released into the circulation as free fatty acids. Only adipocytes have the ability to release free fatty acids into the bloodstream. The free fatty acids bind to albumin for transport to most energy-requiring cells in the body (except red blood cells), where they are oxidized via the TCA cycle for ATP production. In this way, adipose tissue is constantly taking up and releasing fatty acids throughout the day to meet constant energy needs in the face of sporadic energy consumption.

When energy is abundant following a meal, excess nutrients such as monosaccharides and amino acids can be converted to fatty acids for storage in adipose tissue. Alternatively, insufficient dietary energy can cause fatty acids to be converted to ketone bodies, which are necessary to maintain function of certain tissues, including the brain and red blood cells. The various metabolic events involving fatty acids are discussed in the following sections.

Catabolism of Triacylglycerols and Fatty Acids

The complete hydrolysis of triacylglycerols yields glycerol and three fatty acids. In the body, this hydrolysis occurs largely through the coordinated activity of three lipases: adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL). The three enzymes each hydrolyze one fatty acid from the glycerol backbone in sequence. ATGL preferentially hydrolyzes

fatty acids at the *sn*-2 position (ATGL can also hydrolyze at the *sn*-1 position to a lesser extent). Next, HSL hydrolyzes the fatty acid at the *sn*-3 position and MGL targets the remaining fatty acid at the *sn*-1 position (or *sn*-2, depending on the initial action of ATGL). Regulation of triacylglycerol hydrolysis within the adipocyte is significantly more complicated than once thought. A cascade of events leading to enzyme activation (by phosphorylation) is controlled by the hormone epinephrine, considered a master regulator of lipolysis [27]. The following events occur in the main regulatory pathway:

- Epinephrine binds to adrenergic receptors on the cell surface ($\beta_{1,2}$ -AR).
- Activated receptors interact with adenylyl cyclase.
- Activated adenylyl cyclase converts intracellular ATP to cAMP.
- Increased intracellular cAMP activates protein kinase A.
- Activated protein kinase A phosphorylates HSL, causing HSL translocation to the lipid droplet surface.
- Activated protein kinase A also phosphorylates the protein perilipin 1 on the lipid droplet surface, promoting the release of another protein (comparative gene identification-58), which stimulates ATGL.

In addition to epinephrine, lipolysis is stimulated by natriuretic peptides. The cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) increase in the circulation during exercise and are important stimulating factors of lipolysis. The following events occur in the signaling pathway mediated by natriuretic peptides:

- ANP and BNP bind to type-A natriuretic peptide receptors.
- Activated receptors interact with guanylyl cyclase.
- Activated guanylyl cyclase converts intracellular GTP to cGMP.
- Increased intracellular cGMP activates protein kinase G.
- Activated protein kinase G phosphorylates HSL and perilipin 1, causing their activation in a manner similar to protein kinase A.

In addition to epinephrine and natriuretic peptides, other factors can act as regulators either directly by receptor-mediated signaling or indirectly by affecting the lipolytic cascade. These factors include adrenocorticotrophic hormone, thyroid-stimulating hormone, growth hormone, tumor necrosis factor α , and glucocorticoids [27].

After the complete hydrolysis of triacylglycerols, the liberated free fatty acids are secreted by the adipocyte and bind to albumin in the circulation for transport to energy-requiring tissues. The remaining glycerol cannot be metabolized by adipose tissue and is secreted into the circulation. The glycerol can be used for energy by the liver

and by certain other tissues that have the enzyme glycerokinase, which converts glycerol to glycerol phosphate. Glycerol phosphate can enter the glycolytic pathway at the level of dihydroxyacetone phosphate, from which point either energy oxidation or gluconeogenesis can occur (review Figure 3.20 in Chapter 3).

Fatty acids are a rich source of energy; on an equal-weight basis they surpass carbohydrates in this property. This occurs because fatty acids exist in a more reduced state than that of carbohydrate and therefore undergo a greater extent of oxidation en route to CO_2 and H_2O . Many tissues are capable of oxidizing fatty acids by way of a mechanism called β -oxidation, described later in this chapter. When the fatty acid enters the cell, it is first “activated” by coenzyme A to acyl-CoA, in an energy-requiring reaction catalyzed by cytosolic fatty acyl-CoA synthetase (Figure 5.24). The reaction consumes two high-energy phosphate bonds to yield AMP. This is equivalent to using two ATPs. The pyrophosphate that is produced is quickly hydrolyzed, which ensures that the reaction is irreversible.

Mitochondrial Transfer of Acyl-CoA

The oxidation of fatty acids occurs primarily within the mitochondria and produces energy through oxidative phosphorylation (see Chapter 3). Short-chain fatty acids can pass directly into the mitochondrial matrix and form acyl-CoA derivatives in the matrix. Long-chain fatty acids and their CoA derivatives are incapable of crossing the inner mitochondrial membrane (but can cross the permeable outer membrane), so a membrane transport system is

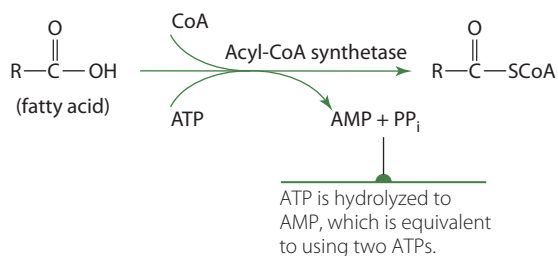


Figure 5.24 Activation of fatty acid by coenzyme A.

necessary. The carrier molecule for this system is carnitine (see Chapter 6). Carnitine can be synthesized in humans from lysine and methionine and is found in high concentration in muscle. The activated fatty acid (acyl-CoA) is joined covalently to carnitine at the cytosolic side of the outer mitochondrial membrane by the transferase enzyme carnitine acyltransferase I (CAT I). Carnitine:acylcarnitine transferase moves the acyl-carnitine across the inner membrane; then a second transferase, carnitine acyltransferase II (CAT II), located on the inner face of the inner membrane, releases the acyl-carnitine to form acyl-CoA and carnitine (Figure 5.25).

β -Oxidation of Fatty Acids

The oxidation of activated fatty acids occurs primarily in mitochondria through a degradative pathway called β -oxidation. The series of enzymatic reactions cleaves two carbons at a time—in the form of acetyl-CoA—with each passage through the pathway. Cleavage of each acetyl-CoA occurs at the carboxyl end of the fatty acid. The reactions of β -oxidation are sometimes referred to as a cycle, but it is more accurate to view β -oxidation as a repeating series of reactions.

Saturated Fatty Acids Figure 5.26 illustrates the oxidation of palmitic acid (16:0), the most abundant saturated fatty acid in the food supply. The activated palmitoyl-CoA is acted upon by the enzyme acyl-CoA dehydrogenase, which introduces a double bond between Δ^2 and Δ^3 (the α - and β -carbons, respectively). There are four such dehydrogenases, each specific to a range of chain lengths. The enzymes specific for longer chain lengths are bound to the inner membrane and those for shorter chain lengths are free in the matrix. The reaction creates a *trans* double bond, so the resulting product is an unsaturated acyl-CoA. The reaction also generates one molecule of FADH_2 that enters the electron transport chain, yielding on average 1.5 ATPs.

The next step is a hydration reaction that adds a water molecule across the double bond to form a β -hydroxyacyl-CoA. The reaction is catalyzed by the enzyme enoyl-CoA hydratase, sometimes called crotonase. The β -hydroxy

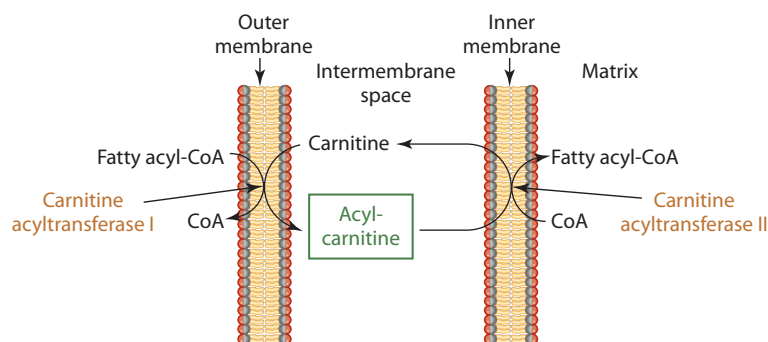


Figure 5.25 Membrane transport system for transporting fatty acyl-CoA across the inner mitochondrial membrane.

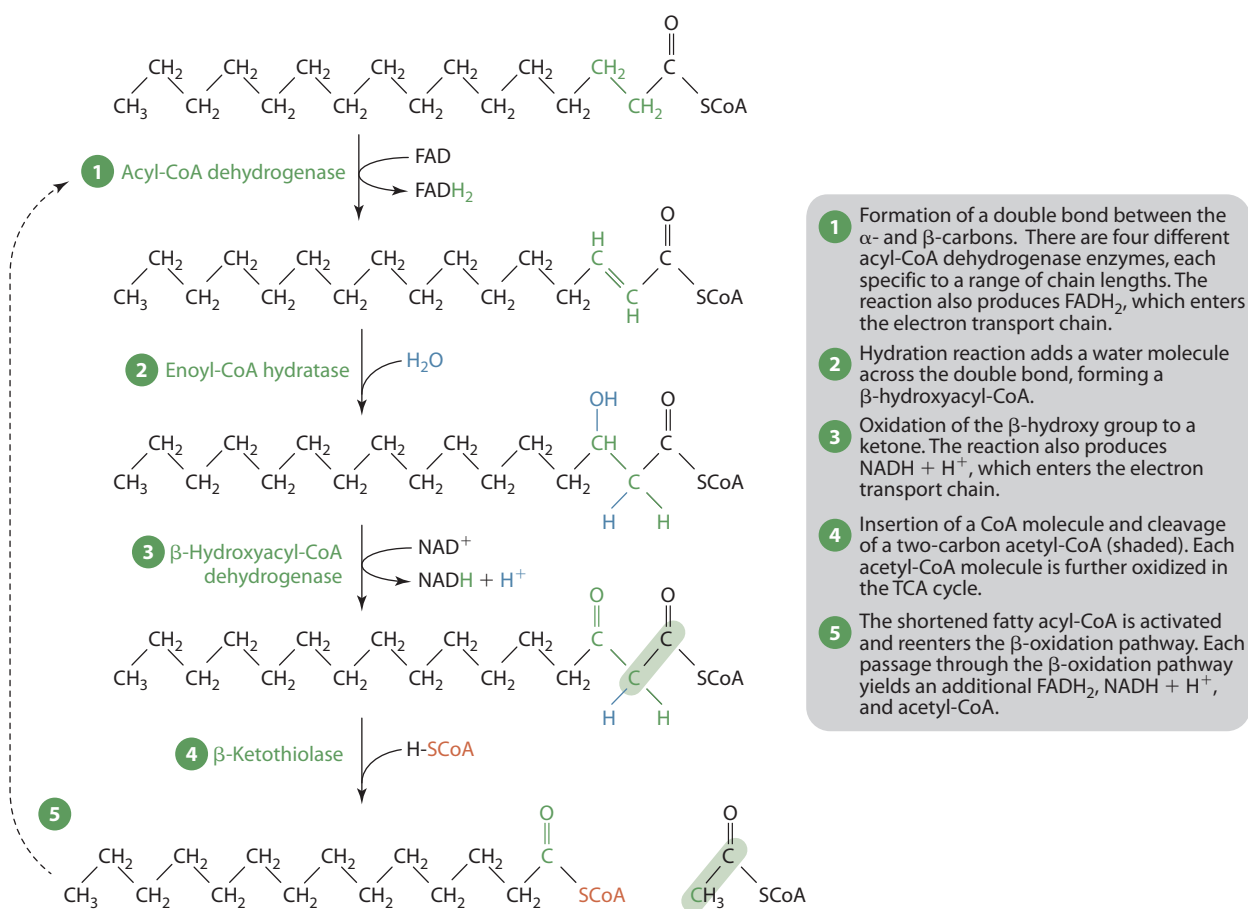


Figure 5.26 The mitochondrial β -oxidation of a saturated fatty acid, palmitic acid.

group is then oxidized to a ketone by the NAD^+ -requiring enzyme β -hydroxyacyl-CoA dehydrogenase, producing an $\text{NADH} (+ \text{H}^+)$ that can enter the electron transport chain to yield on average 2.5 ATPs. The β -ketoacyl-CoA is cleaved by β -ketothiolase, resulting in the insertion of another CoA and cleavage at the β -carbon. The products of this reaction are acetyl-CoA and a shortened saturated CoA-activated fatty acid that has two fewer carbons than the original fatty acid. The acetyl-CoA enters the TCA cycle for further oxidation. The remaining fatty acid, with two fewer carbons, continues through β -oxidation, losing two carbons with each passage through the pathway.

Unsaturated Fatty Acids The presence of double bonds in fatty acids presents a challenge in β -oxidation. Unlike saturated fatty acids, in which the first reaction introduces a double bond, unsaturated fatty acids with preexisting double bonds are not a substrate for acyl-CoA dehydrogenase. For example, oleic acid (18:1) has a double bond between Δ^9 and Δ^{10} , so sequential removal of acetyl-CoAs occurs three times until the double bond is positioned between Δ^3 and Δ^4 . The problem is that enoyl-CoA hydratase, the second step in the β -oxidation pathway, requires the double bond to be between Δ^2

and Δ^3 . An additional enzyme, enoyl-CoA isomerase, is therefore needed to shift the double bond so that enoyl-CoA hydratase can continue normally.

Polyunsaturated fatty acids present another challenge due to multiple double bonds. Using linoleic acid (18:2) as an example, sequential removal of acetyl-CoAs occurs three times until a shortened fatty acid remains with the first double bond now located at the Δ^3/Δ^4 position (Figure 5.27). Enoyl-CoA isomerase shifts the first double bond to the Δ^2/Δ^3 position, leaving the second double bond between Δ^6 and Δ^7 . After removal of an acetyl-CoA, the remaining double bond is now between Δ^4 and Δ^5 . The action of acyl-CoA dehydrogenase proceeds normally with the creation of a new double bond between Δ^2 and Δ^3 . However, the proximity of the two double bonds prevents β -oxidation from continuing, so another enzyme called 2,4-dienoyl-CoA reductase is needed to reduce the two double bonds to one. β -oxidation can now proceed to completion.

Odd-Chain Fatty Acids Most fatty acids are composed of an even number of carbon atoms, although a small proportion of fatty acids having an odd number of carbon atoms are consumed and metabolized for energy. β -oxidation occurs normally as described above, with the

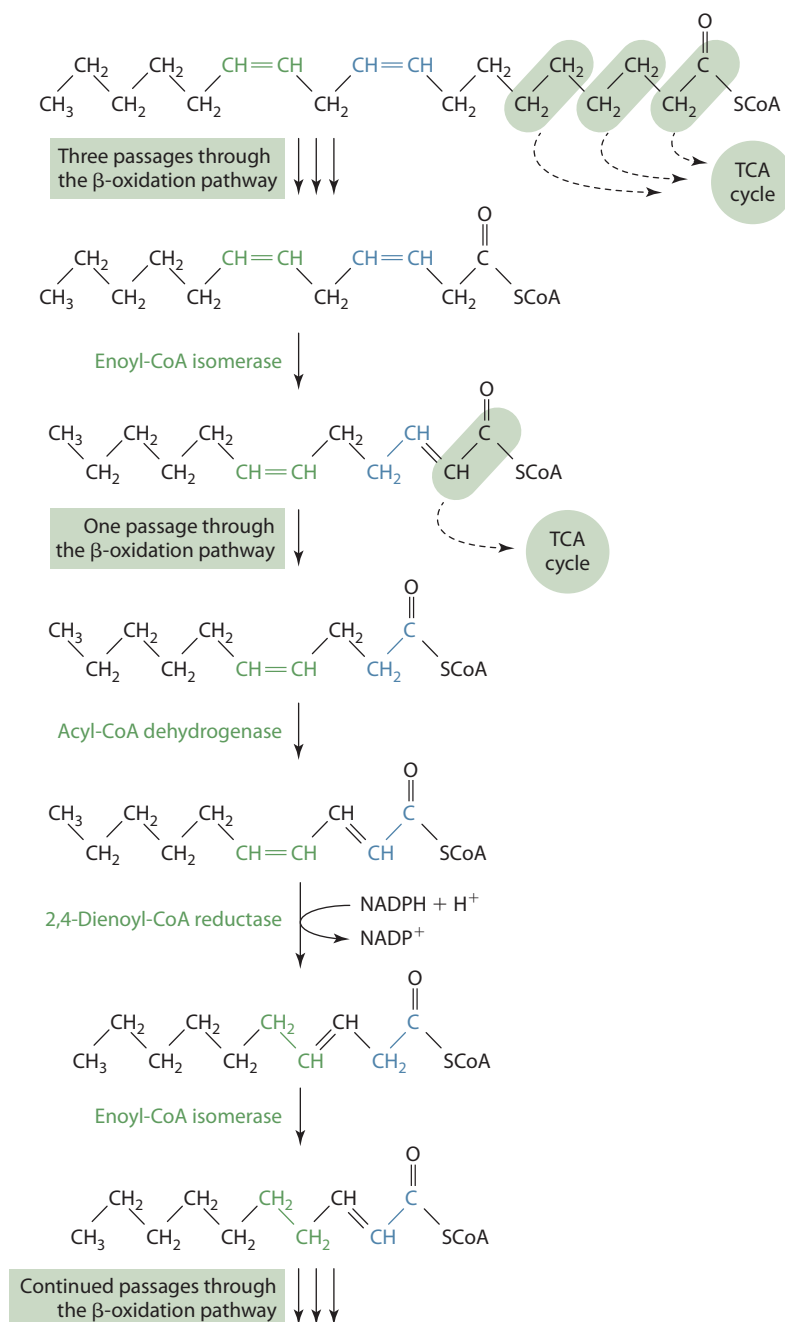


Figure 5.27 The mitochondrial β -oxidation of a polyunsaturated fatty acid, linoleic acid.

final products being acetyl-CoA and the three-carbon propionyl-CoA. The subsequent oxidation of propionyl-CoA requires additional enzymes that use the vitamins biotin and B_{12} in a coenzymatic role (Figure 5.28). Because the succinyl-CoA formed in these reactions can be converted into glucose, the odd-chain fatty acids are uniquely glucogenic among all the fatty acids.

Branched-Chain Fatty Acids β -oxidation is the primary catabolic pathway for branched-chain fatty acids. Methyl branch points generally do not interfere with the enzymes

in β -oxidation, although additional reactions may be required depending on the location of the methyl group. As acetyl-CoA molecules are cleaved during β -oxidation, the position of methyl groups along the fatty acid chain moves closer to the carboxyl end. When the acyl-CoA dehydrogenase enzyme encounters a methyl branch point at the Δ^2 position, the reaction will proceed as usual. In this case, the cleaved product of the β -ketothiolase reaction is a three-carbon propionyl-CoA (rather than acetyl-CoA). Propionyl-CoA is then oxidized as shown in Figure 5.28.

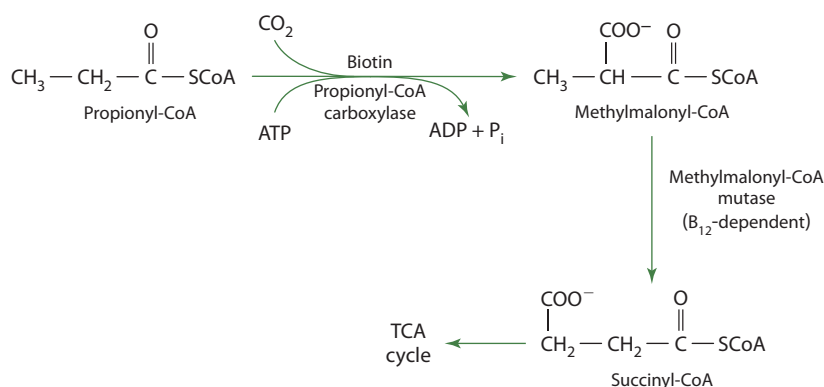


Figure 5.28 Oxidation of propionyl-CoA.

However, acyl-CoA dehydrogenase will not function if the methyl group is located on the Δ^3 carbon. In this case, removal of the first carbon atom—the Δ^1 carboxyl carbon—is required to shorten the fatty acids and move the methyl group from Δ^3 to the Δ^2 position. Removal of the Δ^1 carbon is accomplished by a series of reactions called α -oxidation that occurs in peroxisomes. One of the reactions in the α -oxidation pathway requires thiamin as a coenzyme, which is discussed in detail in Chapter 9.

Most branched-chain fatty acids consumed by humans have a single methyl branch point near the omega terminus, so β -oxidation is sufficient to completely oxidize the fatty acids. In contrast, phytanic acid is a multibranched fatty acid that has a methyl group at the Δ^3 carbon, thus requiring both α -oxidation and β -oxidation to completely oxidize the fatty acids (Figure 5.29). The oxidation of phytanic acid takes place in both peroxisomes and mitochondria. The products of α -oxidation are pristanoyl-CoA (shortened by one carbon) and formyl-CoA. The formyl-CoA is further oxidized to CO_2 . The pristanoyl-CoA molecule then goes through β -oxidation three times while still in the peroxisome, yielding one molecule of acetyl-CoA, two molecules of propionyl-CoA, and one molecule of 4,8-dimethylnonanoyl-CoA. Each of these products is converted to carnitine conjugates (see Figure 5.25) and transported into mitochondria. Here, they are further oxidized to CO_2 and H_2O , thus providing the energy for ATP production.

Energy Yield in Fatty Acid Oxidation

The complete β -oxidation of one 16-carbon palmitic acid molecule requires seven passages through the pathway and produces eight acetyl-CoA, seven FADH_2 , and seven NADH molecules. The FADH_2 and NADH directly enter the electron transport chain and yield on average 1.5 ATP/mole FADH_2 and 2.5 ATP/mole NADH by oxidative phosphorylation. The acetyl-CoAs are completely oxidized to CO_2 and H_2O by the TCA cycle and oxidative phosphorylation, with an average yield of 10 ATP/mole acetyl-CoA

(see Chapter 3). Using the example of palmitic acid, we can summarize the yield of ATP as follows:

7 FADH_2	$7 \times 1.5 = 10.5$
7 NADH	$7 \times 2.5 = 17.5$
8 acetyl-CoA	$8 \times 10 = 80$
Total ATPs produced	108
2 ATPs for activation	−2
Net ATPs	106

Unsaturated fatty acids are catabolized by β -oxidation in the mitochondrion in nearly the same way as their saturated counterparts, except that additional steps are required, as discussed in the previous section. β -oxidation of unsaturated fatty acids releases slightly less energy because of the preexisting double bonds. Each time the acyl-CoA dehydrogenase reaction is skipped, one less molecule of FADH_2 is produced and, consequently, 1.5 fewer ATP are produced.

Formation of Ketone Bodies

Normally, the concentration of the ketone bodies in the blood is very low but will increase in situations of accelerated fatty acid oxidation that occurs during body fat reduction (consuming low-energy, low-carbohydrate diets) or in uncontrolled type 1 diabetes. Under such conditions, an abundance of free fatty acids is released by adipocytes into the circulation, which exceeds the ability of tissues to oxidize them. Furthermore, glucose-requiring tissues, including the brain and red blood cells, cannot use fatty acids for energy and their need for alternative fuels increases. Fortunately, the liver is able to handle excess free fatty acids by converting them to the so-called **ketone bodies** in a process called **ketogenesis**.

Following β -oxidation, the liver converts the excess acetyl-CoA to acetoacetate, β -hydroxybutyrate, and acetone (Figure 5.30). Acetoacetate and β -hydroxybutyrate are not

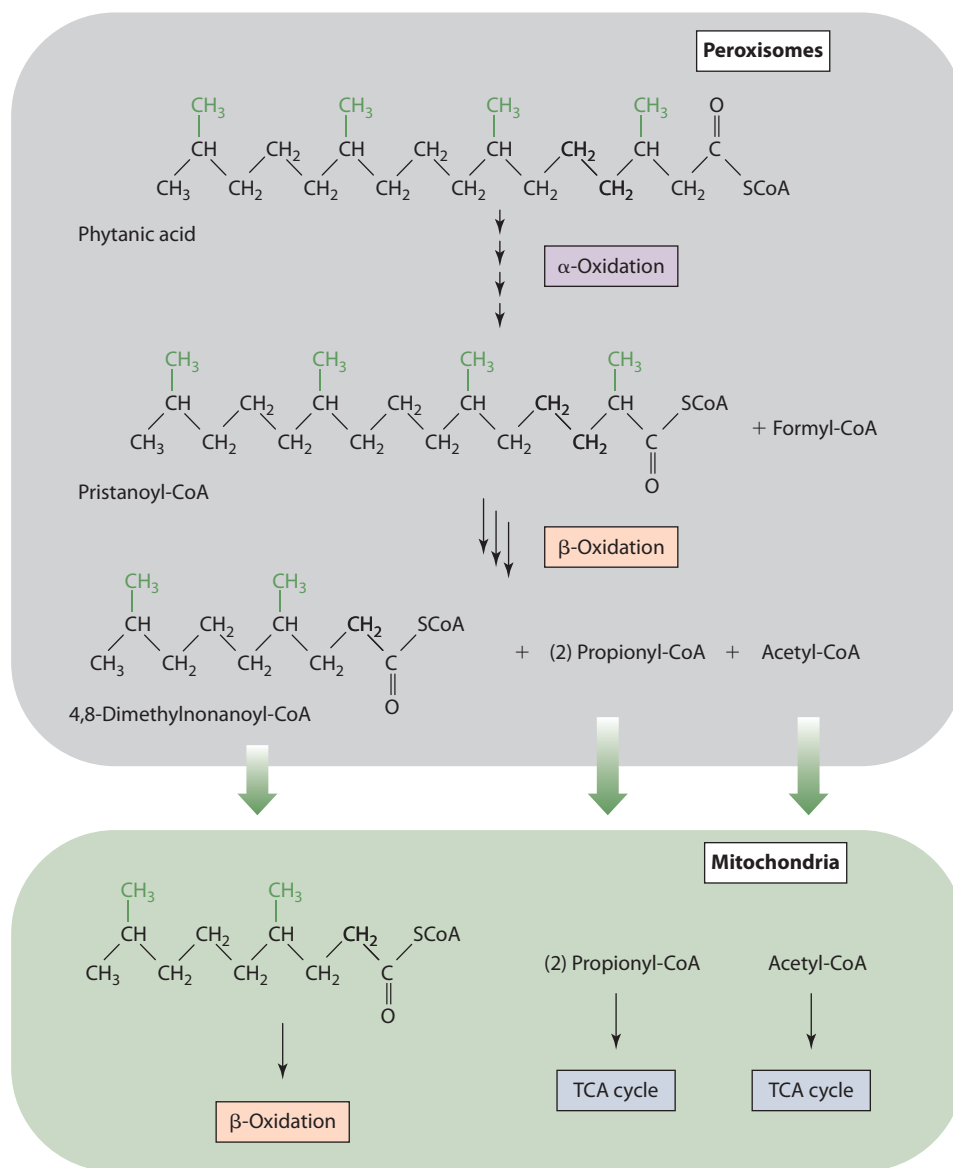


Figure 5.29 Oxidation of phytanic acid.

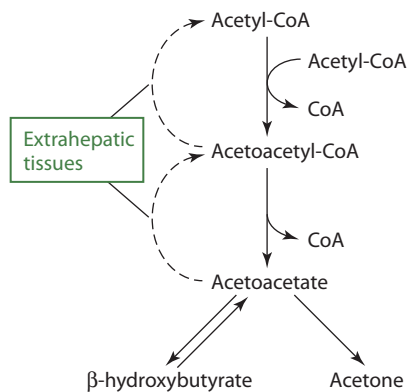


Figure 5.30 Steps in hepatic ketone body formation.

oxidized further in the liver but instead are transported by the blood to peripheral tissues, where they can be converted back to acetyl-CoA and oxidized through the TCA cycle. Acetone is a minor player and arises in the blood by spontaneous decarboxylation of acetoacetate. The reactions in ketone body formation occur only in the mitochondria. The reversibility of the β -hydroxybutyrate dehydrogenase reaction, together with enzymes present in extrahepatic tissues that convert acetoacetate to acetyl-CoA (shown by the dashed arrows in Figure 5.30), reveals how the ketone bodies can serve as a source of fuel in these tissues. Ketone body formation is considered an “overflow” pathway for acetyl-CoA use, providing another way for the liver to distribute fuel to peripheral cells.

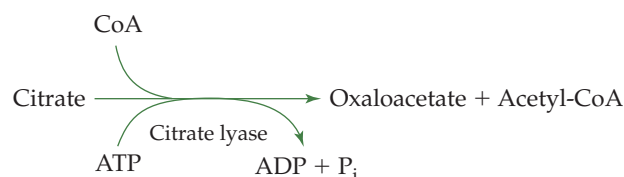
Fat loss strategies often include consumption of low-carbohydrate and/or low-energy diets. Mild increases in ketone bodies, called **ketosis**, is to be expected during fat loss and usually poses no harm. However, in uncontrolled diabetes mellitus, in starvation, or with prolonged consumption of a very low-carbohydrate diet, ketone bodies can rise to dangerous levels that lower the pH of the blood, resulting in **ketoacidosis** (sometimes called diabetic ketoacidosis in someone with diabetes). Recall from Chapter 3 that for the TCA cycle to function, the supply of four-carbon molecules must be adequate. These TCA cycle intermediates are formed mainly from pyruvate, the end product of glycolysis. When the supply of carbohydrate is inadequate, there is insufficient glucose for glycolysis to occur at a normal rate and less pyruvate is produced. Thus, the pool of oxaloacetate (made directly from pyruvate), with which the acetyl-CoA normally combines for oxidation in the TCA cycle, is reduced. As carbohydrate use by cells diminishes, oxidation of fatty acids accelerates to provide substrate (acetyl-CoA) for the TCA cycle. This shift to fat catabolism, coupled with reduced oxaloacetate availability, results in an accumulation of acetyl-CoA. As would be expected, an increase in ketone body formation follows. On one hand, the liver's ability to deliver ketone bodies to peripheral tissues such as the brain and muscle is an important mechanism for providing fuel in periods of prolonged energy deficit. On the other hand, untreated ketoacidosis can lead to low blood pressure, dehydration, coma, and death.

Synthesis of Fatty Acids

Except for the essential fatty acids linoleic acid and α -linolenic acid, most human cells are capable of synthesizing fatty acids from acetyl-CoA. The major sites of synthesis are the liver, lungs, adipose tissue, lactating mammary glands, brain, and kidneys. The initial reaction, a carboxylation reaction, occurs in the cytosol and is catalyzed by acetyl-CoA carboxylase. The vitamin biotin serves as a coenzyme for the carboxylase reaction, as discussed in Chapter 9. ATP furnishes the energy needed to attach the new carboxyl group to acetyl-CoA (Figure 5.31).

Nearly all acetyl-CoA needed for fatty acid synthesis is produced in the mitochondrial matrix. It is formed there from the oxidation of pyruvate, which may arise from the oxidation of glucose and fructose (and possibly fatty acids)

and from the degradation of the carbon skeletons of some amino acids (see Chapter 6). Some acetyl-CoA is formed in the cytosol directly from amino acid catabolism. The synthesis of fatty acids occurs in the cytosol, but acetyl-CoA produced within the mitochondrial matrix is unable to exit through the mitochondrial membrane. The major mechanism for the transfer of acetyl-CoA to the cytosol is its reaction with oxaloacetate to form citrate, which can pass through the mitochondrial membranes. In the cytosol, citrate lyase converts the citrate back to oxaloacetate and acetyl-CoA. This reaction, shown here, is essentially the reversal of the citrate synthetase reaction of the TCA cycle, except that it requires expenditure of ATP.



The enzymes involved in fatty acid synthesis are arranged in a complex called the fatty acid synthase system, located in the cytosol. Key components of this complex are the acyl carrier protein (ACP) and the condensing enzyme, both of which possess free sulfhydryl ($-\text{SH}$) groups to which the acetyl-CoA and malonyl-CoA building blocks attach. ACP is structurally similar to CoA (see Figure 9.19 in Chapter 9). Both possess a 4'-phosphopantetheine component (pantothenic acid coupled through β -alanine to thioethanolamine) and phosphate. The thioethanolamine contributes the free $-\text{SH}$ group to the complex. The free $-\text{SH}$ of the condensing enzyme is contributed by the amino acid cysteine.

Before the actual steps in the elongation of the fatty acid chain can begin, the two $-SH$ groups must be “loaded” correctly with malonyl and acetyl groups. Acetyl-CoA is transferred to ACP, with the loss of CoA, to form acetyl-ACP. The acetyl group is then transferred again to the $-SH$ of the condensing enzyme, leaving available the ACP-SH, to which malonyl-CoA attaches, again with the loss of CoA. This loading of the complex can be represented as in Figure 5.32. The extension of the fatty acid chain then proceeds through the following sequential steps, which are also shown schematically in Figure 5.33 along with the enzymes and cofactors catalyzing their actions. The enzymes catalyzing these reactions are also part of the fatty acid synthase complex, along with ACP and condensing enzyme.

The first step is the coupling of the carbonyl carbon of the acetyl group to the C-2 of malonyl-ACP with the elimination of the malonyl carboxyl group as CO_2 . The β -ketone is then reduced, with NADPH serving as hydrogen donor. (The NADPH is generated by the pentose phosphate pathway in the cytosol, as discussed in Chapter 3.)

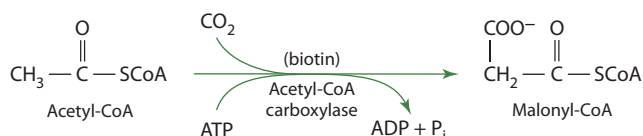


Figure 5.31 Formation of malonyl-CoA from acetyl-CoA and CO_2 (carboxylation reaction).

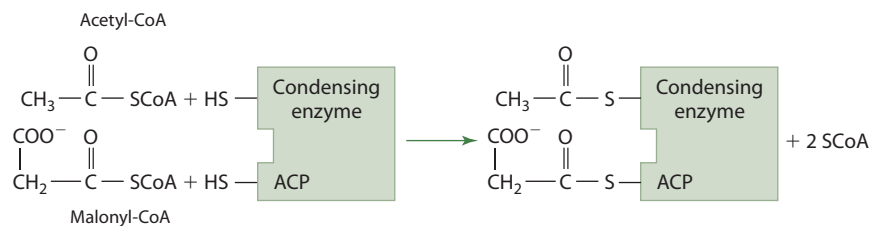


Figure 5.32 “Loading” of sulfhydryl groups into the fatty acid synthase system.

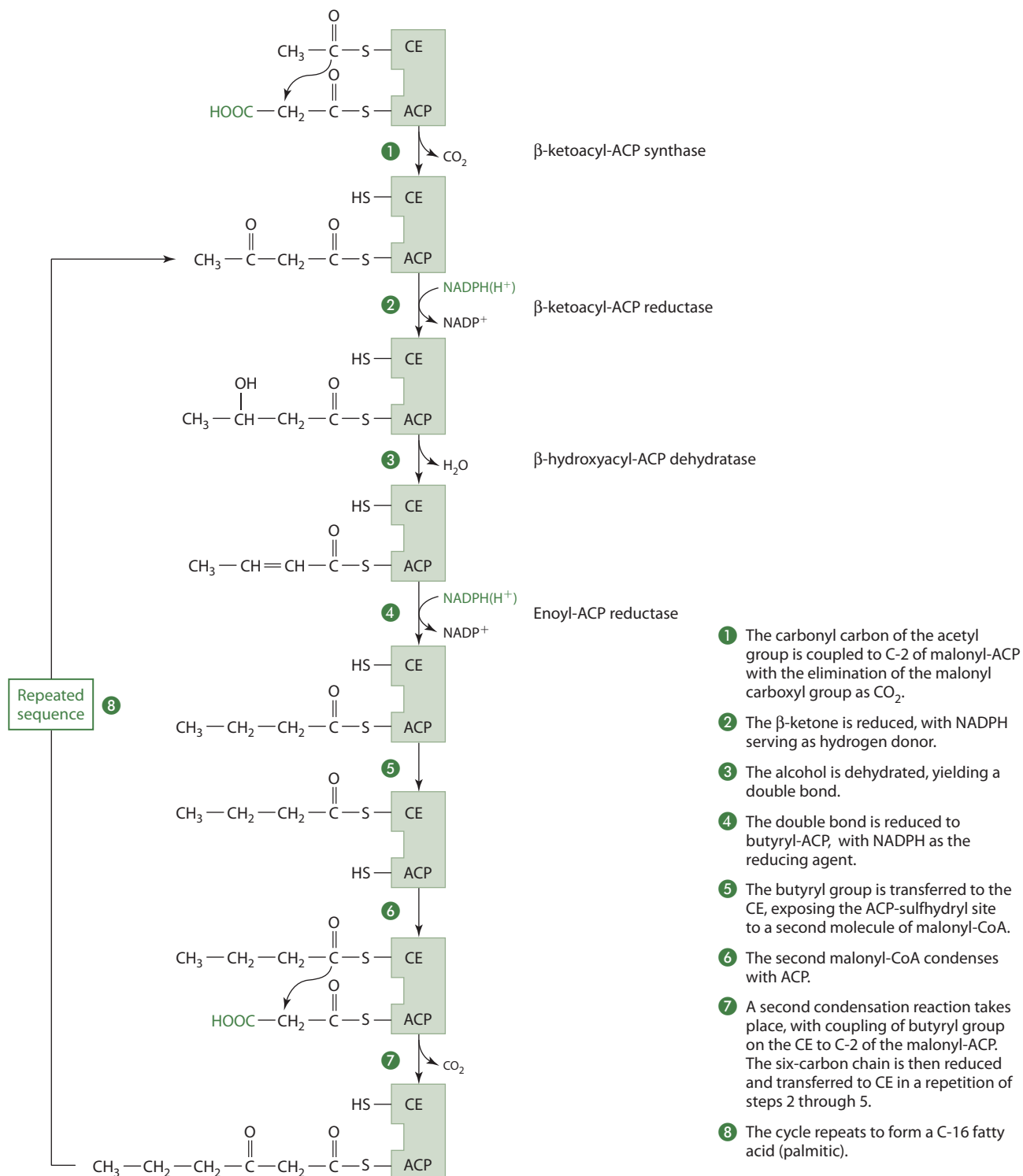


Figure 5.33 Fatty acid synthesis. Condensing enzyme (CE) and acyl carrier protein (ACP) are members of a complex of enzymes referred to as the fatty acid synthase system.

The resulting hydroxyl group is dehydrated, yielding a double bond. The double bond is reduced to butyryl-ACP, again with NADPH acting as reducing agent. The butyryl group is transferred to the condensing enzyme, exposing the ACP sulfhydryl site, which accepts a second molecule of malonyl-CoA. A second condensation reaction takes place, coupling the butyryl group on the condensing enzyme to C-2 of the malonyl-ACP. The six-carbon chain is then reduced and transferred to the condensing enzyme in a repetition of steps 2 through 5. A third molecule of malonyl-CoA attaches at ACP-SH, and so forth.

The completed fatty acid chain is hydrolyzed from the ACP without transfer to the condensing enzyme. The normal product of the fatty acid synthase system is palmitate, 16:0. It can in turn be lengthened by fatty acid **elongation** systems to stearic acid, 18:0, and even longer saturated fatty acids. Elongation occurs by the addition of two-carbon units at the carboxylic acid end of the chain. Furthermore, by **desaturation** reactions, palmitate and stearate can be converted to their corresponding Δ^9 mono-unsaturated fatty acids, palmitoleic acid (16:1) and oleic acid (18:1), respectively. Fatty acid desaturation reactions are catalyzed by enzymes referred to as mixed-function oxidases, so called because two different substrates are oxidized: the fatty acid (by removal of hydrogen atoms to form the new double bond) and NADPH. Oxygen is the terminal hydrogen and electron acceptor to form H_2O .

Essential Fatty Acids

Recall that human cells cannot introduce additional double bonds beyond the Δ^9 carbon because they lack enzymes called Δ^{12} and Δ^{15} desaturases. That is why linoleic acid (18:2 $\Delta^{9,12}$) and α -linolenic acid (18:3 $\Delta^{9,12,15}$) are essential fatty acids and must be obtained from the diet (plant sources). The Western diet is replete with n-6 linoleic acid and once it is consumed, longer, more highly unsaturated fatty acids can be formed from it by a combination of elongation and desaturation reactions. When n-3 α -linolenic acid is consumed, it is also subject to elongation and desaturated in parallel reactions. Figure 5.34 outlines the synthesis of various PUFAs from linoleic acid and α -linolenic acid, listing their chemical and common names. The biologically active compounds derived from these PUFAs are also shown, along with the enzymes involved. These compounds include eicosanoids—prostaglandins, **thromboxanes**, and **leukotrienes**—produced from both n-6 and n-3 pathways. Note that the eicosanoids derived from each pathway are different and generally have opposing biological effects, as discussed below. Resolvins and neuroprotectins come from docosahexaenoic acid (DHA) [28].

Elongation and desaturation of the essential fatty acids occurs in the smooth ER. Linoleic acid undergoes a desaturation by the enzyme delta-6-desaturase (d-6-d) to form γ -linolenic acid (18:3 n-6). The next step is an elongation

catalyzed by the enzyme elongase (ELG) to form dihomogamma-linolenic acid (20:3 n-6). Arachidonic acid (20:4 n-6) is then formed by a second desaturation. α -Linolenic acid (n-3) undergoes comparable reactions to form eicosapentaenoic acid (EPA; 20:5 n-3). Since the n-6 and n-3 fatty acids follow the same pathway with the same enzymes, they compete, and an excess of one family causes a significant change in the conversion of the other family [29]. The eicosanoids (both n-6 and n-3) are esterified with the glycerol backbone to form phospholipids or triacylglycerols and incorporate into membranes. Arachidonic acid is predominant in membranes, so n-6 synthesis predominates. Arachidonic acid and EPA go through further elongation and desaturations in the smooth ER to form tetracosapentaenoic acid (24:5 n-6) and tetracosahexaenoic acid (24:6 n-3). These fatty acids are transferred to the peroxisome, where they undergo β -oxidation to form docosapentaenoic acid (22:5 n-6) and docosahexaenoic acid (DHA; 22:6 n-3).

Humans of all ages require linoleic and α -linolenic acid (or their derivatives) in the diet for normal growth and cellular metabolism. The n-6 and n-3 fatty acids are metabolized by the same series of desaturases and elongases to longer-chain polyunsaturated fatty acids as described previously. Deficiency symptoms for the n-6 series that have been identified in adults and children include poor growth and skin abnormalities, including dry or scaly skin, raised bumps, and hair loss (Figure 5.35). Deficiency symptoms for the n-3 series include neurological and visual abnormalities.

Infants have been observed to have similar neurological abnormalities when maintained on a regimen that was lacking in n-3 α -linolenic acid. Human milk contains more of the essential fatty acids (though the level varies), as well as the elongated derivatives EPA and DHA, than do most infant formulas. There is evidence that n-3 essential fatty acids are necessary for neural tissue and retinal photoreceptor membranes. Both term and preterm infants can convert n-3 essential fatty acids to the long-chain polyunsaturated fatty acids, but whether they can convert them at an adequate rate to meet their needs is unclear. Essential n-3 fatty acid deficiency appears to be more common among preterm infants than term infants. Infant formulas containing n-3 EPA and n-6 arachidonic acid are now available.

Eicosanoids: Fatty Acid Derivatives of Physiological Significance

As long-chain arachidonic acid, α -linolenic acid, EPA, and DHA are synthesized, they incorporate into phospholipids (and triacylglycerols) and thus become an integral part of cell membranes. The higher the degree of unsaturation among the fatty acids within a membrane, the greater the fluidity of that membrane. The membrane's fluidity is an important determinant for cell

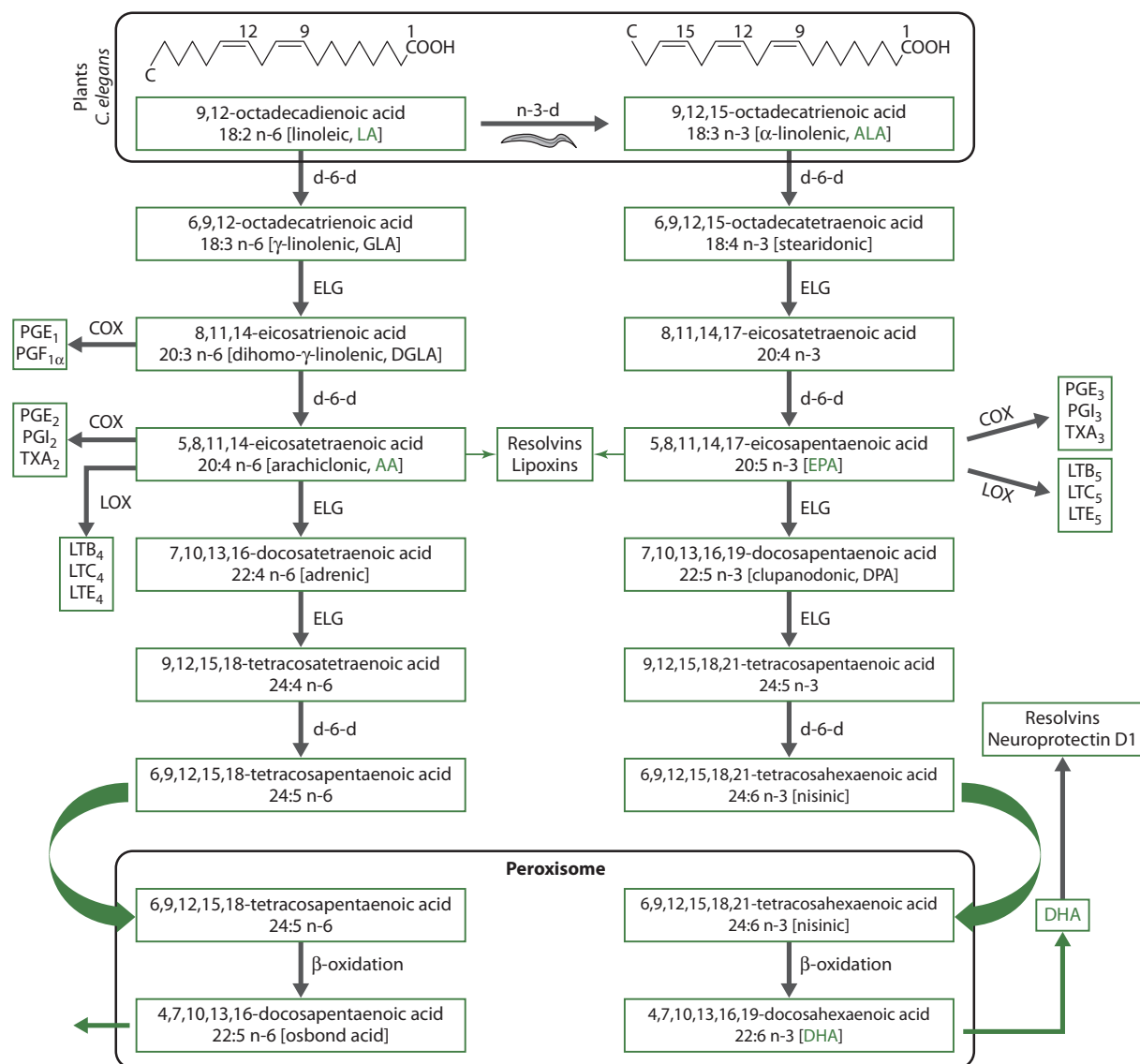


Figure 5.34 PUFA biosynthesis. The IUPAC names (all-*cis*) and the common names (in square brackets) with abbreviations are reported. ELG indicates elongase, while d-6-d and d-5-d indicate delta desaturases. In plants, a n-3 desaturase (n-3-d) converts LA to ALA. Mammals convert LA and ALA to long-chain fatty acids using a series of desaturation and elongation reactions in the ER. However, the synthesis of DHA from 24:6 n-3 and osbond acid (22:5 n-6) from 24:5 n-6 requires the synthesis of 24:6 n-3 and 24:5 n-6 in the ER, and their passage into the peroxisome, where they undergo one passage through beta-oxidation to produce DHA and osbond acid, which move back to the ER (red arrows). Formation of resolvins and protectins from DHA is also shown.

Source: Russo, G.L., Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implication in cardiovascular prevention. *Biochemical Pharmacology*, 2009; 77:937–46.

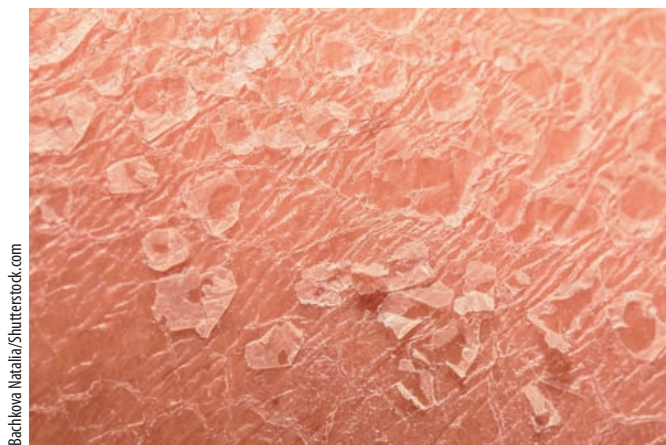


Figure 5.35 Desquamation of skin due to essential fatty acid deficiency.

receptors and membrane-bound enzymes. For example, it has been hypothesized that insulin resistance might be associated with the development of cells with a rigid membrane, which limits the expression of insulin receptors and reduces their number.

When eicosanoids are synthesized, the polyunsaturated fatty acid precursors are mobilized from the phospholipids or triacylglycerols by phospholipase A₂. Further reactions can then produce the biologically active eicosanoids, as shown in Figure 5.34. Note that n-6 arachidonic acid produces the eicosanoids in what is called the 2 and 4 series, while EPA produces eicosanoids in the 1 and 3 series. Cyclo-oxygenases and lipoxygenases convert AA to the prostaglandin-2-, thromboxane-2-, and leukotriene-4-series; various hydroperoxy- and hydroxyl-eicosatetraenoic acid (HPETE and

HETE) derivatives; and lipoxin A₄. EPA is metabolized to the eicosanoids of the prostaglandin-3-, leukotriene-5-, and thromboxane-3-series. DHA can be metabolized to other biologically active compounds that include resolvins, docosatrienes, and neuroprotectins.

Opposing Effects of n-6 and n-3 Fatty Acid–Derived Eicosanoids

Table 5.6 highlights the effects of individual messengers derived from n-6 arachidonic acid (AA) and n-3 EPA and DHA. Note that most of the arachidonic acid–derived messengers are proinflammatory or show other disease-propagating effects, whereas n-3 derivatives oppose these effects. Mediators of the n-6 prostaglandin family are proarrhythmic, while the messengers derived from n-3 EPA and DHA are antiarrhythmic, anti-inflammatory, or vasodilators. Thromboxanes (TXB₂) produced from n-6 AA activate platelets (which promote blood clots) and cause vasoconstriction (which raises blood pressure); in opposition, the n-3 thromboxane (TXB₃) inhibits platelets and causes vasodilation. Similarly, whereas n-6 leukotriene (LTB₄) from arachidonic acid is proinflammatory and leads to the production of inflammatory cytokines, the corresponding n-3 leukotriene (LTB₅) from EPA and DHA

is anti-inflammatory and actually blocks the biosynthesis of the inflammatory leukotriene derived from arachidonic acid. Other anti-inflammatory derivatives of EPA include resolvins (RV1 or RVD) and nuclear receptors (NF).

The cardioprotective effects of n-3 fatty acids (especially EPA and DHA) are the basis of the recommendation for the increased consumption of fish, particularly deep-water fish such as herring, salmon, and tuna. Benefits have been shown most consistently when meals featuring fish are consumed at least twice per week rather than fish oil supplements. n-3 fatty acids also benefit the nervous system, where DHA is concentrated and appears to function in photoreceptors and synaptic membranes. DHA thus plays roles in vision, neuroprotection, successful aging, and memory in addition to its anti-inflammatory and inflammation-resolving properties as compared to n-6 PUFAs. A recent review addresses the importance of DHA in brain health [30].

Impact of Diet on Fatty Acid Synthesis

Following a carbohydrate-rich meal, *de novo* fatty acid synthesis (lipogenesis) increases mainly in the liver, but also in many other tissues. In the fed state, the amount of carbohydrate consumed usually exceeds immediate energy needs,

Table 5.6 n-3 and n-6 Fatty Acid–Derived Messengers and Their Physiological Effects

Messenger Classes	Arachidonic Acid (n-6)–Derived Messengers	Physiological Effects	EPA- and DHA (n-3)–Derived Messengers	Physiological Effects
Prostaglandins	PGD ₂		PGD ₃	
	PGE ₂	Proarrhythmic	PGE ₃	Antiarrhythmic
	PGF ₂		PGF ₃	
	PGI ₂	Proarrhythmic	PGI ₃	Antiarrhythmic
Thromboxanes	TXA ₂	Platelet activator	TXA ₃	Platelet inhibitor
	TXB ₂	Vasoconstriction	TXB ₃	Vasodilation
Leukotrienes	LTA ₄		LTA ₅	
	LTB ₄	Proinflammatory	LTB ₅	Antiinflammatory
	LTC ₄		LTC ₅	
	LTE ₄		LTE ₅	
	LTD ₄		LTD ₅	
Epoxyeicosatrienoic derivatives	5,6-EET			
	8,9-EET			
	11,12-EET	Proinflammatory		
	14,15-EET			
Hydroxyleicosatetraenoic derivatives	5-HETE			
	12-HETE			
	15-HETE			
Lipoxins	LXA ₄			
Resolvins			RVE1	Antiinflammatory
			RVD	Antiinflammatory
Neuroprotectin			NPD1	Antiinflammatory

Source: Based on data from Heird W, Lapillonne A, The role of essential fatty acids in development. *Annu Rev Nutr*. 2005;25:549–71.

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so the excess is first stored as glycogen in liver and muscle (see Chapter 3). When glycogen stores reach capacity, the remaining carbohydrates are converted to fatty acids and triacylglycerols. Some tissues such as cardiac muscle, skeletal muscle, and adipose tissue can store triacylglycerols at the site of lipogenesis. In contrast, the liver packages newly synthesized triacylglycerols in VLDL and releases them into the circulation for delivery to tissues that express lipoprotein lipase, including the adipose tissue, cardiac muscle, skeletal muscle, lactating mammary gland, brain, kidney, and, to lesser extent, lung and spleen tissue.

The main carbohydrates that reach the liver after a meal are glucose (from starch and simple sugars) and fructose (from simple sugars). The presence of glucose and insulin in the systemic circulation stimulates the lipogenic enzymes. The regulatory role of fructose is less certain, but it may stimulate lipogenesis by insulin-independent mechanisms [31]. Nevertheless, both dietary glucose and fructose not immediately used for energy in the liver can be converted to fatty acids and triacylglycerols, then transported out in VLDL. Prolonged intake of diets excessively high in glucose and fructose can lead to triacylglycerol accumulation in adipose tissue and in the liver, resulting in nonalcoholic fatty liver disease (NAFLD), although the dietary level of sugars that constitutes “excess” is a matter of debate. Whether fructose alone affects hepatic lipogenesis and NAFLD independently of excessive energy intake remains uncertain [31].

Synthesis of Triacylglycerols and Phospholipids

The synthesis of triacylglycerols and phospholipids share common precursors and are considered together in this section. The precursors are CoA-activated fatty acids and glycerol-3-phosphate, the latter produced either from the reduction of dihydroxyacetone phosphate or from the phosphorylation of glycerol. These and subsequent reactions of the pathways are shown in Figure 5.36, which depicts two pathways for phosphatidylcholine synthesis from diacylglycerol. The *de novo* pathway of phosphatidylcholine synthesis is the major route. However, the importance of the salvage pathway increases when a deficiency of the essential amino acid methionine exists. Triacylglycerols synthesized in the liver are assembled into VLDL and secreted into the circulation for delivery to tissues expressing lipoprotein lipase (mainly adipose tissue and cardiac and skeletal muscle). Triacylglycerols synthesized in extrahepatic tissues can be stored at the site of lipogenesis.

Synthesis, Catabolism, and Whole-Body Balance of Cholesterol

Unlike the triacylglycerols and fatty acids, cholesterol is not an energy-containing nutrient, nor is it required in the diet since all cells can synthesize it. Another unusual feature

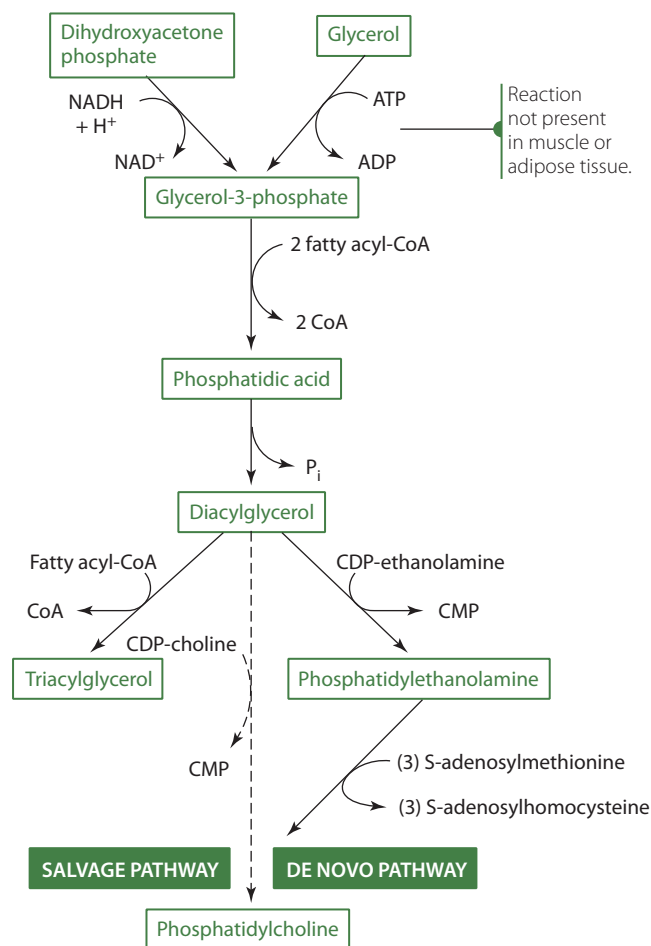


Figure 5.36 Synthesis of triacylglycerols and phosphatidylcholine showing that precursors are shared. In phosphatidylcholine formation, three moles of activated methionine (*S*-adenosylmethionine) introduce three methyl groups in the *de novo* pathway, and choline is introduced as cytidine diphosphate (CDP)–choline in the so-called salvage pathway.

of cholesterol is that no degradative (oxidative) enzymes exist in mammals, so cholesterol catabolism depends on its conversion to other molecules and its elimination from the body through biliary excretion. Its four-ring core structure remains intact in the course of its catabolism.

The concept of *whole-body cholesterol balance* was developed many years ago as a useful way to describe cholesterol homeostasis in which “input” includes synthesis and dietary intake, whereas “output” includes biliary excretion as free cholesterol or bile salts (after hepatic conversion from cholesterol). While cholesterol can also be converted to hormones and 7-dehydrocholesterol (which can then be used to synthesize vitamin D) (see Figure 5.10), these pathways are quantitatively small compared to biliary output and are not usually considered in models of whole-body cholesterol balance. The discussion in this section begins with the liver and its ability to mediate cholesterol output through bile, followed by cholesterol synthesis and the coordination of events necessary to maintain cholesterol homeostasis.

The liver is the central organ that mediates the elimination of cholesterol from the body. Cholesterol, primarily in the form of its ester, is delivered to the liver as a component of chylomicron remnants, LDL, and HDL. The cholesterol ester that is destined for excretion is either hydrolyzed by cholesterol esterase to its free form, which is secreted directly into the bile canaliculi, or it is first converted into bile salts before entering the bile. As shown in Figure 5.11, conversion of cholesterol to bile salts involves addition of hydroxyl groups to the ring structure and conjugation of the side chain with glycine or taurine. The effect of these reactions is to enhance the water solubility of the sterol, facilitating its secretion into bile. Up to 10 times more bile salts than cholesterol is present in bile, although the proportions can change depending on many factors that include a person's diet and interaction of bile salts with intestinal microbiota [32].

Recall that bile salts and cholesterol can be reabsorbed and returned to the liver as part of the enterohepatic circulation. Their absorption efficiency can influence their rate of fecal excretion and thereby affect whole-body cholesterol balance. Bile salts returning to the liver from the intestine repress the formation of an enzyme that catalyzes the rate-limiting step in their conversion from cholesterol. If the bile salts are prevented from returning to the liver, the activity of this enzyme increases, stimulating the conversion of cholesterol to bile acids and leading to their excretion. The removal of bile salts is, in fact, a therapeutic treatment for hypercholesterolemia that employs an unabsorbable, cationic resin (cholestyramine) to bind bile salts in the intestinal lumen and prevent them from returning to the liver. Preventing the reabsorption of cholesterol is also a strategy for treating hypercholesterolemia, which can be achieved by drugs (ezetimibe) and diet (phytosterols), both strategies having been discussed earlier in this chapter. Recall that cholesterol present in the intestinal lumen can come from both the liver and diet, although the majority is from the liver. Consequently, blocking cholesterol absorption can be an effective treatment for hypercholesterolemia, even for vegans who consume no animal products.

Changes in biliary excretion of bile salts and cholesterol are compensated for by changes in the rate of whole-body cholesterol synthesis. Nearly all tissues in the body are capable of synthesizing cholesterol from acetyl-CoA. The liver accounts for about 20% of endogenous cholesterol synthesis. Among the extrahepatic tissues, which are responsible for all other cholesterol synthesis, the intestine is probably the most active. Endogenous synthesis accounts for most (and perhaps all, in vegans) of cholesterol “input” and can quickly adjust in response to changes in cholesterol and bile salt absorption, the efficiency of lipoprotein cholesterol transport, and the cholesterol needs of cells throughout the body.

At least 26 steps are known to be involved in the formation of cholesterol from acetyl-CoA. The individual steps

are not provided here, but the synthesis of cholesterol can be thought of as occurring in three stages:

- 1 A cytosolic sequence by which 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) is formed from 3 moles of acetyl-CoA
- 2 The conversion of HMG-CoA to squalene, including the important rate-limiting step of cholesterol synthesis, in which HMG-CoA is reduced to mevalonic acid by HMG-CoA reductase
- 3 The formation of cholesterol from squalene.

The rate of cholesterol synthesis is controlled in each cell by a negative feedback regulation of the HMG-CoA reductase reaction. A family of drugs called statins are HMG-CoA reductase inhibitors and are widely used to block endogenous cholesterol synthesis, which forces cells (particularly in the liver) to increase the number of LDL receptors to recruit needed cholesterol from the circulation. The net effect is a significant reduction in serum LDL cholesterol concentration. A brief scheme of hepatic cholesterol synthesis and its regulation is shown in Figure 5.37.

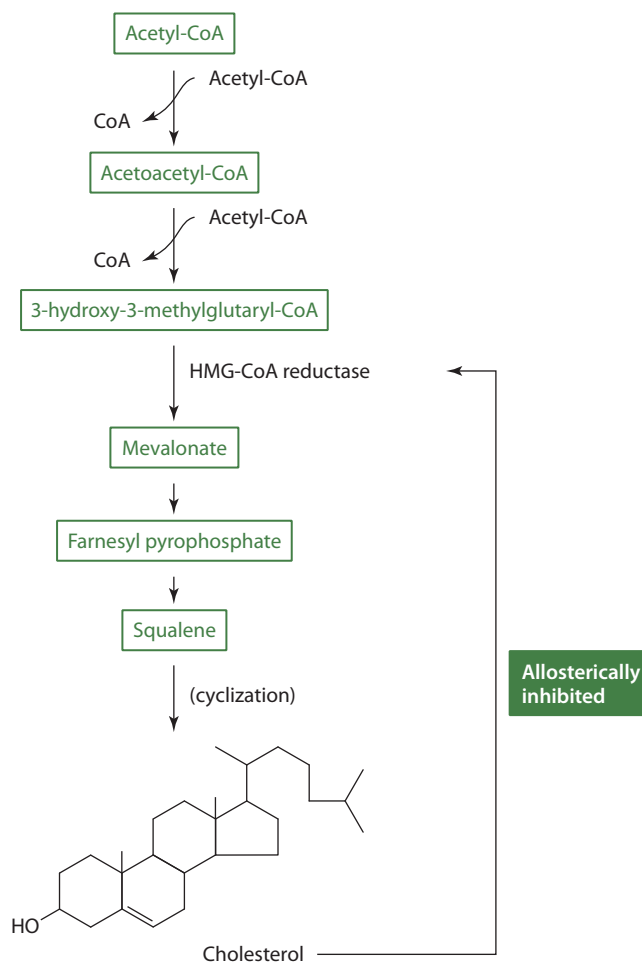


Figure 5.37 Cholesterol biosynthesis in hepatocytes indicating the negative regulatory effect of cholesterol on the HMG-CoA reductase reaction. Not all reactions are shown.

5.8 REGULATION OF LIPID METABOLISM

Fatty Acids

The regulation of fatty acid synthesis and oxidation is closely linked to carbohydrate status—synthesis increases when adequate carbohydrates are consumed, whereas oxidation increases in carbohydrate deficit. Fatty acids formed in the cytosol of lipogenic cells can either be converted into triacylglycerols and phospholipids or be transported via carnitine into the mitochondrion for oxidation. The enzyme carnitine acyltransferase I, which catalyzes the transfer of fatty acyl groups to carnitine (see Figure 5.25), is specifically inhibited by malonyl-CoA. Recall that malonyl-CoA is the first intermediate in the synthesis of fatty acids. Therefore, it is logical that an increase in the concentration of malonyl-CoA would promote fatty acid synthesis while inhibiting fatty acid oxidation. Malonyl-CoA concentration increases whenever a person is well supplied with carbohydrate. Excess glucose that cannot be oxidized through the glycolytic pathway or stored as glycogen is converted to fatty acids then stored as triacylglycerols.

Blood glucose levels also affect lipolysis and fatty acid oxidation. Hyperglycemia triggers the release of insulin, which promotes glucose transport into adipocytes for conversion to fatty acids. Insulin also inhibits lipolysis in adipocytes by antagonizing the effects of hormones that stimulate lipases, particularly hormone-sensitive lipase (HSL), as discussed earlier in this chapter.

The extent to which adipocytes convert glucose to fatty acids has been a matter of debate. While the lipogenic enzymes are highly expressed in human adipocytes, methods used to measure the conversion rates are inadequate and likely underestimate the true contribution. Hypoglycemia, on the other hand, results in a reduced intracellular supply of glucose, thereby suppressing lipogenesis. Furthermore, the low level of insulin accompanying the hypoglycemic state would favor lipolysis, with a flow of free fatty acids into the bloodstream. Low blood glucose (and therefore low intracellular levels) also stimulates fatty acid oxidation. In this case, accelerated oxidation of fatty acids follows the reduction in TCA cycle activity, which in turn results from inadequate oxaloacetate availability.

An important allosteric enzyme involved in the regulation of fatty acid synthesis is acetyl-CoA carboxylase, which forms malonyl-CoA from acetyl-CoA (see Figure 5.31). This enzyme is positively stimulated by citrate in the cytosol, but is barely active in the absence of citrate. Recall that citrate is part of the shuttle for moving acetyl-CoA from the mitochondria (a major site of production) to the cytosol, where fatty acids are synthesized. Citrate

is continuously produced in the mitochondrion as a TCA cycle intermediate, but its concentration in the cytosol is normally low. When mitochondrial citrate concentration increases, it can escape to the cytosol by way of a transport protein called the citrate carrier. In the cytosol, citrate acts as a positive allosteric signal to acetyl-CoA carboxylase, thereby increasing the rate of formation of malonyl-CoA, resulting in lipogenesis. Recent studies have reported that diets rich in PUFA, but not SFA or MUFA, inhibit citrate transport into the cytosol by decreasing transcription and translation of the citrate carrier, with subsequent decreases in acetyl-CoA carboxylase and fatty acid synthase activity. These results suggest an interesting mechanism whereby PUFA-rich diets may protect against hepatic fat accumulation and possibly serve as treatment for NAFLD [33].

Acetyl-CoA carboxylase can be modulated negatively by palmitoyl-CoA, which is the end product of fatty acid synthesis. This situation would most likely arise when free fatty acid concentrations increase because of insufficient glycerol-3-phosphate, with which fatty acids must combine to form triacylglycerols (see Figure 5.36). Deficient glycerol-3-phosphate levels would likely stem from inadequate carbohydrate availability. In such a situation, regulation would logically favor fatty acid oxidation rather than synthesis.

Cholesterol

The liver is the central organ responsible for maintaining cholesterol homeostasis. Cholesterol metabolism in hepatocytes is unique among the body's cells because of its ability to

- synthesize cholesterol;
- accept cholesterol from the circulation via lipoprotein receptors;
- store excess cholesterol as cholesterol esters;
- package cholesterol esters into VLDL and secrete them into the circulation;
- convert cholesterol to bile salts; and
- release bile salts and free cholesterol via bile into the small intestine for excretion from the body.

The combined results of these coordinated events help to regulate the serum concentration of LDL and HDL cholesterol, as well as whole-body cholesterol balance.

The size of the intracellular cholesterol pool is key to the regulation of each of the metabolic pathways that contribute to cholesterol homeostasis. The intracellular cholesterol pool exists primarily as free cholesterol within membranes of the endoplasmic reticulum (ER) where its presence can

influence the activity of regulatory proteins (enzymes, receptors, transports, etc.). When the intracellular pool increases, regulatory events are engaged that normalize the level of free cholesterol in the cell. First, cholesterol synthesis is inhibited by down-regulation of HMG-CoA reductase (see Figure 5.37). Second, LDL receptor production is decreased and the receptors are translocated away from the cell surface to prevent further uptake of LDL from the circulation (Step 4 in Figure 5.21). Third, cholesterol esterification is accelerated by up-regulation of acyl-CoA:cholesterol acyltransferase (ACAT). The cholesterol esters may be stored in cytosol vesicles or they may be secreted by the cell as a component of VLDL. Fourth, bile acid synthesis is increased by up-regulation of the rate-limiting enzyme cholesterol-7 α -hydroxylase (CYP7A1). All of the enzymes mentioned above reside in the ER and are therefore directly controlled by the regulatory pool of cholesterol. As in the case of LDL receptors, they are synthesized in the ER and are subject to the same direct control.

In contrast, as the intracellular cholesterol pool decreases, the opposite regulatory events occur. HMG-CoA reductase is up-regulated to synthesize more cholesterol. LDL receptor production and translocation to the cell surface increases to recruit more LDL cholesterol from the circulation. Cholesterol esters stored in cytosolic vesicles are hydrolyzed by cholesterol ester hydrolase (and possibly hormone-sensitive lipase) to yield more free cholesterol. CYP7A1 is down-regulated and fewer bile acids are synthesized, although this does not negatively affect bile function because of the relatively large amount of bile salts produced throughout the day.

5.9 BROWN FAT THERMOGENESIS

Brown adipose tissue (or brown fat) is metabolically active and derived from a different embryological origin than white fat. Brown fat is found in greater abundance in the neck area of adults and is associated with lower adiposity. In contrast to energy-storing white fat, energy-burning brown fat contributes to increasing energy expenditure and insulin sensitivity. Brown fat obtains its name from its high degree of vascularity and the abundant mitochondria present in its adipocytes. Recall that the mitochondria are pigmented, owing to the cytochromes and perhaps other oxidative pigments associated with the electron transport chain. Not only do brown fat cells contain larger numbers of mitochondria than do white fat cells, but the mitochondria are also structurally different and contain uncoupling protein 1 (UCP1) to promote **thermogenesis** (heat production) rather than producing ATP.

Brown fat mitochondria have special H^+ pores in their inner membranes, formed by UCP1 as an integral membrane protein. UCP1 is a translocator of protons, which allows the external H^+ pumped out of the mitochondrial matrix by electron transport to flow back into the matrix and thus become unavailable to drive ATP synthase, the site of phosphorylation. Recall that the H^+ gradient creates the energy potential for the phosphorylation of ADP to produce ATP. Figure 5.38 illustrates how the proposed mechanism of brown fat thermogenesis relates to the chemiosmotic theory of oxidative phosphorylation.

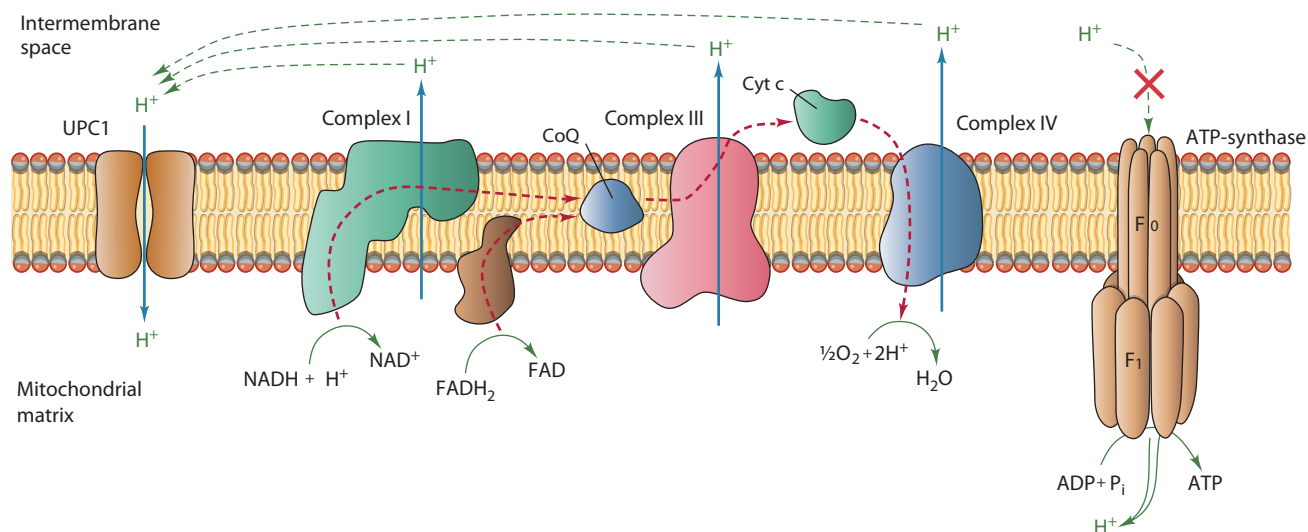


Figure 5.38 Action of uncoupling protein 1 (UCP1) in brown adipose tissue. Normally, the electron transport chain causes protons (H^+) to move into the intermembrane space of mitochondria, thus creating the energy gradient for ATP synthesis. The activation of UCP1 generates heat by allowing H^+ to move back into the mitochondrial matrix without ATP synthesis.

Membrane pores of brown fat mitochondria allow protons to pass back into the mitochondrial matrix, which lowers the proton concentration in the inner membrane space. The overall result is heat generation rather than ATP production.

Three types of external stimuli trigger thermogenesis: (1) ingestion of food, (2) prolonged exposure to cold temperature, and (3) exercise through the muscle-derived hormones. The first two events stimulate the tissue via sympathetic innervation via the hormone norepinephrine. The sympathetic signal has a stimulatory and hypertrophic effect on brown adipose tissue. This effect enhances expression of the UCP1 in the inner membrane of the mitochondrion and accelerates synthesis of lipoprotein lipase and glucose transporters to make more fatty acids and glucose available to meet the higher metabolic demand. It stands to reason, then, that body weight reduction should accompany a higher activity of brown fat and, indeed, an association between obesity and deficient brown fat cell function has been established [34]. Obese individuals seem to have less brown fat than nonobese individuals. Better understanding of the brown adipose tissue's activity may increase the potential for enhancing energy expenditure by increasing its quantity or activity through new drugs.

5.10 ETHYL ALCOHOL: METABOLISM AND BIOCHEMICAL IMPACT

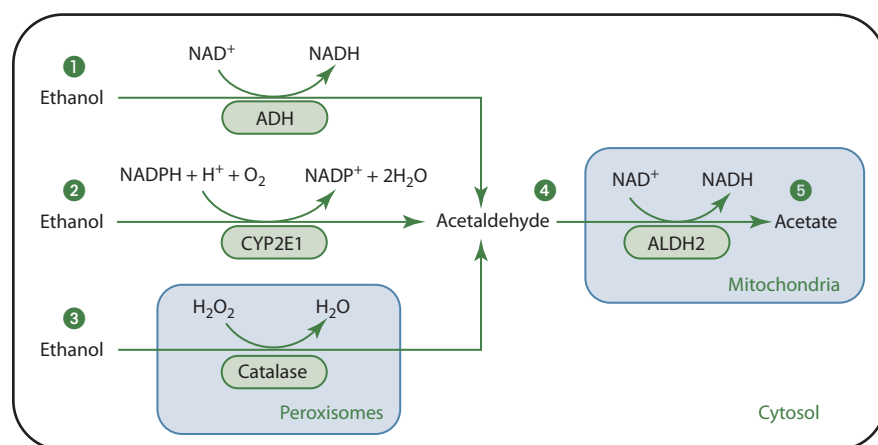
Ethyl alcohol (ethanol) is neither a carbohydrate nor a lipid. Though empirically ethanol's structure ($\text{CH}_3-\text{CH}_2-\text{OH}$) most closely resembles a carbohydrate, its metabolism

most closely resembles fatty acid catabolism. We have chosen to review it in this chapter for several reasons. First, it is a common dietary component, being consumed in the form of alcoholic beverages such as beer, wine, and distilled spirits. Second, the pathways that oxidize ethyl alcohol also oxidize (or detoxify) other exogenous substances in the body. Although ethanol has been part of the human diet for centuries, it provides no metabolic benefits (other than energy) and is considered “empty calories.” Furthermore, prolonged consumption of excessive amounts can lead to health problems, most notably liver damage. Each gram of ethanol yields 7 kcal, and ethanol may account for up to 10% of the total energy intake of moderate consumers and up to 50% for alcoholics. Because of its widespread consumption and relatively high caloric potency, it commands attention in a nutrition textbook.

Ethanol is readily absorbed into the blood through the entire GI tract. It is transported unaltered in the circulation and then oxidatively degraded in tissues, primarily the liver. Ethanol is first oxidized to acetaldehyde and then to acetate, which can enter the circulation. In most tissues, the acetate subsequently is converted to acetyl-CoA and oxidized via the TCA cycle. As depicted in Figure 5.39, at least three enzyme systems are capable of ethanol oxidation:

- The alcohol dehydrogenase (ADH) system
- The microsomal ethanol oxidizing system (MEOS)
- The catalase system.

Of these, the catalase system is the least active, probably accounting for <2% of *in vivo* ethanol oxidation. While most ingested ethanol is oxidized by the hepatic and, to some extent, the gastric MEOS and ADH system, it can also be metabolized in nonhepatic tissues that do



- 1 The majority of ethanol is oxidized by ADH in the cytosol. Reduced NADH, a byproduct of the reaction, accumulates when excessive ethanol is consumed.
- 2 The microsomal ethanol oxidizing system (MEOS) accounts for up to 20% of ethanol oxidation. Located in the ER, the CYP2E1 enzyme is a cytochrome P450 mixed-function oxidase. NADPH is concurrently oxidized in the reaction.
- 3 The catalase system in peroxisomes plays a minor role in total ethanol oxidation. The reaction requires H₂O₂.
- 4 The initial product of ethanol oxidation, acetaldehyde, is transported to mitochondria and further oxidized to acetate by ALDH2. Reduced NADH accumulates in mitochondria when excessive ethanol is consumed.
- 5 The final product of ethanol oxidation, acetate, will be metabolized to acetyl-CoA and used for energy via the TCA cycle or for fatty acid synthesis.

Figure 5.39 Ethanol oxidation in hepatocytes.

not contain ADH, such as the brain, by the MEOS and catalase system.

The Alcohol Dehydrogenase Pathway

Alcohol dehydrogenase is a soluble enzyme functioning in the cytosol of hepatocytes. It is an ordinary NAD^+ -requiring dehydrogenase and can oxidize ethanol to acetaldehyde. The acetaldehyde is then transported to the mitochondria and further oxidized to acetate in a reaction catalyzed by acetaldehyde dehydrogenase (ALDH2). The NADH formed by ADH can be oxidized by mitochondrial electron transport by way of the NADH shuttle systems (see Chapter 3), thereby giving rise to ATP formation by oxidative phosphorylation. The K_m of ADH for ethanol is approximately 1 millimolar, or about 5 mg/dL. (K_m is reviewed in Chapter 1, in the section dealing with enzymes.) This means that at this cellular concentration of ethanol, ADH is functioning at half its maximum velocity. At concentrations three or four times the K_m , the enzyme is saturated with the ethanol substrate and is catalyzing at its maximum rate. Concentrations of ethanol in the cell more than four times the K_m level cannot be completely oxidized by ADH.

Because ethanol is an exogenous dietary ingredient, there is no “normal” concentration of ethanol in the cells or the bloodstream. The so-called toxic level of blood ethanol, however, is considered to be in the range of 50–80 mg/dL and is defined by its pharmacological actions. The high lipid solubility of ethanol allows it to passively enter cells with ease. If its cellular concentration reaches a level even one-third or one-fourth of that of the blood at toxic levels, ADH becomes saturated by the substrate and will be functioning at its maximum velocity. The excess ethanol then must be metabolized by alternate systems, the most important being the MEOS. The depletion of NAD^+ brought about by the high level of activity of ADH can also force the shift to the MEOS, which does not require NAD^+ for its oxidative reactions. The depletion of NAD^+ increases the NADH:NAD^+ ratio and impairs NAD^+ -requiring reactions such as the TCA cycle, gluconeogenesis, and fatty acid oxidation. The buildup of acetyl-CoA encourages fatty acid synthesis and TAG accumulation in the liver, as discussed later in the “High NADH:NAD^+ Ratio” section.

ADH is also active in gastric mucosal cells. Interestingly, there appears to be a significant gender difference in the level of its activity in these cells. Young (premenopausal) females develop higher blood alcohol levels than male counterparts with equal consumption and consequently display a lower tolerance for alcohol and are at greater risk of toxic effects in the liver. Several physiological differences between males and females are thought to be contributing factors, including lower levels of ADH in females [35].

The Microsomal Ethanol Oxidizing System

The MEOS is able to oxidize a wide variety of compounds in addition to ethanol, including fatty acids, **aromatic** hydrocarbons, steroids, and barbiturate drugs. The oxidation is mediated by a cytochrome P450 enzyme, CYP2E1, and involves a system of electron transport, similar to the mitochondrial electron transport system described in detail in Chapter 3. Because the MEOS is microsomal (isolated ER) and associated with the smooth endoplasmic reticulum, it is sometimes referred to as the microsomal electron transport system. Another distinction of the system is its requirement for a special cytochrome called cytochrome P450, which acts as an intermediate electron carrier. Cytochrome P450 is not a single compound but rather exists as a family of structurally related cytochromes, the members of which share the property of absorbing light that has a wavelength of 450 nm.

Ethanol oxidation by the MEOS involves several linked reduction-oxidation reactions that result in the simultaneous oxidation of NADPH and the reduction of molecular oxygen to H_2O (Figure 5.39). Because two substrates (ethanol and NADPH) are oxidized concurrently, the enzymes involved are commonly called mixed-function oxidases. Both oxygen atoms are reduced to H_2O , and therefore two H_2O molecules are formed in the reactions. Acting as intermediate carriers of electrons from NADPH to oxygen are FAD, FMN, and a cytochrome P450 system (not shown in the figure).

An important feature of the MEOS is that certain of its enzymes, including the cytochrome P450 units, are inducible by ethanol—particularly at higher concentrations of ethanol. With increased synthesis of these enzymes, the hepatocytes can metabolize ethanol much more effectively, thereby establishing a state of metabolic tolerance. Compared with a normal (nondrinking or light-drinking) subject, an individual in a state of metabolic tolerance to ethanol can ingest larger quantities of the substance before showing the effects of intoxication. When enzyme induction occurs, however, it can also accelerate the metabolism of other substances metabolized by the microsomal system. In other words, tolerance to ethanol induced by heavy drinking can render a person tolerant to other substances as well.

The Catalase System

The enzyme catalase is located in peroxisomes and, in the presence of hydrogen peroxide (H_2O_2), is capable of oxidizing ethanol to acetaldehyde. In view of the high capacity of hepatic ADH to oxidize ethanol, the catalase system is considered a minor pathway except in tissues, such as the brain, which lack ADH. Chronic alcohol consumption

may increase H_2O_2 in the liver and thus increase catalase activity.

Alcoholism: Biochemical and Metabolic Alterations

Excessive consumption of ethanol can lead to alcoholism, defined by the National Council on Alcoholism as consumption that is capable of producing pathological changes. Alcoholism is a serious socioeconomic and health problem, as excessive ethanol consumption is a leading preventable cause of death. In the U.S., the majority of ethanol-attributable deaths are caused by chronic metabolic conditions, whereas less than 8% are due to traffic accidents [36]. The well-known consequences of alcoholism—fatty liver, hepatic disease (cirrhosis), lactic acidemia, and metabolic tolerance—can be explained by the manner in which ethanol is metabolized. The consequences of excessive alcohol intake are explainable by the metabolic effects of (1) acetaldehyde toxicity, (2) high NADH:NAD^+ ratio, (3) substrate competition, and (4) induced metabolic tolerance.

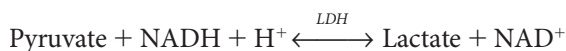
Acetaldehyde Toxicity

All pathways of ethanol oxidation produce acetaldehyde, which is believed to exert direct adverse effects on metabolic systems. For example, acetaldehyde is able to attach covalently to proteins, forming protein adducts. Should the adduct involve an enzyme, the activity of that enzyme could be impaired. Acetaldehyde has been shown to impede the formation of microtubules in liver cells and to cause the development of perivenular fibrosis, either of which is believed to initiate the events leading to cirrhosis. Acetaldehyde may also contribute to cancer development [37].

High NADH:NAD^+ Ratio

The oxidation of ethanol increases the concentration of NADH at the expense of NAD^+ , thereby elevating the NADH:NAD^+ ratio. This occurs because both ADH in the cytosol and ALDH2 in the mitochondria use NAD^+ as a cosubstrate. NADH is an important regulator of certain dehydrogenase reactions. The rise in concentration of NADH represents an overproduction of reducing equivalents, which in turn acts as a signal for a metabolic shift toward reduction—namely, hydrogenation. Such a shift can account for the fatty liver (through the anabolic activity producing fatty acids) and lactic acidemia (high blood-lactate levels resulting from increased reduction of pyruvate to lactic acid) that often accompany alcoholism. For example, lactic acidemia can be attributed in part to the direct effect of NADH in shifting the lactate dehydrogenase (LDH) reaction toward the formation of lactate.

The reaction, which follows, is driven to the right by the high concentration of NADH :



Lipids accumulate in most tissues in which ethanol is metabolized, resulting in fatty liver, fatty myocardium, fatty renal tubules, and so on. The mechanism appears to involve both increased lipid synthesis and decreased lipid removal and can be explained in part by the increased NADH:NAD^+ ratio. As NADH accumulates, it slows dehydrogenase reactions of the TCA cycle, such as the isocitrate dehydrogenase and α -ketoglutarate dehydrogenase reactions, thereby slowing the overall activity of the cycle. This results in an accumulation of citrate, which positively regulates acetyl-CoA carboxylase. Acetyl-CoA carboxylase, which converts acetyl-CoA into malonyl-CoA by the attachment of a carboxyl group, is the key regulatory enzyme for the synthesis of fatty acids from acetyl-CoA. The high NADH:NAD^+ ratio therefore directs metabolism away from TCA cycle oxidation and toward fatty acid synthesis.

Also contributing to the lipogenic effect of alcoholism is the effect of NADH on the glycerophosphate dehydrogenase (GPDH) reaction. This reaction, shown in Figure 5.40, favors the reduction of dihydroxyacetone phosphate to glycerol-3-phosphate if NADH concentration is high. Glycerol-3-phosphate provides the glycerol component in the synthesis of triacylglycerols. Therefore, a high NADH:NAD^+ ratio stimulates the synthesis of both the fatty acids and the glycerol components of triacylglycerols, contributing to the cellular fat accumulation that develops in alcoholism.

A rise in NADH concentration also affects the glutamate dehydrogenase (GluDH) reaction (Figure 5.41), resulting in impaired gluconeogenesis. The GluDH reaction is extremely important in gluconeogenesis because of the role it plays in the conversion of amino acids to their carbon skeletons by transamination and in the release of their amino groups as NH_3 . A shift in the reaction toward glutamate because of the elevated NADH depletes the availability of α -ketoglutarate, which is the major acceptor of amino groups in the transamination of amino acids.

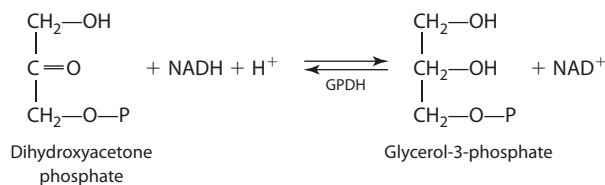


Figure 5.40 Reduction of dihydroxyacetone phosphate to glycerol-3-phosphate. GPDH , glycerophosphate dehydrogenase.

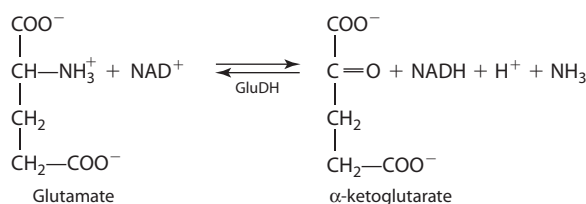


Figure 5.41 Reversible reaction of glutamate to α -ketoglutarate. GluDH, glutamate dehydrogenase.

Source: Based on data from Heird W, Lapillonne A. The role of essential fatty acids in development. *Annu Rev Nutr.* 2005; 25:549–71.

Substrate Competition

A well-established nutritional problem associated with excessive alcohol metabolism is a deficiency of vitamin A. Two aspects of ethanol interference with normal metabolism probably can account for this problem. One is the effect of ethanol on retinol dehydrogenase, the cytosolic enzyme that converts retinol to retinal. Retinal is required for the synthesis of photo pigments used in vision. Retinol dehydrogenase is thought to be identical to ADH, and therefore ethanol competitively inhibits the hepatic conversion of retinol to retinal. In addition to this substrate competition effect, ethanol may interfere with retinol metabolism through induced metabolic tolerance.

Induced Metabolic Tolerance

As explained earlier, ethanol can induce enzymes of the MEOS, causing an increased rate of metabolism of substrates oxidized by this system. Retinol, like ethanol, spills over into the MEOS when ADH is saturated and NAD^+ stores are low because of heavy ingestion of ethanol. Ethanol induction of retinol-metabolizing enzymes, most notably CYP2E1, can then occur. Although induction accelerates the hepatic oxidation of retinol, the oxidation product is not retinal but other polar, inert products of oxidation. The hepatic depletion of retinol can therefore be attributed to its accelerated metabolism, which is secondary to ethanol induction of a metabolizing enzyme. In effect, the alcoholic subject becomes tolerant to vitamin A, necessitating a higher dietary intake of the vitamin to maintain normal hepatocyte concentrations.

Alcohol in Moderation: The Brighter Side

Alcohol is a nutritional “Jekyll and Hyde,” and which face it flaunts is clearly a function of the extent to which it is consumed. We focused earlier on the effects of alcohol at high intake levels and the negative impact of alcoholism on metabolism and nutrition. Epidemiological data from worldwide studies consistently demonstrate there is an inverse association between moderate alcohol or wine intake and cardiovascular disease risk. Positive effects of alcohol or wine intake on oxidative stress, insulin insensitivity, diabetes mellitus, and autoimmune diseases have been shown [37].

Despite the association between moderate ethanol consumption and lower risk of cardiovascular disease, the metabolic explanation is not well understood. Recent studies suggest that ethanol's benefit may be mediated through G protein–coupled receptor 43 (GPCR43), which is highly expressed in adipose tissue. GPCR43 binds acetate and, as a result, suppresses insulin signaling in adipocytes, thus decreasing triacylglycerol accumulation. Ethanol is metabolized to acetate. Consequently, increased acetate that occurs with ethanol consumption could activate GPCR43 and thus regulate fat accumulation, serum lipoprotein profiles, and cardiovascular disease [38].

Among the three classes of alcoholic beverages—wine, beer, and distilled spirits—the strongest correlations are with the amount of ethanol consumed and not the individual types of beverages. The common belief is that distilled spirits and beer have about equal benefits and wine has somewhat more, but scientific data that substantiates that belief has not been reported. Wine, particularly red wine, contains many substances with antioxidant properties. Most of the antioxidant chemicals in grapes are found in the skin and seeds. The skin is included in the production of red wine. The polyphenols and a variety of other antioxidants that can reduce reactive oxygen species (ROS) are present. ROS contribute to the oxidative stress that causes inflammation and contributes to cardiovascular disease [39].

SUMMARY

The hydrophobic character of lipids makes them unique among the major nutrients, requiring special handling in the body's aqueous milieu.

- Ingested fat must be finely dispersed in the intestinal lumen to present a sufficiently large surface area for enzymatic digestion to occur.
- In the bloodstream, reassembled lipids must be associated with proteins to form lipoproteins, thus

ensuring their solubility for transport in the aqueous environment.

- The major sites for the formation of lipoproteins are the intestine, which produces them from diet-derived lipids, and the liver, which forms lipoproteins from endogenous lipids.
- Central to the processes of lipid transport and storage is adipose tissue, which accumulates fat as triacylglycerols

when the intake of energy-producing nutrients is greater than the body's caloric needs. When energy demands so dictate, fatty acids are released from storage and transported to other tissues for oxidation. The mobilization follows the adipocyte's response to specific hormonal signals that stimulate the activity of the intracellular lipases.

Fatty acids are a rich source of energy. Their mitochondrial oxidation furnishes large amounts of acetyl-CoA for TCA cycle catabolism.

- In situations of low carbohydrate intake or utilization, as occurs in starvation or diabetes, the rate of fatty acid oxidation increases significantly with concomitant acetyl-CoA accumulation.
- Excess acetyl-CoA causes an increase in the level of ketone bodies, organic acids that can be deleterious through their disturbance of acid–base balance but that also are beneficial as sources of fuel to tissues such as the muscle and brain in periods of starvation.

Although the lipids are thought of first and foremost as energy sources, they have some regulatory functions, including blood pressure alteration, platelet aggregation, enhancement of immunological surveillance, and cell signaling.

- These potent bioactive substances include the eicosanoids (prostaglandins, thromboxanes, and leukotrienes), all of which are derived from the fatty acids arachidonic acid (n-6), eicosatetraenoic acid (n-3), and docosahexaenoic acid (n-3).

- Steroid hormones include corticosteroid hormones (e.g., cortisol) and sex hormones (e.g., estradiol and testosterone).

Dietary lipids have been implicated in atherogenesis, the process leading to development of degenerative cardiovascular disease. Major considerations in preventing and controlling atherosclerosis have been the concentration of cholesterol in the blood and the relative hypo- or hypercholesterolemic effect of certain diets.

- Saturated and *trans* fatty acids are generally believed to be hypercholesterolemic.
- Polyunsaturated *cis* fatty acids tend to lower serum cholesterol.
- Monounsaturated *cis* fatty acids, representing the most abundant fatty acids in the food supply, have neutral effects on serum cholesterol.

Fatty acids can be synthesized by cytosolic enzyme systems when energy provided by carbohydrate is adequate.

- Synthesis begins with simple precursors such as acetyl-CoA and can be triggered by hormonal signals or by elevated levels of citrate, which acts as a regulatory substance.
- Blood glucose concentration also acts as a sensitive regulator of lipogenesis, which is stimulated when a hyperglycemic state exists.

Ethanol is catabolized ultimately to acetyl-CoA, furnishing energy through its TCA cycle oxidation. The nutritional complexities of alcohol abuse were discussed.

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Perspective

THE ROLE OF LIPOPROTEINS AND INFLAMMATION IN ATHEROSCLEROSIS

Atherosclerosis is a disease of the arteries in which lipid-filled plaques develop within the artery wall, causing the arterial lumen to narrow (stenosis), thereby restricting blood flow and the supply of oxygen (ischemia). Atherosclerotic plaque is the root cause of coronary heart disease (also called ischemic heart disease), ischemic stroke, and peripheral artery disease. These conditions belong to the larger category of atherosclerotic cardiovascular diseases (ASCVD), along with all other non-atherosclerotic diseases of the cardiovascular system. Atherosclerotic diseases account for nearly 20% of all deaths in the United States, second only to cancer [1].

Atherosclerosis is a chronic inflammatory disease as well as a disorder of lipid metabolism [2]. Chapter 5 covered the role played by disorders in lipid and lipoprotein metabolism in the development of ASCVD. There are still many aspects of atherogenesis we do not fully understand, but considerable insight has been gained beyond the classical view of a couple of decades ago. The classical view postulated an ever-increasing plaque with a concomitant decrease in size of the arterial lumen, much like the accumulation of rust in a water pipe, caused by alterations in lipid metabolism. It is now recognized that plaque development is caused by the combination of inflammation and dyslipidemia. Under normal circumstances, the components of blood—red cells, white cells, and platelets—flow past the inner lining without adhering to it. Fatty streaks are prevalent but asymptomatic in young people; they may at some point cause symptoms or just disappear. So what happens to start the process that causes a plaque to form, and what triggers a plaque to produce a blood clot (thrombosis)? Just how is inflammation involved? This Perspective explores the current understanding of atherosclerosis.

Inflammation is just one part of the body's innate immune response to tissue

damage or a foreign object. Inflammation is a nonspecific response to injury in which phagocytic cells, neutrophils, and macrophages play an important role. The inflammation response is similar regardless of the cause of the damage. Typically, when there is an injury, the local macrophages release cytokines, which are chemicals released by leukocytes that function as mediators for the inflammation response [3].

Larger arteries are considered to be elastic—they contain the protein elastin—and do not restrict flow to any great extent. The medium- to small-size arteries, often called arterioles, make up most of the arterial bed that restricts blood flow and increases blood pressure. Of these, smaller coronary arteries of the heart are a special example. Unlike peripheral arteries, coronary arteries fill when the heart is in diastole (relaxed) and empty during systole (contracted). If a coronary artery contains an atherosclerotic plaque and develops a clot, a myocardial infarct occurs and can cause death of the cardiac muscle [4]. These smaller arteries do not contain the elastin protein but do contain more smooth muscle. Their walls are made up of three layers: the inner layer or tunica intima, the middle layer or tunica media, and the outer layer or adventia. This outer layer contains connective tissue, nerve endings, mast cells, fibroblasts, and micro-vessels. The innermost layer, the intima, includes a monolayer of endothelial cells that are in contact with the blood plus a small layer of smooth muscle cells under the endothelial cells. The middle layer also contains smooth muscle cells along with a variety of other cell types.

When these arteries are subjected to any of several negative stimuli associated with risk factors for ASCVD such as hypertension, dyslipidemia, or proinflammatory mediators, the endothelial cells produce proteins on their outer matrix that act as an adhering factor. The platelets, the first blood component to interact with the

endothelial cells, further change the cell surface, allowing leukocytes (white blood cells) to begin to attach to the endothelial cells. The first cells to attach are the macrophages, which release cytokines, which in turn call in additional leukocytes. The most common type of leukocyte, and the most numerous among the leukocytes adhering to the endothelial cells, is the monocyte. The monocytes are directed into the intima by proinflammatory effectors such as cytokines or tumor necrosis factor (TNF). The monocytes differentiate into macrophages and take up the cholesterol-rich LDL particles by endocytosis. When they have engulfed sufficient lipid they are called foam cells because of their microscopic appearance. The cytokines and TNF attract additional monocytes into the intima and media. Other leukocytes such as T-cells and mast cells also accumulate in the media. As the **atheroma** progress, additional smooth muscle cells from the media are attracted into the intima and then proliferate in response to platelet-derived growth factors. The smooth muscle cells produce the extracellular proteins collagen and elastin, forming a fibrous cap that covers the plaque [4].

The plaque can enlarge and thus narrow the lumen of the artery so that it begins to impede the blood flow; it may or may not progress to clot formation. The balance between inflammatory and anti-inflammatory activity controls the progression of the atherosclerosis [2]. The thrombus (clot) is most likely to form when the fibrous cap is physically disrupted, which can be caused by several mechanisms [4]. The immune cells that have been attracted to the plaque, including activated macrophages, T-cells, and mast cells, produce a variety of molecules that can destabilize the plaque. These include inflammatory cytokines, activated oxygen species, proteases, coagulation factors, and vasoactive molecules [2]. Some of the smooth muscle cells and macrophages die, leaving lipid droplets and crystals of

cholesterol that enhance the plaque's lipid core. The polyunsaturated fatty acids and cholesterol can be oxidized by the reactive oxygen species, producing epoxy fatty acids or oxysterols. The macrophages also produce procoagulant factors that make the lipid core more thrombogenic.

Known risk factors for ASCVD can initiate the pathogenic pathway. For instance, hypertension can increase arterial wall tension, leading to a disturbed repair process and aneurysm formation. Also, angiotensin is a major pressor hormone that alters the endothelial membrane and can be the first factor to initiate the leukocyte adherence. Cigarette smoking and diabetes likewise alter endothelial function, which increases the risk for plaque development, but the mechanism is unknown. Once the process begins with leukocyte adherence the cascade of events described previously follows, and plaque development is the result. However, if LDL levels are reduced, the plaque formation is retarded.

Recent research demonstrates that inflammation plays a key role in atherogenesis within small- to mid-size arteries,

including coronary arteries. Immune cells and proinflammatory effectors are prominent in the early formation of the atherosclerotic plaque. The plaque would not be such a problem if it were not for its tendency to rupture and the subsequent clot formation. The known ASCVD risk factors also alter the expression of certain proinflammatory genes. Certain areas in the genome are associated with myocardial infarction [2].

HDL plays an important role in modifying plaque formation and development. HDL particles are involved in reverse cholesterol transport, as described in the previous chapter. HDL particles also have anti-inflammatory actions. The cholesterol ester transfer protein (CETP) facilitates the exchange of cholesterol esters in HDL for triacylglycerol in the LDL particles, which removes some of the oxidized lipid. The anti-inflammatory properties of HDL reverse some of the inflammation that drives the deposition of new lipid.

In summary, atherosclerosis was once thought to be due primarily to dyslipidemia, but it is now known that inflammation

plays a major role in its development. With this increased understanding of the role of inflammation in atherosclerosis, the prevention and treatment of ASCVD can go beyond managing dyslipidemia to focus on reducing inflammation as well.

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PROTEIN

LEARNING OBJECTIVES

- 6.1 Describe how amino acids are classified.
- 6.2 Explain how dietary proteins are digested and absorbed.
- 6.3 Describe factors influencing protein synthesis.
- 6.4 Describe the functional roles of proteins and nitrogen-containing nonprotein compounds in the body.
- 6.5 Describe the role of the urea cycle and the cycle's common intermediates with the TCA cycle.
- 6.6 Identify common intermediates generated during the catabolism of amino acids.
- 6.7 Describe the roles and contributions of the intestine, liver, muscle, and kidneys in amino acid metabolism.
- 6.8 Explain the contributions that glutamine and alanine play in the liver and muscle.
- 6.9 Describe the systems responsible for protein degradation.
- 6.10 Describe the changes that occur in lean body mass with aging.
- 6.11 Describe the differences between animal and plant sources of protein.
- 6.12 Identify the methods used to determine protein recommendations as well as other methods used to assess amino acid and protein needs and protein quality.
- 6.13 Describe the manifestations of protein deficiency.

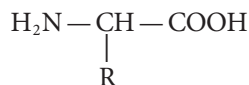
THE IMPORTANCE OF PROTEIN IN NUTRITION AND HEALTH CANNOT BE OVEREMPHASIZED. It is quite appropriate that the Greek word chosen as a name for this nutrient is *proteos*, meaning “primary” or “taking first place.” Proteins are found throughout the body, with over 40% of body protein found in skeletal muscle, over 25% found in body organs, and the rest found mostly in the skin and blood. Proteins are essential nutritionally because of their constituent amino acids, which the body must have to synthesize its own variety of proteins and nitrogen-containing molecules that make life possible. Each body protein is unique in the characteristics and sequence pattern of the amino acids that comprise its structure. This chapter focuses first on classifications of amino acids. Next, sources and digestion of protein to provide amino acids to the body are reviewed, along with how the amino acids are subsequently absorbed and metabolized in cells. The body's use of amino acids to make proteins and nitrogen-containing nonprotein compounds as well as the functional roles of these proteins and compounds are also presented. Lastly, changes in the body's protein (lean) mass with aging are covered, as are recommended intakes of protein, protein quality, assessment of protein and amino acid needs, and protein deficiency.

6.1 AMINO ACID CLASSIFICATION

Amino acids may be classified in a variety of ways, including by structure, net charge, polarity, and essentiality. This section addresses each of these four classifications.

Structure

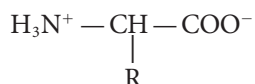
Structurally, amino acids have a central carbon (C), at least one amino group (—NH_2), at least one carboxy (acid) group (—COOH), and a side chain (R group) that makes each amino acid unique. The generic amino acid may be represented as follows:



However, depending on the pH of the environment, the amino and carboxy groups can accept or donate H^+ , and thus the amino group may be represented as $^+\text{NH}_3$ and the carboxy group as COO^- . The distinctive characteristics of the side chains of the amino acids that make up a polypeptide bestow on a protein its structure and influence its functional role in the body. These same distinctive characteristics determine whether certain amino acids can be synthesized in the body or must be ingested. Furthermore, these characteristics program the various amino acids for their specific metabolic pathways in the body. The differences among the side chains of the amino acids commonly found in body proteins are shown in Table 6.1. This division of amino acids based on structural similarities is one approach used to classify amino acids; dividing amino acids based on the presence or absence of a net charge is another.

Net Electrical Charge

Table 6.1 shows the structures of amino acids as they exist in an aqueous solution at the physiological pH, approximately 6–8, of the human body. Amino acids in an aqueous solution are ionized. The term **zwitterion**, or dipolar ion, is applied to amino acids with no carboxy or amino groups in their side chain to generate an additional charge to the molecule. Zwitterions have no net electrical charge because their side chains are not charged, and the one positive and one negative charge from the amino and carboxy groups, respectively, in their base structure cancel each other out. Amino acids with no net charge, also called neutral amino acids (and listed in Table 6.2A), do not migrate substantially if placed in an electric field.



Two groups of amino acids (Table 6.2B) exhibit a net charge. Because of the presence of an additional carboxy group in the side chain, the dicarboxylic (also called acidic) amino acids aspartic acid and glutamic acid exhibit a net negative charge at pH 7; these forms of the amino acids are called aspartate and glutamate. Dicarboxylic amino acids

or proteins with a high content of dicarboxylic amino acids migrate toward the anode if placed in an electric field. In contrast, because of the presence of an additional amino group in the side chain, the basic (also called dibasic) amino acids (lysine, arginine, histidine) exhibit a net positive charge at pH 7. These amino acids or proteins rich in them will migrate toward the cathode if placed in an electrical field.

Polarity

The tendency of an amino acid to interact with water at physiological pH—that is, its polarity—represents another means of classifying amino acids. Polarity depends on the side chain or R group of the amino acid. Amino acids are classified as polar or nonpolar, although they can have varying levels of polarity. Polar-charged amino acids include both the dicarboxylic (aspartic acid and glutamic acid) and basic (lysine, arginine, histidine) amino acids, as shown in the second column in Table 6.2C. Polar-charged amino acids interact with aqueous environments, can form salt bridges, and can interact with electrolytes/minerals such as potassium, chloride, and phosphate.

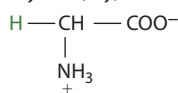
The neutral amino acids interact with water to different degrees and can be divided into polar, nonpolar, and relatively nonpolar categories, as listed in the first, third, and last columns in Table 6.2C. The side chains of polar neutral amino acids (first column in Table 6.2C) contain functional groups—such as the hydroxyl group for serine and threonine, the sulfur atom for cysteine, and the amide group for asparagine and glutamine—that can interact through hydrogen bonds with water (the aqueous environment of cells). Polar amino acids are generally found on the surfaces of proteins. If not found on the surface, they are oriented inward and function at a protein's (such as an enzyme's) binding site.

In contrast, the amino acids listed in the third column in Table 6.2C contain side chains that do not interact with water and are categorized as nonpolar or hydrophobic (water fearing). Hydrophobicity tends to increase as the length of the side chain increases (i.e., with more carbons found in the side chain). The term *aliphatic* is often used when discussing nonpolar amino acids; amino acids that are aliphatic contain carbons and hydrogens in their side chains but no functional groups like hydroxyl groups.

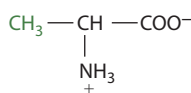
As the last column in Table 6.2C reveals, the aromatic amino acids are considered relatively nonpolar. Tyrosine, for example, because of its hydroxyl group on the phenyl ring, can to a limited extent form hydrogen bonds with water—hence the term *relatively nonpolar*. Because they do not interact with water, the nonpolar (and often the relatively nonpolar) amino acids are typically found tightly coiled in proteins or compacted (e.g., attracted by van der Waal forces) and oriented toward or within the central region or core portion of proteins.

Table 6.1 Structural Classification of Amino Acids1. *With aliphatic/nonpolar side chains*

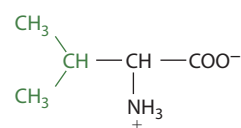
Glycine (Gly)



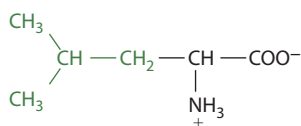
Alanine (Ala)



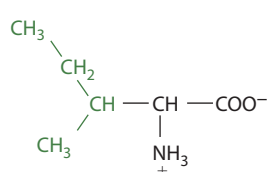
Valine (Val)



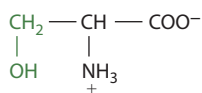
Leucine (Leu)



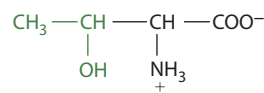
Isoleucine (Ile)

2. *With side chains containing hydroxylic (OH) groups**

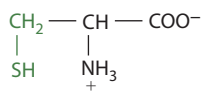
Serine (Ser)



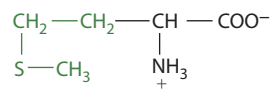
Threonine (Thr)

3. *With side chains containing sulfur atoms*

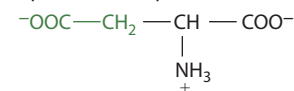
Cysteine (Cys)



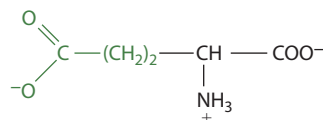
Methionine (Met)

4. *With side chains containing acidic groups or their amides*

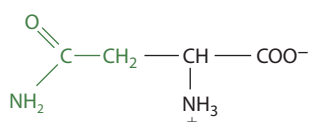
Aspartic acid (Asp)



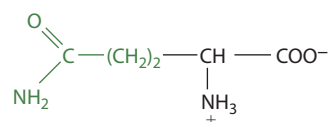
Glutamic acid (Glu)



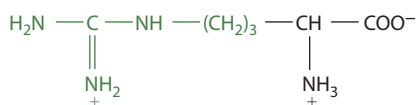
Asparagine (Asn)



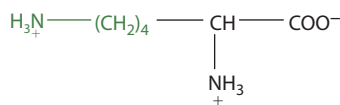
Glutamine (Gln)

5. *With side chains containing basic groups*

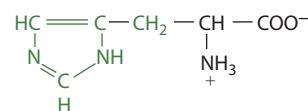
Arginine (Arg)



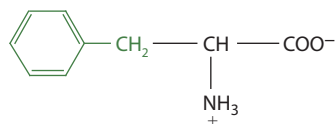
Lysine (Lys)



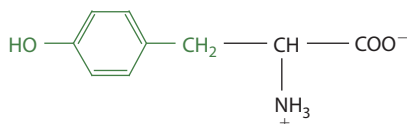
Histidine (His)

6. *With side chains containing aromatic ring*

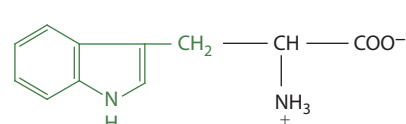
Phenylalanine (Phe)



Tyrosine (Tyr)



Tryptophan (Trp)

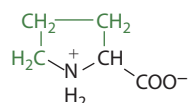


*Although tyrosine contains a hydroxyl group, it is classified as an amino acid containing an aromatic ring (see Group 6).

Table 6.1 Structural Classification of Amino Acids (*Continued*)

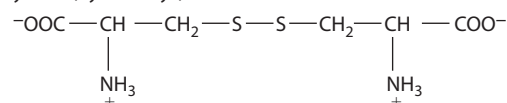
7. Imino acids

Proline (Pro)

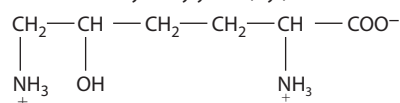


8. Amino acids formed posttranslationally

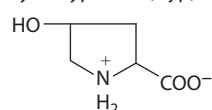
Cystine (Cys-S-S-Cys)



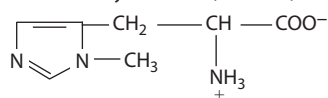
Hydroxylysine (Hyl)



Hydroxyproline (Hyp)



3-methylhistidine (3-meHis)



Essentiality

While amino acids can be classified based on structure or properties such as net charge and polarity, in 1957 Rose [1] categorized the amino acids found in proteins as nutritionally essential (indispensable) or nutritionally nonessential (dispensable). In the context of nutrition, the term *essential*

or *indispensable* means that the body cannot make the nutrient (in this case the amino acid) and that it must be supplied by the diet. Back in the 1950s, only eight amino acids—leucine, isoleucine, valine, lysine, tryptophan, threonine, methionine, and phenylalanine—were considered essential for adult humans. Histidine was later added as an essential amino acid. Table 6.2D lists the essential amino acids.

Table 6.2A Neutral Amino Acids (one-letter code)

Alanine (A)	Glycine (G)	Phenylalanine (F)	Tryptophan (W)
Asparagine (N)	Isoleucine (I)	Proline (P)	Tyrosine (Y)
Cysteine (C)	Leucine (L)	Serine (S)	Valine (V)
Glutamine (Q)	Methionine (M)	Threonine (T)	

Table 6.2B Amino Acids (one-letter code) Exhibiting a Net Charge

Negatively Charged Amino Acids	Positively Charged Amino Acids
Aspartic acid (D)	Arginine (R)
Glutamic acid (E)	Histidine (H)
	Lysine (K)

Table 6.2C Polar and Nonpolar Amino Acids

Polar Neutral Amino Acids	Polar Charged Amino Acids	Nonpolar Neutral Amino Acids	Relatively Nonpolar Amino Acids
Asparagine	Arginine	Alanine	Phenylalanine
Cysteine	Lysine	Glycine	Tryptophan
Glutamine	Histidine	Isoleucine	Tyrosine
Serine	Glutamate	Leucine	
Threonine	Aspartate	Methionine	
		Proline	
		Valine	

Table 6.2D Essential/Indispensable Amino Acids

Phenylalanine	Methionine	Isoleucine
Valine	Tryptophan	Leucine
Threonine	Histidine	Lysine

Table 6.2E Conditionally Indispensable Amino Acids and Their Precursors

Amino Acid	Precursor(s)
Tyrosine	Phenylalanine
Cysteine	Methionine, serine
Proline	Glutamate
Arginine	Glutamine or glutamate, aspartate
Glutamine	Glutamate, ammonia

Identifying amino acids strictly as nonessential/dispensable or essential/indispensable is an inflexible classification, however, that allows no gradations, even in decidedly different or changing physiological circumstances. Therefore, a third category exists: conditionally or acquired indispensable amino acids. A dispensable amino acid may become indispensable if, for example, there are rate limitations to its synthesis such as may occur if precursor availability is limited. Similarly, if an organ does not function properly then amino acid metabolism may not proceed normally. For example, neonates born prematurely often have immature organ function. Immature liver function or liver disease (cirrhosis) impairs both phenylalanine and methionine metabolism, which occurs primarily in the liver. Consequently, the amino acids tyrosine and cysteine, normally synthesized via phenylalanine and methionine catabolism, respectively, become indispensable until normal organ function is established. Arginine represents another example of a conditionally essential amino acid. While it can be synthesized *de novo* in the body, its production is not sufficient to meet the body's needs during early development nor during periods of infection or inflammation. Moreover, arginine can become essential if changes in intestine or renal functions disrupt synthesis of the amino acid.

Inborn errors of amino acid metabolism, which result from inherited mutations in genes coding for enzymes needed for amino acid use, represent another situation in which dispensable amino acids may become indispensable. Phenylalanine hydroxylase, the enzyme that converts phenylalanine to tyrosine, exhibits little to no activity in people with the inborn error of metabolism known as classic phenylketonuria (PKU). Without adequate hydroxylase activity, tyrosine is not synthesized in the body and must be provided completely by the diet; in other words, tyrosine is indispensable for those with PKU. Medical conditions such as trauma also affect amino acid needs. For example, with trauma, endogenous arginine synthesis is typically not sufficient, and thus this amino acid is considered conditionally essential in conditions of trauma. Some examples of conditionally indispensable amino acids are listed in Table 6.2E, along with their usual amino acid precursors.

6.2 SOURCES OF AMINO ACIDS

Amino acids are derived from protein. Both dietary (exogenous) and endogenous proteins provide the body with amino acids. Dietary sources of protein include:

- Animal products such as meat, poultry, fish, eggs, and dairy (with the exception of butter, sour cream, and cream cheese)
- Plant products such as grains, grain products, legumes (including lentils, beans, and peas), nuts, seeds, and vegetables.

Adult men and women (age 20–70 years) in the United States ingest about 98 g and 68 g of protein per day, respectively, and adults over 70 years ingest about 66 g per day [2]. Protein intake is typically greatest (sometimes over 60%) at the evening meal and smallest at the morning meal [2].

Following ingestion, exogenous proteins serve as sources of the essential amino acids, nonessential amino acids, and additional nitrogen needed to synthesize more nonessential amino acids, nitrogen-containing compounds, and protein in the body. The differences between animal and plant proteins are discussed in the section of this chapter on protein quality and protein synthesis.

Endogenous proteins presented to the digestive tract represent another source of amino acids and nitrogen. Endogenous proteins include:

- Desquamated mucosal cells
- Digestive enzymes and glycoproteins.

The digestive enzymes and glycoproteins are derived from secretions of the salivary glands, stomach, intestine, liver, and pancreas. The digestive tract's mucosal cells contain a variety of proteins (such as apoproteins, structural proteins, and cytosolic enzymes) that when sloughed into the gastrointestinal (GI) tract are degraded. Most of these endogenous proteins, which typically total 70 g or more per day, are digested and provide amino acids that are available for absorption. Digestion of these proteins and the absorption of subsequently generated amino acids are crucial for protein nutrition.

6.3 DIGESTION

This next section of the chapter addresses protein digestion within the GI tract organs (Figure 6.1 and Table 6.3) and emphasizes the major enzymes responsible for protein digestion. Chapter 2 provides detailed information on the digestive tract, its organs, and digestive processes. As no appreciable digestion of protein occurs in the mouth or esophagus, this discussion focuses first on the stomach.

Stomach

The digestion of protein begins in the stomach with the action of hydrochloric acid (HCl), which is found in gastric juice. The hydrochloric acid content of the gastric juice results in a gastric pH less than 3 and enables denaturation (disruption) of the quaternary, tertiary, and secondary structures of protein (shown later in Figures 6.17, 6.18, and 6.19). Denaturants such as hydrochloric acid break apart hydrogen and electrostatic bonds to unfold or uncoil the protein; however, peptide bonds are not affected by the hydrochloric acid. Hydrochloric acid does, however, begin pepsin activation from pepsinogen, which is secreted as

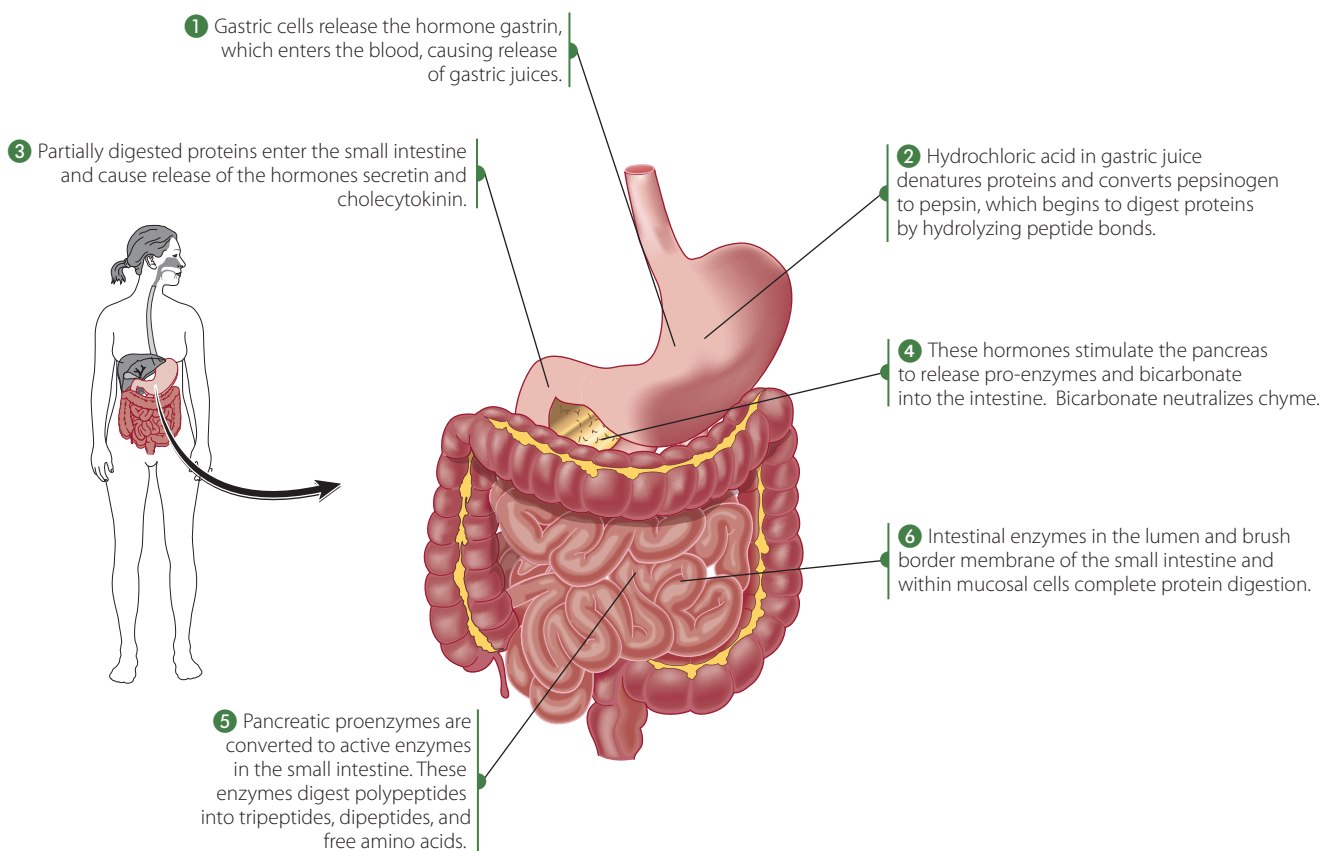


Figure 6.1 An overview of protein digestion.

Source: Derived from Beerman/McGuire, *Nutritional Sciences*, 1/e. © Cengage Learning.

Table 6.3 Major Enzymes Responsible for Protein Digestion

Zymogen	Enzyme or Activator	Enzyme	Site of Activity	Substrate (peptide bonds adjacent to)	End Product(s)
Pepsinogen	HCl or pepsin →	Pepsin	Stomach	Most amino acids, including aromatic, dicarboxylic, leu, met	Peptides
Trypsinogen	Enteropeptidase or Trypsin →	Trypsin	Intestine	Basic amino acids	Smaller peptides, some free amino acids
Chymotrypsinogen	Trypsin →	Chymotrypsin	Intestine	Aromatic amino acids, met, asn, his	Smaller peptides, some free amino acids
Procarboxypeptidases	Trypsin →	Carboxypeptidase	Intestine		
		A		C-terminal neutral amino acids	Free amino acids
		B		C-terminal basic amino acids	Free amino acids
		Aminopeptidases	Intestine	N-terminal amino acids	Free amino acids

a zymogen (inactive enzyme) by gastric chief cells. Pepsin, once formed, is catalytic against pepsinogen as well as other proteins.



Pepsin functions as an **endopeptidase** (meaning that it hydrolyzes interior peptide bonds within proteins or polypeptides) at a $\text{pH} < \sim 3.5$. Specifically, pepsin attacks peptide bonds adjacent to the carboxy end of a relatively wide variety of amino acids (i.e., pepsin has

low specificity), including leucine, methionine, the aromatic amino acids (phenylalanine, tyrosine, and tryptophan), and the dicarboxylic amino acids (glutamate and aspartate).

The end products of gastric protein digestion include primarily large polypeptides, along with some oligopeptides (short chains of amino acid peptide bonded to each other) and free amino acids. These end products are emptied in an acidic chyme through the pyloric sphincter into the duodenum (the proximal or upper part of the small intestine) for further digestion.

Small Intestine

The chyme–nutrient mixture that is delivered into the duodenum further stimulates the release of regulatory hormones and peptides such as secretin and cholecystokinin; these regulatory hormones and peptides facilitate further digestion of proteins and polypeptides. For example, secretin and cholecystokinin are carried by the blood to the pancreas, where selected pancreatic cells are stimulated to secrete zymogens and pancreatic juice containing bicarbonate, electrolytes, and water. In addition to pancreatic juice, the Brunner's glands of the small intestine release mucus-rich secretions needed for digestion.

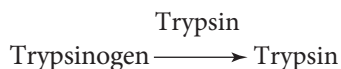
Zymogens secreted by the pancreas into the intestine and further responsible for protein and polypeptide digestion include:

- Trypsinogen
- Chymotrypsinogen
- Procarboxypeptidases A and B
- Proelastase.

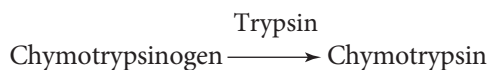
Within the small intestine, these zymogens are chemically altered to be converted into their respective active enzymes capable of protein hydrolysis. The activation of trypsinogen by enteropeptidase is important since the formation of trypsin facilitates activation of other zymogens. Yet, while trypsinogen activation is important in the intestine, extensive damage would occur should trypsinogen become active within the pancreas. To prevent this, the pancreas produces a compound called trypsin inhibitor.



Enteropeptidase (an endopeptidase formerly known as enterokinase), which is secreted from the enterocyte in response to cholecystokinin and secretin, converts trypsinogen to trypsin. Once trypsin is formed, it can convert (like enteropeptidase but to a lesser extent) other trypsinogen molecules and other zymogens to their respective active **proteolytic** enzymes (proteases).



Trypsin, for example, activates the zymogen chymotrypsinogen, as shown here:



Trypsin and chymotrypsin are both endopeptidases. Trypsin is specific for peptide bonds at the carboxy end of basic amino acids (lysine and arginine). Excess trypsin also acts by negative feedback to inhibit trypsinogen synthesis by pancreatic cells, thereby regulating pancreatic zymogen secretion. Chymotrypsin is specific for peptide bonds at

the carboxy end of aromatic amino acids (phenylalanine, tyrosine, and tryptophan) and for peptide bonds adjacent to methionine, asparagine, and histidine.

Procarboxypeptidases are also converted to carboxypeptidases by trypsin and function as **exopeptidases**.



These exopeptidases attack peptide bonds at the carboxy (C)–terminal end of polypeptides to release free amino acids. Carboxypeptidases are zinc dependent, specifically requiring zinc at the enzyme's active site. Carboxypeptidase A hydrolyzes peptides with C-terminal aromatic or aliphatic (nonpolar) neutral amino acids. Carboxypeptidase B cleaves basic amino acids from the C-terminal end to generate free basic amino acids as end products.

Elastase, once activated by trypsin, functions as an endopeptidase to hydrolyze bonds adjacent to aliphatic amino acids, including those in elastin. Elastin is a component of connective tissues and is found especially in meats.

Several peptidases are produced by enterocytes, including those in the ileum, enabling peptide digestion and amino acid absorption to occur in the distal small intestine. Some of these intestinal peptidases include the following:

- Aminopeptidases, which vary in specificity, cleave amino acids from the amino/(N)-terminal end of oligopeptides.
- **Dipeptidyl aminopeptidases**, some of which are magnesium dependent, hydrolyze dipeptides.
- Tripeptidases, which are specific for selected amino acids, hydrolyze tripeptides to yield a dipeptide and a free amino acid.

Not all tripeptides, however, undergo additional digestion to produce free amino acids at the brush border of enterocytes. Triglycine and proline-containing peptides tend to be absorbed intact and hydrolyzed within the enterocyte. The digestive process is influenced to some extent by the free amino acid end products, which can sometimes inhibit the activity of brush border peptidases (a process called end product inhibition) to diminish digestion.

Protein digestion yields two main end products: peptides (principally dipeptides and tripeptides) and free amino acids. To be used by the body, these end products must next be absorbed.

6.4 ABSORPTION

Absorption is the process by which the end products of digestion are transported from the lumen of the GI tract, most often the small intestine, into the body. To get into the blood for transport to tissues, amino acids must cross

two intestinal membranes, the brush border (also called apical) membrane and the basolateral (also called serosal) membrane. Of the 100+ g of amino acids present each day in the small intestine, the equivalent of about 10 g is not absorbed (and excreted and assayed as fecal nitrogen). This section of the chapter addresses transporters found in cell membranes that carry the end products of protein digestion into and out of cells.

Intestinal Cell Absorption

Amino Acid Absorption across the Intestinal Brush Border Membrane

Amino acid absorption occurs along the entire small intestine, but most amino acids are absorbed in the duodenum and proximal jejunum. The absorption of amino acids into enterocytes requires carriers (integral membrane proteins); however, paracellular absorption—that is, absorption via passage through the tight junctions of enterocytes or transcellular endocytosis—can occur in occasional situations in which large volumes of hypotonic fluids containing some amino acids have been ingested.

Transport systems for amino acids have been traditionally designated using a lettering system based on the preferred substrate(s) and functional characteristics of the carriers, with a further distinction that uppercase letters be used for sodium dependence and lowercase letters for sodium independence; however, not all systems (e.g., the L and T, which are sodium independent) follow this rule. The newer nomenclature is based on gene sequences and categorized by solute carrier families. Table 6.4 lists some of the transport systems responsible for carrying amino acids across the brush border membrane into the intestinal cell and some examples of amino acids that are carried by each of these transport systems. The older nomenclature has been retained in this text because it facilitates associations between the transporter names and the amino acids carried by the transporters.

The transporters vary in mechanism of action. Some amino acid transporters, such as y^+ , are passive and function as uniporters; they bind to one amino acid and transport it into the cell with its concentration gradient. Symporters simultaneously carry two substances into the cell. For example, basic amino acids may be carried into the cell along with neutral amino acids via the y^+L system. Lastly, some amino acids enter cells via an antiport mechanism in which two substances move across the enterocyte membrane in opposite directions. The movement of one substance across the enterocyte membrane and down its concentration gradient provides the means for the transport of a second substance against its gradient in the opposite direction. For example, with the X_{AG}^- antiport transporter, glutamate, H^+ , and three Na^+ enter the cell in exchange for one K^+ , and with the N system, glutamine and Na^+ enter the cell in exchange for H^+ . Transport of leucine and the other branched-chain amino acids isoleucine and valine into some cells (such as muscle) may involve the bidirectional transport of glutamine. Most amino acids are thought to be transported across the enterocyte brush border membrane by sodium-dependent transporters, as shown and described in Figure 6.2.

The affinity (K_m) of a carrier for an amino acid is influenced both by the hydrocarbon mass of the amino acid's side chain and by the net electrical charge of the amino acid. Larger amino acids (by mass), such as methionine, phenylalanine, tryptophan, tyrosine, and the branched-chain amino acids, are typically absorbed faster than smaller amino acids. Neutral amino acids also tend to be absorbed at higher rates than basic or acidic amino acids. Essential (indispensable) amino acids are absorbed faster than nonessential (dispensable) amino acids, with methionine, leucine, isoleucine, and valine being the most rapidly absorbed [3,4]. The most slowly absorbed amino acids are the two acidic amino acids, glutamate and aspartate, both of which are nonessential [3]. Changes in *de novo* synthesis of specific amino acid carriers help to ensure adequate capacity.

Table 6.4 Some Systems Transporting Amino Acids across the Intestinal Cell Brush Border Membrane

Transport Systems	Requirements and Mechanism	Amino Acid Substrates
B^0	Na^+ Symport	Most longer-chain neutral amino acids, especially methionine, phenylalanine, leucine
$B^0, +$	Na^+ and Cl^- Symport	Neutral and basic amino acids, β -alanine
$b^0, +$	Stimulated by Na^+ Antiport	Basic amino acids (arginine, lysine, ornithine, cystine) and neutral amino acids, β -alanine
ASC (ASCT2)	Na^+ Antiport	Neutral amino acids, primarily alanine, serine, cysteine as well as threonine, glutamine, asparagine, methionine
IMINO	Na^+ and Cl^- Symport	Proline, hydroxyproline, glycine, alanine, β -alanine
Imino-glycine	H^+ Symport	Short-chain amino acids, especially proline, glycine, alanine, β -alanine
β	Na^+ and Cl^- Symport	Taurine and β -alanine
X_{AG}^-	Na^+ and H^+ Symport in exchange for K^+ Antiport	Acidic amino acids—aspargate, glutamate
X_C^-	- Antiport	Acidic amino acids—aspargate, glutamate
N	Na^+ Symport in exchange for intracellular H^+ Antiport	Primarily glutamine, asparagine, histidine, serine, glycine

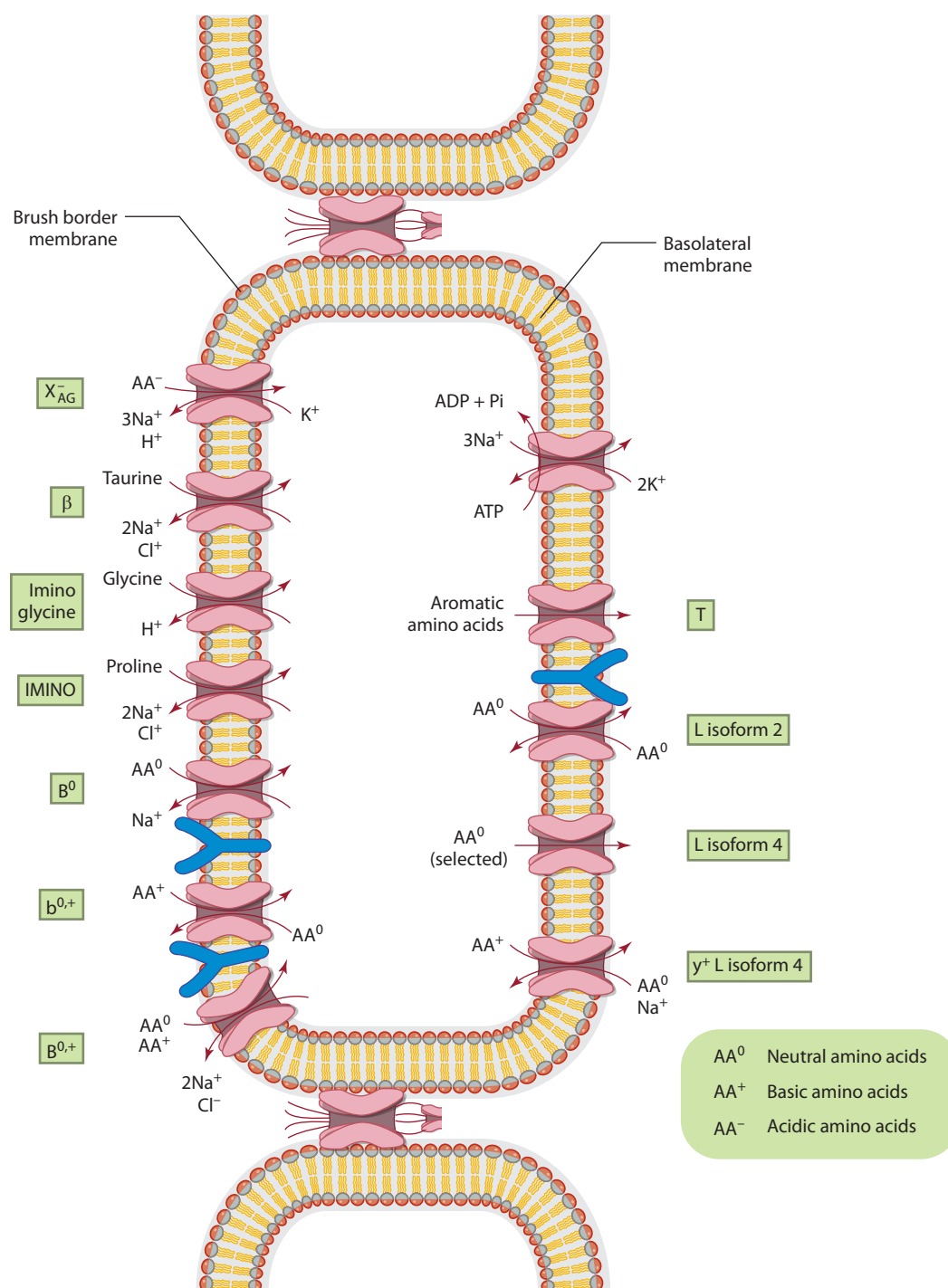


Figure 6.2 Intestinal cell amino acid transporters.

However, ingesting large quantities of one amino acid or a particular group of amino acids that use the same carrier system may create, depending on the amount ingested, competition among the amino acids for absorption. The result may be that the amino acid present in highest concentration is absorbed but also may impair the absorption of the other, less concentrated amino acids carried by that same system. Thus, amino acid supplements may result in impaired or imbalanced amino acid absorption. Moreover, the absorption of peptides (discussed in the next section) is more rapid than the absorption of an equivalent mixture of free amino acids. These differences in absorption

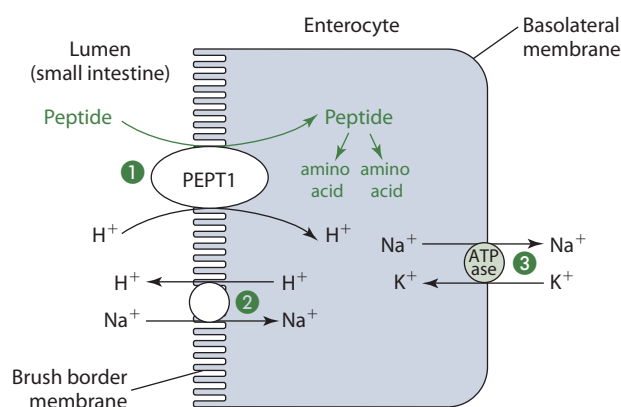
impact protein synthesis, as discussed in the “Protein Synthesis” section of this chapter. And, in those with intestinal resection or damage, ingestion of protein-containing foods (vs. free amino acids) may improve growth of remaining enterocytes and enhance peptide transporter expression [5].

Peptide Absorption across the Intestinal Brush Border Membrane

Peptide (primarily dipeptide and tripeptide) transport across the brush border membrane of the enterocyte is accomplished by a transport system different from those that transport amino acids. The transport system peptide

transporter 1, designated PEPT1, appears to transport all di- and tripeptides and is found throughout the entire length of the small intestine. This transport of peptides across the brush border membrane using PEPT1 is associated with the comovement of protons (H^+) and thus depolarization of the brush border membrane. An area of low pH lying adjacent to the brush border surface of the enterocyte provides the driving force for the H^+ gradient. Thus, as shown in Figure 6.3, as the dipeptide or tripeptide is transported into the enterocyte, an H^+ ion also enters the enterocyte. The transport of the H^+ into the enterocyte results in an intracellular acidification. The H^+ ions are pumped back out into the lumen in exchange for Na^+ ions. A Na^+/K^+ -ATPase allows for Na^+ extrusion at the basolateral membrane to maintain the gradient

Although peptides, like amino acids, compete with one another for transporters, peptide transport appears to occur more rapidly than amino acid transport and is thought to represent the primary means by which most amino acids enter the intestinal cell. In other words, the majority of amino acids are absorbed as peptides. Peptides, once within the enterocytes, are generally hydrolyzed by cytosolic peptidases to generate free intracellular amino acids. Intact peptides, however, can be found occasionally in circulation, and this is thought to result from entry of peptides into the body via paracellular (also called intercellular) routes. With illnesses, especially those affecting the intestines (such as inflammatory bowel diseases or celiac disease), the gastrointestinal tract can become more permeable, thus increasing the likelihood of peptides appearing intact in the blood.



- ① Peptides are transported into the intestinal cell along with H^+ .
- ② The H^+ are pumped back into the intestinal lumen in exchange for Na^+ .
- ③ A Na^+, K^+ -ATPase pumps Na^+ out of the cell in exchange for K^+ across the basolateral membrane.

Figure 6.3 Peptide transport across the brush border membrane of the intestinal cell.

Peptides found in the blood can be hydrolyzed by peptidases or proteases in the plasma or at the cell membrane (especially in the liver, kidneys, and muscle). Intracellular hydrolysis of the peptide, if absorbed intact, may also occur in the cytosol or in various organelles. Peptide transport into renal tubular cells is influenced by the net charge of the amino acid at the amino (N-) and the carboxy (C-) terminals. Peptides containing either basic or acidic amino acids at either the N- or C-terminal have lower affinity for transport than peptides with neutral side chains at these positions. The ability to administer peptides directly into the blood as in parenteral nutrition is of nutritional significance since some amino acids (e.g., tyrosine, cysteine, and glutamine) are insoluble or unstable in their free form. Administration of the peptide form, since it can be used by tissues, allows nutrients to be provided in situations in which traditional free amino acid parenteral mixtures are ineffective.

Amino Acid Absorption across the Intestinal Basolateral Membrane

For amino acids to enter the blood and be used by other body tissues, the amino acids must be transported across the basolateral (serosal) membrane of the enterocyte and into interstitial fluid, where they enter the blood (through capillaries of the villi) for transport into the portal vein leading to the liver. The carriers found in the enterocyte's basolateral membrane are generally sodium independent and similar to those found in other cell membranes (see Table 6.5). Some of these carriers also transport amino acids from the blood back into the enterocytes; this is especially true for glutamine, which functions as a major energy source for intestinal cells.

Defects in Amino Acid Absorption

The significance of the amino acid transporters becomes extremely apparent when genetic mutations prevent the synthesis of functional transporters. Lysinuric protein intolerance, for example, results from defects in the basic amino acid transporters in the intestine, liver, and kidneys. The defects cause poor absorption of lysine, arginine, and ornithine and consequently low plasma concentrations and availability of these amino acids for protein synthesis and for urea cycle activity. Symptoms of the disorder include hyperammonemia, growth retardation, muscle weakness, hepatomegaly, and hypotonia, among other problems. Nutrition support involves a protein-restricted diet to help minimize the hyperammonemia and supplements of citrulline to help improve arginine and ornithine production.

Similarly, the critical role of transporters is illustrated by another condition called **Hartnup disease**, an autosomal recessive genetic disorder that affects absorption (likely via the B transport system) of tryptophan and other neutral amino acids into intestinal and kidney cells. Hartnup

Table 6.5 Some Systems Transporting Amino Acids across the Intestinal Cell Basolateral Membrane

Transport System	Requirements and Mechanism	Amino Acids Substrates
L	- Antiport	Mostly leucine, methionine, phenylalanine, isoleucine, and other neutral amino acids except proline; multiple isoforms with LAT1 transporting most essential amino acids—leucine, isoleucine, phenylalanine, methionine, histidine, tryptophan, valine, and tyrosine
T	- Antiport and Uniport	Aromatic amino acids—phenylalanine, tyrosine, tryptophan
X _c ⁻	- Antiport	Glutamate and cystine
A	Na ⁺ Symport	Primarily neutral amino acids (mainly alanine, glycine, serine, proline, cysteine, methionine), asparagine and histidine; it also transports glutamine from the blood into the enterocyte
ASC1	- Antiport	Primarily short-chain amino acids (glycine, alanine, serine, cysteine, and threonine)
y ⁺	- Uniport	Arginine, lysine, histidine, ornithine
y ⁺ L	Neutral amino acid and Na ⁺ Symport exchanged for basic amino acid Antiport	Basic amino acids (lysine, arginine, histidine) along with neutral amino acids (mainly methionine, leucine, alanine, cysteine); two isoforms.
Gly	Na ⁺ and Cl ⁻ Symport	Bidirectional transport of glycine
N	Na ⁺ Symport in exchange for intracellular H ⁺ Antiport	Primarily glutamine, asparagine, histidine

disease causes malabsorption of tryptophan along with some other amino acids. Niacin deficiency can occur because of insufficient availability of tryptophan, which serves as a precursor of niacin (see Chapter 9).

Mutations in other genes coding for other amino acid carriers have also been identified. One such mutation is characterized by large quantities of methionine along with branched-chain amino acids in the feces. Intestinal tryptophan malabsorption, due to a suspected defect in the gene coding for the T system in the basolateral membrane, is characterized by blue diaper syndrome, failure to thrive, nephrocalcinosis, and hypercalcemia. Increased fecal tryptophan concentrations are found along with indoles generated by bacterial tryptophan degradation. The indoles, following colon cell absorption and transport to the liver, are converted to indoxylsulfate. The indoxylsulfate is then excreted from the body via the kidneys and feces. However, when indoxylsulfate is exposed to air (as with changing the diaper), it oxidizes to a blue (indigo) color, hence the name blue diaper syndrome.

Extraintestinal Cell Absorption

After amino acids are transported out of the enterocyte, they enter portal blood for transport to tissues. Uptake of the amino acids into liver cells (hepatocytes), as well as cells of the kidneys and other organs, occurs by some carrier systems similar to those found in the intestinal cell membranes. The sodium-dependent N system is especially prominent in the periportal cells of the liver and functions as an antiporter to take up glutamine and sodium in exchange for H⁺. The process occurs in reverse in the perivenous hepatic cells; glutamine is released in exchange for H⁺. Hormones and cytokines, such as interleukin-1 and tumor necrosis factor α , influence amino acid transport. System A in hepatocytes, for example, is induced by glucagon and provides amino acid substrates

for gluconeogenesis. System GLY is sodium dependent and specific for glycine; two sodium ions are transported for each glycine. The γ -glutamyl cycle is thought to be involved in amino acid transport through membranes of renal tubular cells, erythrocytes, and perhaps neurons. In the γ -glutamyl cycle, glutathione acts as a carrier of neutral amino acids into cells. The cycle is depicted in detail in Figure 6.4. Briefly, glutathione reacts with γ -glutamyl transpeptidase located in cell membranes, forming a γ -glutamyl enzyme complex, which binds a neutral amino acid at the cell surface and transports it into the cytosol for use.

6.5 AMINO ACID CATABOLISM

The liver is the primary site for the uptake of most amino acids following ingestion of a protein-containing meal. The liver is thought to monitor the absorbed amino acids and to adjust the rate of their metabolism (including catabolism or breakdown of amino acids and anabolism or use of amino acids for synthesis) according to the needs of the body. In this section of the chapter, an overview of amino acid catabolism is presented first. The specific catabolism of individual amino acids, along with some other uses of individual amino acids, is reviewed next. Later sections discuss the anabolic uses of amino acids for the synthesis of proteins and nitrogen-containing compounds, along with other roles of amino acids affecting protein utilization.

Catabolism of amino acids occurs to varying degrees in different tissues both during fasting periods and immediately after eating (the postprandial period). Liver cells have a high capacity for the uptake and catabolism of amino acids. In fact, after a meal, the liver takes up about 50–65% of amino acids from portal blood. The liver is the main site for the catabolism of the indispensable amino acids, with

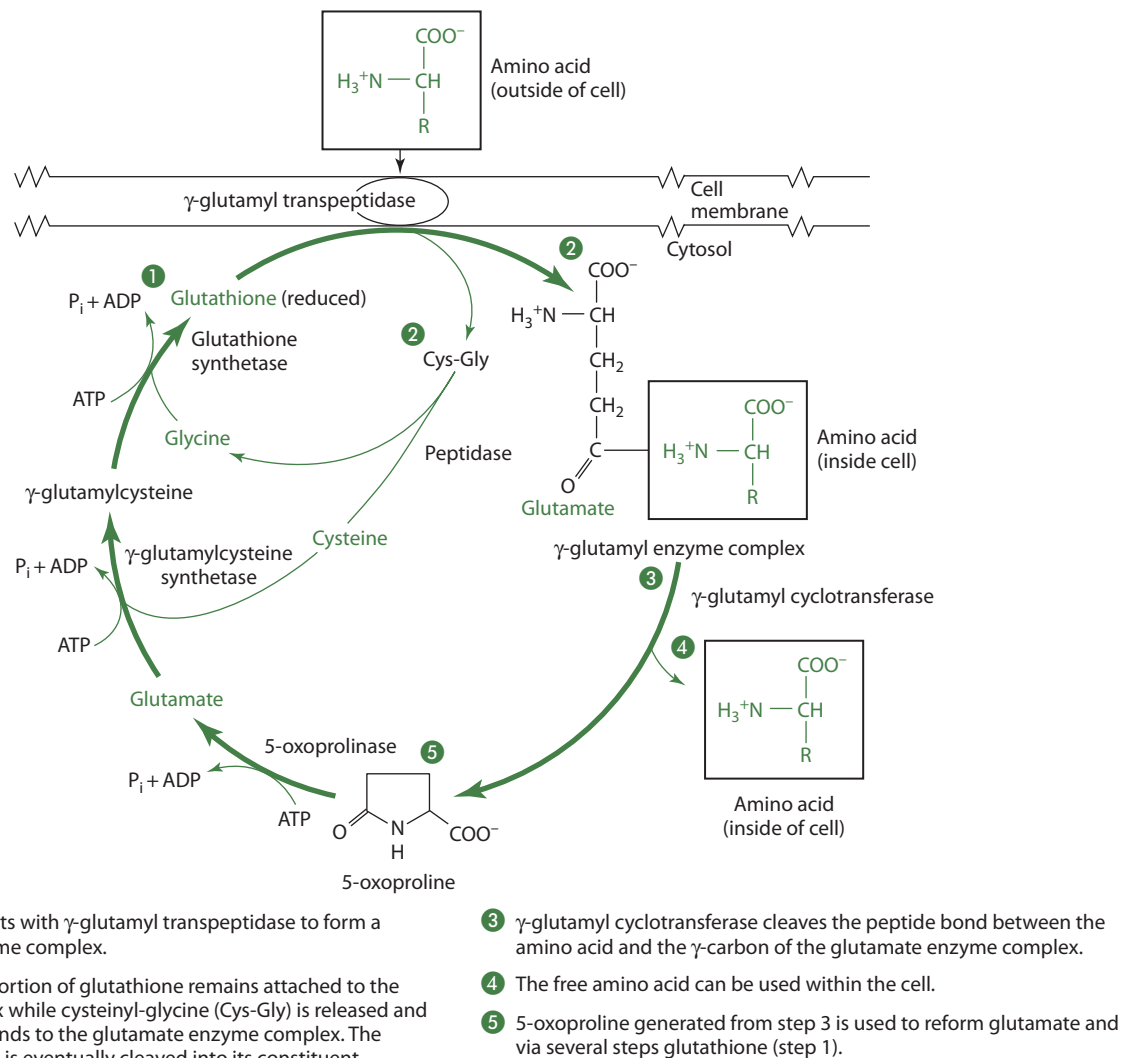


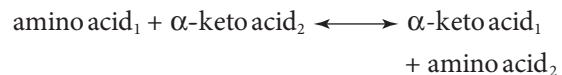
Figure 6.4 The γ -glutamyl cycle for transport of amino acids.

the exception of the branched-chain amino acids, which tend to be utilized to a greater extent by muscle and other organs such as the heart. Within the liver, the periportal hepatocytes catabolize most amino acids with the exception of glutamate and aspartate, which are metabolized to a greater extent by perivenous hepatocytes. The liver derives up to 50% of its energy (ATP) from amino acid oxidation; the energy generated may in turn be used for gluconeogenesis or urea synthesis, among other needs, depending on the body's state of nutriture. This section on amino acid catabolism focuses on the reactions that occur as amino acids are broken down in cells, including first the transamination and/or deamination of amino acids and then the disposal of ammonia. It next discusses the uses of the carbon skeleton of amino acids, as well as some other uses of amino acids.

Transamination of Amino Acids

Frequently (but not always), the first step in amino acid catabolism is the transfer or removal of an amino acid's amino group. The process occurs by transamination and/

or deamination. Transamination reactions involve the transfer of an amino group from one amino acid to an α -keto acid (also referred to as an amino acid carbon skeleton). The carbon skeleton/ α -keto acid that gains the amino group becomes an amino acid, and the amino acid that loses its amino group becomes an α -keto acid. A generic transamination reaction can be written as:



These reactions are important for the synthesis of many of the body's dispensable amino acids.

Transamination reactions are catalyzed by enzymes called aminotransferases/transaminases. These enzymes typically require vitamin B₆ in its coenzyme form, pyridoxal phosphate (PLP). Some examples of aminotransferases include tyrosine aminotransferase, branched-chain aminotransferases, alanine aminotransferase (ALT; formerly called glutamate pyruvate transaminase and abbreviated GPT), and aspartate aminotransferase (AST; formerly called glutamate oxaloacetate transaminase and abbreviated GOT).

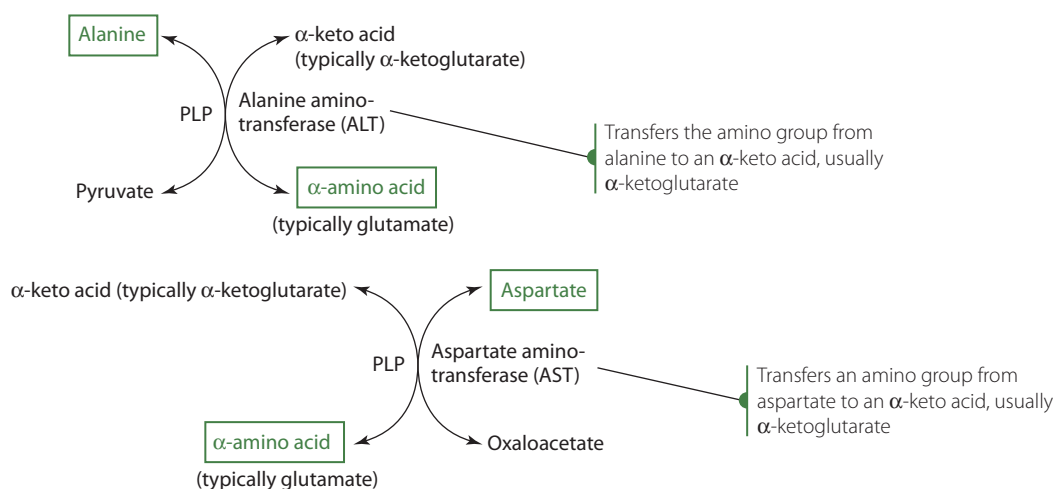


Figure 6.5 Transamination reactions.

These last two aminotransferases (ALT and AST) are among the body's most active and involve three key amino acids and α-keto acids—alanine and its α-keto acid pyruvate, glutamate and its α-keto acid α-ketoglutarate, and aspartate and its α-keto acid oxaloacetate—as shown and described in Figure 6.5. Specifically, ALT transfers amino groups from alanine to an α-keto acid (e.g., α-ketoglutarate), forming pyruvate and another amino acid (e.g., glutamate), respectively. Similarly, AST transfers amino groups from aspartate to an α-keto acid (e.g., α-ketoglutarate), yielding oxaloacetate and another amino acid (e.g., glutamate), respectively. These reactions are reversible, and because glutamate and α-ketoglutarate readily transfer and/or accept amino groups, these compounds play central roles in amino acid metabolism.

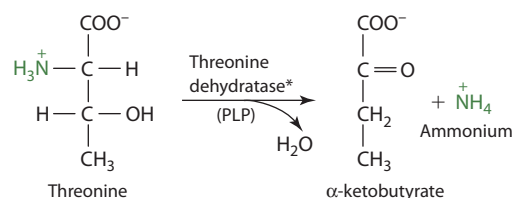
Aminotransferases are found in varying concentrations in different tissues. For example, AST is found in higher concentrations in the heart than in the liver, muscle, and other tissues. In contrast, ALT is found in higher concentrations in the liver than in the heart but is also found in moderate amounts in the kidneys and small amounts in other tissues. Normal serum concentrations of these enzymes are low; however, with injury or disease to an organ, serum enzyme concentrations rise and can serve as an indicator of organ damage. For example, with liver damage, higher than normal blood concentrations of AST and ALT as well as other enzymes such as alkaline phosphatase and lactate dehydrogenase (that are normally found in the liver) are observed. With heart damage (as may occur with a heart attack), enzymes that are normally found in the heart, such as AST, “leak” out into the blood and serve as indicators of heart cell damage.

Interestingly, α-keto acids are sometimes used nutritionally. In kidney failure, nitrogenous compounds that are normally excreted in the urine accumulate in the blood. The provision of α-keto acids of some of the essential amino acids to someone with kidney failure allows some of the body's excess nitrogen to be used to aminate the α-keto acids. This results in the lowering of blood nitrogen

concentrations while also providing the individual with essential nutrients. Three amino acids (lysine, histidine, and threonine), however, cannot undergo transamination to any appreciable extent; thus, these amino acids cannot be given effectively as α-keto acids.

Deamination of Amino Acids

In contrast to transamination reactions, **deamination** reactions involve only the removal of an amino group from an amino acid, with no transfer of the amino group to another compound. The amino group is released as ammonia, but at the body's physiological pH, ammonia is typically converted (in a reversible reaction) to the ammonium ion. Some amino acids that are more commonly deaminated include glutamate, histidine, serine, glycine, and threonine; however, many of these same amino acids can also be transaminated. The enzymes carrying out the deamination reactions are generally lyases, dehydratases, or dehydrogenases and produce an α-keto acid and ammonia or an ammonium ion. Figure 6.6 shows the deamination of the amino acid threonine by threonine dehydratase (which deaminates and dehydrates threonine) to form α-ketobutyrate and ammonium ion. The next section addresses the disposal of ammonia by the body.



*The enzyme is called *dehydratase* rather than *deaminase* because the reaction proceeds by loss of elements of water. In the deamination, the amino group from the amino acid is removed. Vitamin B₆ as PLP is required by the enzyme.

Figure 6.6 The deamination of the amino acid threonine. In the deamination, the amino group from the amino acid is removed.

Disposal of Ammonia

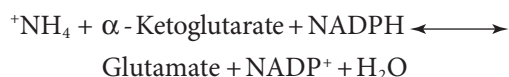
The ammonia (or ammonium ions) generated by deamination reactions is not the only source of ammonia found in the body. Major sources of ammonia and/or ammonium ions in the body include:

- Formation in the body from chemical reactions such as deamination
- Generation by the deamidation of the amide groups from glutamine and asparagine
- Ingestion and absorption from foods (e.g., cheeses, processed meats)
- Generation by the bacterial lysis of urea and amino acids in the GI tract and subsequent absorption into the body.

Typically, three enzymes—glutamate dehydrogenase, glutamine synthetase, and carbamoyl phosphate synthetase I (as part of the urea cycle)—assist in the removal of the ammonia/ammonium ions from body cells. These enzymes are found in high concentrations in the liver but are also found in the kidneys among other organs.

Glutamate and Glutamine Synthesis

Glutamate dehydrogenase readily uses an ammonia or ammonium ion ($^+\text{NH}_4$) and α -ketoglutarate to synthesize the amino acid glutamate, as shown here:



The glutamate generated in this reversible reaction can then release the ammonia or ammonium ion for the synthesis of either urea or a dispensable amino acid.

Glutamine synthetase can also use ammonia or an ammonium ion for the amidation of glutamate's gamma carboxy group to form glutamine in an ATP-dependent reaction that also requires magnesium or manganese as follows:



Glutamine's functions, including its role in ammonia transport, are discussed further in this chapter in the "Interorgan Flow of Amino Acids and Organ-Specific Metabolism" section.

While the liver's perivenous cells and other body tissues readily synthesize glutamate and glutamine from ammonia or ammonium ions, the periportal hepatocytes are active in ureagenesis using (from portal blood) ammonia that was ingested in foods or obtained from bacterial synthesis in the intestine. These same periportal cells are responsible for almost all amino acid catabolism, so ammonia and ammonium ions generated during amino acid degradative reactions can also be immediately used for urea synthesis.

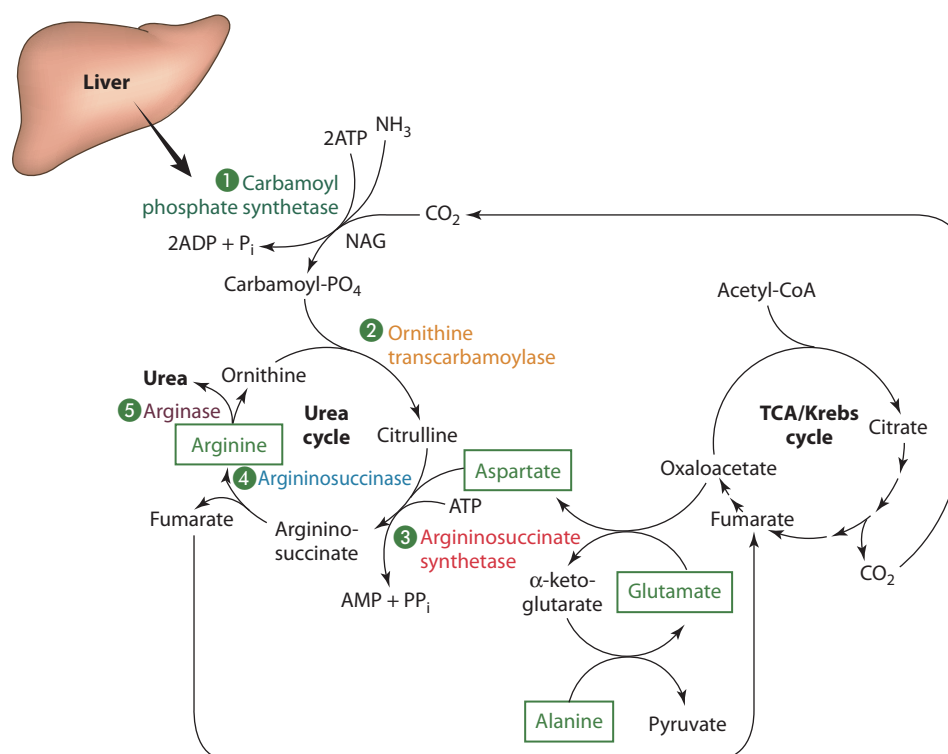
The Urea Cycle

The urea cycle, discovered by Sir Hans Krebs, functions in the liver and is extremely important for the removal of ammonia (and ammonium ions) from the body. Figure 6.7 reviews key compounds of the urea cycle and shows its relationship with amino acids and the tricarboxylic acid (TCA), also known as the Krebs, cycle. The five reactions of the urea cycle are also presented hereafter:

- 1 Ammonia (NH_3) (or an ammonium ion) combines with CO_2 (which can also be present as HCO_3^-) to form carbamoyl phosphate in a reaction catalyzed by mitochondrial carbamoyl phosphate synthetase I and using 2 mol of ATP and Mg^{2+} . N-acetylglutamate (NAG), made in the liver and intestine, is required as an allosteric activator to allow ATP binding.
- 2 Carbamoyl phosphate reacts with ornithine in the mitochondria to form citrulline using the enzyme ornithine transcarbamoylase (OTC). Citrulline in turn inhibits OTC activity.
- 3 Aspartate reacts with citrulline once it has been transported into the cytosol to form argininosuccinate in a reaction catalyzed by argininosuccinate synthetase. This reaction is the rate-limiting step of the cycle. ATP (two high-energy bonds) and Mg^{2+} are required for the reaction. Argininosuccinate, arginine, and $\text{AMP} + \text{PP}_i$ inhibit the enzyme.
- 4 Argininosuccinate is cleaved by argininosuccinase in the cytosol to form fumarate and arginine. Both fumarate and arginine inhibit argininosuccinase activity. Argininosuccinase is found in a variety of tissues throughout the body, especially the liver and kidneys. High concentrations of arginine increase the synthesis of N-acetylglutamate (NAG), which is needed in the reaction for the synthesis of carbamoyl phosphate in the mitochondria.
- 5 Urea is formed and ornithine is re-formed from the cleavage of arginine by arginase, a manganese-requiring hepatic enzyme. Arginase activity is inhibited by both ornithine and lysine and may become rate limiting under conditions that limit manganese availability or that alter its affinity for manganese.

Overall, the urea cycle uses four high-energy bonds. The urea molecule derives one nitrogen from ammonia, a second nitrogen from aspartate, and its carbon from CO_2 (or as HCO_3^-). Once formed, urea typically travels in the blood to the kidneys for excretion in the urine; however, up to about 25% of urea may be secreted from the blood into the intestinal lumen, where it may be degraded by bacteria to yield ammonia.

Activities of urea cycle enzymes fluctuate with diet and hormone concentrations. For example, with low-protein



- 1 Ammonia (NH_3) combines with CO_2 to form carbamoyl phosphate in a reaction catalyzed by mitochondrial carbamoyl phosphate synthetase I. N-acetyl-glutamate (NAG) allosterically activates the enzyme to allow ATP binding.
- 2 Carbamoyl phosphate reacts with ornithine using ornithine transcarbamoylase to form citrulline.
- 3 Aspartate reacts with citrulline once it has been transported into the cytosol. This step is catalyzed by argininosuccinate synthetase. ATP (two high-energy bonds) and Mg^{2+} are required for the reaction, and argininosuccinate is formed.
- 4 Argininosuccinate is cleaved by argininosuccinase in the cytosol to form fumarate and arginine.
- 5 Urea is formed and ornithine is re-formed from the cleavage of arginine by arginase, a manganese-requiring hepatic enzyme.

Figure 6.7 Interrelationships of amino acids and other compounds of the urea and TCA/Krebs cycles in the liver.

diets or acidosis, urea synthesis diminishes and urinary urea nitrogen excretion decreases significantly. Thus, substrate availability results in short-term changes in the rate of ureagenesis. In the healthy individual with a normal protein intake, blood urea nitrogen (BUN) concentrations range from about 8–20 mg/dL, and urinary urea nitrogen represents about 80% of total urinary nitrogen. Glucocorticoids and glucagon, which promote amino acid degradation, typically increase mRNA for the urea cycle enzymes.

Several defects (genetic mutations) have been identified in genes coding for urea cycle enzymes. Urea cycle enzyme defects typically result in high blood levels of ammonia. Ammonia in high concentrations in the blood is toxic, causing initially lethargy but ultimately coma and possibly death. Treatment of urea cycle disorders necessitates a protein-restricted diet and, depending on the enzyme that is defective, supplementation of citrulline and/or arginine (among other nutrients) may be prescribed. Urea synthesis is also diminished and blood ammonia concentrations increased in those with advanced liver disease. The elevated blood ammonia

concentrations observed in liver disease are thought to contribute to hepatic encephalopathy, characterized in part by brain dysfunction including coma. One aspect of medical treatment for encephalopathy involves decreasing blood ammonia concentrations. Drugs such as lactulose are given to acidify the GI tract contents and promote the diffusion of the ammonia out of the blood and into the GI tract. Furthermore, antibiotics are prescribed that promote the destruction of bacteria in the intestinal tract that generate ammonia.

Carbon Skeleton/ α -Keto Acid Uses

As outlined in Figure 6.8, once an amino group has been removed from an amino acid, the remaining part is called a carbon skeleton or α -keto acid.



Carbon skeletons of amino acids can be further metabolized with the potential for multiple uses in the cell, depending on the original amino acid from which they were derived and the body's physiological and nutritional state.

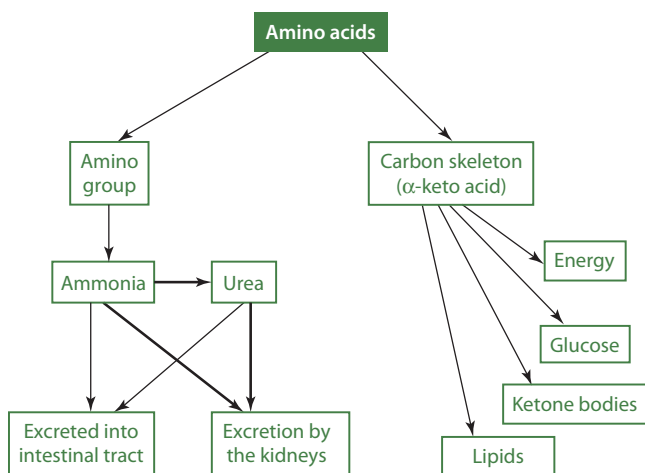


Figure 6.8 Possible fates of amino acids upon catabolism.

An amino acid's carbon skeleton, for example, depending on the particular amino acid, can be used to produce energy, glucose, ketone bodies, cholesterol, and fatty acids.

Energy Production

The complete oxidation of amino acids generates energy, along with water, $\text{CO}_2/\text{HCO}_3^-$, and ammonia/ammonium ions. Amino acids are used for energy production when the diet is inadequate in energy. The oxidation of selected amino acids is presented in later subsections of this chapter.

Glucose Production

The production of glucose from a noncarbohydrate source such as amino acids is known as gluconeogenesis. Gluconeogenesis occurs primarily in the liver but also in the kidneys and small intestine; it is discussed in detail in Chapter 3. The carbon skeletons of several amino acids can be used to synthesize glucose. For example, oxaloacetate (the carbon skeleton of aspartate) and pyruvate (the carbon skeleton of alanine) may be used to produce glucose in body cells. In addition, the carbon skeleton of asparagine can be converted into oxaloacetate, and the carbon skeletons of glycine, serine, cysteine, tryptophan, and threonine can be converted into pyruvate for glucose production. Valine and methionine are also glucogenic, yielding succinyl-CoA. Thus, to be considered a glucogenic amino acid, catabolism of the amino acid must yield pyruvate or intermediates of the TCA cycle.

The conversion of amino acids to glucose is accelerated by a high blood glucagon-to-insulin ratio and by high blood cortisol concentrations. Glucagon concentrations are generally elevated in the blood when blood glucose concentrations are low, as may occur in between meals or with a fast in which liver glycogen stores have been depleted. Blood glucagon (and in some cases cortisol along with epinephrine) is also elevated in the presence of infection or trauma/injury and in certain disease states such as untreated diabetes mellitus and liver disease, to name a few.

Ketone Body Production

For an amino acid to be considered ketogenic, the catabolism of the amino acid must generate acetyl-CoA or acetoacetate, which are used for the formation of ketone bodies (also referred to as ketones). Some amino acids are both glucogenic and ketogenic. Phenylalanine and tyrosine, for example, can be degraded to form fumarate (an intermediate of the TCA cycle), which can be used to form glucose, but also acetoacetate, which can be used to synthesize ketone bodies. Isoleucine is partially glucogenic, generating succinyl-CoA, but also ketogenic, yielding acetyl-CoA as well upon its catabolism. Threonine is partially glucogenic, yielding succinyl-CoA or pyruvate depending on its pathway of degradation, and partially ketogenic when degraded by another pathway to acetyl-CoA. Tryptophan is also considered partially ketogenic and partially glucogenic. Tryptophan yields acetyl-CoA as well as pyruvate upon catabolism. Leucine and lysine are the only totally ketogenic amino acids and upon catabolism generate acetyl-CoA. Figure 6.9 shows the general fates of amino acid carbon skeletons with respect to key intermediates of metabolism.

Lipid (Cholesterol and Fatty Acid) Production

The oxidation of several amino acids—including isoleucine, leucine, lysine, tryptophan, and threonine—yields acetyl-CoA, which can be metabolized to produce cholesterol (Figure 6.9). Leucine, however, is also the only amino acid whose catabolism directly generates β -hydroxy β -methylglutaryl-CoA, an intermediate (shown later in Figure 6.36) in cholesterol synthesis. Moreover, leucine oxidation produces another metabolite, β -hydroxy β -methylbutyrate (HMB), which appears to promote *de novo* cholesterol synthesis in muscle, enabling cell growth and function. This topic is further discussed in the “Skeletal Muscle” section and more specifically in the “Isoleucine, Leucine, and Valine Catabolism” section. Cholesterol synthesis is discussed in detail in Chapter 5.

In times of excess energy and protein intakes coupled with adequate carbohydrate intake, the carbon skeleton of amino acids may be used to synthesize fatty acids. Leucine's carbon skeleton, for example, is used to synthesize fatty acids in adipose tissue. Fatty acid synthesis is discussed in detail in Chapter 5.

Hepatic Catabolism and Uses of Aromatic Amino Acids

The details of the metabolism of selected amino acids and the formation of TCA cycle and non-TCA cycle intermediates are discussed in the following sections. The catabolism of the amino acids is categorized primarily according to their structural classification, although also by net charge for the basic amino acids. The aromatic amino acids are discussed first, followed by the sulfur-containing amino acids,

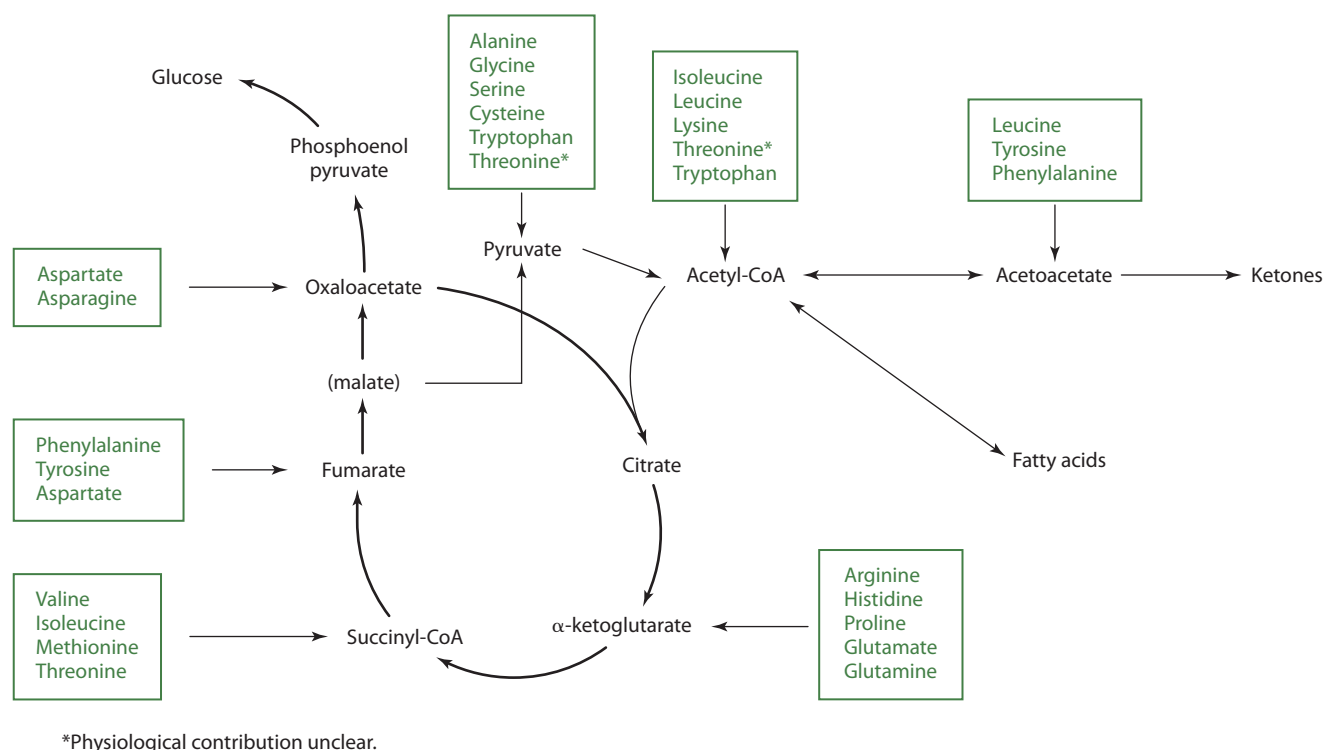


Figure 6.9 The fate of amino acid carbon skeletons. Ketogenic: Lysine and leucine. Partially ketogenic and glucogenic: Phenylalanine, isoleucine, threonine, tryptophan, and tyrosine. Glucogenic: alanine, glycine, cysteine, aspartate, asparagine, glutamate, glutamine, arginine, methionine, valine, histidine, and proline.

the branched-chain amino acids, the basic amino acids, and lastly other selected amino acids.

Amino acid catabolism occurs in most body tissues, although the liver plays a primary role for some amino acids (such as the aromatic and sulfur-containing amino acids) more than for others. For example, in advanced liver disease, the inability of the liver to take up and catabolize certain amino acids is evidenced by the increased plasma concentrations of both the aromatic amino acids—phenylalanine, tyrosine, and tryptophan—and the sulfur-containing amino acids methionine and cysteine.

Phenylalanine and Tyrosine

As shown in Figures 6.9 and 6.10, phenylalanine and tyrosine are partially glucogenic because they are degraded to fumarate. In addition, phenylalanine and tyrosine are catabolized to acetoacetate and are thus partially ketogenic.

- The first step in the degradation of phenylalanine (Figure 6.10) is specific to the liver and the kidneys. Phenylalanine is converted to tyrosine by the enzyme phenylalanine hydroxylase, also called a monooxygenase. This enzyme is iron dependent, and tetrahydrobiopterin functions as a cosubstrate in the reaction. Enzyme activity is regulated by phosphorylation/dephosphorylation, with glucagon promoting phosphorylation and enzyme activity. Insulin has the opposite effect.

- Tyrosine degradation (Figure 6.10) begins with transamination by a vitamin B₆ (as PLP)–dependent tyrosine aminotransferase to yield p-hydroxyphenylpyruvate. Higher tyrosine concentrations as well as high cortisol levels promote increased tyrosine aminotransferase activity. The compound p-hydroxyphenylpyruvate, once formed, is then decarboxylated by a dioxygenase to generate homogentisate. Homogentisate dioxygenase converts homogentisate to maleylacetoacetate, which is then isomerized to fumarylacetoacetate. A hydrolase converts fumarylacetoacetate into fumarate (a TCA cycle intermediate) and acetoacetate, which may be further metabolized to acetyl-CoA for energy, fatty acid, or ketone body production.

Tyrosine can also be used to synthesize other compounds. Some of these uses are mentioned hereafter and shown in Figure 6.10 as well as later in Figure 6.16.

- In neurons and the adrenal medulla, tyrosine is used for the synthesis of neurotransmitters and hormones, respectively. The initial reaction involves tyrosine hydroxylase (also called monooxygenase), an iron-dependent enzyme that hydroxylates tyrosine to generate 3,4-dihydroxyphenylalanine (L-dopa). Subsequent reactions utilizing L-dopa yield the catecholamines (dopamine, norepinephrine, and epinephrine). The catecholamines function as neurotransmitters in the nervous system; however, in circulation they (especially

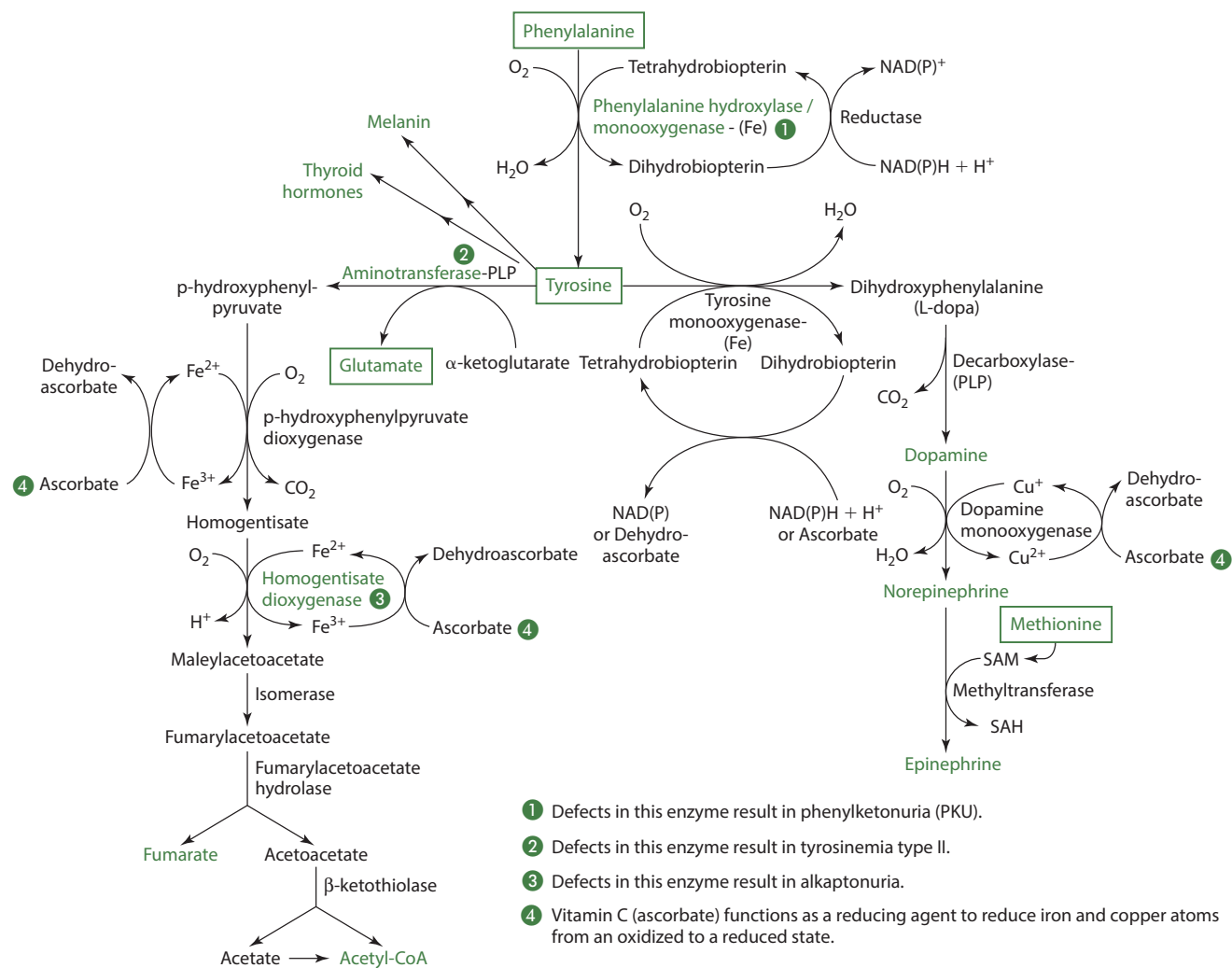


Figure 6.10 Phenylalanine and tyrosine metabolism.

epinephrine) function as hormones and have major effects on nutrient metabolism.

- In melanocytes in the skin, eye, and hair cells, tyrosine is converted through multiple reactions into melanin. The reactions occur within melanosomes, membrane-bound organelles found in the melanocytes. Melanin is a pigment that gives color to skin, eyes, and hair.
- In the thyroid gland, tyrosine is taken up and used with iodine to synthesize thyroid hormones. These reactions are discussed in the section on iodine in Chapter 13.

Disorders of Phenylalanine and Tyrosine Metabolism

Several inborn errors have been identified in phenylalanine and tyrosine metabolism (Figure 6.10). Phenylketonuria, an autosomal recessive genetic disorder, occurs when the activity of phenylalanine hydroxylase, which converts phenylalanine to tyrosine, is impaired. It is one of the most prevalent disorders of amino acid metabolism, with an incidence of about 1 in 10,000 in the United States. This enzymatic defect results in a buildup of phenylalanine and phenylalanine metabolites (phenyllactate, phenylpyruvate,

and phenylacetate) in the blood and other body fluids. In addition, because phenylalanine's conversion to tyrosine is impaired, blood tyrosine concentrations diminish. If untreated, PKU causes neurologic problems such as seizures and hyperactivity, among other problems. The disorder is treated with a phenylalanine-restricted diet, which means that the ingestion of natural protein-containing foods must be extremely limited, and tyrosine must be added to the diet because it cannot be made in the body. In addition, labels on products that contain aspartame (brand name Equal®) must have a warning indicating that the product contains phenylalanine and thus its use must be restricted by those with PKU.

Impaired activity of the enzyme tyrosine aminotransferase, which converts tyrosine to p-hydroxyphenylpyruvate, results in the inborn error of metabolism known as tyrosinemia type II. Tyrosinemia type II, with a worldwide incidence of about 1 in 250,000, is characterized by high plasma tyrosine concentrations, skin and eye lesions, and impaired mental development. Treatment requires a diet restricted in both phenylalanine and tyrosine.

Another genetic disorder involving tyrosine degradation is alkaptonuria. The condition is not common worldwide (affecting 1 in 250,000 to 1 million); however, in Slovakia it affects about 1 in 19,000. Homogentisate dioxygenase, which is defective in alkaptonuria, normally converts homogentisic acid to maleylacetoacetate. Alkaptonuria is characterized by high concentrations of homogentisic acid in body tissues and fluids including urine. When homogentisic acid oxidizes, it turns a dark color, thus making the urine appear black when exposed to air. People with alkaptonuria often experience joint problems (such as arthritis) as the homogentisic acid accumulates in connective tissues. Dietary treatment is not usually prescribed.

Tryptophan

Another aromatic amino acid metabolized principally by the liver is tryptophan (Figure 6.11). Tryptophan is partially glucogenic because it is catabolized to form pyruvate; it is also partially ketogenic, forming acetyl-CoA as shown in Figure 6.9.

- The first step in tryptophan catabolism is catalyzed by the heme-iron-dependent tryptophan dioxygenase and produces N-formylkynurenine. Tryptophan dioxygenase is induced by glucagon as well as cortisol.
- Further catabolism of N-formylkynurenine yields formate and kynurenine. Kynurenine is metabolized by a monooxygenase to 3-hydroxykynurenine. Kynureninase, a vitamin B₆ (PLP)-dependent enzyme, converts 3-hydroxykynurenine to 3-hydroxyanthranilate and alanine. This alanine can be transaminated to form pyruvate, hence the glucogenic nature of tryptophan.
- Further catabolism of 3-hydroxyanthranilate forms 2-amino 3-carboxymuconic 6-semialdehyde. This compound is further metabolized to produce many additional compounds, including picolinate (a possible binding ligand for minerals), and 2-aminomuconic 6-semialdehyde, which is further metabolized in several reactions to acetyl-CoA (a ketogenic intermediate).

Tryptophan also has other fates in the body, as shown in Figure 6.11 and later in Figure 6.16. For example:

- Tryptophan metabolism through N-formylkynurenine generates the B vitamin niacin as nicotinamide as well as its coenzyme form nicotinamide adenine dinucleotide phosphate (NADP). Deficient protein intake and tryptophan malabsorption limit niacin synthesis in the body.
- Tryptophan is also used for the synthesis of serotonin (5-hydroxytryptamine) and melatonin (N-acetyl 5-methoxyserotonin). Melatonin is made primarily in the pineal gland, which lies in the center of the brain. Melatonin synthesis and release correspond with darkness; the hormone is thought to be involved mainly with the regulation of circadian rhythms and sleep. Supplement use has been helpful for some people

with jet lag. Serotonin functions throughout the body including in the GI tract. It promotes vasoconstriction and smooth muscle contraction. It also functions as a neurotransmitter.

Disorders of Tryptophan Metabolism Inherited disorders have been identified in tryptophan degradation. One disorder, α -ketoacidic aciduria, results from the defective activity of α -ketoacidic dehydrogenase, which converts α -ketoacidate to glutaryl-CoA. With this disorder, lysine, tryptophan, α -aminoacidate, α -ketoacidate, and α -hydroxyacidate build up in the blood and other body fluids. Affected infants become hypotonic, acidotic, and experience seizures and motor and developmental problems. Another autosomal recessive condition, glutaric aciduria (or acidemia) type 1, results from the defective activity of the riboflavin (as FAD)-dependent enzyme glutaryl-CoA dehydrogenase, which converts glutaryl-CoA to glutaconyl-CoA. As with α -ketoacidic aciduria, the enzyme glutaryl-CoA dehydrogenase is critical to the catabolism of two amino acids, tryptophan (Figure 6.11) and lysine (shown later in Figure 6.13). In glutaric aciduria type 1, glutaryl-CoA builds up and is converted to glutaric acid, which also accumulates in body fluids. Over time, affected infants develop acidosis, **ataxia**, seizures, and macrocephaly, among other problems. Treatment of these two conditions requires a diet restricted in both lysine and tryptophan (because the reactions are common in both tryptophan and lysine degradation). For some individuals with glutaric aciduria type 1, riboflavin supplements have been shown to enhance residual glutaryl-CoA dehydrogenase activity.

Hepatic Catabolism and Uses of Sulfur-Containing Amino Acids

The catabolism of methionine, a sulfur (S)-containing essential amino acid, occurs to a large extent in the liver and generates cysteine, another S-containing nonessential amino acid.

Methionine

Methionine is a glucogenic amino acid with its oxidation generating the TCA cycle intermediate succinyl-CoA (Figure 6.9). Methionine metabolism, shown in Figure 6.12, is described briefly here, and some of its uses are depicted later in Figure 6.16.

- The first step in methionine catabolism is the conversion of methionine to S-adenosyl methionine (SAM) by methionine adenosyl transferase (present in high concentrations in the liver) in an ATP-requiring reaction. SAM, the body's principal methyl donor, has many functions.
 - SAM provides methyl groups for the synthesis of nonprotein nitrogen-containing compounds, including carnitine and creatine.

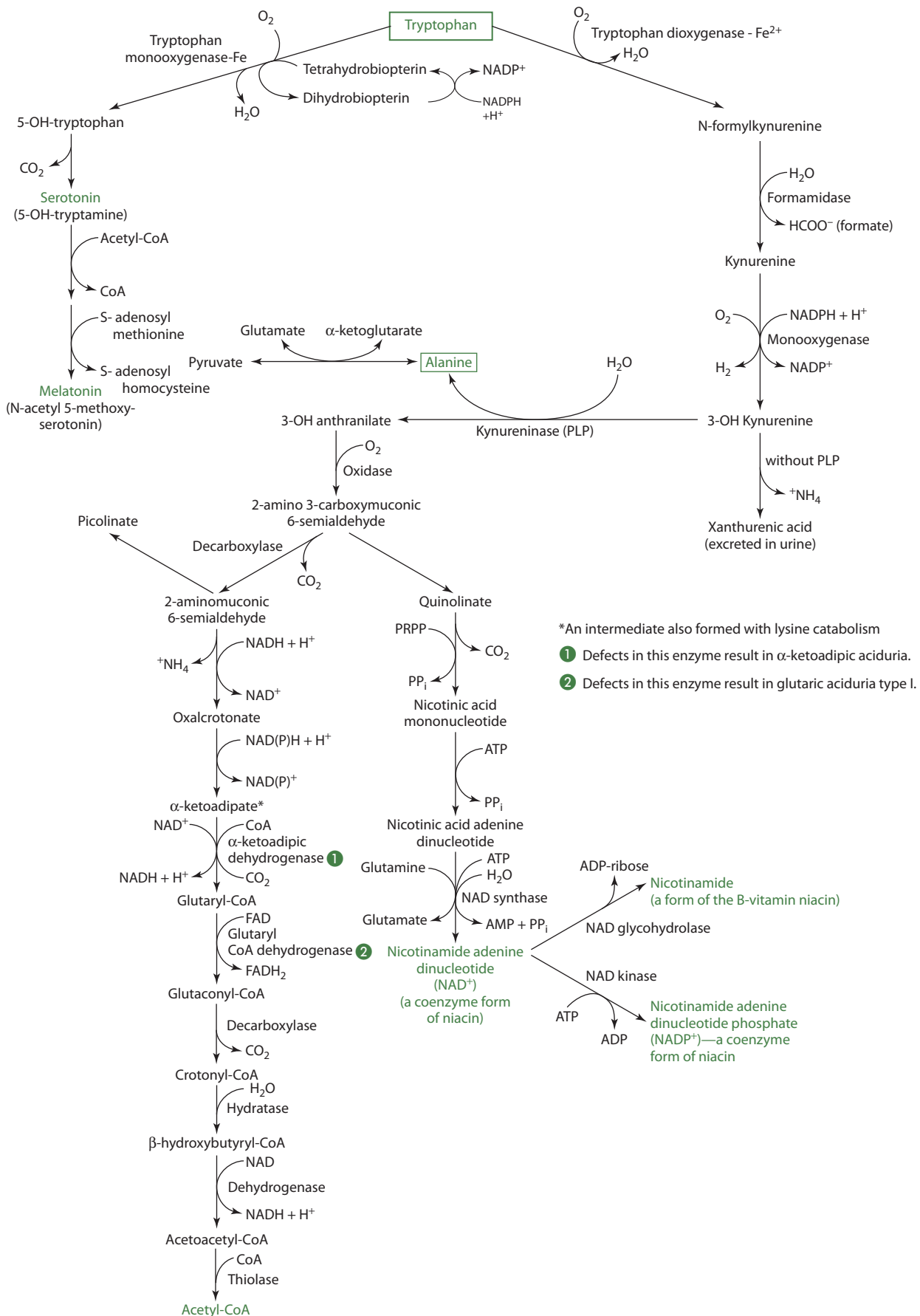


Figure 6.11 Tryptophan metabolism.

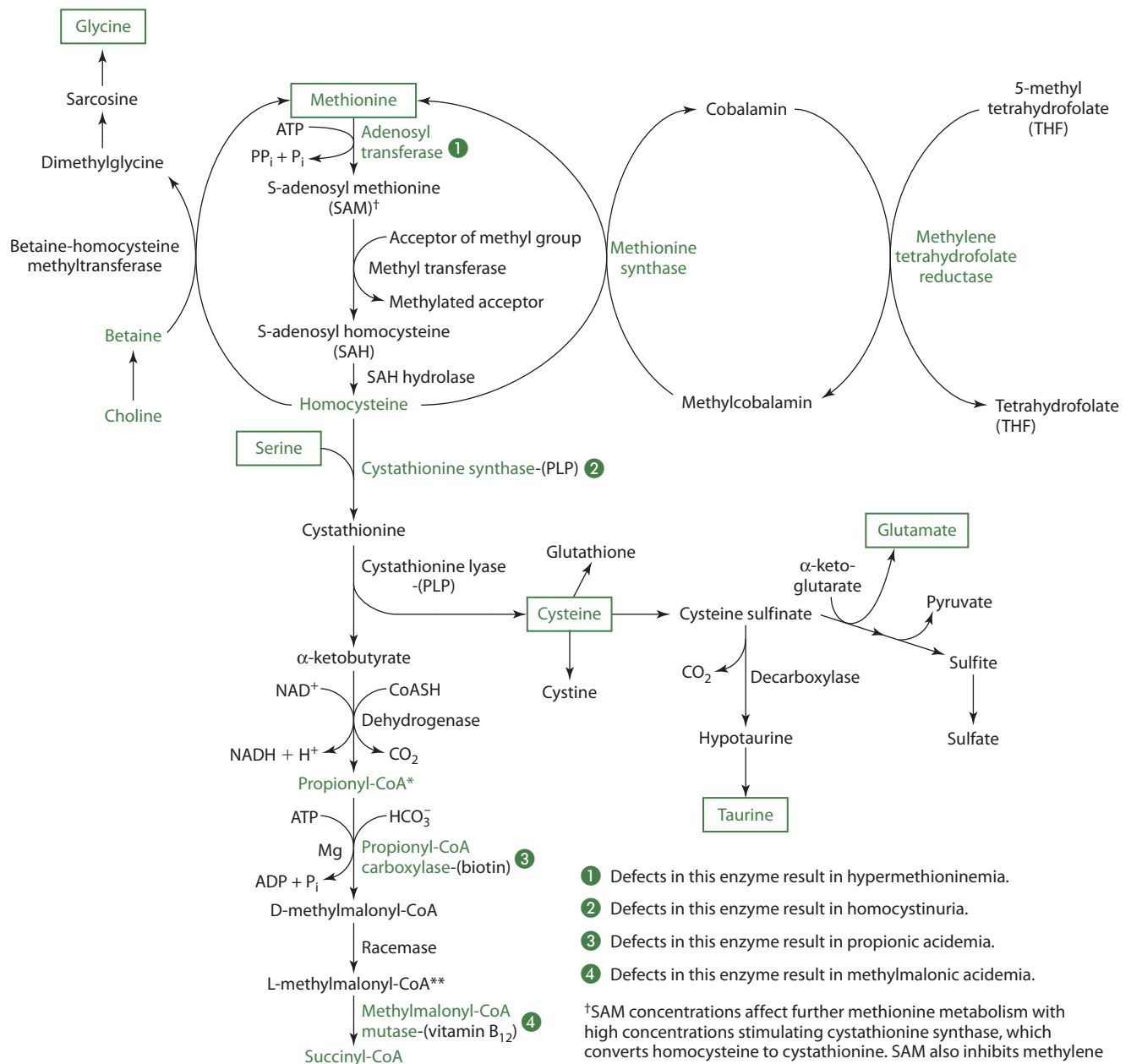


Figure 6.12 Methionine and cysteine metabolism.

- SAM's methyl groups are needed for the synthesis of hormones such as epinephrine and melatonin.
- SAM is needed for the metabolism of arsenic.
- SAM's methyl groups are needed to maintain myelin. Inadequate methylation of myelin disrupts nerve cell functions and ultimately may lead to neuropathy and ataxia, among other problems. Myelin, which is made from proteins and various lipids, surrounds and insulates the axon of a neuron, enabling faster conduction of nerve impulses.
- SAM's methyl groups are used to methylate DNA and histone proteins and thus affect gene expression.
- SAM affects membrane fluidity by providing methyl groups for the methylation of phospholipids in cell membranes.
- SAM may be decarboxylated to form S-adenosyl methylthiopropylamine, an intermediate in the synthesis of the polyamines—putrescine, spermidine, and spermine. Polyamines are important in cell division and growth.

- The removal or donation of the methyl group from SAM yields the compound S-adenosyl homocysteine (SAH). SAH is converted to homocysteine and adenosine by S-adenosyl homocysteine hydrolase in a reversible reaction that favors SAH synthesis. SAH or a low SAM-to-SAH ratio diminishes methylation reactions. SAH competes with SAM for the active site on the methyltransferase.
- Homocysteine, once formed, can be converted back to methionine either in a betaine-dependent reaction or in a vitamin B₁₂- and folate-dependent reaction.
- Betaine, obtained from the diet or generated in the liver from choline oxidation, provides a methyl group that is transferred to homocysteine by the hepatic enzyme betaine homocysteine methyltransferase. With the loss of the methyl group, betaine becomes dimethylglycine. Dimethylglycine can be further demethylated to generate glycine.
- The methylation reaction to form methionine via methionine synthase (also called homocysteine methyltransferase) requires cobalamin (vitamin B₁₂) as a coenzyme. Cobalamin receives the methyl group that is needed from 5-methyl THF, a derivative of folate. Failure to form methionine due to deficiencies in folate or vitamin B₁₂ can lead to elevated plasma homocysteine concentrations, which have been shown to interfere with collagen cross-linking in bone and increase fracture risk. High plasma homocysteine concentrations are also a risk factor for heart disease and stroke.
- To be further metabolized, homocysteine reacts with the amino acid serine, forming cystathionine through the action of cystathionine (β) synthase. The enzyme requires vitamin B₆ in its PLP coenzyme form. A deficiency of vitamin B₆, like folate and vitamin B₁₂, can lead to elevated plasma homocysteine concentrations.
- Further catabolism of cystathionine requires cystathionine β lyase, another vitamin B₆ (as PLP)-dependent enzyme, and forms the dispensable amino acid cysteine. Also generated in the reaction is α-ketobutyrate, which is further decarboxylated to propionyl-CoA. The conversion of homocysteine to cysteine by cystathionine synthase and cystathionine lyase is sometimes called the transsulfuration pathway. These reactions occur in the liver but also in the kidneys, intestine, and pancreas.
- Propionyl-CoA (made from α-ketobutyrate) is next converted to D-methylmalonyl-CoA by the biotin-dependent enzyme propionyl-CoA carboxylase. D-methylmalonyl-CoA is then converted to L-methylmalonyl-CoA by a racemase. Then L-methylmalonyl-CoA is converted by methylmalonyl-CoA mutase, a vitamin B₁₂-dependent enzyme, to the TCA cycle intermediate succinyl-CoA. See Figure 6.12.

Disorders of Methionine Metabolism Mutations in the gene for methionine adenosyl transferase, the enzyme that converts methionine to S-adenosyl methionine (SAM), result in hypermethioninemia. This condition's high blood methionine concentrations necessitate treatment with a diet restricted in methionine but increased in cysteine.

The genetic disorder homocystinuria results from defects in cystathionine β synthase, which converts homocysteine to cystathionine. The condition, which affects about 1 in 200,000–300,000 people worldwide, but about 1 in 65,000 in Ireland, is characterized by high blood homocysteine and methionine and low blood cysteine concentrations. The high plasma homocysteine concentrations can promote blood clot (thrombi) formation as well as increase the risk for heart disease. Other selected manifestations include skeletal problems including osteoporosis, ocular changes, and impaired mental development. Treatment requires a diet low in methionine (and thus low intakes of protein-containing foods), added cysteine, and in some cases supplements of betaine and folate.

The genetic disorder propionic acidemia (with an incidence of about 1 in 35,000–70,000 but as many as 1 in 1,000 in Greenland and 1 in 2,000–3,000 in Saudi Arabia) results from mutations in the gene coding for propionyl-CoA carboxylase, a biotin-dependent enzyme. Another disorder caused by genetic errors in the same pathway is methylmalonic acidemia, which results from impaired methylmalonyl-CoA mutase activity and affects about 1 in 48,000. Propionic acidemia is characterized by the accumulation of propionic acid in body fluids, and in methylmalonic acidemia, both propionic and methylmalonic acids (as well as other compounds such as methylcitrate, 3-hydroxy propionate, and tiglic acid) accumulate in body fluids. Infants exhibit excessive vomiting, ketoacidosis, hypertonia, failure to thrive, and respiratory difficulties, among other problems. Because propionyl-CoA and thus methylmalonyl-CoA are generated from not only methionine, as shown in Figure 6.12, but also from the degradation of both threonine (shown later in Figure 6.15), isoleucine, and valine (see later Figure 6.36), treatment of both conditions requires restriction of these amino acids. In addition, odd-chain fatty acids and polyunsaturated fatty acids (in excessive amounts) generate propionyl-CoA and thus foods containing large amounts of these fatty acids must be restricted. In some cases, biotin supplements may improve the activity of propionyl-CoA carboxylase, but a restricted diet is still typically needed. Similarly, vitamin B₁₂ supplements can sometimes improve methylmalonyl-CoA mutase activity in those with methylmalonic acidemia.

Cysteine

Cysteine is a nonessential amino acid. Hepatic concentrations of free cysteine appear to be tightly controlled. Cysteine is used like other amino acids for protein synthesis. It is also used to synthesize glutathione (discussed further in later sections of the chapter).

- Cysteine is metabolized by cysteine dioxygenase to cysteine sulfinate, which is used to produce the amino acid taurine (Figure 6.12). Taurine, a β -amino sulfonic acid, is made in the liver from cysteine but concentrated in muscle and the central nervous system; it is also found in smaller amounts in the heart, liver, and kidneys, among other tissues. Although taurine is not involved in protein synthesis, it is important in the retina, where it maintains membrane stability and photoreceptor cell function through its antioxidant abilities (such as scavenging peroxidative [e.g., oxychloride] products). Taurine also is found in the liver and intestine as a bile salt, taurocholate, and in the central nervous system as an inhibitory neurotransmitter.
- Cysteine degradation (Figure 6.12) yields pyruvate and sulfite. Sulfite is converted by sulfite oxidase (an iron- and molybdenum-dependent enzyme) to sulfate, which can be excreted in the urine or used to synthesize sulfolipids and sulfolipoproteins. Sulfate is also found in foods and is absorbed, via a sodium-sulfate co-carrier protein NaSl, in the small intestine.

Hepatic Catabolism and Uses of Branched-Chain Amino Acids

The liver plays only a minor role in the initial catabolism of the three branched-chain amino acids— isoleucine, leucine, and valine. Transaminase activity needed to remove the amino groups is minimal in the liver, although hepatic transferases increase in response to glucocorticoids (cortisol), as may occur with infection, burns, trauma, or prolonged fasting. Thus, under normal circumstances, the branched-chain amino acids typically remain in circulation and are taken up and transaminated primarily by the skeletal muscle, but also by the heart, kidneys, diaphragm, and adipose tissue, if needed. Alpha-keto acids of the branched-chain amino acids, generated from branched-chain amino acid transamination, may be used within the tissues or released into circulation. The liver, among other organs, can further catabolize these α -keto acids. Additional information on branched-chain amino acid metabolism is found under “Skeletal Muscle” in the “Interorgan ‘Flow’ of Amino Acids and Organ-Specific Metabolism” section of this chapter.

Hepatic Catabolism and Uses of Basic Amino Acids

Lysine

The catabolism of lysine, a totally ketogenic amino acid, generates acetyl-CoA, as shown in Figures 6.13 and 6.9. The main pathways responsible for the degradation of lysine include the saccharopine pathway and the pipecolic acid pathway. While these pathways differ initially,

they converge with the formation of α -amino adipic semialdehyde. This later compound is next metabolized via α -amino adipic semialdehyde dehydrogenase to create α -amino adipic acid, which is then used to produce α -ketoadipate. Lysine and tryptophan degradation both produce α -ketoadipate, which can be referred to as a common intermediate in both pathways, and thus the amino acids share some common reactions in their metabolism.

Lysine has other important uses in the body. After being methylated using SAM (made from methionine), lysine is used in the synthesis of carnitine (shown later in Figure 6.23), which is needed for fatty acid oxidation. In addition, within collagen, the amino acid (along with proline) is hydroxylated, forming hydroxylysine. These hydroxylated amino acids within collagen facilitate cross-linking among collagen strands to improve the strength of collagen.

Disorders of Lysine Metabolism Defects in lysine degradation due to mutation in the genes coding for glutaryl-CoA dehydrogenase and α -ketoadipate dehydrogenase result in glutaric aciduria type 1 and α -ketoadipic aciduria, respectively, as discussed in the “Disorders of Tryptophan Metabolism” section and shown in Figure 6.11. In addition, mutations in the gene coding for α -amino adipic semialdehyde dehydrogenase, which converts α -amino adipic semialdehyde to α -amino adipic acid (Figure 6.11), leads to the accumulation of both α -amino adipic semialdehyde and piperidine 6-carboxylate and to the inactivation of pyridoxal phosphate (PLP), the main coenzyme form of vitamin B₆. The inactivation of the coenzyme is thought to result from a reaction that occurs between PLP and piperidine 6-carboxylate. Individuals with defective dehydrogenase activity experience seizures and developmental delays. Supplementation with vitamin B₆ can sometimes help to ameliorate some manifestations.

Arginine

Arginine is metabolized mostly in the liver and kidneys but also in the intestine, lungs, and leukocytes. It is a glucogenic amino acid, as its catabolism generates the TCA cycle intermediate α -ketoglutarate (Figure 6.9). Arginine, however, also has several other uses, as shown in Figure 6.14 and later in Figure 6.16.

- In the liver, arginine is primarily degraded as part of the urea cycle to form urea and ornithine. Ornithine may be decarboxylated to form polyamines (putrescine, spermine, and spermidine), or it may be transaminated by ornithine aminotransferase to form, through a series of reactions, the amino acid proline. Polyamines are thought to function in cell signaling, growth, and proliferation.

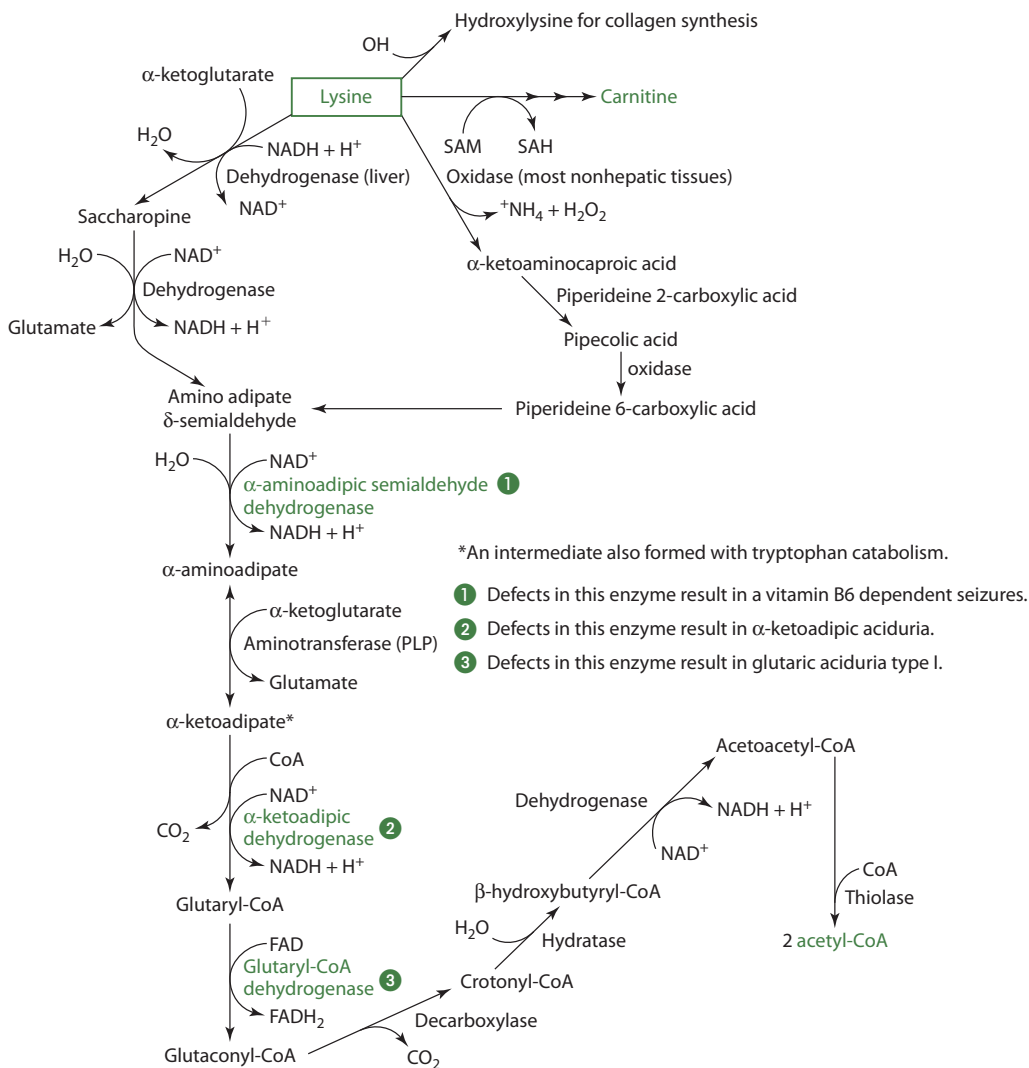


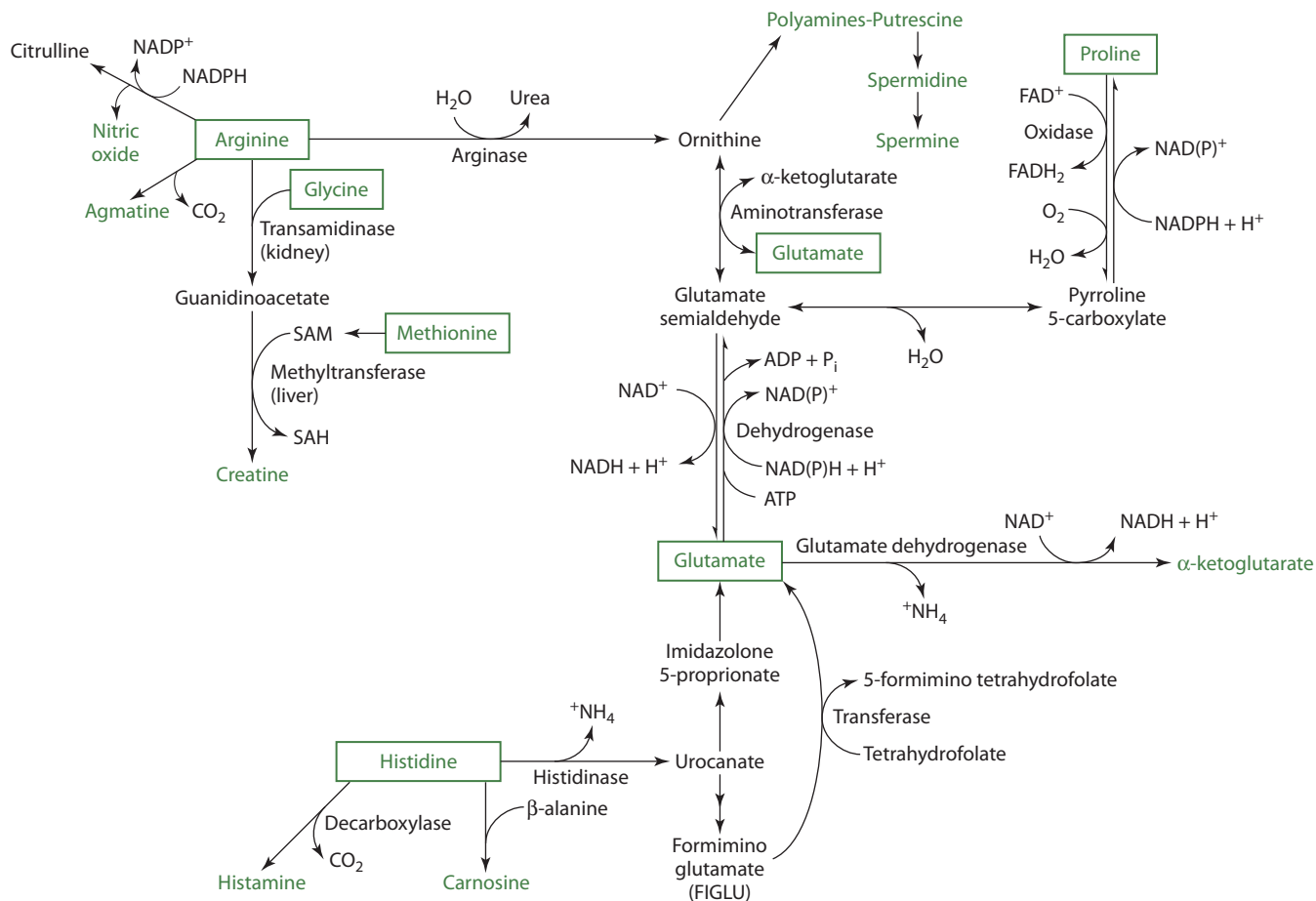
Figure 6.13 Lysine metabolism.

- In the kidneys, arginine is used with glycine to produce guanidinoacetate in a reaction catalyzed by arginine glycine amidotransferase. Guanidinoacetate is then released into the blood and travels to the liver, where it is converted into creatine. It is the kidneys that are also the primary site of arginine synthesis from citrulline, which was produced in the intestinal cells.
- In the kidneys, arginine can also react with lysine (instead of glycine), with the resulting production of homoarginine and ornithine via the actions of arginine glycine amidotransferase. Increased plasma homoarginine concentrations have been associated inversely with mortality in individuals who have suffered a stroke. Homoarginine is speculated to inhibit the activities of arginase and nitric oxide synthase [6].
- Agmatine, made in the brain and central nervous system, among other organs, from the decarboxylation of arginine via arginine decarboxylase, is involved in neuromodulatory functions.
- In endothelial cells, cerebellar neurons, neutrophils, and splanchnic tissues, arginine is used for nitric oxide production in a reaction catalyzed by nitric oxide synthase. Arginase activity influences arginine availability, with higher enzyme activity limiting arginine availability for nitric oxide synthesis.
- Methylation of arginine residues occurs in some proteins by arginine methyl transferases. When these proteins are degraded, various methylated arginine forms are released including asymmetric (ADMA) and symmetric dimethylarginine (SDMA) and N^G-monomethyl arginine. These are then excreted by the kidney intact or with some additional metabolism. Increased plasma ADMA concentrations have been associated with increased risk of cardiovascular disease and hypertension. While the mechanism of action is still being elucidated, ADMA is thought to inhibit nitric oxide synthase activity and/or the nitric oxide function. Some of the roles of nitric oxide are provided in the boxed region on page 211.

oxide, a compound known to stimulate the glutathionylation of proteins. This posttranslational modification is thought to affect the function and stability of many cellular proteins.

- Through a vitamin B₆ (as PLP)–dependent decarboxylation reaction, the amine histamine can be formed from histidine. Histamine functions in the brain as a neurotransmitter. In the GI tract, it has many roles including the stimulation of gastric secretions such as hydrochloric acid. In mast cells (found throughout the body in locations such as within the nose and mouth, blood vessels, and internal body surfaces) and in white blood cells (especially basophils), histamine exhibits immunologic roles. It stimulates constriction of bronchial smooth muscle and causes dilation or increased permeability of capillaries to facilitate white blood cell

- Histidine may combine with β -alanine to generate carnosine, which is discussed in more detail in the



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infiltration and **phagocytosis** of foreign antigens. This increased permeability may result in flushing (redness) of the skin and in the escaping of fluid into tissues, causing a runny nose and watery eyes.

- Another interesting fate of histidine relates to its post-translational modification. Specifically, histidine, in some body proteins like actin in muscle, becomes methylated and when the protein is broken down, the methylated histidine, called 3-methylhistidine (see Table 6.1), is released but cannot be reused to synthesize another protein. Because it cannot be reused, the 3-methylhistidine is excreted in the urine and is often used as an index of muscle catabolism. This topic is also discussed in the “Skeletal Muscle” section later in this chapter.

Hepatic Catabolism and Uses of Other Selected Amino Acids

Threonine

Threonine can be metabolized (as shown in Figure 6.15) by three different pathways and consequently is both glucogenic and ketogenic (Figure 6.9).

- One of the more commonly used pathways of degradation is through cytosolic threonine dehydratase to generate α -ketobutyrate, which is further catabolized to propionyl-CoA, then to D-methylmalonyl-CoA, L-methylmalonyl-CoA, and ultimately succinyl-CoA.

These latter steps in the catabolism are shared with methionine and isoleucine and valine.

- Alternately, threonine may be degraded by mitochondrial threonine dehydrogenase to form aminoacetone, which is converted to methylglyoxal and then pyruvate. This pathway is thought to be used if cytosolic threonine concentrations are relatively high.
- In a third pathway, the mitochondrial threonine cleavage complex (composed of a dehydrogenase and a ligase) converts threonine to glycine and acetaldehyde; acetaldehyde is further metabolized to acetate and then to acetyl-CoA in an ATP- and CoA-dependent reaction. Glycine is discussed in the next section.

Threonine is found in fairly high concentrations relative to other amino acids in the glycoproteins of mucus. Consequently, in situations of intestinal inflammation characterized by excess mucus production, threonine needs are thought to be elevated.

Disorders of Threonine Metabolism Defects in two steps of threonine metabolism from propionyl-CoA to succinyl-CoA can lead to propionic acidemia and methylmalonic acidemia, as shown in Figure 6.15 and previously discussed in the “Disorders of Methionine Metabolism” section.

Glycine and Serine

Glycine and serine can be produced from one another in a reversible reaction that uses folate as tetrahydrofolate (THF) and 5,10 methylene THF (also called N⁵ N¹⁰

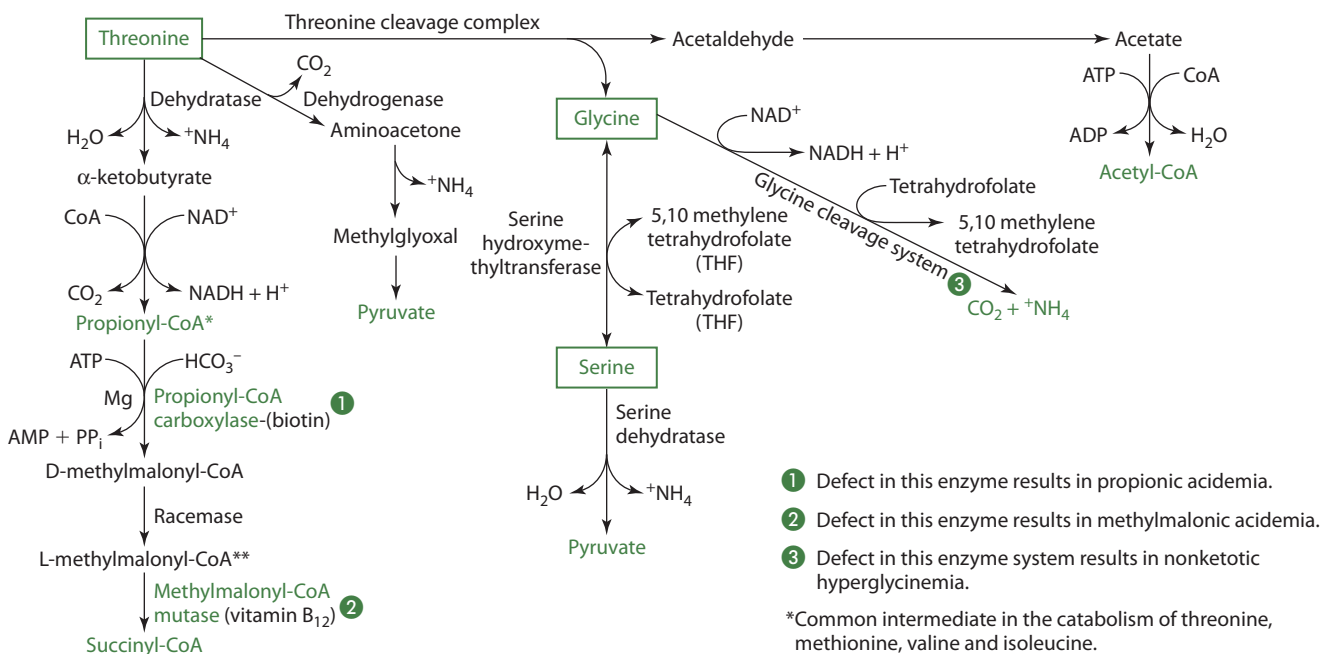


Figure 6.15 Threonine, glycine, and serine metabolism.

methylene THF; Figure 6.15) as cosubstrates. The reaction, which is catalyzed by the vitamin B₆ (as PLP)–dependent enzyme serine hydroxymethyltransferase, occurs in the liver and kidneys. Serine, if not needed for glycine production, can be deaminated/dehydrated to form pyruvate. Glycine, if not needed for serine production, can be catabolized via the glycine cleavage system to carbon dioxide and an ammonium ion in a folate (as THF)–dependent and niacin (as NAD⁺)–dependent reaction. Glycine is also used for the synthesis of glutathione, creatine, porphyrins, and the bile salt glycocholate. Serine is used for the synthesis of ethanolamine and choline for phospholipids.

Disorders of Glycine Metabolism Impaired glycine catabolism, secondary to mitochondrial glycine cleavage system defects, results in nonketotic hyperglycinemia. Infants with this autosomal recessive condition exhibit seizures, neurologic deterioration, flaccidity, and lethargy, among other problems. Blood and other body fluids contain increased glycine concentrations. Treatment requires a protein-restricted diet.

Figure 6.16 provides a summary of uses of some amino acids. The next section of the chapter further elaborates on anabolic uses of amino acids in the body.

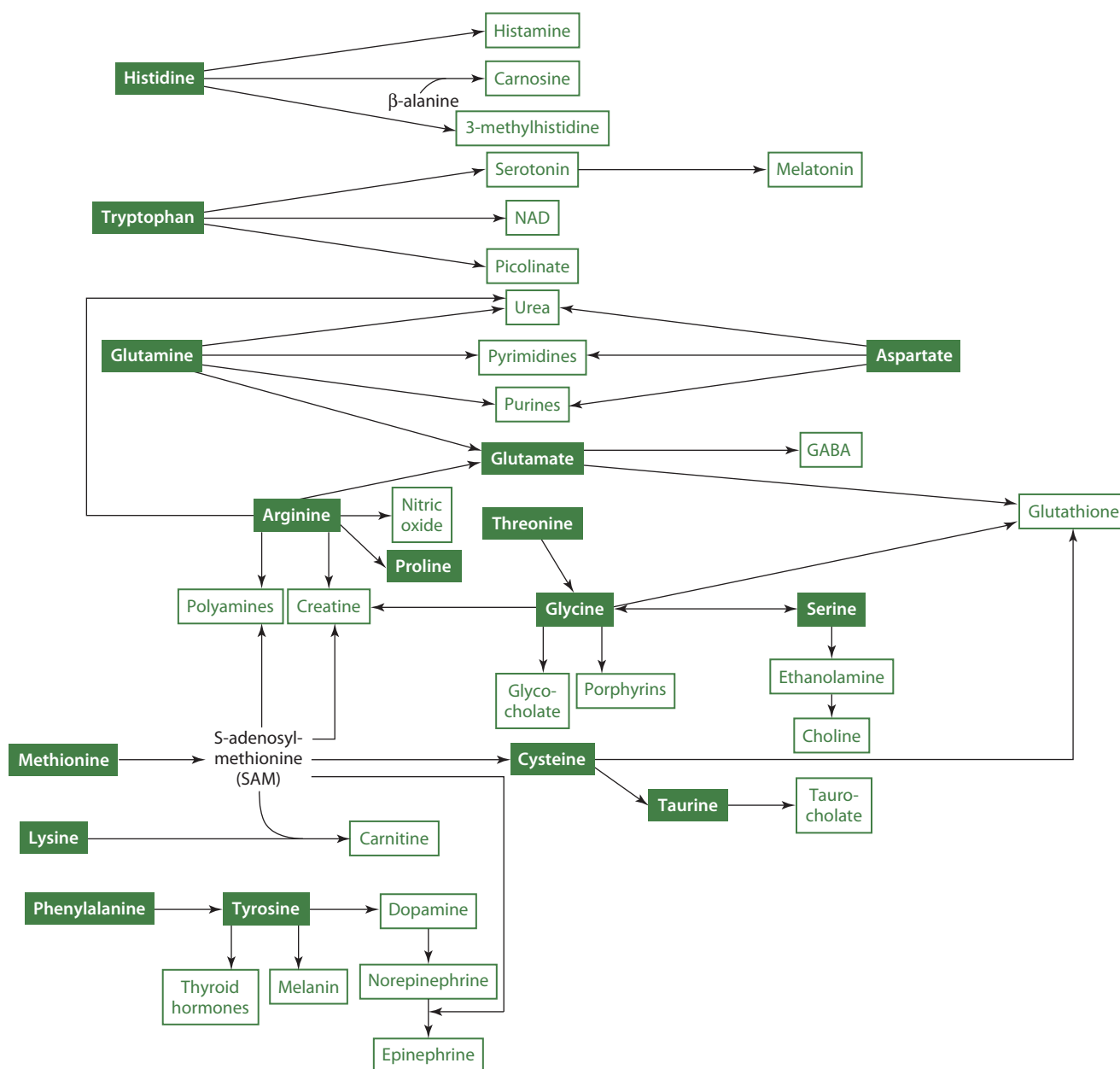


Figure 6.16 A summary of the uses of selected amino acids for the synthesis of nitrogen-containing compounds and selected biogenic amines, hormones, and neuromodulators.

6.6 PROTEIN SYNTHESIS

Anabolism, including protein synthesis, increases in tissues following the ingestion of food, which provides the amino acids and energy necessary to build body proteins. Each cellular protein exhibits a specific and characteristic rate of synthesis that is influenced by multiple, interdependent but converging actions. On a cellular level, some amino acids, for example, may directly affect gene expression through interactions with amino acid-responsive elements in the promoter regions of genes. Translation within cells is affected by the amount and stability of mRNA, the ribosome number (amount of ribosomal RNA, or rRNA), the activity of the ribosomes (rapidity of translation or peptide formation), the presence of amino acids in the appropriate concentrations to attach to the tRNA (referred to as charged tRNA), and the hormonal environment, which in turn can be influenced by nutrients. On a more systemic level, increased plasma amino acids, increased blood flow, and hormone release, especially insulin, generally enhance muscle protein synthesis, which occurs for several hours following food ingestion. Physical activity (exercise) further enhances protein synthesis in muscle.

Slow versus Fast Proteins

The form and nature of the ingested dietary protein influence its digestion and absorption, the rate of appearance of the amino acids in the plasma, and the subsequent utilization of the amino acids within cells. For example, amino acids released following ingestion and digestion of “fast proteins,” which include whey protein, some soy proteins, amino acid mixtures, and protein hydrolysates (e.g., usually partially hydrolyzed whey protein), appear to be utilized differently in the body when compared with amino acids released following ingestion and digestion of “slow proteins” such as casein. Ingestion of fast proteins better stimulates muscle protein and whole-body protein synthesis than slow proteins both at rest and following resistance exercise. Ingestion of foods providing slow proteins, however, is important as the lower and more prolonged plasma amino acid concentrations that result from ingestion of these proteins help reduce protein breakdown, which predominates in the postabsorptive state (between meals and overnight). Thus, consuming foods (such as milk/dairy products) that provide a combination of “fast” and “slow” proteins (whey and casein, respectively) enable protein synthesis while reducing protein degradation. Other factors, however, also significantly impact digestion and absorption rates, including the presence and nutrient composition of other foods co-consumed with the protein source. Delays in gastric emptying secondary to ingestion

of higher-fat foods/beverages or higher-fiber foods, for example, would in turn slow the digestive and absorptive processes and alter the plasma amino acid response and in turn impact protein synthesis rates.

Plant versus Animal Proteins

Plant-based protein sources are typically limiting in one or more essential amino acids, most often methionine and/or lysine, and have lower digestibility when compared with animal-based protein sources. (See the later sections of the chapter on “Protein Quality” and “Protein and Amino Acid Needs.”) Animal-based protein sources are also usually higher in leucine (helpful in promoting muscle protein synthesis, as discussed later in this section under “Hormonal Effects” and “mTor, Intracellular Signaling, and Amino Acids”) than plant-based protein sources. These dietary differences can affect muscle protein synthesis, with lower synthesis typically observed when plant-based diets are consumed versus animal-based diets. Postprandial concentrations of amino acids that do not contain enough of all the essential amino acids (as may occur with consumption of solely plant-based dietary proteins) are thought to reduce amino acid availability to tissues for protein synthesis and stimulate hepatic oxidation of amino acids and ureagenesis [7]. Higher consumption of animal-based protein foods has been associated with greater gains in lean body mass in individuals participating in resistance/strength-training exercises, and with better maintenance of muscle mass in individuals not engaged in resistance training [8]. Ingestion of higher amounts of plant foods at each meal or supplementation of plant foods with selected amino acids or some animal-based protein foods at meals may better promote anabolism [8]. Much additional research, however, is needed since relatively few protein sources have been evaluated for their effects on muscle and whole-body protein synthesis.

Hormonal Effects

Hormones play a major role in amino acid utilization for protein synthesis. During prolonged periods in which food is not eaten, such as during the overnight hours or a more prolonged fast, protein synthesis still occurs but at a much lower rate (than occurs postprandially), and protein degradation predominates. The degradative processes are stimulated by epinephrine and cortisol release and by the higher glucagon-to-insulin ratio in the blood. This higher glucagon-to-insulin ratio diminishes insulin's ability to inhibit protein degradation and diminishes the overall rate of protein synthesis. Yet, while skeletal muscle experiences more extensive protein

degradation and limited protein synthesis during postabsorptive periods, the glucagon-to-insulin ratio favoring glucagon stimulates the hepatic synthesis of some proteins, such as enzymes for gluconeogenesis and ureagenesis. Higher blood cortisol concentrations (which typically rise with depletion of hepatic glycogen stores occurring with fasting or with injury, sepsis, burns, etc.) further promote muscle protein catabolism and hepatic use of the amino acids for gluconeogenesis and ureagenesis.

In contrast to the general catabolic nature of glucagon, epinephrine, and cortisol, other hormones, such as insulin, are anabolic. Insulin increases protein synthesis and decreases protein degradation. (*Note:* Insulin is secreted in response to a rise in incretins [glucose-dependent insulinotropic peptide and glucagon-like peptide 1 released in response to glucose in the digestive tract], a rise in blood glucose, and a rise in some blood amino acid concentrations [as occurs with food consumption].) This effect typically occurs to a greater extent if both carbohydrate- and protein-containing foods are coingested versus ingestion of either carbohydrate or protein alone.

Insulin, upon binding to its receptors in cell membranes, exhibits multiple actions to promote protein synthesis. Insulin, for example, generally stimulates the transcellular movement of amino acid transporters to the cell membrane for use in amino acid uptake and increases the overall activity of amino acid transporters, including systems A, ASC, and N in the liver, muscle, and other tissues. Insulin antagonizes the activation of some enzymes responsible for amino acid oxidation (degradation); the phosphorylation and thus activation of phenylalanine hydroxylase (which degrades phenylalanine), for instance, is inhibited by insulin. Thus, insulin promotes the uptake of the amino acids into the tissues and inhibits the enzymes that are responsible for the degradation of the amino acids. Such actions of insulin facilitate the use of the amino acids for protein synthesis.

Further actions of insulin to promote protein synthesis include its stimulatory effects on nitric oxide synthase; this enzyme increases nitric oxide production, which through multiple mechanisms, enhances the flow of amino acid-rich blood to tissues. Insulin, along with the amino acid leucine (which by itself acts as an insulin secretagogue [i.e., it stimulates insulin secretion]), also plays other roles in stimulating protein synthesis. While a more in-depth discussion of all the mechanisms of insulin's actions upon binding to insulin receptors on cell membranes is beyond the scope of this section, some of insulin's intracellular effects result in the stimulation of the mammalian target of rapamycin (mTOR) (see the next section).

mTOR, Intracellular Signaling, and Amino Acids

mTOR is a large protein kinase complex that consists of two units: complex 1 (mTORC1) and 2 (mTORC2); each complex is further made up of several components. mTORC1 functions as part of a signal transduction pathway, primarily stimulating protein synthesis and overall anabolism through multiprotein complexes and activities. mTORC1 activity is influenced by multiple factors including insulin, leucine (both in the plasma and intracellularly, and perhaps a metabolite of leucine, β -hydroxy- β -methylbutyrate abbreviated HMB), and G-protein-coupled receptor activation, among other factors. (A complete understanding of the regulation of mTOR remains unclear.)

mTORC1 and another protein kinase called general control nonderepressible 2 (GCN2) appear to serve as amino acid sensors and link the information on amino acid availability to protein synthesis (translation). GCN2 becomes activated by higher concentrations of uncharged transfer RNAs (tRNAs), a situation that occurs when insufficient amino acids are present in the cell. mTORC1 is also inactivated under conditions of amino acid deprivation; the signaling to mTORC1 is thought to arise, at least in part, from G-protein-coupled receptor activity, which is influenced by the binding of arginine to the receptor. GCN2 kinase down-regulates mTORC1 kinase activity through binding and phosphorylation of one of mTORC1's regulatory subunits. In the presence of higher amounts of amino acids, mTORC1 kinase activity increases.

mTORC1 actions/signaling promote the synthesis of regulatory components and key enzymes required for mRNA translation and protein synthesis. S6 kinase 1 (S6K1) also acts on target proteins that regulate the translation of specific mRNAs. The specific mRNAs affected by S6K1 code for proteins needed for the translation of ribosomal proteins. Thus, the production of the synthetic machinery for translation is influenced by both insulin and leucine. mTORC1 signal activation occurs within about 30 minutes of meal consumption, and maximum protein synthesis occurs at about 1–1½ hours after eating and then declines [9].

Amino acids also affect two phases, initiation and elongation, of mRNA translation for protein synthesis. Initiation of translation involves numerous eukaryotic initiation factors (eIFs), which are needed for the delivery of Met-tRNA^{MET} (the initiation transfer RNA) to the 40S ribosomal subunit and for the formation of a pre-initiation complex. One of the eIFs needed for the binding of Met-tRNA^{MET} to the ribosomal subunit is eIF2, whose activity is enhanced by amino acid availability and diminished with amino acid depletion within cells.

Additionally, in initiation, the binding of the mRNA to the preinitiation complex relies on several additional eIFs. Amino acids, especially leucine, and insulin through interactions with mTOR increase the phosphorylation of binding protein(s) that in turn enhance some of the eIFs' actions directed at mRNA-preinitiation complex binding. These effects in turn stimulate protein synthesis.

Another example of the role of leucine is in the elongation phase of translation. In elongation, translocation of the ribosomes along the mRNA must occur following the addition of an amino acid to the growing peptide. Eukaryotic elongation factor 2 (eEF2), which mediates the translocation, is regulated by a kinase whose activity appears to be affected by leucine, insulin, and mTORC1.

Protein Intake, Distribution, and Quantity at Meals

Recent studies on protein synthesis have focused on dietary approaches to maximize muscle protein synthesis and improve muscle mass, strength, and function. Older adults have been the focus of much of this research due to reductions that occur in muscle mass, strength, and function with aging (see the section “Changes in Body Mass with Age”). Protein should be ingested in sufficient

quantities on a daily basis for health (including muscle health). Specific recommended amounts of protein per meal to maximize muscle protein synthesis vary with the protein source and with age (younger vs. older adults). In addition, a more even (vs. skewed) distribution of sufficient quantities of protein among meals has also been shown to promote better muscle protein synthesis over a 24-hour period and, long term, better maintenance of muscle mass, strength, and physical function in older adults [8–10]. More specific recommendations for protein intake are discussed in the sections “Changes in Body Mass with Age” and “Recommended Protein and Amino Acid Intakes.”

6.7 PROTEIN STRUCTURE AND ORGANIZATION

Proteins begin to fold and take “shape” as they are synthesized on the ribosomes. The shape or structure and organization of proteins are designated as primary, secondary, tertiary, and quaternary, although not all proteins have this fourth level (see Figures 6.17–6.20). The primary structure of a protein represents the amino acid sequence of the protein. The secondary structure is the coiling, folding, and/or bending of the protein. Various interactions

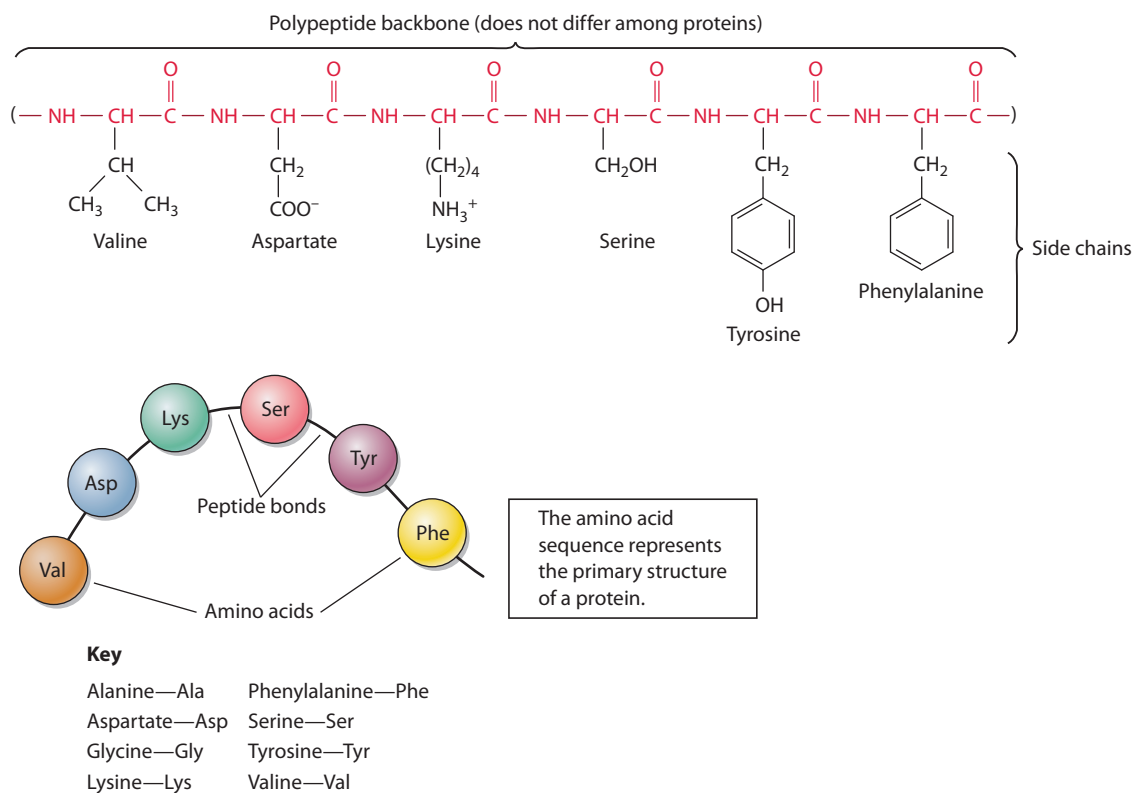


Figure 6.17 The primary structure of a protein.

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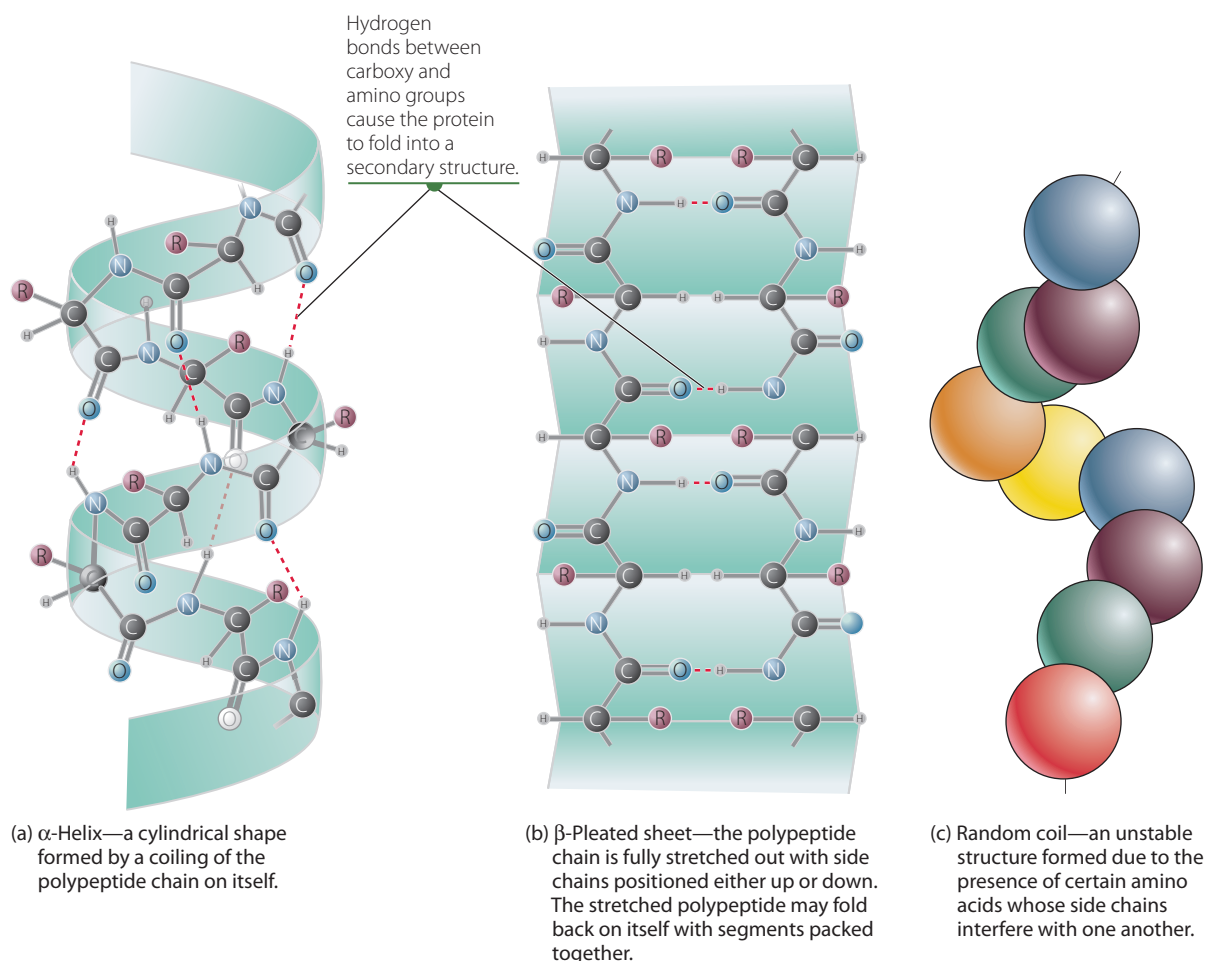


Figure 6.18 Secondary structure of proteins.

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between and among amino acids within the proteins contribute to the overall levels of organization. For example, hydrogen bonds between amino acids contribute to the secondary structures of proteins. Hydrogen (H) bonds are weak electrical attractions that can occur between hydrogen atoms and negatively charged atoms such as oxygen or nitrogen. Electrostatic attractions, also called ionic attractions or salt bonds, occur between oppositely charged side chains of amino acids, such as lysine and glutamate, to impact the secondary structure. In addition, hydrophobic interactions occur between the side chains of nonpolar amino acids. These interactions can generate particular structures, such as the α -helix and β -pleated sheet; the presence of these structures provides important attributes, including added stability, strength, and rigidity, to proteins. The α -helix and β -pleated sheet are particularly abundant in proteins with structural roles, such as collagen, elastin, and keratin.

The overall or total three-dimensional configuration of the protein is referred to as the tertiary structure.

For example, the tertiary structures of globular proteins, which are named for their spherical shape, generally contain multiple α -helices and β -pleated sheets. Myoglobin, calmodulin, and many enzymes and serum proteins are globular proteins. The quaternary structure results from two or more polypeptide chains interacting. Some of the same interactions contributing to the secondary structure also contribute to these additional levels of organization and include the clustering of hydrophobic amino acids toward the center of the protein and the electrostatic attraction of oppositely charged amino acids. In addition to the weak noncovalent hydrogen, electrostatic, and hydrophobic bonding, strong covalent bonding (involving electron sharing) may occur. One of the more commonly formed covalent bonds occurs between cysteine residues where the $-\text{SH}$ groups are oxidized to form disulfide bridges ($-\text{S}-\text{S}-$), as shown in Figure 6.19b. Together, the interactions among the amino acid side chains determine the protein's overall shape and, therefore, influence the protein's function in the body.

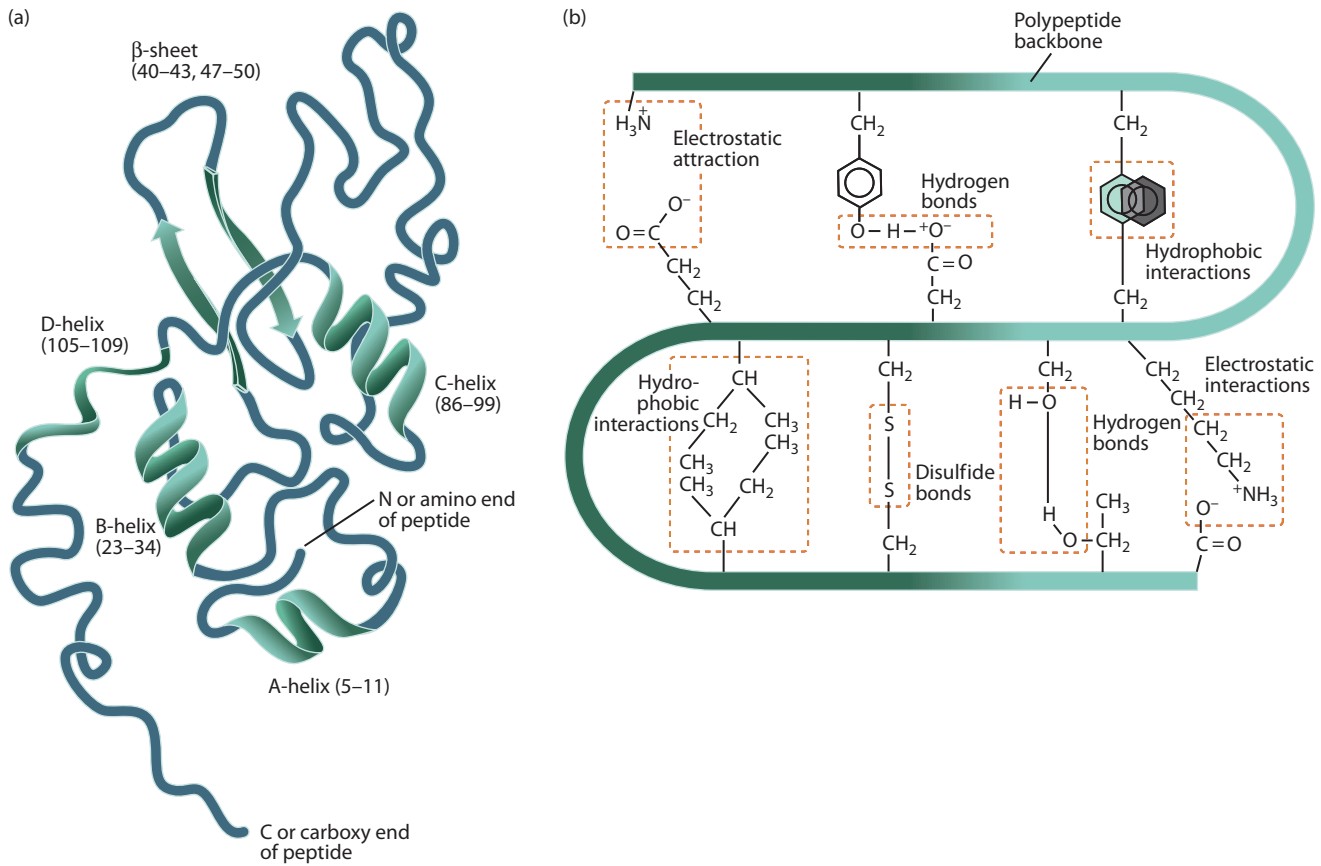


Figure 6.19 (a) The tertiary structure of the protein α -lactalbumin. (b) Examples of interactions found in tertiary structures.

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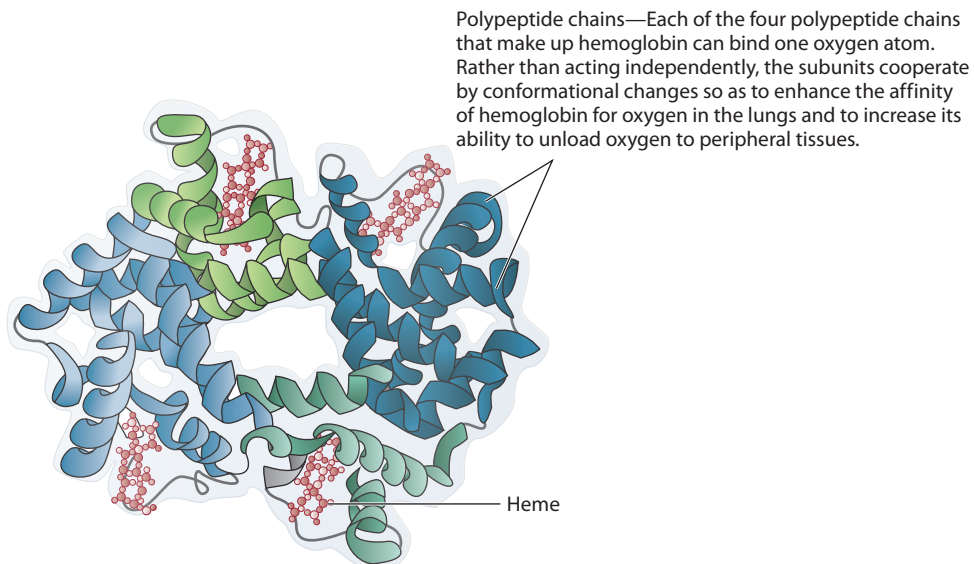


Figure 6.20 Quaternary structure of the hemoglobin protein. Quaternary proteins are characterized by interactions between two or more (usually two or four) polypeptide chains. The aggregated form is called an oligomer. The polypeptide chains (called subunits) making up the oligomer are held together by hydrogen bonds and electrostatic salt bridges.

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6.8 FUNCTIONAL ROLES OF PROTEINS

The molecular architecture and activity of living cells depend largely on proteins, which make up over half of the solid content of cells and which show great variability in size, shape, and physical properties. Their physiological roles are also quite variable and, because of this variability, categorizing proteins according to their functions can be helpful in the study of human metabolism.

Catalysts

Enzymes are protein molecules (generally designated by the suffix *-ase*) that act as catalysts; they change the rate of reactions occurring in the body. Enzymes are necessary for sustaining life and are found in the body both intracellularly and extracellularly (e.g., in the blood).

Enzymes are constructed so that they combine selectively with other molecules (called substrates) in the cell. The active site on the enzyme (a small region usually in a crevice of the enzyme) is where the enzyme and substrate bind and the product is generated. Some enzymes, however, require a cofactor or coenzyme to carry out the reaction. Minerals such as zinc, iron, and copper function as cofactors for some enzymes. *Metalloprotein* is the name typically used for proteins to which minerals are complexed. Some, but not all, metalloproteins have enzymatic activity. B vitamins serve as coenzymes for many enzymes. *Flavoprotein* is the term generally used for protein enzymes bound to flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), coenzyme forms of the B vitamin riboflavin. Most human physiological processes require enzymes to promote chemical changes that could not otherwise occur. Some examples of different types of enzymes include dehydrogenases, which remove or transfer hydrogens; kinases, which add phosphate groups; and isomerases, which transfer atoms within a molecule. Some examples of physiological processes that depend on enzyme function include digestion, energy production, blood coagulation, and excitation and contraction of neuromuscular tissue. The section titled “Catalytic Proteins” in Chapter 1 provides further information on enzymes.

Messengers

Some proteins are hormones. **Hormones** act as chemical messengers in the body. They are synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs, where they bind to protein receptors on membranes. Hormones generally regulate metabolic processes, for example, by promoting enzyme synthesis or affecting enzyme activity.

Whereas some hormones are derived from cholesterol and classified as steroid hormones, others are derived from one or more amino acids. The amino acid tyrosine, for example, is used along with the mineral iodine to synthesize the thyroid hormones. Tyrosine is also used to synthesize the catecholamines, including dopamine, norepinephrine, and epinephrine. The hormone melatonin is derived in the brain from the amino acid tryptophan. Other hormones are made up of one or more polypeptide chains. Insulin, for example, consists of two polypeptide chains linked by a disulfide bridge. Glucagon, parathyroid hormone, and calcitonin each consist of a single polypeptide chain. Many other peptide hormones, such as adrenocorticotropic hormone (ACTH), somatotropin (growth hormone), and vasopressin (also known as antidiuretic hormone, or ADH), have important roles in human metabolism and nutrition. These hormones are discussed throughout this chapter and the book.

Structural Elements

Several proteins have structural roles in the body. Two groups of structural proteins include:

- Contractile proteins
- Fibrous proteins.

The two main contractile proteins, actin and myosin, are found in cardiac, skeletal, and smooth muscles. Skeletal muscle is found throughout the body and is under voluntary control. It is made of myosin (thick filaments) and actin (thin filaments). Contraction is calcium induced and involves not only actin and myosin but also troponin and tropomyosin. Smooth muscle is found in many tissues including, for example, blood vessels, the lungs, the uterus, and the GI tract. Smooth muscle is under involuntary control and contracts in response to calcium-induced phosphorylation of the structural protein myosin.

Fibrous proteins, which tend to be somewhat linear in shape, include collagen, elastin, and keratin and are found in bone, teeth, skin, tendons, cartilage, blood vessels, hair, and nails. Collagen is a group of well-studied proteins. Each type of collagen is made of three polypeptide (tropocollagen) chains that are cross-linked for strength. These chains, rather than forming specific secondary structures (α -helices or β -pleated sheets, discussed in the protein structure section), form a helical arrangement. The amino acid composition of the chains is rich in the amino acids glycine and proline. In addition, collagen contains two hydroxylated amino acids—hydroxylysine and hydroxyproline—that are not found in other proteins. Collagen polypeptides are also attached to carbohydrate chains and are thus considered to be glycoproteins. Other structural proteins, such as elastin, are associated with proteoglycans.

Both glycoproteins and proteoglycans are conjugated proteins and are discussed further in the “Other Roles” section. Elastin, rich in glycine and alanine, is a component of connective tissues, especially blood vessels, ligaments, and the dermis. Keratins, a group of proteins typically found in the cytoskeletal structure of some cells (especially hair and some epithelial cells), help maintain cell integrity and stability. Keratin molecules interact, forming strong bundles referred to as intermediate filaments. Keratin’s high cysteine content allows for the formation of strong disulfide bridges between keratin molecules.

Buffers

Proteins, because of their constituent amino acids, can serve as buffers in the body and thus help to regulate acid–base balance. A **buffer** is a compound that ameliorates a change in pH that would otherwise occur in response to the addition of alkali or acid to a solution. The pH of the blood and other body tissues must be maintained within an appropriate range. Blood pH ranges from about 7.35 to 7.45, whereas cellular pH levels are often more acidic. For example, the pH of red blood cells is about 7.2, and that of muscle cells is about 6.9. The H^+ concentration within cells is buffered by both the phosphate system and the amino acids in proteins. The protein hemoglobin, for example, functions as a buffer in red blood cells. In the plasma and extracellular fluid, proteins and the bicarbonate system serve as buffers. The buffering ability of proteins can be illustrated by the reaction $H^+ + \text{protein} \leftrightarrow \text{Hprotein}$ and is shown in Figure 6.21.

Fluid Balancers

In addition to acid–base balance, proteins influence fluid balance through their presence in the blood and in cells. More specifically, proteins help attract and keep water inside a particular area and contribute to **osmotic pressure**. Diminished blood/plasma concentrations of proteins result in a decrease in plasma osmotic pressure. When protein concentrations in the blood are less dense than normal, fluid “leaks” out of the blood and into interstitial spaces and causes swelling (and if severe even pitting edema). Restoring adequate concentrations of protein in the blood (e.g., by infusing the protein albumin intravenously) and within cells where proteins are also found (e.g., by providing sufficient dietary protein and energy to enable protein synthesis) promotes diffusion of water from the interstitial space back into the blood and back within cells.

Immunoprotectors

Immunoprotection is provided to the body in part by a group of proteins called **immunoproteins**, also called immunoglobulins (Ig) or antibodies (Ab). These immunoproteins, of which there are five major classes (IgG, IgA, IgM, IgE, and IgD), are Y-shaped proteins made of four polypeptide chains (two small chains called light [L] chains and two large chains called heavy [H] chains). The immunoglobulins are produced by plasma cells derived from B-lymphocytes, a type of white blood cell. Immunoglobulins function by binding to antigens—which typically consist of foreign substances, such as bacteria or viruses that

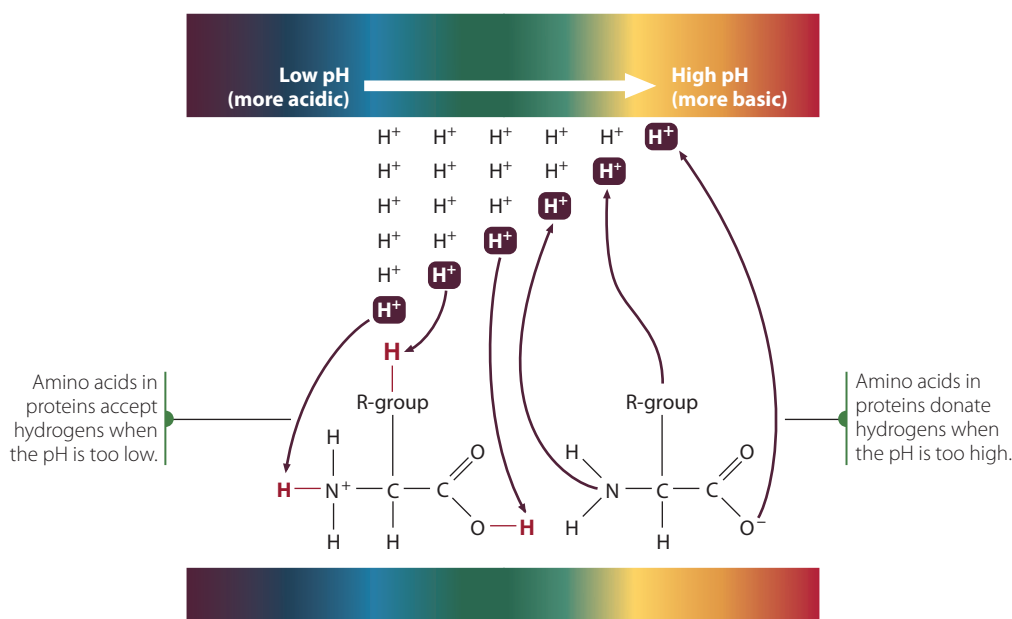


Figure 6.21 The role of the amino acid in pH balance.

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have entered the body. By complexing with antigens, immunoglobulins create immunoprotein–antigen complexes that can be recognized and destroyed through reactions with either complement proteins or cytokines. Complement proteins (which are part of the complement pathway and made primarily by the liver) are vital to the protection of the body against foreign microbes/organisms or substances after recognition by antibodies. The pathway includes about 35 proteins, which, when activated, form interactions with the surfaces of invading foreign substances to facilitate immune system recognition and activation and destruction of the foreign substance by phagocytosis. Cytokines are produced by white blood cells such as T-helper (CD4) cells and macrophages. In addition, white blood cells such as macrophages and neutrophils also destroy foreign antigens through the process of phagocytosis.

Transporters

Transport proteins are a diverse group of functional proteins that provide a means of carrying substances, such as vitamins, minerals, and other nutrients, within the blood, into cells, out of cells, or within cells. In the blood, for example, the protein hemoglobin, found in red blood cells, transports oxygen and carbon dioxide. In cell membranes, some membrane-spanning proteins provide pores or channels through which substances such as sodium, potassium, chloride, and calcium may gain entry. Some of these pores may be gated open or closed in response to alterations in membrane potential (voltage gated) and/or the presence of a ligand. Ligands from outside the cell—such as hormones or neurotransmitters—or ligands from within the cytosol—such as calcium, cAMP, among others—initiate changes to affect gate function. Other proteins act as carriers of which there are several types. Uniporters carry only one substance across cell membranes; for example, some amino acid transporters are uniporters or symporters. Symporters carry more than one substance simultaneously across the cell membrane; in the intestinal brush border a glucose transporter carries both glucose and sodium into cells. Antiporters, another type of cell membrane protein transporter, function by exchanging one substance for another.

Of the hundreds of proteins in the blood, several serve as transporters. The concentration of total protein in human plasma typically totals up to about 7.5 g/dL. Lipoproteins, for example, transport cholesterol and triacylglycerol in the blood; the proteins in the lipoproteins are actually a group of about 10 different apoproteins that both enable lipid transport and direct the lipoproteins to cells for use. An apoprotein is a protein that is complexed or part of a molecule that also contains a nonprotein portion.

A few transport proteins of clinical significance include albumin, transthyretin, and retinol-binding protein. Albumin, the most abundant of the plasma proteins, transports nutrients such as tryptophan, fatty acids, and vitamin B₆;

some minerals including zinc, calcium, and small amounts of copper; and some drugs. The protein is synthesized by the liver and released into the blood; changes in osmotic pressure and osmolality as well as inflammatory mediators affect its rate of synthesis. A healthy person makes about 9–12 g of albumin per day. Albumin is often used in the absence of inflammation to assess an individual's protein status, specifically visceral (internal organ) protein status. Because of albumin's relatively long half-life (~14–18 days), however, it is not as good or as sensitive an indicator of visceral protein status as some of the other plasma proteins. The half-life is the time it takes for 50% of the amount of a protein such as albumin (or nonprotein compound) to be degraded.

Two other proteins synthesized by the liver and released into plasma are transthyretin (also called prealbumin) and retinol-binding protein. Retinol-binding protein, as its name implies, transports retinol (a form of vitamin A) but also thyroid hormone. (see also the section on vitamin A in Chapter 10 for more about retinol-binding protein). Transthyretin and retinol-binding protein, like albumin, are also used as biochemical indicators of visceral protein status in the absence of inflammation and, in the case of retinol-binding protein, also in the absence of zinc deficiency. Because transthyretin and retinol-binding protein have relatively shorter half-lives (~2 days and 12 hours, respectively) than albumin, they are more sensitive indicators of changes in visceral protein status. The concentrations of albumin, prealbumin, and retinol-binding protein diminish in the blood over varying time periods (depending on their half-life) in people, for example, who have ingested inadequate dietary protein. Typically, plasma concentrations of albumin < 3.5 g/dL, prealbumin (transthyretin) < 18 mg/dL, and retinol-binding protein < 2.1 mg/dL suggest inadequate visceral protein status. A diet high in energy (kcal) and protein is needed to promote improvements (assuming the liver is healthy) in status.

Some transport proteins found in the blood are classified according to size and charge using the procedure protein electrophoresis. Globulins, a heterogeneous group of proteins with diverse transport functions, are identified using this method. Most globulins are synthesized in the liver, with the exception of the gamma globulins (antibodies), which are made mostly by plasma cells (mature B-lymphocytes). The four classes of globulins are listed here, along with some examples of proteins comprising each class and their transport roles:

- α 1-globulins: glycoproteins and high-density lipoproteins (for lipid transport)
- α 2-globulins: glycoproteins, haptoglobin (for free hemoglobin transport), ceruloplasmin (for copper transport and oxidase activity), prothrombin (for blood coagulation), and very-low-density lipoproteins (for lipid transport)

- β -globulins: transferrin (for iron and other mineral transport) and low-density lipoproteins (for lipid transport)
- γ -globulins: immunoglobulins or antibodies (for immunoprotection).

Some hospital laboratories report serum albumin concentrations relative to globulin concentrations. Normally, albumin concentrations exceed globulin concentrations slightly.

Acute-Phase Responders

Another heterogeneous group of proteins, called acute-phase or positive acute-phase reactant proteins, are made in the liver in response especially to acute (sudden), critical illnesses such as infection (sepsis) and injury/trauma. The body's reaction to such situations is referred to as an inflammatory response, and the magnitude of the response varies with the severity of the illness/situation. Some examples of these acute-phase proteins are C-reactive protein, fibronectin, orosomucoid (also called α 1-acid glycoprotein), haptoglobin, serum amyloid A, α 2-macroglobulin, ceruloplasmin, and metallothionein. Collectively, these proteins perform a variety of functions that protect the body, such as stimulating the immune system, promoting wound healing, and chelating and removing free iron from circulation to prevent its use by bacteria for growth. C-reactive protein is used clinically to evaluate inflammation in patients, and its concentration rises dramatically in the blood within a few hours of infection and inflammation. Diminishing plasma concentrations of C-reactive protein suggest the possibility of a less catabolic state.

The body generates a group of proteins called stress or heat shock proteins (abbreviated *hsp*). Heat shock proteins also function in cytoprotection and cell survival primarily by facilitating protein folding or refolding, trafficking, repair, assembly, and degradation. Heat shock proteins are categorized into families based on molecular weight (e.g., hsp 60, hsp 70, hsp 90). Some are synthesized constitutively while others are synthesized in response to stress, including heat stress and oxidative stress such as with exercise and other physical activity in warm environments. The amino acid glutamine also appears to enhance the expression of some heat shock proteins during critical illness (oxidative stress). More specifically, glutamine is thought to be necessary for the activation of specific transcription factors required for heat shock protein synthesis. The Perspective at the end of this chapter provides some additional information about the body's response to stress.

Other Roles

Proteins carry out many additional roles in the body. For example, some serve to transmit signals into and out of the cell. Between adjacent cells, proteins function in cell

adhesion. A group of claudin proteins function in intestinal barrier regulation, with some claudins forming charge-selective pore channels, some enabling paracellular absorption, and others functioning to keep cell junctions tightly closed. The protein opsin is important for vision (as discussed under the section on vitamin A in Chapter 10). Proteins also serve as receptors on cell membranes and can function in storage roles. For example, some minerals such as copper, iron, and zinc are stored in body tissues bound to proteins; these proteins are often called metalloproteins.

Many proteins in the body are conjugated proteins—that is, proteins that are joined to nonprotein components. **Glycoproteins**, one type of conjugated protein, represent a huge group of proteins with multiple functions. Glycoproteins consist of a protein covalently bound to a carbohydrate component. The carbohydrates in glycoproteins generally include short chains of glucose, galactose, mannose, fucose, N-acetylglucosamine, N-acetylgalactosamine, and acetylneuraminic (sialic) acid at the terminal end of the oligosaccharide chain. The carbohydrate portion of the glycoprotein can make up as much as 85% of the glycoprotein's weight. The carbohydrate component is bound typically through an N-glycosidic linkage with asparagine's amide group in its side chain or through an O-glycosidic linkage with the hydroxy group in serine's or threonine's side chain. Glycoproteins are found in the blood, on the outer surface of plasma membranes, and in association with the extracellular matrix (which surrounds and supports some body cells). In the extracellular matrix of bone, for example, glycoproteins play structural roles. Mucus, which is found in body secretions, is rich in glycoproteins. Mucus both lubricates and protects epithelial cells in the body. Some of the body's hormones (such as thyrotropin) and blood proteins (such as transthyretin and immunoglobulins) are glycoproteins.

Another group of conjugated proteins is the proteoglycans, which are found in every tissue of the body. Most proteoglycans are associated with the extracellular matrix but also with other structural matrix components, where they form cross-links to enhance strength and resilience and to modulate adhesion and communication between cells and between the extracellular matrix and cells. **Proteoglycans** are macromolecules consisting of a core protein covalently conjugated, typically by O-glycosidic or N-glycosylamine linkages, to one or more **glycosaminoglycans**. Glycosaminoglycans consist of long chains of repeating disaccharides, comprise up to 95% of the weight of the proteoglycan, and are the main site of the proteoglycan that interacts with cell surface proteins or extracellular matrix proteins. Examples of glycosaminoglycans include hyaluronic acid (found in high concentrations in cartilage), chondroitin sulfate (found in high concentrations in bone and cartilage), keratan sulfate and dermatan sulfate (found in the cornea of the eye), and heparan sulfate (found in high concentrations in plasma cell membranes).

6.9 FUNCTIONAL ROLES OF NITROGEN-CONTAINING NONPROTEIN COMPOUNDS

In addition to their use in the synthesis of body proteins, amino acids are used to synthesize nitrogen-containing compounds that are not proteins but nonetheless play important roles in the body. This next section addresses the roles of some nutritionally significant nitrogen-containing nonprotein compounds (Table 6.6). Not included in this review, however, are a number of biogenic amines, neurotransmitters, and neuropeptides that are synthesized from amino acids in many glands, tissues, and organs throughout the body. A discussion of these compounds is found in this chapter in the “Brain and Accessory Tissues” section. Some of the compounds are also mentioned in sections that discuss the metabolism of amino acids.

Glutathione

Glutathione (Figure 6.22) is a tripeptide synthesized from three amino acids—glycine, cysteine, and glutamate—in most body cells. Its synthesis occurs in two steps, both ATP dependent. First, the γ carboxy group of glutamate is attached to the amino group of cysteine by γ glutamyl cysteine synthetase to form a peptidic γ linkage. In the second step, glutathione synthetase creates a peptide bond between the amino group of glycine and the carboxy group of cysteine to produce glutathione. The availability of cysteine appears to be the major factor influencing glutathione synthesis, although synthesis can be affected by administration of any of the precursor substrates or via activity of glutathione peroxidase and reductase, which catalyze other glutathione-dependent reactions.

Glutathione is referred to as a thiol because it contains a sulfhydryl ($-\text{SH}$) group in its reduced form (designated GSH). Glutathione can also be found in cells in its oxidized form (designated GSSG) and attached to proteins (up to about 15%). Normally, the ratio of GSH to GSSG in cells is >10 to 1; the GSH-to-GSSG ratio represents an indicator of the cell's redox state. In fact, the ratio of GSH to GSSG is thought to be the most important regulator of the cellular redox potential.

Table 6.6 Sources of Nitrogen for Some Nitrogen-Containing Nonprotein Compounds

Nitrogen-Containing Nonprotein Compound	Constituent Amino Acids
Glutathione	Cysteine, glycine, glutamate
Carnitine	Lysine, methionine
Creatine	Arginine, glycine, methionine
Carnosine	Histidine, β -alanine
Choline	Serine

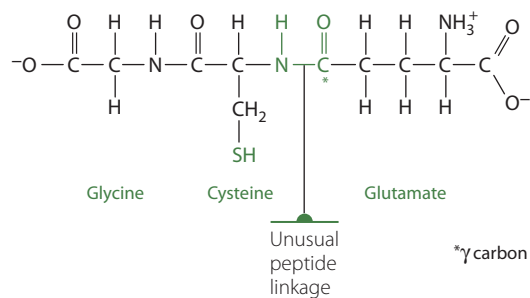


Figure 6.22 The structure of glutathione in its reduced form (GSH).

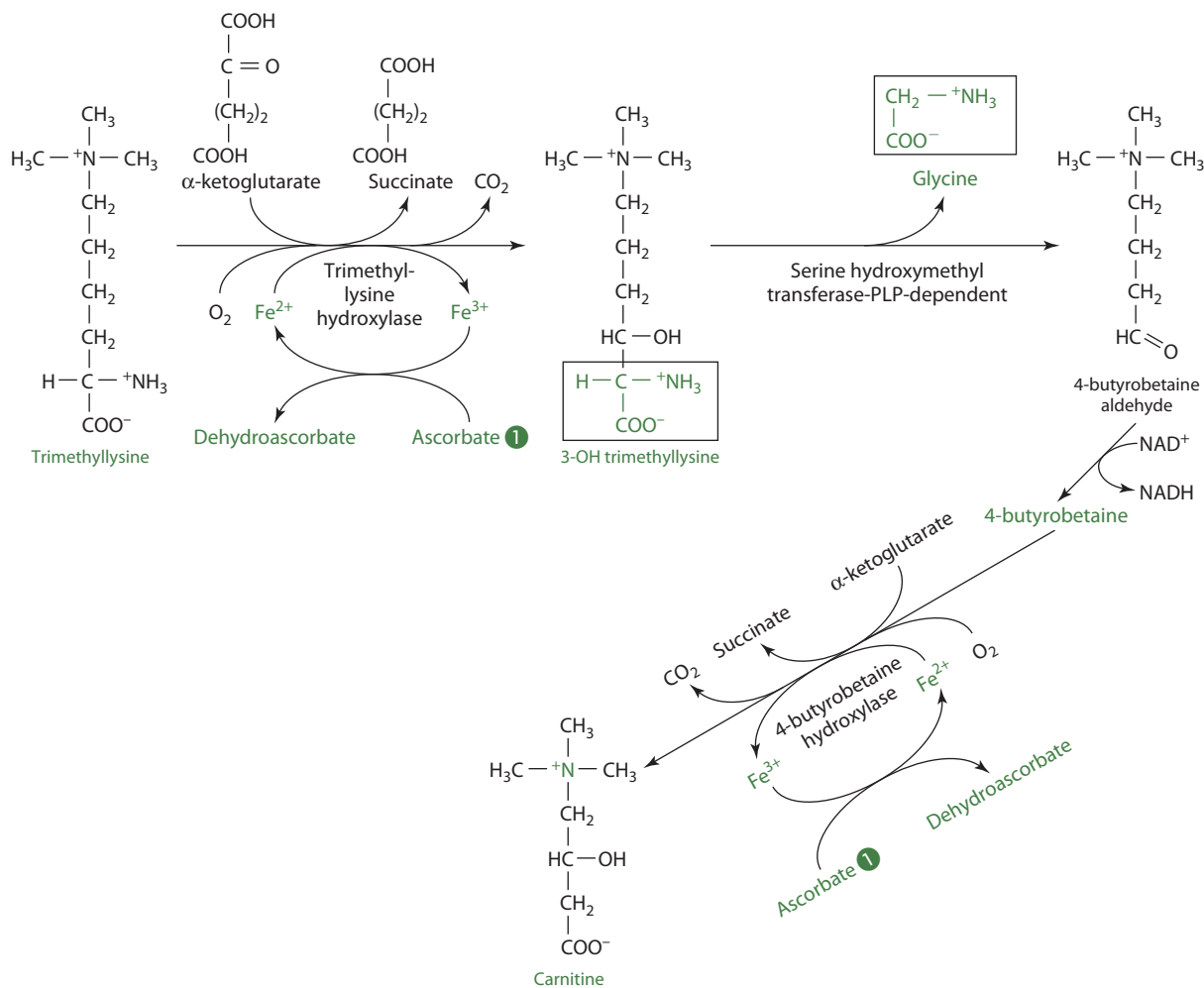
Glutathione is found in the cytosol of most cells, but small amounts are also found within cell organelles and in the plasma. Glutathione has several functions. It is a major antioxidant with the ability to scavenge free radicals ($\text{O}_2^\bullet$ and $\text{OH}^\bullet$), thereby protecting critical cell components including SH-containing proteins against oxidation. With the enzyme glutathione peroxidase, glutathione protects cells by reacting with hydrogen peroxides (H_2O_2) and lipid hydroperoxides (LOOHs) before they can cause damage. Glutathione also transports amino acids as part of the γ -glutamyl cycle (Figure 6.4) in some tissues. It participates in the synthesis of leukotriene (LT) C₄, which mediates the body's response to inflammation. Glutathione is also involved in the conversion of prostaglandin H₂ to prostaglandins D₂ and E₂ by endoperoxide isomerase. Glutathione can conjugate with nitric oxide to form S-nitrosoglutathione.

Glutathione synthesis is sensitive to protein intake and pathological conditions. Hepatic, intestinal, and systemic GSH concentrations decline with poor protein intake as well as during inflammation and disease; this decline negatively impacts the body, necessitating strategies to enhance or at least maintain GSH concentrations. Glutathione is discussed further in the section on selenium in Chapter 13.

Carnitine

Carnitine, another nitrogen-containing compound, is made from the amino acid lysine that has been methylated (Figure 6.23); the methyl groups are derived from S-adenosyl methionine (SAM), which is made in the body from the oxidation of the amino acid methionine. Following lysine methylation, trimethyllysine undergoes hydroxylation at the 3 position to form 3-OH trimethyllysine. Hydroxytrimethyllysine is further metabolized to generate γ -butyrobetaine and subsequently carnitine. Iron, vitamin B₆ (as PLP), vitamin C, and niacin (as NAD^+) are needed for carnitine synthesis.

In addition to being synthesized in the liver and kidneys, carnitine is found in primarily animal foods, especially milk, fish, poultry, and meats. In these foods, carnitine may be free or bound (as acylcarnitine) to long- or short-chain fatty acid esters. Carnitine from food or



① Ascorbate functions as a reducing agent in two reactions. In both reactions for carnitine synthesis, the vitamin is needed to reduce the iron atom that has been oxidized (Fe³⁺) in the reaction back to its reduced (Fe²⁺) state.

Figure 6.23 Carnitine synthesis.

supplements is absorbed in the proximal small intestine by sodium-dependent active transport and passive diffusion; diffusion typically predominates with ingestion of supplements, providing about 0.5–6 g. Approximately 54–87% of carnitine intake is absorbed. Intestinal absorption of carnitine is thought to be saturated with intakes of about 2 g. However, selected bacteria in the colon metabolize carnitine, generating trimethylamine; this compound, its metabolism to trimethylamine N-oxide, and its negative associations with disease are discussed further under the section “Choline.”

Muscle represents the primary carnitine pool, although no carnitine is made there. Intramuscular concentrations of carnitine are generally 50 times greater than usual plasma concentrations. Carnitine homeostasis is maintained principally by the kidneys, with >90% of filtered carnitine and acylcarnitine being reabsorbed.

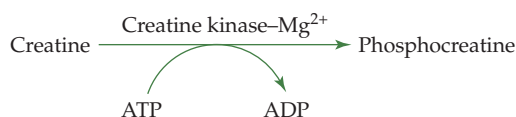
Carnitine, found in most body tissues, is needed for the transport of fatty acids, especially long-chain fatty acids, across the inner mitochondrial membrane for β-oxidation. The inner mitochondrial membrane is impermeable to long-chain (10 or more) fatty acyl-coenzyme (Co) A. This role of carnitine is discussed in more detail in Chapter 5. Carnitine is also needed for ketone catabolism for energy. Carnitine also forms acylcarnitines from short-chain acyl-CoAs. These acylcarnitines may serve to buffer the free CoA pool.

Carnitine deficiency, though rare, results in impaired energy metabolism. Carnitine supplementation increases plasma and muscle carnitine concentrations and has been beneficial for some people with specific cardiac problems and diabetes. Supplementation with carnitine does not, however, “burn fat,” as suggested in some advertisements.

Creatine

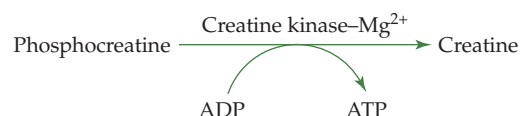
Creatine, a key component of the energy compound creatine phosphate, also called phosphocreatine, can be obtained from foods (primarily meat and fish) or synthesized from three amino acids in the body. Creatine synthesis, which is shown in Figure 6.24, begins first in the kidneys and requires arginine and glycine. The second step occurs in the liver and involves the methylation of guanidinoacetate using SAM (S-adenosyl methionine).

Once synthesized, creatine is released into the blood for transport to tissues. About 95% of creatine is in muscle, with the remaining 5% in organs such as the kidneys and brain. In tissues, creatine is found both in free form as creatine and in its phosphorylated form. The phosphorylation of creatine to form phosphocreatine is shown here.



Phosphocreatine functions as a “storehouse for high-energy phosphate.” In fact, over half of the creatine in muscle at rest is in the form of phosphocreatine. Phosphocreatine replenishes ATP in a muscle that is rapidly contracting. Remember, muscle contraction requires

energy. This energy is obtained with the hydrolysis of ATP. However, the ATP in muscle can suffice for only a fraction of a second. Phosphocreatine, stored in the muscle and possessing a higher phosphate group transfer potential than ATP, can transfer a phosphoryl group to ADP, thereby forming ATP or assisting in ATP regeneration, providing energy for muscular activity. Creatine kinase, also called creatine phosphokinase (abbreviated CK or CPK), catalyzes the phosphate transfer in active muscle, as shown here.



Creatine kinase is made up of different subunits in different tissues. For example, in the heart, creatine kinase is made up of two subunits designated M and B. (The brain and muscle also have creatine kinase, but in these tissues the enzyme is made up of the BB and MM subunits, respectively.) Damage to the heart, as with a heart attack, causes the enzyme to “leak” out of the heart and reach elevated concentrations in the blood. Thus, an elevation in CK-MB in the blood along with other indicators is used to diagnose a heart attack. Similarly, damage to skeletal muscle, as may occur with trauma, results in elevations of CK-MM in the blood.

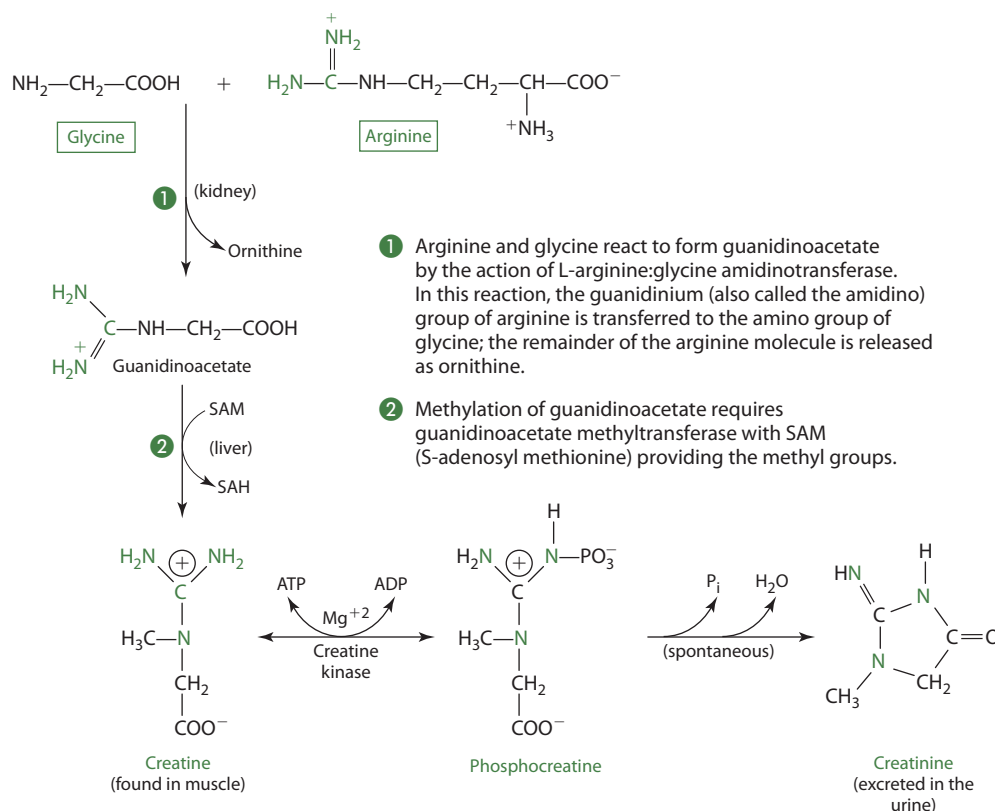


Figure 6.24 Creatine synthesis and use.

The availability of phosphocreatine and its use by muscle are thought to delay the breakdown of muscle glycogen stores, which upon further catabolism can also be used by muscle for energy. Creatine and creatine phosphate do not remain indefinitely in muscle; rather, both slowly but spontaneously cyclize (as shown in Figure 6.24) because of nonreversible, nonenzymatic dehydration. This cyclization of creatine and phosphocreatine forms creatinine. Once formed, creatinine leaves the muscle, passes across the glomerulus of the kidneys, and is excreted like other nitrogenous waste products (e.g., urea, ammonia, uric acid) in the urine. Creatinine clearance is sometimes used as a means of estimating kidney function. The urinary excretion of creatinine is used as an indicator of existing muscle mass, as discussed later in the section under “Skeletal Muscle” titled “Indicators of Muscle Mass and Muscle/Protein Catabolism.” Not all creatinine, however, gets excreted in the urine. Small amounts may be secreted into the gut and, like urea, metabolized by intestinal bacteria. The effects of creatine supplementation on athletic performance are discussed in the Perspective for Chapter 7.

Carnosine

Carnosine (also called β -alanyl histidine; Figure 6.25) is made from the amino acid histidine and β -alanine in an energy-dependent reaction catalyzed by carnosine synthetase. In the body, carnosine is synthesized and found largely in the cytosol of skeletal and cardiac muscle, but also in the brain, kidneys, and stomach. Related compounds include a methylated form of carnosine known as anserine (β -alanyl methylhistidine) and homocarnosine (γ -aminobutyryl histidine), among others. Carnosine is also found in foods, primarily meats, and may be digested into histidine and β -alanine in the intestine or possibly absorbed intact by peptide transporters. While not all of the functions of carnosine have been identified, some studies have shown that carnosine acts as both a buffer and an antioxidant within muscle cells; it may also reduce calcium needs for muscle contractility. The use of β -alanine supplements (about 3–6 g per day) increases muscle carnosine concentrations; the effects of supplementation on athletic performance are discussed in the Perspective for Chapter 7.

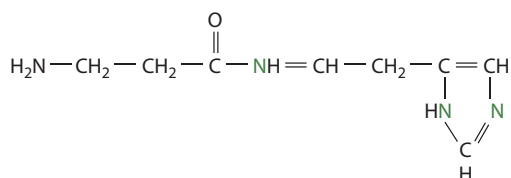


Figure 6.25 Carnosine.

Choline

Choline (Figure 6.26) is made in the body primarily in the liver through the methylation (involving S-adenosyl methionine, or SAM) of the phospholipid phosphatidylethanolamine when linked with the catabolism of phosphatidylcholine. The formation of phosphatidylserine from phosphatidylcholine, involving the replacement of choline with serine by phosphatidylserine synthase 1, also releases choline for other use in the body.

In foods, choline is found free (unattached) in small amounts but is more commonly found bound as part of phosphatidylcholine (also called lecithin) and sphingomyelin, among other forms. Foods rich in lecithin include eggs, meats (especially liver and other organ meats), shrimp, cod, salmon, wheat germ, and legumes such as soybeans and peanuts. Lecithin is also added to many foods as an emulsifier. Intake is estimated at about 6–10 g/day [11]. Pancreatic enzymes hydrolyze some choline from its bound forms. Free choline is absorbed in the small intestine by diffusion and carrier-mediated uptake and is transported via the blood to tissues. Choline existing as phosphatidylcholine and sphingomyelin are incorporated into chylomicrons for transport to tissues. The liver and kidneys store choline to a limited extent.

Choline is a nitrogen-containing compound that is also often presented and/or discussed with the B vitamins, although it is not defined as a vitamin. It has several functions. Most choline is used to synthesize phosphatidylcholine and sphingomyelin, major components of cell membranes. Phosphatidylcholine also functions in intracellular signaling and in the secretion of very-low-density lipoproteins from the liver. Sphingomyelin is a component of myelin that functions as a sheath around nerves and is important in nerve conduction. Choline is also used in the formation of platelet aggregating factor and for the neurotransmitter acetylcholine. To be converted to acetylcholine, free choline crosses the blood–brain barrier and enters cerebral cells from the plasma through a specific choline transport system. Within the presynaptic terminal of the neuron, acetylcholine is formed by the action of choline acetyltransferase as follows:



The acetyl-CoA needed for the reaction is thought to arise from glucose metabolism by neural glycolysis and the action of the pyruvate dehydrogenase complex. Concentrations of choline in cholinergic neurons typically are below the K_m of choline acetyltransferase; thus, the enzyme is not normally saturated. Choline from acetylcholine can be reused following

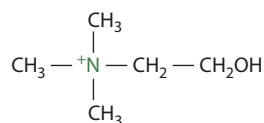


Figure 6.26 Choline.

synaptic transmission; the enzyme acetylcholinesterase hydrolyzes the neurotransmitter. Phospholipases can also liberate choline from lecithin and sphingomyelin as needed.

Choline is oxidized in the liver and kidneys (see Figure 6.12). In the liver, choline oxidation generates betaine, a compound also found in plant foods and that functions as a methyl donor in the generation of methionine from homocysteine. Further metabolism of betaine (also called trimethylglycine) generates dimethyl glycine (also called sarcosine) and subsequently glycine; the reactions require folate as tetrahydrofolate and generate another folate derivative, 5,10-methylene tetrahydrofolate. These reactions are shown in the section in Chapter 9 on folate (specifically, the amino acid metabolism of serine and glycine).

Experimental diets devoid of choline can decrease plasma choline and phosphatidylcholine concentrations. In some cases, insufficient dietary choline intakes promote muscle damage and the development of a fatty liver accompanied by altered liver enzymes and some hepatic necrosis. Low intakes of both choline and betaine have been associated with inflammation. Because *de novo* synthesis does not consistently meet the body's needs for choline, the Food and Nutrition Board has suggested an Adequate Intake of 425 mg and 550 mg of choline daily for adult females and males, respectively [11]. Such intakes are easily obtained through dietary consumption of animal products and foods containing fats.

A Tolerable Upper Intake Level of 3.5 g of choline daily also has been set [11]. The **Tolerable Upper Intake Level** represents the highest level of daily intake that is likely to pose no risks of adverse health effects to most people in the general population [11]. Adverse effects associated with ingestion of large doses of choline include excessive sweating, salivation, vomiting, and a fishy body odor. Intakes of 7.5 g of choline have caused small hypotensive effects [11] and, more recently, choline has been linked with an increased risk of several diseases secondary to the actions of the gut microbiome. Within the GI tract, selected species of gut bacteria metabolize choline (as well as dietary phosphatidylcholine, betaine, and carnitine), producing trimethylamine. The trimethylamine (which is also present in some fish) is subsequently absorbed by passive diffusion in the intestine and taken up by the liver where it is converted (oxidized) to a bioactive compound called trimethylamine N-oxide (TMAO). High plasma TMAO concentrations have been positively associated with chronic kidney disease, diabetes, and cardiovascular conditions including heart attack and stroke (see [12] for review of TMAO and its deleterious effects on health). The compound is excreted from the body in urine, sweat, and the breath.

Purine and Pyrimidine Bases

Nitrogenous bases, along with a five-carbon sugar and phosphoric acid, are needed for the synthesis of two nucleic acids, deoxyribonucleic acid (DNA) and ribonucleic acid

(RNA), in the body. It is amino acids that provide the source for the nitrogen in these bases. The nitrogenous bases can be divided into two categories: pyrimidines and purines. The pyrimidines are six-membered rings containing nitrogen atoms in positions 1 and 3. The pyrimidine bases include uracil, cytosine, and thymidine. Deoxycytidine and thymidine (also called deoxythymidine) are found in DNA. Cytidine and uridine are present in RNA. The purines are made up of two fused rings with nitrogen atoms in positions 1, 3, 7, and 9. The purine bases include adenine and guanine and are found in DNA as deoxyadenosine and deoxyguanosine and in RNA as adenosine and guanosine. A brief review of purine and pyrimidine synthesis and degradation follows.

The synthesis of the nitrogen-containing bases used to make nucleic acids and nucleotides occurs for the most part *de novo* in the liver. The individual steps in pyrimidine synthesis are shown in Figure 6.27. First, synthesis of the pyrimidines uracil, cytosine, and thymine (or in nucleotide form UTP, CTP, and TTP, respectively) is initiated by the formation of carbamoyl phosphate from the amino acid glutamine, CO_2 , and ATP. The enzyme carbamoyl phosphate synthetase II catalyzes this reaction in the cytosol and is distinct from carbamoyl phosphate synthetase I, which is needed in the initial step of urea synthesis and is found in the mitochondria. Second, carbamoyl phosphate reacts with the amino acid aspartate to form N-carbamoyl aspartate. Aspartate transcarbamoylase catalyzes the reaction, which is the committed step in pyrimidine biosynthesis. Following several additional reactions, detailed in Figure 6.27, uridine monophosphate (UMP) is synthesized. Reductions in the activity of either OMP decarboxylase used to make UMP (reaction 6 in Figure 6.27) or orotate phosphoribosyl transferase (reaction 5 in Figure 6.27) due to genetic mutations result in orotic aciduria (the excretion of large amounts of orotic acid in the urine). This condition is also characterized by megaloblastic anemia, leukopenia, and retarded growth.

The interconversions among the pyrimidine nucleoside triphosphates are shown in Figure 6.28 and are discussed next. Once uridine monophosphate (UMP) is formed, it may react with other nucleoside di- and triphosphates. UMP can be converted to uridine diphosphate (UDP) utilizing ATP. UDP can be converted to uridine triphosphate (UTP) also using ATP, and UTP can be converted to cytosine triphosphate (CTP) using ATP and an amino group from glutamine. Alternately, UDP can be reduced to deoxy(d)UDP by ribonucleotide reductase; this reaction requires riboflavin as FADH_2 and the protein thioredoxin. DeoxyUDP can then be converted to dUMP. The formation of deoxythymidine (also called thymidine) monophosphate (dTMP or TMP, also called thymidylate) from dUMP is catalyzed by thymidylate synthetase; the reaction requires the cosubstrate folate as 5,10 methylene tetrahydrofolate and forms another folate derivative

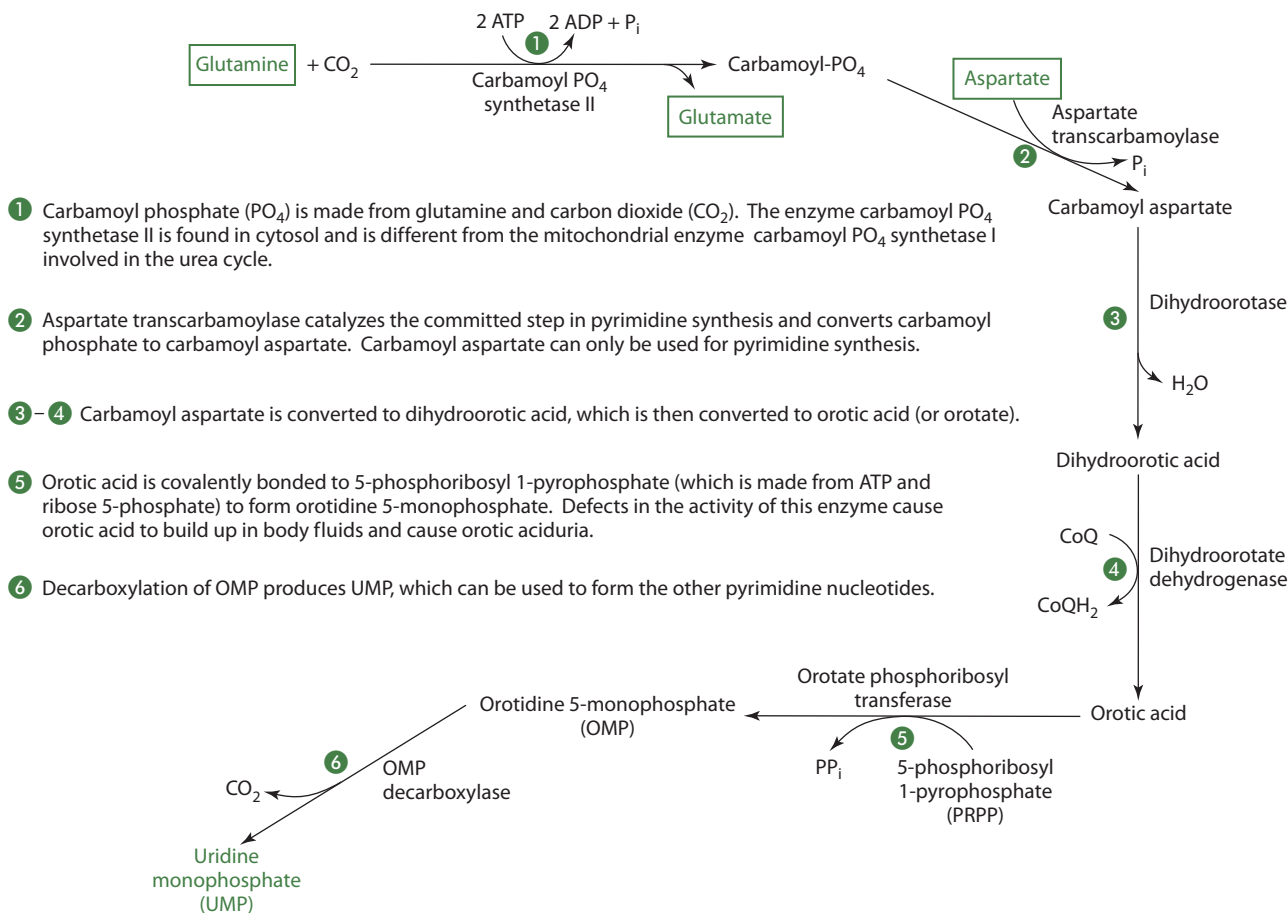


Figure 6.27 The initial reactions of pyrimidine synthesis.

dihydrofolate (DHF). Dihydrofolate reductase is needed to convert DHF to tetrahydrofolate, which is then converted to 5,10 methylene tetrahydrofolate and thus allows for dTMP synthesis. DeoxyTMP can be phosphorylated to form deoxythymidine diphosphate (dTDP) and then phosphorylated again to produce deoxythymidine triphosphate (dTTP or TTP). Thus, through these reactions, CTP, (d)TTP, and UTP have been generated and can be used for the synthesis of DNA and RNA. The pyrimidine ring structure and its sources of carbon and nitrogen atoms along with the structures of the pyrimidine bases are shown in Figure 6.29. CTP is also used in phospholipid synthesis, and UTP is used to form activated intermediates in the metabolism of various sugars. Drugs used to treat cancer often target key enzymes needed for the synthesis of purines or pyrimidines, which are needed by both healthy and cancer cells to grow and multiply. The drug methotrexate, for example, inhibits dihydrofolate reductase activity and thereby decreases dTMP (and thus TTP) formation. Rapidly dividing cells such as cancer cells are more susceptible to the effects of these drugs.

The purine bases adenine and guanine (Figure 6.29) are synthesized *de novo* as nucleoside monophosphates by sequential addition of carbons and nitrogens to

ribose-5-phosphate that has originated from the hexose monophosphate shunt. As shown in Figure 6.30, in the initial reaction, ribose 5-phosphate reacts with ATP to form 5-phosphoribosyl 1-pyrophosphate (PRPP). Glutamine then donates a nitrogen to form 5-phosphoribosylamine. This step represents the committed step in purine nucleotide synthesis. Next in a series of reactions, nitrogen and carbon atoms from glycine are added, formylation occurs by tetrahydrofolate, another nitrogen atom is donated by the amide group of glutamine, and ring closure occurs. Another set of reactions involving the addition of carbons from carbon dioxide and from 10-formyl THF (from folate) and a nitrogen from aspartate occurs. The net result of all of these reactions is the formation of a purine ring. The ring (Figure 6.29) is thus derived from components of several amino acids, including glutamine, glycine, and aspartate, as well as from folate and CO_2 .

The formation of purine nucleoside triphosphates for DNA and RNA synthesis is shown in Figure 6.31. Inosine monophosphate (IMP) is used to synthesize adenosine monophosphate (AMP) and guanosine monophosphate (GMP). AMP and GMP are phosphorylated to ADP and GDP, respectively, by ATP. The deoxyribotides are formed at the diphosphate level by converting ribose to

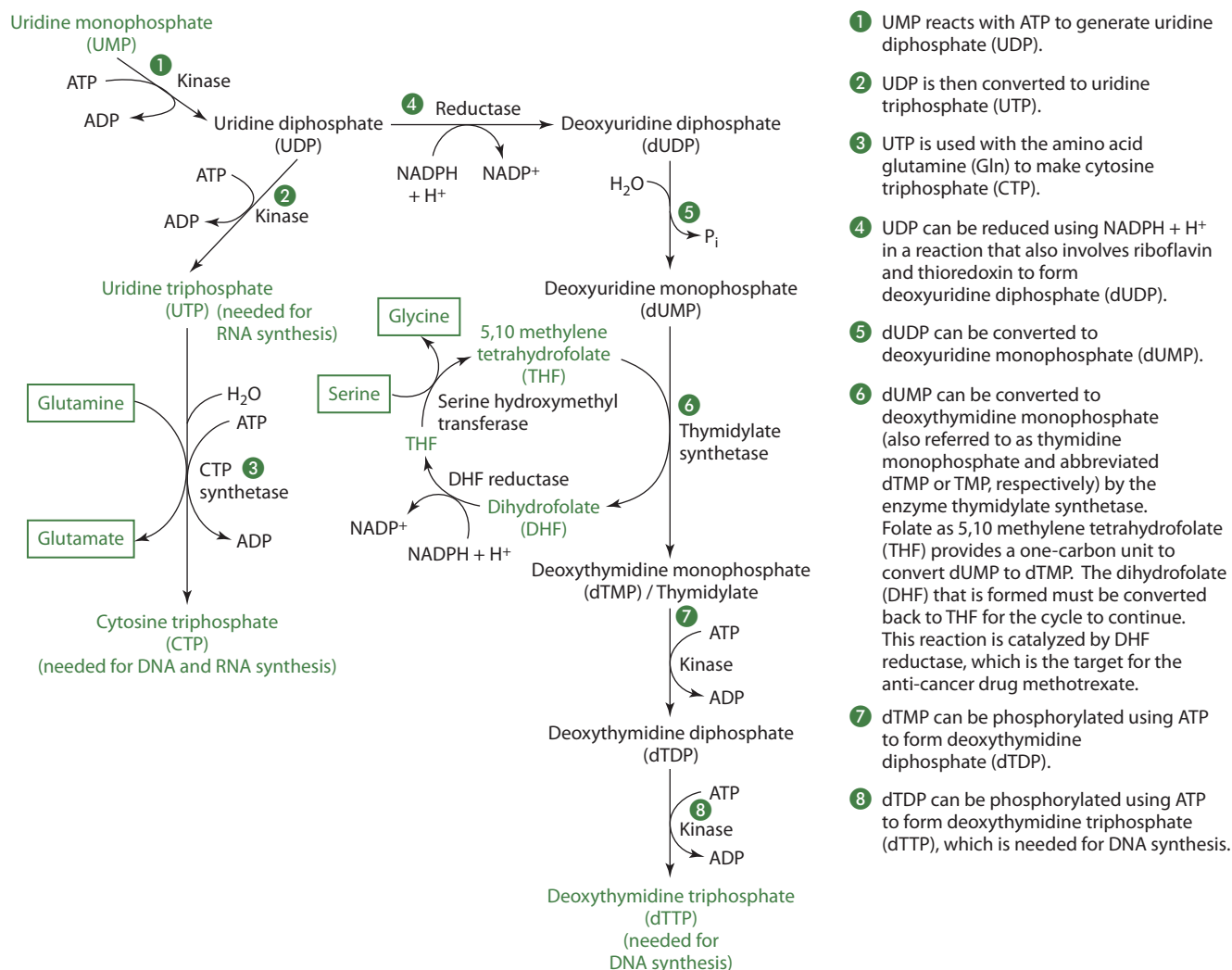


Figure 6.28 The formation of the pyrimidine nucleoside triphosphates UTP, CTP, and TTP for DNA and RNA synthesis.

deoxyribose, thereby producing dADP and dGDP. ADP can be phosphorylated to ATP by oxidative phosphorylation; the remaining nucleotides are phosphorylated to their triphosphate form by ATP.

Purine nucleotides can also be synthesized by the salvage pathway, which requires much less energy than *de novo* synthesis. In the salvage pathway, the purine base adenine reacts with PRPP to form AMP + PP_i in a reaction catalyzed by adenine phosphoribosyl transferase. The purine guanine can also react with PRPP to form GMP + PP_i. Hypoxanthine can react with PRPP to form IMP + PP_i. These last two reactions are catalyzed by hypoxanthine–guanine phosphoribosyl transferase. Defects in the gene for this enzyme cause the disorder Lesch-Nylan syndrome, a genetic X-linked condition characterized most notably by self-mutilation, such as the biting off of one's fingers, and premature death. Other symptoms include mental retardation and the accumulation of hypoxanthine, phosphoribosyl pyrophosphate, and uric acid in body fluids.

Degradation of pyrimidines involves the sequential hydrolysis of the nucleoside triphosphates to mononucleotides, nucleosides, and, finally, free bases. This process can be accomplished in most cells by lysosomal enzymes. During catabolism of pyrimidines, the ring is opened with the production of CO₂ and ammonia from the carbamoyl portion of the molecule. The ammonia can be converted into urea and excreted. Malonyl-CoA and methylmalonyl-CoA, produced from the remainder of the ring, follow their normal metabolic pathways, thus requiring no special excretion route.

Purines (GMP and AMP) are progressively oxidized for degradation primarily in the liver, yielding xanthine, which is converted to uric acid for excretion (Figure 6.32). Xanthine oxidoreductase, a molybdenum- and iron-dependent flavoenzyme, converts hypoxanthine (generated from AMP) to xanthine and also converts xanthine (made from both AMP and GMP) to uric acid. The oxidase form of the enzyme uses molecular oxygen and generates hydrogen peroxide, while the dehydrogenase form

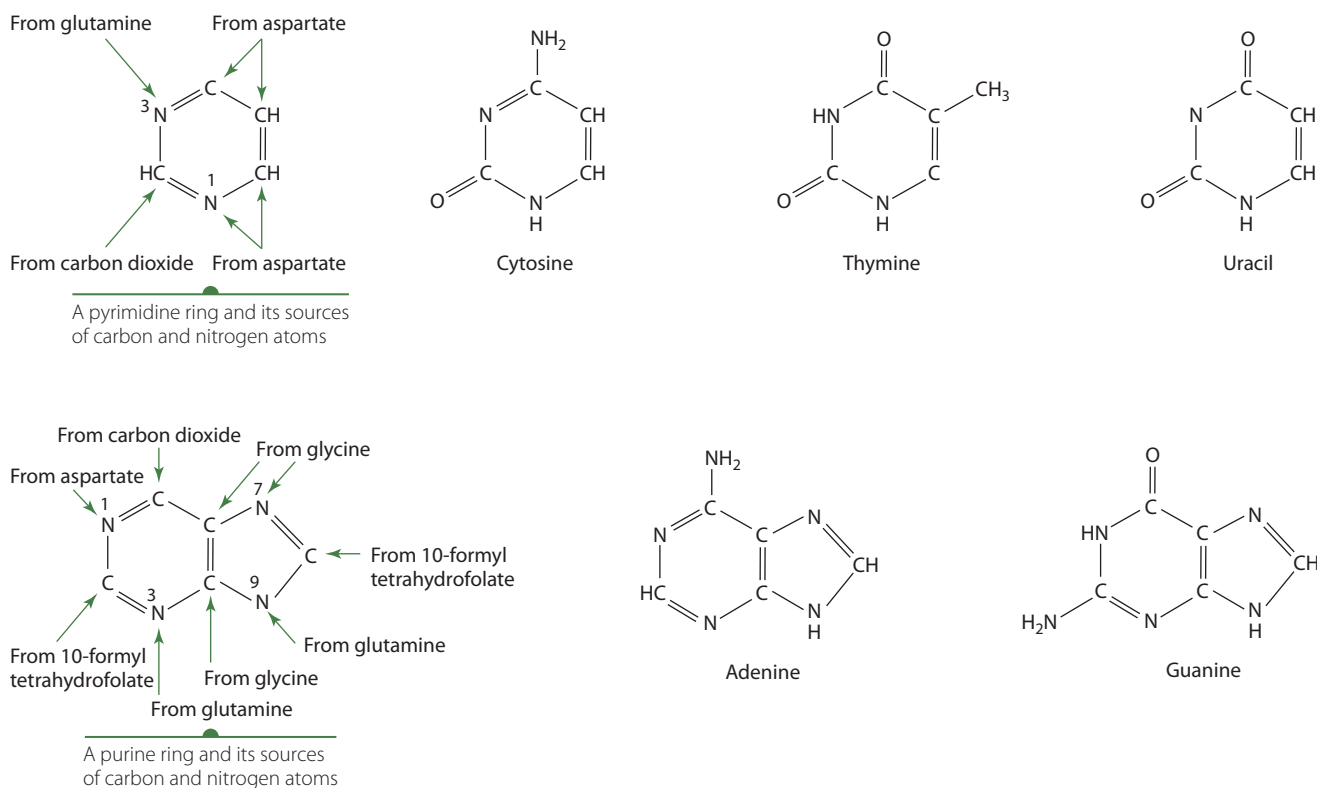
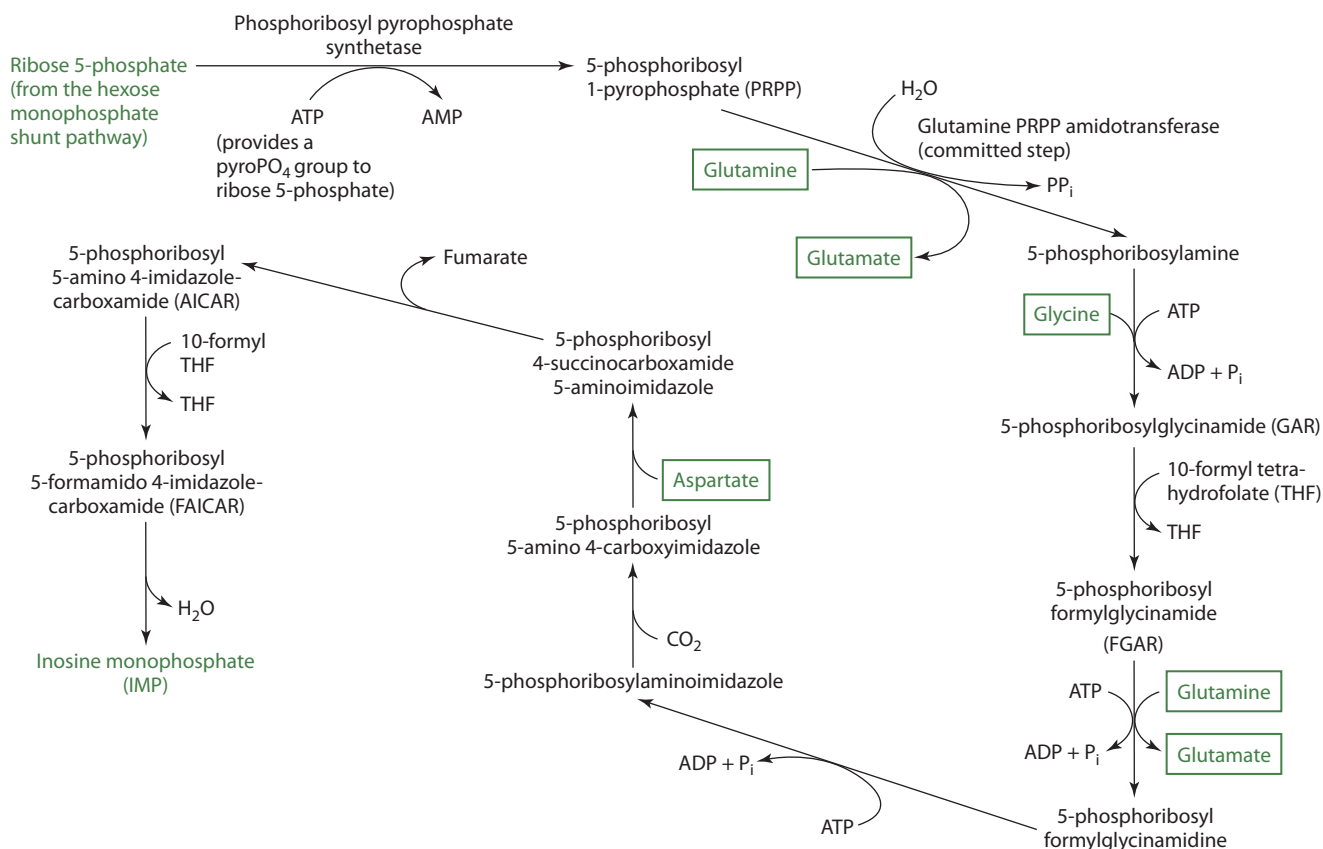


Figure 6.29 The pyrimidine and purine ring structures and the pyrimidine and purine bases. Cytosine, adenine, and guanine are found in both DNA and RNA. Thymine is found in DNA and uracil only in RNA.



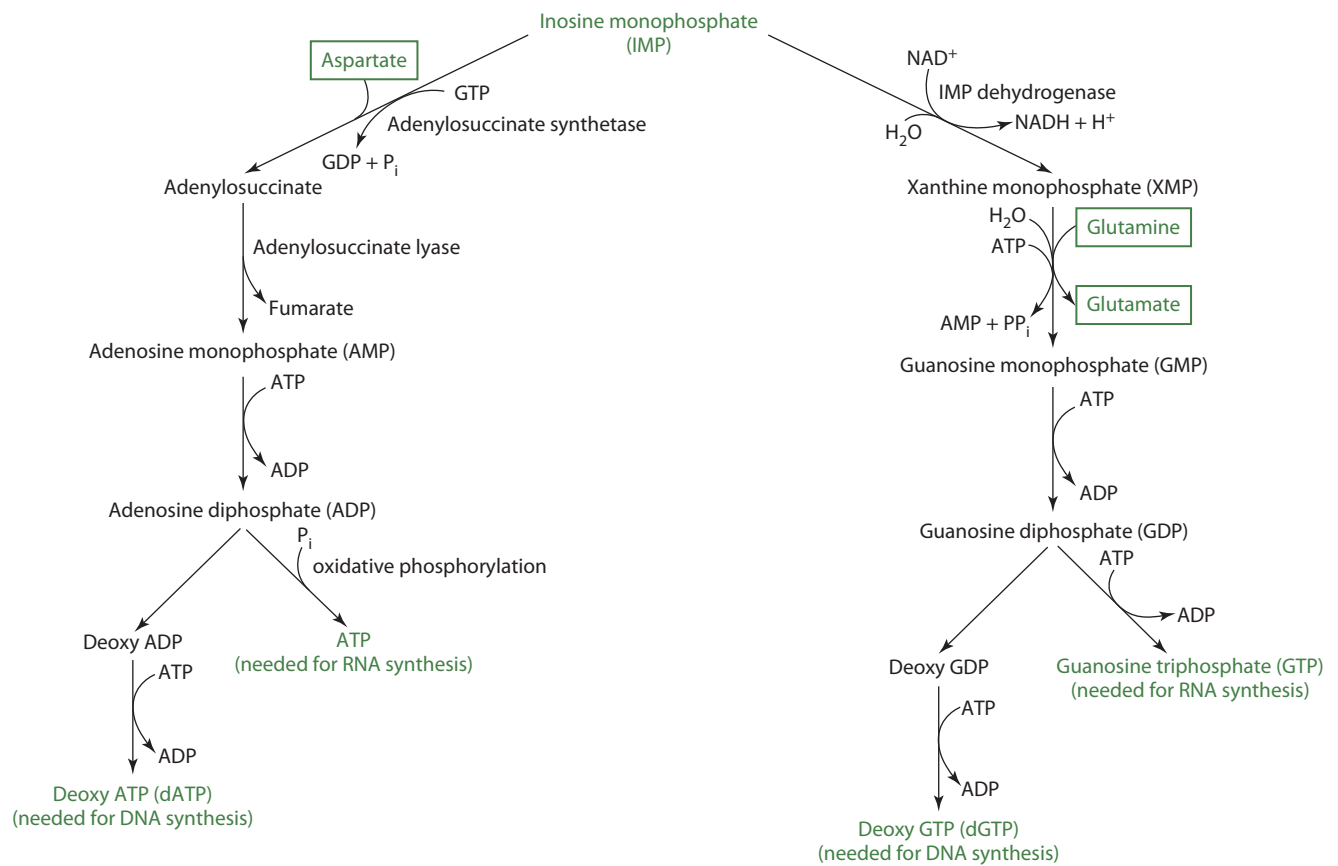


Figure 6.31 The formation of purines and nucleoside triphosphates needed for DNA and RNA synthesis.

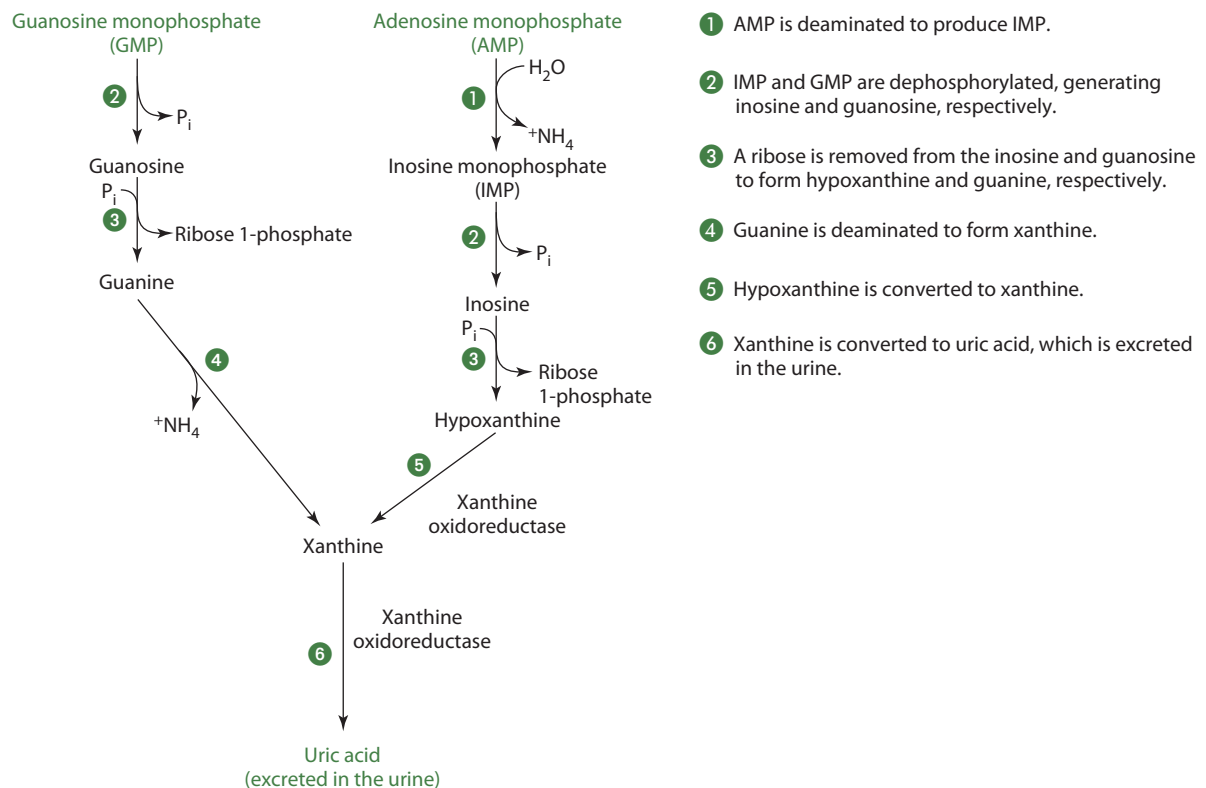


Figure 6.32 The degradation of the purines AMP and GMP generates uric acid.

uses NAD^+ and forms $\text{NADH} + \text{H}^+$. The uric acid that is produced is normally excreted in the urine, although up to 200 mg may also be secreted into the digestive tract. In the disorder gout and in renal failure, uric acid accumulates in the body, causing painful joints, among other problems. Allopurinol is one of several drugs used to treat gout; it works by binding to the enzyme to prevent its interaction with xanthine and hypoxanthine and thus diminish uric acid production. The oxidase (rather than the dehydrogenase) form of the enzyme predominates in several body tissues under conditions of oxygen deprivation (as with a heart attack). A problem in this situation is that when oxygen delivery relieves this deprivation, hydrogen peroxide and free-radical production both increase and may further damage the injured tissues. Research involving introduction of enzymes and antioxidant nutrients to help minimize tissue damage with reoxygenation is ongoing.

6.10 INTERORGAN “FLOW” OF AMINO ACIDS AND ORGAN-SPECIFIC METABOLISM

While tissues and organs use amino acids to synthesize proteins and some nitrogen-containing compounds, the metabolism of the amino acids varies to some extent among the different organs. In many instances, the products generated from amino acid metabolism in one organ may be needed by another organ, creating a dependence between organs. This interdependence begins with the intestinal cells, which are the first cells of the body to receive dietary amino acids. The first part of this section covers amino acid metabolism by intestinal cells, followed by a discussion of amino acids in the plasma and then the specific roles that glutamine and alanine play among body tissues. Lastly, specific uses of amino acids by other selected tissues and organs such as skeletal muscle, the kidneys, and the brain are presented.

Intestinal Cell Amino Acid Metabolism

Intestinal cells use amino acids for energy production as well as for the synthesis of proteins and nitrogen-containing compounds. Some of the uses of amino acids in enterocytes include:

- Structural proteins
- Nucleotides
- Apoproteins necessary for lipoprotein (chylomicron) formation
- New digestive enzymes
- Hormones
- Nitrogen-containing compounds.

Amino acids may be totally or partially metabolized within intestinal cells. It is estimated that the intestine (which represents about 3–6% of the body weight) uses 30–40% and splanchnic tissues use up to 50% of some of the essential amino acids absorbed from the diet. Nonessential amino acids, especially glutamate, are also utilized to varying degrees by intestinal cells. The next five subsections discuss the metabolism of glutamine, glutamate, aspartate, arginine, and methionine in intestinal cells. Figure 6.33 provides a partial overview of intestinal cell amino acid metabolism.

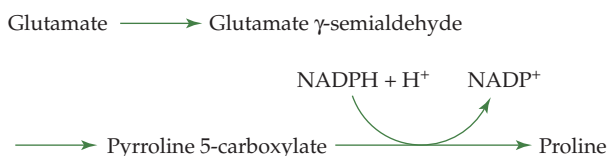
Intestinal Glutamine Metabolism

Glutamine serves several roles in the intestines. It is degraded extensively by intestinal cells, providing a primary source of energy. It has also been shown to have trophic (growth) effects, stimulating gastrointestinal mucosa cell proliferation. Consequently, glutamine helps to prevent both atrophy of gut mucosa and bacterial translocation. In addition, glutamine has been shown to enhance the synthesis of heat shock proteins. It is also needed in large quantities along with threonine for the synthesis of mucins found in mucus secretions in the GI tract. These roles of glutamine in the GI tract have prompted several companies to enrich enteral and parenteral (intravenous) nutrition products with glutamine. When glutamine is provided through tube feedings, over 50% of glutamine is extracted by the splanchnic (visceral) bed. It is estimated that the human GI tract uses up to 10 g of glutamine per day, and that the cells of the immune system use over 10 g per day. In addition to dietary glutamine, much of the body's glutamine that is produced by the skeletal muscles (and to lesser extents by the lungs, brain, heart, and adipose tissue) is released and taken up, mostly by the intestinal cells.

Glutamine not used for energy production within the intestine may also be partially catabolized to generate ammonia and glutamate. The ammonia enters the portal blood for uptake by the liver or may be used within the intestinal cell for carbamoyl phosphate synthesis. The glutamate thus formed is discussed next.

Intestinal Glutamate Metabolism

In the intestinal cell, glutamate arises directly from glutamine metabolism or directly from the diet. About 50 g of dietary protein contains about 2–6 g of glutamate and about 90% of this absorbed dietary glutamate is metabolized within the enterocyte. Glutamate is often transaminated with pyruvate to form α -ketoglutarate and alanine (Figure 6.33); the alanine typically enters portal blood for transport to the liver. Glutamate not used for alanine synthesis is often used with glycine and cysteine to make glutathione, or it may be used to synthesize proline, as shown here:



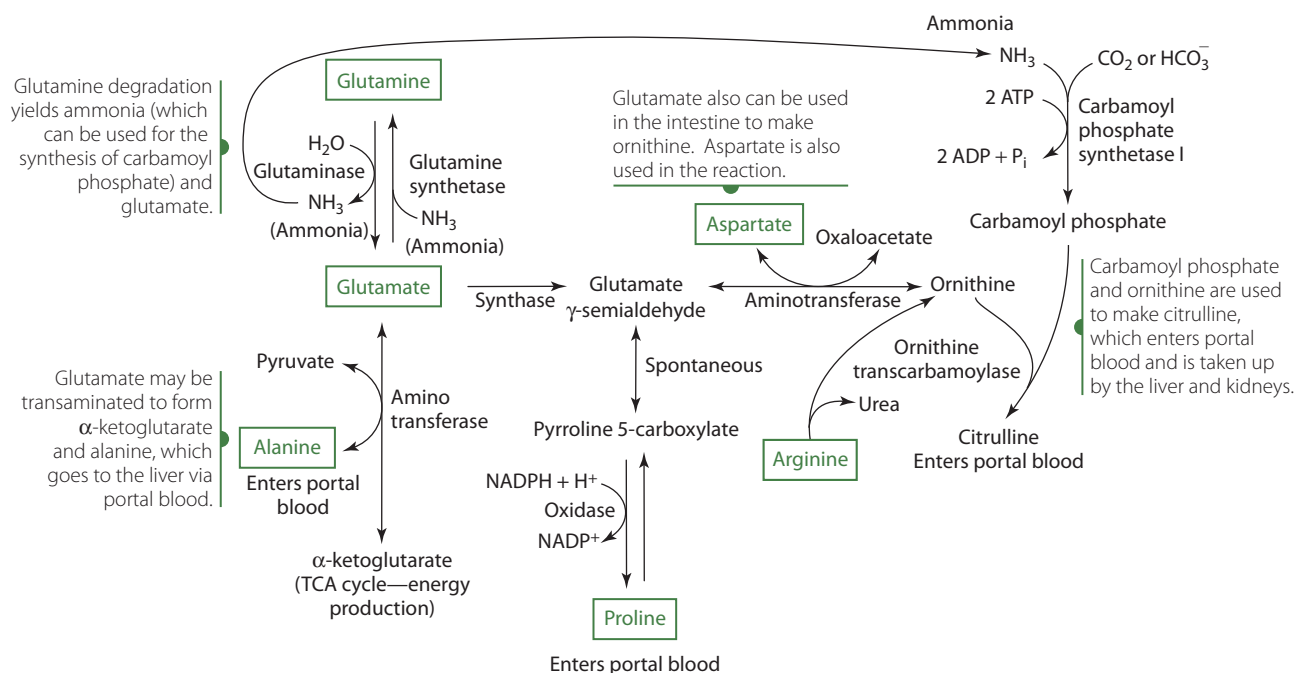


Figure 6.33 A partial overview of amino acid metabolism in the intestinal cell.

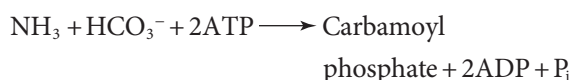
The majority of proline synthesis is thought to occur through intestinal cell glutamate metabolism. Proline is then released into portal blood for delivery to the liver. Lastly, glutamate may be used along with aspartate to synthesize ornithine, which in turn may be released into portal blood or can be used to make citrulline (Figure 6.33). Thus, very little glutamate leaves the intestinal cell as glutamate and enters portal blood.

Intestinal Aspartate Metabolism

In addition to metabolism of glutamine and glutamate, metabolism of aspartate from the diet generally occurs within intestinal cells. Aspartate most often undergoes transamination to generate oxaloacetate; aspartate's amino group in turn is used to synthesize ornithine. Very little aspartate (like glutamate) leaves the intestinal cells as aspartate and is found in portal blood.

Intestinal Arginine Metabolism

Arginine is also used by intestinal cells. Up to 40% of dietary arginine is oxidized in enterocytes, yielding citrulline and urea [6]. Carbamoyl phosphate is synthesized in intestinal cells by the action of carbamoyl phosphate synthetase I using ammonia (NH_3), carbon dioxide (CO_2) or bicarbonate (HCO_3^-), and 2ATP, as shown in Figure 6.33 and here:



The carbamoyl phosphate in turn is used along with ornithine to synthesize citrulline in a reaction catalyzed by ornithine transcarbamoylase, as follows:



Citrulline that is made in the enterocytes is released into blood and then typically taken up, mostly by the kidneys, which use it for arginine synthesis (this pathway is referred to as the intestinal–renal axis of arginine synthesis and is not thought to be affected by dietary arginine ingestion). The liver may also take up the citrulline as needed for the urea cycle. Because of the role of the intestine in citrulline synthesis and the need for citrulline in arginine synthesis, arginine production can be impaired in individuals with intestinal injury. In such a situation, arginine becomes a conditionally essential amino acid and either arginine or citrulline must be supplemented in the diet.

Intestinal Methionine (and Cysteine) Metabolism

Methionine is also metabolized by intestinal cells. Studies suggest over 50% of methionine intake is metabolized in the intestinal cells [4]. Cysteine, generated from methionine or obtained directly from the diet, is used in the intestinal cells to make glutathione. Alternately, cysteine is metabolized primarily (70–90%) to taurine and to a lesser extent (10–30%) to pyruvate and sulfite. These reactions can be reviewed in Figure 6.12.

Amino Acids in the Plasma

After ingestion of a protein-containing meal, amino acid concentrations typically rise in the plasma for several hours, then return to basal concentrations. In basal situations and between meals, plasma amino acid concentrations are relatively stable and are species specific; however, absolute concentrations of specific amino acids in the plasma vary from person to person.

Amino acids circulating in the plasma and found within cells arise from digestion and absorption of dietary (exogenous) protein as well as from the breakdown of existing body (endogenous) tissues. These endogenous amino acids intermingle with exogenous amino acids to form a “pool” totaling about 150 g. The pool includes amino acids in the plasma as well as amino acids in the cytosol of body cells. Reuse of endogenous amino acids is thought to represent the primary source of amino acids for protein synthesis. Despite differences in protein intake and in degradation rates of tissue proteins, the pattern of the amino acids in the amino acid pool appears to remain relatively constant, although the pattern is quite different from that found in body proteins.

The total amount of the essential amino acids found in the pool is less than that of the nonessential amino acids. The essential amino acids found in greatest concentrations are lysine and threonine. Of the nonessential amino acids, those found in greatest concentrations are alanine, glutamate, aspartate, and glutamine. In fact, up to 80 g of glutamine can be found in the body’s amino acid pool.

Amino acids within the pool, regardless of source, are taken up by tissues and metabolized in response to various stimuli such as hormones and physiological state. Tissues extract amino acids for energy production or for the synthesis of nonessential amino acids, protein, nitrogen-containing nonprotein compounds, biogenic amines, neurotransmitters, neuropeptides, hormones, glucose, fatty acids, or ketones, depending on the nutritional status and hormonal environment.

Glutamine and the Muscle, Intestine, Liver, and Kidneys

Glutamine has several major roles in the body, one of which is in ammonia transport. Whereas ammonia arising in the liver from amino acid reactions is typically shuttled into the urea cycle, this is not true in other tissues. In extrahepatic tissues, especially muscle but also the lungs, heart, brain, and adipose, glutamine synthetase catalyzes the utilization of ammonia or ammonium ions with glutamate in an ATP-dependent reaction to form glutamine. It is estimated that the body produces 40–80 g glutamine per day. Ammonia is typically generated in these cells by amino acid deamination and deamidation. In muscle it also forms from AMP deamination; AMP is generated in the muscle with ATP degradation as occurs rapidly with exercise. Glutamate is formed in muscle and other cells from the transamination of the branched-chain amino acids with α -ketoglutarate to form branched-chain α -keto acids and glutamate, respectively. As shown in Figure 6.34, ammonia generated from AMP deamination combines with the glutamate to produce glutamine.

The glutamine that is formed in the muscle is released into the blood and transported for use by other tissues.

Whereas the cells of the GI tract as well as the immune system (such as lymphocytes, monocytes, and macrophages) rely on glutamine catabolism for energy production, glutamine in the liver and kidneys is utilized differently. In the absorptive state (or with **alkalosis**), liver glutaminase activity increases, yielding ammonia for the urea cycle. Hepatic enzyme activity is stimulated by epinephrine and glucagon. In an acidotic state, the use of glutamine for the urea cycle diminishes, and the liver releases glutamine into the blood for transport to and uptake by the kidneys for use in acid–base balance. In the renal tubular cells, glutamine is catabolized by glutaminase to yield ammonia and glutamate. The glutamate may be further catabolized by glutamate dehydrogenase to yield α -ketoglutarate plus another ammonia. Ammonia reacts with H^+ to form an ammonium ion in the lumen of the kidney tubule; the ammonium ion is then excreted in the urine. Renal glutaminase activity and ammonia excretion increase with acidosis and decrease with alkalosis.

Glutamine use by cells increases dramatically with hypercatabolic conditions such as infection and trauma. In these conditions muscle glutamine release increases but cannot meet other cellular demands. Thus, glutamine “stores” can become depleted and some cell functions may become impaired. Remember, glutamine plays several roles that are especially critical with illness/injury. To briefly review, glutamine is used extensively by immune system cells. Glutamine promotes proliferation of these cells and glutamine metabolites are used directly by these cells, for example, for purine and pyrimidine synthesis. Purines and pyrimidines are required in large quantities by activated lymphocytes and macrophages. Expression of cell surface activation markers and production of cytokines such as interferon and tumor necrosis factor α by lymphocytes and lymphokine-activated killer cell activity also depend on glutamine. Furthermore, phagocytes require adequate glutamine availability. Glutamine also promotes the synthesis of heat shock/stress proteins, which help protect body cells. Glutamine prevents atrophy of the intestine, protects against intestinal bacterial translocation, and serves as the major substrate for energy production for intestinal cells. Finally, glutamine, along with alanine, uptake into cells promotes increases in cell volume with possible associated regulatory roles in intermediary metabolism. Glutamine supplementation, about 20–25 g/day, typically normalizes plasma glutamine concentrations and improves outcomes in critically ill patients. Administration of glutamine as a dipeptide (alanyl-glutamine or glycyl-glutamine) is needed in either an intravenous or enteral solution because the amino acid is not stable in aqueous solutions used in feeding. Dipeptidases on the surface epithelium of blood vessels are thought to hydrolyze the dipeptide so that the glutamine is available for use.

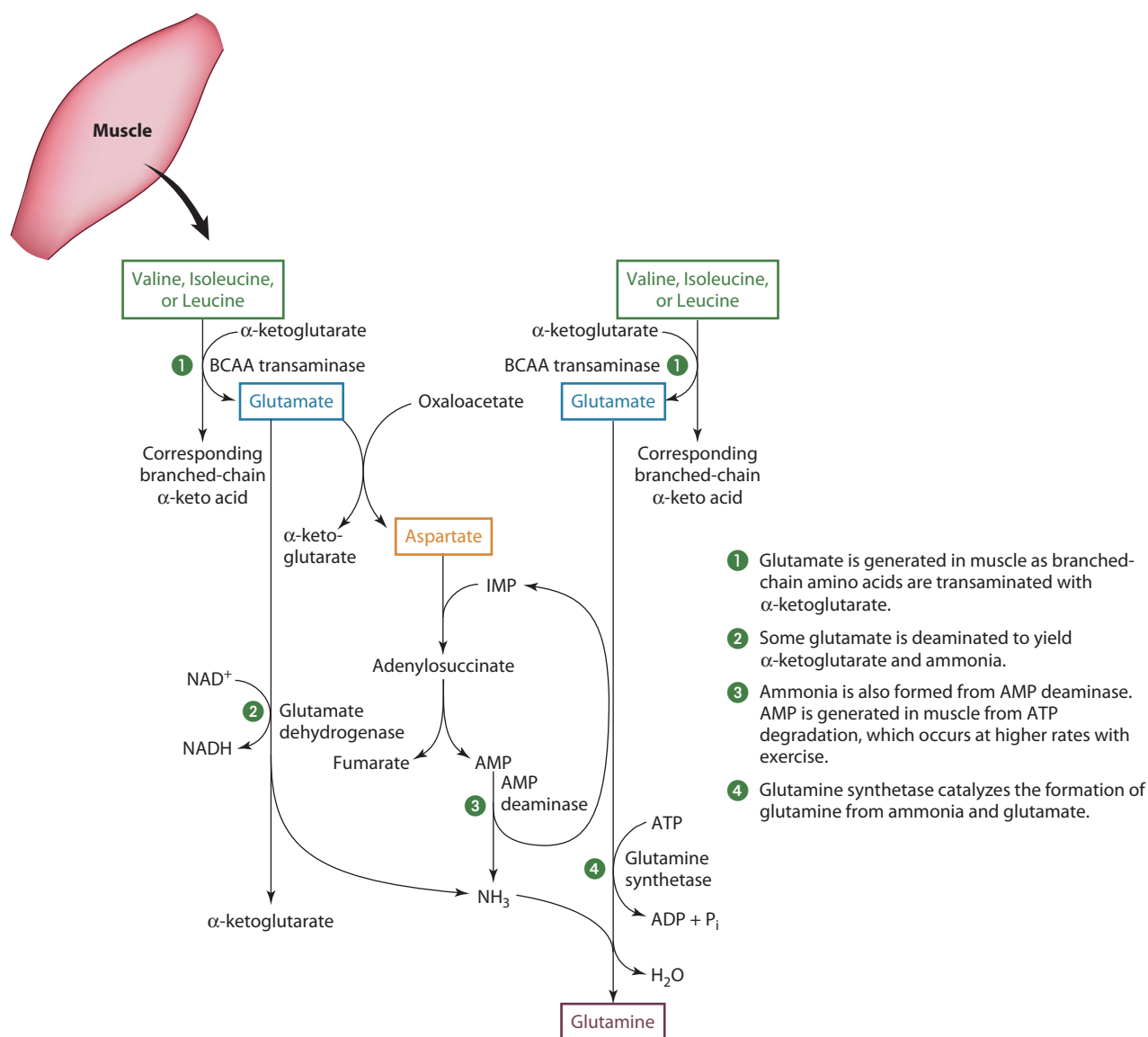


Figure 6.34 Some pathways of glutamine generation in muscle.

Alanine and the Liver and Muscle

In addition to glutamine, the amino acid alanine is also important in the intertissue (between tissues) transfer of amino groups generated from amino acid catabolism. As discussed in the previous section, transamination reactions in muscle generate glutamate, which is used, especially in a fed state/after eating, to synthesize glutamine for release into the blood. In several situations (between meals, with excessive glucose needs, with illness characterized by increased release of epinephrine and cortisol, or in situations such as fasting marked by low hepatic glycogen stores and a glucagon-to-insulin ratio favoring glucagon), glutamate typically transfers its amino group to pyruvate, generated from glucose oxidation via glycolysis, to form α -ketoglutarate and alanine, respectively. Once made, the alanine is released from the muscle into the blood for travel to the liver. Within the liver, alanine undergoes transamination back to pyruvate, which is then

used to remake glucose. The glutamate that is generated with transamination can undergo deamination to provide ammonia for urea synthesis. These reactions are known as the glucose–alanine or alanine–glucose cycle and are shown in Figure 6.35. The glucose that is generated from the alanine is subsequently released into the blood, where it is available to be taken up and used by muscle. Muscle cells use the glucose through glycolysis and generate pyruvate. The formed pyruvate is again available for transamination to re-form alanine. This alanine–glucose cycle serves to transport nitrogen to the liver for conversion to urea while also allowing needed substrates to be regenerated.

Skeletal Muscle Use of Amino Acids

About 40% of the body's protein is found in muscle, and skeletal muscle mass represents up to about 43% of the body's mass. Uptake of amino acids by the skeletal muscles readily occurs following ingestion of food, especially

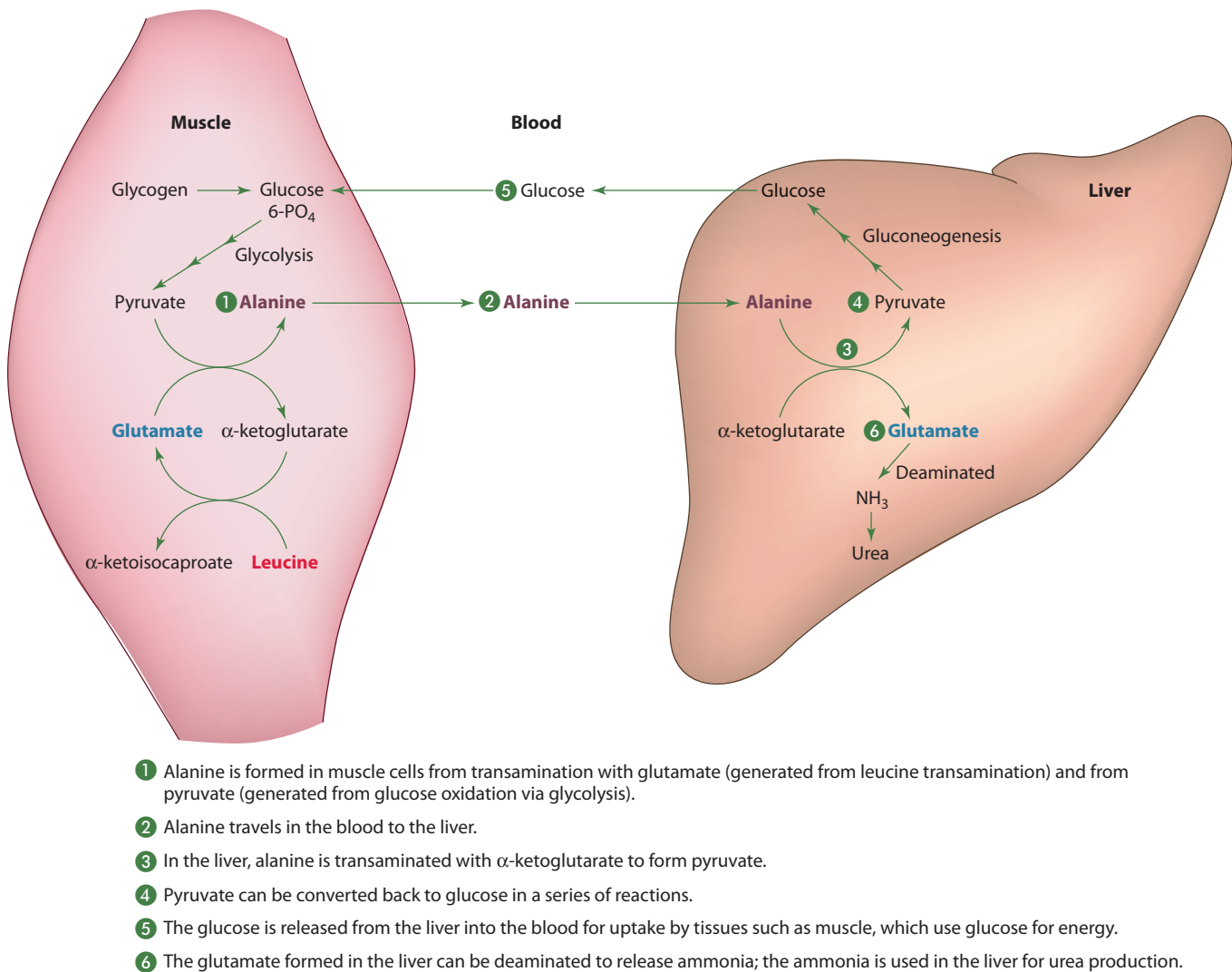


Figure 6.35 The alanine–glucose cycle: alanine generation in muscle and glucose generation in the liver.

a mixed meal rich in protein. Exercise further encourages amino acid uptake by muscles (see the “Exercise and Nutrition” section in Chapter 7). After eating, skeletal muscles exhibit net protein synthesis (i.e., protein synthesis is greater than protein degradation).

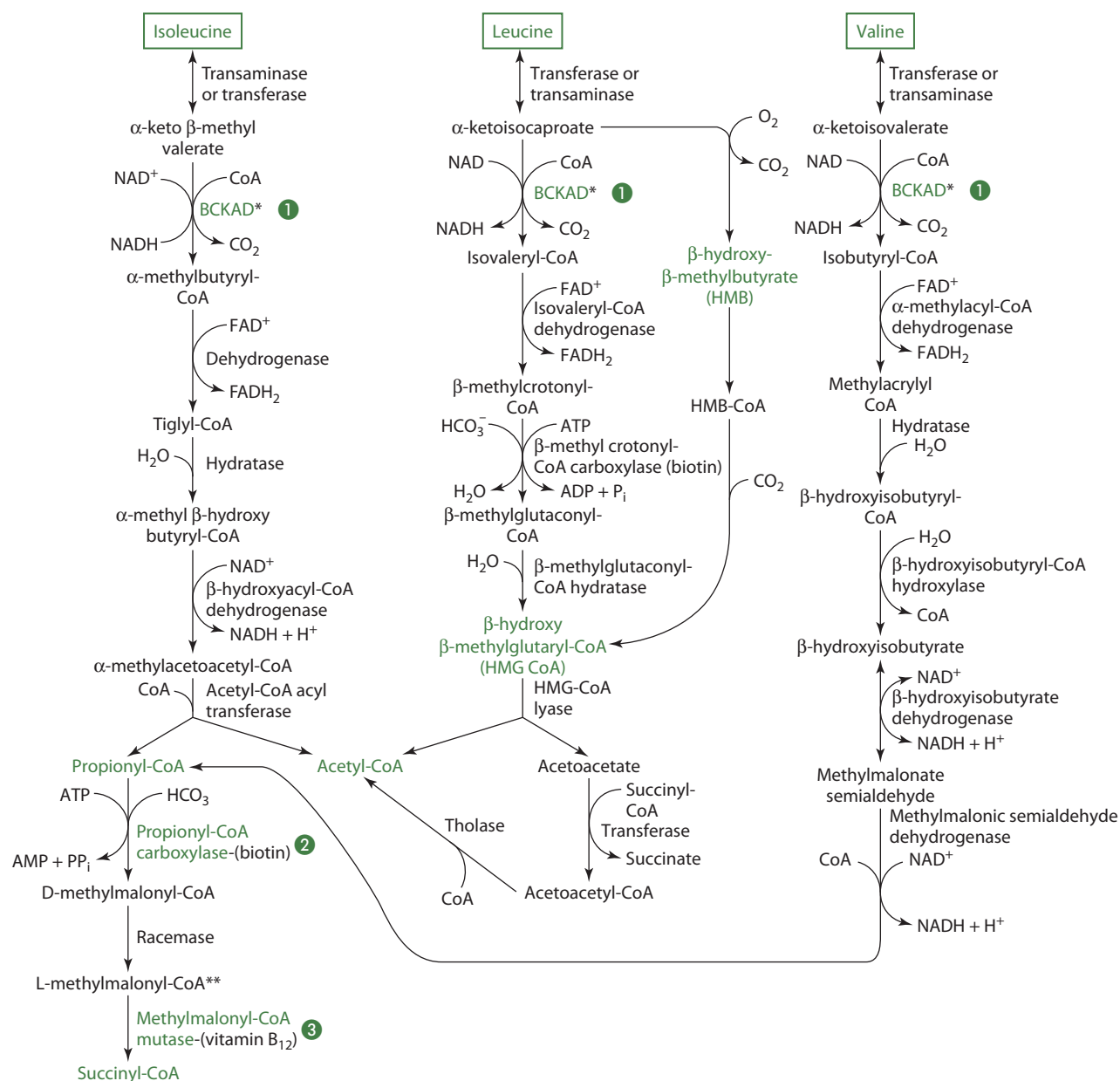
In a postabsorptive state such as between meals or in a fasting situation, the reverse is true. Protein degradation predominates over synthesis, and amino acids may be released into the blood for use by other tissues. While alanine is released in the greatest concentration, other amino acids (including phenylalanine, methionine, lysine, arginine, histidine, tyrosine, proline, tryptophan, threonine, and glycine) are released in lesser quantities.

Muscle protein degradation is also associated with exercise. Cortisol, secreted by the adrenal glands, in response to exercise-induced stress, promotes, in part, this muscle and amino acid catabolism (see the “Hormonal Regulation of Metabolism” and “Exercise and Nutrition” sections in Chapter 7).

Like other tissues, muscles preferentially catabolize some amino acids more than others; six amino acids (aspartate, asparagine, glutamate, leucine, isoleucine, and valine) appear to be catabolized to greater extents in the skeletal muscle than other tissues. This use of amino acids by muscle as well as leucine’s role in promoting protein synthesis has prompted the consumption of branched-chain amino acid supplements by some athletes. The catabolism of the branched-chain amino acids (isoleucine, leucine, and valine) is discussed in the following subsection and is shown in Figure 6.36.

Isoleucine, Leucine, and Valine Catabolism

Muscle, as well as the heart, kidneys, diaphragm, adipose tissue, and other organs (except, for the most part, the liver), possesses branched-chain aminotransferases, located in both the cytosol and mitochondria and responsible for the transamination of all three branched-chain amino acids. Following transamination, the α-keto acids



*Branched-chain α-keto acid dehydrogenase (BCKAD), requiring thiamin as TDP/TPP, niacin as NADH, and Mg^{2+} and CoA from pantothenate.

**Common intermediate in the catabolism of methionine, threonine, isoleucine, and valine.

① Defect in this enzyme complex causes maple syrup urine disease.

② Defect in this enzyme results in propionic acidemia.

③ Defect in this enzyme results in methylmalonic acidemia.

Figure 6.36 Branched-chain amino acid metabolism.

of the branched-chain amino acids either remain within muscle or may be transported (bound to albumin) in the blood to other tissues (including the liver) for use.

Further catabolism of the branched-chain α-keto acids occurs by decarboxylation in an irreversible reaction catalyzed by the branched-chain α-keto acid dehydrogenase (BCKAD) complex. BCKAD is a large multienzyme complex made up of three subunits: E1α, E1β, and E2. This enzyme complex is found in the mitochondria of many

tissues, including liver, muscle, heart, kidneys, intestine, and brain. It is highly regulated through phosphorylation (inactivation) and dephosphorylation (activation) mechanisms involving kinase and phosphatase proteins that act on the E1α subunit and act through end-product inhibition. This enzyme complex operates in a fashion similar to the pyruvate dehydrogenase complex (see Chapter 3) in that it requires thiamin in its coenzyme form TDP, niacin as NADH, and Mg^{2+} and CoA from pantothenic acid.

The details of the oxidation of the three branched-chain amino acids are shown in Figure 6.36. As with other amino acids, the complete oxidation of branched-chain amino acids yields products that are glucogenic and/or ketogenic. Valine oxidation yields succinyl-CoA and is thus considered glucogenic. The end products of isoleucine catabolism are succinyl-CoA and acetyl-CoA, which are glucogenic and ketogenic, respectively. The oxidation of leucine results in acetyl-CoA and acetoacetate formation; acetoacetate may be further metabolized to form acetyl-CoA. Leucine is thus totally ketogenic.

Other common intermediates are formed during branched-chain amino acid oxidation. Isoleucine and valine, for example, generate propionyl-CoA, which is a common intermediate in the degradative pathways of methionine and threonine.

Leucine's metabolism also generates β -hydroxy β -methylbutyrate (HMB) (Figure 6.36). HMB is important for the production of β -hydroxy β -methylglutaryl (HMG)-CoA, a precursor for *de novo* cholesterol synthesis in the muscle and that enables repair and regeneration of damaged cells. It appears that with some illnesses and with muscle damage, HMG-CoA concentrations may be inadequate to support cholesterol synthesis. Supplementation with HMB, usually as calcium HMB monohydrate (about 3 g per day given in three 1-g doses), provides cells with a source of HMG-CoA to maintain cholesterol synthesis and thus cell function. In addition, HMB appears to attenuate both muscle proteolysis and depression of muscle protein synthesis to improve muscle mass. Atrophy of muscle with muscle damage or secondary to conditions such as cancer, sepsis, and acquired immune deficiency syndrome (AIDS), among others, is due in large part to the activity of the ubiquitin–proteasome pathway (see the “Catabolism of Tissue Proteins” section); HMB appears to inhibit this pathway and the autophagy-lysosomal protein degradation system as well as to stimulate, along with leucine, protein synthesis through mTOR. HMB's effects have been demonstrated in healthy individuals as well as in those with conditions typically associated with muscle loss such as cancer, AIDS, and in some with sarcopenia. Other forms of HMB, such as a free-acid form in a gel, are under investigation and may prove superior to the more commonly available form as a calcium salt. However, studies are also needed to identify if any adverse or toxic effects occur from supplementation.

Leucine is one of the few amino acids that is completely oxidized in the muscle for energy. Leucine is oxidized in a manner similar to fatty acids, and its oxidation results in the production of 1 mol of acetyl-CoA and 1 mol of acetoacetate. Complete oxidation of leucine generates more ATP molecules on a molar basis than complete oxidation of glucose. Leucine appears to be preferentially oxidized during fasting situations. During fasting, leucine concentrations rise in the blood and muscle, and the capacity of the muscle to

degrade leucine increases concurrently. This rise in capacity supplies the muscle with the equivalent of 3 mol of acetyl-CoA per molecule of leucine oxidized; the acetyl-CoA produces energy for the muscle while simultaneously inhibiting the oxidation of pyruvate, which is derived from glucose oxidation via glycolysis. Pyruvate is then transaminated to alanine and transported via the blood to the liver (see the previous section “Alanine and the Liver and Muscle”).

Disorders of Isoleucine, Leucine, and Valine Metabolism

Maple syrup urine disease (MSUD) results from genetic mutations in genes coding for the BCKAD complex. The condition affects about 1 in 225,000 individuals worldwide, but in the Mennonite population in the United States it impacts about 1 in 150. MSUD, if untreated, results in an accumulation of the branched-chain amino acids and their α ketoacids in the blood and body fluids. The condition is characterized by acidosis, vomiting, lethargy, and frequently coma and death. High plasma leucine concentrations (vs. high plasma isoleucine and valine) are more neurotoxic, and thus one aspect of management involves maintaining plasma concentrations of especially leucine but also isoleucine and valine in the normal range. A diet restricted in leucine, isoleucine, and valine intakes is required; large doses of thiamin are also tried to see if supplementation enhances residual BCKDC activity (remember thiamin is a coenzyme for the BCKDC).

Mutations in genes coding for some enzymes required for leucine degradation have also been documented (see Figure 6.36). Reductions in isovaleryl-CoA dehydrogenase activity result in isovaleric acidemia. Although fairly rare, it is one of the more prevalent disorders of leucine metabolism, affecting about 1 in 250,000 worldwide but about 1 in 62,000 in Germany. Defects in β -methyl crotonyl-CoA carboxylase cause β -methyl crotonylglycinuria. Impaired activity of β -methylglutaconyl-CoA hydratase causes β -methyl-glutaconic aciduria and altered activity of β -hydroxyl β -methylglutaryl (HMG)-CoA lyase causes β -hydroxyl β -methylglutaric aciduria. Each of these disorders results in the production and accumulation of numerous acids and other compounds in body fluids, causing acidosis, dehydration, neurological problems, seizures, coma, and mental retardation, among other problems. A leucine-restricted diet is typically prescribed for these conditions. In some cases, to prevent the accumulation of toxic compounds, supplements of carnitine and glycine may be useful. Dietary fat restriction is also needed for those with HMG-CoA lyase deficiency.

Impaired propionyl-CoA carboxylase and methylmalonyl-CoA mutase activities result in propionic acidemia and methylmalonic acidemia, respectively. These enzymes in addition to affecting valine and isoleucine oxidation are also common to methionine and threonine catabolism. Refer back to the section “Disorders of Methionine Metabolism.”

Indicators of Muscle Mass and Muscle/Protein Catabolism

While muscle proteolysis generates amino acids that are released into the plasma for circulation to and use by other tissues, changes in plasma amino acid concentrations do not reflect changes in muscle mass. Instead, two previously mentioned compounds, creatinine and 3-methylhistidine, are used as indicators of existing muscle mass and muscle degradation, respectively. Urinary creatinine excretion is used to assess muscle mass because creatinine is the degradation product of creatine, which constitutes a fairly standard proportion of muscle (approximately 0.3–0.5% of muscle mass by weight). Urinary creatinine excretion reflects about 1.7% of the total creatine pool per day and is expressed per 24 hours, as a coefficient based on weight or height; however, because of variation in muscle creatine content, urinary creatinine is not always an accurate indicator of muscle mass.

The urinary excretion of 3-methylhistidine is used as an indicator of muscle catabolism (degradation). As mentioned under the section on histidine in “Hepatic Catabolism and Uses of Basic Amino Acids,” the amino acid histidine is found in high concentrations as 3-methylhistidine in the muscle protein actin. Because 3-methylhistidine cannot be reused for protein synthesis following protein degradation and is excreted in the urine, its urinary excretion can be measured and serves as an indicator of muscle breakdown. A drawback to its use, though, is that actin is not found only in muscle but appears to occur in other body tissues, including the intestine and platelets, which have high turnover rates. Thus, urinary 3-methylhistidine excretion may also represent an index of protein breakdown for many nonmuscle tissues in the body.

Amino Acid Metabolism in the Kidneys

The kidneys preferentially take up and metabolize a number of amino acids and nitrogen-containing compounds (Figure 6.37). The kidneys’ roles include:

- Glutamine catabolism for acid–base balance
- Glycine catabolism for acid–base balance
- Serine synthesis from glycine
- Arginine and glycine use to form guanidinoacetate for creatine synthesis
- Glutathione catabolism
- Arginine synthesis from citrulline
- Tyrosine synthesis from phenylalanine
- Histidine generation from carnosine degradation.

In fact, the kidneys are considered to be the major site in the body for arginine, histidine, serine, and perhaps tyrosine production [13].

Glutamine uptake by the kidneys has been estimated at 7–10 g per day [13] but uptake increases dramatically with acidosis, whereas glutamine uptake by the intestine, liver, and other organs diminishes. Especially in acidotic conditions, glutamine and then glutamate are deamidated and deaminated, respectively, in the kidneys, resulting in two ammonias. In the kidney’s tubular lumen (see Figure 12.8), the ammonias combine with H^+ ions and form ammonium ions, which are excreted in the urine. H^+ ions enter the tubular lumen in exchange for Na^+ . In the lumen, the H^+ ions may also react with bicarbonate (HCO_3^-) to form water and carbon dioxide and with dibasic phosphate (HPO_4^{2-}) to form monobasic phosphate ($H_2PO_4^-$). Glycine utilization by the kidneys under acidotic conditions is similar to glutamine utilization; glycine is degraded, forming ammonia and carbon dioxide. The ammonia then enters into the tubular lumen, where it reacts with H^+ ions, forming ammonium ions that are excreted in the urine. The loss of the H^+ from the body serves to increase blood pH from an acidotic state to a value ideally within the normal range of about 7.35–7.45.

Under healthy (nonacidotic) conditions, glycine is used by the kidneys (proximal tubule) for the synthesis of the amino acid serine. The kidneys also use glycine along with arginine for the synthesis of guanidinoacetate; this compound then travels to the liver, where it is used to generate creatine. The kidneys are thought to take up about 1.5 g of glycine per day [13]. Glycine, however, is also generated from glutathione catabolism in the proximal tubules of the kidneys.

Most arginine that is made in the body for tissue use is made in the kidneys from citrulline that was generated in the intestines and has been extracted from the blood; remember, the arginine made in the liver is immediately degraded to form urea and is thus not available to body tissues. It is estimated that the kidneys extract about 1.5 g of citrulline per day from the blood and release about 2–4 g of arginine daily [13].

Phenylalanine catabolism to tyrosine in the kidneys has also been demonstrated. It is estimated that the kidneys take up about 0.5–1 g of phenylalanine from the blood each day and releases about 1 g of tyrosine [13]. In addition to phenylalanine degradation, carnosine is oxidized by the kidneys, releasing histidine for use by other body tissues.

The kidneys can also generate glucose for the body. The kidneys, like the liver and to some extent like the small intestine, have the enzymes necessary for gluconeogenesis. See Chapter 3 for a detailed description of these reactions.

The role of the kidneys in nitrogen metabolism cannot be overemphasized. The organ is responsible for ridding the body of nitrogenous wastes that would otherwise accumulate in the blood plasma. Kidney glomeruli act as filters of blood plasma, and all the constituents in plasma, with the exception of plasma proteins, move into

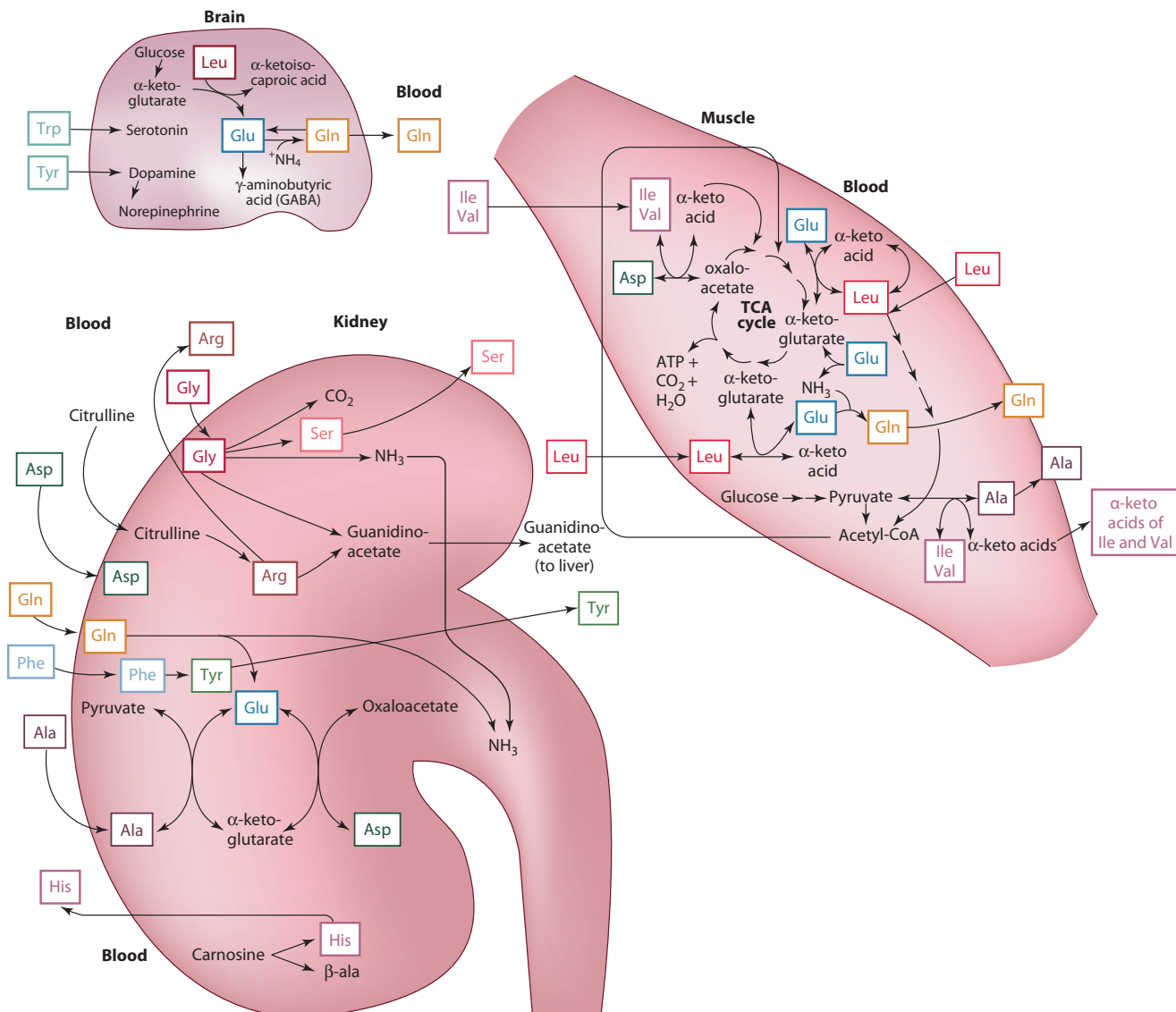


Figure 6.37 Amino acid metabolism in selected organs.

the filtrate. Essential nutrients such as sodium, amino acids, and glucose are actively reabsorbed as the filtrate moves through the tubules. Many other substances are not actively reabsorbed and must either move along an electrical gradient or move osmotically with water to enter the tubular cells. The amount of these substances that enters the tubular cells, then, depends on how much water moves into the cells and how permeable the cells are to the specific substances. The cell membranes are relatively impermeable to urea and uric acid and are particularly impermeable to creatinine, little to none of which is typically reabsorbed.

Nitrogenous wastes found in the urine are listed in Table 6.7. About 80% of nitrogen is lost in the urine as urea under normal conditions. In acidotic conditions, urinary urea nitrogen losses decrease and urinary excretion

of ammonium ions rises. In addition to urea and ammonia, usual nitrogenous wastes found in the urine include creatinine and uric acid, with lesser or trace amounts of creatine (<100 mg/day), protein (<100 mg/day), amino acids (<700 mg/day), and hippuric acid (<100 mg/day). Hippuric acid results from the conjugation of the amino acid glycine and benzoic acid, which is generated mostly in the liver from the catabolism of aromatic compounds. Because the benzoic acid is not water soluble, it must be conjugated for excretion. Trace amounts of other nitrogen-containing compounds such as porphobilinogen and metabolites of tryptophan also may be present in the urine. In addition to urinary nitrogen losses, nitrogen may be lost in the feces and sweat and with the loss of hair and skin cells. These losses are referred to as insensible nitrogen losses.

Table 6.7 Nitrogen-Containing Waste Products Excreted in the Urine

Compound	Approximate Amount Excreted/Day	
	g/day	mmol/N
Urea	5–20	162–650
Creatinine	0.6–1.8	16–50
Uric acid	0.2–1.0	4–20
Ammonia	0.4–1.5	22–83

Brain and Accessory Tissues and Amino Acids

The brain has a high capacity for the active transport of amino acids. In fact, the brain has transport systems for neutral, basic, and acidic amino acids. The transporters for some of the amino acids are almost fully saturated at normal plasma concentrations; this is especially true of the transporters for the large neutral amino acids like the branched-chain and the aromatic amino acids, which can compete with each other for the common carriers. The effects of this competition become especially apparent in conditions in which the blood concentrations of any of the branched-chain or aromatic amino acids become elevated. For example, in untreated PKU, elevations in blood phenylalanine result in increased uptake of this amino acid by the brain. In untreated MSUD, elevations in blood leucine, isoleucine, and valine result in the increased uptake of these amino acids (at the expense of the aromatic amino acids) into the brain. Moreover, in liver disease, the concentrations of the aromatic amino acids exceed those of the branched-chain amino acids and cause increased uptake of the aromatic amino acids by the brain. The elevations of amino acids in the brain alter brain function, causing a variety of neurologic problems such as impaired brain development and altered behavior and mental function, among other manifestations.

While it is clear that conditions like liver disease and inborn errors of amino acid metabolism can alter the brain's uptake of selected amino acids and cause neurological and behavioral changes, such effects have not been demonstrated consistently in healthy individuals who attempt to alter behavior by altering dietary intakes of nutrients. For example, ingestion of carbohydrate (without ingestion of protein) has been shown to increase the brain's uptake of tryptophan and raise serotonin concentrations but does not always result in the expected behavioral effects (such as feeling calm and relaxed) of elevated serotonin. In other words, varying nutrient intakes, including carbohydrate and protein, by eating selected foods is thought to have little effect on the brain's serotonergic function.

The brain and nervous system tissue use several amino acids for the synthesis of neuropeptides, biogenic amines, and neurotransmitters (discussed further in the next

section). Glutamate serves multiple roles—as an excitatory neurotransmitter and as a substrate for the production of the inhibitory neurotransmitter γ -amino butyric acid (GABA) (discussed in the next section) and as a means of ridding the brain of ammonia. While glutamate serves this critical role, little glutamate is actually transported across the blood–brain barrier and into the brain. Rather, glucose that has been transported into the brain can be metabolized to α -ketoglutarate, which can be converted to glutamate through reductive amination. More commonly, however, leucine, following transport across the blood–brain barrier, undergoes transamination with α -ketoglutarate to produce the glutamate and α -ketoisocaproate (which undergoes further metabolism) in the neuron. Leucine, valine, and isoleucine are thought to provide about 33% of the amino groups used by the brain for glutamate synthesis [14].

Glutamate, following its neuronal synthesis, diffuses into the synaptic cleft and is taken up by astrocytes. Within the astrocyte, the glutamate readily reacts with any excess ammonia to form glutamine by the action of glutamine synthetase, which is highly active in neural tissues. The glutamine can be transported back into the neuron where it reforms glutamate by the action of glutaminase. Alternately, the glutamine can freely diffuse into the blood or cerebrospinal fluid, thereby allowing the removal of 2 mol of toxic ammonia from the brain. This glutamate–glutamine cycle (shown in Figure 6.37) is a major means of regulating neuronal glutamate levels and accounts for about 80% of glutamine production [14].

Neurotransmitters and Biogenic Amines

Neurotransmitters are compounds generated in the body that transmit signals from a neuron to a target cell across a synapse. Neurotransmitters are stored or packaged in the nerve axon terminal as vesicles or granules until stimuli arrive to effect their release into the synaptic cleft and allow for their binding to receptors on the postsynaptic side of the synapse. Neurotransmitter action on the cell membranes typically elicits an action or electrical potential. Several amino acids act directly as neurotransmitters, including:

- Glycine, which acts primarily in the spinal cord as an inhibitory neurotransmitter
- Taurine, which is thought to function as an inhibitory neurotransmitter
- Aspartate, which is derived chiefly from glutamate through aspartate aminotransferase activity common in neural tissue and is thought to act as an excitatory neurotransmitter in the central nervous system
- Glutamate, which acts primarily in the brain and spinal cord as an excitatory neurotransmitter. Glutamate can also be decarboxylated in the brain in a vitamin B₆ (PLP)–dependent reaction to produce GABA (Figures 6.37 and 6.38). GABA functions in the brain as an inhibitory neurotransmitter.

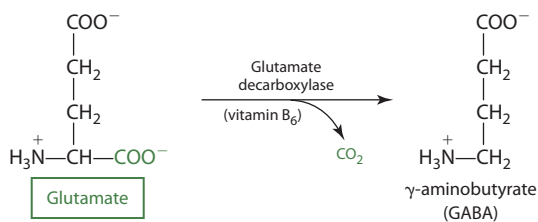


Figure 6.38 GABA synthesis from the amino acid glutamate.

Amino acids that are excitatory stimulate receptors on postsynaptic membranes; this stimulation in turn propagates the nerve impulse. In contrast, amino acids that are inhibitory retard the postsynaptic neuron from propagating nerve impulses.

Many amino acids are catabolized within the brain and nervous system to generate biogenic amines. These biogenic amines may also function as neurotransmitters. Some of these amino acids and the amines they produce include:

- Tryptophan, which is used to synthesize serotonin (Figures 6.37 and 6.39). Serotonin functions as an excitatory neurotransmitter (biogenic amine) in the central nervous system and in circulation as a potent vasoconstrictor and stimulator of smooth muscle contractions. Serotonin affects sleep, mood, and appetite as well as memory and learning (e.g., cognitive functions).
- Tyrosine, which is used in sympathetic neurons to make catechol derivatives, collectively called catecholamines

(dopamine, norepinephrine, and epinephrine; Figures 6.37 and 6.40). In the brain and neurons, the catecholamines function as neurotransmitters. Dopamine affects a variety of behaviors as well as coordination of movement. Norepinephrine plays roles in alertness and sleep. Epinephrine is found in low concentrations in the brain; however, in circulation, it functions as a hormone with major (primarily catabolic) effects on nutrient metabolism.

- Histidine, which is decarboxylated to generate histamine (shown previously in Figure 6.14). The neurotransmitter histamine mediates attention and alertness, among other possible roles.

Once neurotransmitters and biogenic amines have exerted their actions, the fastest mechanism for their inactivation is uptake by adjacent cells or synaptic terminals. Enzymes responsible for catecholamines and serotonin degradation include monoamine oxidase and aldehyde dehydrogenase; catechol-O-methyltransferase (which is found in the liver, kidneys, and smooth muscle but not the neurons) can also methylate the catecholamines to effect their slower degradation after transport via the blood. The well-known interaction between medications known as monoamine oxidase inhibitors and foods high in amines such as tyramine is discussed in the Chapter 13 Perspective on nutrient–drug interactions. Other neurotransmitters are degraded by other pathways; histamine, for example, is catabolized by diamine oxidase.

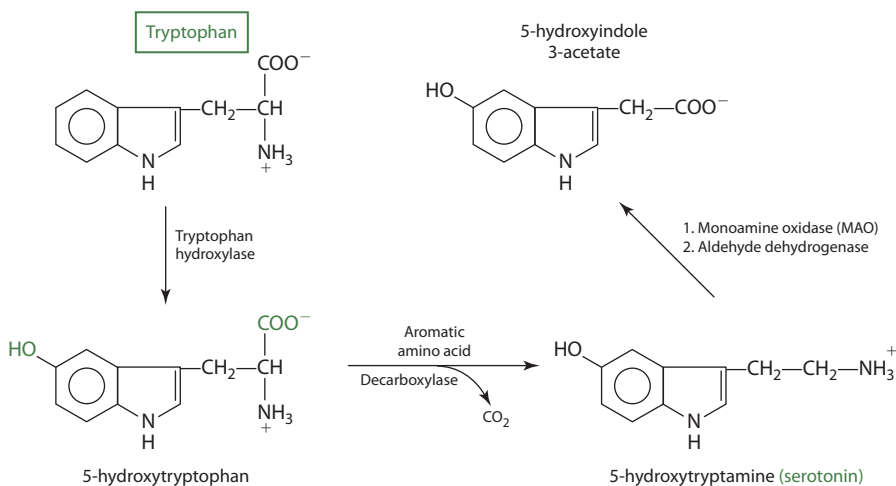


Figure 6.39 Serotonin synthesis (from tryptophan) and degradation.

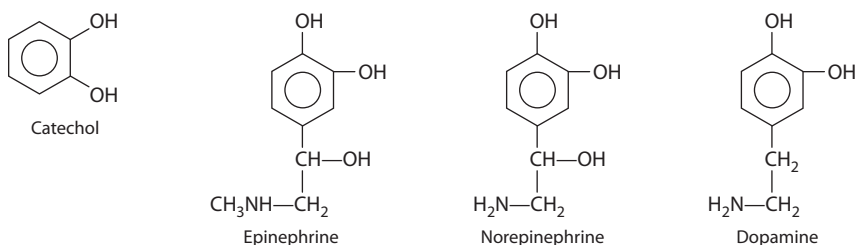


Figure 6.40 The structures of the catecholamines. The synthesis of these compounds is shown in Figure 6.10.

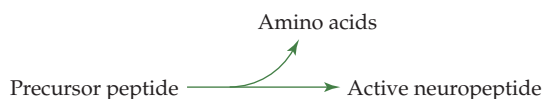
Neuropeptides

Neuropeptides (also referred to as neuroactive peptides) are small protein-like compounds that are similar to neurotransmitters but have more diverse effects. They are derived from amino acids but are not necessarily biogenic amines. The central nervous system abounds in neuropeptides; in fact, many of the same peptides that were discussed in Chapter 2 in association with the intestinal tract are also found associated with the central nervous system.

Neuropeptides perform a variety of functions. Some peptides act as hormone-releasing factors; ACTH, for instance, is involved with cortisol release. Some, such as somatotropin or growth hormone, have endocrine effects. Others, such as the enkephalins, have modulatory actions on transmitter functions, mood, or behavior. The enkephalins and endorphins, though similar to natural opiates, possess a wide range of functions, including affecting pain sensation, blood pressure, body temperature, body movement, hormone secretion, feeding, and modulation of learning ability. Some additional examples of neuropeptides include alpha melanocyte-stimulating hormone, neuropeptide Y, agouti-related peptide, ghrelin, and neurotensin, to name a few.

The neurosecretory cells of the hypothalamus are foremost in the secretion of the neuropeptides. Those that have hormone action move out of the axons of the nerve cells into the pituitary, from which they are secreted. This linkage between the nervous system and the pituitary is of great significance in the overall control of metabolism because the pituitary gland is primary in coordinating the various endocrine glands scattered throughout the body.

Neuropeptides are expressed and released by neurons. Because the nucleus and ribosomes in neurons are found in the cell body and dendrites, the neuropeptides, once made, must travel to the end of the axon to be stored in vesicles for future release. The neuropeptides are typically stored as inactive precursor polypeptides, which must be cleaved to generate an active neuropeptide, as shown here:



Following synthesis of the active neuropeptide, it is released by exocytosis to perform its function at the membrane. After performing its function, the neuropeptide is hydrolyzed to its constituent amino acids.

6.11 CATABOLISM OF TISSUE/CELL PROTEINS AND PROTEIN TURNOVER

Protein synthesis and protein degradation (i.e., protein turnover) are under independent controls but together account for about 10–25% of resting energy expenditure.

Rates of synthesis can be high, as with protein accretion during growth. Alternately, protein degradation can predominate, as during illness/injury. Rates of protein turnover also vary among the body tissues, as is evidenced in the more rapid turnover of visceral protein as compared with skeletal muscle. Yet, because of its mass, muscle accounts for about 25–35% of all protein turnover in the body. Total body protein turnover represents about 1–2% of body protein each day and may total over 300 g.

The degradation of proteins yields amino acids that are mostly reused by body tissues. Proteins are degraded within cells primarily by the action of proteases, which are compartmentalized primarily in lysosomes and proteasomes; however, some are also found in the cytosol. The contributions of the different systems to overall proteolysis vary depending on the tissue and the physiological status. Nonetheless, the constant degradation of proteins is of prime importance because it ensures a flux of amino acids through the cytosol that can be used for cellular growth and/or maintenance.

The degradation of damaged proteins (and other cellular components) is also critical for cell survival. Several mechanisms are in place within cells to monitor for the production of aberrant mRNA. There are also mechanisms that assist with protein folding processes on the ribosomes to enable detection and subsequent removal of misfolded proteins. These mechanisms, and others, help to prevent the accumulations of damaged and misfolded or unfolded proteins. Malfunctioning of the cell's degradation systems are known contributors to aging as well as to the development of some chronic diseases, including neurodegenerative disorders.

Multiple signals influence the degradation of proteins within cells. mTOR integrates signals from insulin and leucine, among other molecules, to influence protein turnover. Inhibition of mTORC1 along with the presence of other compounds/events stimulates protein degradation. Cellular levels of amino acids are also influential, with limited or imbalanced concentrations enhancing protein degradation. Changes in hormone concentrations (such as higher glucagon relative to insulin) as well as the presence of inflammatory cytokines enhance protein degradation. In addition, enzymes, such as kinases among others, regulated via phosphorylation/dephosphorylation mechanisms, and transcription factors targeting promoter regions in specific genes influence the protein turnover. The mechanisms by which protein degradation is controlled within cells are being actively studied; see Suggested Readings at the end of this chapter for more detailed information on this subject. The main protein degradation pathways/systems are presented hereafter.

Autophagy-Lysosome Systems

Three main types of **autophagy** (“self-eating”) are found in cells—microautophagy, macroautophagy, and chaperone-mediated autophagy, although it is the latter two types

that account for most protein degradation. Autophagy typically provides for the removal of larger-sized cytosolic substances such as dysfunctional organelles, lipid droplets, protein polymers or aggregates, and even some invading bacteria; it is sometimes referred to as “bulk degradation.” Longer-lived cytosolic proteins (small/monomeric but especially larger ones) are degraded primarily by autophagy. The system does, however, adapt to the cellular environment, with increased activity exhibited in “stressed” situations (such as nutrient deprivation, hypoxia, and reductions in growth factors, among others). Lysosomes are involved in each type of autophagy, but the methods used to deliver the substances in need of removal to the lysosomes varies among the types.

Lysosomes are cell organelles (about 0.2–0.5 μ m in diameter) that act like a cellular garbage digestive system to degrade proteins, nucleic acids, lipids, and carbohydrates, among other compounds. Lysosomes are found in all mammalian cells, with the exception of red blood cells, but in varying numbers. For example, skeletal muscles contain few lysosomes, whereas hepatocytes are particularly rich in lysosomes. The organelles contain a variety of enzymes including protein-digesting endopeptidases and exopeptidases, known as cathepsins. Examples of **cathepsins** (designated by letters), which vary in specificity, include B, H, and L (cysteine proteases) and cathepsin D (an aspartate protease). Lysosomal enzymes are active only at an acidic pH, which is achieved by a proton pump in the lysosomal membrane. This pump actively transports protons (H^+) from the cytosol into the lysosome to lower the lysosomal pH. Once activated, the proteases and other enzymes digest cellular proteins and components.

Lysosomes acquire proteins and other substances for degradation primarily from the cell’s plasma membrane and from intracellular locations. In macroautophagy the lysosomes acquire substances for degradation after endocytosis of a portion of the cell’s plasma membrane. The endocytosed substances are sequestered in a phagosome (also called an autophagosome), which then fuses with lysosomes using cytosolic microtubules. Lysosomal enzymes, mentioned in the previous paragraph, digest the contents. Resulting amino acids are released into the cytosol for reuse.

The process of microautophagy is somewhat similar to macroautophagy but involves the direct invagination of the lysosomal (vs. plasma) membrane and the engulfing of cytosolic components. The lysosome’s autophagic tubules mediate the invagination and vesicle formation processes, which trap cytosolic components within the lysosome. Scission (pinching or cutting off) from the autophagic tubules enables the vesicle containing the engulfed cellular components to be released within the lumen/interior section of the lysosome. Degradation of vesicle contents by enzymes within the lysosome follows. A more selective autophagy is also present whereby autophagy receptors

recognize targeted proteins or other cytosolic components and attach them to specific regions on autophagosome membranes.

The degradation of proteins by chaperone-mediated autophagy relies on heat shock protein 70 (HSPA8/HSC70, hereafter abbreviated Hsp70) and lysosomal-associated membrane protein (LAMP) type 2A. Hsp70 is found within both the cytosol and lysosomes. Proteins degraded by this system are usually soluble, can be unfolded, and contain specific exposed sequences (motifs) that are recognized by hsp70 as well as other chaperones. Hsp70, upon binding to both the targeted protein and to LAMP type 2A in the lysosomal membrane, facilitates the unfolding of the targeted protein and its movement into the lumen of the lysosome via the formation of a small invagination in the lysosomal membrane. The target protein undergoes degradation from the actions of a variety of lysosomal proteases. The amino acids released from lysosomal proteolysis can be reused by the cell for protein synthesis or degraded based on cellular needs. Hsp70 is also released and can be reused (i.e., recycled).

Ubiquitin Proteasomal Pathway

In addition to lysosome-mediated cellular protein degradation, proteasomes (protease systems or complexes) are present in all cells to degrade proteins. Proteasomal degradation is thought to be responsible for the removal of relatively small misfolded or damaged proteins as well as regulatory proteins that typically have short half-lives (often less than 30 minutes). Regulatory proteins play major roles in multiple cellular processes including transcription, cell signaling, cell-cycle progression, and cell survival, among others. Proteasomal degradation of proteins increases during starvation as well as in pathological conditions such as sepsis, cancer, and trauma.

Short-lived proteins that will be degraded by this system appear to be identified through the presence of “signals” at their amino end (N-terminal). The signals, referred to as N-degrons, include the presence of specific modifications such as acetylation, deamidation, leucylation, and formylation, among others. Changes to the N-end of proteins are also utilized in other ways, such as enabling the protein’s binding to membrane receptors, to facilitate removal of a protein from the blood or cellular environment.

Proteasomes are large, oligomeric structures with a central “barrel-like” cavity or core where degradation occurs. They are found in both the cytosol and nucleus of cells in two forms: 20S and 26S. The 20S proteasome consists of several subunits, of which three are responsible for protein degradation via capase-, trypsin-, and chymotrypsin-like activities that provide for the cleavage of peptide bonds on the carboxy sides of acidic, basic, and hydrophobic amino acids. The 20S proteasome can be found bound by a 19S regulatory complex or cap to form a 26S proteasome,

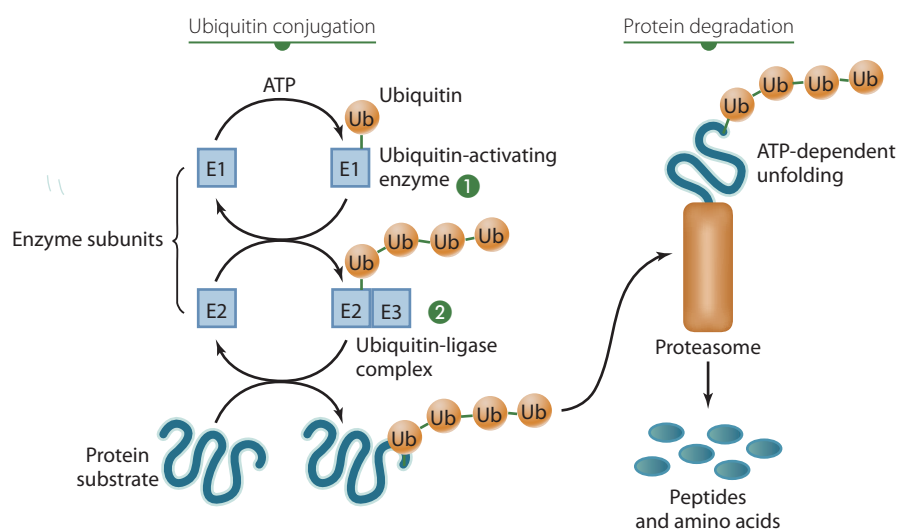
the form responsible for energy- and ubiquitination-dependent roles. However, the 20S proteasome can also operate alone, specifically in the situations when high concentrations of oxidized proteins are present, as would occur with oxidative stress. The 20S proteasome selectively identifies and degrades these oxidized proteins.

Ubiquitination is an ATP-dependent process by which proteins that are to be degraded are ligated to **ubiquitin**, a 76-amino acid polypeptide, as shown in Figure 6.41. Other proteins, including heat shock protein chaperones, may facilitate the ubiquitination process as well as the transport of the ubiquitin-linked protein to the proteasome. While the attachment of ubiquitin marks a protein for degradation, before the ubiquitin can be linked to a protein, it must first be activated. Enzyme E1 is responsible for ubiquitin activation. The activated ubiquitin is transferred to E2, ubiquitin-conjugating enzymes. Next, the carboxy end of ubiquitin is ligated by E3 to the protein substrate that is ultimately to be degraded. E3 has two distinct sites that interact with a targeted protein's N-terminal amino acid. One or more (typically five) ubiquitin proteins bind to a targeted protein substrate; at least four ubiquitin proteins appear to be needed to ensure recognition and degradation by the 26S proteasome. ATP is required to unfold the tertiary and secondary structures of the proteins. Once ubiquitins are ligated to the protein to be degraded and the protein structure permits, proteases present as part of the proteasome degrade the ubiquitinated proteins in a series of reactions. Following proteolysis, ubiquitin is released for reuse, and the amino acids from the degraded protein can be reused.

Calpains

While the proteasomal system accounts for the majority of proteolysis in skeletal muscle, another group of proteases, called **calpains**, also play a role in protein degradation in muscle and perhaps other tissues (including neurons and the brain). Calpains are calcium-dependent cysteine proteases; they are designated by number with calpain1 also known as micro- or μ -calpain, and calpain2 as milli- or m-calpain. Calpain3 is also found in muscle. This micro and milli nomenclature is indicative of the different concentrations of calcium needed for activation. Calpains are not found in lysosomes or in proteasomes; they are present in an inactive form within the cytosol and move to membranes and become activated as intracellular calcium concentrations rise. Subsequent changes in calpain structure, and in some cases dimerization with other calpains, ultimately leads to the binding of the calpain to target proteins.

In muscle, the calpain proteases appear to work in conjunction with the proteasomal pathway, whereby the calpain proteases initiate the release of damaged/oxidized myofibrillar proteins. Myofibrils are composed of several proteins including sarcomeric (i.e., contractile proteins actin and myosin) and cytoskeletal (filamin, desmin, vimentin, and dystrophin) proteins. The calpain-assisted released proteins are then ligated to ubiquitin for further degradation by the ubiquitin proteasomal pathway. Ongoing research is serving to better characterize protein degradation systems, their regulation, and their roles in the



- 1 The attachment of activated ubiquitin by E1, a subunit of the ubiquitin enzyme system, which hydrolyzes ATP to form a thiol ester with the carboxy end of ubiquitin. This activated ubiquitin is transferred to another enzyme protein, E2, referred to as ubiquitin-conjugating enzymes.
- 2 The carboxy end of ubiquitin is ligated by E3 to the protein substrate that is ultimately to be degraded. E3 has two distinct sites that interact with a targeted protein's N-terminal amino acid.

Figure 6.41 Ubiquitin proteasomal degradation of a protein.

Source: Adapted from "The ubiquitin-proteasome proteolysis pathway: potential for target of disease intervention" by Breen, H.B. and Espat, N.J., *Journal of Parenteral and Enteral Nutrition* 2004; 28:272–277. Copyright © 2004 by Sage Publications. Reprinted by permission of SAGE Publications.

disease including cancers, Huntington disease, a variety of muscular conditions, Alzheimer’s disease, and other neurodegenerative disorders, among others.

6.12 CHANGES IN BODY MASS WITH AGE

Body composition differs between males and females and changes with age. The reference numbers shown in Table 6.8, first developed in the 1970s, provide information on body composition, including muscle mass, based on average physical dimensions from measurements of thousands of people who participated in various surveys. These numbers are a frame of reference with which to examine gender differences in body composition; they are not representative of an “ideal” body composition. As seen in Table 6.8, the reference man weighs 29.26 lb (13.3 kg) more and is 4 inches (~10 cm) taller than the reference woman (nonpregnant). Muscle accounts for 44.7% and body fat 15% (with 3% essential fat) of body weight in the male, whereas muscle is only 36% and body fat is 27% (12% essential fat) of body weight in the female. Essential fat is fat associated with bone marrow, the central nervous system, viscera (internal organs), and cell membranes and, in females, essential fat also includes fat in mammary glands and the pelvic region.

The difference in body composition is influenced not only by gender but also by other factors, including age, race, heredity, and stature. The influence of gender on body composition appears to exist from birth but becomes dramatically evident at puberty and continues throughout life. In both sexes, serum testosterone levels rise during adolescence; however, the increase is much greater in boys, whose testosterone values approach 10 times those of girls. As a result of this higher testosterone production and a growth spurt of longer duration, boys gain considerably more lean body mass than girls. Increased estrogen and progesterone concentrations and a shorter growth spurt

duration contribute to greater gains in fat mass in females versus males during adolescence. The female achieves maximum lean body mass by about age 18 years, whereas the male continues accretion of lean body mass until about age 20 years. Such differences in lean body mass are largely responsible for the gender difference in nutrient requirements.

After 25 years of age, weight gain usually results from body fat accretion. As an example, healthy young men (about age 25) may average 20% body fat, while 55-year-old healthy men more likely average 30% body fat, and those who are 75 years old average 35% body fat. More marked increases occur in females than in males. In addition to the fat gains and changes in the distribution of body fat (adipose tissue), lean body mass decreases with aging. Adults who maintain their weight may still gain fat and lose muscle. The decline in muscle mass occurs predominantly after age 50 years at a rate of about 1–2% per year, but may begin to occur after age 30 years. Skeletal muscle fiber numbers diminish (with reductions affecting type II fast-twitch muscle fibers to a greater extent than type I slow twitch) and the cross-sectional area of the remaining muscle fibers become reduced. Lipids, adipose tissue, and fibrotic tissue typically accumulate and infiltrate within the muscle, reducing muscle quality. A further effect of the decreased muscle mass with aging is a decrease in total body water, which is greater in females than in males. More specifically, extracellular fluid volume remains virtually unchanged, but interstitial fluid decreases and plasma volume increases. Atrophy of organs as well as loss of bone mass also occurs with aging. The loss of bone mass is discussed in the Perspective at the end of Chapter 11.

Loss of Muscle Mass and Disease

The loss of muscle (strength, physical function, and mass), if of sufficient magnitude, can result in a condition known as sarcopenia. Sarcopenia (*sarx* referring to “flesh” in

Table 6.8 Body Composition of Reference Man and Woman

Reference Man	Reference Woman
Age: 20–24 yr	Age: 20–24 yr
Height: 68.5 in (174 cm)	Height: 64.5 in (164 cm)
Weight: 154 lb (70 kg)	Weight: 125 lb (56.8 kg)
Total fat: 23.1 lb (10.5 kg) (15.0% body weight)	Total fat: 33.8 lb (15.4 kg) (27.0% body weight)
Storage fat: 18.5 lb (8.4 kg) (12.0% body weight)	Storage fat: 18.8 lb (8.5 kg) (15.0% body weight)
Essential fat: 4.6 lb (2.1 kg) (3.0% body weight)	Essential fat: 15.0 lb (6.8 kg) (12.0% body weight)
Muscle: 69 lb (31.4 kg) (44.7% body weight)	Muscle: 45 lb (20.5 kg) (36.0% body weight)
Bone: 23 lb (10.4 kg) (14.9% body weight)	Bone: 15 lb (6.8 kg) (12.0% body weight)
Remainder: 38.9 lb (17.7 kg) (25.3% body weight)	Remainder: 31.2 lb (14.2 kg) (25.0% body weight)

Sources: Adapted from Behnke A.R., Wilmore J.H., *Evaluation and Regulation of Body Build and Composition*. Englewood Cliffs, NJ: Prentice Hall, 1974; and Katch F.I., McArdle W.D., *Introduction to Nutrition, Exercise, and Health*, 4th ed., Philadelphia: Lea & Febiger, 1993, p. 235.

Greek and *penia* meaning “loss” or “low”) typically occurs after age 50 years; the likelihood of sarcopenia increases with age. Reductions in muscle strength and physical function may precede changes in mass.

The causes of sarcopenia are not completely understood but are likely multifactorial. Age-related loss of alpha motor neuron input to muscle is thought to be one major cause of the condition; this innervation is vital to muscle mass maintenance and strength. Age-associated oxidative damage in muscle also contributes, as it results in atrophy and loss of muscle fibers and muscle function. Moreover, with aging, the oxidized proteins generated in muscle may not be completely removed; this accumulation of “oxidized debris” diminishes muscle function and strength. Another cause of sarcopenia is likely the diminished concentrations of estrogen and testosterone, which have anabolic effects on muscle. Chronic low-grade inflammation associated with increased production of inflammatory cytokines (e.g., interleukins [IL] 1 and 6, tumor necrosis factor α) causes further catabolic effects. Insulin resistance (and its negative impacts on mTOR), altered insulin and growth hormone concentrations, as well as increased activation of ubiquitin-mediated proteolysis are also thought to play roles in the development of sarcopenia. Age-associated reductions in food intake (which diminish protein and nutrient intakes) and physical inactivity also contribute and represent potentially modifiable risk factors.

The loss of muscle mass (as well as strength and physical function) has extraordinary and widespread impacts on well-being. Sarcopenia causes significant functional limitations, diminishes quality of life, and increases the risks for frailty and falls. In those with chronic conditions, such as cardiovascular, respiratory, and renal diseases, and in those being treated for cancer, the loss of muscle mass increases the risk for premature death. In those who are hospitalized and undergoing surgery, the presence of reduced muscle mass increases the length of hospital stay, postoperative complications, and hospital readmission. Risk of mortality rises with increased loss of lean body (muscle) mass such that a

- 10% lean body (muscle) mass loss is associated with decreased immunity and increased infection risk and 10% mortality risk;
- 20% lean body (muscle) mass loss is associated with the medical risks listed above along with increased muscle weakness and decreased wound healing and 30% mortality risk;
- 30% lean body (muscle) mass loss is associated with the medical risks listed above along with being too weak to sit, further reductions in wound healing, increased risk of pressure ulcers, greater risk of infection, and 50% mortality risk; and
- 40% lean body (muscle) mass loss is associated with 100% mortality usually from infection (pneumonia).

While preventing deleterious changes in muscle with aging may not be possible, the changes are thought to be reduced with exercise and with changes to diet, especially protein. Protein intake at meals needed to maximize muscle protein synthesis is higher in older versus younger adults due to anabolic resistance [15]. This anabolic resistance represents a reduction in muscle protein synthesis that occurs in response to the ingestion of protein (tested in studies usually by the provision of a bolus protein dose). Multiple impairments are thought to contribute to the observed resistance, including diminished activation of mTORC1 and signaling molecules and decreased tissue perfusion and amino acid transport into muscle, among others. Research on prevention and treatment is ongoing; however, consumption of adequate energy and increased consumption of high-quality protein-rich foods to overcome the anabolic resistance (perhaps distributed evenly among meals and supplemented with essential amino acids) are thought to be needed. Of the very few proteins that have been studied, whey protein appears to be superior to others when ingested as the sole food source (note that this is not typical of real-life food consumption). However, the protein dose (i.e., amount consumed, especially the amount of provided leucine) is also influential. In fact, some of the observed differences in protein synthesis responses among various protein sources have been attributed to differences in the protein’s leucine content. Plant proteins are composed of about 6–8% leucine (except maize, which has up to 12%), whereas animal proteins contain 10% or more leucine. A relatively high postprandial (after eating) plasma leucine concentration (resulting from a higher food leucine content) is important for the activation of protein synthesis, as discussed earlier in the chapter under the section “mTORCids, Intracellular Signaling, and Amino Acids.” However, a sustained (prolonged) elevation in plasma leucine (along with other essential amino acids) following the consumption of higher amounts of some (but not all) protein sources can also promote muscle protein synthesis to the same extent as lower doses of whey protein [16].

Recommendations for protein intake aimed at maximizing muscle protein synthesis among older adults and reducing risk of sarcopenia encourage protein intakes greater than 1 g/kg body weight, and typically closer to 1.2 g protein/kg body weight/day, although intakes up to 1.6 g protein/kg body weight/day have also been recommended for those who also have chronic health conditions [17–19]. To better maximize muscle protein synthesis and maintain muscle mass, strength, and function with aging, a more even distribution of protein among meals has also been recommended. Minimum recommendations suggest an intake of at least 20 g high-quality (discussed in the next section) protein per meal but range up to 35 g protein/meal; per-meal recommendations at least 0.4 g protein per kg of body weight are also suggested [9,17–21]. It has been

further suggested that, if protein consumption is solely from plant-based foods, higher protein intakes at each meal may be needed or the meal may need to be supplemented with animal protein and perhaps small amounts (2.5–3 g) of leucine [22].

6.13 PROTEIN QUALITY AND PROTEIN AND AMINO ACID NEEDS

The quality of a protein depends to some extent on its digestibility as well as its indispensable amino acid composition—both the specific amounts and the proportions of these amino acids. Protein-containing foods can be divided into two categories: high-quality or complete proteins and low-quality or incomplete proteins.

A **complete protein**, or high-quality protein, contains all the indispensable amino acids in the approximate amounts needed by humans. Sources of complete proteins are mostly foods of animal origin such as milk, yogurt, cheese, eggs, meat, fish, and poultry. Exceptions include gelatin, which is of animal origin but lacks the indispensable amino acid tryptophan, and collagen, which can also be of animal origin but is relatively low in tryptophan and the branched-chain amino acids.

Incomplete proteins, or low-quality proteins, are found in plant foods including legumes, lentils, peas, nuts, seeds, vegetables, and cereals/grains. Most plant foods tend to have too little of one or more indispensable amino acids. The exceptions are soy protein and quinoa, which are of plant origin but are complete proteins. The term **limiting amino acid** is used to describe the indispensable amino acid that is present in the lowest quantity in the food. Table 6.9 lists various plant proteins and the limiting amino acid(s).

In addition to having lesser quantities of some indispensable amino acids, plant proteins also have lower digestibility than animal proteins. The digestibility of a protein is a measure of the amounts of amino acids that are absorbed following ingestion of the given protein.

Animal proteins have been found to be 90–99% digestible. Meat and cheese, for example, have a digestibility of 95%. In contrast, plant proteins are usually less than 80% digestible with some less than 50%. For example, cooked split peas are about 70% digestible, and peanut butter is 46% digestible. Several factors contribute to the lower digestibility of plant proteins including the presence of fiber and other components like phytic acid (found in plant- but not in animal-based foods), which hamper the ability of digestive enzymes to physically access and hydrolyze the protein (needed for subsequent absorption of the amino acids). Protease inhibitors are also found in some plants and limit enzyme activity. Thus, of the amino acids that are present in the plant protein, because of the lower digestibility, less of these amino acids are available for absorption.

Unless carefully planned, a diet containing only low-quality proteins may result in inadequate availability of selected indispensable amino acids and may reduce the body's ability to synthesize its own body proteins. The body cannot make a protein if an amino acid is limited or missing. When all the amino acids, especially essential amino acids, are not available within the cell at the time of protein synthesis, amino acid oxidation (degradation) increases. Muscle protein synthesis is maximal during the postprandial period (i.e., right after eating).

To ensure that the body cells receive all the indispensable amino acids, certain plant protein foods should be ingested together or combined so that their amino acid patterns become complementary. This practice or strategy is called mutual supplementation. For example, legumes, with their high content of lysine but low content of sulfur-containing amino acids (e.g., methionine, cysteine), complement the grains, which are more than adequate in methionine and cysteine but limited in lysine. Other combinations of foods with complementary amino acid patterns are shown in Table 6.9.

The lacto-ovo vegetarian does not usually have a problem with protein adequacy if they consume milk, yogurt, cheese, or eggs—even in small amounts—with plant foods, the indispensable amino acids are supplied in adequate amounts. One exception is the combination of milk with legumes. Although milk contains more methionine and cysteine per gram of protein than do the legumes, it still fails to meet the standard of the ideal pattern for the sulfur-containing amino acids. Most, but not all, agree that the complementary proteins should be consumed at the same meal to help maximize protein synthesis. Such practices may be particularly important for older adults.

Table 6.9 Limiting Amino Acids in Plant Foods

Food Group	Limiting Amino Acid(s)	Complementary Food Groups
Legumes	Methionine	Grains, Nuts, and Seeds
Lentils	Methionine	Grains, Nuts, and Seeds
Peas	Methionine	Grains, Nuts, and Seeds
Vegetables	Methionine	Grains, Nuts, and Seeds
Grains (most)*	Lysine and threonine	Legumes
Nuts and Seeds	Lysine	Legumes

*Limiting amino acids in corn (maize) include lysine and tryptophan

Evaluation of Protein Quality

Several methods are available to determine the protein quality of foods. A few of these methods are discussed in this section.

Protein Digestibility Corrected Amino Acid Score

The protein digestibility corrected amino acid score (PDCAAS) is a commonly used indicator of protein quality. In fact, foods intended for individuals over 1 year of age or with health claims must use the PDCAAS method to provide information on the product's food label. This method involves comparing the amount of the limiting amino acid for a test protein to the amount of the same amino acid in 1 g of a reference protein (usually egg or milk). This value is then multiplied by the test protein's digestibility, with the final PDCAA value truncated to 1.0 (or 100%). The formula is shown below.

$$\text{PDCAAS}(\%) = \frac{\text{Amount (mg) of limiting amino acid in 1 g of test protein}}{\text{Amount (mg) of same amino acid in 1 g of reference protein}} \times \text{True digestibility}(\%)$$

The digestibility of the protein is determined by examining fecal nitrogen. This fecal measurement has been criticized because it includes not only nitrogen from the test protein but also from endogenous proteins such as from digestive secretions and cells. Moreover, bacterial fermentation of some proteins in the colon also overestimates digestibility and suggests a greater delivery of dietary amino acids to the body. Examples of foods with a PDCAAS of 100 include milk proteins (casein and whey), egg white, ground beef, and tuna, along with some other animal products. Foods with a PDCAAS <100% do not meet the body's essential amino acids requirements. Soybean protein has a PDCAAS of 94, although soy protein isolate has a PDCAA of 100%. Grain scores range from about 40 to 56%, and the scores for various lentils, peas, and legumes range from <50 to about 75%. Chickpeas have a score of 74%, and peanut butter has a score of 45%.

An alternate approach to this method involves a comparison of the amino acid composition of a test protein with an amino acid reference *pattern* (as opposed to reference protein). The reference pattern that has been selected for use for all people (except infants) is the amino acid requirements of preschool children age 1–3 years. The requirements of preschool children, which include needs for growth and development, are higher for each amino acid than are those of adults (who are not undergoing growth and development) and thus are considered to meet or exceed the needs of older people. The scoring pattern, expressed as (mg amino acid) / (g protein), is calculated by dividing the requirements of individual indispensable amino acids (in mg) for children by the protein requirements (in g). The scoring pattern for foods intended for children age 1 year or older and for older age groups is

Table 6.10 Amino Acid Scoring Patterns and Whole-Egg Pattern [23]

Amino Acid	Infants (mg/g protein)	Children, age 1–3 years (mg/g protein)	Adults (mg/g protein)	Whole Egg (mg/g protein)
Histidine	23	18	17	22
Isoleucine	57	25	23	54
Leucine	101	55	52	86
Lysine	69	51	47	70
Methionine + cysteine	38	25	23	57
Phenylalanine + tyrosine	87	47	41	93
Threonine	47	27	24	47
Tryptophan	18	7	6	17
Valine	56	32	29	66

shown in Table 6.10, along with the recommended amino acid scoring pattern for infant formulas and foods, which is based on the amino acid composition of human milk. Table 6.10 also shows the whole-egg pattern.

Digestible Indispensable Amino Acid Score

The digestible indispensable amino acid score (DIAAS) represents a newer method to assess protein quality. DIAAS considers the amount, profile, and digestibility of each of the indispensable amino acids in protein-containing foods. Specifically, as shown in the formula below, it compares the amount of a digestible indispensable amino acid in 1 g of a dietary test protein to the amount of the same indispensable amino acid in 1 g of the reference protein.

$$\text{DIAAS} = \frac{\text{mg of indispensable amino acid in 1 g of a dietary test protein} \times \text{amino acid digestibility}}{\text{mg of the same indispensable amino acid in 1 g of the reference protein}} \times 100$$

For regulatory purposes, two scoring patterns have been recommended as the reference protein: for infants the amino acid composition of human milk and for other age groups the pattern for young children age 6 months to 3 years (see Table 6.11). The ratio is best calculated for each indispensable amino acid, with the lowest value used as an indicator of the protein's dietary quality.

Unlike the PDCAAS, this method assesses the digestibility of the amino acids in the ileum versus the colon. Given amino acids are primarily absorbed in the upper small intestine and not in the colon, the measurement of ileal digestibility more accurately predicts utilization by humans and eliminates the impact of protein utilization by colonic microbiota. The DIAAS also allows for a ranking of different protein sources based on their ability to meet indispensable amino acid requirements. Since it does not truncate scores for individual foods as does the PDCAAS method, the technique allows distinction among

Table 6.11 Amino Acid Scoring/Reference Patterns Used for Regulatory Purposes*

Amino Acid	Infants (mg/g protein human milk)	Children and Adults (mg/g protein)
Histidine	21	20
Isoleucine	55	32
Leucine	96	66
Lysine	69	57
Methionine + cysteine	33	27
Phenylalanine + tyrosine	94	52
Threonine	44	31
Tryptophan	17	8.5
Valine	55	43

*Report of an FAO Expert Consultation. Diet protein quality evaluation in human nutrition. FAO Food and Nutrition paper #92. 2011. Available at <http://www.fao.org/ag/humannutrition/35978-02317b979a686a57aa4593304ffc17f06.pdf>

protein sources that the PDCAAS method identified as having the same values. For example, many dairy protein sources and dairy components used in nutritional products (such as whey protein isolate) have scores greater than 100%. Scores that are over 100% mean that the protein source is high quality and that the protein is relatively high in a particular indispensable amino acid; this “extra” amino acid content could then complement another protein source that is lower in that particular indispensable amino acid.

Protein Efficiency Ratio

The protein efficiency ratio (PER) represents body weight gained on a test protein divided by the grams of protein consumed. This method of assessing protein quality is used by food manufacturers for infant formulas and baby foods and is reported on the product’s food label. To calculate the PER of proteins, young growing animals are typically placed on a standard diet with about 10% (by weight) of the diet as test protein. Weight gain is measured for a specific time period and compared to the amount of the protein consumed. The PER for the protein is then calculated using the following formula:

$$\text{PER} = \frac{\text{Gain in body weight (g)}}{\text{Grams of protein consumed}}$$

To illustrate, the PER for casein (a protein found in milk) is 2.5; thus, rats gain 2.5 g of weight for every 1 g of casein consumed. However, a food with a PER of 5 does not have double the protein quality of casein, with a PER of 2.5. Furthermore, although the PER allows determination of which proteins promote weight gain (per gram of protein ingested) in growing animals, no distinction is made regarding the composition (fat or muscle/organ) of the weight gain.

In addition to protein digestibility corrected amino acid score and protein efficiency ratio, which are used for

nutrition labeling purposes, other methods—chemical or amino acid score, biological value, and net protein utilization—may be used to determine protein quality. A discussion of these methods follows.

Chemical or Amino Acid Score

The chemical score (also called the amino acid score) involves determination of the amino acid composition of a test protein. This procedure is done in a chemical laboratory using either an amino acid analyzer or high-performance liquid chromatography techniques. Only the indispensable amino acid content of the test protein is determined. The value is then compared with that of the reference protein, for example, the amino acid pattern of egg protein (considered to have a score of 100). The amino acid/chemical score of a food protein can be calculated as follows:

$$\text{Score of test protein} = \frac{\text{Indispensable amino acid in food protein (mg/g protein)}}{\text{Content of same amino acid in reference of protein (mg/g protein)}}$$

The amino acid with the lowest score on a percentage basis in relation to the reference protein (egg) becomes the first limiting amino acid, the one with the next lowest score is the second limiting amino acid, and so on. For example, if after testing all amino acids, lysine was found to be present in the lowest amount relative to the reference protein (e.g., 85%), the test protein’s chemical score would be 85. The amino acid present in the lowest amount is the limiting amino acid and determines the amino acid or chemical score for the protein. Table 6.10 gives the amino acid pattern in whole egg. Comparison of the quality of different food proteins against the standard of whole-egg protein can be valuable but probably is not nearly as important to adequate protein nutriture as comparison with reference patterns for the various population groups.

Biological Value

The biological value (BV) of proteins is another method used to assess protein quality. BV is a measure of how much nitrogen is retained in the body for maintenance and growth rather than absorbed. BV is most often determined in experimental animals, but it can be determined in humans. Subjects are fed a nitrogen-free diet for a period of about 7–10 days and then fed a diet containing the test protein in an amount equal to their protein requirement for a similar time period. Nitrogen that is excreted in the feces and in the urine during the period when subjects consumed the nitrogen-free diet is analyzed and compared to amounts excreted when the subjects consumed the test protein. In other words, the change in urinary and fecal nitrogen excretion between the two diets is calculated. The

BV of the test protein is determined through the use of the following equation:

$$\begin{aligned}\text{BV of test protein} &= \frac{I - (F - F_0) - (U - U_0)}{I - (F - F_0)} \times 100 \\ &= \frac{\text{Nitrogen retained}}{\text{Nitrogen absorbed}} \times 100\end{aligned}$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Foods with a high BV are those that provide the amino acids in amounts consistent with body amino acid needs. The body retains much of the absorbed nitrogen if the protein is of high BV. Eggs, for example, have a BV of 100, meaning that 100% of the nitrogen absorbed from egg protein is retained. Although the BV provides useful information, the equation fails to account for losses of nitrogen through insensible routes such as the hair and nails. This criticism is true of any method involving nitrogen balance studies. A further consideration is that proteins exhibit a higher BV when fed at levels below the amount necessary for nitrogen equilibrium, and retention decreases as protein intake approaches or exceeds adequacy.

Net Protein Utilization

Another measure of protein quality, similar to nitrogen-balance studies, is net protein utilization (NPU). NPU measures retention of food nitrogen consumed rather than retention of food nitrogen absorbed. NPU is calculated from the following equation:

$$\begin{aligned}\text{NPU of test protein} &= \frac{I - (F - F_0) - (U - U_0)}{I} \times 100 \\ &= \frac{\text{Nitrogen retained}}{\text{Nitrogen consumed}} \times 100\end{aligned}$$

where I is intake of nitrogen, F is fecal nitrogen while subjects are consuming a test protein, F_0 is endogenous fecal nitrogen when subjects are maintained on a nitrogen-free diet, U is urinary nitrogen while subjects are consuming a test protein, and U_0 is endogenous urinary nitrogen when subjects are maintained on a nitrogen-free diet.

Although NPU can be measured in humans through nitrogen-balance studies in which two groups of well-matched experimental subjects are used, a more nearly accurate measurement is made on experimental animals through direct analysis of the animal carcasses. In either case, one experimental group is fed the test protein, while the other group receives an isocaloric, protein-free diet. When experimental animals are used as subjects, their carcasses can be analyzed for nitrogen directly (total carcass

nitrogen, or TCN) or indirectly at the end of the feeding period. The indirect measurement of nitrogen is made by water analysis. Given the amount of water removed from the carcasses, an approximate nitrogen content can be calculated. NPU involving animal studies is calculated from the following equation:

$$\text{NPU} = \frac{\text{TCN on test protein} - \text{TCN on protein-free diet}}{\text{Nitrogen consumed}}$$

Proteins of higher quality typically cause a greater retention of nitrogen in the carcass than poor-quality proteins and thus have a higher NPU.

Net Dietary Protein Calories Percentage

The net dietary protein calories percentage (NDpCal%) can be helpful in the evaluation of human diets in which the protein-to-calorie ratio varies greatly. The formula is as follows: $\text{NDpCal\%} = \text{Protein kcal/Total kcal intake} \times 100 \times \text{NPU}_{\text{op}}$, where NPU_{op} is NPU when protein is fed above the minimum requirement for nitrogen equilibrium.

Protein Information on Food Labels

Food labels are required to indicate the amount (quantity) of protein in grams and the % Daily Value for protein in a serving of food. As previously mentioned, the protein efficiency ratio (PER) is used to calculate the % Daily Value for infant formulas and baby foods. The U.S. Food and Drug Administration (FDA) specifies the use of the milk protein casein as a standard for comparison of protein quality based on the PER. Specifically, for infant formulas and baby foods, if a test protein has a protein quality equal to or better than that of casein—that is, if the PER is ≥ 2.5 —then 45 g of protein is considered equivalent to 100% Daily Value. If a test protein is lower in quality than casein—that is, if the PER is < 2.5 —then 65 g of protein is needed to provide 100% Daily Value. For foods other than baby foods, the PDCAAS method is used to establish the protein quality for % Daily Value on food labels. Specifically, 50 g of protein is considered sufficient if the food protein has a PDCAAS equal to or higher than that of milk protein (casein). However, 65 g of protein is needed if the protein is of lower quality than milk protein.

Assessing Protein and Amino Acid Needs

To prevent deficiencies, people need to achieve adequate intakes of energy and protein as well as other essential nutrients. Two techniques—nitrogen balance and indicator amino acid oxidation—are commonly used to assess the adequacy of protein and amino acid intakes.

Nitrogen Balance/Nitrogen Status

Nitrogen-balance studies involve the evaluation of dietary nitrogen intake and the measurement and summation of nitrogen losses from the body. They can be conducted when subjects consume a diet with a protein (nitrogen) intake that is at or near a predicted adequate amount, less than (including protein-free nitrogen) a predicted adequate amount, or greater than a predicted adequate amount. This technique or modified versions of it are often used with hospitalized patients to determine whether protein intake is adequate.

To determine nitrogen balance or status, nitrogen intake and output must be assessed. Assessment of nitrogen intake is based on protein intake. Protein contains approximately 16% nitrogen. Thus, to calculate grams of nitrogen consumed from grams of protein, we can do the following calculation: $0.16 \times \text{protein ingested (measured in grams)} = \text{nitrogen (measured in grams)}$. Expressed alternately, $\text{ingested protein (g)} / 6.25 = \text{ingested nitrogen (g)}$. So, for example, 70 g of protein intake provides 11.2 g of nitrogen. To reverse the calculations and convert grams nitrogen into grams of protein: $\text{protein (g)} = \text{nitrogen (g)} \times 100/16$, or $\text{protein (g)} = \text{nitrogen (g)} \times 6.25$.

Nitrogen losses are measured in the urine (U), feces (F), and skin (S). For example, in the urine, nitrogen is found mainly as urea but also as creatinine, amino acids, ammonia, and uric acid. In the feces, nitrogen may be found as amino acids and ammonia. To calculate nitrogen balance/status, nitrogen losses are summed and then subtracted from nitrogen intake (In). Thus, $\text{nitrogen balance/status} = \text{In} - [(\text{U} - \text{U}_e) + (\text{F} - \text{F}_e) + \text{S}]$. The subscript *e* (in U_e and F_e) in the equation stands for *endogenous* (also called obligatory) and refers to losses of nitrogen that occur when the subject is on a nitrogen-free diet.

In clinical settings, nitrogen losses are often estimated. Fecal and insensible (including skin, nail, and hair) losses of nitrogen are thought to account for about 1 g of nitrogen each for a total of 2 g. (Losses should not be estimated in individuals with larger than normal fecal nitrogen losses, as with diarrhea, or large insensible nitrogen losses, as from the skin with burns or high fever.) Urinary losses of nitrogen are measured either as total urinary nitrogen (UN), which gives the most accurate value, or as urinary urea nitrogen (UUN). If UUN is measured, 2 g of nitrogen is usually added to this value to account for the urinary losses of other nitrogenous compounds such as creatinine, uric acid, ammonia, and so on. Thus, $\text{nitrogen balance/status} = [\text{protein intake (g)} / 6.25] - [\text{UN} + 2 \text{ g}]$, whereby the 2 g accounts for the fecal and insensible nitrogen losses, or $\text{nitrogen balance/status} = [\text{protein intake (g)} / 6.25] - [\text{UUN} + 2 \text{ g} + 2 \text{ g}]$, whereby the first 2 g accounts for the losses in the urine of nonurea nitrogen compounds and the other 2 g accounts for the fecal and insensible nitrogen losses.

Nitrogen-balance studies have been criticized for their overall underestimation of protein needs with

overestimations of intake and underestimations of losses. In addition, nitrogen balance does not necessarily mean amino acid balance; that is, a person may be in nitrogen balance but in amino acid imbalance.

Indicator Amino Acid Oxidation

Studies assessing protein and amino acid requirements have relied on the indicator amino acid oxidation (IAAO) technique. This method involves feeding test amino acids individually to a person in graded amounts in the presence of an indicator amino acid. The amounts of the test amino acid that are provided include quantities below, at, and above the expected requirement. The method is based on a few principles, including (1) if a test amino acid is not provided, oxidation of the indicator amino acid will be maximal and protein synthesis will be minimal; (2) at an intake above the expected requirement of the test amino acid, oxidation of the indicator amino acid will diminish; and (3) at an intake that is the requirement for the test amino acid, oxidation of the indicator amino acid will be fairly constant. Thus, changes in the oxidation of the indicator amino acid reflect metabolism of the limiting amino acid in the body since if one amino acid is limiting for protein synthesis, all other amino acids (including the indicator amino acid) would be “extra” and oxidized. As the intake of the limiting amino acid increases, the rate of oxidation of the other amino acids decreases, with higher amounts of amino acids being used for protein synthesis. Additionally, any “losses” of the amino acid associated with digestion, absorption, and cellular metabolism are accounted for. Most studies have relied on ($1\text{-}^{13}\text{C}$) phenylalanine in the presence of excess tyrosine, lysine, and leucine as the indicator amino acid. The technique has enabled the examination of the requirements for protein as well as the essential amino acids and some conditionally essential amino acids. Mean protein requirements using this technique have been typically higher than those determined using nitrogen-balance methodology.

Recommended Protein and Amino Acid Intakes

Protein and amino acid requirements of humans are influenced by age, body size, and physiological state, as well as by the level of energy intake. Multiple studies using multiple methods, especially indicator amino acid oxidation, nitrogen-balance studies, and the factorial method, have been used over the years to determine protein and amino acid needs. The Estimated Average Requirement for protein for adults (men and women age 19 years and older) is 0.66 g of protein per kg of body weight, or 105 mg of nitrogen per kg of body weight per day [23]. This value represents the lowest continuing dietary protein intake necessary to achieve nitrogen equilibrium or a zero-nitrogen balance in a healthy adult [23]. The Recommended Dietary Allowance (RDA) for protein for adults is

set at 0.8 g of protein per kg of body weight per day [23]. The protein RDAs for children, adolescents, and adults, including women during pregnancy and lactation, are provided on the inside cover of this book. Instead of RDAs, the recommendations for protein for infants from birth to 6 months of age are given as an Adequate Intake (AI). The AI was derived from data from infants fed human milk as the primary nutrient source for the first 6 months [23].

While the RDA includes a safety variance of over 20%, it is thought to be too low for older adults, not providing for maximum protein utilization (especially for maintenance of muscle mass and perhaps for bone health) [9, 17–19, 22, 24]. High-quality protein intakes in amounts in excess of 1.0 and usually closer to 1.2 g of protein/kg body weight have been proposed to promote muscle health in older (greater than age 65 years) adults [17–19]. Yet many adults, especially older adults, do not consume enough dietary protein. The current RDA for protein is also not thought to be adequate to support new muscle protein synthesis, to repair muscle damage, and to maintain lean body mass in athletes who are strenuously training and competing [25]. Further information about protein and exercise is provided in Chapter 7.

In addition to the RDA for protein, RDAs for the indispensable amino acids have also been established (see Figure 6.42) based on a variety of methods including amino acid balance and indicators of amino acid oxidation studies. Similar recommendations for the indispensable amino acids have been proposed by the World Health Organization and Food and Agricultural Organization. The reader is directed to the Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Protein, and Amino Acids [23] for in-depth information on the methods used in determining the recommendations for the amino acids and protein.

While the RDA for protein is calculated based on body weight, the Institute of Medicine's Acceptable Macronutrient Distribution Range for protein is a function of energy intake, with the recommendation of 10–35% of daily energy intake coming from protein. Most Americans consume about 14–16% of energy from protein, which is well below the upper end of the Acceptable Macronutrient Distribution Range for protein [23, 26]. Use of this distribution range is appropriate as long as energy intake is adequate. If, for example, a person only ingests 800 kcal per day, then 10–35% of energy as protein equals 80–280 kcal; since protein provides 4 kcal/g, this translates into 20–70 g of protein. An intake of 20 g of protein is not sufficient for an adult to maintain nitrogen balance; however, depending on the age, gender, and body weight of the individual, 70 g may be more than adequate. In contrast, an intake of 35% of energy as protein (at the upper end of the recommendation) usually translates into protein intakes for adults exceeding 100 g of protein/day. For example, based on a person consuming a rather low 1,200-kcal (but higher than the 800 kcal used in the previous example) diet, the math can be depicted as follows: $1,200 \text{ kcal} \times .35 = 420 \text{ kcal}$ as protein; $420 \text{ kcal} / 4 \text{ kcal/g} = 105 \text{ g}$ of protein. This example serves to illustrate that consumption of 100 g or more of protein is not “necessarily” high when basing calculations on the Acceptable Macronutrient Distribution Range.

No Tolerable Upper Intake Level for protein has been established, as no adverse consequences (including cancer, kidney disease, kidney stones, and osteoporosis) were identified from studies of high-protein diets reviewed by the Food and Nutrition Board of the Institute of Medicine [23]. Similarly, indispensable amino acids are thought to have relatively high safety limit intakes. The most commonly cited concerns from ingesting diets high in protein

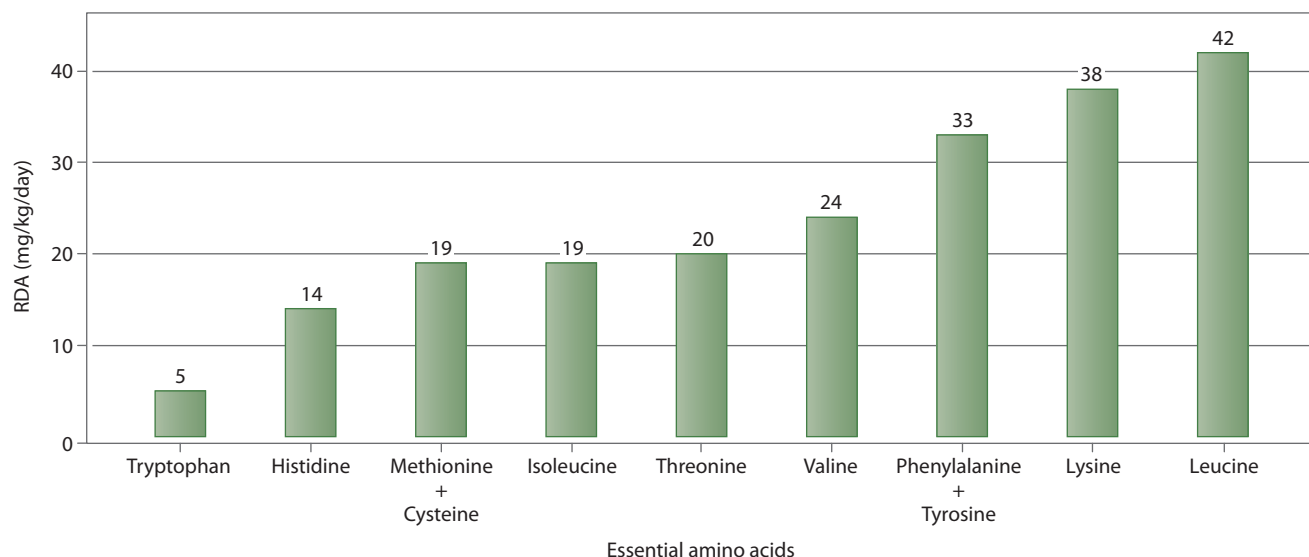


Figure 6.42 Recommended Dietary Allowances for indispensable amino acids for adults.

Source: *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Food and Nutrition Board, Institute of Medicine, Washington DC, National Academic Press, 2005, p. 680. Reprinted with permission from the National Academies Press.

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are possible increased risk of dehydration and detrimental effects on the kidneys and bones. Yet, neither effect has been substantiated. Dehydration is not caused by the excretion of urea and other nitrogenous wastes from protein catabolism. Similarly, increased urea production and changes in glomerular filtration rate do not promote renal damage in healthy individuals. A systematic review of the literature showed no association between changes in renal function and protein intakes within the accepted macronutrient distribution range (i.e., up to 35% of energy), which included individuals ingesting protein in amounts in excess of 2.5 g of protein per kg body weight [26]. With respect to bone health, intake of protein must be adequate (along with calcium and other nutrients) to build and maintain bone mass. Protein intakes of at least 1.2 g of protein/kg body weight (similar to proposed recommendations for muscle health) have been recommended for bone health. Most studies report positive associations between bone mass and higher dietary protein; this relationship is better when the diet is also adequate in other nutrients and derived from dairy, meats, grains, fruits, and vegetables.

To help guide decisions in choosing good sources of protein, the U.S. Department of Agriculture published the Food Patterns and MyPlate, which include five major food groups. MyPlate is designed for the consumer and can be accessed at www.choosemyplate.gov. From this site, an individual can determine the appropriate amount of foods recommended from each of the food groups. The amounts vary based on a person's gender and age. Generally, however, the recommended quantities for adults from the meat, poultry, and fish range from 5 to 6.5 oz per day, and the recommended amount from the dairy group is 3 cups per day. The amounts of legumes, lentils, peas, nuts, and seeds that must be consumed to obtain an equivalent amount of protein as found in meats, poultry, and fish varies among these different plant sources. Typically, higher amounts of plant foods, and in somewhat specific combinations (as discussed under protein quality), need to be eaten at meals to obtain sufficient amounts of dietary protein and indispensable amino acids. Foods ingested from these protein-rich food groups should also be low in saturated fat to promote heart health. Grains also provide some protein. Choices from this group should be high in fiber and low in saturated fat; recommended amounts from the grain group for adults range from 6 to 8 oz or the equivalent per day, with the further recommendation that 50% of intake should be whole grains. One slice of bread, 1 cup of ready-to-eat cereal, or $\frac{1}{2}$ cup of cooked cereal, pasta, or rice is equal to 1 oz equivalent.

In addition to MyPlate, dietary protein-related guidelines by the American Heart Association promote the consumption of fish, especially oily fish rich in omega-3 fatty acids, at least twice a week, and lean meats and meat alternatives. Similarly, dietary protein-related guidelines by the American Cancer Society address foods rich in protein, specifically suggesting that intakes of processed

meat (such as ham, salami, bacon, hot dogs, chorizo, sausage, and bologna) and red meat (such as beef, veal, some pork, lamb, mutton, and goat) be limited to less than three servings per week. Consumption of fruits, vegetables, and whole grains is advocated by both organizations. Meals higher in dietary protein have been associated with greater satiety in comparison with meals higher in carbohydrates or fats. Higher satiety may result in reduced food consumption, and thus energy intake with implications in diet planning to promote weight loss. The protein leverage hypothesis suggests that food intake is affected by the protein density of the diet via signaling pathways to the hypothalamus.

Protein Deficiency/Malnutrition

Malnutrition is associated with increased morbidity and mortality. The condition is classified as nonsevere (moderate) or severe within the context of three etiologies: acute injury/illness, chronic illness, or social/environmental circumstances (i.e., reduced access to food or knowledge/beliefs that reduce food intake) [27]. Assessment of individuals for malnutrition includes identification of inflammation (such as by increased plasma C-reactive protein concentrations) for determination of an etiologic-based diagnosis, along with evidence of reduced dietary intake, unintended weight loss, loss of subcutaneous fat, loss of muscle, localized or generalized fluid accumulation, and reduced physical functional status (such as grip strength) [27].

Prolonged or chronic reduced access to food may result in **marasmus**, also referred to as wasting. Individuals with marasmus appear extremely thin (emaciated or underweight) with wasted (depleted) muscle mass and adipose tissue. Bones are prominent in appearance and the skin often droops or hangs from the body. Hair in young children is typically sparse and brittle. In older children and adults, there may be areas of depigmentation in the hair, resembling a “flag” and excessive hair loss.

Kwashiorkor or edematous malnutrition represents a form of protein malnutrition in which protein intake is typically insufficient, but energy consumption (usually as carbohydrates) is adequate. It is often seen in children in developing countries. Inadequate quantities of proteins in the blood and in cells (due to insufficient dietary protein intake) cause water to diffuse out of the blood (the intravascular space) and out of the cells (intracellular space) into interstitial (intercellular) spaces, causing edema (swelling). The edema usually appears first in the legs but may also be present in the face or more generalized all over the body (called anasarca).

With chronic disease-related malnutrition, mild to moderate inflammation may also be present. The condition occurs with a variety of illnesses such as rheumatoid arthritis and organ failure, to name a few. Other conditions such as burns, sepsis (infection), and trauma result

in marked inflammation and are often associated with acute disease/injury-related malnutrition. Individuals with inflammation (acute or chronic) often do not physically look malnourished, which is why assessment of food energy and nutrient intakes and changes in weight, body composition, and functional status need to be assessed. Systemic inflammation diminishes protein synthesis and enhances protein catabolism to negatively impact muscle

mass and strength; these effects are mediated in large part by the presence of increased release of proinflammatory cytokines. Recommendations for dietary protein in critical care patients with severe injury, illness, and/or malnutrition may exceed 2 g of protein/kg body weight [18, 28]. The effects of inflammation and stress, such as that occurring with burns, sepsis, and trauma, on protein are discussed in the Perspective following this chapter.

SUMMARY

Proteins in foods become available for use by the body after they have been broken down into their component amino acids.

- Nine of these amino acids are considered essential; therefore, the quality of dietary proteins correlates with their content of these indispensable amino acids.

In the body, proteins play many vital roles including functions in structural capacities and as enzymes, hormones, transporters, and immunological protectors, among other roles.

An important concept in protein metabolism is that of amino acid pools, which contain amino acids of dietary origin plus those contributed by the breakdown of body tissue. The amino acids comprising the pools are used in a variety of ways:

- for synthesis of new proteins for growth and/or replacement of existing body proteins;
- for production of nonprotein nitrogen-containing compounds;
- for oxidation as a source of energy; and

- for synthesis of glucose, ketones, or lipids.

The liver is the primary site of amino acid metabolism, but no clear picture of the body's overall handling of nitrogen can emerge without considering amino acid metabolism in a variety of tissues and organs. Of particular significance is the metabolism of the branched-chain amino acids in the skeletal muscle, the role of the intestine in citrulline production, and the role of the kidneys in the production of dispensable amino acids, nitrogen-containing compounds, and glucose as well as in the elimination of nitrogenous wastes.

Of the nonessential amino acids, glutamine, glutamate, and alanine assume particular importance because of their versatility in overall metabolism. Glutamate and its α -keto acid make possible many crucial reactions in various metabolic pathways for amino acids. An appreciation for the functions performed by glutamine and glutamate and the numerous amino acids functioning as or used to synthesize neurotransmitters, biogenic amines, and neuropeptides makes one realize that the term “dispensable” as applied to many amino acids may be quite misleading.

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Perspective

STRESS AND INFLAMMATION: IMPACT ON PROTEIN

In the healthy adult, protein synthesis approximately balances protein degradation. However, in conditions affected by a stress and/or an inflammatory response, such as with sepsis (the presence of a pathogenic microorganism or its toxin in the blood and/or body tissues), burns, and injury/trauma (including surgery), protein synthesis and protein degradation are not in balance. The body systems basically prioritize wound repair and host defense (such as from infection) at the expense of body tissues, in essence gambling that convalescence or a return to health will occur before tissue depletion threatens survival.

A coordinated set of actions occurs in the body in response to “insults” such as that occurring with sepsis/infection, burns, injury/trauma including surgery, and some diseases. The endocrine system responds with increased secretion of catecholamines (especially epinephrine) as well as glucagon, cortisol, growth hormone, aldosterone, and antidiuretic hormone (also called vasopressin). The immune system responds, releasing inflammatory cytokines that augment and exacerbate the stress response.

Some of these hormones impact organ functions to help restore homeostasis. For example, aldosterone promotes renal sodium and fluid reabsorption and thus increased blood volume, and antidiuretic hormone (ADH) inhibits diuresis (urination) and thus increases blood volume, diminishes fluid losses, and helps to restore circulation if it has been depressed by shock, fever, burns, and/or hemorrhage. Metabolic rate also increases (referred to as a hypermetabolic state) in an attempt to restore homeostasis with the rise driven by elevations in catecholamines, glucagon, and cortisol. Some of these same hormones also impact nutrient metabolism and body tissues (Figure 1).

The actions of catecholamines, glucagon, and cortisol result in catabolism of body proteins (primarily muscle mass by the ubiquitin system), fat, and carbohydrate (glycogen) (Figure 2) and disrupt signaling pathways that normally promote protein synthesis after food ingestion. The catecholamines, especially epinephrine, stimulate adipose tissue lipolysis and, along with growth hormone, stimulate

glycogenolysis. Glucagon and especially cortisol (considered the body’s major stress hormone) promote muscle proteolysis and gluconeogenesis. Glutamine and alanine release from tissues rises substantially, with alanine used for gluconeogenesis and glutamine used by immune system and intestinal cells for energy and by the kidneys for acid–base balance. While insulin is produced, the body’s tissues become resistant to its action, and hyperglycemia persists. Hyperglycemia further increases inflammation, increases risk for infection, reduces wound healing, and increases length of hospital stay and mortality. Growth hormone contributes to hyperglycemia by exerting anti-insulin effects. Cortisol, like epinephrine, also stimulates adipose tissue lipolysis. However, unlike with starvation, the fatty acids generated from lipolysis do not produce ketones (ketogenesis is inhibited by the presence of insulin). With limited availability of ketones, body proteins, especially those from white fast-twitch muscle, continue to be degraded to supply amino acids for gluconeogenesis and for the synthesis of critical acute-phase proteins.

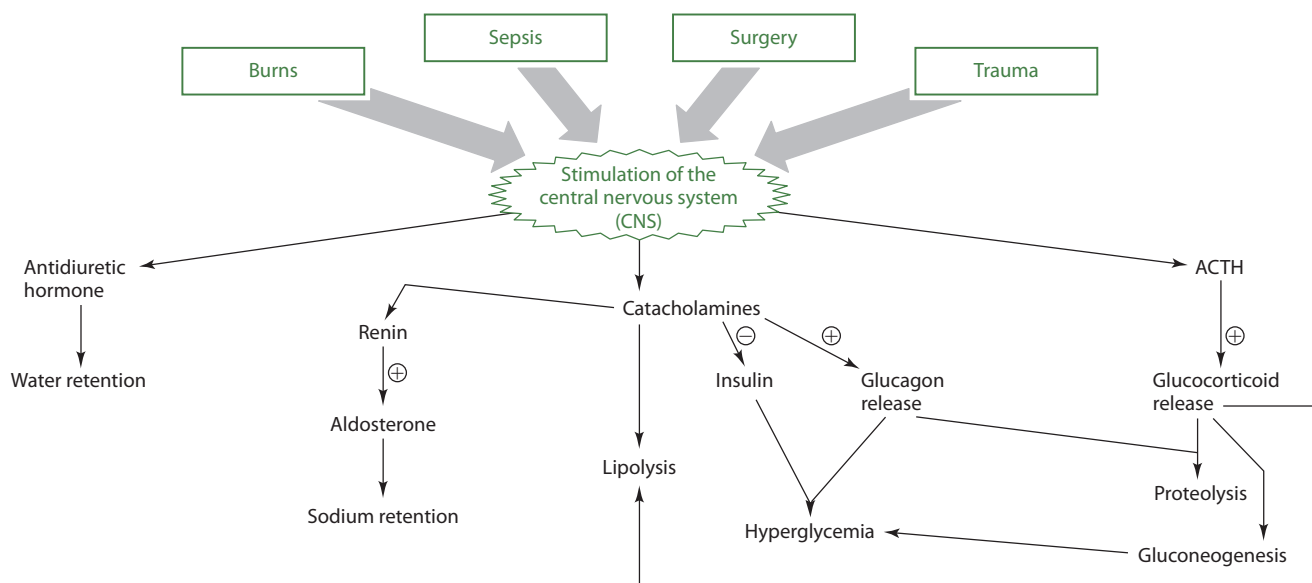


Figure 1 Response to metabolic stress.

Sepsis/trauma

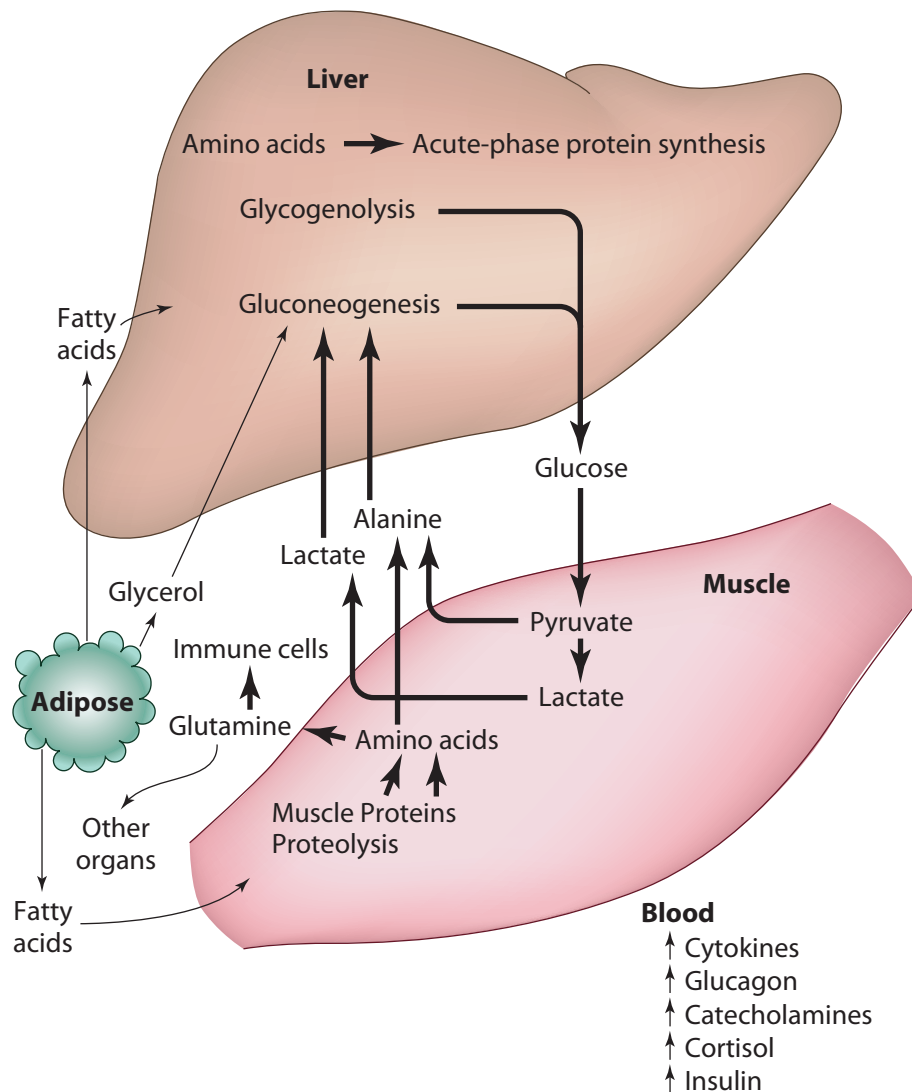


Figure 2 Substrate utilization during metabolic stress with increased responses shown by heavier black arrows.

Exacerbating the catabolism of body proteins associated with hypermetabolic states is bedrest or physical inactivity. Bedrest further impairs existing muscle function and diminishes signaling via mTOR to promote muscle protein synthesis. Bedrest, even in the absence of illness, decreases whole-body protein synthesis that normally follows the ingestion of food by about 15–20% and results in loss of muscle mass [1].

Evidence of protein catabolism is apparent with measurements in the urine of 3-methylhistidine and nitrogen. Urinary nitrogen losses frequently total 30 g or more per day. Each gram of nitrogen lost can be translated into the breakdown of *approximately 30 g of hydrated lean tissue*

(whereby 1 g nitrogen = 6.25 g protein; muscle is about 80% water, so 80% of 30 g = 24 g, and 30 g muscle = 24 g water + 6.25 g protein).

The inflammatory response begins with tissue injury or cell death and the release of damage-associated molecular patterns (DAMPs) from the necrotic or damaged cells, more specifically their intracellular components such as mitochondrial DNA, histones, and high-mobility group box 1 (nuclear factors bound to DNA), among others. DAMPs, through binding to pattern recognition receptors such as Toll-like receptors found on immune system cells, activate an immune response. With a bacterial infection, pathogen-associated

molecular patterns (PAMPs)—small molecular components of bacteria including, for example, bacterial lipopolysaccharide (an endotoxin found on bacterial cell membranes)—initiate a similar inflammatory immunocascade as DAMPs.

The body response to such insults is complex, but a brief overview is provided. The bone marrow responds by increasing production of white blood cells, especially neutrophils (phagocytic cells). DAMPs and PAMPs trigger the production of proinflammatory cytokines by macrophages, T-cells, and monocytes, among others. Two cytokines, interleukin (IL)-1 and tumor necrosis factor (TNF) α , are thought to orchestrate much of the inflammatory response.

Fibroblasts and endothelial cells magnify the response by secreting IL-6 in addition to more IL-1 and TNF α . IL-6 levels typically correspond to the severity of the insult. IL-1, IL-6, and TNF α increase body temperature (fever) and, with other cytokines, accelerate the production of white blood cells, especially neutrophils. Metabolic rate is also elevated above normal, secondary to fever and increased oxygen consumption needed for phagocytosis by activated white blood cells. Increases in body temperature also promote the synthesis of heat shock proteins. Cytokines, similar to cortisol's effects, promote adipose tissue lipolysis and muscle protein degradation (via the ubiquitin-proteasomal pathway); resulting increases in plasma free fatty acids contribute to tissue insulin resistance. IL-1 and TNF α facilitate white blood cell infiltration into damaged tissues. Similarly, IL-8 recruits neutrophils into inflamed tissues. Edema, redness, and pain occur at the site of injury as a result from vasodilation and increased capillary permeability around the damaged tissues. The vasodilation and increased capillary permeability facilitate entry of white blood cells into the damaged/infected area.

Inflammatory states range from acute and severe to chronic and low grade depending on the nature of the insult and its persistence. Low-grade inflammatory states may be present in several chronic conditions, such as Crohn's disease, heart disease, some connective tissue disorders, diabetes, and obesity (especially excess body fat in the abdominal/visceral region). Moreover, low-grade inflammation is thought to play a role in the pathology of many chronic diseases through its effects on immune system components (especially cytokines) and nutrient metabolism and utilization. Even the brain can be affected, for example, with higher (vs. lower) plasma concentrations of TNF α and IL-6 associated with greater cognitive decline. And, with obesity, the adipose tissue and the stroma vascular fraction (a group of cells—including preadipocytes, fibroblasts, macrophages, and histocytes, among others—associated with white adipose tissue) secrete cytokines, growth factors, and adipokines (molecules secreted by white adipose tissue, such as resistin, visfatin, and adiponectin), which either directly or indirectly promote inflammation. Moreover, macrophages that become

embedded within the adipose tissue also induce inflammation. (As an aside, this association between inflammation and diet has resulted in classifications of foods, food components and diets as “anti”- or “pro”-inflammatory, and the development of a dietary inflammatory index.)

The cytokines, like many of the hormones, released with inflammation also affect nutrient utilization. Specifically, the hepatic synthesis of proteins such as albumin, retinol-binding protein, and prealbumin is preferentially reduced. Moreover, proteins such as albumin may migrate from the blood, resulting in further reductions in the blood. In contrast, the synthesis of some proteins, primarily acute-phase reactant/response proteins, is stimulated. Acute-phase reactant/response protein synthesis increases mostly by the liver, but other cells such as macrophages, lymphocytes, and fibroblasts also generate these proteins. One acute-phase response protein with extensive roles is C-reactive protein (CRP).

CRP is made primarily by hepatocytes, but also can be synthesized by macrophages, lymphocytes, endothelial cells, smooth muscle cells, and adipocytes. It is released as a pentameric (five-subunit) protein, known as native CRP (nCRP), under usual (noninflammatory) conditions by the liver. The subunits forming nCRP, however, can dissociate irreversibly to form monomeric CRP (mCRP) with the two forms exhibiting different functions. With inflammation, IL-6 (a predominantly proinflammatory cytokine with a major role in regulating the acute phase response) stimulates CRP production. nCRP displays generally more anti-inflammatory activities in contrast to mCRP, which typically elicits more proinflammatory actions. nCRP activates the complement pathway and promotes phagocytosis; it can also opsonize apoptotic cells and induce phagocytosis of damaged cells. Some roles of mCRP include enhancing chemotaxis and circulating leukocyte recruitment to infection sites via stimulatory effects on two cytokines, IL-8 and monocyte chemoattractant protein 1. (Note: IL-8 serves as a chemoattractant of neutrophils and stimulates neutrophil degranulation with the release of antimicrobial agents. Monocyte chemoattractant protein 1 influences monocyte and macrophage migration and infiltration and enhances T-cell activity.) Plasma CRP

concentrations (sometimes written as hsCRP) in healthy individuals are usually less than 0.08 mg/dL; however, they can rise up to 1,000-fold at sites of severe inflammation or with bacterial infection. In the absence of significant inflammation or infection, plasma CRP concentrations > 0.3 mg/dL may suggest low-grade inflammation and have been associated with increased risk of disease, primarily heart disease. Some examples of other acute-phase response proteins and their functions include:

- Orosomucoid (α 1-acid glycoprotein): a protein important in wound healing and immunomodulatory functions. Orosomucoid concentrations rise about two- to fivefold with inflammation.
- Serum amyloid A: a protein that displaces apolipoprotein A1 on high-density lipoproteins and facilitates cholesterol delivery to cells and cholesterol removal from damaged tissue. The protein also recruits immune cells (neutrophils and monocytes) to inflammatory sites and induces extracellular matrix degrading enzymes. Concentrations may rise 20- to 1,000-fold with inflammation.
- Fibrinogen: a protein that contributes to blood viscosity and can be converted to fibrin by thrombin to promote blood clotting; increased plasma fibrinogen may increase arterial thromboembolism (blood clot formation and dislodgement) risk.
- Fibrinectin: a glycoprotein functioning in cell adhesion and wound healing.
- Haptoglobin: a protein that binds hemoglobin that has been released into the blood due to red blood cell hemolysis and inhibits microbial use of iron.
- Ceruloplasmin: a copper-containing protein with the ability to scavenge free radicals and with oxidase activity to promote iron oxidation and thus inhibit microbial iron use.
- α 2 Macroglobulin: a protease inhibitor that, for example, inhibits blood coagulation and fibrinolysis.

In addition to the synthesis of these proteins, more metallothionein (a zinc-containing protein) and ferritin (an

iron-containing protein) are made in the liver with inflammation. Consequently, hepatic zinc and iron concentrations increase, while plasma zinc and iron concentrations decrease. Such changes diminish the likelihood of microorganisms utilizing the body's zinc and iron for their own proliferation. Another nutrient affected by inflammation is vitamin A; plasma retinol concentrations typically decrease with both infection and trauma.

Restoration of homeostasis following an inflammatory response involves a group of anti-inflammatory compounds including endogenous lipid mediators like resolvins, protectins, lipoxins, and maresins, as well as anti-inflammatory cytokines like IL-10, transforming growth factor β , and cytokine antagonist IL1Ra. While a discussion of the resolution to inflammation and stress is beyond the scope of this Perspective, it is important to note that inadequate energy and nutrient intakes impair the ability to rebuild lost muscle mass as well as diminish the immune and antioxidant defense

responses. Research is focused on determining optimal nutrition support required for the treatment of stress and inflammatory conditions, including the identification of nutrients that may serve as immunomodulators. (However, the benefits of physical activity [early ambulation] should not be ignored.) Higher protein intakes (greater than 2 g protein/kg body weight) may be recommended for critically ill patients with severe sepsis, extensive burns, and multiple trauma [2]. Supplementation of the diet with essential amino acids, including sufficient amounts of leucine, has been shown to offset the catabolic effects associated with bedrest and acute hypercortisolemia [3]. However, the absolute quantities of protein as well as other nutrients needed to reverse the catabolic state associated with stress and inflammation have not yet been determined.

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INTEGRATION AND REGULATION OF METABOLISM AND THE IMPACT OF EXERCISE

LEARNING OBJECTIVES

- 7.1 Define energy homeostasis.
- 7.2 Explain the role of glucose, amino acids, and fatty acids in energy homeostasis.
- 7.3 Describe the distribution of fuel molecules in the fed state, the postabsorptive state, the fasting state, and the starvation state.
- 7.4 Explain hormonal regulation of energy metabolism.
- 7.5 Distinguish between anaerobic and aerobic production of ATP.
- 7.6 Describe fuel sources for skeletal muscle during exercise.

METABOLISM IS OFTEN DEFINED AS ALL CHEMICAL REACTIONS AND PATHWAYS THAT OCCUR IN A LIVING ORGANISM TO MAINTAIN LIFE. Such a broad definition may seem overwhelming, although a closer look at metabolism reveals a highly coordinated set of events that occur around the central theme of energy homeostasis. Humans require frequent input of energy to perform mechanical work, including cardiac and skeletal muscle contractions; active transport of molecules and ions; and synthesis of complex molecules from simple precursors. The demand for energy by cells and the intake of energy from food are rarely synchronized, so the body is constantly adjusting metabolic pathways to maintain energy homeostasis. Despite the complexity, metabolic integration is achieved by the cells' ability to use a common energy currency (i.e., ATP) and surprisingly few intermediates (e.g., pyruvate and acetyl-CoA) that tie together metabolic pathways.

Chapters 3, 5, and 6 featured carbohydrate, lipid, and protein metabolism. Those chapters discussed how the pathways are regulated at the enzyme level by substrate availability, allosteric mechanisms, and covalent modifications such as phosphorylation. This chapter focuses on the integration of carbohydrate, lipid, and protein metabolism as it occurs in different organs and tissues—and the interconnections among them. The important topics discussed are: (1) the homeostasis of cellular energy and the control between catabolism and anabolism; (2) how the major organs and tissues interact through integration of their metabolic pathways to redistribute energy; (3) hormonal regulation of these metabolic processes; and (4) examples of the body's ability to maintain homeostasis under the daily events of fasting, refeeding, and physical activity. This chapter also discusses **exercise**—planned, structured physical activity to enhance physical fitness—and sports nutrition.

7.1 ENERGY HOMEOSTASIS IN THE CELL

Metabolic pathways generally belong to two broad categories: those that yield energy (degradative; catabolic) and those that require energy (synthetic; anabolic). The main purpose of catabolic reactions is to break down macronutrients so their inherent energy can be released and transformed into ATP. To a lesser extent, other high-energy molecules such as GTP and UTP are formed, although ATP is ubiquitous and the primary cellular energy carrier. Anabolic reactions, on the other hand, synthesize complex molecules from simple precursors by utilizing the energy from ATP and, in some key reactions, GTP and UTP.

Despite the thousands of reactions that occur in the body, there are only a few common pathways in the metabolic roadmap that control whether a cell or organ is engaged in catabolism or anabolism. As depicted in Figure 7.1, pyruvate and acetyl-CoA are critical junctions in the roadmap that connect the metabolism of carbohydrate, lipid, and protein. The energy status of the cell largely determines the direction in which molecules flow. If cellular energy (ATP) is needed, the pyruvate from glycolysis is sent to the mitochondria, decarboxylated to acetyl-CoA, and oxidized via the TCA cycle to produce ATP through oxidative phosphorylation (see Chapter 3). Similarly, fatty acids may be catabolized to acetyl-CoA in mitochondria, resulting in the production of ATP via the TCA cycle and oxidative phosphorylation. And when carbohydrates and lipids are in short supply, amino acids are converted to pyruvate and acetyl-CoA, thus providing needed energy for ATP production. Amino acids are also used to replenish many of the TCA cycle intermediates to ensure the cycle's continued operation.

Pyruvate and acetyl-CoA may be used to produce more complex molecules when the cellular energy status favors anabolic reactions (Figure 7.1). Pyruvate is converted to glucose via gluconeogenesis, whereas acetyl-CoA is mostly used for fatty acid synthesis. Note that the conversion of pyruvate to acetyl-CoA is an irreversible reaction, preventing appreciable amounts of acetyl-CoA from being used for gluconeogenesis. Other metabolic intermediates, particularly those of the TCA cycle, can be diverted into anabolic pathways as needed. TCA cycle intermediates used for anabolic reactions are largely replenished by the conversion of pyruvate to oxaloacetate, although a variety of molecules are available to ensure the TCA cycle continues to function. Examples of TCA cycle intermediates entering anabolic pathways include the following:

- Citrate can move from the mitochondria into the cytosol, where citrate lyase cleaves it into oxaloacetate and acetyl-CoA, the latter being used for fatty acid synthesis.
- Malate, in the presence of NADP⁺-linked malic enzyme, may provide a portion of the NADPH required for reduction reactions in fatty acid synthesis.
- Succinyl-CoA can combine with glycine in the mitochondria to form Δ -aminolevulinic in initial step in heme synthesis (see Figure 13.7).
- Oxaloacetate may be used for conversion to amino acids or it may enter the gluconeogenesis pathway.
- CO₂ produced by the TCA cycle is a source of cellular carbon for carboxylation reactions that initiate fatty acid synthesis and gluconeogenesis. This CO₂ also supplies the carbon of urea and certain portions of the purine and pyrimidine rings (see Figures 6.7, 6.27, and 6.31).

It is important to remember that anabolic reactions generally require NADPH to provide reductive power for synthesis of complex molecules. NADPH is the major electron donor in cells that drives anabolic reactions. Most of the needed NADPH is supplied by the pentose phosphate pathway, which also produces ribose-5-phosphate used in the synthesis of nucleotides (see Chapter 3).

Regulatory Enzymes

Maintaining the balance between catabolism and anabolism is achieved by the regulation of distinct enzymes that are highly sensitive to changes in energy status within the cell. Regulatory enzymes are located at strategic points in metabolic pathways and are mostly unidirectional (irreversible). The majority of regulatory enzymes is controlled allosterically and respond immediately to cellular signals, although some are controlled by covalent modification, usually phosphorylation, in response to hormonal signals. Glycogen synthase and phosphorylase are examples of covalently modified regulatory enzymes (see Chapter 3). Table 7.1 summarizes the cellular signals and key enzymes that are controlled allosterically in response to immediate changes in cellular energy status. The changes occur because of metabolic activities in a particular cell or tissue (such as exercising muscle) or during eating and fasting. Metabolic events related to the fed-fast cycle are discussed later in this chapter.

As the energy status of the cell declines, so does the concentration of acetyl-CoA, citrate, and ATP due to decreased glycolysis, lipolysis (β -oxidation), and TCA cycle reactions. At the same time, increased amounts of ADP and AMP indicate that ATP has been used up in anabolic reactions and more ATP is needed. Furthermore, decreased concentration of malonyl-CoA reflects little or no fatty acid synthesis occurring during low energy status. Each of these cellular signals will trigger the allosteric stimulation of regulatory enzymes to increase glycolysis, β -oxidation, and the TCA cycle to replenish ATP.

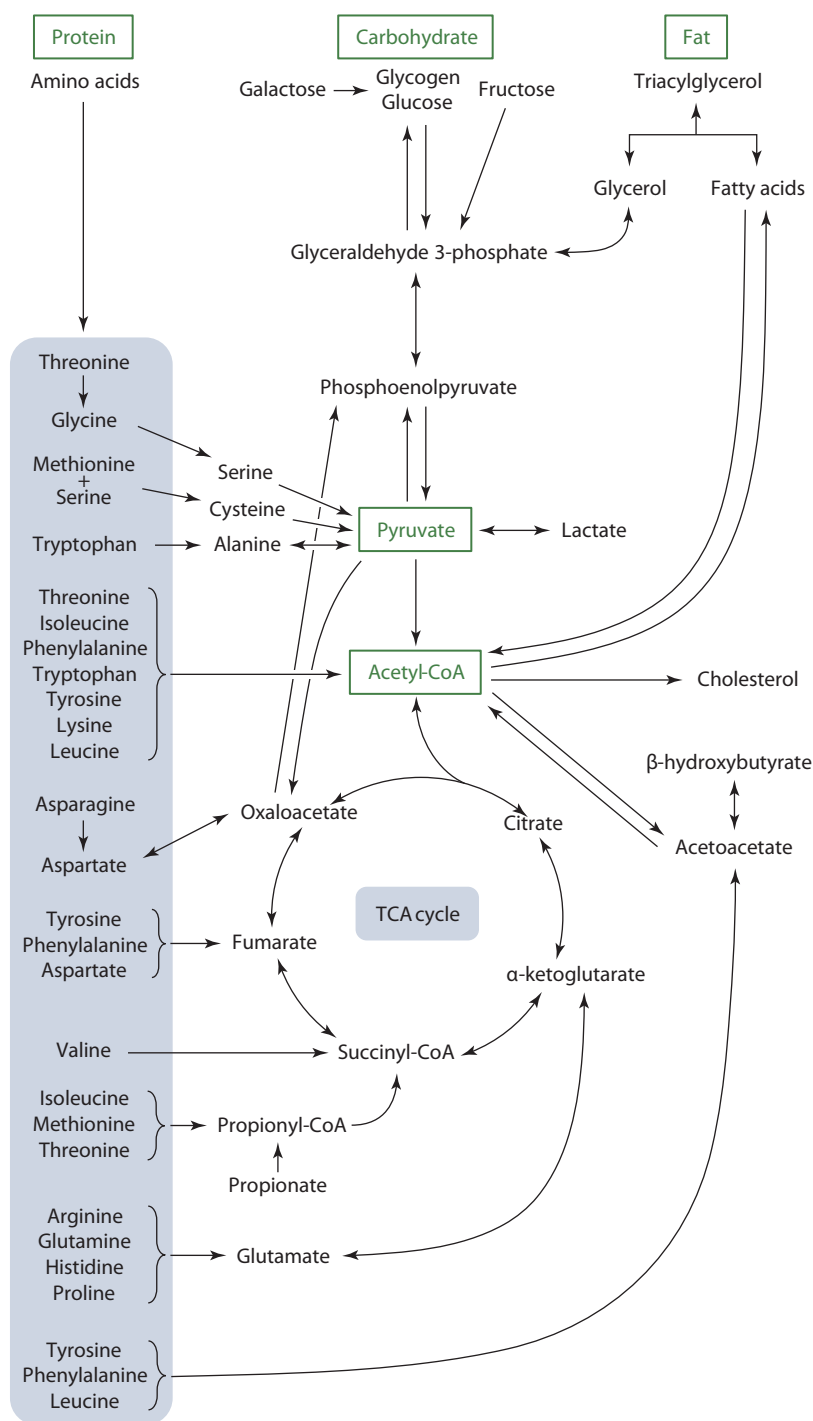


Figure 7.1 Metabolic pathways involved in the maintenance of energy homeostasis. Bidirectional pathways with separate arrows indicate separate regulatory enzymes controlling each direction. Not all pathway intermediates are shown.

When cellular ATP is abundant, the concentration of ATP, acetyl-CoA, and citrate are relatively high. These molecules act to allosterically inhibit regulatory enzymes that govern glycolysis and the TCA cycle while stimulating

gluconeogenesis and fatty acid synthesis as a way of capturing and storing the energy for later use. Each of the regulatory enzymes highlighted in Table 7.1 have been discussed in more detail in Chapters 3 and 5.

Table 7.1 Allosteric Regulation of Enzymes in Response to Cellular Energy Status.

Low Cellular Energy		
Cellular Signals	Regulated Enzyme	Metabolic Response
↑ AMP, ↓ ATP, ↓ citrate, ↓ acetyl-CoA	↑ Phosphofructokinase	↑ Glycolysis
↓ ATP, ↓ acetyl-CoA	↑ Pyruvate kinase	
↑ ADP, ↑ pyruvate	↑ Pyruvate dehydrogenase	↑ TCA cycle
↑ ADP	↑ Isocitrate dehydrogenase	
↑ AMP	↓ Fructose-1,6-bisphosphatase	↓ Gluconeogenesis
↓ Malonyl-CoA	↑ Carnitine acyltransferase I	↑ Fatty acid β -oxidation
Abundant Cellular Energy		
Cellular Signals	Regulated Enzyme	Metabolic Response
↑ ATP, ↑ citrate	↓ Phosphofructokinase	↓ Glycolysis
↑ ATP	↓ Isocitrate dehydrogenase	↓ TCA cycle
↑ ATP	↓ α -Ketoglutarate dehydrogenase	
↑ Acetyl-CoA	↑ Pyruvate carboxylase	↑ Gluconeogenesis
↑ Citrate	↑ Fructose-1,6-bisphosphatase	
↑ Citrate	↑ Acetyl-CoA carboxylase	↑ Fatty acid synthesis
↑ Malonyl-CoA	↓ Carnitine acyltransferase I	

Role of Malonyl-CoA

Malonyl-CoA deserves special mention because of its role in both fatty acid synthesis and β -oxidation. Its regulatory role is expressed through its allosteric control of carnitine acyltransferase I (CAT I), although the cellular levels of malonyl-CoA are mediated by the phosphorylation of AMP-activated protein kinase (AMPK). Recall from Chapter 5 that CAT I is required to transport activated fatty acids (as fatty acyl-CoA) into the mitochondria for oxidation. Increased cellular concentration of malonyl-CoA blocks CAT I and prevents transport and subsequent oxidation, whereas the absence of malonyl-CoA allows CAT I to function. Figure 7.2 illustrates the cellular signals that control the concentration of malonyl-CoA.

Note that malonyl-CoA and acetyl-CoA are part of a rapid cycle in the cytosol that is mediated by two opposing enzymes, acetyl-CoA carboxylase (ACC) and malonyl-CoA decarboxylase (MCD), as depicted in the shaded area in Figure 7.2. If energy is not needed by the cell, the acetyl-CoA will be carboxylated to form malonyl-CoA, which is the first step of fatty acid synthesis (see Figure 5.31). Malonyl-CoA levels are highest in the fed state and decline with fasting (the fed-fast cycle is discussed in detail later in this chapter). The amount of malonyl-CoA in skeletal muscle is increased by glucose and insulin, resulting in a decrease in β -oxidation of fatty acids due to the inactivation of AMPK. In lipogenic tissues such as the liver, adipose tissue, and lactating mammary glands, malonyl-CoA is a cosubstrate for the cytosolic fatty acid synthase system for the *de novo* synthesis of palmitic acid (see Chapter 5).

When the energy status of the cell is low, recruitment of fatty acids from the circulation increases the cytosolic fatty acyl-CoA concentration (Figure 7.2). The need for ATP production is accelerated by fasting and muscle contraction. Under these conditions, increased levels of fatty acyl-CoA and glucagon activate AMPK by phosphorylation, which in turn activates MCD and inactivates ACC by phosphorylation. In cardiac muscle 50–80% of the energy is derived from fatty acids. Fatty acids provide less energy following consumption of a high-carbohydrate meal and more following a high-fat meal. Malonyl-CoA is also thought to function as one of the signals for β -cells of the pancreas to secrete insulin in response to elevated blood glucose levels. The elevated malonyl-CoA levels in β -cells inhibit the transfer of fatty acids into the mitochondria, and the increased fatty acid levels in the cytosol act as a coupling factor for insulin secretion.

Malonyl-CoA is also associated with the restraint of food intake. It acts through the hormone leptin released by adipose tissue to signal that triacylglycerol storage in adipose is adequate. Leptin is discussed in detail in Chapter 8.

Role of AMP-Activated Protein Kinase

The previous section described how AMPK participates in fatty acid metabolism. AMPK appears to play a much larger role in metabolism and can be viewed as a master energy sensor, controlling both catabolic and anabolic pathways involving all the macronutrients [1]. Regulatory systems that are responsive to AMPK represent another common mechanism of regulation that links carbohydrate, lipid, and protein metabolism. AMPK is activated by an increasing cellular AMP and declining ATP (high

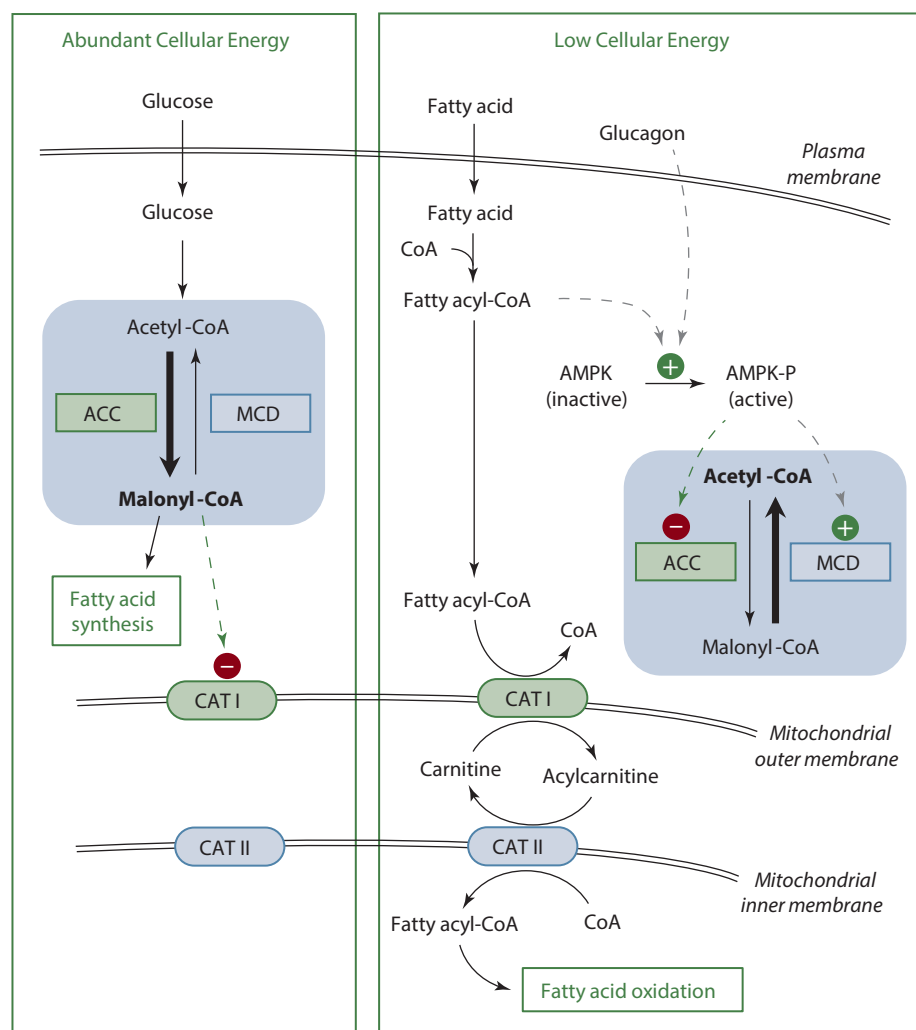


Figure 7.2 Role of malonyl-CoA in fatty acid synthesis and oxidation. Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; CAT, carnitine acyltransferase (I and II); and MCD, malonyl-CoA decarboxylase.

AMP:ATP ratio), indicating low energy status of the cell. This can be caused by declining cellular glucose that occurs during fasting, increased ATP utilization that occurs with muscle contraction, and metabolic stresses that interfere with ATP production such as hypoxia. Several dietary phytochemicals can activate AMPK, including capsaicin in peppers, resveratrol in red wine and grapes, curcumin in turmeric, and epigallocatechin gallate in green tea.

AMPK influences glucose metabolism in several ways. In response to increasing AMP:ATP ratio, activated AMPK in turn activates a transporter protein in adipocytes and muscle cells involved in the translocation of GLUT4 to the plasma membrane, thus increasing the uptake of glucose into the cell. A common drug used to treat type 2 diabetes, metformin, exerts part of its hypoglycemic effect by activating AMPK and increasing the translocation of GLUT4 to the cell surface by mechanisms independent of insulin. AMPK also promotes glucose uptake in cells that express only GLUT1 by activating the transporter that is already located in the plasma membrane (see Table 3.2).

AMPK stimulates glycolysis mainly in cardiac muscle by phosphorylating phosphofructokinase-2, which produces 2,6-bisphosphate, a potent allosteric activator of phosphofructokinase (reaction 3 in Figure 3.20). Furthermore, activated AMPK prevents cellular energy from being diverted into anabolic pathways by inhibiting glycogen synthase (see Figure 3.16) and the gluconeogenic enzymes phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (see Figure 3.32).

Another role of AMPK in lipid metabolism when the cellular AMP:ATP ratio is high includes the promotion of fatty acid uptake into cardiac muscle by increasing the translocation of the fatty acid transporter CD36 to the plasma membrane. Activated AMPK also inhibits acyltransferases involved in triacylglycerol and phospholipid synthesis (see Figure 5.36), and HMG-CoA reductase, the rate-limiting step in cholesterol synthesis (see Figure 5.37). With regard to protein metabolism, AMPK appears to inhibit protein synthesis by phosphorylating at least two enzymes involved in the translation of mTOR,

itself a protein kinase that controls many cellular processes including protein synthesis [1].

AMPK also plays a role in energy metabolism through its action in the hypothalamus, the primary appetite control center of the body. Details of the role of AMPK and hormones in appetite regulation are discussed in Chapter 8.

7.2 INTEGRATION OF CARBOHYDRATE, LIPID, AND PROTEIN METABOLISM

The chapter thus far has focused mainly on individual pathways and regulation of those pathways by specific enzymes. We now shift our attention to a broader view of metabolism in which many pathways are coordinated simultaneously through common cellular and extracellular signals. In fact, the entire human body must be continually synchronized for normal metabolism to occur. This expanded view of metabolism reveals a type of “communication” within cells, between cells, and even among tissues and organs. The constantly changing metabolic status of various tissues throughout the body requires communication across great distances, facilitated by the nervous, endocrine, and vascular systems. Described here is an overview of how fuel molecules can be interconverted and redistributed among the body’s tissues to maintain energy homeostasis. The impact of the fed-fast cycle and skeletal muscle activity (exercise) on these events are discussed later in this chapter.

Interconversion of Fuel Molecules

The body needs a constant supply of energy from macronutrients to function optimally. The amount of food consumed and the frequency of intake have profound effects on metabolic pathways that maintain the balance between catabolism and anabolism both short term (minutes, hours) and long term (days, weeks, months), as discussed later in this chapter. Dietary carbohydrate and lipid provide the majority of energy for ATP production. Amino acids are also used for energy, although the high demand for body proteins generally diverts most food-derived amino acids into protein synthesis. Amino acids may be called upon for energy when carbohydrate and lipid are insufficient.

Figure 7.1 illustrates how macronutrients are catabolized to common intermediates (pyruvate and acetyl-CoA), which can be resynthesized into glucose, triacylglycerol, and amino acids as warranted by the metabolic status of the cell. Pyruvate and acetyl-CoA represent key intersections in the metabolic roadmap where interconversion among the nutrients can occur. For example, as explained in Chapter 6, certain amino acids can be synthesized in the

body from carbohydrates or fatty acids; conversely, most amino acids can serve as precursors for glucose or fatty acid/triacylglycerol synthesis. Carbohydrates can be used to synthesize fatty acids and triacylglycerols. Fatty acids, in contrast, cannot be used to make glucose because humans lack the necessary enzymes to convert acetyl-CoA to pyruvate or any intermediate along the gluconeogenic pathway. Not evident from the figure, but important to recall, is that the TCA cycle is an **amphibolic pathway**, meaning that it not only functions in the oxidative catabolism of carbohydrates, fatty acids, and amino acids but also provides precursors for many biosynthetic pathways, particularly gluconeogenesis (see Figure 3.33). Amino acids can be converted into several TCA cycle intermediates. When needed, α -ketoglutarate, succinate, fumarate, and oxaloacetate can be used as gluconeogenic precursors.

When ATP is needed, glucose and amino acids may be catabolized to pyruvate, which is translocated from the cytosol into the mitochondria while simultaneously decarboxylating it to acetyl-CoA. The acetyl-CoA can be oxidized to CO_2 and H_2O to produce ATP by the TCA cycle and oxidative phosphorylation. Another fate of pyruvate is its reduction in the cytosol to lactic acid (Figure 7.1). The lactate can be transported to other tissues, converted back to pyruvate and oxidized in the muscle, or used for gluconeogenesis in the liver. Most of the acetyl-CoA is produced in the mitochondria through the β -oxidation of fatty acids. When acetyl-CoA is involved in anabolic reactions, it has to be translocated back to the cytosol across the mitochondrial membrane, which is not permeable to it. Therefore, the acetyl-CoA in the mitochondria combines with oxaloacetate to form citrate (as in the TCA cycle), to which the mitochondrial membrane is freely permeable. The citrate moves into the cytosol and can break down again to oxaloacetate and acetyl-CoA. The acetyl-CoA may undergo a carboxylation reaction catalyzed by acetyl-CoA carboxylase to form malonyl-CoA (see Figure 5.31), the first step of fatty acid synthesis.

Glucose is the precursor for the glycerol moiety of triacylglycerol in adipose tissue. It can be formed from dihydroxyacetone phosphate, a three-carbon intermediate in glycolysis (see Figure 3.20). Reduction of dihydroxyacetone phosphate by glycerol-3-phosphate dehydrogenase and NADH produces glycerol-3-phosphate (see Figure 5.40). The fatty acid components of triacylglycerols in adipose tissue can come from the diet, from adipose tissue (via lipolysis), or from the liver, where they are synthesized and packaged for delivery to adipocytes by VLDL. Triacylglycerols are synthesized by the reaction of the glycerol-3-phosphate with CoA-activated fatty acids (Figure 5.33). Recall that muscle and adipose tissue lack the glycerol kinase that can phosphorylate glycerol directly and must obtain the glycerol-3-phosphate through glycolysis.

While carbohydrate can be converted into both the glycerol and the fatty acid components of triacylglycerols, only

the glycerol portion of triacylglycerols can be converted into carbohydrate. The conversion of fatty acids into carbohydrate is not possible because the pyruvate dehydrogenase reaction is not reversible. This fact prevents the direct conversion of acetyl-CoA, the sole catabolic product of fatty acids with an even number of carbons, into pyruvate for gluconeogenesis. In addition, gluconeogenesis from acetyl-CoA as a TCA cycle intermediate cannot occur because for every two carbons in the form of acetyl-CoA entering the TCA cycle, two carbons are lost by decarboxylation in early reactions of the cycle (see Figure 3.21). Consequently, there can be no net conversion of acetyl-CoA to pyruvate or to the gluconeogenic intermediates of the TCA cycle. Acetyl-CoA produced from any source must be used for ATP production, lipogenesis, cholesterol synthesis, or ketogenesis (Figure 7.1).

In contrast to fatty acids that have an even number of carbons, fatty acids with an odd number of carbon atoms are partially glucogenic. The so-called odd-chain fatty acids can be partially converted to glucose because propionyl-CoA ($\text{CH}_3\text{—CH}_2\text{—COSCoA}$), ultimately formed by β -oxidation, is carboxylated and rearranged to succinyl-CoA, a glucogenic TCA cycle intermediate (see Figure 5.28). Odd-chain fatty acids are not abundant in the diet, although ruminant meat and milk fat and some fish are known sources.

Metabolism of the amino acids gives rise to a variety of amphibolic intermediates, some of which produce glucose (glucogenic), while others produce ketone bodies (ketogenic) by their conversion to acetyl-CoA or acetoacetyl-CoA. Only the amino acids leucine and lysine are purely ketogenic. The dispensable (nonessential) glucogenic amino acids can be converted to carbohydrate, but like the ketogenic amino acids, they can also be converted indirectly into fatty acids by undergoing oxidation to acetyl-CoA. Fatty acids cannot be converted into the glucogenic amino acids for the same reason that fatty acids cannot be converted into glucose—namely, the irreversibility of the pyruvate dehydrogenase reaction. Although metabolically possible, the conversion of the glucogenic amino acids into fatty acids is rather uncommon. Only when protein is supplying a high percentage of calories would glucogenic amino acids be expected to be used in fatty acid synthesis. All the amino acids producing acetyl-CoA directly—iso-leucine, threonine, phenylalanine, tryptophan, tyrosine, lysine, and leucine—are indispensable. Tyrosine is conditionally indispensable because it is formed by hydroxylation of phenylalanine. The catabolism of the individual amino acids is covered in Chapter 6.

Energy Distribution among Tissues

The ability to interconvert fuel molecules is crucial for maintaining energy homeostasis in the body, where metabolism in every tissue is unique and markedly

different. The metabolic events that occur in one tissue will significantly affect metabolism in other tissues. In this way, fuel molecules are constantly being interconverted and transported among tissues via the bloodstream under all metabolic conditions to provide energy where needed. The following discussion highlights the unique metabolic features and primary differences of tissues that participate in energy distribution.

Liver

The liver plays a central role in metabolism. Most nutrients absorbed by the small intestine first pass through the liver, and many fuel molecules released by extrahepatic tissues travel to the liver for additional processing. Figures 7.3, 7.4, and 7.5 illustrate the fate of glucose-6-phosphate, amino acids, and fatty acids in the liver. In these figures, anabolic pathways are shown pointing up; catabolic pathways are pointing down; and distribution to other tissues is running horizontally. The pathways indicated are described in detail in Chapters 3, 5, and 6, which deal with carbohydrate, lipid, and protein metabolism, respectively.

Glucose entering hepatocytes from the hepatic portal vein and, to a lesser extent, the systemic circulation is phosphorylated by glucokinase to glucose-6-phosphate. Dietary galactose is also phosphorylated and rearranged to glucose-6-phosphate. Figure 7.3 shows the possible metabolic routes available to glucose-6-phosphate. The liver uses relatively little glucose-6-phosphate for its own energy needs and, instead, stores a significant amount as glycogen for times when glucose is in short supply. Glycogen synthesis occurs when blood glucose levels are high and the liver takes in more glucose, especially after a meal. About two-thirds of the glucose-6-phosphate entering glycogenesis is derived from glucose absorbed by the small intestine. The remaining glucose-6-phosphate entering glycogenesis is derived, paradoxically, from newly synthesized glucose because gluconeogenesis continues to function under all metabolic conditions. This is due to the constant flow of lactate into systemic circulation that the liver must convert to glucose-6-phosphate to keep blood lactate levels in check. The lactate comes from extrahepatic tissues, notably skeletal muscle and red blood cells.

Figure 7.4 reviews the particularly active role of the liver in amino acid metabolism. The liver is the site of synthesis of many different proteins, both structural and plasma-borne, from amino acids. The liver can also convert amino acids into nonprotein products such as nucleotides and porphyrins. Catabolism of amino acids can take place in the liver, where most are transaminated and degraded to acetyl-CoA and other TCA cycle intermediates. These substances in turn can be oxidized for energy or converted to glucose or fatty acids. Glucose formed from gluconeogenesis can be transported to muscle, brain, nerve cells, red blood cells, and other tissues for energy utilization. Newly synthesized fatty acids can be

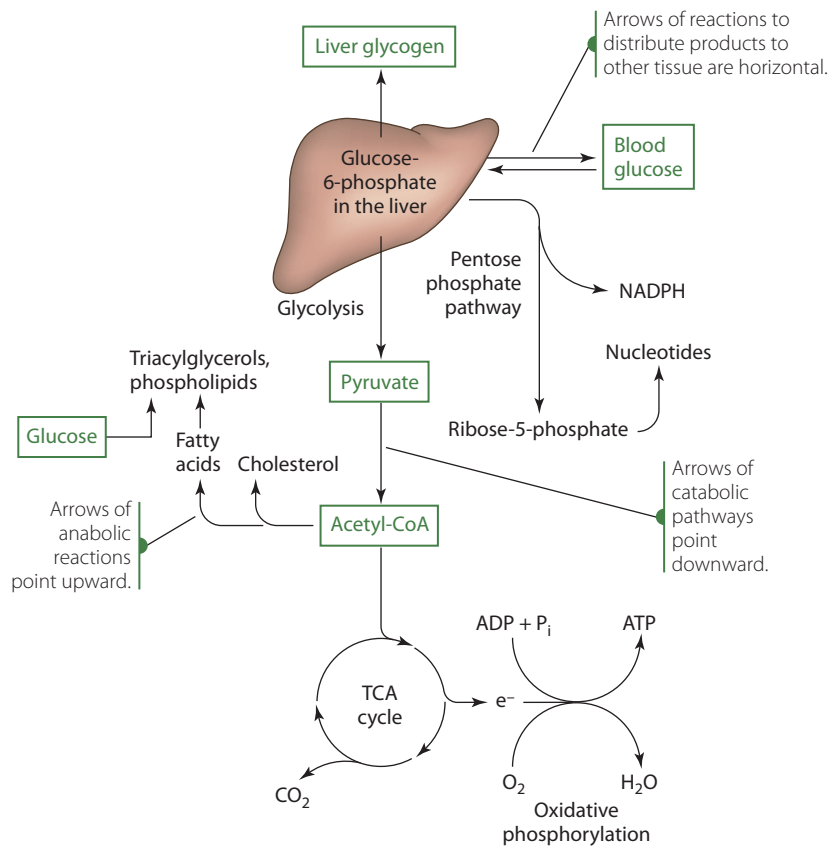


Figure 7.3 Pathways of glucose-6-phosphate metabolism in the liver.

transported to adipose tissue for storage or used as fuel primarily by cardiac and skeletal muscle. Hepatocytes are the exclusive site for the formation of urea, the major excretory form of amino acid–derived nitrogen.

The fate of fatty acids in the liver is outlined in Figure 7.5. Hepatic fatty acids are derived from chylomicron remnants and from *de novo* synthesis. In humans, most fatty acid synthesis takes place in the liver rather than in adipose tissue. Fatty acids can be assembled into liver triacylglycerols and released into the circulation as plasma VLDL. Circulating VLDL interact with tissues expressing lipoprotein lipase, namely muscle and adipose tissue, where the triacylglycerols are delivered. Adipocytes store the triacylglycerols, whereas muscle will mostly hydrolyze the triacylglycerols and oxidize the resulting fatty acids for ATP production. Under most circumstances, fatty acids are a major fuel supplying energy to the liver via the TCA cycle and oxidative phosphorylation. The acetyl-CoA that cannot be used for energy may be converted to ketone bodies, which are important fuels for certain peripheral tissues such as the brain and heart muscle, particularly during periods of prolonged fasting.

Muscle

Fatty acids and glucose are the major fuels for both skeletal and cardiac muscle. Muscle can also use ketone bodies for energy when the availability of fatty acids and glucose

is insufficient. Cardiac muscle requires a continuous supply of energy, whereas skeletal muscle's demand for fuel molecules is quite low when at rest but will increase as muscle contractions increase. The relative contribution of fatty acid and glucose use in skeletal muscle can change dramatically depending on the duration and intensity of physical activity (discussed later in this chapter).

Skeletal and cardiac muscle express GLUT4 on the cell surface when the blood concentration of glucose and insulin are elevated such as following a carbohydrate-rich meal. Recall that GLUT4 is the only GLUT protein whose function is dependent on insulin (see Table 3.2). Consequently, muscle cells can take up large amounts of glucose and will quickly phosphorylate it to glucose-6-phosphate by the enzyme hexokinase. Cardiac muscle has a very limited capacity to synthesize glycogen and therefore uses the glucose-6-phosphate for immediate energy needs. Skeletal muscle, on the other hand, can store large amounts of glycogen for use at a later time. Skeletal and cardiac muscle express other GLUT proteins and can take up glucose from the circulation during fasting or when energy demands exceed incoming dietary sources of carbohydrate. Under these conditions, the blood glucose originates from the liver via glycogenolysis or gluconeogenesis. The liver releases glucose into the circulation after dephosphorylation by the enzyme glucose-6-phosphatase. Muscle cells lack this enzyme and cannot export glucose;

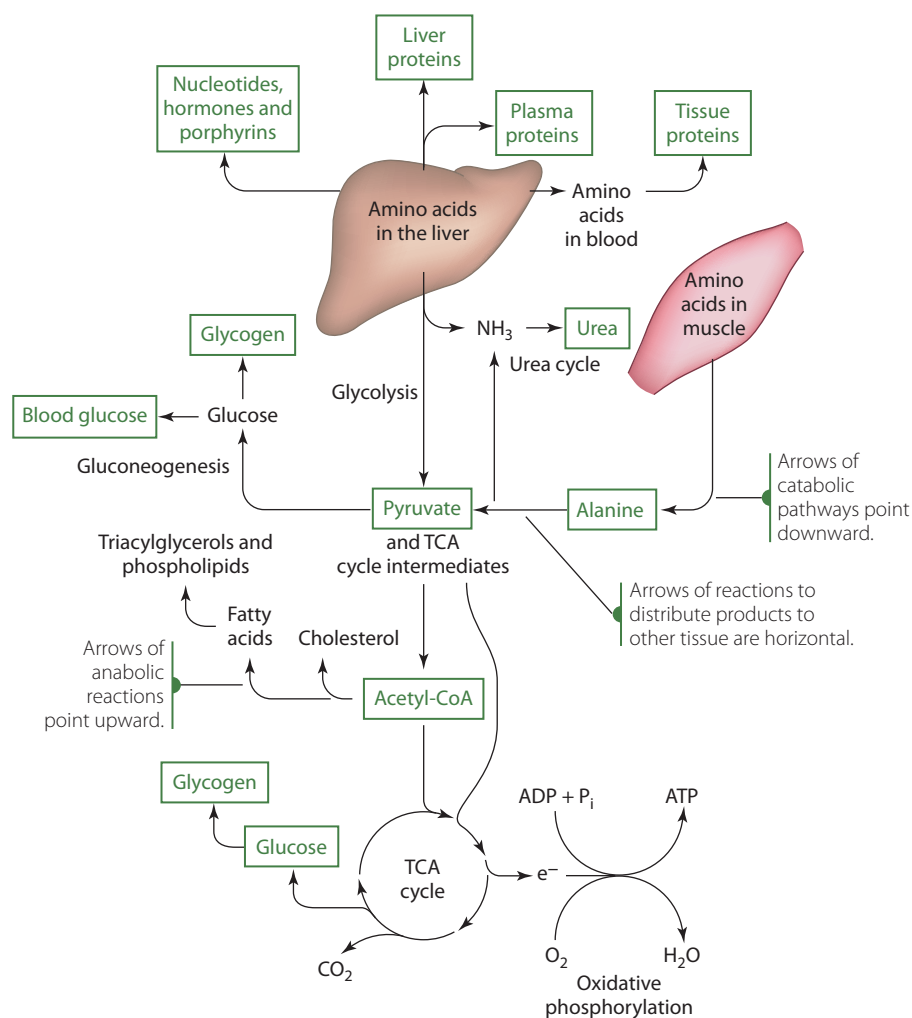


Figure 7.4 Pathways of amino acid metabolism in the liver.

therefore, glucose stored in muscle as glycogen is used for glycolysis.

Fatty acids are a primary fuel source for cardiac muscle and resting skeletal muscle. β -oxidation of fatty acids is entirely aerobic, so it is not surprising that cardiac muscle has a high concentration of mitochondria. Cardiac muscle will increase its use of glucose when glucose is abundant, but still favors fatty acids as the main fuel source. Similarly, resting skeletal muscle can increase its use of glucose in the fed state when there is ample glucose, insulin, and GLUT4. The use of fatty acids by active skeletal muscle can be augmented by the ability of muscle to store moderate amounts of triacylglycerol adjacent to mitochondria. However, skeletal muscle relies increasingly on glucose for energy as physical activity increases. Most fatty acids used for energy by muscle are derived from the circulation. Cardiac and skeletal muscle express lipoprotein lipase on the cell surface that will bind to circulating chylomicrons (derived from the intestine following a meal) and VLDL (derived from the liver). The triacylglycerols transported by chylomicrons and VLDL are hydrolyzed by

lipoprotein lipase and the fatty acids are transferred into the cell. Muscle cells can also utilize free fatty acids released by adipose tissue and transported by serum albumin.

Adipose Tissue

Adipose tissue has the ability to store huge amounts of triacylglycerols and thus serves as an energy reservoir in the body. Triacylglycerols derived from the diet are transported by chylomicrons to adipose tissue where lipoprotein lipase hydrolyzes the triacylglycerols, facilitating the transfer of fatty acids into the cell. In a similar manner, triacylglycerols derived from the liver are transported by VLDL to adipose tissue. Triacylglycerols secreted by the liver come from the catabolism of chylomicron remnants as well as hepatic synthesis of fatty acids from nonlipid precursors, including “excess” dietary glucose and fructose. Immediately following the uptake of fatty acids into adipocytes, the fatty acids are esterified with glycerol-3-phosphate to form triacylglycerols. The action of lipoprotein lipase does not facilitate the transfer of glycerol into the cell, so adipocytes rely on the presence of glucose

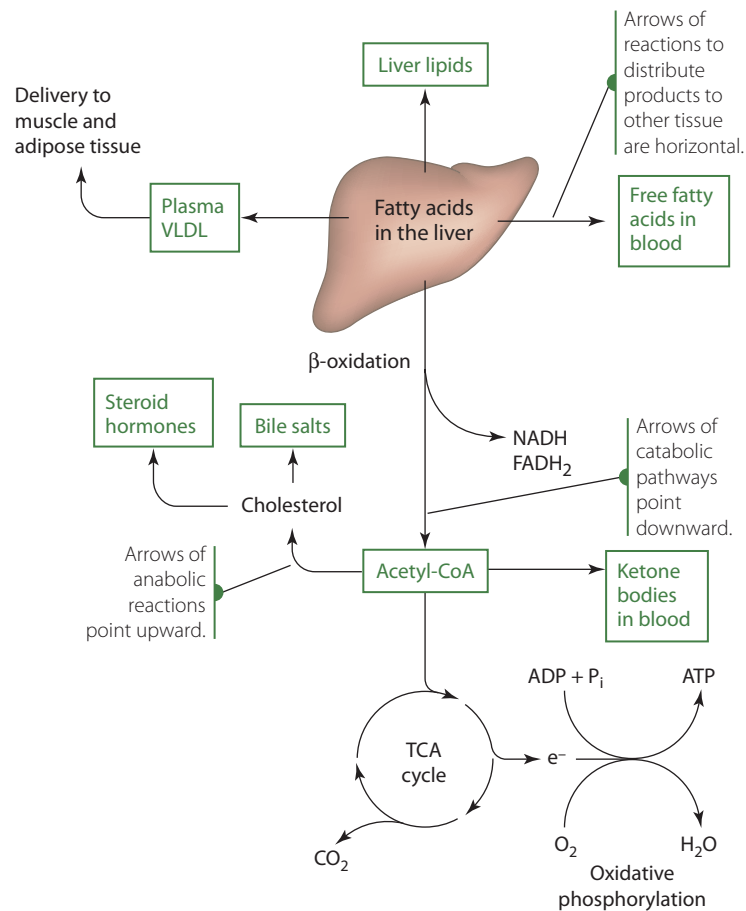


Figure 7.5 Pathways of fatty acid metabolism in the liver.

as the source of glycerol-3-phosphate. Free glycerol may be transported to the liver and used as a precursor for gluconeogenesis.

Adipocytes express GLUT4 on the cell surface, which promotes glucose uptake when blood glucose levels are elevated. Glucose is converted to glucose-6-phosphate and rapidly enters glycolysis. The glycolytic pathway provides glycerol-3-phosphate for triacylglycerol assembly (as mentioned above) and pyruvate that can be converted to acetyl-CoA. Some acetyl-CoA may be oxidized via the TCA cycle to address the energy needs of the cell, while the remaining is used for fatty acid synthesis. Recall that lipogenesis requires the reducing power of NADPH. Some of the glucose-6-phosphate is directed into the pentose phosphate pathway to supply the necessary NADPH. The rate of lipogenesis is higher in the liver than in adipose tissue when measured on a gram-per-gram basis. However, the mass of adipose tissue can be many times greater than liver, especially in obese individuals, demonstrating that an overabundance of carbohydrate can contribute significantly to adiposity.

When dietary energy is in short supply, the triacylglycerols in adipose tissue are hydrolyzed and released as free fatty acids into the circulation where they bind to albumin for transport to other tissues. Many tissues in the body

use fatty acids as a fuel, most notably cardiac and skeletal muscle.

Brain

Glucose is the primary fuel used by the brain and nerve cells. Under normal conditions, glucose is the sole energy source, but the brain can adapt to using ketone bodies during prolonged energy deficit as occurs when consuming energy-restricted diets and in starvation. The brain cannot use fatty acids because these large molecules do not transport across the blood–brain barrier. The brain requires a constant and relatively large supply of glucose. Unlike skeletal muscle whose energy requirements are highly variable, mental activity does not increase energy utilization by the brain. The brain accounts for about 20–25% of total energy used in the body; it also accounts for the majority of glucose removed from the blood when at rest.

Maintaining adequate blood glucose levels is imperative for normal brain function. In the short term when the diet is unable to supply adequate amounts of glucose, the liver releases glucose into the circulation. Liver glucose is derived from the breakdown of glycogen and from gluconeogenesis using noncarbohydrate precursors (lactate, glycerol, and certain amino acids). Prolonged energy deficit leads to accelerated breakdown of triacylglycerols in adipose tissue,

causing an overabundance of fatty acids that the liver oxidizes to acetyl-CoA. The acetyl-CoA is converted to ketone bodies that the brain and other tissues can convert back to acetyl-CoA and use for ATP production via the TCA cycle and oxidative phosphorylation.

Red Blood Cells

Red blood cells rely exclusively on glucose as their only energy source under all metabolic conditions. During their development, red blood cells lose their organelles, including mitochondria. Without mitochondria, anaerobic glycolysis is the only means of producing ATP. The metabolic advantage is that red blood cells are unable to consume any of the oxygen they transport. The disadvantage is that glycolysis is an inefficient means of producing ATP from glucose. However, the end product of glycolysis (pyruvate) is quickly converted to lactate, released into the blood plasma and taken up by the liver. The lactate is then used to synthesize glucose via gluconeogenesis and released back into the circulation.

Kidneys

The kidneys require about 10% of the total energy used by the body. Their main function is to produce urine and, in the process, remove metabolic waste products from the blood plasma. The kidneys filter the plasma approximately 60 times per day. Most of the constituents of plasma filtered by the kidney are desirable, such as glucose and water, and need to be retained. Reabsorbing these constituents in the kidney tubules requires substantial amounts of energy.

The role of the kidneys in helping maintain energy homeostasis has not been studied as thoroughly as other major organs, as their contribution appears to have been underappreciated. It is useful to think of the kidneys as two separate organs, with glucose utilization occurring mostly in the renal medulla and glucose synthesis and secretion occurring in the renal cortex [2]. These separate activities are the result of different enzymes located within each region of the kidney. The renal medulla is similar to the brain in that it requires glucose for energy, whereas the renal cortex uses fatty acids as a primary fuel source under normal conditions. Cells of the renal medulla are able to convert glucose to glucose-6-phosphate for entry into glycolysis; however, they lack glucose-6-phosphatase and are unable to release glucose into the circulation. In contrast, cells of the renal cortex possess gluconeogenic enzymes—they lack phosphorylating capacity and cannot synthesize glycogen—and therefore can make and release glucose. The renal cortex increases glucose synthesis during prolonged starvation and may contribute up to half of the circulating glucose. Interestingly, any lactate produced as a result of glycolysis in the renal medulla can be used by the renal cortex for gluconeogenesis.

7.3 THE FED-FAST CYCLE

The best way to appreciate the integration of metabolic pathways and the involvement of different organs and tissues in metabolism is to understand the fed-fast cycle. Humans are “meal eaters” and typically consume food at routine times, separated by periods of not eating. Most meals provide significantly more energy than is needed at that moment, which triggers regulatory hormones and enzymes designed to capture and store the excess energy largely as glycogen and triacylglycerols in tissues equipped to handle such molecules. Because glucose is a major fuel for tissues, it is important that glucose homeostasis be maintained, whether the person has just consumed food or is in a fasting state. If the period since the last meal is short (less than 18 hours), the mechanisms used to maintain glucose homeostasis are different from those used if the fasting state is prolonged. The extent to which different organs are involved in carbohydrate, fatty acid, and amino acid metabolism varies within the fed-fast cycles that underlie the eating habits of humans. A fed-fast cycle can be divided into four states, or phases:

- The fed state, lasting about 3 hours after a meal is ingested and characterized by insulin secretion
- The postabsorptive state, occurring from about 3 to 18 hours following the meal and accompanied by a rise in glucagon secretion
- The fasting state, lasting from 18 hours to about 2 days without additional intake of food and accompanied by further increases in glucagon
- The starvation state or long-term fast, a fully adapted state of food deprivation lasting longer than about 2 days.

The time frames assigned to each phase are only approximate and are strongly influenced by factors such as activity level, the caloric value and nutrient composition of the meal, and the person's metabolic rate. While somewhat variable, the established time frames represent distinctive metabolic events that characterize each phase. The following discussion highlights these hallmark events that occur as time extends beyond a person's last meal. In a normal eating routine, only the fed and postabsorptive states will apply, although prolonged energy deprivation will occur in extreme dieting, involuntary starvation, and certain metabolic diseases.

The Fed State

Figure 7.6 illustrates the distribution of glucose, fat, and amino acids among the major tissues during the fed state, sometimes called the postprandial state. A primary indicator of the fed state is the release of insulin by the β -cells of the pancreas in response to increased blood glucose levels

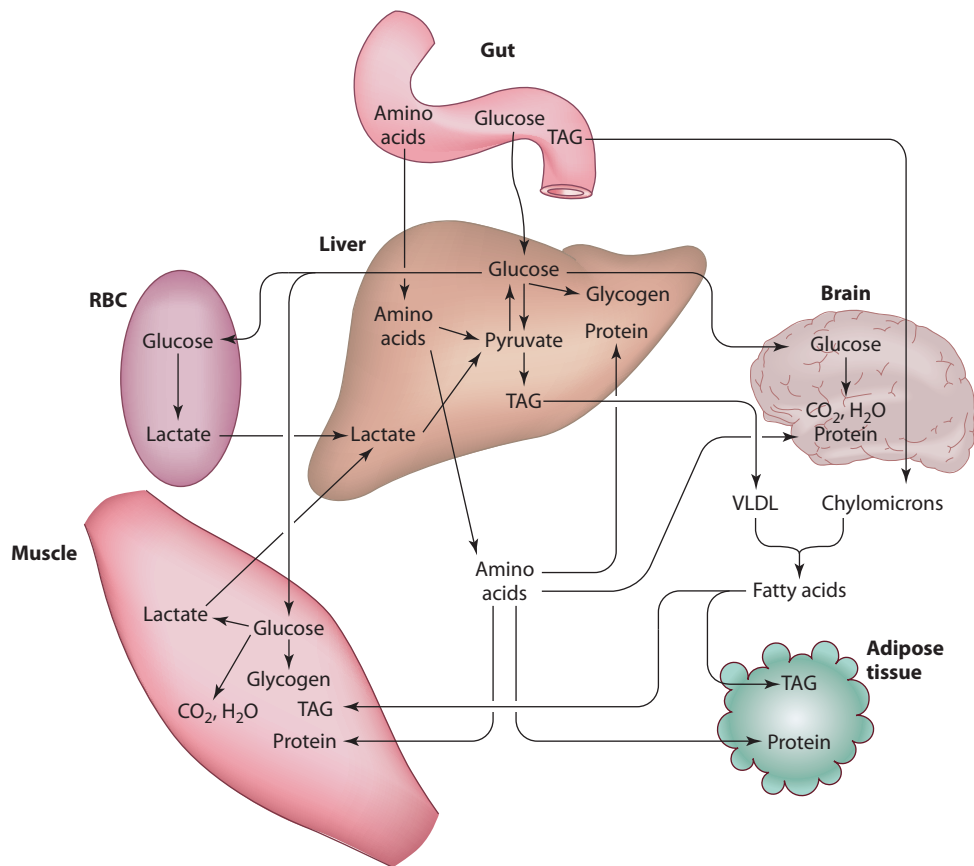


Figure 7.6 Distribution of dietary glucose, amino acids, and triacylglycerols in the fed state. Abbreviations: RBC, red blood cells; TAG, triacylglycerols.

(discussed later in this chapter). The liver is the first tissue to have the opportunity to use dietary glucose. Only some glucose is retained in the hepatocyte on first pass, while about two-thirds passes into the systemic circulation. Glucose that is retained may enter glycolysis or, to a lesser extent, be converted into glycogen. Liver glycogen is preferentially made from newly synthesized glucose. Even in the fed state, when ample dietary glucose is present, gluconeogenesis continues to function because of lactate returning to the liver from glycolysis constantly occurring in red blood cells and, under certain conditions, skeletal muscle (discussed later in this chapter). Red blood cells do not have mitochondria and therefore cannot oxidize fatty acids or glucose aerobically; they can oxidize glucose only anaerobically and produce lactate. The preferential use of gluconeogenic precursors for glycogen synthesis is further encouraged by the low phosphorylating activity (high K_m) of hepatic glucokinase at physiological concentrations of glucose.

Most dietary glucose enters the systemic circulation and is delivered to red blood cells, skeletal muscle, the brain and nervous tissue, adipose tissue, and other tissues of the body. Red blood cells and the brain rely on glucose for energy and have no metabolic mechanisms by which

glucose or fatty acids can be converted to energy stores. These tissues cannot make glycogen or store triacylglycerols. Glucose available to these tissues is oxidized immediately to produce ATP. On the other hand, skeletal muscle can store glucose as glycogen and a moderate amount of fatty acids and triacylglycerols in the fed state. With the exception of red blood cells, all of the tissues included in Figure 7.6 actively catabolize glucose for energy by glycolysis and the TCA cycle.

When available glucose or its gluconeogenic precursors exceed the glycogen storage capacity of the liver, the excess glucose can be converted to fatty acids (and triacylglycerols), as shown in Figure 7.3. The conversion of glucose to fatty acids appears to occur only in the fed state when energy intake exceeds energy expenditure. Chronic overconsumption of carbohydrate can therefore lead to triacylglycerol accumulation in the liver as well as increased secretion of triacylglycerol-rich VLDL into the circulation. The VLDL deliver their lipid cargo to adipose tissue for storage and may contribute to increased body fat. Adipose tissue itself uses glucose as a precursor for both the glycerol and fatty acid components of triacylglycerols, although most triacylglycerols are delivered to adipocytes from circulating lipoproteins.

The Postabsorptive State

With the onset of the postabsorptive state, tissues can no longer derive energy directly from ingested macronutrients, but instead must begin to depend on fuel sources already in the body (Figure 7.7). During the short period of time marking this phase (3–18 hours after eating), hepatic glycogenolysis is the major provider of glucose to

the blood, which transports it to other tissues for use as fuel. When glycogenolysis is occurring, the synthesis of glycogen and triacylglycerols in the liver is diminished, and the *de novo* synthesis of glucose (gluconeogenesis) becomes a more important contributor in maintaining blood glucose levels. Each of these events is controlled largely by glucagon secreted by the pancreas in response to declining blood glucose.

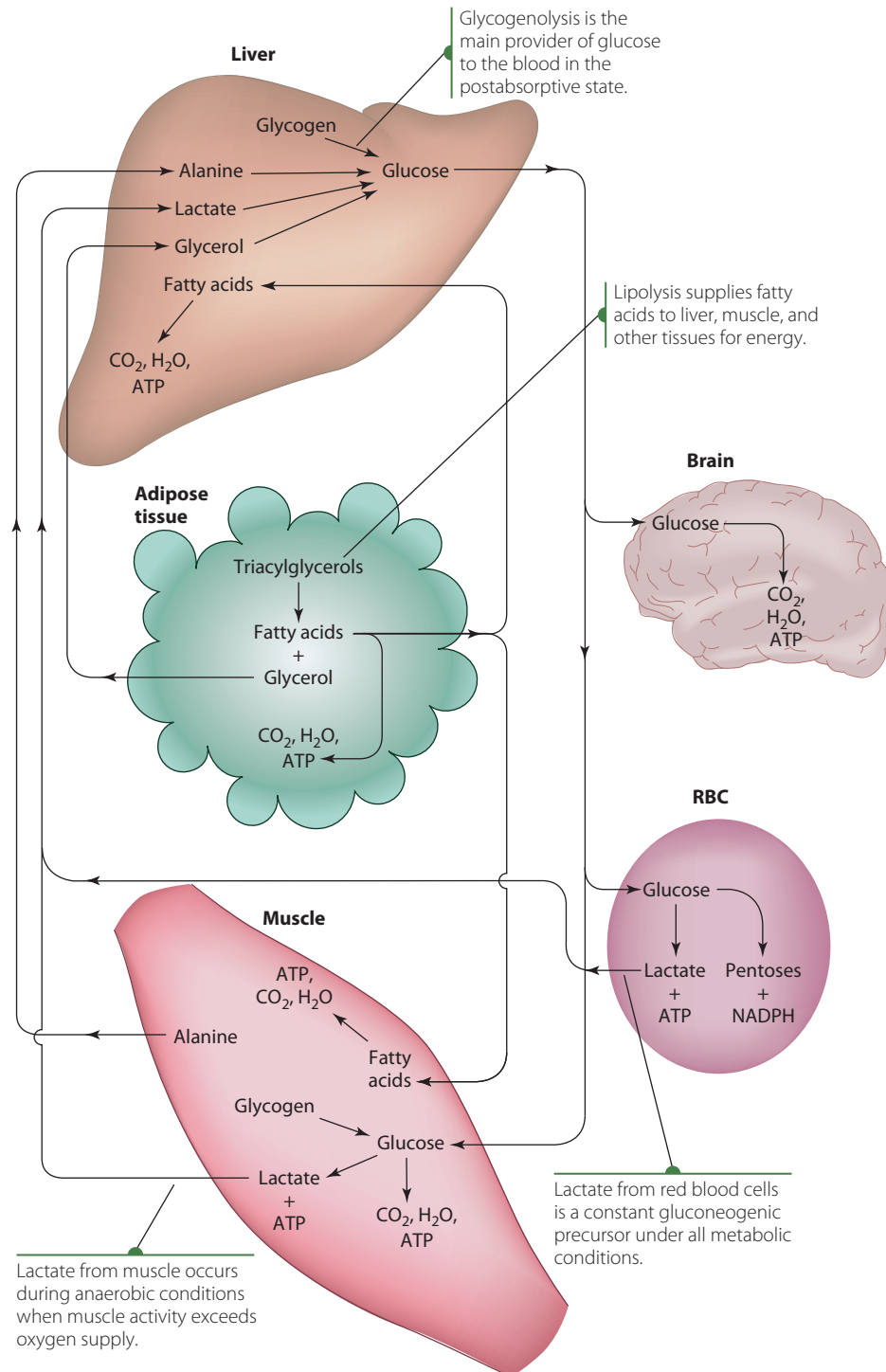


Figure 7.7 Distribution of fuel molecules in the postabsorptive state. Abbreviations: RBC, red blood cells; TAG, triacylglycerols.

Source: Modified from Zakim D, Boyer T, eds, *Hepatology: A Textbook of Liver Disease*. 4th ed. Philadelphia: WB Saunders.

Lactate, formed in and released by red blood cells, is a constant noncarbohydrate carbon source for hepatic gluconeogenesis. Skeletal muscle may also contribute lactate resulting from anaerobic glycolysis. The glucose–alanine cycle, in which alanine returns to the liver from muscle cells, also becomes important (see Figure 6.35). The alanine is then converted to pyruvate by the transfer of the amino group to α -ketoglutarate as the first step in the gluconeogenic conversion of alanine in the liver. Alanine cannot be converted to glucose in skeletal muscle. In the postabsorptive state, glucose provided to the muscle by the liver comes primarily from the recycling of lactate and alanine and, to a lesser extent, from hepatic glycogenolysis. Muscle glycogenolysis provides glucose as fuel only for muscle cells in which the glycogen is stored because muscle lacks the enzyme glucose-6-phosphatase, which converts glucose-6-phosphate to free glucose. Once phosphorylated in the muscle, glucose is trapped there and cannot leave except as lactate or alanine.

The brain is an extravagant consumer of glucose, oxidizing it for energy and releasing no gluconeogenic precursors in return. At rest, the brain uses about 20–25% of the available energy even though it is only about 2% of the body by weight. Mental activity does not increase energy utilization by the brain. The rate of glucose use in the postabsorptive state is greater than the rate of glucose production by gluconeogenesis, and thus the stores of liver glycogen begin to diminish rapidly. In the course of sleeping through the night, nearly all reserves of liver glycogen are depleted.

Fatty acids released from adipose tissues are another valuable source of energy for tissues that can oxidize fatty acids via the TCA cycle. The brain, nerve cells, and red blood cells are unable to use fatty acids, but both cardiac and skeletal muscle are well adapted to using fatty acids. The liver can also oxidize fatty acids for energy in the absence of insulin, which promotes fatty acid synthesis rather than oxidation. The glycerol that results from triacylglycerol hydrolysis in the adipose tissue is released into the circulation and used as a gluconeogenic precursor by the liver. Free glycerol—that which is not phosphorylated or attached to fatty acids—is not used in the adipocyte and is released to the blood. Plasma free glycerol levels have thus been used as an indication of triacylglycerol turnover in adipose tissue.

The Fasting State

The postabsorptive state evolves into the fasting state after 18–48 hours of no food intake. Particularly notable in the liver is the increase in gluconeogenesis that occurs in the wake of hepatic glycogen depletion (Figure 7.8). Amino acids from muscle protein breakdown provide the chief substrates for gluconeogenesis during this time, although the glycerol from lipolysis and the lactate from red blood cells (and skeletal muscle if exercising anaerobically)

continue to provide gluconeogenic precursors. The release of fatty acids from adipose tissues continues to occur during the early fasting state at about the same or slightly higher rate as during the postabsorptive state. This supplies many tissues with fatty acids for ATP production, while the glycerol is converted to glucose in the liver.

The shift to gluconeogenesis using amino acids during the fasting state is mediated by the increased secretion of glucagon and cortisol. Proteins are hydrolyzed in muscle cells at an accelerated rate, providing amino acids for gluconeogenesis. The high rate of breakdown of muscle protein is accompanied by large daily losses of nitrogen through the urine. Of all the amino acids, only leucine and lysine cannot directly contribute to gluconeogenesis because they are ketogenic. However, these two amino acids can nonetheless be used for energy due to their conversion to acetyl-CoA and ketone bodies (acetoacetate and β -hydroxybutyrate), thus providing a source of energy for the brain, heart, and skeletal muscle.

An amino acid of particular significance during the fasting state is alanine, which is involved in the alanine–glucose cycle (see Figure 6.35). During fasting, as muscle protein breaks down to amino acids, the nitrogen from the amino acids is transaminated to α -ketoglutarate (formed in the TCA cycle) to make glutamate. The α -amino group from glutamate is then transaminated to pyruvate (formed from glycolysis) to make alanine. The alanine enters the bloodstream and is transported to the liver, where it again transaminates its amino group to α -ketoglutarate. Alanine is converted to pyruvate, and α -ketoglutarate is converted to glutamate. This cycle serves several functions. It removes the nitrogen from muscle during a period of high proteolysis and transports it to the liver in the form of alanine. This process also transfers the carbon structure of pyruvate to the liver, where it can be made into glucose through gluconeogenesis. The synthesized glucose can be transported back to the muscle and used for energy by that tissue.

Glutamine also plays a central role in transporting and excreting amino acid nitrogen, which is greatly increased during the fasting state. Many tissues, including the brain, form glutamine from glutamate and generate ammonia. In the form of glutamine, the ammonia can then be released from the tissues and carried through the blood to the liver or kidneys for excretion as urea or ammonium ion, respectively. Figure 7.9 gives an overview of organ cooperation and other aspects of amino acid metabolism. See also Chapter 6, “Interorgan ‘Flow’ of Amino Acids and Organ-Specific Metabolism,” for a more detailed discussion of amino acid metabolism, particularly Figure 6.37.

The Starvation State

If the fasting state persists and progresses into a starvation state (often referred to as a long-term fast), a more dramatic metabolic fuel shift occurs, this time in an effort

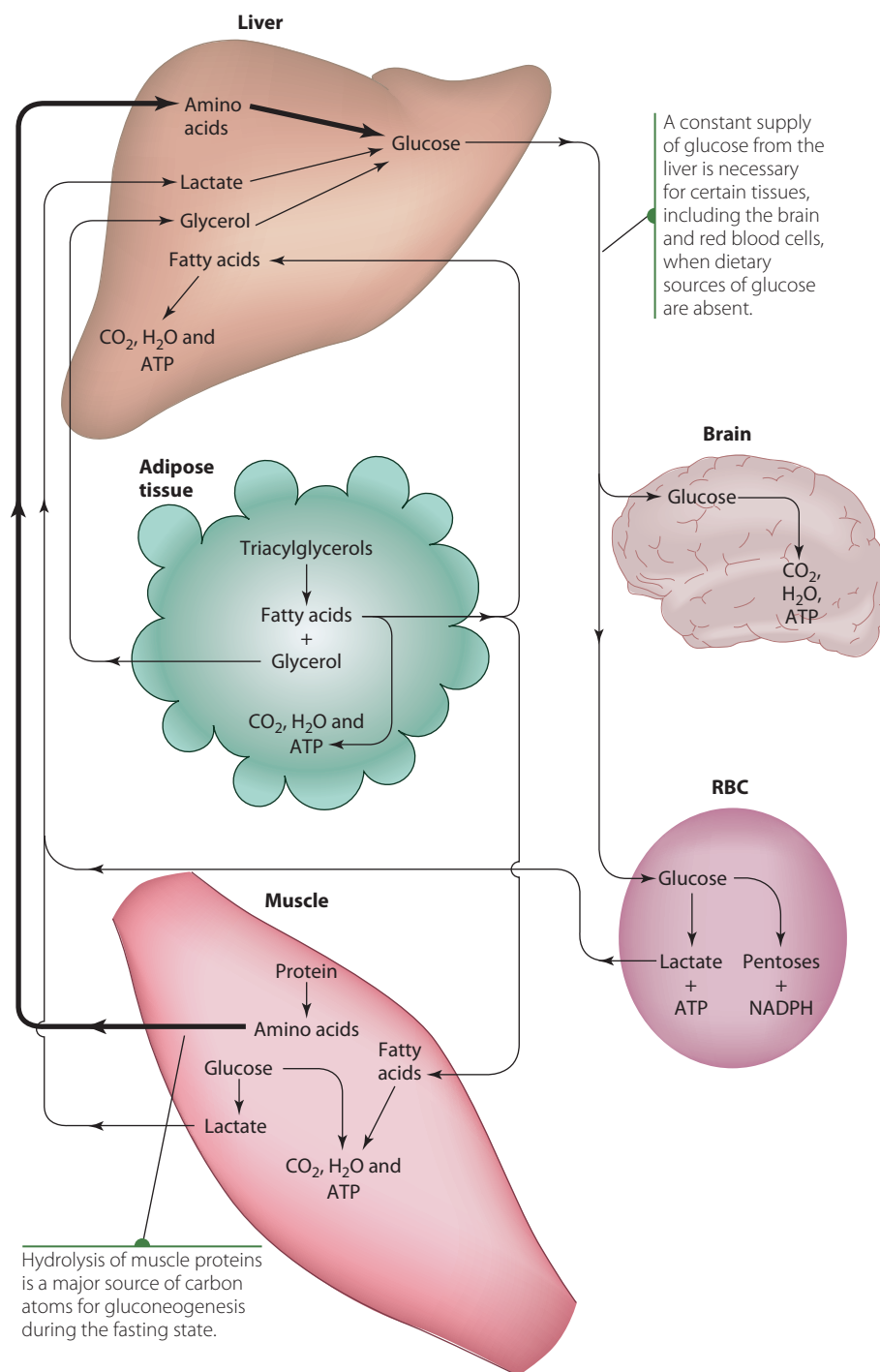


Figure 7.8 Distribution of fuel molecules in the fasting state.

to spare body protein. This new priority is justified by the vital physiological importance of many body proteins such as hemoglobin, which is necessary for the transport of oxygen to tissues. Several important changes in metabolism characteristic of the starvation state occur in order to spare protein: (1) accelerated lipolysis, (2) increased use of fatty acids as fuel in certain tissues, (3) increased use of glycerol for gluconeogenesis, and (4) increased ketone body synthesis and utilization (Figure 7.10).

The protein-sparing shift to lipolysis makes use of the ample triacylglycerol stores in most people. Free fatty acids are released by adipose tissue, becoming the primary fuel for the kidneys, liver, heart, and skeletal muscle. Hydrolysis of triacylglycerols also provides glycerol, now the primary source of carbon atoms for gluconeogenesis in the liver. Lactate is still a gluconeogenic precursor because red blood cells continually produce lactate from glycolysis under all metabolic conditions. And while muscle protein

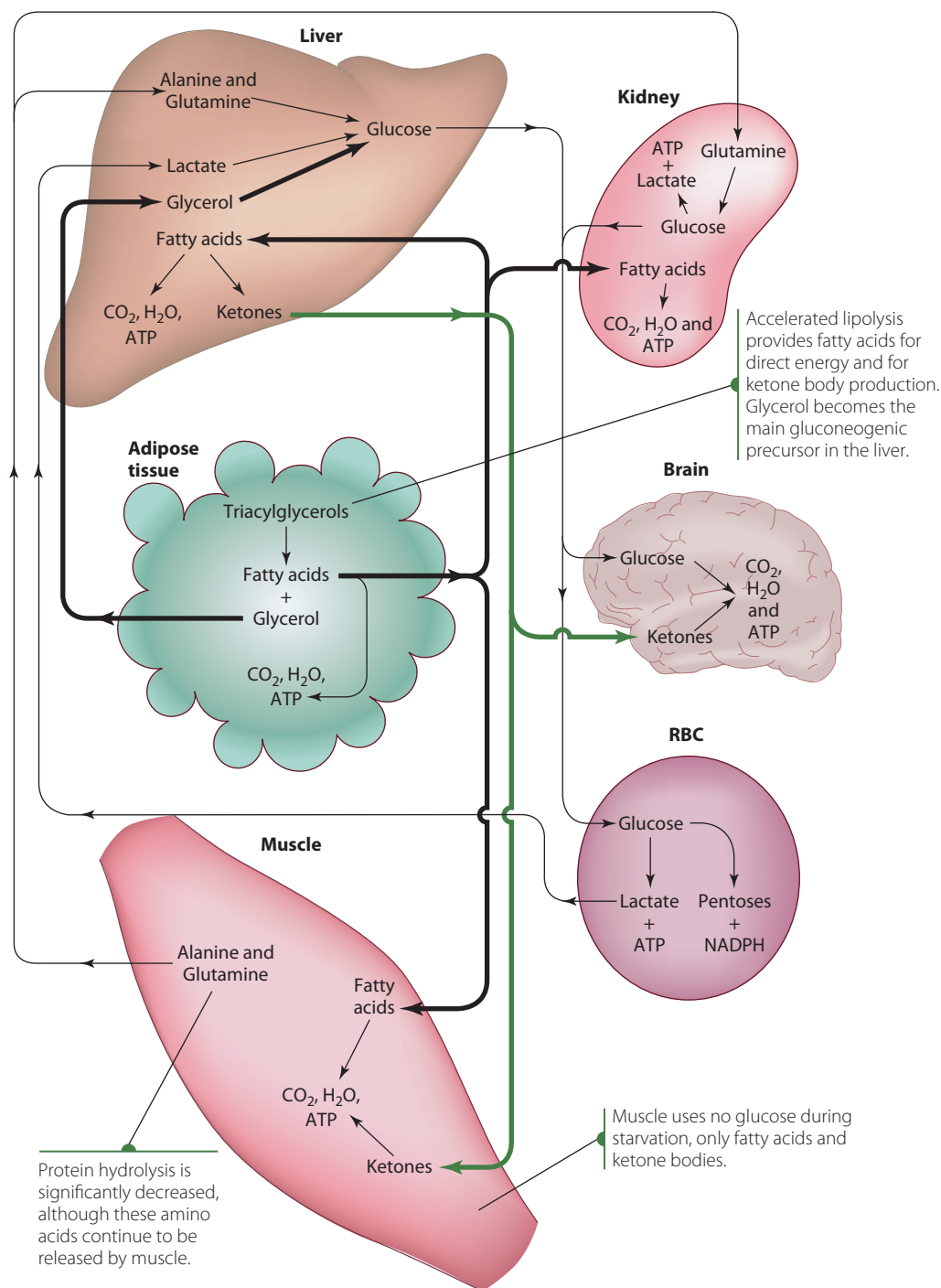


Figure 7.10 Distribution of fuel molecules in the starvation state.

oxidize the ketone bodies instead of glucose via the TCA cycle. In fact, after several weeks of starvation, about two-thirds of the biological fuel for the brain comes from β -hydroxybutyrate and acetoacetate [3]. Table 7.2 shows the dramatic increase in ketone body utilization that occurs during many days of starvation. The kidneys also produce ammonia (NH_3), which helps neutralize the acidity associated with ketone bodies. Furthermore, although

cardiac and skeletal muscle can use fatty acids, they also shift to using ketone bodies when available. These shifts away from using glucose help to conserve the blood glucose for tissues that depend solely on glucose as a fuel source. As long as ketone bodies are maintained at a high concentration by increased lipolysis and hepatic fatty acid oxidation, the need for glucose and gluconeogenesis is reduced, thus sparing valuable protein.

Table 7.2 Fuel Metabolism in Starvation

Fuel Exchanges and Consumption	Amount Formed or Consumed in 24 Hours (g)	
	Day 3	Day 40
Fuel Use by the Brain		
Glucose	100	40
Ketone bodies	50	100
All other use of glucose	50	40
Fuel Mobilization		
Adipose tissue lipolysis	180	180
Muscle protein degradation	75	20
Fuel Output of the Liver		
Glucose	150	80
Ketone bodies	150	150

Source: Adapted from Berg JM, Tymoczko JL, Stryer L. *Biochemistry*. 6th ed. New York: Freeman. 2007. p. 773.

Survival time in starvation depends mostly on the quantity of triacylglycerols stored before starvation. Stored triacylglycerols in the adipose tissue of a person of normal weight and adiposity can provide enough fuel to sustain basal metabolism for about 3 months. A very obese adult probably has enough fat calories stored to endure a fast of more than a year, but physiological damage and even death could result from the accompanying extreme ketoacidosis. When triacylglycerol reserves are gone, the body begins to use essential protein, leading to the loss of liver and muscle function.

To summarize, Figure 7.11 illustrates the changes that occur in plasma concentration of fuel molecules following a single meal. The gradual decrease in glucose during the postabsorptive state stimulates glucagon and inhibits insulin secretion by the pancreas. In normal eating patterns, the postabsorptive phase would be reversed by food consumption and thus prevent the more dramatic changes

that occur with prolonged food deprivation. Continued declines in glucose during fasting and starvation states cause greater secretion of glucagon. Increased lipolysis in adipose tissue and subsequent rise in free fatty acids cause the liver to produce significant amounts of ketone bodies.

7.4 HORMONAL REGULATION OF METABOLISM

The organs of the endocrine system secrete hormones that play a major role in regulating metabolism. Tissues and cells that respond to hormones are called target tissues and cells because they express membrane receptors to which the hormones bind. The act of binding triggers a series of intracellular reactions (referred to as signal transduction) leading to a metabolic response. The metabolic events resulting from insulin binding to its receptor is a classic example of a hormone signaling pathway (see Figure 3.12).

Hormones that control metabolism may be generally categorized as those promoting anabolic reactions and energy storage (insulin) versus those that promote catabolic reactions and energy utilization (glucagon, epinephrine, and cortisol). Some hormones elicit both anabolic and catabolic responses depending on the target tissue (growth hormone). Table 7.3 summarizes the role of key regulatory hormones and the target tissues most affected. Regulatory hormones involved in nutrient digestion and absorption were discussed in Chapter 2 and are not elaborated on further here.

Insulin

Insulin is the major anabolic hormone that impacts glucose, fatty acids, and protein synthesis and storage [4]. It is a protein secreted by the β -cells of the pancreas in response

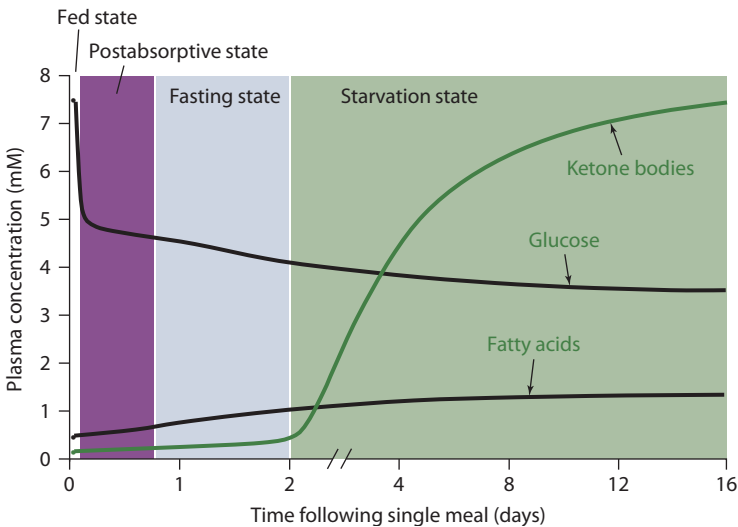


Figure 7.11 Changes in plasma concentration of fuel molecules following a single meal.

Table 7.3 Hormonal Regulation of Energy Metabolism

	Insulin	Glucagon	Epinephrine	Cortisol	Growth hormone	Adiponectin
Gluconeogenesis	↓ Liver	↑ Liver		↑ Liver		↓ Liver
Glycogenolysis		↑ Liver	↑ Liver, skeletal muscle	↑ Liver, skeletal muscle	↑ Liver	↓ Liver
Glycogenesis	↑ Liver, skeletal muscle	↓ Liver	↓ Liver, skeletal muscle			
Glycolysis	↑ Liver, skeletal muscle	↓ Liver	↑ Skeletal muscle			↑ Skeletal muscle
Glucose uptake by GLUT4	↑ Liver, heart, skeletal muscle					↑ Skeletal muscle
Fatty acid oxidation		↑ Liver				↑ Skeletal muscle
Fatty acid synthesis	↑ Adipose tissue, skeletal muscle, liver	↓ Adipose tissue, liver	↓ Adipose tissue, skeletal muscle, liver			
Fatty acid uptake from plasma lipoproteins	↓ Skeletal muscle; ↑ Adipose tissue		↑ Skeletal muscle		↑ Skeletal muscle, liver	↑ Skeletal muscle
Triacylglycerol synthesis	↑ Adipose tissue	↓ Liver				
Triacylglycerol breakdown (lipolysis)	↓ Adipose tissue	↑ Adipose tissue	↑ Adipose tissue, skeletal muscle	↑ Adipose tissue	↑ Adipose tissue	
Protein synthesis (translation)	↑ Skeletal muscle, liver				↑ Skeletal muscle, liver	
Protein breakdown (proteolysis)	↓ Skeletal muscle			↑ Skeletal muscle	↓ Skeletal muscle	
Ketone body production		↑ Liver				

to rising blood glucose and has a half-life in the circulation of 4–6 minutes. The impact of insulin is critical in the fed state when large amounts of blood glucose must be removed to prevent hyperglycemia. Insulin promotes the uptake of glucose into muscle and adipose tissue by stimulating the translocation of GLUT4 from storage vesicles to the cell surface (see Figure 3.12). It also increases glycogen synthesis in the liver and skeletal muscle and inhibits gluconeogenesis in the liver. With regard to controlling blood glucose levels, insulin is a master hormone without peer. Failure of insulin to function properly causes chronic

hyperglycemia and increases the risk of cardiovascular disease (discussed in detail in Chapter 8).

Insulin also stimulates fatty acid synthesis—using excess glucose and fructose as precursors—that leads to increased triacylglycerol assembly for energy storage. Newly synthesized triacylglycerols in the liver are packaged in VLDL and shipped out to adipose tissue. As an anabolic hormone, insulin inhibits lipolysis in adipose tissue and proteolysis in muscle, while promoting protein synthesis in muscle, liver, and many other tissues expressing insulin receptors.

HOW IS TYPE 1 DIABETES SIMILAR TO STARVATION?

Type 1 diabetes is a metabolic condition in which the pancreas produces little or no insulin. As a consequence, GLUT4 is unable to transport blood glucose into muscle and adipose cells for storage and energy utilization. The result is markedly elevated blood glucose levels (hyperglycemia). The inaccessibility of glucose causes the body to utilize triacylglycerol at an accelerated rate. If type 1 diabetes is left untreated,

the primary metabolic responses include increased lipolysis; increased use of fatty acids as fuel in most tissues; increased use of glycerol for gluconeogenesis; and increased ketone body production for energy in tissues that cannot use fatty acids. The same metabolic adjustments occur in starvation.

Both type 1 diabetes and starvation cause increased blood levels of free fatty acids

and ketone bodies. The liver is the site of ketone body production. The liver converts excess acetyl-CoA (from accelerated fatty acid catabolism) into β -hydroxybutyrate and acetoacetate. These ketone bodies are released into the circulation for delivery to tissues that can use them for energy. But in contrast to starvation, type 1 diabetes can cause ketone body production to spiral out of control. Because ketone bodies are weak

(Continued)

acids, they acidify the blood, leading to diabetic ketoacidosis.

The metabolic adjustments due to type 1 diabetes and starvation are similar because cells are deprived of glucose.

But the main difference between type 1 diabetes and starvation is the root cause of glucose deprivation. In starvation, glucose is simply not being ingested. In type 1 diabetes, despite abundant dietary glucose,

muscle and adipose cells lack the ability to transport glucose into the cell. Not surprisingly, a hallmark of type 1 diabetes—but not starvation—is hyperglycemia.

Glucagon

All metabolic effects of glucagon reflect the need to liberate stored energy for ATP production while maintaining blood glucose levels in the absence of dietary carbohydrate. The metabolic responses elicited by glucagon oppose those of insulin. Therefore, it is a prominent hormone in nonfed states and its concentration in the blood increases as starvation approaches.

Glucagon is a protein secreted by the α -cells of the pancreas when blood glucose levels decline and has a half-life in the circulation of 3–6 minutes. The main tissues expressing glucagon receptors are the liver and adipose tissue. In the liver, glucagon causes an increase in gluconeogenesis and glycogenolysis, while inhibiting glycogen synthesis. The main result is that more glucose can be released into the circulation and thus reverse the effects of insulin (Table 7.3). Additional effects include increased lipolysis in adipose tissue for release of free fatty acids into the circulation, with increased fatty acid oxidation and ketone body production in the liver as starvation progresses. Glucagon also stimulates thermogenesis in brown adipose tissues, presumably to maintain body heat during periods of low or no food intake [5].

Skeletal muscle does not make glucagon receptors and is unresponsive to the hormone. The kidneys do have receptors, although the effect of glucagon in the kidney is not well studied. It is possible that glucagon contributes to the increased gluconeogenesis known to occur in the kidney during starvation.

Epinephrine

Epinephrine is a catecholamine produced in the adrenal medulla from the amino acids phenylalanine and tyrosine (see Figure 6.10). It functions both as a neurotransmitter in the nervous system and as a stress hormone in the circulation. Epinephrine has a half-life in the circulation of 1–2 minutes. It binds to two classes of adrenergic receptors on cell membranes, α and β receptors. The receptors function as part of a cAMP signal transduction cascade, an example of which is shown in Figure 1.9. As a stress hormone, epinephrine can increase cardiac muscle contractions and increase vasodilation and blood flow to skeletal muscle and the liver. Epinephrine levels are known to increase during exercise.

The binding of epinephrine to α receptors in the pancreas inhibits insulin secretion; in the liver and skeletal muscle it stimulates glycogen breakdown and inhibits glycogen synthesis; and in skeletal muscle it stimulates glycolysis. Epinephrine binding to β receptors in the pancreas stimulates glucagon secretion; and in adipose tissue and skeletal muscle it stimulates lipolysis and inhibits fatty acid synthesis. Each of these responses leads to increased blood glucose and free fatty acids, allowing stored fuels to be used when dietary sources are insufficient.

Cortisol

Cortisol is a corticosteroid hormone produced in the adrenal cortex from cholesterol (see Figure 5.10). It is released from the adrenal cortex in response to low blood glucose levels and has a half-life of about 1 hour in the circulation. Cortisol travels in the circulation bound to albumin and corticosteroid-binding globulin (also called transcortin). After being delivered to target cells, cortisol passes freely through plasma membranes, then binds to intracellular cortisol receptors residing in the cytosol.

In the liver, cortisol stimulates gluconeogenesis and glycogenolysis. It also increases the activity of glucose-6-phosphatase, thus promoting the release of free glucose into the circulation. In skeletal muscle, cortisol stimulates glycogenolysis and inhibits the translocation of GLUT4 to the cell membrane. Cortisol also stimulates lipolysis in adipose tissue, thus providing free fatty acids for energy use in the liver, kidneys, and cardiac and skeletal muscle. Persistently high levels of cortisol, as seen in prolonged fasting (starvation) and vigorous exercise, stimulate protein breakdown in skeletal muscle so amino acids may be used for gluconeogenesis.

Growth Hormone

Growth hormone (GH), also known as somatotropin, is a protein hormone produced by the anterior pituitary gland. It is transported in the circulation bound to GH-binding protein and has a half-life of 12–16 minutes. GH is secreted in response to a variety of stimuli, including fasting and strenuous exercise. GH receptors are present in the liver, adipose tissue, heart, skeletal muscle, kidney, brain, and pancreas.

In adipose tissue, GH stimulates lipolysis and the release of fatty acids into circulation. This lipolytic action occurs predominantly in the visceral adipose tissue and to a lesser extent in the subcutaneous adipose tissue. It also stimulates lipoprotein lipase in skeletal muscle, thus promoting triacylglycerol uptake from circulating VLDL. This may seem paradoxical in view of increased lipolysis in adipocytes, but GH is tissue-specific and has no effect on lipoprotein lipase in adipose tissue.

In the liver, GH increases triacylglycerol uptake from VLDL by inducing the expression of lipoprotein lipase and hepatic lipase. Once again, the action of GH seems paradoxical since the liver produces VLDL. Apparently increased lipase activity in the liver occurs mainly in starvation as a way to recapture energy from the circulation. GH also functions to conserve protein by inhibiting protein breakdown while stimulating protein synthesis [6].

Adiponectin

Adiponectin is a protein hormone produced primarily by adipose cells. It exists in the plasma as a trimer, hexamer, or higher-order multimer. Because of its multimeric forms, plasma adiponectin has a circulating half-life ranging from about 30 minutes to 1.5 hours. The major target organs are the liver and skeletal muscle, although receptors for adiponectin are found in the hypothalamus, pancreas, smooth muscle, and a variety of other tissues.

In skeletal muscle, adiponectin activates AMP-activated protein kinase (AMPK), thus increasing β -oxidation of fatty acids for energy utilization (Figure 7.2). Adiponectin increases the expression and activity of lipoprotein lipase on the cell surface, which promotes the hydrolysis of VLDL triacylglycerols and uptake of the resulting fatty acids for energy utilization (see Figure 5.20). Adiponectin promotes blood glucose uptake by increasing GLUT4 translocation to the cell surface (see Figure 3.12). In the liver, adiponectin suppresses glycogenolysis and gluconeogenesis, thus helping to maintain blood glucose concentration within the normal range.

Obese individuals, despite having greater fat mass, have low circulating adiponectin concentrations. This observation has led to much research focused on adiponectin as an important molecule linking obesity (and low adiponectin) to several conditions, including elevated glucose, elevated triacylglycerols, and other factors related to metabolic syndrome (discussed further in Chapter 8).

7.5 EXERCISE AND NUTRITION

Movement of the human body requires the contraction of skeletal muscle. Significant amounts of energy may be needed to support muscle function, especially in people who are physically active. Some people may be physically

active due to the nature of their jobs, whereas others engage in exercise—defined as planned, structured physical activity to enhance physical fitness. Whether it be an average person wishing to stay physically fit or an elite athlete, the amount and type (and timing) of nutrient intake can influence health and performance outcomes. Only in recent years has the connection between exercise and nutrition been fully appreciated, which has led to an increase of research on the topic. The following sections address the energy demands of skeletal muscle, the fuel sources available to muscle under different types of exercise, and the special application of sports nutrition.

Muscle Function

Skeletal muscle is composed of striated cells (called myocytes or muscle fibers) that generally extend the length of the muscle. The main proteins in muscle are actin and myosin; upon stimulation, myosin ATPase hydrolyzes ATP that provides the energy for muscle contraction. Muscle fibers also contain myoglobin that can store oxygen to be quickly used when needed. The typical red color of muscle is due to the presence of myoglobin. On the basis of their metabolic characteristics, muscle fibers are classified as type I, type IIa, and type IIx. Type I muscle fibers are also called oxidative, slow-twitch fibers. Type I fibers contain a large number of mitochondria and a relatively high concentration of myoglobin, both features designed to support aerobic metabolism. These fibers are surrounded by more capillaries than other fiber types in order to facilitate oxygen transport. Type I fibers are capable of oxidizing fatty acids and glucose to CO_2 and H_2O via the TCA cycle and oxidative phosphorylation. Because of their reliance on aerobic metabolism, the speed of contraction of type I fibers is considered slow but resistant to fatigue.

In contrast are type IIx fibers, also called glycolytic, fast-twitch fibers. Type IIx fibers have significantly fewer mitochondria and less myoglobin, giving these fibers a white appearance. This type of muscle fiber has increased myosin ATPase and an active glycolytic pathway for rapid ATP replenishment in the absence of oxygen. Type IIx fibers have an increased ability to store glycogen and higher phosphofructokinase activity to support glycolysis.

The metabolic characteristics of type IIa fibers lie between those of types I and IIx fibers, having both glycolytic (fast) and oxidative (slow) function. Type IIa fibers are red in appearance and contain intermediate levels of both mitochondria and myoglobin. They are resistant to fatigue but have relatively high myosin ATPase activity and can contract rapidly when necessary.

The combination of fibers is an important property of skeletal muscle that allows the human body to respond to a variety of physical demands. The presence of fast-twitch fibers allows for a rapid and intense muscle contraction, whereas the presence of slow-twitch, fatigue-resistant

fibers allows for muscular endurance. When a low amount of force is required, muscle contraction involves predominantly type I fibers. Increasing force requirements will recruit progressively more type IIa fibers and lastly type IIx fibers when the greatest force is required. Each muscle in the body exhibits different proportions of fiber types depending on its function. For example, muscles involved in maintaining posture engage in prolonged but relatively low-force contractions and thus have a high proportion of type I fibers. Muscles that engage in rapid or high-force contractions, such as jaw muscles, have a high proportion of type IIa and IIx fibers. The proportion (relative number) of each type of muscle fiber a person has is genetically determined; however, with appropriate exercise training, the metabolic potential of muscle can be influenced by effecting changes in fiber size and its components.

Exercising muscle causes several changes in hormones and other regulatory molecules. Such changes are required to support the increasing energy demands of muscle while simultaneously maintaining blood glucose levels for other tissues that rely on glucose for energy. It is well known that exercise increases the circulating levels of epinephrine, cortisol, and growth hormone. Collectively these regulatory molecules increase gluconeogenesis, glycogenolysis, and lipolysis, thus promoting the release of more glucose and fatty acids into the bloodstream. Most of the fatty acids and some glucose can be taken up by muscle and used for energy, whereas the remaining glucose is intended for the brain and red blood cells. Exercise of higher intensity also activates AMPK, resulting in increased lipolysis and fatty acid oxidation, as well as increased GLUT4 translocation to the muscle cell surface for glucose uptake independent of insulin (discussed earlier in this chapter). Furthermore, physical inactivity is associated with chronic inflammation and related conditions, including atherosclerosis and insulin resistance. Research indicates that exercise, even a single bout of exercise in untrained adults, stimulates the secretion of cytokines from skeletal muscle that promotes the clearance of glucose and lipoproteins from the circulation and may improve insulin sensitivity [7].

An important tool used to measure exercise capacity is the concept of maximum oxygen consumption ($\text{VO}_2 \text{ max}$). As physical work increases, the volume of oxygen taken up by the body also increases. The $\text{VO}_2 \text{ max}$ is defined as the point at which a further increase in the intensity of the exercise no longer results in an increase in the volume of oxygen uptake. $\text{VO}_2 \text{ max}$ is unique for each person and is generally expressed in milliliters of oxygen consumed per kilogram of body mass per minute ($\text{mL} \times \text{kg}^{-1} \times \text{min}^{-1}$). As a person goes from an untrained state to a trained state, the $\text{VO}_2 \text{ max}$ increases. A sedentary (untrained) person may have a $\text{VO}_2 \text{ max}$ of 30 or 40, whereas a trained runner may have a $\text{VO}_2 \text{ max}$ of 80 or 90. Consequently, $\text{VO}_2 \text{ max}$ can be used as a measure of cardiovascular fitness. Another application of $\text{VO}_2 \text{ max}$ is in quantifying the intensity of

exercise. If the $\text{VO}_2 \text{ max}$ for an individual is known, then the intensity of exercise can be expressed as a percentage of $\text{VO}_2 \text{ max}$. When at rest, the relative intensity is $< 10\% \text{ VO}_2 \text{ max}$; exercise intensity during light physical activity such as slow walking might correspond to $25\% \text{ VO}_2 \text{ max}$. Techniques used to measure $\text{VO}_2 \text{ max}$ are discussed in Chapter 8.

Energy Sources in Resting Muscle

Energy utilization by skeletal muscle varies greatly depending on the level of physical activity. Even at rest, skeletal muscle needs a minimum of energy to maintain basal function that includes active transport, replenishing glycogen and triacylglycerol stores, and the continuous synthesis and breakdown (turnover) of proteins. Skeletal muscle, despite comprising nearly half of the body's mass in nonobese people, accounts for only 20–25% of the body's energy use when at rest. These energy needs are met primarily by the oxidation of fatty acids and glucose, although their relative contribution depends on the phase of the fed-fast cycle. Glucose is the preferred fuel source in the fed state when ample glucose is available. Blood glucose is the major source rather than stored glycogen. In the postabsorptive and fasting states, resting skeletal muscle will shift to using fatty acids, as glucose becomes more precious to other tissues. Free fatty acids released from adipose tissues provide the major energy source under these conditions, although resting muscle can also obtain some fatty acids from circulating VLDL via lipoprotein lipase on the cell surface. The shift in fuel sources that occurs in resting muscle is regulated by factors induced by the fed-fast cycle, as previously discussed in this chapter.

Muscle ATP Production during Exercise

Contracting muscle fibers require ATP. Skeletal muscle is unique among the body's tissue because its need for ATP is highly variable and entirely dependent on the intensity and duration of muscle contraction. In order to accommodate such wide-ranging energy demands, skeletal muscle possesses three energy systems that supply ATP:

- the ATP-phosphocreatine system
- the lactic acid system (anaerobic glycolysis)
- the oxidative system (aerobic metabolism).

The ATP-Phosphocreatine System

The ATP-phosphocreatine system is a cooperative system in muscle cells using the high-energy phosphate bond of phosphocreatine to quickly regenerate ATP (see Figure 3.25 and Figure 6.24). When the body is at rest, energy needs of skeletal muscle are fulfilled by glucose and fatty acid oxidation because the low demand for oxygen can easily be met by oxygen exchange in the lungs and by the

oxygen carried to the muscle by the cardiovascular system. At the onset of physical activity, the energy requirement of contracting muscle is initially met by existing ATP. However, stores of ATP in muscle fibers are limited, providing enough energy for only a few seconds of maximal exercise. As ATP levels diminish, they are replenished rapidly by the transfer of high-energy phosphate from phosphocreatine to ADP to regenerate ATP. The muscle fiber concentration of phosphocreatine is only four to five times greater than that of ATP, and therefore most energy furnished by this system is diminished after the first 15–25 seconds of strenuous exercise. As the ATP-phosphocreatine system is exhausted, the lactic acid system (anaerobic glycolysis) increases to produce more ATP. Performance demands of high intensity and short duration such as weightlifting, 100-m sprinting, gymnastics, and various short-duration field events benefit most from the ATP-phosphocreatine and lactic acid systems. Lower-intensity activity may allow skeletal muscle to use the combined ATP-phosphocreatine and lactic acid systems for several minutes.

The Lactic Acid System

This system involves the glycolytic pathway, which anaerobically produces ATP through substrate phosphorylation by the incomplete breakdown of one molecule of glucose into two molecules of lactate in skeletal muscle (see Figure 3.20). The sources of glucose are primarily muscle glycogen and, to a lesser extent, circulating glucose. The system can generate ATP quickly for high-intensity exercise. The rapid rise in cellular AMP resulting from ATP hydrolysis is a strong allosteric stimulator of phosphofructokinase, the most prominent regulatory enzyme in glycolysis (Table 7.1). As pointed out in Chapter 3, the lactic acid system is not efficient from the standpoint of the quantity of ATP produced. However, because the process is so rapid, the relatively small amount of ATP is produced quickly and supplies important energy for a short duration.

The lactate produced by this system rapidly crosses the muscle cell membrane into the bloodstream, from which it can be cleared by other tissues (including the liver) for aerobic production of ATP or gluconeogenesis. If the rate of production of lactate exceeds its rate of clearance, blood lactate accumulates. The quantity of lactate released at the onset of strenuous exercise is low, but when lactate accumulates, it lowers the pH of the blood and is one cause of fatigue. Under such circumstances, exercise cannot continue for long periods.

Muscle fibers engage the lactic acid system to provide a rapid source of energy in the absence of oxygen. When an inadequate supply of oxygen prevents the aerobic system from furnishing sufficient ATP to meet the demands of exercise, the lactic acid system will continue to function for a brief time, resulting in what is called “oxygen debt.” Although the lactic acid system is operative as soon as strenuous exercise begins, it becomes the primary

supplier of energy only after phosphocreatine stores in the muscle are depleted, which occurs after about 15–25 seconds at maximal exercise. As a backup to the ATP-phosphocreatine system, the lactic acid system becomes important in high-intensity anaerobic power events that last from about 20–75 seconds, such as sprints of up to 800 meters and swimming events of 100 or 200 meters. During such events, the anaerobic lactic acid system and the aerobic oxidative system each supply about 50% of the energy at maximal exercise [8].

The Oxidative System

The oxidative system involves the TCA cycle and oxidative phosphorylation to completely catabolize glucose, fatty acids, and some amino acids to CO_2 and H_2O . During the onset of maximal exercise, glucose and fatty acids provide nearly all of the aerobic energy for ATP production. The source of glucose and fatty acids may be drawn from storage in skeletal muscle or it may be taken up from the circulation.

The oxidative system is highly efficient from the standpoint of the quantity of ATP produced. Because oxygen is necessary for the system to function, a person’s level of cardiovascular fitness, as measured by VO_2 max, becomes an important factor in exercise capacity. Contributing factors to VO_2 max include the ability of blood to deliver oxygen, glucose, and fatty acids to exercising muscle; pulmonary ventilation; oxygenation of hemoglobin; and release of oxygen from hemoglobin at the muscle. Inefficiencies in any of these metabolic processes become rate limiting for long-duration exercise at maximal output. The oxidative system is the predominant supplier of energy for forms of exercise lasting longer than 2 or 3 minutes, depending on the intensity of the exercise. Many types of endurance exercise meet these criteria, including distance running, distance swimming, and cross-country skiing.

The contribution of the three energy systems during the first 5 minutes of exercise at maximal output is depicted in Figure 7.12. All systems function at all times, but to varying degrees depending on the intensity and duration of exercise. At the onset, the anaerobic systems (the ATP-phosphocreatine and lactic acid systems) dominate in order to provide “quick energy” for skeletal muscles engaged in immediate bursts of activity. This represents an evolutionary adaptation, called flight or fight, which provides skeletal muscle with readily available ATP to allow an individual to escape danger. The ATP-phosphocreatine system is most important during the first few seconds, followed by the lactic acid system. The oxidative system becomes increasingly more important as exercise duration increases. Not surprisingly, the speeds achieved by runners in 100 m are faster than 800 m simply because the oxidative system requires more time to completely metabolize glucose and fatty acids to CO_2 and H_2O , being fully dependent on the body’s ability to deliver oxygen to muscle cells.

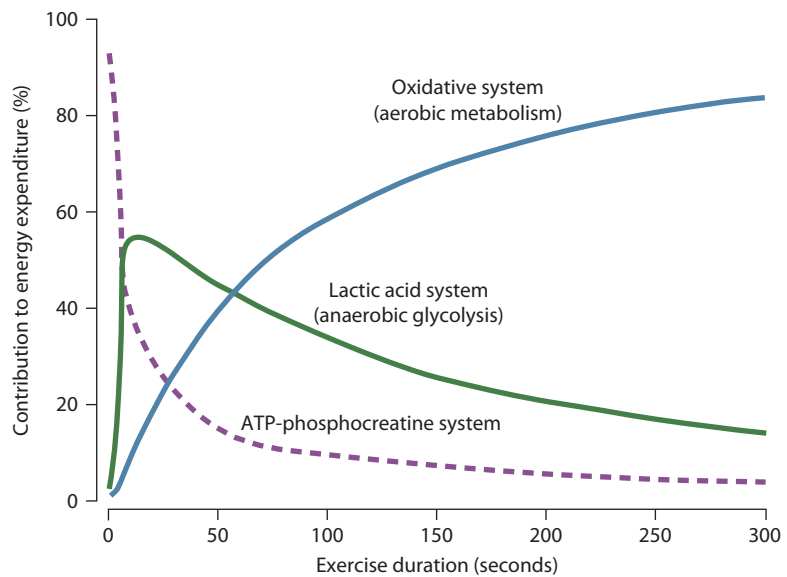


Figure 7.12 Relative contribution of energy systems during the onset of exercise.

Fuel Sources during Exercise

Exercise Intensity and Duration

The intensity and duration of exercise dictates which fuel sources are used. Skeletal muscle utilizes mostly glucose and fatty acids but will use amino acids as the exercise conditions warrant. Recall that resting muscle uses blood glucose as the preferred fuel source in the fed state but shifts to using circulating fatty acids released from adipose tissue in the postabsorptive and fasting states. When normal daily physical activity is light (20–40% VO_2 max), skeletal muscle preferentially uses fatty acids as the primary fuel, much like in the nonfed states when at rest as a way to conserve blood glucose and muscle stores of glycogen and triacylglycerols. Continued energy expenditure at this low activity level causes adipose tissue to release free fatty acids into the circulation. The rate of lipolysis may increase three times the basal rate when at rest. Triacylglycerol synthesis is also inhibited, further promoting fatty acid release into plasma to meet the energy demand while conserving glucose. As illustrated in Figure 7.13, fatty acid utilization at 25% VO_2 max provides 80–90% of total energy expenditure [9].

As exercise intensity increases to 65% VO_2 max, skeletal muscle relies heavily on stored glycogen and triacylglycerols to meet the increased energy demand (Figure 7.13). Plasma fatty acids from the circulation still contribute significant energy, although it is slightly reduced. With the added contribution of muscle triacylglycerols, fatty acids from both sources provide about half of the total energy for skeletal muscles at this level of exercise intensity. Fatty acids are the favored substrates for exercise intensities of up to about 50% VO_2 max. Within the exertion range of 60–75% VO_2 max, fatty acids are typically being oxidized at their maximum rate due to the limited transfer

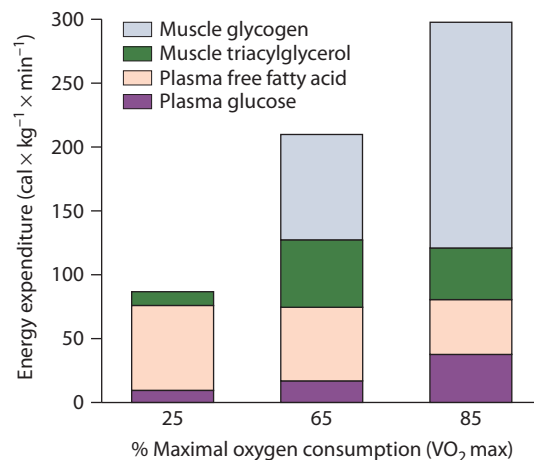


Figure 7.13 Utilization of fuel sources after 30 minutes of exercise at different exercise intensity.

Source: Adapted from Coyle EF. Substrate utilization during exercise in active people. *Am J Clin Nutr.* 1995; 61:968S–79S.

rate of fatty acids into mitochondria, and therefore glucose becomes an important fuel as exercise intensity increases. Muscle glycogen, rather than plasma glucose, becomes the principal fuel at exercise intensity of 65% VO_2 max, which is equivalent to playing basketball or swimming at a vigorous pace. Although plasma glucose utilization does increase somewhat, it is still a minor contributor to total energy expenditure at this level of exercise.

As exercise intensity increases to 85% VO_2 max, the relative contribution of carbohydrate oxidation to total metabolism increases sharply (Figure 7.13). The degradation of muscle glycogen, because of its immediate availability, continues to increase and supplies the majority of fuel at 85% VO_2 max. Exercise intensity at this level is very high, as seen in cross-country skiers and middle-distance runners, and cannot be sustained for prolonged periods

due to the depletion of glycogen. The contribution of plasma glucose increases approximately three to four times compared to low-intensity exercise as a result of increased hepatic glycogenolysis and gluconeogenesis.

Maintenance of blood glucose levels is always a priority, even during high-intensity exercise, and several gluconeogenic substrates are available to the liver. Because the high rate of muscle contraction at 85% VO_2 max requires greater involvement of anaerobic glycolysis, lactate accumulates in muscle when oxygen levels are insufficient for the complete oxidation of pyruvate to CO_2 and H_2O . The lactate is released into the circulation and travels to the liver where it is converted back to pyruvate and used for glucose synthesis. The ability of the liver to convert muscle-derived lactate to glucose, and for muscle to take up that glucose and use it in glycolysis, constitutes the Cori cycle (see Chapter 3). In addition, lipolysis in adipose tissue generates glycerol that the liver converts to glucose. Once released into the blood, the glucose can be taken up by the muscle and used for energy. Finally, skeletal muscle produces alanine during normal metabolism that the liver converts to glucose as part of the alanine–glucose cycle (see Figure 6.35).

The use of fatty acids at an exercise intensity of 85% VO_2 max declines. Fewer fatty acids are released by adipose tissue into the plasma, resulting in a decreased concentration of plasma fatty acids. This decrease occurs despite a continued high rate of lipolysis in adipose tissues, which causes free fatty acids to accumulate in adipocytes. The buildup of fatty acids has been attributed to insufficient blood flow and albumin delivery of fatty acids from adipose tissue into the systemic circulation [10]. The cessation of exercise is followed by a rapid rise in plasma fatty acid levels after adequate blood flow and albumin transport is restored.

The duration of exercise at different levels of intensity also influences the fuel sources used by skeletal muscles. As mentioned earlier, normal daily activities generally represent light work (< 25% VO_2 max), and skeletal muscle preferentially uses fatty acids as the primary fuel. High-intensity exercise at 65% VO_2 max for 30 minutes causes muscle to utilize both glucose (glycogen) and fatty acids (triacylglycerols) stored in muscle, as shown in Figure 7.13. In comparison, Figure 7.14 depicts the changes in fuel sources during prolonged exercise lasting 4 hours at a high intensity of 65–75% VO_2 max. Muscle stores of glycogen and triacylglycerols provide about two-thirds of the energy during the first 30 minutes. After 4 hours of high-intensity exercise, glycogen is completely depleted and half of the muscle triacylglycerol stores are used up. This means that for high-intensity exercise to be sustained for prolonged periods, plasma sources of fuel are critical. Plasma fatty acids released from adipose tissue become the main fuel for high-intensity exercise lasting more than 1 hour, as is typical of marathon runners. Plasma glucose also becomes a critical fuel source, although hepatic gluconeogenesis

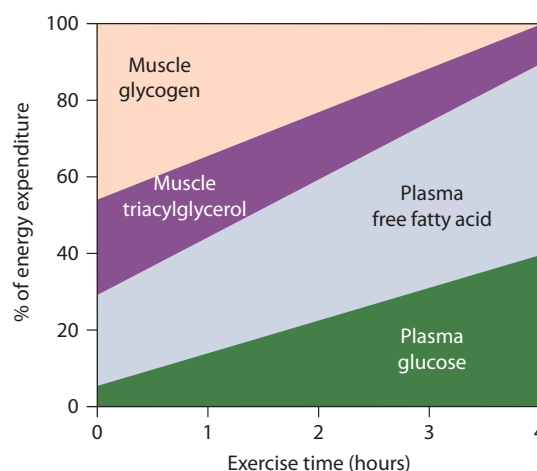


Figure 7.14 Utilization of fuel sources during prolonged exercise at 65–75% VO_2 max. Carbohydrate ingestion is needed after 2 hours to maintain plasma glucose concentration and oxidation.

Source: Adapted from Coyle EF. Substrate utilization during exercise in active people. *Am J Clin Nutr.* 1995; 61:968S–79S

alone cannot keep pace and ingestion of carbohydrates is necessary to sustain high-intensity exercise [9].

Skeletal muscle is limited in its ability to directly use amino acids for energy. However, muscle liberates amino acids as a result of normal protein turnover in the body. Skeletal muscle is a large depot of protein and accounts for 25–35% of all protein turnover, as discussed in detail in Chapter 6. Some of these amino acids can be converted to TCA cycle intermediates (mostly branched-chain amino acids), whereas some can be transported to the liver for gluconeogenesis (mostly alanine and glutamine). Moreover, exercise can affect muscle protein breakdown and synthesis. Endurance exercise tends to increase muscle protein breakdown (possibly through the action of cortisol), while decreasing protein synthesis. Eating protein immediately after endurance exercise can augment protein synthesis. Eating carbohydrate after exercise causes an increase in insulin, which in turn stimulates protein synthesis and inhibits breakdown (see Table 7.3). Resistance training has little effect on protein turnover during exercise, but both protein synthesis and breakdown are accelerated after strenuous resistance training. Based on these observations, it seems logical to consume both protein and carbohydrate to optimize the benefits of resistance training.

Fatigue

Muscle fatigue has a variety of causes, some of which are related to substrate availability. For example, fatigue occurs when the supply of glucose is inadequate, such as with muscle glycogen depletion or hypoglycemia. Thus, the consumption of glucose is necessary in prolonged exercise such as marathon running and may temporarily delay fatigue. As muscle fatigue begins to set in, exercise intensity must be reduced to match the muscle's ability to oxidize

predominantly fatty acids, possibly as low as 30% VO_2 max. The reason for this limitation, and thus the dependence of muscle upon glucose as an energy source, may be based on two factors: (1) oxidation of fatty acids is limited by the enzyme carnitine acyltransferase I (CAT I), which catalyzes the transport of fatty acids across the mitochondrial membrane, and (2) CAT I is known to be inhibited by malonyl-CoA. When availability of glucose to the muscle is high, fatty acid oxidation may be reduced by the inhibition of CAT I by glucose-derived malonyl-CoA [10].

Benefits of Exercise Training

Endurance training increases the ability to perform more aerobically at the same absolute exercise intensity. Several factors aid in this increase. First, endurance-trained muscle exhibits an increase in the number and size of mitochondria. Cardiovascular and lung capacity also increase, and type I muscle hypertrophies. The activity of oxidative enzymes in endurance-trained subjects has been shown to be 100% greater than in untrained subjects at 65% VO_2 max. Endurance training also results in an increased use of fatty acids as an energy source during submaximal exercise. In skeletal muscle, fatty acid oxidation inhibits glucose uptake and glycolysis. For this reason, the trained athlete benefits from the carbohydrate-sparing effect of enhanced fatty acid oxidation during competition because muscle glycogen and plasma glucose are depleted more slowly. This effect largely accounts for the training-induced increase in endurance for prolonged exercise.

Trained athletes tend to have lower plasma fatty acid concentrations and exhibit less adipose tissue lipolysis than untrained counterparts at similar exercise intensity. This finding suggests that the primary source of fatty acids used by the trained athlete is intramuscular triacylglycerol stores, rather than adipocyte triacylglycerols. After exercise, the intramuscular triacylglycerols are replaced, utilizing plasma fatty acids supplied by lipolysis in adipose

tissues. This process can result in a reduction in the size of the adipose tissue.

Endurance training appears to result in an increased capacity for muscle glycogen storage. Therefore, the trained athlete benefits not only from a slower use of muscle glycogen (as explained earlier), but also from the capacity to have higher glycogen stores at the onset of competition. High muscle glycogen levels allow exercise to continue longer at a submaximal workload. Even in the absence of carbohydrate loading (see the following section), a strong positive correlation exists between initial glycogen level and time to exhaustion, level of performance, or both during exercise periods that last more than 1 hour. The correlation does not apply at low levels of exertion (25–35% VO_2 max) or at high levels of exertion for short periods because glycogen depletion is not a limiting factor under these conditions. It has been suggested that the importance of initial muscle glycogen stores is related to the inability of glucose and fatty acids to cross the cell membrane rapidly enough to provide adequate substrate for mitochondrial respiration [9].

Carbohydrate Loading (Supercompensation)

Carbohydrate loading is a dietary and exercise strategy to maximize the storage of glycogen in muscle and liver for the purpose of enhancing endurance performance. Glycogen depletion is strongly associated with muscle fatigue, and carbohydrate loading provides greater energy reserves for individuals engaged in prolonged endurance events such as distance running and cross-country skiing. Figure 7.15 illustrates graphically the amount of muscle glycogen formed as a result of the classical regimen and a modified regimen.

The classical regimen for carbohydrate loading resulted from investigations in the late 1960s by Scandinavian scientists [11]. This regimen involved two sessions of intense exercise to exhaustion to deplete muscle glycogen

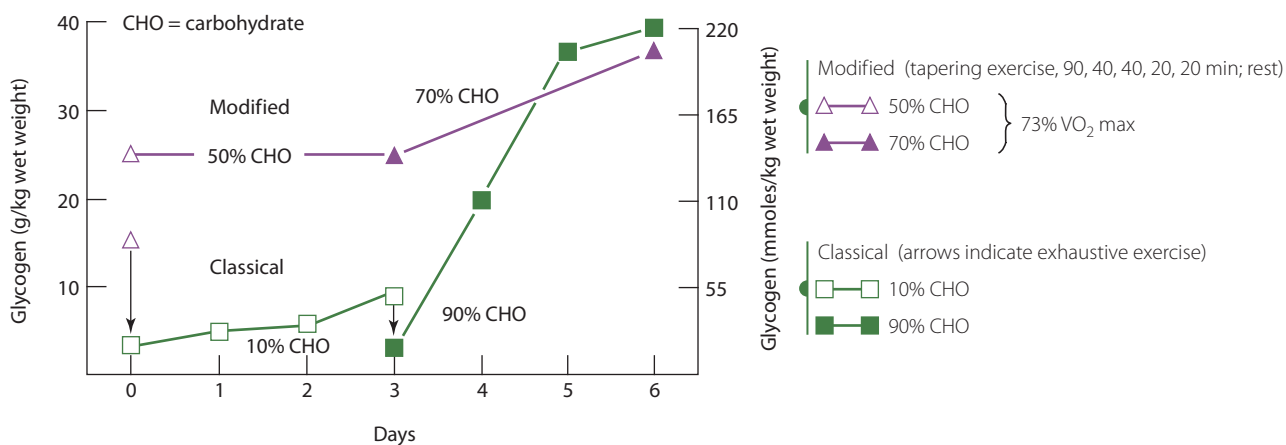


Figure 7.15 Schematic representation of the “classical” and modified regimens of muscle glycogen supercompensation.

Source: From Sherman WM, Carbohydrate, muscle glycogen, and muscle glycogen supercompensation, In Williams MH, *Ergogenic Aids in Sport*. Champaign, IL: Human Kinetics. 1983. p. 14.

stores, separated by 2 days of a low-carbohydrate diet (< 10%) to “starve” the muscle of carbohydrate. This interval was followed by 3 days of a high-carbohydrate diet (> 90%) and rest. The event would be performed on day 7 of the regimen. The classical method yielded muscle glycogen levels approaching 220 mmol/kg wet weight, more than double the athlete’s resting level. However, because of various undesirable side effects of the classical regimen, such as irritability, dizziness, and a diminished exercise capacity, a less stringent regimen of diet and exercise has evolved that produces comparably high muscle glycogen levels. In the modified regimen, runners perform tapered-down exercise sessions over the course of 5 days, followed by 1 day of rest. During this time, 3 days of a 50% carbohydrate diet are followed by 3 days of a 70% carbohydrate diet, generally achieved by consuming large quantities of pasta, rice, or bread.

The modified regimen, which can increase muscle glycogen stores 20–40% above normal, has been shown to be as effective as the classical approach, with fewer adverse side effects [12].

In contrast to carbohydrate loading, emerging research suggests that a low-glycogen approach to endurance training may also enhance performance. The strategy is to deliberately train in conditions of low carbohydrate intake to limit glycogen storage. This promotes adaptations in skeletal muscle that increases mitochondria and improves oxidative capacity, particularly fatty acid oxidation. Then a high-carbohydrate meal is consumed immediately prior to an important competition. Some athletes have claimed success using a “train low, compete high” approach, although strategies that create optimal conditions are unknown and a common protocol has not been established [13].

SUMMARY

- Metabolic pathways are constantly adjusting in response to the energy status of cells, tissues and organs, and the whole body.
- All cells of the body require energy to function. The liver, cardiac and skeletal muscle, kidneys, and adipose tissue can use both glucose and fatty acids for energy. The brain and nerve cells cannot use fatty acids and rely on glucose but can adapt to ketone bodies made from fatty acids during long-term fasting. Red blood cells lack mitochondria and have no oxidative capacity, so they depend solely on glucose and anaerobic glycolysis for energy.
- Humans require frequent input of energy from dietary sources to perform mechanical work, including active transport at the cell level, synthesis of complex molecules, and muscle contractions. Dietary carbohydrates and fats (triacylglycerols) are the primary fuel molecules, although amino acids from dietary protein can also be used for energy when necessary.
- In the fed state, ample energy is consumed in excess of immediate needs, resulting in energy storage as triacylglycerols in adipose tissue and muscle and as glycogen in liver and muscle. The fed state is characterized by high insulin levels that stimulate anabolic reactions by allosteric regulation of key enzymes.
- In the postabsorptive state, insulin diminishes and glucagon increases, which causes the release of stored molecules to provide the energy for cellular function between meals or sleeping through the night.
- Long-term energy deprivation that occurs in starvation can result in severe loss of body fat and muscle mass

as the body sacrifices protein to meet critical energy needs.

- Energy demands during exercise are strongly influenced by the intensity and duration of exercise. Contracting muscles that require an immediate burst of energy depend on ATP and phosphocreatine inherently present in the muscle fibers, then shift to anaerobic glycolysis as required in the first several seconds of maximal activity. Aerobic oxidation of fatty acids and glucose becomes the major source of energy as muscle contractions continue beyond 2 or 3 minutes.
- Skeletal muscle engaged in normal daily activities at low intensity uses primarily fatty acids derived from adipose tissue for energy. As exercise intensity increases, the muscle uses glycogen stores until it is depleted. Skeletal muscle engaged in long-duration, high-intensity exercise becomes increasingly dependent on plasma glucose for energy and continues to use free fatty acids released from adipose tissue.

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Perspective

THE ROLE OF DIETARY SUPPLEMENTS IN SPORTS NUTRITION BY KARSTEN KOEHLER, PhD

At least since the Ancient Olympics, athletes have been exploring possible ways to gain a competitive edge. While the diet provides ample opportunities to maximize performance and recovery during training and competition, it has become an appealing option for many athletes to supplement their diet with isolated nutrients in highly concentrated form. Supplements, particularly those with claimed ergogenic effects, appear as a legal and healthy alternative to performance-enhancing drugs, which are prohibited by the strict anti-doping regulations in competitive sports. Who would not be intrigued by “miracle pills” that promise one to run faster, jump higher, and be stronger, all while being safe and legal?

Considering this appeal, it is not surprising that athletes are much more prone to using supplements than the average population. In fact, depending on the sport and the level of competition, it may be hard to find a single athlete who does not use supplements on a regular basis [1]. Many athletes use supplements for the obvious motive of improving their performance and health, but supplement use is also often done in an attempt to emulate the behavior of opponents and peers [2]. These trends can be observed not only in competitive athletes but also in the world of recreational sports, which is eminent by the ever-increasing market of “sport supplements.” This market has been expanding both in revenue as well as in the number of products on the market. Due to varying product definitions and categorizations, it is difficult to estimate the true size of the market, but it is safe to assume that annual revenue from sport supplements is in the multibillion-dollar range.

However, contrary to popular beliefs about supplements among competitive and recreational athletes, scientific evidence for potential ergogenic effects is rather scarce. In fact, only a few selected

substances are unanimously considered as performance enhancing, and their ergogenic properties are limited to certain sports and activities. Furthermore, for most substances available on the market, research has failed to demonstrate the claimed ergogenic effects or, more importantly, scientifically valid studies on these effects are completely lacking. Despite the lack of concrete evidence, many supplements are heavily advertised using anecdotal reports from athletes or pseudoscientific publications, which makes it difficult for the layperson to tell fact from fiction. Considering that many athletes obtain their supplement knowledge from coaches, athletic trainers, physical therapists, or team physicians (and not from peer-reviewed scientific journals), it is not surprising that most athletes are inadequately educated about the true effects of supplements [1].

SUPPLEMENTS WITH CONFIRMED ERGOGENIC EFFECTS

Only for a handful of substances available as supplements is there sufficient scientific evidence available to confirm performance-enhancing effects: caffeine, creatine, buffering agents, and nitrate-containing, carbohydrate, and protein and amino acid supplements.

Caffeine

Caffeine is probably the most accepted dietary compound used to enhance performance, even in the nonathletic population. Caffeine is available in various forms, including food and beverages as well as supplements and drugs. As an adenosine receptor antagonist, caffeine has numerous central, neuronal, and metabolic effects. The ergogenic properties of caffeine are most likely modulated through neuromuscular effects that include improved neuromuscular coupling, increased recruitment of motor units, and reduced fatigue. To a lesser extent, caffeine may also influence metabolism by

increasing the rate of lipolysis. At doses of approximately 3 mg/kg body weight and higher, caffeine improves endurance exercise performance. Ergogenic effects are likely for other modes of exercise, such as team and racquet sports as well as sports involving prolonged high-intensity exercise, even though scientific data is limited [3]. Despite the well-documented performance-enhancing effects, caffeine is currently not banned by the World Anti-Doping Agency [4]. Possible side effects of caffeine include insomnia, gastrointestinal bleeding, muscle tremor, and coordinative impairments. The diuretic properties of caffeine are minimal during exercise.

Creatine

Creatine, a nonessential nutrient and a component of phosphocreatine involved in intracellular energy storage, is a very popular supplement among strength athletes. In doses of 3 g/d and greater, creatine supplementation is associated with improved contractile performance during high-intensity exercise as well as muscle **hypertrophy**. Furthermore, creatine may also improve intramuscular glycogen storage [5]. For healthy individuals, creatine supplementation is considered safe, even though larger doses of 20–30 g/d, which are frequently endorsed for initial “charging” or “loading” phases, are not recommended [6].

Buffering Agents

During high-intensity exercise, buffering agents may improve performance by increasing fatigue resistance. Extracellular buffers with demonstrated ergogenic properties include sodium bicarbonate, which improves performance during repeated high-intensity interval exercise at doses of 0.3 g/kg and greater [7]. However, sodium bicarbonate supplementation is frequently associated with severe gastrointestinal distress, including nausea and vomiting. Beta-alanine, a precursor

of the intracellular buffer carnosine, has been shown to increase performance and attenuate fatigue during short, high-intensity interval exercise at doses of 4–6 g/day. Potential side effects of beta-alanine supplementation include **paresthesia** [8].

Nitrate-Containing Supplements

The ingestion of nitrate, either from supplementation or through nitrate-rich foods such as beetroot juice, is associated with increased nitric oxide generation and an increase in metabolic efficiency during sub-maximal exercise. However, it remains to be determined whether these effects translate into meaningful improvements in athletic performance, particularly in highly trained individuals [9].

Carbohydrate Supplements

The ingestion of carbohydrates during prolonged aerobic exercise can improve endurance performance, and performance benefits are maximized at intakes of 60–80 g/h when multiple absorbable carbohydrates such as glucose and fructose are utilized. Carbohydrates are obviously not limited to supplements, as many sport-specific products such as beverages, bars, or gels as well as conventional foods can provide similar amounts and types of carbohydrates, and the ergogenic effects appear to be independent of their form of presentation. However, the ingestion of carbohydrates in highly concentrated form has been linked to increased gastrointestinal distress [10].

Protein and Amino Acid Supplements

Protein and amino acids supplements are extremely popular among athletes who wish to increase their muscle mass or strength. It is well established that the ingestion of protein or essential amino acids in association with resistance training can support anabolic adaptations to training. However, there is currently no scientific evidence to suggest that protein or amino acid supplementation is superior to protein from conventional food sources [11].

POPULAR SUPPLEMENTS WITHOUT SCIENTIFIC EVIDENCE OF ERGOGENIC EFFECTS

With a strong desire to improve health and performance, many products have become popular, but without compelling

scientific evidence of their benefit. Among these products are ribose and β -hydroxy- β -methylbutyrate (HMB), which are popular among strength athletes, as well as L-carnitine and medium-chain triglycerides (MCT), which are claimed to improve fatty acid oxidation and promote weight loss. Other popular supplements with mixed or limited findings include sodium citrate, phosphates, quercetin, exotic berries, glutamine, and glucosamine. For many other supplements, sound scientific data is mostly lacking. As new products enter the supplement market almost on a daily basis, a list of supplements without sufficient evidence will always remain incomplete.

CHALLENGES IN THE EVALUATION OF ERGOGENIC EFFECTS

Research addressing the effects of supplements on performance is often complicated by the fact that most studies are exclusively laboratory based and typically employ standardized tasks such as treadmill running, bicycle ergometry, or isometric strength tests to assess physical and physiological measures of performance. While these tests are mostly validated and well accepted, they may not adequately reflect true sports performance in a competitive setting [12]. It has further been questioned whether these laboratory-based tests as well as the statistical approaches employed in laboratory-based research are sufficiently sensitive to detect differences in performance that decide between victory and defeat, which can be as small as hundredths of a second or millimeters. Another caveat is that most research is conducted in moderately trained or untrained subjects, whereas scientific studies explicitly conducted in elite athletes are scarce. For several supplements, including buffering agents and nitrate-containing supplements, the ergogenic properties appear to be more pronounced in untrained or moderately trained individuals. As such, study results may not be transferable across the whole fitness spectrum.

POTENTIAL NEGATIVE EFFECTS OF SUPPLEMENTATION

It has further been questioned whether the use of certain supplements could potentially impair athletic performance. For example, antioxidant supplementation has been shown to attenuate beneficial

effects of exercise training in untrained or moderately trained individuals. However, it remains to be determined whether antioxidant supplementation is similarly detrimental in trained athletes [13].

The consumption of supplements further bears the risk of ingesting substances that are not adequately declared on the label. Despite their form of presentation (i.e., pills, tablets, capsules, or powders), dietary supplements are regulated as food in the United States as well as in many other countries. As such, they are controlled less tightly than pharmaceuticals. There have been numerous cases in which supplements were found to contain substances that were harmful or that represented doping agents. For example, numerous rapid weight loss or muscle gain supplements were found to contain large amounts of prohibited stimulants (e.g., ephedrine, sibutramine) or anabolic steroids [14]. In addition, there have also been findings of supplements containing trace amounts of doping agents, most likely due to cross-contamination during the production process. Even though these minute doses were rarely pharmacologically relevant, they were sufficient to trigger a positive doping test [14]. Consequently, athletes enrolled in antidoping programs must be particularly cautious when using supplements. Several countries have fortunately adopted programs in recent years to better protect athletes from adulterated and contaminated supplements.

SUMMARY

Based on current scientific evidence, there is only a handful of dietary supplements, including caffeine, creatine, buffering agents, and nitrate-containing products, that can improve performance during certain sporting events. In addition, dietary supplements may also serve to improve nutrient intake in situations of special needs, such as during travel or weight loss, as well as in athletes with dietary insensitivities or severe dietary restrictions. Furthermore, supplements may also improve athletic performance through placebo effects. However, the use of supplements may also be associated with adverse events that include physical side effects as well as the unintentional uptake of prohibited substances. Therefore, supplements should only be used following a careful benefit–risk

analysis, and athletes are encouraged to limit their use of supplements to specific situations [12,15].

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ENERGY EXPENDITURE, BODY COMPOSITION, AND HEALTHY WEIGHT

LEARNING OBJECTIVES

- 8.1** Describe how energy expenditure is measured when at rest and during exercise.
- 8.2** Explain the relationships between body weight, body fat, and health.
- 8.3** Describe the advantages of various field and laboratory methods used to measure body composition.
- 8.4** Explain how intestinal microflora participate in regulation of body weight.
- 8.5** Define metabolic syndrome and its diagnosis criteria.
- 8.6** Describe how obesity, type 2 diabetes, and insulin resistance are connected.

ENERGY IS CONSTANTLY BEING USED BY EVERY CELL IN THE BODY. Consequently, humans must consume food on a regular basis to meet energy demands. When the amount of food energy matches energy expenditure over time, a person is in **energy balance**. A person who habitually consumes energy in excess of energy needs is said to be in positive energy balance and will convert the unused energy into triacylglycerols for storage as body fat. The previous chapter discussed how the body automatically adjusts to the daily inconsistencies in energy intake and energy expenditure by redistributing fuel molecules among tissues during the fed-fast cycle and during exercise. Over longer periods of time, however, maintaining whole-body energy balance is largely under external control, influenced by how much we eat and how much we exercise. These controllable factors inevitably form the basis of recommendations and interventions aimed at reducing the prevalence of obesity in the United States and other developed countries. This chapter addresses the common methods used to measure energy expenditure and body composition. The chapter also discusses energy balance, the concept of healthy weight, and the genetic and hormonal factors that regulate appetite and body composition.

8.1 MEASURING ENERGY EXPENDITURE

Techniques for measuring energy expenditure have been important tools for health professionals in developing dietary and exercise strategies for maintaining healthy weight and improving athletic performance. Energy expenditure can be assessed through direct or indirect calorimetry. Another method utilizes doubly labeled water that compares well with calorimetric methods and is considered by many to be a “gold standard” for determining total energy expenditure. These methods have provided data that have been used to develop formulas by which energy expenditure can be quickly calculated based on body weight, height, gender, and age. Each of these methods of assessment is explained in the following sections.

Direct Calorimetry

Recall from Chapter 1 that metabolic processes in the body result in the production of heat. Figure 1.12 illustrates how the metabolic oxidation of a typical fatty acid releases more energy as heat than is captured in ATP molecules. Consequently, energy expenditure can be quantified by measuring heat dissipated by the body. The technique of **direct calorimetry** is highly accurate and includes both sensible heat loss and heat of water vaporization. Although the concept of direct calorimetry is relatively simple, direct measurement of body heat loss is expensive, impractical, cumbersome, and usually rather unpleasant for the subject or subjects involved. Direct calorimetry is seldom used and has been replaced by the indirect methods discussed in the following sections.

Indirect Calorimetry

In addition to heat production, metabolic processes also consume oxygen in a quantifiable manner. Therefore, the heat released by metabolic oxidation can be calculated indirectly by measuring the consumption of oxygen. **Indirect calorimetry** is used most often to assess energy expenditure because the required instrumentation can be portable and, under most conditions, does not interfere with physical activities. The expiration of carbon dioxide is also measured so that the ratio of carbon dioxide produced relative to oxygen consumed (termed the *respiratory quotient*) can be determined. While carbohydrate and fat are the major fuels used in the body, it is recommended

that urinary nitrogen excretion also be measured to account for the contribution of protein oxidation to energy expenditure.

Oxygen consumption and carbon dioxide production are measured using either portable equipment (Figure 8.1) that can be placed on a person, enabling collection and analysis of gases while mobile, or stationary equipment, often referred to as a metabolic cart (Figure 8.2). The relative ease of indirect calorimetry makes it a widely used method in research settings when measured data is desired rather than calculated estimates based on body weight.

The Respiratory Quotient

Measuring gas exchange in indirect calorimetry provides additional information about the fuel sources used in the body. Carbohydrates, fats, and proteins each requires different amounts of O_2 to completely oxidize to CO_2 and water because of primary differences in their chemical structures. Thus, the ratio of CO_2 produced relative to O_2 consumed, called the **respiratory quotient (RQ)**, is characteristic for each fuel source. The RQ for carbohydrate, fat, and protein is 1.0, 0.70, and 0.82, respectively. An RQ value that falls somewhere between the lowest (0.70) and highest (1.0) value indicates a mixture of fuels were used for energy.

Measuring gas exchange over a known period of time provides the necessary data to calculate not only total energy expenditure, but also the relative contribution of fuel sources. It is assumed that no proteins are oxidized for energy during short-duration activity. Over longer periods, the amount of protein being oxidized can be



Figure 8.1 A portable device to measure oxygen consumption and carbon dioxide production. The headgear contains oxygen and carbon dioxide sensors on the left side and the flow sensor on the right side, all tethered to an electronics box (to the left of the headgear) that fits into a small wearable pack.

Photo courtesy of the NASA John H. Glenn Research Center



Courtesy of CareFusion Respiratory Diagnostics

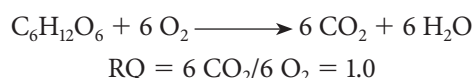
Figure 8.2 A metabolic cart (shown here with bicycle ergometer) measures oxygen consumption and carbon dioxide exhaled.

estimated from the amount of urinary nitrogen excreted, and the remainder of the metabolic energy must be made up of a combination of carbohydrate and fat. Should the principal fuel source shift from mainly fat to carbohydrate, the RQ correspondingly increases, and a shift from carbohydrate to fat lowers the RQ.

Table 8.1 includes the thermal (caloric) equivalents of oxygen consumed at RQ values between 0.70 and 1.0, assuming no contribution of proteins to energy expenditure. The use of RQ to calculate energy expenditure also assumes that gas exchange in the lungs reflects the ratio of oxygen consumption and carbon dioxide production at the cell level.

RQ and Substrate Oxidation

An RQ equal to 1.0 suggests that carbohydrate is being oxidized because the amount of oxygen required for the combustion of glucose equals the amount of carbon dioxide produced, as shown here:



The RQ for fat is <1.0 because fatty acids, compared to carbohydrates, require more oxidation relative to the number of carbon atoms when producing CO_2 and water. For example, a triacylglycerol such as tristearin, shown in the following reaction, requires 163 mol of oxygen and produces 114 mol of carbon dioxide per two tristearin molecules:

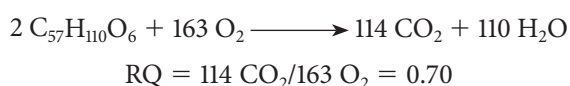
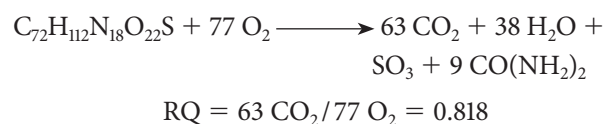


Table 8.1 Thermal Equivalent of O_2 and CO_2 for Nonprotein RQ

Nonprotein RQ	Caloric Value of O_2 (kcal/L)	Caloric Value of CO_2 (kcal/L)	Source of Calories	
			Carbohydrate (%)	Fat (%)
0.707	4.686	6.629	0	100
0.71	4.690	6.606	1.10	98.9
0.72	4.702	6.531	4.76	95.2
0.73	4.714	6.458	8.40	91.6
0.74	4.727	6.388	12.0	88.0
0.75	4.739	6.319	15.6	84.4
0.76	4.751	6.253	19.2	80.8
0.77	4.764	6.187	22.8	77.2
0.78	4.776	6.123	26.3	73.7
0.79	4.788	6.062	29.9	70.1
0.80	4.801	6.001	33.4	66.6
0.81	4.813	5.942	36.9	63.1
0.82	4.825	5.884	40.3	59.7
0.83	4.838	5.829	43.8	56.2
0.84	4.850	5.774	47.2	52.8
0.85	4.862	5.721	50.7	49.3
0.86	4.875	5.669	54.1	45.9
0.87	4.887	5.617	57.5	42.5
0.88	4.899	5.568	60.8	39.2
0.89	4.911	5.519	64.2	35.8
0.90	4.924	5.471	67.5	32.5
0.91	4.936	5.424	70.8	29.2
0.92	4.948	5.378	74.1	25.9
0.93	4.961	5.333	77.4	22.6
0.94	4.973	5.290	80.7	19.3
0.95	4.985	5.247	84.0	16.0
0.96	4.998	5.205	87.2	12.8
0.97	5.010	5.165	90.4	9.58
0.98	5.022	5.124	93.6	6.37
0.99	5.035	5.085	96.8	3.18
1.00	5.047	5.047	100	0

Source: Adapted from McArdle WD, Katch FI, Katch VL, *Exercise Physiology*. 2nd ed. Philadelphia: Lea & Febiger; 1986. p. 127.

Calculating the RQ for protein oxidation is more complicated because metabolic oxidation of amino acids requires removing the nitrogen and some oxygen and carbon as urea, a compound excreted in the urine. Urea nitrogen represents a net loss of energy to the body, and only the remaining carbon structure of the amino acid can be oxidized in the body. The following reaction illustrates the oxidation of a small protein molecule into carbon dioxide, water, sulfur trioxide, and urea:



RQ values of 1.0, 0.70, and 0.82 are the generally accepted values for carbohydrate, fat, and protein, respectively. The RQ for an individual consuming an ordinary mixed diet consisting of all three macronutrients will range between 0.70 and 1.0. But, as mentioned earlier, the main fuels are carbohydrate and fat, with relatively small amounts of protein being oxidized as fuel. An RQ of 0.82, as indicated in Table 8.1, represents the metabolism of a mixture of 40% carbohydrate and 60% fat. RQ values that approach 1.0 indicate a higher contribution of carbohydrate for fuel, whereas an RQ closer to 0.70 indicates more fat being used for fuel. In clinical practice, an $RQ < 0.8$ suggests that a patient may be underfed. An $RQ < 0.7$ suggests starvation; consumption of low-carbohydrate, high-fat, calorie-restricted diets; or alcoholism, due to ethanol having an RQ of 0.67 [1].

Occasionally the RQ value can be greater than 1. For instance, when you hyperventilate you exhale more CO_2 without using more O_2 , resulting in an RQ greater than 1. This may also occur when the body is in acidosis, such as following exhaustive exercise when lactic acid builds up. NaHCO_2 neutralizes the lactic acid to form sodium lactate and carbonic acid (H_2CO_3). The carbonic acid is converted to CO_2 and H_2O and exhaled, which results in a loss of CO_2 that is not related to oxygen uptake.

RQ and Energy Expenditure

Once the RQ has been computed from oxygen and carbon dioxide exchange, the calculation of energy expenditure is performed using the established caloric value of oxygen at different RQ values, as shown in Table 8.1. Calculating energy expenditure from RQ is common practice for determining basal metabolic rate (BMR).

For example, if under standard conditions for determining BMR a person consumed 15.7 L of oxygen per hour and expired 12.0 L of carbon dioxide, the RQ would be $12.0/15.7$, or 0.7643. From Table 8.1, the caloric equivalent of 1 L of oxygen at an RQ of 0.76 is 4.751 kcal. Based on the caloric equivalent for oxygen, calories produced per hour are 15.7×4.751 , or 74.6 kcal. If we use 75 kcal/h as the caloric expenditure under basal conditions, the basal energy expenditure for the day would be $75 \text{ kcal/h} \times 24 \text{ h}$, or about 1,800 kcal/day. At an RQ of 0.76, fat is supplying almost 81% of energy expended (Table 8.1).

Because under ordinary circumstances the contribution of protein to energy metabolism is so small, the oxidation of protein is ignored in the determination of the so-called nonprotein RQ. If a truly accurate RQ is required, a minimal correction can be made by measuring the amount of urinary nitrogen excreted over a specified time period. For every 1 g of nitrogen excreted, about 6 L of oxygen are consumed and 4.8 L of carbon dioxide are produced. The amount of oxygen and carbon dioxide exchanged in the release of energy from protein can then be subtracted from the total amount of measured gaseous exchange.

Measurement of the energy expended in various physical activities has also been made primarily through indirect calorimetry. The method for measuring gas exchange, however, differs slightly from that used for determining BMR. The subject performing the activity for which energy expenditure is being determined inhales ambient air, which has a constant composition of 20.93% oxygen, 0.03% carbon dioxide, and 78.04% nitrogen. Air exhaled by the subject is collected in a spirometer (a device used to measure respiratory gases) and is analyzed to determine how much less oxygen and how much more carbon dioxide it contains compared with ambient air. The difference in the composition of the inhaled air and the exhaled air reflects the energy release from the body. A lightweight portable spirometer (Figure 8.1) can be worn during the performance of almost any sort of activity, and thus freedom of movement outside the laboratory is possible. In many laboratories and hospitals, gas exchange is measured using a so-called metabolic cart (Figure 8.2).

Doubly Labeled Water

The doubly labeled water method also enables assessment of total energy expenditure. $^2\text{H}_2$ (deuterium) and $^{18}\text{O}_2$ are stable isotopes of hydrogen and oxygen, respectively. In this technique, stable isotopes of water are given as H_2^{18}O and as $^2\text{H}_2\text{O}$ (or as $^2\text{H}_2^{18}\text{O}_2$). The isotopes equilibrate throughout the water compartments in the body over about 5 hours. The labeled hydrogen can leave the body as water ($^2\text{H}_2\text{O}$) in sweat, urine, and pulmonary water vapor, while the labeled oxygen can leave the body as either labeled water (H_2^{18}O) or C^{18}O_2 . The disappearance of the H_2^{18}O and $^2\text{H}_2\text{O}$ is measured in the blood and urine for about 3 weeks. The disappearance of the H_2^{18}O is representative of the flux of water (i.e., water turnover) and of the production rate of carbon dioxide. Because the $^2\text{H}_2$ can be excreted only as H_2O , the disappearance of the $^2\text{H}_2\text{O}$ represents water turnover alone. Thus, the difference between the disappearance rate of H_2^{18}O and that of $^2\text{H}_2\text{O}$ corresponds to the production rate of carbon dioxide.

The CO_2 production rate is then used to calculate energy expenditure. However, an RQ is needed to determine the caloric value of CO_2 using Table 8.1. Rather than measuring gas exchange, which would defeat the unrestricted character of the doubly labeled water technique, food records are kept throughout the testing period to estimate the metabolic fuel mix from dietary intake. In subjects maintaining body weight, the recorded *food quotient* is equal to the respiratory quotient and can act as a surrogate RQ [2]. Use of the doubly labeled water method to assess total energy expenditure in free-living individuals produces accurate results that correlate well with those of indirect calorimetry. One source of potential error lies with the use of food records, which requires attention to detail and knowledge of portion size to improve accuracy.

HOW TO MEASURE WHAT PEOPLE EAT

Assessment of food intake can be accomplished by direct or indirect methods. Direct methods require the collection of data from individual subjects, whereas indirect methods assess food consumption trends of a population or group of people. The Perspective at the end of

Chapter 3 highlights an example of indirect methods.

Direct methods are frequently used in research, each having strengths and limitations. The accuracy of direct methods depends on the ability of subjects to know the foods they are eating (including

portion size) and to be truthful. And even with the highest level of cooperation, the act of collecting data by direct methods may change a subject's eating behavior. The following direct methods are common research tools used to measure what people eat.

Food Frequency Questionnaire

Strengths	Limitations
• Can be self-administered without prior training • Machine readable • Relatively inexpensive • Ideal for large sample sizes • May be more representative of usual intake than diet records or recalls	• Incomplete list of food choices and portion sizes • Many foods grouped with single listings • May not be culturally sensitive • Depends on ability of respondent to describe diet • Not appropriate for determining absolute (true) nutrient intake in large survey studies

24-Hour Recall

Strengths	Limitations
• Inexpensive • Easy and quick to administer • Requires only short-term memory • Does not alter usual diet • Does not require food diary • Multiple recalls may be used	• One-day recall is often a poor indicator of an individual's usual food consumption • Respondents may withhold or alter information • Relies on memory • Data entry can be laborious and difficult

Food Record or Diary

Strengths	Limitations
• Not dependent on memory • Provides detailed food intake data • Includes information about eating habits and lifestyle • Multiple-day food records more representative of usual intake	• Requires high degree of cooperation and willingness to maintain accurate food record • Requires literate participants • Act of recording may alter diet • Requires training for estimating portion size • Low response rate with large survey studies • Data collection is time-consuming • Data analysis is laborious and expensive

Diet History

Strengths	Limitations
• Assesses usual food intake over extended period of time • Utilizes the advantages of both the 24-hour recall and food record • Assesses other lifestyle habits • Detects seasonal changes	• Lengthy interview process • Requires highly trained interviewers • Difficult to maintain consistency among interviewers • Data collection and analysis are expensive and time-consuming • Requires high degree of cooperation of respondent

8.2 COMPONENTS OF ENERGY EXPENDITURE

Daily total energy expenditure is attributable to three primary components: basal metabolic rate, physical activity, and the thermic effect of food. A fourth component, **thermoregulation**, is sometimes included. The average contribution of energy expenditure among the components is illustrated in Figure 8.3 and is discussed in the following sections.

Basal and Resting Metabolic Rate

Basal metabolic rate (BMR) represents the amount of energy needed to sustain basic life processes such as respiration, heartbeat, renal function, brain and nerve function, blood circulation, active transport, and synthesis of proteins and other complex molecules. Basal metabolism accounts for the majority of energy expenditure in the human body (Figure 8.3). Most of the energy used at rest is attributed to the liver (27%), brain (19%), kidneys (10%), heart (7%), and skeletal muscle (18%), which even at rest require appreciable amounts of energy for protein synthesis and normal cellular function.

Several factors can affect basal metabolism, including body composition and surface area, age and gender, pregnancy and lactation, environmental temperature, and dietary energy restriction. Many of these factors can be attributed to the amount and proportion of lean body mass, which has higher metabolic activity than adipose tissue. People with greater body weight because of increased lean body mass have a higher BMR. In aging, fat mass increases at the expense of fat-free mass, causing BMR to decrease. Women generally have a higher proportion of body fat relative to fat-free mass and, consequently, have a lower BMR than men of the same age, height, and total

body weight. Tall, thin people have more surface area relative to volume, which is associated with greater heat loss and higher BMR. Cold environments can increase BMR due to shivering, which generates internal body heat. Paradoxically, hot environments can also increase BMR, possibly due to increased blood circulation and sweat gland activity. BMR increases during pregnancy and when lactating. BMR decreases during starvation due to the loss of lean body mass. BMR also decreases with aging due to reductions in some body organ functions and mass.

BMR is assessed indirectly by measuring oxygen consumption under carefully controlled conditions that eliminates any contribution of energy expenditure due to physical activity, thermic effect of food, or heat production that occurs in cold environments. BMR is measured when awake and in a postabsorptive state between 12 and 18 hours following food intake, preferably in the morning shortly after waking from sleep. A person must be completely relaxed in a supine position for at least 30 minutes in a thermoneutral environment. Any factors that could influence the person's internal work are minimized as much as possible. Oxygen consumption (recorded as mL per minute) is then measured for at least 10 minutes. The next step is to convert the rate of oxygen consumption into energy expenditure, based on the principle that the oxidation of carbohydrate, fat, and protein yields approximately 5 kcal of energy per liter of oxygen consumed. BMR is often expressed as daily energy expenditure (kcal/day) and, accordingly, is called *basal energy expenditure*.

Measuring BMR accurately requires strictly controlled laboratory conditions, making it difficult to obtain in most people. As an alternative, resting metabolic rate (RMR) is more easily measured and can provide information that is nearly the same as BMR, albeit slightly higher. To measure RMR, an individual needs to fast only 3–4 hours, much less than the more stringent fasting time required for BMR. Resting comfortably just prior to recording oxygen consumption is required, but it is not necessary to conduct the measurements just after waking in the morning. RMR is usually about 10% higher than BMR because of its less stringent conditions of measurement. The term *resting energy expenditure* is used when RMR is converted to daily energy expenditure (kcal/day).

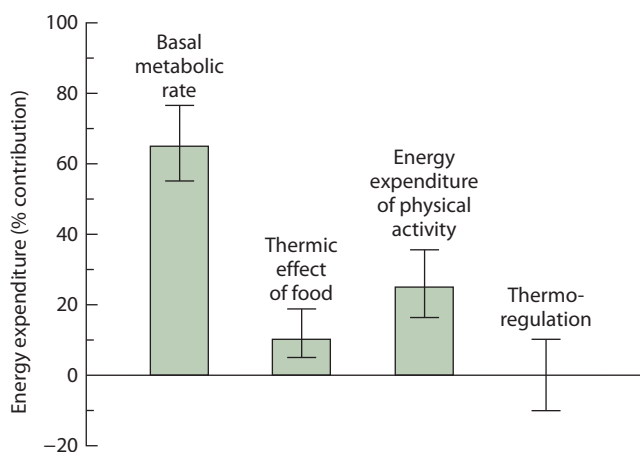


Figure 8.3 Components of energy expenditure and their approximate percentage contribution.

Predictive Equations for RMR

Several equations have been developed that accurately estimate RMR based on body weight, height, age, and gender. These equations do not require specialized equipment or the expertise needed to conduct calorimetric measurements. Many equations have been developed over the past century, although only a few are commonly used today. In general, predictive equations are convenient and yield reasonably accurate estimates of RMR in a variety of populations. However, predictive equations are more variable in older people and tend to overestimate RMR in people with

excess body fat. The following describes a few commonly used equations for adult men and women based on body weight in kilograms (W), height in centimeters (H), and age in years (A).

Harris-Benedict Equations Based on indirect calorimetry, the equations were developed by Harris and Benedict in 1919 using mostly normal-weight white men and women [3]. The Harris-Benedict equations have undergone extensive validation and found to yield reasonably accurate results in nonobese individuals, but the equations overestimate RMR in obese individuals. Separate equations are used for men and women:

$$\text{Men: RMR, kcal/day} = 66.5 + (13.7 \times W) + (5.0 \times H) - (6.8 \times A)$$

$$\text{Women: RMR, kcal/day} = 665 + (9.56 \times W) + (1.85 \times H) - (4.7 \times A)$$

Mifflin-St. Jeor Equations Published in 1990, the Mifflin-St. Jeor equations were developed using indirect calorimetry in normal-weight, overweight, obese, and severely obese individuals to improve the accuracy of RMR measurements in people with excess body fat [4]. The Mifflin-St. Jeor equations are used frequently in clinical settings and can accurately predict RMR within 10% of that measured by indirect calorimetry in both nonobese and obese adults [5]. As with Harris-Benedict estimates, separate Mifflin-St. Jeor equations are used for men and women and require body weight, height, and age as data inputs:

$$\text{Men: RMR, kcal/day} = (9.99 \times W) + (6.25 \times H) - (4.92 \times A) + 5$$

$$\text{Women: RMR, kcal/day} = (9.99 \times W) + (6.25 \times H) - (4.92 \times A) - 161$$

A female who is 35 years old, weighs 125 lb (56.8 kg), and is 5 feet, 5 inches tall (165.1 cm) would have a RMR of 1,339 kcal/day using the Harris-Benedict equation and an RMR of 1,266 kcal/day using the Mifflin-St. Jeor equation.

Weight-Only Equations Several predictive equations based only on body weight have been developed from indirect calorimetry data [6,7]. These equations are less accurate, but work reasonably well when information about height or age are unavailable. Perhaps the most frequently used weight-only equation is not based on established gas exchange methodology, but rather on the principle that BMR (represented by heat production) is correlated with body mass of most vertebrate animal species. The resulting equation for humans is specific for BMR and is written: $\text{BMR, kcal/day} = 70 \times W^{0.75}$.

Using the same 125-lb (56.8-kg) female as an example, her estimated BMR is calculated to be $70 \times 56.8^{0.75} = 1,448$ kcal/day.

Energy Expenditure of Physical Activity

Skeletal muscle requires significant amounts of energy when physically active. Muscle is also involved in maintaining posture when awake, which requires energy in less obvious ways. The energy expenditure of physical activity is highly variable depending on an individual's activity level. Physical activity typically accounts for about 15–30% of total energy expenditure, but it can be considerably less in a truly sedentary person or much more in a very active person. During physical activity that engages large muscles, energy expenditure can greatly exceed RMR at least for a short time. Such high rates of energy usage cannot be sustained, so the daily average of energy expenditure due to physical activity is usually less than RMR in most people. Physical activity includes all exercise and nonexercise activities associated with daily living.

Quantifying the energy expenditure of physical activity requires measuring RMR (or BMR) and total energy expenditure, then calculating the difference. This can be achieved in a clinical setting by measuring gas exchange (oxygen consumed and carbon dioxide expired) or by predictive equations. Alternatively, practitioners can simply estimate the contribution of physical activity to total energy expenditure by multiplying RMR by a factor that approximates the additional energy usage by skeletal muscle [8]. The multiplication factors—called the *physical activity level (PAL)*—are categorized into four different levels, as described in Table 8.2. For comparison, Table 8.2 also shows the number of miles a person would need to walk per day to match each PAL category.

Once again using the 125-lb female as an example, we start with her RMR of 1,266 kcal/day that was calculated using the Mifflin-St. Jeor equation. We know she works at a home improvement store and walks throughout the day, putting her in the “active” PAL category. Therefore, the combined energy expenditure attributed to RMR and physical activity is $1,266 \times 1.75 = 2,216$ kcal/day.

Another way of estimating energy expended during physical activity is the use of data tables in which the amount of energy expended has previously been determined for a variety of activities. Table 8.3, an example of such a table, indicates the amount of energy (kcal) expended per minute per body weight. This table incorporates the basal energy expenditure, whereas some tables provide data for only physical activity. To calculate the energy expended for a given activity, multiply the kcal by your body weight and then by the number of minutes spent performing the activity. Note that every activity performed during a 24-hour period (and possibly for several days) would need to be recorded if total daily energy expenditure is desired.

Table 8.2 Physical Activity Level (PAL) Categories and Walking Equivalence

PAL Category	PAL Range	PAL Average	Walking Equivalence (miles/day at 3–4 mph)		
			Light-Weight Individual (44 kg or 97 lb)	Middle-Weight Individual (70 kg or 154 lb)	Heavy-Weight Individual (120 kg or 264 lb)
Sedentary	1.00–1.39	1.25	~ 0	~ 0	~ 0
Low active	1.40–1.59	1.50	2.9	2.2	1.5
Active	1.60–1.89	1.75	9.9	7.3	5.3
Very active	1.90–2.49	2.20	22.5	16.7	12.3

Source: Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academies Press. 2005.

Table 8.3 Energy Expended on Various Activities

The values listed in this table reflect both the energy expended in physical activity and the amount used for BMR. To calculate kcal spent per minute of activity for your own body weight, multiply ($\text{kcal} \times \text{lb}^{-1} \times \text{min}^{-1}$) or ($\text{kcal} \times \text{kg}^{-1} \times \text{min}^{-1}$) by your exact weight and then multiply by the number of minutes spent in the activity. For example, if you weigh 142 pounds, and you want to know how many kcal you spent doing 30 minutes of vigorous aerobic dance: $0.062 \times 142 = 8.8$ kcal per minute; 8.8×30 minutes = 264 total kcal spent.

Activity	$\text{kcal} \times \text{lb}^{-1} \times \text{min}^{-1}$	$\text{kcal} \times \text{kg}^{-1} \times \text{min}^{-1}$	Activity	$\text{kcal} \times \text{lb}^{-1} \times \text{min}^{-1}$	$\text{kcal} \times \text{kg}^{-1} \times \text{min}^{-1}$
Aerobic dance (vigorous)	.062	.136	Soccer (vigorous)	.097	.213
Basketball (vigorous, full court)	.097	.213	Studying	.011	.024
Bicycling			Swimming		
13 mph	.045	.099	20 yd/min	.032	.070
15 mph	.049	.108	45 yd/min	.058	.128
17 mph	.057	.125	50 yd/min	.070	.154
19 mph	.076	.167	Table tennis (skilled)	.045	.099
21 mph	.090	.198	Tennis (beginner)	.032	.070
23 mph	.109	.240	Vacuuming and other household tasks	.030	.066
25 mph	.139	.306	Walking (brisk pace)		
Canoeing, flat water, moderate pace	.045	.099	3.5 mph	.035	.077
Cross-country skiing, 8 mph	.104	.229	4.5 mph	.048	.106
Gardening	.045	.099	Weightlifting		
Golf (carrying clubs)	.045	.099	Light-to-moderate effort	.024	.053
Handball	.078	.172	Vigorous effort	.048	.106
Horseback riding (trot)	.052	.114	Wheelchair basketball	.084	.185
Rowing (vigorous)	.097	.213	Wheeling self in wheelchair	.030	.066
Running			Wii games		
5 mph	.061	.134	Bowling	.021	.046
6 mph	.074	.163	Boxing	.021	.047
7.5 mph	.094	.207	Tennis	.022	.048
9 mph	.103	.227			
10 mph	.114	.251			
11 mph	.131	.288			

Source: Rolfes, Pinna, Whitney, *Understanding Normal and Clinical Nutrition*, 9/e.

Thermic Effect of Food

A third component of energy expenditure is the thermic effect of food. This represents the metabolic response to food and is also called diet-induced thermogenesis, specific dynamic action, or the specific effect of food. The thermic effect of food represents the increase in energy expenditure associated with the body’s processing of food, including the work associated with the digestion,

absorption, transport, metabolism, and storage of energy from ingested food. The percentage increase in energy expenditure above BMR caused by the thermic effect of food is typically about 10% (see Figure 8.3).

Protein in foods has the greatest thermic effect, increasing energy expenditure 20–30%. Carbohydrates have an intermediate effect, raising energy expenditure 5–10%, and fat increases energy expenditure 0–5%. The value most commonly used for the thermic effect of food is 10%

of the caloric value of a mixed diet averaged over a 24-hour period [8]. Because of its relatively small contribution, the thermic effect of food is usually not included in calculations of total energy expenditure.

Thermoregulation

An additional component of energy expenditure that is of some importance is thermoregulation, also called adaptive, nonshivering, facultative, or regulatory thermogenesis. Thermoregulation refers to the adjustments in metabolism necessary to maintain the body's core temperature of about 98.2–98.6°F. A drop of temperature of 10°F or an increase of just 5°F can be tolerated, but fluctuations beyond this range can result in death. Most people adjust their clothing and environment to maintain comfort and thermoneutrality, although the body can adjust metabolic heat production when needed by hormonal changes controlled by the hypothalamus. Measurements of BMR or RMR are performed in a thermoneutral setting so that the contribution of thermoregulation can be excluded from calculations of energy expenditure.

As a cautionary note, muscular activity can generate significant heat and cause a rapid increase in body core temperature beyond the ability of the body to thermoregulate, especially in hot environments. **Heat stress** is a concern among high school athletes, particularly football players, who have the highest incidence of heat-related deaths [9]. Coaches, parents, and athletes should take the necessary steps to ensure proper hydration and to avoid conditions that increase the risk of heat stress.

8.3 BODY WEIGHT: WHAT SHOULD WE WEIGH?

Monitoring changes in body weight has long been a diagnostic tool of the health practitioner. Whether body weight remains stable, increases, or decreases depends entirely on the extent to which total energy expenditure is being met or exceeded by energy intake. The connections between body weight, body fat, and health were recognized centuries ago; as noted by Hippocrates, “those who are constitutionally very fat are more apt to die earlier than those who are thin” [10].

Today, scientists and health professionals recognize that the risk of many diseases—including heart disease, stroke, diabetes mellitus, hypertension, osteoarthritis, infertility, and some cancers (breast, endometrial, colon, and kidney)—increases with excess body fat. Conversely, low body weight may indicate malnutrition or an eating disorder and may pose risks for other diseases, such as osteoporosis. Many methods have been employed to quantify body fat, as discussed in detail later in this chapter.

Most of these methods require specialized equipment that is expensive and time-consuming to operate. Using body weight as a proxy for body fat is convenient, applicable to most people, and can be used by researchers to collect data in large numbers of subjects. An important caveat, however, should be noted when using only body weight as an indicator of health. Some individuals may increase body weight by adding muscle mass rather than fat, as seen in body-builders and many competitive athletes.

Comparing body weight to height has become a standard measurement among health professionals. In 1846, English surgeon John Hutchinson published a height–weight table based on a sample of 30-year-old Englishmen and urged that future census-taking include such information, which he believed to be valuable in promoting health and detecting disease [11]. The concept of a *desirable body weight* later emerged from the life insurance industry in the early 20th century. In an attempt to find the healthiest body weight, insurance companies began compiling data from their policyholders whose body weights (relative to height) were associated with the lowest mortality. Eventually height–weight tables were developed that showed the most desirable weight from a health standpoint [12]. Health professionals began using these tables in the 1940s for educational purposes for the general public. Data from the tables have also been subjected to regression analysis, resulting in the use of *ideal body weight* formulas for estimating a person's health status. Although the height–weight tables and ideal body weight formulas are able to convey a general notion of disease and mortality risk, they are limited to the demographic populations on which they are based and require a reference population so that the comparative terms *desirable* and *ideal* can apply. Many health experts have abandoned the use of height–weight tables and formulas in favor of body mass index and waist circumference as better indicators of body fat (and thus health status) because the latter measurements do not require a reference population for comparison.

Ideal Body Weight Formulas

Despite falling out of favor, the use of ideal body weight (IBW) formulas may still have a role in certain situations. As previously mentioned, IBW formulas evolved directly from height–weight tables and are intended to provide guidance to health practitioners when determining overall mortality risk in a given population. IBW is more easily understood by the general public than body mass index and may be the method of choice by some health professionals. Table 8.4 describes several common formulas that have been used for calculating IBW [13,14]. Note that the Broca formula provides a range for IBW at a given height, whereas the other formulas provide a single value for IBW for a given height over 60 inches.

Table 8.4 Ideal Body Weight (IBW) Formulas

	Men	Women
Broca formula (1871)	Weight(kg) = height(cm) – 100 ± 10%	Weight(kg) = height(cm) – 100 ± 15%
Hamwi formula (1964)	48.1 kg + 2.7 kg/inch over 60 inches	45.4 kg + 2.3 kg/inch over 60 inches
Devine formula (1974)	50.0 kg + 2.3 kg/inch over 60 inches	45.5 kg + 2.3 kg/inch over 60 inches
Miller formula (1983)	56.2 kg + 1.41 kg/inch over 60 inches	53.1 kg + 1.36 kg/inch over 60 inches
Robinson formula (1983)	51.7 kg + 1.85 kg/inch over 60 inches	48.7 kg + 1.65 kg/inch over 60 inches
Deitel-Greenstein formula* (2003)	61.3 kg + 1.36 kg/inch over 63 inches	54.0 kg + 1.36 kg/inch over 60 inches
Kammerer formula* (2015)	64.5 kg + 1.36 kg/inch over 63 inches + 0.45 kg/inch over 71 inches	54.0 kg + 1.36 kg/inch over 60 inches

*IBW formulas were developed in bariatric surgical patients.

Using the Hamwi formula, the IBW for a male who is 6 feet tall (72 inches) is calculated as 48.1 kg + (2.7 kg × 12 inches over 60) = 80.5 kg or 177 lb. The Hamwi formula yields the highest single-value IBW among all of the formulas in Table 8.4. The Miller formula provides the lowest IBW, at 73.1 kg or 161 lb. If one chooses to use the Broca formula, which provides a range, the IBW is 75–91 kg or 165–200 lb.

Body Mass Index

Body mass index (BMI), first described in the 1860s and known as Quetelet’s Index, is one of the most widely accepted approaches to categorizing weight for a given height. BMI is considered an indication of body adiposity but does not directly measure body fat. BMI is calculated from a person’s height and weight, as shown in this formula:

Body mass index = Weight / Height²

with weight measured in kilograms (kg) and height measured in meters (m) and raised to a power of 2. BMI is expressed in units of kg/m².

An adult male who weighs 165 lb (74.9 kg) and is 5 feet, 11 inches tall (1.803 m) will have a BMI = 74.9/1.803² = 23.0 kg/m². The following conversion factors are used: 1 lb = 0.454 kg and 1 inch = 0.0254 m. Alternatively, BMI can be calculated as lb/inches² × 703 to convert to kg/m². In this case, BMI = 165/71² × 703 = 23.0 kg/m².

BMI is considered a good index of total body fat in both men and women and has generally replaced the practice of classifying people as underweight or overweight compared to a reference weight. Using BMI to categorize overweight and obesity was proposed in 1997 by the World

Health Organization (WHO) to provide a basis for intervention at the individual and population levels [15]. The WHO weight categories, based on BMI, have been widely adapted as clinical guidelines for treating individuals at risk for chronic diseases (Figure 8.4).

BMI is also used to assess weight in children, but through comparison to population standards for sex and age. BMI changes with age in healthy children, as demonstrated by the growth charts for boys and girls 2–20 years of age shown in Figure 8.5. BMI < 5th percentile is underweight; BMI between the 5th and 85th percentiles is considered healthy weight; BMI between the 85th and 95th percentiles are classified as overweight; and BMI > 95th percentile is considered obese [16]. Body weight and recumbent length for boys and girls under 2 years of age are assessed using growth charts similar to those in Figure 8.5.

Although BMI is a valuable tool for categorizing body weight, it does not directly determine body fat. People who have large amounts of lean body mass and a low percentage of body fat may fall into the overweight category. Consequently, intermediate BMI values in the normal and overweight categories are not as strongly correlated with actual body fat percentage as compared to BMI of ≥ 30 kg/m². The relationship between BMI and body fat has also been shown to vary among different age, sex, and racial/ethnic groups. Nevertheless, measurements of BMI sampled from the U.S. population clearly indicate an alarming trend of increasing obesity prevalence in all age groups (Figure 8.6). Note in the figure that BMI ≥ 25 kg/m² includes both the overweight and obese categories and that the obese category (BMI ≥ 30 kg/m²) is shown separately.

Because of the limitations of using BMI alone, many health professionals measure waist circumference in addition to BMI. By utilizing both of these measurements, disease risk relative to normal weight and waist circumference can be determined, as indicated in Table 8.5. Monitoring changes in waist circumference over time is helpful since it

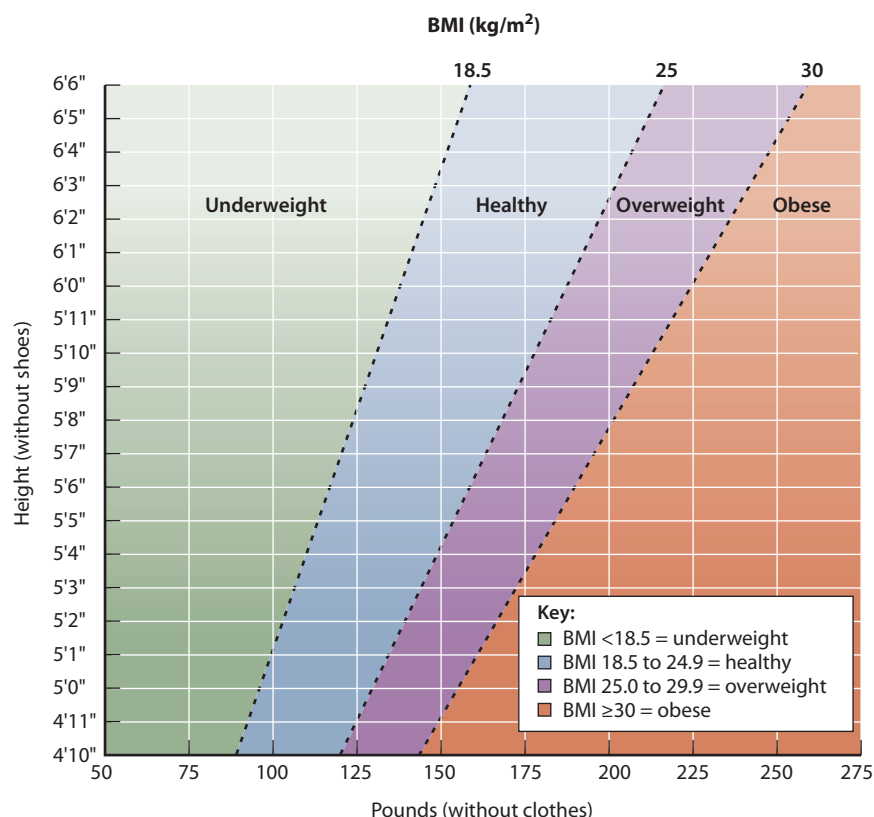


Figure 8.4 BMI values used to categorize weight.

Source: National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Publication No. 98-4083. Bethesda, MD: National Institutes of Health, National Health, Lung, and Blood Institute. 1998.

can be an indicator of abdominal fat even in the absence of a change in BMI. Including waist circumference measurements is particularly useful in people who are categorized as normal or overweight on the BMI scale because it helps distinguish those who have increased muscle mass versus excess body fat. For individuals with a BMI ≥ 35 kg/m², waist circumference adds no further predictive power of disease risk beyond BMI alone; therefore, it is unnecessary to measure waist circumference in people with a BMI ≥ 35 kg/m².

The simplicity and convenience of measuring height, weight, and waist circumference—and the relative ease of calculating BMI and IBW—has contributed to their widespread use as indicators of body fat, disease risk, and overall health. These simple methods work relatively well as an initial screening tool for the general population and in epidemiological research when large sample sizes are studied. However, as previously discussed, methods based on body weight do not directly quantify fat mass or the proportion of body fat relative to lean body mass, which varies greatly in the human population. Therefore, the development of methods that specifically assess body fat and overall body composition has helped researchers and health practitioners better understand the role of body fat in health and disease. The following section discusses some of the methods used to measure body composition in the laboratory and in the field.

8.4 MEASURING BODY COMPOSITION

Early observations linking body weight with disease risk and mortality, as discussed in previous sections, led to the obvious conclusion that excessive body weight was due to the accumulation of body fat. Humans have a tremendous capacity to store triacylglycerols in adipose tissue when in positive energy balance over time. However, using body weight as a proxy for body fat is less accurate at intermediate body weights because of individuals who gain muscle mass rather than body fat. The desire to have a more complete and accurate understanding of body composition prompted the development of methods that directly assess body fat and other components that include bone, muscle, visceral organs, minerals, and water. Assessment of body composition can be approached in different ways. Historically, the gross anatomy and chemical composition of the human body were determined using cadavers. Today, modern techniques allow for detailed analyses that can be performed quickly and accurately.

Conceptual models of body composition are currently used that partition the body into “compartments.” The two-compartment model includes fat mass and fat-free mass, whereas the four-compartment model includes fat mass, fat-free mass, bone mineral, and total body

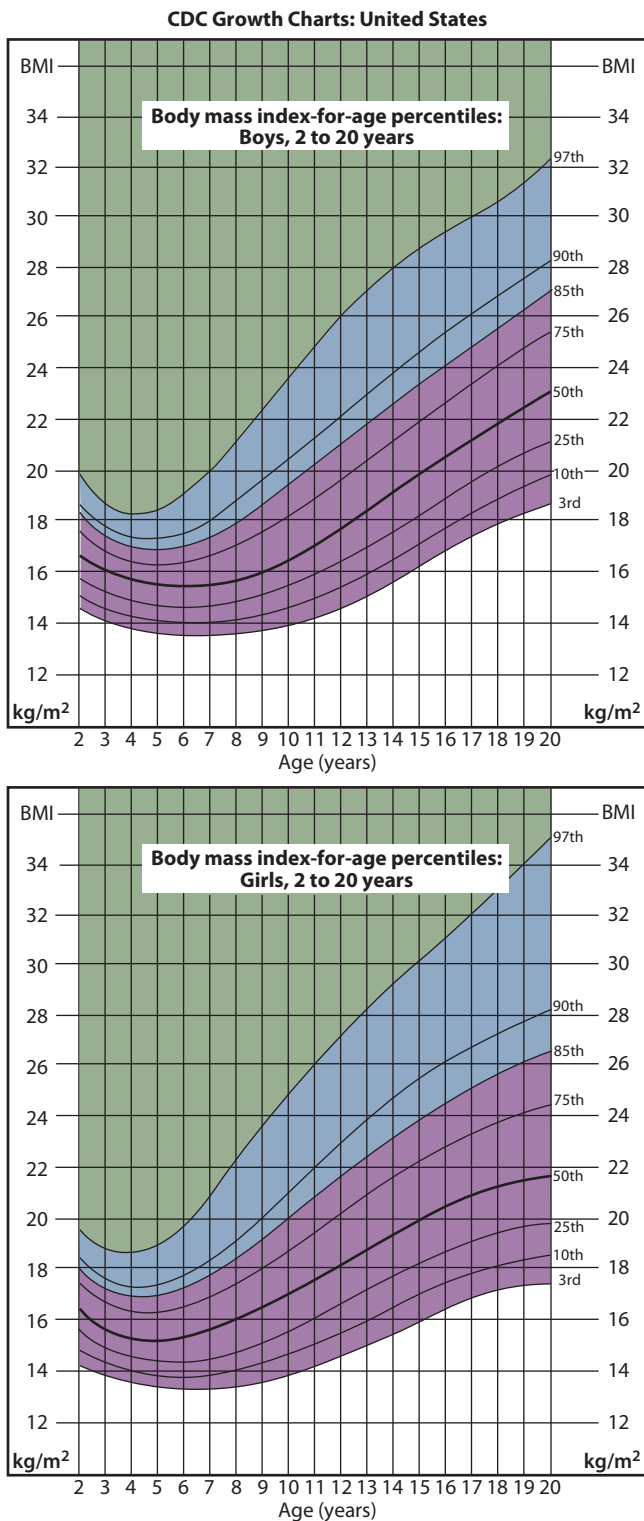


Figure 8.5 Example of growth curves (2–20 years): boys' and girls' body mass index-for-age percentiles.

Source: Centers for Disease Control and Prevention, National Center for Health Statistics. Growth charts. <https://www.cdc.gov/growthcharts/>

water. The four-compartment model is considered the gold standard but requires sophisticated equipment to measure all four components. In some cases, the mass of specific organs and the location of adipose tissue can be determined. The most frequently used methods to assess

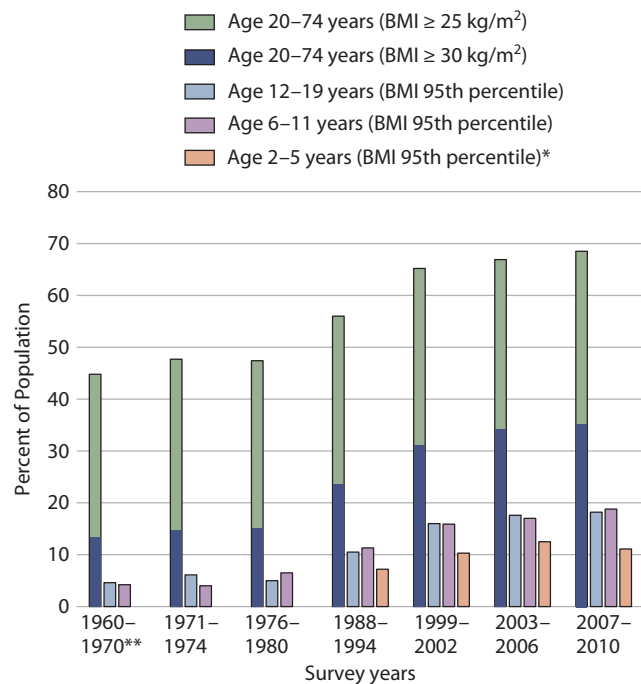


Figure 8.6 Obesity prevalence in the United States.

*Surveys of children age 2–5 years began in 1988.

**Survey years were 1960–1962 for adults age 20–74 years; 1963–1965 for children age 6–11 years; and 1966–1970 for adolescents age 12–19 years.

Source: Centers for Disease Control and Prevention, National Health and Nutrition Examination Survey (NHANES)

body composition consider only two compartments, fat mass and fat-free mass. Fat mass consists mostly of triacylglycerols, with relatively small amounts of water and minerals. Fat-free mass is much more diverse and comprised of muscle, bones, and the intra- and extracellular fluids. Muscle itself contains about 73% water. The differences in the chemical and physical properties of the two compartments—which include variations in density, the electrolyte content, the ability to conduct an electrical current, and the X-ray density—form the basis for many of the methods of determining body composition.

Choosing a method for measuring body composition depends on the purpose, access to the equipment, the number of individuals to be measured, age, and cost. Some methods can be performed only in a laboratory or clinical setting because of the large equipment involved, whereas some methods use portable equipment and can be used in the field. The following discussion highlights some of the common techniques used to measure body composition.

Field Methods

Skinfold Thickness

The use of skinfold measurements as an indicator of body fat is based on the assumption that the thickness of subcutaneous fat directly correlates with total body fat. Skinfold

Table 8.5 Disease Risk* Relative to Normal BMI and Waist Circumference

BMI Categories	Waist Circumference	
	Men ≤ 40 in (102 cm) Women ≤ 35 in (88 cm)	Men > 40 in (102 cm) Women > 35 in (88 cm)
Underweight (< 18.5 kg/m ²)	—	—
Normal (18.5–24.9 kg/m ²)	—	—
Overweight (25.0–29.9 kg/m ²)	Increased	High
Obese, class I (30.0–34.9 kg/m ²)	High	Very high
Obese, class II (35.0–39.9 kg/m ²)	Very high	Very high
Obese, class III (≥ 40 kg/m ²)	Extremely high	Extremely high

Source: National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults. Publication No. 98-4083. Bethesda, MD: National Institutes of Health, National Health, Lung, and Blood Institute. 1998.

*Risk for type 2 diabetes, dyslipidemia, hypertension, and cardiovascular diseases.

measurements can estimate the percentage of body fat and is therefore consistent with the two-compartment model. Skinfold measurements are made at various anatomical sites using a caliper (Figure 8.7). The anatomical sites commonly used for measuring skinfold thickness are the triceps (measured on the back of the upper arm), subscapula (measured just below the tip of the scapula), suprailiac (measured above the hip bone), abdomen (measured 1 inch to the right of the umbilicus), and thigh (measured at the midpoint of the thigh, between the kneecap and the hip). All measurements should be repeated at least two or three times, and the average should be used as the skinfold value. The precision of skinfold thickness measurements depends on the skill of the technician; in general, a precision of within 5% can be obtained by a well-trained and experienced technician. After skinfold thickness is measured, the values are entered into mathematical equations to calculate the percentage of body fat. Because of the convenience and low cost, the use of skinfold measurements is a popular choice of community-based health and wellness practitioners.

Bioelectrical Impedance

Bioelectrical impedance analysis is another commonly used field technique that assesses the two-compartment model. The method is based on the principle that the flow of electricity (conductivity) is facilitated in fat-free tissue high in water and electrolyte content, but is impeded by fat tissue low in water and electrolytes. Electrical conductivity is measured by various techniques. For example, an instrument generates a painless electrical current that flows through the body by means of the electrodes. Other devices are available in which the subject stands barefoot on a scale or grasps a hand-held device that measures electrical conductivity (Figure 8.8). In each case, opposition to the electric current, called impedance, is detected and measured by the instrument. Impedance is the inverse of conductance. The lowest-resistance value of a person is used to calculate conductance and predict lean body mass or fat-free mass. For example, muscle, organs, and blood, which have high water and electrolyte contents, are good conductors. Tissues containing little water and electrolytes (such as adipose tissue) are poor conductors and have a high resistance to the

**Figure 8.7** Measuring triceps skinfold with a Lange caliper to estimate body fat.**Figure 8.8** Hand-held device used to measure bioelectrical impedance.

passage of electrical current. When multiple frequencies are used, the higher frequencies can estimate both intracellular and extracellular water because the higher-frequency current can penetrate cell membranes. At lower frequencies, the flow of the current is blocked and the measured resistance indicates extracellular water.

Bioelectrical impedance is a safe, noninvasive, and rapid means to assess body composition. The equipment is relatively inexpensive, portable, and fairly easy to operate. Bioelectrical impedance readings are affected by hydration and electrolyte imbalances. Thus, the technique is more useful for healthy subjects. Several bioelectrical impedance analysis prediction equations have been developed for various populations. The use of multifrequency techniques provides results that are in good agreement with other body composition methods because this technique estimates both total body water and extracellular water.

Laboratory Methods

Densitometry: Underwater Weighing

The density of body fat is about 0.9 g/mL, whereas the density of fat-free mass is about 1.1 g/mL. Percent body fat of an individual can therefore be calculated if whole-body density is known. The Greek mathematician Archimedes discovered that the volume of an object submerged in water is equal to the volume of water displaced by the object. The density of an object can then be calculated by dividing the object's weight (wt) in air by its loss of weight in water. For example, for a person who weighs 47 kg in air and 2 kg underwater, 45 kg represents the loss of body weight and the weight of the water displaced. After an adjustment for the change in density of water at different temperatures is made, the volume of the person can be calculated. Figure 8.9 illustrates an apparatus for weighing under water. Correction for residual air volume in the lungs (RLV) and gas in the gastrointestinal tract (GIGV) must be made.

$$\text{Body density} = \frac{\text{Wt of body in air}}{\left(\frac{\text{Wt of body in air} - \text{Wt of body underwater}}{\text{Density of water}} \right) - \text{RLV} - \text{GIGV}}$$

Residual lung volume is thought to be about 24% of vital lung capacity. The volume of gas in the gastrointestinal tract is estimated to range from 50 to 300 mL. This volume is typically neglected, or a value of 100 mL may be used in calculations. The density or the weight of water is known for a wide range of temperatures and must be obtained for the calculation. Once density of the human body is known, an estimation of body fat can be determined. At any known body density, estimating the percentage of body fat is possible using established equations.



Figure 8.9 Apparatus for underwater weighing to determine body density.

Underwater weighing is considered a noninvasive and relatively precise method for assessment of percent body fat. The standard error of body fat measurements using densitometry has been estimated at 2.7% for adults and about 4.5% for children and adolescents [17]. Measurements obtained by underwater weighing correlate well in broad populations with those obtained by other techniques. Limitations of underwater weighing include its relatively high equipment cost, the inability to measure gas volume in the gastrointestinal tract, its impracticality for large numbers of subjects, and the high level of cooperation and time required of subjects, who must be submerged and remain motionless for an extended time. Thus, the technique is not suitable for young children, older adults, or subjects in poor health.

Densitometry: Air Displacement

Another way to determine the volume—and thus density—of the body is with air displacement plethysmography. In the commercially available apparatus shown in Figure 8.10 (BOD POD, Cosmed Inc.), the subject is seated in a sealed chamber of known volume, separated from a second chamber by a membrane. The instrument measures the change in pressure caused by the volume occupied by the person. The person is dressed in a tight-fitting bathing suit and wears a bathing cap (to displace pockets of air in the hair). The measurement takes only a few minutes to complete. The apparatus has an advantage in that it can measure the body composition in age groups that are not suitable for underwater weighing, such as older adults or the very young. A similar instrument called the PEA POD is designed for infants and small children. Once the density of the body is obtained, the calculation of percent body fat is the same as with underwater weighing using established equations.

Dual-Energy X-ray Absorptiometry

Dual-energy X-ray absorptiometry (abbreviated DXA or DEXA) involves scanning subjects with X-rays at two different energy levels, illustrated in Figure 8.11. The subject lies



AP Images/Keith Srakocic

Figure 8.10 Air-displacement plethysmography determines body density by measuring the amount of air displaced.

on a table while an X-ray source beneath the table and the detector above the table pass across the subject's body. Attenuation of the beam of X-rays as it passes through the body is calculated by computer. Percentage of fat mass, bone-free fat-free mass, and bone mineral (total body or specific sites) can be calculated based on the restriction in the flux of the X-rays across the fat and the fat-free masses [18].



Stephen Ausmus/USDA/Science Source

Figure 8.11 Dual-energy X-ray absorptiometry (DEXA) uses two X-ray beams of different energy to determine fat-free mass, fat mass, and bone tissue.

DEXA is considered to be the gold standard technique for diagnosing osteoporosis and osteopenia and is a commonly used method for body composition measurements. It is widely available and entails relatively low X-ray exposure: 1–10% that of a chest X-ray [18]. Limitations to the use of absorptiometry include the expense of the equipment and the exposure of subjects to radiation. In addition, trained personnel are required to run the instrument and analyze the scans. DEXA measurements are highly reproducible and correlate with other body composition assessment methods. The technique, however, is not accurate for people with metal implants.

Other Imaging Techniques

Computed tomography, more commonly known as CT or CAT scan, and magnetic resonance imaging (MRI) have been used to measure body composition. Both of these imaging techniques are used extensively in medical diagnostics, but the equipment is very expensive and requires highly trained technicians to operate. Consequently, both imaging methods are used primarily for research purposes.

Knowledge about body composition led to the concept of the reference man and woman as a standard benchmark for educational and research purposes. These reference figures are based on average physical dimensions from thousands of volunteer subjects and provide a frame of reference for comparison. They should not be viewed as “ideal” body composition. The characteristics of the reference man and woman were discussed in Chapter 6 and are summarized in Table 6.8. The reference man has 3% essential fat, 12% storage fat (for a total of 15% body fat), 44.8% muscle, 14.9% bone, and 25.3% other components. The reference woman has 12% essential fat, 15% storage fat (for a total of 27% body fat), 36% muscle, 12% bone, and 25% other components. Essential fat includes the fat that is associated with bone marrow, the central nervous system, internal organs, and the cell membranes. The essential fat in females also includes the fat in mammary glands and the pelvic region. These gender differences must be considered when body composition is evaluated. Normal changes in body composition associated with development and aging are discussed in Chapter 6 in the section “Changes in Body Mass with Age.”

8.5 REGULATION OF ENERGY BALANCE AND BODY WEIGHT

The concept of energy balance has been discussed throughout this chapter, particularly *positive* energy balance that promotes body fat accumulation. Although the concept of energy balance could apply to the daily shifts in metabolic fuels that occur during the fed-fast cycle or bouts of exercise (discussed in Chapter 7), most health

professionals refer to energy balance within the context of body composition and the changes that may occur over weeks, months, or years. When an individual is in positive energy balance over time, the excess energy, regardless of the macronutrient form in which it was consumed, will be stored as triacylglycerols in adipose tissue. Most experts generally agree on this concept, as emphasized in the 2015-2020 *Dietary Guidelines for Americans* that recommend eating “within an appropriate calorie level” and engaging in “regular physical activity in a variety of ways” [19]. Even slight imbalances can have a significant long-term impact on body weight. Consuming an energy excess of just 150 kcal per day—the amount found in one 12 oz. soft drink—can result in the gain of one pound of adipose tissue in just 23 days.

Although once thought to be a simple matter of “energy in versus energy out,” numerous lines of evidence have shown energy balance to be tightly controlled by several hormones working in concert. Additional internal and external factors can influence the function of these hormones and thus food intake, energy expenditure, and body fat deposition. The following discussion highlights some of the key hormones and contributing factors that impact energy balance and body weight.

Hormonal Influences

Hunger and satiety control meal-to-meal eating behavior, and both are under the influence of hormones. The main target of these hormones is the hypothalamus, in particular the **arcuate nucleus** region and its collection of neurons.

There are two distinct populations of neurons in the arcuate nucleus with opposing actions. The first group of neurons express hormone receptors and, upon hormone binding, release **orexigenic** (appetite-stimulating) peptides that include neuropeptide Y (NPY) and agouti-related peptide (AgRP). The other group of neurons produce **anorexigenic** (appetite-inhibiting) peptides that belong to the pro-opiomelanocortin (POMC) family. The released peptides act as neurotransmitters and affect other neurons within the hypothalamus and elsewhere in the brain to produce the feeling of either hunger or satiety [20].

The major hormones involved in appetite control and energy homeostasis are summarized in Table 8.6. The hormonal effects can be thought of as short term, involving daily fluctuations in appetite, and long term, which impacts energy balance and body weight over time. The so-called “gut hormones” include ghrelin, cholecystokinin, peptide YY, and glucagon-like peptide-1. Hormones secreted by the pancreas, namely insulin and pancreatic peptide, are often included in the list of gut hormones because of the intimate connection between the pancreas and the gastrointestinal tract. In addition, adipose tissue secretes leptin and adiponectin.

Leptin

The discovery of leptin by Jeffrey Friedman in 1994 was a milestone event in understanding the relationships of appetite control and obesity [21]. For many years it was suspected that factors in the bloodstream regulated food intake and energy expenditure, and the discovery of leptin provided the first solid evidence. Leptin is a hormone

Table 8.6 Agents of Energy Regulation

Agent	Site of Production	Stimulus	Action	Comments
Leptin	White adipose tissue	Levels increase with overfeeding or increased adipose tissue. Levels decrease with starvation or reduced adipose tissue.	Decreases the urge to eat and increases physical activity to produce a negative energy balance.	Leptin resistance reported in the obese.
Insulin	β-cells of pancreas	Levels increase with elevated blood glucose levels.	Suppresses hunger and stimulates the deposition of triacylglycerols in adipose tissue, which stimulates leptin.	
Adiponectin	Adipocytes	Levels increase with decreased fat mass. Levels decrease with increased fat mass.	Protects against insulin resistance, glucose intolerance, and dyslipidemia.	No role in food intake.
Ghrelin	Stomach and duodenum	Levels increase between meals and decrease after a meal and when absorption begins.	Stimulates hunger and food intake. Promotes digestion.	Only known orexigenic hormone.
Cholecystokinin	Intestine	Levels increase during and after a meal.	Suppresses appetite and promotes satiety.	
Pancreatic polypeptide	Pancreas	Levels increase during a meal.	Suppresses hunger in the short term.	Relatively brief half-life in the circulation.
Glucagon-like peptide-1	Intestine	Levels increase during and after a meal.	Suppresses hunger and inhibits glucagon production.	
Peptide YY	Small and large intestine	Levels rapidly increase after a meal. Levels are low in a fasting state.	Suppresses hunger in the long term.	May influence weight loss following surgery.

secreted by white adipose tissue that interacts with the hypothalamus to reduce hunger. When leptin binds to its receptors, the orexigenic neurons are inhibited and the production of NPY and AgRP declines, while the anorexigenic neurons are stimulated and release POMC peptides. Leptin levels in the circulation are directly correlated with the amount of body fat. As the amount of body fat increases, so does the level of leptin, thus suppressing hunger. In contrast, less body fat and lower leptin levels mean hunger is suppressed to a lesser extent. The correlation between BMI and leptin concentration is 0.9, illustrating the important role of leptin in long-term control of body weight. Moreover, mutations in the genes that code for leptin or leptin receptors result in severe obesity [21].

The elucidation of the action of leptin is hoped to be an important tool in the treatment of obesity, but this has yet to be fully realized. On one hand, the small number of obese individuals in the population who lack leptin because of genetic mutations benefit from leptin injections. On the other hand, the vast majority of obese people have normal or increased leptin levels, but the feeling of hunger persists and they do not decrease food intake. This has given rise to the concept of **leptin resistance** in which increased leptin levels fail to bring about weight loss even though the leptin receptor is functional. The mechanism of this resistance is not fully understood. One hypothesis is that part of the resistance is due to the inability of leptin to reach the receptor because of diminished transport across the blood–brain barrier. Another hypothesis points to receptor overstimulation, and thus activation of negative feedback pathways that block leptin signaling. The precise mechanisms of leptin resistance require further study.

Leptin also exerts rapid effects on glucose and lipid metabolism by activating leptin-responsive relays that are initiated in the hypothalamus and transmitted to other tissues via the sympathetic nervous system. The major tissues affected include the pancreas, skeletal muscle, liver, and adipose tissue. Furthermore, leptin receptors are present in tissues other than the hypothalamus, so leptin can exert direct effects on these tissues independent of its role in the hypothalamus. In skeletal muscle, leptin activates AMP-activated protein kinase (AMPK) and thus promotes fatty acid oxidation and utilization for energy. Recall from Chapter 7 (Figure 7.2) that when AMPK is active (phosphorylated), fatty acid oxidation is stimulated and fatty acid synthesis is inhibited. Direct binding of leptin to tissues also regulates glucose metabolism through its effects on insulin. Leptin inhibits the synthesis and secretion of insulin by the pancreas, while insulin signaling is suppressed in the liver and white and brown adipose tissue [22].

Insulin

In a manner similar to leptin, insulin suppresses hunger through its action on orexigenic and anorexigenic neurons, although its effects are less robust than leptin. Insulin

receptors are distributed throughout the brain, with high concentrations in the arcuate nucleus. Insulin binding stimulates the release of POMC peptides and inhibits the release of NPY and AgRP. Also similar to leptin, blood insulin levels increase in proportion to body fat, which may be the result of insulin resistance, as discussed later in this chapter.

Previous chapters highlighted the role of insulin in anabolic metabolism and energy storage through its direct action in several peripheral tissues such as muscle, adipose tissue, and liver. Insulin binding to hypothalamic receptors also transmits signals to peripheral tissues, which inhibits gluconeogenesis and proteolysis in the liver and stimulates lipogenesis and triacylglycerol accumulation in adipose tissue. The deposition of triacylglycerols, in turn, stimulates the release of leptin by adipocytes. This metabolic coordination of insulin and leptin is not fully understood, although their action clearly impacts long-term energy homeostasis beyond just daily appetite control [22].

Adiponectin

Adiponectin is an adipocyte-derived hormone that plays a role in food intake and energy balance. Adiponectin binds to receptors in the hypothalamus, where it suppresses NPY neurons. Conversely, adiponectin stimulates POMC neurons when blood glucose levels are low, suggesting that the hormone may be less effective at suppressing appetite in diabetic patients with hyperglycemia [23]. Plasma adiponectin levels are negatively correlated with body fat. Circulating levels of adiponectin are low in obese individuals, but increase in response to weight loss.

The precise role of adiponectin is not fully understood, although decreased plasma levels are associated with a number of metabolic conditions that include hypertension, type 2 diabetes, plasma triacylglycerol concentration, body mass index, several inflammatory markers, bone mineral density, and the risk of some cancers. Adiponectin has a protective effect on pancreatic β -cells by maintaining cell mass. The promise of adiponectin as a protective molecule against diabetes, inflammation, atherosclerosis, and cancer has led to dietary and lifestyle strategies that boost the levels of plasma adiponectin levels. Caloric restrictive diets, exercise, and associated weight loss increase adiponectin, as does the consumption of n-3 fatty acids, carotenoids, and polyphenols [24].

Ghrelin

Ghrelin is produced predominantly in the stomach and, unlike the other regulatory hormones, stimulates the feeling of hunger. Ghrelin secretion rises between meals when the stomach is empty. It binds to receptors in the orexigenic neurons of the hypothalamus, causing the release of NPY and AgRP. As a meal is consumed and nutrient absorption begins, ghrelin secretion rapidly diminishes and hunger reduces. The half-life of ghrelin in the serum

is only about 30 minutes. However, in obese individuals, the postprandial decline in ghrelin does not occur and may contribute to overeating [25]. Ghrelin also promotes digestion by stimulating gastric acid secretion and gastric motility prior to food consumption.

Other Anorexigenic Hormones

Cholecystokinin (CCK) An important role of CCK is to stimulate digestive processes when food is consumed (see Chapter 2). CCK is produced in the small intestine in response to food intake, especially lipids and proteins. Another important function is appetite regulation. CCK binds to receptors in the hypothalamus and inhibits NPY production by down-regulating mRNA expression. CCK also binds to receptors in the pylorus, causing contractions that send vagal nerve signals from the stomach to the brain, resulting in decreased hunger and increased satiety.

Pancreatic Polypeptide (PP) The anorexigenic PP is synthesized primarily in the pancreas and secreted in response to food intake. Its mechanisms of action are not well understood, although PP receptors are present in the hypothalamus and binding appears to down-regulate NPY mRNA expression, thus decreasing hunger. PP has a relatively short half-life in the circulation and therefore a brief period of action.

Glucagon-Like Peptide (GLP)-1 Production of GLP-1 occurs along the entire length of the intestine in proportion to caloric intake. GLP-1 release is delayed in obese individuals, who also have lower circulating GLP-1 levels. GLP-1 stimulates the pancreas to secrete insulin while inhibiting secretion of glucagon. It also reduces gastric emptying and intestinal motility, the latter causing signals to be sent via the vagus nerve to the brain, resulting in decreased hunger. GLP-1 receptors are found in the brain, although the role of GLP-1 binding is unclear.

Peptide YY (PYY) The small and large intestine produces PYY and its action lasts longer than the other anorexigenic hormones. As mentioned in Chapter 2, this characteristic is important in countering the effects of CCK by inhibiting intestinal motility and secretion of digestive juices. In addition, PYY binds to receptors in the hypothalamus, although the anorexigenic mechanisms of action are not fully understood. Circulating levels of PYY are lower in obese individuals.

Intestinal Microbiota

The human gastrointestinal tract is host to trillions of microbial cells that participate in nutrient digestion and utilization (see Chapters 2 and 4). Growing evidence indicates that intestinal microbiota are also linked to obesity development and metabolic dysfunction [26]. Normally

the microbial community is dominated by bacteria, with 90% belonging to the phyla *Firmicutes* and *Bacteroidetes*. In obese people, the intestinal microbiota are altered and responds to changes in body weight. Excess body fat is associated with more *Firmicutes* and fewer *Bacteroidetes*. The level of *Bacteroidetes* increases when weight is reduced with energy-restricted diets, although it is unclear whether the microbiota are responding to changes in energy intake or changes in adiposity.

One possible outcome of altered microbiota is their altered ability to ferment non-digestible carbohydrates (fiber) and produce short-chain fatty acids. The microbiota of obese individuals have an increased capacity to ferment fiber and thus “harvest” more energy as short-chain fatty acids that contribute to the total energy absorbed into the body. The additional energy can be stored as body fat. The short-chain fatty acids may also act as signaling molecules in intestinal cells by decreasing the production of the anorexigenic hormones, GLP-1 and PYY, thus removing the feeling of satiety.

Long-term dietary habits have significant impact on intestinal microbiota, as evident in populations that consume different macronutrient profiles [26]. For example, the microbiota resulting from European diets rich in protein and animal fat are quite different compared to high-fiber diets in West Africa, which are rich in cellulose and hemicelluloses. The microbiota in West Africans have adapted to fermenting these fibers to gain maximum energy as short-chain fatty acids.

The interrelationships of intestinal microbiota, diet, and obesity are complex and difficult to study because of the high degree of variability in human microbiota and diet. Nevertheless, it has been established that an individual's diet is a strong determinant of the microbiota composition and that altered microbiota profiles are associated with obesity.

Environmental Chemicals

Exposure to certain chemicals may play a role in body fat accumulation. Recent research suggests that a group of endocrine-disrupting chemicals present in the environment, including the diet, may predispose some people to gain body fat despite appropriate levels of food intake and physical activity [27]. These chemicals (aptly named **obesogens**) interfere with hormone function by binding to hormone receptors and either stimulating or inhibiting the signaling pathway. Obesogens are believed to promote triacylglycerol production and deposition in adipose tissue, as well as disrupting appetite control in the hypothalamus. Some evidence suggests that developmental exposure to obesogens early in life may interfere with epigenetic programming of gene regulation, thus influencing the risk of obesity later in life [27]. Much more research is needed to confirm these findings.

Lifestyle Influences

Diet and physical activity are the most obvious factors that influence energy balance and body weight. Achieving a healthy weight and maintaining energy balance should be a life-long goal. The most successful approaches include establishing both dietary and exercise habits that improve fitness and lower the risk of disease. For the average person, the importance of controlling the amount of food consumed cannot be overstated. For example, an individual can easily consume 2,000 kcal or more in one meal. In comparison, that same individual would need to walk 21 miles at a brisk pace requiring more than 5 hours to expend 2,000 kcal of energy (see Table 8.3). The importance of food intake in maintaining energy balance is further illustrated by the fact that total energy intake in adults has increased approximately 200–300 kcal/day since the 1970s, which closely parallels the increase in obesity as depicted in Figure 8.6 [28].

Daily physical activity and exercise contribute to total energy expenditure and help maintain energy balance. Unfortunately, nearly 30% of adults worldwide are physically inactive, with significantly higher inactivity in affluent countries [29]. In the past 50 years, energy expenditure related to occupations and household activities has declined, thus increasing the importance of planning activities that increase the use of skeletal muscle throughout the day. This includes both regular exercise and nonexercise activities such as walking, climbing stairs, standing, fidgeting, cleaning, and so on that can significantly contribute to daily energy expenditure [30].

Many social factors influence eating behaviors and exercise habits. Research has shown that increased food and energy intake occurs when eating away from home, and people tend to eat more when presented with larger portion sizes. Interestingly, though, there is little evidence that portion size *per se* is associated with body weight gain [31]. Living in a socioeconomically deprived area, having limited access to supermarkets, and having greater access to fast-food outlets are associated with higher BMI, but whether these factors directly *cause* body weight gain is unclear [32]. At present, there are relatively few studies that address the impact of social and behavioral factors on food intake, physical activity, and obesity.

8.6 HEALTH IMPLICATIONS OF ALTERED BODY WEIGHT

The terms *overweight* and *obesity* are often used to indicate different degrees of body “fatness.” The overweight category, as previously mentioned in this chapter, can unintentionally include individuals who have gained muscle mass rather than fat. The obesity category, on the other

hand, includes only those with excess body fat because of physical limits on gaining muscle mass.

Accumulation of body fat is associated with an increased risk of mortality and morbidity due to hypertension, stroke, coronary artery disease, dyslipidemia, type 2 diabetes, sleep apnea, osteoarthritis, and certain cancers. During the past few decades, prevalence of overweight and obesity has risen steadily to epidemic levels (see Figure 8.6). More than 2.1 billion people worldwide are overweight or obese, creating an economic burden of \$2 trillion annually [33].

When assessing the health risks related to excess body fat, the differences in fat distribution between men and women should be considered. Recall from Chapter 6 the concept of the reference man and woman (Table 6.8) in which women have significantly more “essential fat,” referring to the subcutaneous fat that accumulates mostly around the hips and thighs and in mammary tissue. On the other hand, excess fat in both men and women can accumulate within the body core (visceral fat) and subcutaneously around the torso. These observations form the basis of definitions for two patterns of obesity. **Gynoid obesity**, also called pear-shape, is associated primarily with women and refers to the excess fat that accumulates around the hips and thighs. **Android obesity**, or apple-shape, describes the central adiposity resulting from the accumulation of excess visceral fat. Android obesity is more strongly associated with disease risk. Consequently, measuring waist circumference, waist-to-height ratio, or waist-to-hip ratio is a more useful risk assessment tool than body weight or BMI alone.

At the cell level, the deposition of triacylglycerols causes adipocytes to become enlarged (hypertrophy). In addition, new adipocytes can be produced (**hyperplasia**) to accommodate more triacylglycerol molecules. The creation of new fat cells increases most rapidly in late childhood and early puberty whenever a positive energy balance exists, although hyperplasia can occur later in life as well. Obese people have more, and larger, fat cells than nonobese people. If body fat is lost, the number of fat cells does not decrease; they just get smaller.

Metabolic Syndrome

The definition of *syndrome* is a clustering of factors that occur together more often than expected based on chance alone, and the cause is often uncertain. The concept of **metabolic syndrome** refers to a group of risk factors that are associated with increased risk of cardiovascular disease (CVD). The risk factors for metabolic syndrome include

- central obesity;
- increased fasting plasma glucose;
- increased fasting plasma triglyceride;
- decreased plasma HDL cholesterol; and
- hypertension.

A diagnosis of metabolic syndrome requires a patient to exhibit three of the five conditions as defined in Table 8.7. There is some debate, however, whether the underlying cause of metabolic syndrome uniting these factors is obesity or, more specially, insulin resistance (discussed in the next section).

Metabolic syndrome diagnosis holds promise as a predictor of disease: it is associated with a two-fold increase in risk for CVD, myocardial infarction, stroke, and CVD-related mortality [34]. Metabolic syndrome diagnosis is associated with a 1.5-fold increase in risk of all-cause mortality. Still to be determined, however, is whether a diagnosis of metabolic syndrome is a better predictor of risk than the sum of the individual risk factors. Furthermore, central (android) obesity has consistently been recognized as a key component of the syndrome and is nearly always one of the diagnostic criteria. This has led to considerable debate about the usefulness of metabolic syndrome as a practical clinical tool in identifying patients in need of treatment. In fact, the American Diabetes Association and the European Association for the Study of Diabetes issued a joint statement emphasizing that the risk of CVD associated with metabolic syndrome diagnosis is no greater than the sum of each factor, calling into question the medical value of diagnosing the syndrome [35]. In many cases, reduction in body weight alone will improve each of the other diagnostic factors.

Insulin Resistance

Insulin resistance is generally defined as the inability of target tissues to respond to insulin, causing elevated blood glucose (hyperglycemia). This often leads to the diagnosis of type 2 diabetes. The pancreas may release more insulin in an effort to maintain normal blood glucose levels. Elevated insulin levels confirm the type 2 diabetes diagnosis and distinguish it from type 1 diabetes in which insulin is very low or absent. There is no single cause for type

2 diabetes, although many studies have identified excess body fat as the most important factor predicting it, with nearly 90% of patients with type 2 diabetes falling into the overweight or obese category [36].

The insensitivity to insulin is primarily seen in muscle and adipose tissue. Within insulin-resistant muscle, insulin loses its ability to stimulate glucose uptake; in adipose tissue, it no longer inhibits free fatty acid release. These observations can explain the elevated blood glucose and free fatty acid levels that accompany insulin resistance. The liver and kidney retain their sensitivity to insulin, and the elevated insulin levels stimulate liver triacylglycerol synthesis using the excess free fatty acids. As a consequence, the assembly and secretion of VLDL increases, resulting in elevated fasting serum triacylglycerol. Triacylglycerol levels in the liver also increase, leading to nonalcoholic fatty liver disease. The kidney responds to the elevated insulin levels by increasing renal sodium retention and decreasing uric acid clearance. This response results in an increased prevalence of essential hypertension and higher plasma uric acid concentrations.

Each of these metabolic conditions related to insulin resistance—excess body fat, elevated blood glucose, elevated triacylglycerols, and high blood pressure—are diagnostic criteria (along with low HDL cholesterol) for metabolic syndrome. It is easy to see why metabolic syndrome has been called insulin resistance syndrome and why the measurement of insulin resistance was once considered as a diagnostic criterion. However, no simple test exists to determine who is insulin resistant and who is not. Fasting insulin levels, fasting plasma glucose levels, and triacylglycerol:HDL-C ratios have all been used as indicators for insulin resistance, with varying degrees of success.

Considerable evidence demonstrates that if a person loses weight, insulin sensitivity improves. Fortunately, the elevated insulin levels do not prevent weight from being lost. On the contrary, free fatty release from adipocytes is accelerated, so it is vital that positive energy balance be avoided for weight reduction to occur. Interesting to note is that variations in the macronutrient content of isocaloric diets have little effect on insulin sensitivity.

One common weight loss strategy is to lower the lipid content of the diet and replace it with carbohydrate to lower overall energy intake. The problem with a low-fat, high-carbohydrate diet for a person with insulin resistance, however, is that the additional carbohydrate requires more insulin to be secreted from the pancreas to maintain glucose homeostasis. If the person is insulin resistant, and the pancreas is functioning properly, insulin levels will be elevated further. Another dietary strategy is to substitute saturated fat with polyunsaturated or monounsaturated fat, although the role of unsaturated fatty acids in mitigating insulin resistance is not completely understood [37].

Table 8.7 Criteria for Clinical Diagnosis of Metabolic Syndrome

Measure	Categorical Cut Points
Elevated waist circumference*	≥ 102 cm (40 in) for men ≥ 88 cm (35 in) for women
Elevated triacylglycerols	≥ 150 mg/dL (1.7 mmol/L)
Reduced HDL-C	< 40 mg/dL (1.0 mmol/L) in men < 50 mg/dL (1.3 mmol/L) in women
Elevated blood pressure	Systolic ≥ 130 and/or diastolic ≥ 85 mm Hg
Elevated fasting glucose	≥ 100 mg/dL

Source: Modified from Alberti KGMN, Eckel RH, Grundy SM, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009; 120:1640–45.

*Current recommended thresholds for the U.S. adult population. Thresholds vary among countries.

Weight-Loss Methods

Each year many people “go on diets” for the purpose of losing body fat. Depending on the nature of the caloric restriction and the level and type of exercise, both body fat and fat-free mass may be lost. Limited information is available on the proportion of fat and muscle lost on different weight-loss regimens.

Losing body fat requires a negative energy balance over an extended period of time. In a systematic review of weight-loss programs lasting at least 6 months, the authors concluded that moderate weight loss (5–10 kg) that consists of both fat mass and fat-free mass can be achieved by calorie restriction alone [38]. Exercise alone is generally considered to be less effective than calorie restriction with

exercise. If exercise is added to the weight-loss program, some or all of the fat-free mass can be spared. Most studies have used an aerobic exercise component, and in those that combined calorie restriction with exercise, most of the weight lost was fat mass and very little (0–1.5 kg) was fat-free mass. Resistance training results in only small losses or even a gain in fat-free mass.

Research has also explored the relationship between diet composition and weight loss. Some controversy remains as to the proper combination of low fat, low carbohydrate, and/or high protein. However, it appears the most important factor in successful weight loss is the intensity of behavior modification, irrespective of the macronutrient composition of the energy-restrictive diet [39].

SUMMARY

To maintain a state of energy balance, the amount of food energy a person consumes must equal energy expenditure in the body. Techniques have been developed to measure energy expenditure based on the principle that all energy-requiring metabolic processes ultimately produce heat while utilizing oxygen.

- Methods using direct calorimetry measure heat production by the body.
- Methods using indirect calorimetry measure oxygen consumed and carbon dioxide expired. The ratio of CO_2 produced relative to O_2 consumed is called the respiratory quotient and reflects the relative proportion of carbohydrate and fat being used as metabolic fuel.

Daily total energy expenditure is attributable to three primary components: basal metabolic rate, physical activity, and the thermic effect of food.

- Basal metabolic rate represents the amount of energy needed to sustain basic life processes such as respiration, heartbeat, renal function, brain and nerve function, blood circulation, active transport, and synthesis of proteins and other complex molecules.
- Physical activity requires energy to support skeletal muscle contraction and is the most highly variable component of total energy expenditure. Physical activity includes all exercise and nonexercise activities associated with daily living.
- The thermic effect of food represents the increase in energy expenditure needed to process food, including the work associated with the digestion, absorption, transport, metabolism, and storage of energy from ingested food.

Consuming more energy than needed puts a person in a state of positive energy balance, resulting in body fat

accumulation. Health practitioners have long used body weight as proxy for body fat.

- The concept of a desirable or ideal body weight is based on height–weight tables developed by the life insurance industry that indicate body weights associated with the lowest mortality.
- Height–weight tables have largely been abandoned as a diagnostic tool in favor of the body mass index (BMI) in conjunction with measurements of waist circumference.
- BMI and waist circumference correlate reasonably well with disease risk and are used extensively as an initial screening tool for the general population and in epidemiological research.

Body composition and fat mass can be assessed by several methods.

- Field methods are portable and relatively easy to use, including skinfold thickness, which measures subcutaneous fat, and bioelectrical impedance, which is based on the principle that a low electrical current is facilitated in fat-free tissue high in water and electrolyte content, but is impeded by fat tissue.
- Laboratory methods include measuring body volume and density by underwater weighing or by air displacement. Computer-based imaging techniques include dual-energy X-ray absorptiometry, computed tomography, and magnetic resonance imaging.

Energy intake is driven by appetite and is influenced by many social and environment factors. Hunger and satiety control meal-to-meal eating behaviors as a result of hormone regulation.

- Ghrelin is an orexigenic hormone that stimulates hunger.

- Anorexigenic hormones that inhibit hunger include leptin, insulin, adiponectin, cholecystokinin, pancreatic polypeptide, glucagon-like peptide-1, and peptide YY.
- Long-term energy balance may be influenced by intestinal microbiota, environmental endocrine-disrupting chemicals (obesogens), exercise habits and food choices, and socioeconomic conditions.

Excess body fat is associated with an increased risk of disease and mortality. Obesity prevalence has increased dramatically during the past 50 years, leading to the development and use of *metabolic syndrome* as a diagnostic tool. Insulin resistance is thought to be an underlying cause of metabolic syndrome. In many cases, weight loss improves insulin sensitivity.

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Perspective

EATING DISORDERS

Few things can create as large a sensation in the media as a new weight-reduction diet guaranteed to remove unwanted fat. The authors of the sensational new diet are interviewed on television talk shows, the news media give publicity to the new diet (and its authors), and the book joins its companions on bookstore shelves. The fact that the new diet book has so many companions on the bookshelves attests to the fact that none of these “new and revolutionary” diets is successful in helping people reduce weight and keep it off. Nevertheless, the new diet gives hope that weight loss may finally be possible, especially for women and girls who dream of achieving their ideal body image.

The desire to be thin can be very compelling. Children as young as 9 years of age have been reported curtailing their food intake to avoid becoming fat [1]. A female's body size too often affects her self-worth and self-esteem. Body image distress results when people become dissatisfied with their weight and, if people believe that their weight and shape are central to their self-worth as a person, an eating-disorder mindset often ensues. Eating disorders affect over 30 million in the United States, with 10–15% being male [2].

The American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition (DSM-5), is commonly used to define eating disorders [3]. The main eating disorder categories listed in DSM-5 are anorexia nervosa, bulimia nervosa, and eating disorder not otherwise specified. This third category includes all other disorders affecting eating.

ANOREXIA NERVOSA

Being too thin is dangerous, even deadly. Anorexia nervosa is a chronic, relapsing illness for many individuals. Of the many psychiatric disorders, anorexia nervosa possesses the highest mortality rate. If left untreated, up to one-fifth of people with

the condition die, often before 30 years of age, and many, despite treatment, die from eating disorder–related complications or suicide.

Anorexia nervosa, described over 100 years ago as a loss of appetite caused by a morbid mental state, is actually misnamed because its victims do not typically experience a loss of appetite. People with anorexia nervosa have a distorted body image and an irrational fear of weight gain. This distorted body image is a perception that they are fat even though they are extremely thin. Furthermore, those with anorexia are extremely critical of their body as a whole and are often more critical about selected body areas (such as thighs, stomach, etc.). Thus, they become obsessed with weight loss and relentlessly pursue thinness, often eating diets providing less than 800 kcal per day.

Eating patterns of people with anorexia nervosa mostly fall into one of two categories: the restricting type or the binge eating–purging type. Individuals with anorexia with the restricting type eat to a very limited

extent without regularly inducing vomiting or misusing laxatives or diuretics. People with the binge eating–purging type alternate between restricting food intake and bouts of binge eating or purging behavior with laxative or diuretic misuse or self-induced vomiting [3]. However, in addition to these controlled eating behaviors, those with anorexia often exercise excessively to further weight loss efforts, to prevent possible weight gain, and to try to correct perceived imperfections in body size and shape. Exercise is considered excessive if its postponement is accompanied by intense guilt or when it is undertaken solely to influence weight or shape.

Some of the diagnostic criteria for anorexia nervosa (Table 1) based on DSM-5 include refusal to maintain body weight at or above minimally normal weight for age and height (e.g., at least 85% of expected weight for height; or, from the *International Classification of Diseases*, a body mass index of at least 17.5 kg/m²), intense fear of gaining weight or being fat, and **amenorrhea** (absence of at least three consecutive

Table 1 Diagnostic Criteria for 307.1 Anorexia Nervosa

- | | |
|----|--|
| A. | Refusal to maintain body weight at or above a minimally normal weight for age and height (e.g., weight loss leading to maintenance of body weight less than 85% of that expected; or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected). |
| B. | Intense fear of gaining weight or becoming fat, even though underweight. |
| C. | Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or denial of the seriousness of the current low body weight. |
| D. | In postmenarcheal females, amenorrhea, i.e., the absence of at least three consecutive menstrual cycles. (A woman is considered to have amenorrhea if her periods occur only following hormone, e.g., estrogen, administration.) |

Specify type:

Restricting Type: during the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Binge-Eating/Purging Type: during the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior (i.e., self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Source: *Diagnostic and Statistical Manual of Mental Disorders*, Fifth Edition, Text Revision, 2013.

menstrual cycles). Self-worth based on weight or shape, preoccupation with food, and abnormal food consumption patterns are also typical of those with anorexia nervosa [4].

The causes of anorexia nervosa are unknown, but the disease is thought to be multifactorial. Genetic vulnerability as well as anxiety, obsessive–compulsive personality disorders, and perfectionism traits are typically present in those who develop anorexia nervosa [5]. Individuals with anorexia may also exhibit depression and substance abuse. In addition, those who develop anorexia nervosa often have a poor self-image and want to please others because their perceived self-worth is heavily dependent upon the words and actions of others (such as teachers, coaches, or instructors). Other traits associated with the development of this eating disorder include issues concerning food and body weight, issues concerning relationships with oneself and with others, conflict regarding maturation, and problems with separation, sexuality, self-esteem, and compulsivity [5].

The initial weight loss of the person suffering from anorexia may not always result from a deliberate decision to diet; initial weight loss may occur unintentionally, for example, as the result of the flu or a gastrointestinal disorder. However, following the initial weight loss, whatever its cause, additional diet restriction (and excessive exercise) is deliberate. Weight loss or control of body weight becomes the overriding goal in life, especially during stressful periods when pressures become overwhelming. The person learns the caloric contents of foods and the energy expenditure associated with various activities. Because those with anorexia have such a disturbed body image and such an intense fear of becoming fat, they may continue starving themselves to emaciation and even death should intervention be delayed too long.

The effects of anorexia nervosa on the body are similar to the effects of hypometabolic states (such as starvation, protein-calorie malnutrition, or marasmus) and affect all parts of the body. Table 2 lists some potential consequences of anorexia nervosa. Growth and development slow. Adipose tissue, lean body mass, and bone mass are lost. Organ mass may be lost, and organ function may become impaired. Loss of heart muscle can weaken the heart and

Table 2 Some Potential Medical Complications of Anorexia Nervosa

Gastrointestinal	Hematologic
• Gastric distention	• Anemia
• Constipation	Skeletal
Cardiovascular	• Stress fractures
• Heart muscle atrophy	• Premature osteoporosis
• Bradycardia	Muscle
• Hypotension	• Depleted muscle mass
• Arrhythmias	Brain
• Mitral valve prolapse	• Abnormal electrical activity
• Peripheral edema	• Confusion
Endocrine/ Metabolic	
• Amenorrhea	
• Hypothermia	

cause, among other serious complications, an irregular heartbeat or a prolonged QT interval (the time that it takes for the heart to contract and refill with blood; with a prolonged QT interval, the heart takes longer to recharge between beats in preparation for the next heartbeat). The gastrointestinal tract atrophies such that peristalsis is slowed, gastric emptying is delayed, and intestinal transit time is lengthened. The secretion of digestive enzymes and of digestive juices is also diminished. Constipation often results, along with abdominal distention after eating just small amounts of food. Hormone and nutrient levels in the blood become altered. Skin typically becomes dry, hair loss from the head occurs while **lanugo**-type (soft woolly) hair may appear on the sides of the face and arms, and body temperature drops. A long-term consequence of the bone loss that occurs with anorexia nervosa is osteopenia and ultimately osteoporosis, which occurs much earlier in those who have (or have had) anorexia nervosa than in those who have not had the condition [6].

Treatment of anorexia nervosa is multidisciplinary (involving a physician, dietitian, nurse, psychologist, psychiatrist, and family therapist, among others) and may be accomplished through outpatient or inpatient care, depending on the severity of the condition. Assessment for inpatient treatment generally includes an evaluation of the person's mental status, how much the person is eating, current weight (inpatient treatment is warranted if weight is <

25–30% of ideal), speed of weight loss, motivation and adherence to treatment, family support, purging behavior, and comorbid complications, especially those affecting the heart [7]. Whether the patient is treated as an inpatient or as an outpatient, goals for the patient's health are established, often with a written contract signed by the patient as well as by members of the health care team.

Summaries of treatment outcomes for anorexia nervosa show that about 40–50% recover completely, about 30% improve, 20–25% continue to experience chronic problems with the condition, and another 10–15% die from medical complications, suicide, or malnutrition. Mortality is typically highest among people who have sustained severe weight loss, who have had the condition for a prolonged duration, and who developed the condition at an older age [8–10].

BULIMIA NERVOSA

Bulimia nervosa, another eating disorder, is a condition characterized by recurring binge eating coupled with self-induced vomiting and misuse of laxatives, diuretics, or other medications to prevent weight gain. Binge eating is marked by a sense of lack of control over eating during the binge episode. A binge is defined as eating an amount of food larger than most people would eat during a similar time period and under similar circumstances [3]. Bulimia denotes a ravenous appetite (or “ox hunger”) associated with powerlessness to control eating. Criteria for the diagnosis of bulimia nervosa are given in Table 3.

Bulimia occurs primarily in young women, especially college-age women who are of normal weight or are slightly overweight. The typical person with bulimia, rather than being overly concerned with losing weight and becoming very thin (like the person with anorexia nervosa), seeks to be able to eat without gaining weight. Other factors associated with the development of bulimia include a history of sexual abuse, psychoactive substance abuse or dependence, a family history of depression or alcoholism, obsessive–compulsive disorder, negative self-evaluation, and a high use of escape-avoidance coping [8].

Bulimia often starts with dieting attempts in which hunger feelings get out of control. These dieting attempts, usually

Table 3 Diagnostic Criteria for 307.51 Bulimia Nervosa

- A.** Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following:
- (1) eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat during a similar period of time and under similar circumstances.
 - (2) a sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).
- B.** Recurrent inappropriate compensatory behavior in order to prevent weight gain, such as self-induced vomiting; misuse of laxatives, diuretics, enemas, or other medications; fasting; or excessive exercise.
- C.** The binge eating and inappropriate compensatory behaviors both occur, on average, at least twice a week for 3 months.
- D.** Self-evaluation is unduly influenced by body shape and weight.
- E.** The disturbance does not occur exclusively during episodes of anorexia nervosa.

Specify type:

Purging Type: during the current episode of bulimia nervosa, the person has regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Nonpurging Type: during the current episode of bulimia nervosa, the person has used other inappropriate compensatory behaviors, such as fasting or excessive exercise, but has not regularly engaged in self-induced vomiting or the misuse of laxatives, diuretics, or enemas.

Source: *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision, 2013.*

based on food abstinence or excessive food restriction, lead to binge eating. Once binge eaters discover that they can undo the consequences of their overeating by vomiting the ingested food, they begin to binge not only when they are hungry but also when they are experiencing any distressing emotion [8]. Most binge eating is done privately in the afternoon or evening, with an intake of about 3,500 kcal; purging behaviors reduce retention of energy to about 1,200 kcal [11]. Favorite foods for bingeing are usually dessert and snack foods very high in carbohydrates.

Diagnosis is usually dependent upon self-reported symptoms or on treatment for related problems or conditions. Conditions that may develop as the result of bulimia are listed in Table 4. The gastrointestinal tract is greatly affected by repeated vomiting and the use of laxatives. Repeated vomiting also causes other problems, including skin lesions or calluses on the dorsal side of the hands (especially over the joints), severe dental erosion, swollen enlarged neck glands (due to salivary or parotid gland enlargement), reddened eyes, headache, and fluid and electrolyte imbalances. Laxative misuse may exacerbate fluid and electrolyte losses and, when coupled with vomiting, may lead to heart arrhythmias and heart failure. The presence of lesions or calluses on the hands (due to the scraping

of teeth against the skin while self-inducing vomiting), swollen neck glands, and frequent trips to the bathroom after meals are often recognized by health professionals and family or friends and facilitate detection and diagnosis of the problem.

The treatment of bulimia, like that of anorexia nervosa, is multidisciplinary. Goals typically focus on eliminating binge-purge behaviors, normalizing eating habits, maintaining weight, and resuming normal menses, if they are affected. The patient is most likely to be hospitalized with problems such as electrolyte imbalance, drug (e.g., laxatives, diuretics) dependence, severe

depression, or suicidal tendencies [12]. The prognosis of those suffering from bulimia nervosa is generally more favorable than for those with anorexia nervosa; over 50% of those with bulimia nervosa fully recover or achieve good outcomes, while fewer than 10% have poor outcomes [13].

BINGE EATING DISORDER

The American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders* includes a provisional eating disorder diagnosis, binge eating disorder, which is not associated with purging as in bulimia nervosa [3]. Binge eating disorder is characterized by binge eating at least twice a week for at least a 6-month period with no compensatory behaviors. The binge eating episode typically involves eating (usually with a general sense of lack of control) large amounts of highly energy-dense foods (such as dessert and snack food-type items) more rapidly than normal, and it continues despite the individual feeling uncomfortably full.

Factors associated with binge eating include repeated exposure to negative comments about eating, shape, and weight; depression; negative self-evaluation; and vulnerability to obesity [14]. For those with a binge eating disorder, food often provides comfort and a sense of emotional well-being, especially if the person is feeling stressed, anxious, unhappy, or depressed. Thus, the binge usually occurs when the person is not physically hungry but is emotionally unhappy. The binge usually happens in private because of embarrassment and, following the binge, feelings of disgust, guilt, and depression are common.

Table 4 Some Potential Medical Complications of Bulimia Nervosa

Gastrointestinal	Cardiovascular
● Erosion of the teeth ● Dental caries ● Sore throat ● Swollen parotid glands ● Esophageal rupture or tears ● Stomach tear ● Gastroesophageal reflux disease ● Constipation ● Cathartic colon	● Arrhythmias
	Respiratory and Skeletal
	● Aspiration pneumonia ● Rib fracture
	Endocrine/Metabolic
	● Irregular menses or amenorrhea ● Electrolyte imbalance

EATING DISORDERS NOT OTHERWISE SPECIFIED

Eating disorders (Table 5) other than anorexia nervosa, bulimia nervosa, and binge eating disorder are categorized by the American Psychiatric Association as eating disorders not otherwise specified [3]. The characteristics of those with disordered eating are similar to those of individuals with anorexia nervosa and bulimia nervosa and include fear of being fat, restrained eating, binge eating, purging behavior, and distorted body image; however, people with disordered eating do not meet the criteria for anorexia nervosa or bulimia nervosa

Table 5 307.50 Eating Disorder Not Otherwise Specified diseases.

The Eating Disorder Not Otherwise Specified category is for disorders of eating that do not meet the criteria for any specific Eating Disorder. Examples include

1. For females, all of the criteria for anorexia nervosa are met except that the individual has regular menses.
2. All of the criteria for anorexia nervosa are met except that, despite significant weight loss, the individual's current weight is in the normal range.
3. All of the criteria for bulimia nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur at a frequency of less than twice a week or for a duration of less than 3 months.
4. The regular use of inappropriate compensatory behavior by an individual of normal body weight after eating small amounts of food (e.g., self-induced vomiting after the consumption of two cookies).
5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.
6. Binge-eating disorder: recurrent episodes of binge eating in the absence of the regular use of inappropriate compensatory behaviors characteristic of bulimia nervosa.

Source: *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision, 2013.*

(Tables 1 and 3). Disordered eating is likely more common than anorexia and bulimia nervosa and is seen in both males and females, especially female athletes, where it often exists as part of the female athlete triad.

The Female Athlete Triad

The female athlete triad, first described in 1992 by the American College of Sports Medicine, is defined as the combination of disordered eating, amenorrhea, and osteopenia. The condition is described as a complex interrelationship between menstrual status, bone health, and energy availability. The condition appears most often in women participating in sports in which physique and body image are important and extra body weight is undesirable. While all female athletes are considered at risk, women most at risk include long-distance runners, figure skaters, gymnasts, ballet dancers, swimmers, and divers [15].

Inadequate energy intakes among athletes is common, reported as high as 58% among endurance athletes [16]. The reasons for inadequate energy intake among athletes may be due to poor nutritional knowledge, intentional energy restriction to achieve a specific weight or physique, or disordered eating.

The causes of amenorrhea in female athletes with eating disorders are not clearly understood. They are thought to relate to synergistic effects of excessive amounts of physical activity and training, constant stress or anxiety, low amounts of body fat, weight fluctuations, and poor diet, especially an extreme energy (caloric) deficit [15]. The effect of these various factors is to evoke changes in the release of hormones such as follicular

stimulating and luteinizing hormones that then lead to diminished release of estrogen and progesterone (among other hormones), which in turn can cause amenorrhea. The amenorrhea—more specifically, the diminished serum estrogen concentrations—in turn negatively impacts the skeletal system, similar to what is observed in those with anorexia nervosa [6]. Premature bone loss, inadequate bone formation, or both with resulting low bone mass, stress fractures, and other orthopedic problems are common among female athletes; in fact, over 50% of athletes with amenorrhea have low bone mass or bone densities at least one standard deviation below the mean [8]. High levels of cortisol in the blood, common in athletes, and extensive training regimens can also contribute to bone loss along with poor energy and nutrient (especially vitamin D and calcium) intakes. Some of the American College of Sports Medicine's recommendations for the prevention and treatment of the female athlete triad emphasize optimizing energy and nutrient availability and providing medications, as needed, to improve bone health and for psychological problems, although the full reversal of low bone mineral density is thought to be unlikely [17].

SUMMARY

The early identification and treatment of the female athlete triad as well as eating disorders is crucial if serious complications are to be avoided. Just as obesity is associated with increased risk for a variety of diseases, eating disorders are associated with a multitude of risks, including death. Combating eating disorders is difficult; not only must the victims be treated, but the values

of society, including images of beauty, must also be rehabilitated.

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WATER-SOLUBLE VITAMINS

LEARNING OBJECTIVES

- 9.1** Identify particularly good food sources of the water-soluble vitamins.
- 9.2** Explain how the water-soluble vitamins are digested, absorbed, transported in the blood, and stored.
- 9.3** Describe the metabolism of the water-soluble vitamins in the small intestine, liver, and kidneys.
- 9.4** Describe the functions and mechanisms of action of the water-soluble vitamins.
- 9.5** Identify the means by which the water-soluble vitamins are excreted.
- 9.6** Describe recommended intakes, deficiencies, and toxicities associated with the water-soluble vitamins.
- 9.7** Identify measures used for water-soluble vitamin status.
- 9.8** Compare the water-soluble versus fat-soluble vitamins in terms of absorption, storage, and likelihood for toxicity.

THE EARLY PART OF THE 20TH CENTURY was the most exciting era in the history of nutrition science. It was during this time that the discovery of vitamins, or “accessory growth factors,” began. Researchers found that for life and growth, animals required something more than a chemically defined diet consisting of purified carbohydrate, protein, fat, minerals, and water. The first of these dietary essentials was discovered as a result of a search to cure a “condition.” An “antiberiberi substance,” which cured the “condition” now called “**beriberi**,” was isolated from rice polishings by Casimir Funk, a Polish biochemist. Funk gave the substance the name *vitamine* because the substance was chemically an amine and was necessary for life (*vita* means “life” in Latin). Very shortly thereafter McCollum and Davis extracted a factor from butter fat that they called fat-soluble A to distinguish it from the previously isolated water-soluble substance. These two essential factors became known as *vitamine A* and *vitamine B*.

Much of the initial research used to identify vitamins relied on the historical method, which seeks to explain the cause of past events and to interpret current happenings on the basis of these findings. Sources of information for the historical researcher are primarily documentary, existing in the form of written records and accounts of past events as well as literary productions and critical writings. The researcher relies, if possible, only on primary data, that is, data that are “firsthand” and therefore minimally distorted by the channels of communication. Generally, information gathered by historical research does not need to be analyzed by any form of statistical treatment or data analysis.

As each additional vitamin was discovered, it was assigned a letter. The *e* on *vitamine* was dropped to give the general name *vitamin* because only a few of these essential substances were found to be amines. As the chemical structure of a vitamin became known through its isolation and synthesis, it was given a chemical name. Each chemical name assigned was assumed to only apply to one substance, with one specific activity. We now know that a vitamin may have a variety of functions and that vitamin activity may be found in several

closely related compounds known as vitamers. An excellent example of this range of activity is vitamin A, which has several seemingly unrelated functions and encompasses not only retinol but also retinal and retinoic acid. Additionally, we know that some vitamins, like vitamin D and the B-vitamins, need to be chemically modified in body tissues to function.

Vitamins are organic compounds with regulatory functions. They are required (usually in small quantities) in the diet because of the inability to synthesize them. Thus, vitamins are “essential,” with inadequate consumption causing specific syndromes or manifestations. Although the clinician should be able to recognize the deficiency syndrome caused by a lack of a particular vitamin, in a

country with an abundant and varied food supply such as the United States, the nutrition professional should think in terms of what specific functions a vitamin performs in the body and to a lesser extent what disease it prevents. Table 9.1 provides a list of the water-soluble vitamins along with their functions and other aspects associated with each vitamin. Unfortunately, relating the function of the vitamin directly to its deficiency syndrome is often difficult. Moreover, some of the signs and symptoms associated with the deficiency of one vitamin may also occur with a deficiency of another vitamin and/or mineral. Thus, diagnosis of deficiency is best based on an assessment of the larger clinical picture, including biochemical parameters versus just one or two signs or symptoms. Table 9.2 summarizes

Table 9.1 The Water-Soluble Vitamins: Coenzyme Forms, Functions, Deficiency Syndromes, Food Sources, and Recommended Intake

Vitamin	Main Coenzyme(s)	Biochemical or Physiological Function(s)	Deficiency Syndrome, Symptoms, or Affected Systems	Major Food Sources	RDA* or AI†
Thiamin (B ₁)	Thiamin diphosphate (TDP)	Oxidative decarboxylation of α -keto acids, interconversion of phosphorylated sugars, nervous system functions	Beriberi — Nervous system and cardiac dysfunction	Pork, sunflower seeds, legumes	1.1 mg* 1.2 mg
Riboflavin (B ₂)	Flavin adenine dinucleotide (FAD); flavin mononucleotide (FMN)	Electron (hydrogen) transfer reactions for nutrient metabolism and energy production	Ariboflavinosis — Cheilosis, glossitis, angular stomatitis, edema oral cavity, dermatitis	Meats, eggs, yogurt, cheese, milk	1.1 mg* 1.3 mg
Niacin (B ₃) (nicotinic acid, nicotinamide)	Nicotinamide adenine dinucleotide (NAD); nicotinamide adenine dinucleotide phosphate (NADP)	Electron (hydrogen) transfer reactions for nutrient metabolism and energy production; ADP ribose transfer	Pellagra — Diarrhea, dermatitis, mental confusion	Fish, meats, peanut butter	14 mg* 16 mg
Pantothenic acid	Coenzyme A (CoA) and 4' phosphopantetheine	Acyl transfer reactions; acetylation/acylation of proteins, sugars, and other substrates; gene expression	Burning foot syndrome — burning of the feet, neuritis	Widespread in foods	5 mg†
Biotin	Carboxybiotin	CO ₂ transfer/carboxylation reactions for nutrient metabolism and energy production; gene expression	Dermatitis, hypotonia, nervous system dysfunction, alopecia	Liver, soybeans, eggs	30 μ g†
Vitamin B ₆ (pyridoxine, pyridoxal, pyridoxamine)	Pyridoxal phosphate (PLP)	Variety of reactions for nutrient metabolism and energy production; gene expression	Dermatitis, cheilosis, glossitis, angular stomatitis, nervous system dysfunction	Beef, fish, legumes, potato, banana, whole grains	1.3 mg*
Folate	Tetrahydrofolate (THF) derivatives: 5,10-methylene THF, 10-formyl THF, 5-formimino THF, 5,10-methylenyl THF, 5-methyl THF	One-carbon transfer reactions for nutrient metabolism and energy production; gene expression; purine and pyrimidine synthesis for DNA and RNA; hematopoiesis	Megaloblastic anemia — enlarged immature red blood cells, diarrhea	Green vegetables — spinach, asparagus, and greens; legumes; fortified grain products	400 μ g*
Vitamin B ₁₂ (cobalamin)	Methylcobalamin, adenosylcobalamin	Nutrient metabolism; energy production; hematopoiesis	Megaloblastic anemia, degeneration of peripheral nerves	Animal products — meat, fish, shellfish, poultry, milk	2.4 μ g*
Ascorbic acid (vitamin C)	None	Antioxidant; cosubstrate for some hydroxylation and amidation reactions	Scurvy — hyperkeratosis of hair follicles, psychological manifestations Impaired collagen — bleeding gums, ruptured capillaries	Citrus fruits and juices, noncitrus fruits, broccoli, Brussels sprouts, green peppers	75 mg* 90 mg

Table 9.2 Some Common Signs Associated with Water-Soluble Vitamin Deficiencies

System/Sites	Common Selected Signs	Possible Vitamin Deficit
Gastrointestinal tract	Cheilosis and/or glossitis	B ₂ , B ₃ , B ₆ , folate
Oral cavity	Nausea and vomiting and/or diarrhea	B ₁ , B ₂ , biotin, pantothenic acid
Stomach and intestines		
Skin	Petechiae	C
	Pigmentation changes, erythema	B ₂ , B ₃
	Dermatitis	B ₂ , B ₃ , B ₆ , biotin
	Collagen disturbances	C
Skeletal	Pain and impairments	C
Bone and teeth		
Cardiovascular and pulmonary	Cardiomegaly, tachycardia	B ₁
	Shortness of breath	Folate, B ₁₂
Blood vessels		C, B ₆
Hematopoiesis	Megaloblastic anemia	Folate, B ₁₂
Nervous and muscular* systems	Neuropathy	B ₁ , B ₂ , B ₆ , B ₁₂
	Changes in reflexes	B ₁ , B ₆ , B ₁₂
	Nervous system — general effects	B ₁ , B ₂ , B ₃ , B ₆ , B ₁₂ , biotin, pantothenic acid
	Psychological manifestations	C, B ₁ , B ₃ , B ₆ , biotin
	Hypotonia	Biotin
	Ataxia	B ₁ , B ₆ , biotin
Ocular	Ophthalmoplegia, nystagmus	B ₁
	Photophobia	B ₂

* Muscle weakness and/or fatigue have been reported with deficiencies of B₁, B₆, B₁₂, biotin, folate, and pantothenic acid.

some of the signs associated with deficiencies of water-soluble vitamins.

Vitamins, for the most part, are not related chemically and differ in their physiological roles. The broad classifications of water-soluble vitamins and fat-soluble vitamins

are made because of certain properties common to each group. The body handles the water-soluble vitamins differently from the way it handles the fat-soluble vitamins (discussed in Chapter 10). Water-soluble vitamins are absorbed into portal blood, in contrast to fat-soluble vitamins, which initially enter the lymphatic system as part of a chylomicron. Furthermore, with some exceptions, water-soluble vitamins are generally excreted in the urine whenever plasma levels exceed renal thresholds; they are also not stored in large quantities in body tissues. Water-soluble vitamins, with the exception of vitamin C, are members of the B-complex. Most of the B-complex group can be further divided according to some of their general functions such as energy production and nutrient metabolism, hematopoiesis, and gene expression (Figure 9.1).

In this chapter, discussions of the vitamins are presented similarly. Each vitamin is considered (when precise information is available) in terms of structure, sources, absorption (also digestion where applicable), transport, tissue uptake, storage, functions and mechanisms of action, metabolism and excretion, recommended intake, deficiency, toxicity, and assessment of nutriture. Specific interrelationships with other nutrients are also noted for selected vitamins. Table 9.3 provides an overview of aspects of water-soluble vitamin absorption, transport in systemic circulation, and storage. From this table, it is clear that most water-soluble vitamins (exception vitamin B₁₂) are absorbed primarily in the duodenum and jejunum (proximal small intestine), and many organs contain small amounts of the vitamins.

Foods, as opposed to supplements, are the preferred sources for the intake of vitamins. Diets rich in fruits, vegetables, and whole grains are associated with reductions in risks for heart disease, type 2 diabetes, metabolic syndrome, and some cancers, among other conditions. Whole grains

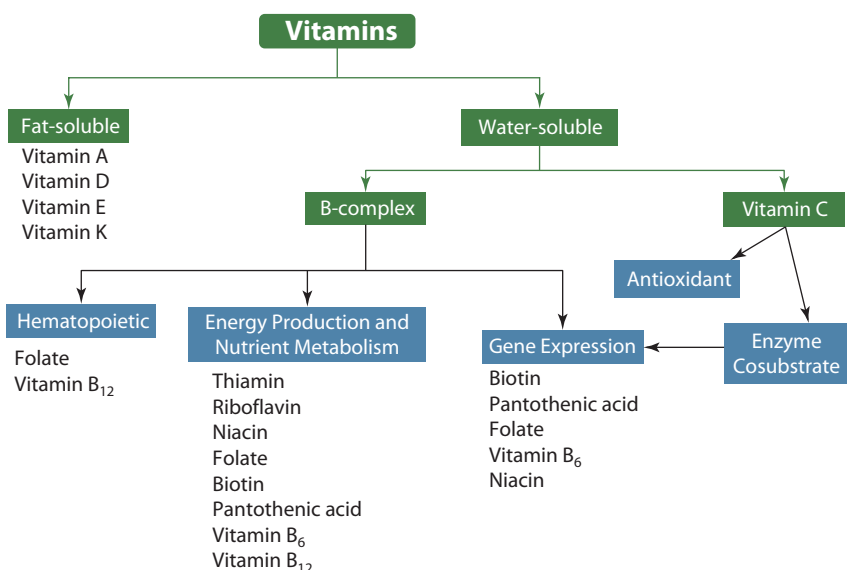
**Figure 9.1** The vitamins, including some functional roles of the water-soluble vitamins.

Table 9.3 Gastrointestinal Tract Absorption, Form in Systemic Circulation, and Storage Sites for the Water-Soluble Vitamins

Vitamin	Absorption Site(s)	Main Method(s)	Form(s) in Blood	Storage Sites(s)
C	Small intestine, jejunum	SVCT1*, SVCT2, GLUT1, diffusion [†]	Free ascorbic acid, dehydroascorbic acid (minor)	Adrenal and pituitary glands, eyes, brain, white blood cells, neurons
Thiamin	Duodenum, jejunum, colon	ThT1*, ThT2, diffusion	Free thiamine, TDP	Muscle, heart, brain, kidneys, liver
Riboflavin	Duodenum, jejunum, colon	RFVT1, RFVT2, RFVT3, diffusion	Protein-bound riboflavin, FMN, and FAD	Liver, kidneys, heart
Niacin	Small intestine, colon	Carrier (unclear), diffusion	Free nicotinamide and nicotinic acid, protein-bound nicotinic acid	Liver, other tissues
Pantothenic acid	Jejunum, duodenum, colon	SMVT, diffusion	Free pantothenic acid	Liver, adrenal glands, kidneys, brain, heart
Biotin	Proximal small intestine, colon	SMVT, diffusion	Free and protein-bound biotin	Liver, muscle, brain
Folate	Duodenum, jejunum, colon	PCFT*, RFC, diffusion	Free and protein-bound THF, 5-methyl THF, 10-formyl THF	Liver, kidneys, other
Vitamin B ₁₂	Ileum	IF receptor-mediated, diffusion	Protein-bound methyl-, adenosyl-, cyano-, and hydroxocobalamins	Liver, muscle, pituitary gland, bone, kidneys, heart, brain, spleen
Vitamin B ₆	Jejunum	Diffusion	Free and protein-bound PL and PLP	Muscle, liver, brain, kidneys, spleen

Abbreviations: SVCT, sodium-dependent vitamin C transporter; ThT, thiamin transporter; TDP, thiamin diphosphate; RFVT, riboflavin vitamin transporter; SMVT, shared multivitamin transporter; PCFT, proton couple folate transporter; RFC, reduced folate carrier; IF, intrinsic factor; PL, pyridoxal; PLP, pyridoxal phosphate.

*Thought to be the primary carrier

provide dietary fiber and several micronutrients including vitamin E, thiamin, riboflavin, niacin, pantothenic acid, and vitamin B₆ along with minerals, phytochemicals and bioactive compounds. Fruits and vegetables are major sources of carotenoids, vitamin C, vitamin E, vitamin K, folate, riboflavin, and niacin, as well as a host of minerals, dietary fiber, and thousands of phytochemicals and bioactive compounds. These nutrients are likely working synergistically or antagonistically through different mechanisms to reduce disease risk. However, scientific evidence supports the consumption of foods (such as fruits, vegetables, whole grains, low-fat dairy products and meats, seafood, nuts, seeds, and legumes) versus the use of supplements to meet

nutrient needs and to reduce the risk of diseases including cancer, cardiovascular disease, cardiovascular disease events (stroke, heart attack, cardiac revascularization, or cardiovascular disease mortality) and all-cause mortality [1–6].

Several different types of research methodologies are used to arrive at recommendations for food groups and individual nutrients. The Perspective at the end of this chapter provides more information on some of the different types of research methodologies and their limitations. Information in the feature Dietary Reference Intakes (DRIs) provides information on nutrient recommendations as well as Daily Values for nutrients which are found on food and supplement facts labels.

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Suggested Readings

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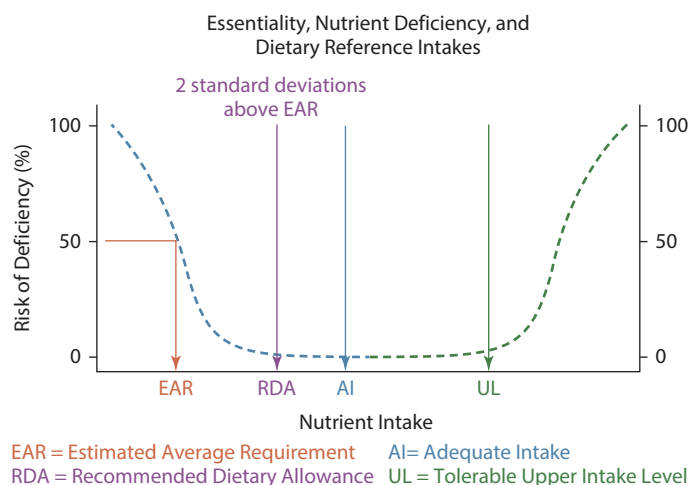
DIETARY REFERENCE INTAKES (DRIs)

Dietary Reference Intakes (DRIs) represent quantitative approximations of nutrient needs for the purpose of planning and assessing the diets of healthy people. DRIs include **Estimated Average Requirements (EARs)**, **Recommended Dietary Allowances (RDAs)**, **Adequate Intakes (AIs)**, **Tolerable Upper Intake Levels (ULs)** (also sometimes abbreviated *TULs*), and Chronic Disease Risk Reduction Intakes. The recommendations are established by the Food and Nutrition Board of the U.S. National Academy of Sciences.

RDAs were first developed in 1942 as a goal for good nutrition; recommendations were provided for energy and nine nutrients, including protein, iron, calcium, vitamins A and D, thiamin, riboflavin, niacin, and vitamin C. The Food and Nutrition Board has continued to re-evaluate nutrient recommendations over the years with subsequent publications every 10 or so years. The 10th edition of the RDAs was published in 1989. Between 1997 and 2001, RDAs for several nutrients were reevaluated and published. The latest RDAs were published for vitamin D and calcium in 2011, and new AIs were released for sodium and potassium in 2019.

RDAs represent the average daily dietary intake level that is sufficient to meet the nutrient requirements of about 97% of healthy people. They are based on EARs, which are the amounts of nutrients thought to meet the nutrient requirements of 50% of the healthy people in a specified age and gender group. RDAs are set higher than EARs by either two standard deviations or a coefficient of variation for the EAR. Consequently,

- The intake of a nutrient is likely inadequate if it is significantly less than the EAR.



- An intake of a nutrient above the EAR but less than the RDA may still be inadequate.

AIs are provided for nutrients instead of RDAs when scientific data are insufficient to calculate the EARs. AIs are based on nutrient intake levels of healthy people (with adequate nutritional status) and are typically thought to exceed the requirement for the nutrient.

- Nutrient intakes are likely adequate if they meet or exceed the AI.
- Nutrient intakes may or may not be adequate if they are less than the AI.
- Nutrient intakes that are greater than the RDA but less than the UL are likely to be adequate.

ULs provide the highest intake level for a nutrient that is unlikely to cause any risk of adverse health to almost all people in the age- or gender-specified groups. ULs are viewed as maximum amounts, especially for those consuming fortified foods or supplements in large quantities. For some nutrients, the UL is not known, but the lack of a UL does not mean that large doses of the nutrient are harmless. Moreover, while

the ULs provide intake levels that are not likely to result in adverse health effects, the ULs do not include risks for chronic diseases.

In addition to EARs, RDAs, AIs, and ULs, some countries have also adopted recommendations aimed at preventing chronic disease (vs. preventing deficiency) for some nutrients. In 2017, in the United States, the National Academies published a report that established the DRI category, Chronic Disease Risk Reduction Intakes (CDRRs). The CDRRs recommendations are designated for nutrients in which it is well established that excessive consumption of that nutrient contributes to chronic disease risk. CDRRs may also be established when sufficient evidence demonstrates that the intake of a nutrient in excess of its current reference value may help prevent chronic diseases. Further information on the CDRRs is available from the National Academies of Sciences, Engineering, and Medicine. Guiding Principles for Developing Dietary Reference Intakes Based on Chronic Disease. Washington, DC: National Academies Press. 2017. doi: 10.17226/24828

DAILY VALUES AND PERCENTAGE DAILY VALUES

Percentage (%) Daily Values (DVs) for nutrients are found on Nutrition and Supplement Facts labels, regulated by the U.S. Food and Drug Administration. Percentage DVs enable comparisons of nutrients across different foods and, to a lesser extent, allow for an evaluation of a food's contribution to dietary nutrient needs.

A nutrient's DV is based on its highest Recommended Dietary Allowance (RDA) among population groups ranging from children age 4 years to adults. Because the DV is based on the highest of a nutrient's RDA, the DVs for some nutrients may be higher than the nutrient's RDA for a *particular* group. An explanation and example using iron is described hereafter.

The %DV, shown on the food and nutrition facts labels, for a particular vitamin or mineral indicates the amount of the particular nutrient provided by a serving of the food (or supplement) compared with the nutrient's DV. For example, if one serving (1 oz) of cheese contains 260 mg calcium, the %DV is 20% (i.e., 260 mg calcium in the serving of cheese/1300 mg, which is the DV for calcium, $\times 100$).

The DVs for nutrients were updated in January 2020; prior to this time the DVs

Source: United States Food and Drug Administration

Nutrition Facts	
8 servings per container	
Serving size	2/3 cup (55g)
Amount per serving	
Calories	230
% Daily Value*	
Total Fat 8g	10%
Saturated Fat 1g	5%
Trans Fat 0g	
Cholesterol 0mg	0%
Sodium 160mg	7%
Total Carbohydrate 37g	13%
Dietary Fiber 4g	14%
Total Sugars 12g	
Includes 10g Added Sugars	20%
Protein 3g	
Vitamin D 2mcg	10%
Calcium 260mg	20%
Iron 8mg	45%
Potassium 235mg	6%

* The % Daily Value (DV) tells you how much a nutrient in a serving of food contributes to a daily diet. 2,000 calories a day is used for general nutrition advice.

The Daily Value for iron is 18 mg and is based on the RDA for adult women (of childbearing age). A serving of this product provides 8 mg iron, as shown here. The %Daily Value (shown on the right side of the facts label) is calculated by dividing the amount in the serving (8 mg) by the Daily Value for iron of 18 mg. Thus, $8/18 \times 100$ (and rounded up) = 45%.

However, while women (of childbearing age) would receive 45% of their daily needs from a serving, adult men who have a lower RDA of 8 mg would actually be receiving 100% of their needs.

were based on RDAs/AIs from 1968. All vitamins and minerals have DVs; however, they are not all required to be listed on a food's nutrition label and supplement facts label. Labels must include information on three minerals, calcium, iron, and potassium, and on one vitamin, vitamin D. Vitamins A and C, which have been required to appear on the label in the past,

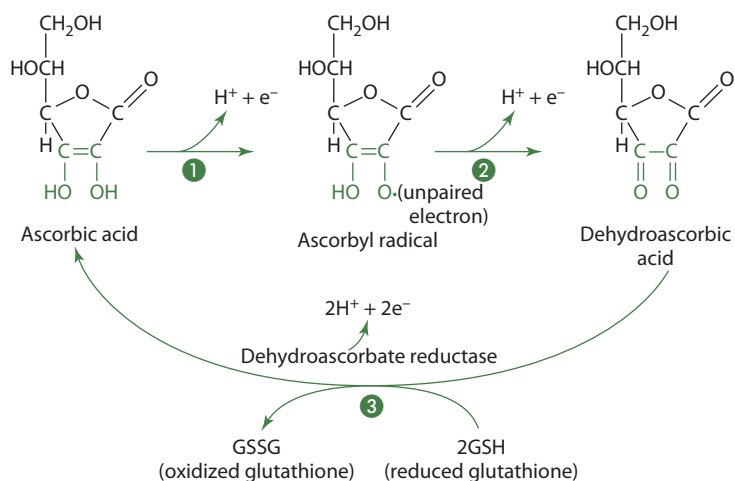
may still be listed but are not required. Other vitamins and minerals must be included on the label if the food has been fortified with the particular vitamin or mineral or if the product displays a nutritional claim about the particular vitamin or mineral. Other nutrients, however, may be listed on the label on a voluntary basis by the food manufacturer.

9.1 VITAMIN C (ASCORBIC ACID)

Vitamin C is also known as ascorbic acid (AH_2). At physiological pH, the vitamin loses a hydrogen and is usually in its ionized form (AH^-), referred to as ascorbate (also sometimes called ascorbate [mono]anion or monodehydroascorbate). Oxidation of ascorbate generates the ascorbyl radical $\text{A}^{\cdot-}$, which has one electron and one proton less than ascorbate; this form of the vitamin is unreactive and usually dismutates (i.e., undergoes simultaneous oxidation and reduction) to form ascorbate and dehydroascorbic acid. Dehydroascorbic acid is the oxidized form of the vitamin (with two electrons and

two protons/hydrogens removed); it has vitamin activity because it can be reduced back to form ascorbate in body cells. These forms of the vitamin are shown in Figure 9.2.

Vitamin C was isolated in 1928, and its structure was determined in 1933. But this vitamin's deficiency disorder—known as **scurvy**—had been prevalent for centuries. Some of the most notable stories are those of the British sailors who frequently died from scurvy on sea voyages. It was a physician who eventually found that sucking the juice from a lime was protective, and in the late 1790s and early 1800s British sailors at sea began receiving limes (resulting in the nickname “limey” for the sailors) in an effort to prevent scurvy. Szent-Györgyi (1928) and King



- 1 During the oxidation of ascorbic acid, a free radical called ascorbyl radical (also called semidehydroascorbic acid radical, ascorbate free radical, ascorbyl, monodehydroascorbate radical) is formed but has a short half-life and reacts poorly with oxygen, so it does not form reactive oxygen species.
- 2 Oxidation of the radical forms of vitamin C.
- 3 Dehydroascorbic acid can be reduced to ascorbic acid with hydrogens provided by the reduced form of glutathione (GSH).

Figure 9.2 Structures and interconversion of ascorbic acid and dehydroascorbic acid.

(1932) are considered co-discoverers of vitamin C. Szent-Györgyi, who isolated the vitamin, and Haworth, who determined its structure, were awarded the Nobel Prize in 1937 for their work on vitamin C.

Vitamin C exists as both a D- and L-isomer; however, it is the L-isomer of the vitamin that is biologically active in humans. Humans (as well as nonhuman primates, fruit bats, guinea pigs, and some birds) are unable to synthesize vitamin C, which is derived from glucose, due to the lack of gulonolactone oxidase, the last enzyme in the vitamin C synthetic pathway (Figure 9.3).

Sources

Food sources of vitamin C (Table 9.4) include primarily fruits (especially citrus) and vegetables, with a few foods providing the recommended intake of the vitamin in a single serving (note that this is not the case for most vitamins and minerals). Major sources of the nutrient are asparagus, papaya, oranges, orange juice, cantaloupe, cauliflower, broccoli, Brussels sprouts, green pepper, grapefruit, grapefruit juice, kale, lemons, red pepper, strawberries, and potatoes. The camu berry (or camu camu), from Peru, is one of the richest sources with 100 g of the fruit containing up to 2 g of vitamin C. Fortified foods, including breakfast cereals, also

contribute some vitamin C to the diet. The Daily Value for vitamin C, found on food and supplement facts labels, is 90 mg.

In foods, vitamin C is found mostly as ascorbic acid, but small amounts of dehydroascorbic acid may also be present. Supplements usually supply vitamin C as ascorbic acid or mineral ascorbates, typically calcium ascorbate and/or sodium ascorbate. (Note: A 1,000 mg dose of calcium ascorbate contains about 100 mg calcium and 900 mg ascorbic acid, and a 1,000 mg dose of sodium ascorbate provides about 110 mg sodium and 890 mg ascorbic acid.) Ester-C® contains mainly calcium ascorbate along with dehydroascorbate (the ionic form of dehydroascorbic acid) and some vitamin C metabolites. Absorption of these various forms of vitamin C does not appear to differ substantially, thus no one form is thought to be significantly superior to another. Dosages in single-ingredient vitamin C supplements range from about 60 to 5,000 mg with many supplying 500 or 1,000 mg. Dosages in multivitamin/mineral supplements are usually closer to the vitamin's Daily Value (90 mg).

A fat-soluble form of vitamin C, ascorbyl palmitate (ascorbic acid esterified to the fatty acid palmitate), is also available in soft gels. Additionally, skin creams containing the vitamin are available and promoted for skin health because of vitamin C's role in collagen synthesis and as

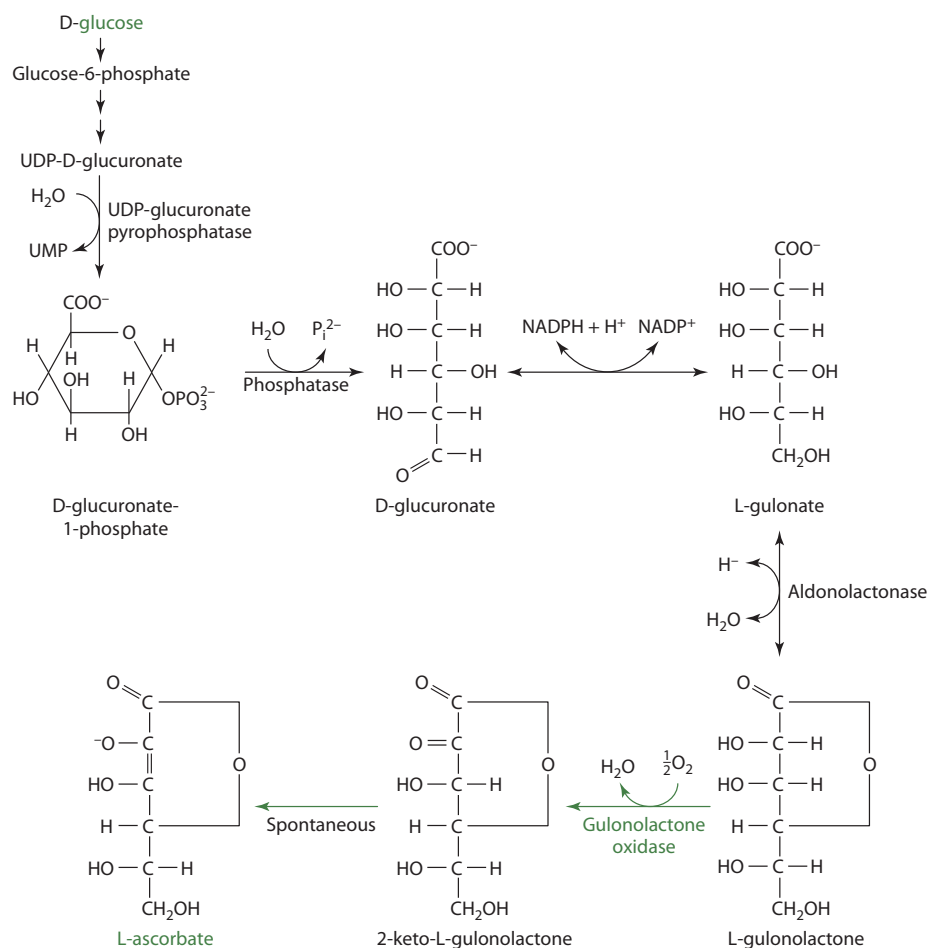


Figure 9.3 Synthesis of ascorbic acid.

Table 9.4 Vitamin C Content of Selected Foods*

Food (serving)	Vitamin C (mg)
Tomato juice, canned (1 c)	170
Orange juice (1 c)	124
Strawberries, sliced (1 c)	98
Papaya, cubed (1 c)	88
Orange, sections (1 c)	87
Grapefruit, sections (1 c)	72
Cantaloupe, cubed (1 c)	68
Kiwi (1)	64
Green pepper, sweet, diced, raw (1/2 c)	60
Broccoli, cooked (1/2 c)	50
Brussels sprouts, cooked (1/2 c)	48
Pineapple, chunks (1/2 c)	46
Avocado, pureed (1 c)	40
Potato, baked with skin (1 large)	29
Cabbage, cooked (1/2 c)	28
Banana (1)	12
Tomato (1 c)	23
Spinach, cooked (1/2 c)	9

A more complete list of vitamin C-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminC-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

an antioxidant (discussed later). Percutaneous absorption of the vitamin is concentration dependent (requiring a topical solution containing at least 10–20% for maximal absorption) and requires an acidic pH (usually < 3.5). Not all forms of the vitamin in these creams, however, effectively penetrate the skin.

Vitamin C is destroyed by heat, light, oxidation, and alkaline solutions but is stable in acidic solutions. Vitamin C is also oxidatively damaged (yielding diketogulonic acid and other products without vitamin C activity) in the presence of oxygen and minerals such as iron and copper. Thus, cooking and storage may reduce the vitamin C content of foods.

Digestion and Absorption

Vitamin C does not require digestion prior to being absorbed into intestinal cells. Absorption of ascorbic acid across the intestinal cell brush border membrane occurs throughout the small intestine, especially in the proximal jejunum, and requires sodium-dependent vitamin C transporters (SVCT) 1 and 2. SVCT1 is the main carrier responsible for intestinal ascorbic acid absorption, carrying two Na^+ and one ascorbic acid molecule. Absorption is down-regulated when the vitamin is present in large

amounts, thus limiting the vitamin's absorption when superphysiological doses are ingested.

The dehydroascorbic acid that is found in foods or that may be formed if ascorbic acid is oxidized in the gastrointestinal tract is absorbed by facilitated diffusion via glucose transporters (GLUT), mainly GLUT 1 and 3. Once absorbed, dehydroascorbic acid is usually rapidly reduced to ascorbate by dehydroascorbic acid reductase in the intestinal cell. Glutathione (GSH), which is required for the reduction of dehydroascorbic acid, is oxidized (to GSSG) in the process as shown in Figure 9.2. NADPH and the dithiol glutaredoxin can also reduce the dehydroascorbic acid.

Over a range of usual vitamin C intake (such as 30–150 mg/day) from foods, vitamin C absorption is about 70–95%. Absorption of the vitamin decreases with increased intake; for example, 16% is absorbed at high intakes (~12 g) versus 98% absorbed at low intakes (~20 mg). At intakes of about 1 g, less than 50% of vitamin C is typically absorbed and, at intakes around 1.25 g, less than 35% is absorbed. Thus, given vitamin C absorption is saturable and dose dependent, more vitamin C is absorbed if several large (1 g) doses of the vitamin are ingested throughout the day than if the same amount is ingested as one single dose.

To enter the blood plasma, vitamin C appears to diffuse out of intestinal cells into the extracellular fluid through volume-sensitive anion channels that create openings or pores in the basolateral membrane. Additionally, vitamin C may exit intestinal cells via calcium-dependent channels, exocytosis, or other unidentified means. Any dehydroascorbic acid that did not get converted to ascorbate within the enterocyte is thought to diffuse into the plasma.

Transport, Tissue Uptake, and Storage

Ascorbic acid and dehydroascorbic acid are transported in the blood primarily in free form; however, only small amounts (about 5%) of dehydroascorbic acid are present due to its rapid cellular uptake. Plasma ascorbic acid concentrations vary, but a typical reference range with adequate vitamin intake is from about 0.6 to 2.0 mg/dL. Higher intakes generate higher peak vitamin concentrations; for example, a vitamin C intake of 1.25 g generated a peak plasma vitamin C concentration of 2.37 mg/dL [1]. (Note: Tissue uptake and fairly rapid urinary excretion of the vitamin reduce plasma concentrations.) Higher plasma vitamin C concentrations can be achieved with intravenous administration of the vitamin (vs. oral ingestion) since intestinal barriers to the vitamin's absorption are circumvented. In the blood, about 70% of the vitamin is found in the plasma and red blood cells and about 30% is found in the white blood cells.

Ascorbic acid uptake into body cells occurs by SVCT1, which exhibits a relatively high capacity but low affinity,

and SVCT2, which displays low capacity but high affinity. SVCT1 is found primarily in the intestine, liver, and kidneys. SVCT2, present in most metabolically active tissues including in the brain, neurons, bone, and endocrine glands, provides predominantly for tissue-specific uptake of the vitamin. Dehydroascorbic acid uptake into cells utilizes GLUT transporters, mainly GLUT 1 and 3. Tissue concentrations of vitamin C usually greatly exceed plasma concentrations, with the magnitude dependent upon the specific tissue. The vitamin C content of white blood cells, for example, can be as much as 80 times greater than plasma concentrations.

High concentrations of vitamin C are found in selected tissues including the adrenal and pituitary glands (with each possessing about 30–50 mg/100 g of wet tissue) as well as in the eyes, brain, white blood cells, and neurons. Intermediate levels of vitamin C (about 10–15 mg/100 g of wet tissue) are found in the liver, spleen, heart, kidneys, lungs, pancreas, and muscle. Skeletal muscle, however, because of its size, may contain about 1,000–1,500 mg of vitamin C. The maximal vitamin C pool (total body vitamin C content) is estimated at about 2 g, although other estimates suggest it may be closer to 5 g [2]. Levels at or below 300 mg are found with deficiency.

Functions and Mechanisms of Action

Vitamin C's most well-known function is as an antioxidant and, related to this role, it also serves as a cosubstrate to maintain enzyme activity. More specifically in reference to this latter role, some enzymes contain a mineral (copper or iron) cofactor and, for some of these metallo-enzymes, vitamin C functions as a reducing agent (electron donor) to maintain the iron and copper atoms in the reduced state. The vitamin also provides for the regeneration of other cosubstrates, such as tetrahydrobiopterin, that are required for some reactions. These cosubstrate roles of vitamin C in primarily hydroxylation and amidation reactions are discussed first (and shown in Table 9.5) and are followed by information on some of the vitamin's other roles.

Collagen Synthesis for Connective Tissue Function and Strength

Vitamin C is necessary for the production of collagen, a structural protein found in skin, bones, dentine, tendons, and cartilage (connective tissues). Collagen is made up of three polypeptide chains that are synthesized by fibroblasts (connective tissue cells). In the initial steps of collagen production, cells generate tropocollagen, which consists of a triple helix of polypeptide chains twisted around each other and linked by hydrogen bonds to form a coil. After these tropocollagen chains are made, vitamin C-dependent hydroxylation reactions occur

Table 9.5 Cosubstrate-Related Functions of Vitamin C

Enzyme	Role of Vitamin C	Significance
Prolyl hydroxylase*	Maintains Fe in reduced state	Collagen synthesis and strength needed for the body's connective tissues
Lysyl hydroxylase	Maintains Fe in reduced state	*Also hypoxia-inducible factor-1 degradation with effects on gene expression
Trimethyllysine hydroxylase	Maintains Fe in reduced state	Carnitine synthesis needed for oxidation of fatty acids for energy production
4-butyrobetaine hydroxylase	Maintains Fe in reduced state	
Para-hydroxyphenylpyruvate hydroxylase	Maintains Fe in reduced state	Energy production from tyrosine catabolism
Homogentisate dioxygenase	Maintains Fe in reduced state	
Tyrosine hydroxylase	Maintains tetrahydrobiopterin in reduced state	L-dopa synthesis from tyrosine
Dopamine β hydroxylase	Maintains Cu in reduced state	Norepinephrine synthesis from dopamine
Peptidylglycine α -amidating monooxygenase	Maintains Cu in reduced state	Hormone and neurotransmitter production
Cholesterol 7 α -hydroxylase	Unclear role; cytochrome P450 stability	Initial and rate-limiting step in bile acid production from cholesterol
	Maintains tetrahydrobiopterin in reduced state	
Ten-eleven translocation methylcytosine dioxygenase	Maintains Fe in reduced state	Gene expression
Jumonji domain-containing histone demethylase	Maintains Fe in reduced state	

post-translationally on specific proline and lysine residues and allow for aggregation and further cross-linking of the chains.

- Proline hydroxylations are catalyzed by prolyl 4-hydroxylase and prolyl 3-hydroxylase (also called dioxygenases). The enzymes hydroxylate specific proline residues (positions 4 and 3) on the newly synthesized chains. Proline and hydroxyproline provide more rigidity to collagen. Prolyl 4-hydroxylase is a member of the Tet family of dioxygenases and is also involved in other functions, discussed later in the section “Gene Expression.”
- Lysine hydroxylation occurs by lysyl hydroxylase (also called dioxygenase). The formation of hydroxyllysyl residues provides for attachment to carbohydrate moieties and allows for additional post-translational modifications, such as glycosylation and phosphorylation, to further strengthen the collagen.

Additionally, a copper-dependent (but not vitamin C-dependent) oxidation reaction catalyzed by lysyl oxidase generates cross-links among collagen chains for added strength.

The cosubstrate role of vitamin C in these hydroxylation reactions relates to the iron cofactor attached to prolyl hydroxylases and lysyl hydroxylase. During the reactions, the hydroxylases incorporate one atom of O_2 into the product and the second atom of O_2 into the α -ketoglutarate to form the new carboxyl group of succinate (Figure 9.4). During these reactions, the iron cofactor in the hydroxylase enzymes is oxidized, that is, it is

converted from a ferrous (2^+) state to a ferric (3^+) state. Vitamin C functions as the reductant, thereby reducing the iron back to its ferrous state (2^+) in the prolyl and lysyl hydroxylases.

Vitamin C is also needed for the formation and maintenance of the basement membranes lining the capillaries, the “intracellular cement” holding together the endothelial cells, and the scar tissue responsible for wound healing. When coupled with its roles in collagen synthesis, it is readily apparent that the vitamin profoundly impacts the normal development and maintenance of skin, tendons, cartilage, bone, and dentine. Moreover, several signs and symptoms of vitamin C deficiency can be related to these roles of the vitamin.

Carnitine Synthesis for Fatty Acid Oxidation/ Energy Production

Vitamin C is involved in two hydroxylation reactions required for the synthesis of carnitine, a nonprotein, nitrogen-containing compound essential for the transport of long-chain fatty acids from the cell cytosol into the mitochondria for β -oxidation (and thus energy production). Carnitine is made from the amino acid lysine, which has been methylated using S-adenosyl methionine. The hydroxylation reactions in carnitine synthesis involving vitamin C are similar to those for proline and lysine hydroxylation. Vitamin C functions as a reducing agent, specifically reducing the iron atom from the ferric state (Fe^{3+}) back to the ferrous state (Fe^{2+}) for the reactions catalyzed by trimethyllysine hydroxylase and 4-butyrobetaine hydroxylase (see Figure 6.23).

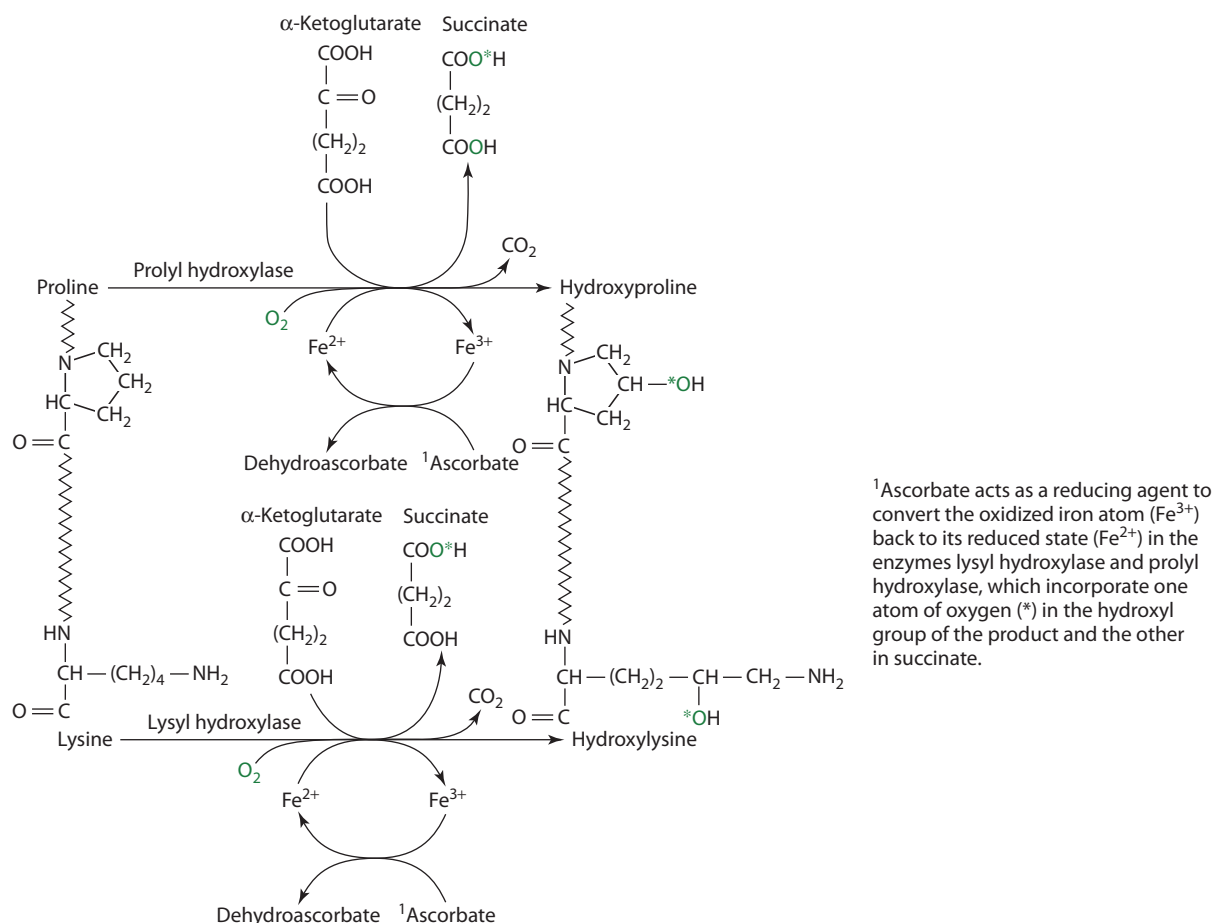
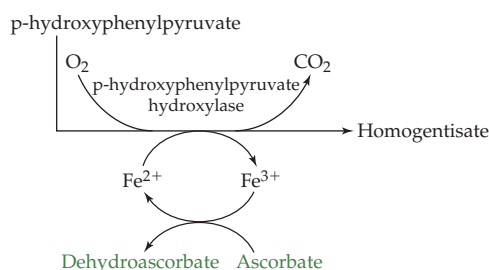


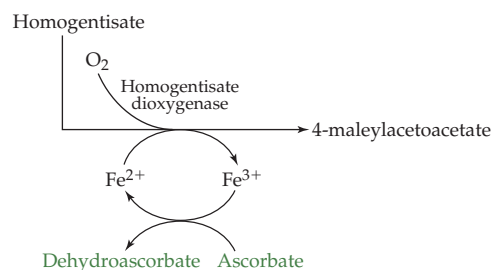
Figure 9.4 Ascorbate functions in the hydroxylation of peptide-bound proline and lysine in the synthesis of collagen.

Tyrosine Catabolism for Energy Production

The amino acid tyrosine, ingested from foods or generated in the body from phenylalanine oxidation, can be degraded (if needed) for energy production. During its catabolism, para (p)-hydroxyphenylpyruvate is produced and then hydroxylated by the iron-dependent enzyme para (p)-hydroxyphenylpyruvate hydroxylase (also called dioxygenase). Vitamin C is a preferred reductant for iron in this reaction, as shown here.



Additionally, in the next step of tyrosine catabolism, vitamin C functions as the reductant for iron as homogentisate is converted to 4-maleylacetoacetate by the iron-dependent enzyme homogentisate dioxygenase.



Tyrosine Metabolism for Hormone and Neurotransmitter Synthesis

Vitamin C also reduces enzyme-associated mineral cofactors that become oxidized during selected reactions in the synthesis of some neurotransmitters, including the catecholamines (dopamine, norepinephrine, and epinephrine) generated in the nervous system and some hormones generated in the adrenal medulla. The amino acid tyrosine serves as the substrate for the production of these neurotransmitters and hormones.

L-Dopa Synthesis Tyrosine is hydroxylated in neuronal cells by tyrosine hydroxylase (also called monooxygenase),

an iron-dependent enzyme, to produce the neurotransmitter 3,4-dihydroxyphenylalanine, commonly called L-dopa. Tetrahydrobiopterin, a cosubstrate, is oxidized during the reaction to dihydrobiopterin. Vitamin C functions in the reduction of dihydrobiopterin back to tetrahydrobiopterin (see Figure 6.10). L-dopa is further metabolized in cells of the nervous system for the production of dopamine, and dopamine (while serving as a neurotransmitter itself with effects on movement and behavior) can also be further metabolized to produce norepinephrine and epinephrine.

Norepinephrine Synthesis Norepinephrine, another neurotransmitter, is generated from the hydroxylation of dopamine's side chain in a vitamin C-dependent reaction. The reaction is catalyzed by dopamine β monooxygenase (also called dopamine β hydroxylase), which contains eight copper (Cu^{1+}) atoms and occurs primarily in the adrenal glands, but also occurs in the nervous system. During the reaction (see Figure 6.10), the cuprous ions (Cu^{1+}) become oxidized to cupric ions (Cu^{2+}) and require vitamin C for reduction.

Amidation of Peptides for Neurotransmitters and Hormone Synthesis

Vitamin C also serves as a reductant, keeping the copper atom in peptidylglycine α -amidating monooxygenase in its reduced state, as shown in Figure 9.5. Although most of the substrate peptides for this enzyme have a terminal glycine residue, the enzyme is also active with peptides terminating in other amino acids. Many of the amidated peptides resulting from this reaction are active as hormones, hormone-releasing factors, or neurotransmitters. Examples include bombesin or gastrin-releasing peptide (GRP), calcitonin, cholecystokinin (CCK), thyrotropin, corticotropin-releasing factor, gastrin, growth hormone-releasing factor, oxytocin, and vasopressin. The enzyme is found in neuroendocrine cells of the pituitary, adrenal, and thyroid glands and in the brain. As a reductant for the amidating enzyme, vitamin C

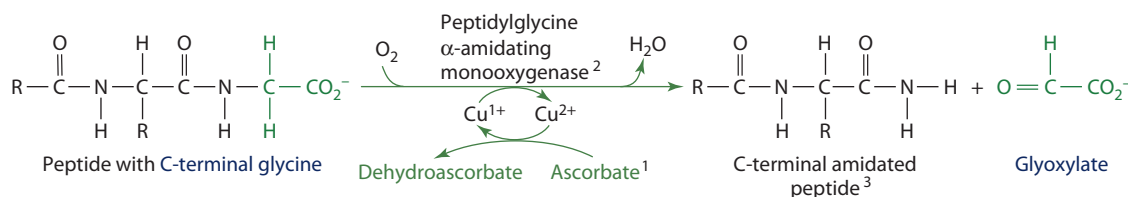
assumes important, although indirect, roles in many regulatory processes.

Cytochrome P450 Function for Steroid and Xenobiotic Metabolism

Cytochrome P450 enzymes include a group of heme-containing mixed-function oxidase enzymes (also called the microsomal metabolizing system) involved in cholesterol metabolism; hormone production and metabolism, especially steroid hormones such as aldosterone and cortisol; and the metabolism of endogenous toxins and **xenobiotics**. The cytochrome P450 enzymes are found primarily, but not solely, in the liver. Vitamin C appears to play roles in the function of a few of the dozens of cytochrome P450 enzymes. *Cholesterol 7 α -hydroxylase*, found in hepatic microsomes, catalyzes the first and rate-limiting step (oxidizing cholesterol in the seventh position using molecular oxygen) in the synthesis of bile acids from cholesterol. While the exact role in the reaction is undefined, vitamin C appears to be involved in the synthesis or catabolism of the enzyme's cytochrome P450 component. Many xenobiotics (*xenos* meaning "stranger" in Greek), which include foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds, are also metabolized by cytochrome P-450 mixed-function oxidases. The metabolism of these substances usually involves hydroxylation reactions followed by conjugation or methylation reactions to produce polar metabolites for excretion. The hydroxylation reactions require oxygen as well as reducing agents such as vitamin C and NAD(P)H; vitamin C's exact role in these reactions has not been established.

Gene Expression

DNA methylation, acetylation, biotinylation, and hydroxylation, among other modifications, represent means of epigenetic regulation of gene activity. Vitamin C plays a role in hydroxylation reactions involving ten-eleven translocation (TET) methylcytosine dioxygenase and Jumonji



¹ Vitamin C functions as a reducing agent to convert copper that has become oxidized during the reaction back to a reduced (Cu^{1+}) form.

² The enzyme cleaves the carboxyl-terminal residue on the peptide substrate. The residue is released as glyoxylate.

³ Many of the amidated peptides are active hormones, hormone-releasing factors, or neurotransmitters, as discussed in the text.

Figure 9.5 Amidation of peptides with C-terminal glycine requires vitamin C.

domain-containing (JmjC) histone demethylases. Both enzymes require iron (in its reduced state) as a cofactor and require 2-oxoglutarate as a cosubstrate. Vitamin C functions to recycle the iron back to its reduced state and thus maintain enzymatic activity. The TET family of dioxygenases catalyze the hydroxylation of multiple substrates, including methylated nucleic acids (such as DNA) and proteins. TET methylcytosine dioxygenase catalyzes the hydroxylation of 5-methylcytosine to form 5-hydroxymethylcytosine in nucleic acids. JmjC histone demethylases are one of two groups of enzymes involved in hydroxylation and subsequent demethylation of histones and other proteins. Thus, vitamin C's role in maintaining the iron cofactor in its reduced state within these enzymes contributes to epigenetic regulation of gene expression. Moreover, given that altered DNA methylation and aberrant histone modifications have been associated with cancer risk, such associations provide for possible roles of vitamin C in epigenetic therapy.

Another link between vitamin C and gene expression involves hypoxia-inducible factor (HIF)-1, a transcription factor (consisting of multiple subunits) that is generated in cells in response to hypoxia (low oxygen). The 1 α -subunit of HIF-1 is controlled by post-translation modification (hydroxylation) that is catalyzed by prolyl 4-hydroxylase. This enzyme, also discussed in the section "Collagen," requires an iron cofactor and 2-oxoglutarate cosubstrate. Vitamin C, as in other reactions, maintains iron in the reduced state, enabling enzyme activity. The importance of the hydroxylation of the HIF-1 α subunit is that it serves as an intracellular signal for HIF degradation via the proteasomal system. And reductions in cellular vitamin C diminish the degradation of HIF, allowing it to continue interacting with DNA and inducing transcription of multiple target genes (including those regulating growth and apoptosis and cell migration, among others).

Antioxidant Activity

In addition to vitamin C's donation of electrons/hydrogen ions to regenerate reduced forms of cosubstrates such as tetrahydrobiopterin and mineral cofactors (iron and copper), vitamin C serves as a reducing agent or electron donor (i.e., provides antioxidant activity/functions) in other situations. Ascorbic acid (which can be thought of as hydrogen ascorbate or AH₂) acts as a reducing agent in aqueous environments such as the blood and within cells. Stated slightly differently, vitamin C is an antioxidant in that it reverses oxidation. Vitamin C's two hydroxyl groups and carbonyl group facilitate its ability to act as a hydrogen or electron donor. Ascorbate (AH⁻) is the ionized form—that is, the form of vitamin C that results when a hydrogen has dissociated from ascorbic acid, as in a solution. Vitamin C readily donates electrons/hydrogen ions to reduce **free radicals** and reactive oxygen and nitrogen species. Free radicals and reactive species, if not “inactivated,”

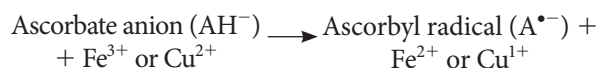
attack nucleic acids in DNA, polyunsaturated fatty acids in phospholipids, and amino acids in proteins. Ascorbic acid has been shown to interact with these radicals in the aqueous phase before they initiate damage. Furthermore, ascorbic acid appears to be superior to other water-soluble antioxidants. The ability of plasma antioxidants to protect, for example, lipids against peroxidation, has been shown to be vitamin C = thiols > bilirubin > uric acid > vitamin E [3]. Vitamin C reduces several:

- oxygen-centered radicals and reactive species, including **hydroperoxyl radical** (HO₂·), **superoxide radical** (O₂·), **alkoxyl radical** (RO·), **peroxyl radical** (RO₂·), **singlet oxygen** (¹O₂), **hydroxyl radical** (·OH), hydrogen peroxide (H₂O₂), and hypochlorous acid (HOCl);
- nitrogen species, such as peroxynitrite radicals (ONOO·) and nitrogen dioxide (·NO₂); and
- antioxidant-derived radicals, like urate (UH·) and thiyl (RS·) radicals.

Vitamin C also readily donates electrons/hydrogen ions to regenerate other antioxidants, such as vitamin E and glutathione (and shown in Figure 10.19). Such regeneration is needed since following interactions with the radicals and reactive oxygen species, antioxidant vitamins and molecules become oxidized and must be returned to their former states for cell protection. To accomplish this regeneration, several different reactions may occur. For example, two ascorbyl radicals may react to form ascorbic acid and dehydroascorbic acid; this is especially likely in situations with high levels of oxidant stress. Alternately, reductases, found in most tissues, can reduce the ascorbyl radical and dehydroascorbic acid to ascorbic acid. Niacin coenzymes (NAD(P)H) and thiols (such as dihydrolipoic acid, glutathione, and thioredoxin) also assist in vitamin C regeneration. Additional reactions involved in the regeneration of vitamin C, as well as more information on the roles of vitamin C and other antioxidants, are discussed in the Perspective at the end of Chapter 10.

Pro-oxidant Activity

Paradoxically, vitamin C may also act as a pro-oxidant by reducing transition metals—for example, cupric ions (Cu²⁺) to cuprous (Cu¹⁺) and ferric ions (Fe³⁺) to ferrous (Fe²⁺) as shown here:



The products—Fe²⁺ and Cu¹⁺—generated from these reactions can cause cell damage by generating reactive oxygen species and free radicals like hydroxyl and superoxide radicals, as shown here:



It is important to note that vitamin C's pro-oxidant activity is thought to be minimal at usual physiological concentrations and that vitamin C reacts with *free* ferric or cupric ions. In the body, iron and copper are both *bound* to various proteins (i.e., not free), thus lessening the likelihood of such interactions.

Other Functions

Many other diverse biochemical functions for vitamin C have been proposed. Possible functions include roles in collagen gene expression; synthesis of bone matrix, proteoglycans, fibronectin, and elastin; and regulation of cellular nucleotide (cAMP and cGMP) concentrations. In the stomach, vitamin C along with vitamin E may react with nitrites (ingested in foods) to deter their conversion to nitrosamines, which are carcinogenic. In cultured cells, vitamin C has been shown to regenerate the cosubstrate tetrahydrobiopterin from dihydrobiopterin to maintain endothelial nitric oxide synthase activity and thus nitric oxide production from arginine. (Note that nitric oxide is a potent biological effector molecule that is involved in a variety of physiological processes including regulation of blood pressure [relaxation of vascular smooth muscle] and intestinal motility, inhibition of platelet aggregation, macrophage function, and signal transduction, among other processes.) Vitamin C is also thought to affect immune system function, including production of complement proteins and white blood cells. The vitamin may also stimulate selected functions of some white blood cells such as promoting chemotaxis and proliferation of immune cells like macrophages and lymphocytes and increasing the activity of natural killer cells. Experimental evidence supporting these functions varies considerably, and the mechanism by which vitamin C may be involved is generally unclear.

Selected Pharmacological Uses/Other Roles

Much attention has also been directed toward vitamin C and its possible effects on diseases ranging from the common cold to cancer and heart disease, among others.

Colds Colds, affecting the average adult about two to six times each year, are caused most often by viruses including adenovirus, rhinovirus, coronavirus, and respiratory syncytial virus, among others. The ability of vitamin C to enhance immune system function (and destroy histamine, associated with some of a cold's symptoms) provides a theoretical basis for the use of vitamin C in the prevention and treatment of colds. Numerous studies, involving tens of thousands of individuals, have been conducted over the last several decades to examine the effectiveness of vitamin C in cold prevention and treatment. Studies continue to find no reduction in the incidence of colds with **prophylactic** (preventative) vitamin C use among the general population. However, regular use of vitamin C (500 mg–2 g daily) reduced the incidence of colds by 50%

among only some subsets of adults, specifically athletes (such as marathon runners and skiers) and soldiers exposed for short time periods to extreme temperatures or participating in extreme physical activities. Regular use of vitamin C supplements (in amounts of 200 mg or more) has been shown to modestly and consistently shorten the duration of cold symptoms by about 8% in adults and 14% in children; however, vitamin C supplementation once cold symptoms are present has not been shown to be beneficial [4].

Cancer Epidemiological studies provide evidence that increased intakes of fruits and vegetables are associated with a decreased risk of some cancers. Studies examining vitamin C status (as opposed to dietary vitamin C intake) and cancer risk also generally report favorable associations. The association between vitamin C and a protective effect against cancer is generally stronger with cancers of the oral cavity (including the pharynx), esophagus, stomach, lung, breast, colon, and rectum. However, reports from studies providing oral vitamin C to prevent or treat cancer are inconsistent, with most showing no overall benefits. Some antitumor effects have been documented, however, with intravenously administered vitamin C in high doses (which generates plasma vitamin C concentrations over 100 times that attained from oral ingestion) as an adjunct to other cancer treatments [5].

Cardiovascular Disease Many (but not all) studies report that increased fruit and vegetable intakes, vitamin C intake, and/or plasma vitamin C concentrations are associated with a decreased risk of cardiovascular disease. Yet, although its antioxidant abilities decrease LDL-oxidation and may enhance endothelial function and reduce monocyte adhesion to endothelial cells lining blood vessels (i.e., factors associated with plaque formation), studies providing supplements of vitamin C, and other antioxidants, have typically not reported beneficial effects in the prevention of heart disease or the reduction of heart disease-related mortality [6,7].

Eye Health It has been suggested that vitamin C is beneficial in diminishing the risks and/or preventing age-related macular degeneration and cataracts, both major causes of blindness, especially in older people. The macula, found in the center of the retina, maintains central vision. Behind the retina is the choroid, which contains blood vessels. In age-related macular degeneration (dry form), cellular debris called drusen accumulates between the retina and the choroid, and in the wet (exudative) form of the condition, abnormal blood vessels grow under the macula. In both forms of age-related macular degeneration, there is a loss of vision in the center of the visual field because of damage to the retina. The condition makes reading and recognition of faces difficult, although peripheral vision remains relatively

unaffected. Unfortunately, vitamin C taken alone or with other antioxidant nutrients does not appear to prevent the development of age-related macular degeneration; however, supplementation with vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg) (as used in the Age-Related Eye Disease Study, abbreviated AREDS, a large randomized, placebo-controlled 5-year clinical trial) may have modest effects in slowing the progression of the condition [8–10]. A second 5-year trial, AREDS2, supplemented those with macular degeneration with a modification of the original formulation (adding omega-3 fatty acids [1,000 mg], lutein [10 mg], and zeaxanthin [2 mg], removing beta-carotene, and reducing zinc [25 mg]), but found no additional benefits [11].

A cataract affects the lens of the eye, causing it to become cloudy. The lens (which functions to focus light onto the retina and adjust eye focus) is made up of over a million lens fiber cells. These cells have high protein contents (especially the protein α -crystallin) and, unlike other tissues, the proteins in the lens do not turn over. α -Crystallin functions to maintain optical clarity by removing damaged proteins from the lens. However, with age, the activity and concentration of α -crystallin diminish. This change results in the accumulation and aggregation of abnormal proteins in the lens. In addition, with aging, the concentrations of antioxidants diminish and lead to oxidative damage in the eye, including lipid peroxidation and protein cross-linking. The damaged proteins aggregate and precipitate, causing the lens to become cloudy and vision to become impaired. Because oxygen and oxyradicals are thought to contribute to the development of cataracts, and because vitamin C functions as an antioxidant, it is logical that vitamin C may help in the prevention of cataracts. However, a review of trials concluded that consumption of fruits and vegetables rich in vitamin C, among other nutrients, as well as dietary and low-dose supplemental vitamin C intake, may be inversely linked with the risk and progression of age-related cataract but not prevention [12,13]. Further studies are needed to establish beneficial effects from supplementation.

Other Conditions Vitamin C in animal studies has been shown to exhibit multiple actions consistent with modulation of the aging process, including ameliorating factors linked with the development of Alzheimer's disease. While the vitamin's antioxidant properties and ability to moderate oxidative stress provide neuroprotective effects, additional studies are needed before the vitamin can be recommended for disease prevention or treatment [14].

Interactions with Other Nutrients

One of vitamin C's most notable interactions is with the mineral iron. Vitamin C enhances the intestinal absorption of nonheme iron most likely by reducing iron ferric

(Fe^{3+}) state to a ferrous (Fe^{2+}) state. Vitamin C's benefits are thought to be maximized at about 75 mg. It is commonly suggested that individuals, especially if iron deficient, consume vitamin C-rich foods (providing at least 25 mg vitamin C) such as orange juice when ingesting nonheme iron-rich foods to promote the iron's absorption.

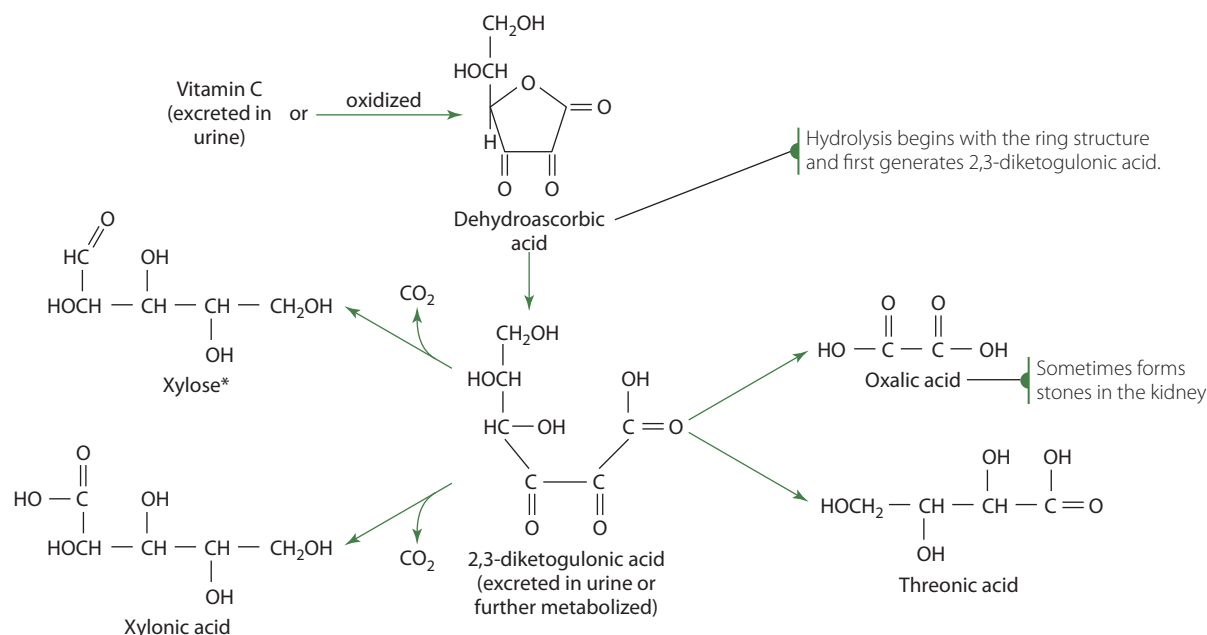
Metabolism and Excretion

Vitamin C in excess of tissue needs and storage capacity is excreted intact or catabolized prior to urinary excretion. As vitamin C intake increases, plasma vitamin C concentrations increase but reach an upper limit as renal handling of the vitamin shifts from active saturable reabsorption by SVCT1 carriers in the proximal renal tubule brush border membrane to a renal threshold in which the maximum reabsorption of the vitamin is achieved. The renal reabsorption threshold occurs generally at a plasma vitamin C concentration of about 1.3–1.8 mg/dL. With ingestion of vitamin C in amounts of about 500 mg (and sometimes less) and with saturated tissue stores, all of the excess ingested vitamin C is usually excreted in the urine within about 6 hours [15].

Vitamin C that is not excreted intact is oxidized primarily in the liver but also to some extent in the kidneys. Oxidation first generates dehydroascorbic acid, which is further degraded with the hydrolysis (opening) of the ring structure to yield 2,3-diketogulonic acid. This metabolite, 2,3-diketogulonic acid, can be excreted in the urine or further hydrolyzed (Figure 9.6). When further hydrolyzed, diketogulonic acid is cleaved by separate pathways into a variety of five-carbon metabolites (xylose, xylonic acid, and lyxonic acid) or into the four-carbon compounds threonic acid and oxalic acid. Vitamin C intakes up to about 200 mg do not alter the urinary excretion of oxalic acid (which is normally less than about 30 mg/day) [16]. The four- and five-carbon sugars that are generated can be converted into other cellular compounds or be oxidized and excreted as CO_2 and water. Other urinary vitamin C metabolites may include 2-O-methyl ascorbate, ascorbate 2-sulfate, and 2-ketoascorbitol.

Recommended Dietary Allowance

Vitamin C requirements for adult men and women, based on nearly maximizing tissue (neutrophil) concentrations and minimizing urinary excretion of the vitamin, are 75 mg and 60 mg, respectively [17]. Vitamin C's Recommended Dietary Allowance (RDA) for adult men and women is 90 mg and 75 mg, respectively, and, for those who are smokers, an additional 35 mg/day is recommended [17]. Smoking accelerates the depletion of the body's ascorbic acid pool. During pregnancy and lactation, recommendations for vitamin C increase to 100 mg and 120 mg, respectively [17]. Higher recommended intakes of vitamin C have been



*Some of the sugars like xylose can be further metabolized before excretion.

Figure 9.6 Vitamin C and the formation of its metabolites excreted in the urine.

suggested based on now-recognized underestimates of the body vitamin C pool (which were used in determining the vitamin's requirements) as well as considerations in replacing daily vitamin C turnover, maintaining adequate plasma concentrations, and perhaps optimizing health (vs. preventing deficiency) [2]. Proposed vitamin C intakes closer to 200 mg/day have been recommended in some countries with a goal of optimizing health [2]. See the inside cover of the book for additional vitamin C recommendations for other age groups.

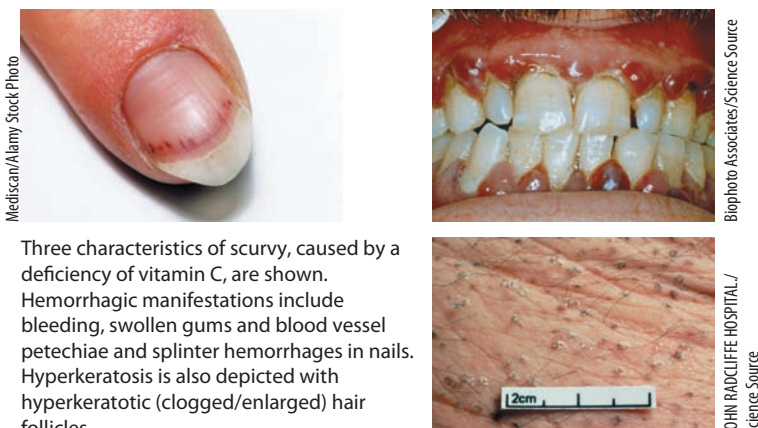
Deficiency: Scurvy

An inadequate intake of vitamin C results in the deficiency condition called scurvy. Scurvy is typically manifested when the total body vitamin C pool falls below about 300 mg and plasma vitamin C concentrations drop to less than about 0.2 mg/dL. Scurvy may develop in as little as 1 month with vitamin C intakes less than 10 mg daily, but the condition is more likely to occur with an inadequate vitamin C intake for a duration of at least 4–6 months.

Scurvy is characterized by a multitude of signs and symptoms. Initial symptoms include fatigue and malaise that may be associated with impaired carnitine synthesis. Impaired hydroxyproline and hydroxylysine synthesis leads to defects in collagen-rich body structures and can diminish vascular wall and bone strength. Blood vessel fragility (rupture of small blood vessels) is visible as small, red skin discolorations called **petechiae** on the skin. Splinter hemorrhages (damaged capillaries under the fingernails) and easy bruising (characterized

by ecchymoses—purple discolorations of the skin due to ruptured blood vessels—and purpurae—dark red to purple spots on the skin due to hemorrhage) may also be apparent (Figure 9.7). An additional early manifestation may be the presence of clogged and enlarged, hyperkeratotic hair follicles (keratin accumulations in the hair follicle), especially on the arms, legs, and buttocks and “corkscrew”-shaping of hair.

Oral changes, also related to impaired collagen synthesis, include swollen, bleeding, necrotic gums; sublingual hemorrhages; and loose and decaying teeth. Additionally, impaired wound healing, easily fractured bones, and bone pain (arthralgia) associated with abnormal collagen formation occur. In some individuals, changes in



Three characteristics of scurvy, caused by a deficiency of vitamin C, are shown. Hemorrhagic manifestations include bleeding, swollen gums and blood vessel petechiae and splinter hemorrhages in nails. Hyperkeratosis is also depicted with hyperkeratotic (clogged/enlarged) hair follicles

Figure 9.7 Some manifestations of a vitamin C deficiency.

behavior/personality also result and may be a result of changes in neurotransmitter production. Scurvy is fatal if untreated. The four Hs—*hemorrhagic* signs, *hyperkeratosis* of hair follicles, *hypochondriasis* (psychological manifestation), and *hematologic* abnormalities (associated mainly with impaired collagen synthesis)—are often used as a mnemonic device for remembering scurvy signs. Scurvy in adults is treated with vitamin C in doses of about 300–1,000 mg daily until symptoms are gone, which usually takes about 3 months.

At Risk for Deficiency

Although scurvy is rare in the United States, low vitamin C status has been observed among smokers, older adults, and individuals with alcoholism or drug abuse. People with malabsorption disorders may exhibit diminished intestinal absorption of the vitamin, and those with conditions such as diabetes mellitus and some cancers are at risk due to increased vitamin C turnover. Critically ill patients also frequently exhibit suboptimal vitamin C status. In those that are critically ill, intravenous administration of vitamin C in doses of 2–3 g/day have been shown to restore normal plasma concentrations, while higher daily doses, 6–16 g of ascorbic acid, have been shown to reduce vascular permeability, preserve endothelial function and microcirculatory flow, improve vasopressor sensitivity and hemodynamic stability, and reduce mortality [18].

Toxicity

Daily intakes of up to 2 g of vitamin C are routinely consumed by many individuals without adverse effects [17]. The most common side effect from the ingestion of large amounts (2 g or more) of the vitamin is gastrointestinal problems characterized by abdominal pain and osmotic diarrhea. The osmotic diarrhea occurs when the unabsorbed vitamin C in the intestinal tract pulls water into the lumen of the digestive tract and is exacerbated by bacteria metabolism of the vitamin within the colon. Based on this side effect, a Tolerable Upper Intake Level of 2 g of vitamin C has been recommended [17].

Two other possible side effects reported from the use of large amounts of vitamin C include an increased risk of (1) kidney stones (nephrolithiasis), especially for those with renal dysfunction or at risk of or who have a history of kidney stones, and (2) iron toxicity for those with disorders of iron metabolism. The link between the vitamin and nephrolithiasis results from vitamin C's metabolism, which generates oxalic acid, a common constituent of kidney stones. For most individuals, urinary oxalic acid excretion typically remains within a normal range with usual vitamin C intakes. However, as intake rises (especially over 500 mg/day), urinary oxalate excretion also rises, and increases in urinary oxalate excretion tend to be higher in those with (vs. without) a history of stone formation [16].

In addition to links with oxalic acid, vitamin C is also associated with uric acid (another constituent of kidney stones). The vitamin competitively inhibits the renal reabsorption of uric acid, thereby increasing uric acid excretion and acidifying the urine, which can promote the precipitation of uric acid crystals and the formation of uric acid kidney stones. Thus, avoiding high intakes of vitamin C may be advisable for those predisposed to either calcium oxalate or uric acid kidney stones.

In addition to increasing the probability of kidney stones, chronic high doses of vitamin C are also purported to be unsafe for people with disorders of iron metabolism (especially iron overload), including **hemo-chromatosis**, **thalassemia**, and **sideroblastic anemia**. Additional studies are needed to more clearly identify safe levels of intake.

Lastly, excessive vitamin C excretion (as occurs with consumption of high doses) can interfere with some clinical laboratory tests. Vitamin C in the urine, for example, may act as a reductive agent and thus interfere with diagnostic tests using redox chemistry such as those used to test for glucose in the urine (testing for diabetes) or for blood in the feces (testing for gastrointestinal tract bleeding).

Assessment of Nutriture

Plasma vitamin C concentrations respond to changes in dietary vitamin C intake and thus are used to assess recent vitamin C intake; however, white blood cell (leukocyte) content of the vitamin better reflects body stores. Plasma concentrations of vitamin C below 0.2 mg/dL are considered to be deficient, while concentrations of 0.2–0.4 mg/dL are considered insufficient. Leukocyte vitamin C concentrations of 10 $\mu\text{g}/10^8$ or less are considered deficient; however, an analysis of vitamin C among the different types of white blood cells is sometimes beneficial due to variations [17].

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9.2 THIAMIN (VITAMIN B₁)

The need for thiamin (vitamin B₁) was first recognized in the late 1800s by a Dutchman, C. Eijkman, when it was discovered that fowl fed a diet of cooked, polished rice (devoid of the outer germ and bran layers and containing primarily the starch-rich endosperm) developed neurologic problems (now called beriberi). The substance initially called thiamine that corrected the problems was later isolated from rice bran in 1912 by Casmir Funk.

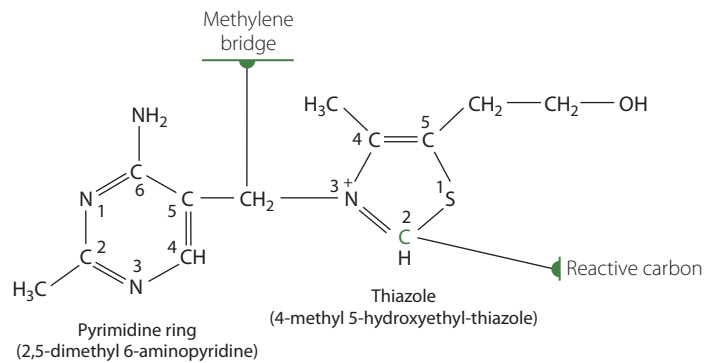


Figure 9.8 Structure of thiamin.

The vitamin's structure was determined by R. Williams from the United States in about the mid-1930s. Thiamin's structure (Figure 9.8) consists of a pyrimidine ring and a thiazole moiety (meaning one of two parts) linked by a methylene (CH₂) bridge. The thiazole moiety contains a sulfur atom.

Sources

Thiamin is widely distributed in foods, with higher quantities found in meats (especially pork), legumes, cereal and grain products (whole, fortified, or enriched). Some other sources of the vitamin include nuts, seeds, yeast, wheat germ, and milk, shown in Table 9.6. Most thiamin in the American diet comes from products that have been

Table 9.6 Thiamin Content of Selected Foods*

Food (serving)	Thiamin (mg)
Pork loin, roasted (3 oz)	0.5
Trout, cooked (3 oz)	0.4
Tuna, bluefin, cooked (3 oz)	0.4
Salmon, cooked (3 oz)	0.2
Chicken, breast, roasted, skinless (3 oz)	0.1
Beef, top sirloin, cooked (3 oz)	0.1
Bean, black, navy, cooked (1/2 c)	0.4
Pasta, macaroni or spaghetti, cooked (1 c)	0.4
Noodles, egg, cooked (1 c)	0.4
Brewer's yeast (<i>Saccharomyces cerevisiae</i>) (2 Tbsp)	0.4
Nuts, macadamia (1/4 c)	0.2
Lentils (1/2 c)	0.2
Seeds, sunflower (1/2 c)	0.2
Rice, white, enriched, cooked (1/2 c)	0.1
Rice, brown, unenriched, cooked (1/2 c)	0.1
Milk, 2% (1 c)	0.1
Soy milk, nonfat (1 c)	0.1
Bread, enriched (1 slice)	0.1

A more complete list of thiamin-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/Thiamin-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

enriched; such products also contribute to the riboflavin, niacin, and iron contents of the diet.

Thiamin in foods can be destroyed (primarily at the methylene bridge) in an alkaline environment (pH of 8 or above) and by heat. Thus, cooking thiamin-rich foods in water can lead to loss of the vitamin. However, other substances (referred to as antithiamin factors) in foods also promote thiamin destruction. Thiaminases found in some raw fish, for example, catalyze the cleavage and destruction of thiamin. These thiaminases, however, are thermolabile, so cooking the fish renders the enzymes inactive. Other antithiamin factors include polyhydroxyphenols such as tannic, chlorogenic, and caffeic acids and are found in coffee, tea, betel nuts, and certain fruits and vegetables such as blueberries, black currants, Brussels sprouts, and red cabbage. These polyhydroxyphenols, which are thermostable, inactivate thiamin by an oxyreductive process that destroys the thiazole ring; the destructive process can be facilitated by the presence of divalent minerals such as calcium and magnesium. Thiamin destruction may be prevented, however, by the presence of reducing compounds such as vitamin C.

In multivitamin supplements, thiamin is provided in amounts of about 1.5 mg, mainly as thiamin hydrochloride or thiamin mononitrate salt. Single-ingredient thiamin supplements often provide up to about 500 mg of the vitamin. The Daily Value, which is found on food and supplement facts labels, for thiamin is 1.2 mg.

Thiamin and one of its phosphorylated forms, thiamin diphosphate (TDP), formerly called thiamin pyrophosphate (TPP), are produced by bacteria in the large intestine. However, the amount of bacterially produced thiamin that is absorbed from the colon is unclear.

Digestion and Absorption

Thiamin exists in a free (nonphosphorylated) form in plant foods. In animal products, about 95% of thiamin is phosphorylated, primarily as TDP, and about 5% exists as thiamin monophosphate (TMP) and thiamin triphosphate (TTP). Digestion of these phosphorylated forms by intestinal phosphatases occurs before absorption. It is primarily free thiamin that appears to be absorbed into the intestinal cells.

Absorption of free thiamin occurs both via diffusion and carrier-mediated active transport. Diffusion takes place throughout the small intestine, with oral intakes of the vitamin in doses of up to 1,500 mg shown to progressively increase blood thiamin concentrations [1]. Carrier-mediated absorption also occurs throughout the digestive tract, including the colon, but mainly in the duodenum and jejunum. Overall, most thiamin absorption is thought to occur within the jejunum.

Carrier-mediated thiamin transport into intestinal cells is accomplished by two carriers, ThTr1 and ThTr2. ThTr1

is more highly expressed and has a higher capacity for thiamin than ThTr2; however, ThTr2 has a higher specificity for thiamin than ThTr1 [2]. In the intestine, ThTr1 is present on both the brush border and basolateral membranes, and ThTr2 is found on the basolateral membrane. Thiamin is actively transported into intestinal cells in exchange for H^+ ions as part of an antiport carrier system. Transport appears to become saturated with thiamin intakes of about 5 mg [1]. ThTr2 synthesis is enhanced with low thiamin intake. Alcohol inhibits the intestinal expression of both ThTr1 and ThTr2 and thus impairs thiamin absorption. This alcohol-associated inhibitory effect on absorption also extends to colonic cells, but the mechanism of the inhibition is unclear. A transporter for thiamin diphosphate has also been identified in the colon among other nonintestinal tract tissues.

Several mutations have been characterized in genes coding for proteins involved in thiamin transport and metabolism. Defects, for example, in the gene *SLC19A2*, which codes for ThTr1, cause a thiamin-responsive deficiency disorder known as Rogers syndrome. The condition is also referred to as thiamin-responsive megaloblastic anemia and thiamin metabolism dysfunction syndrome. This rare autosomal recessive disorder is characterized primarily by megaloblastic anemia, deafness, and glucose intolerance (or diabetes). Pharmacological doses (about 25–100 mg) of thiamin improve but do not totally correct the manifestations of the syndrome [3].

Within intestinal cells, thiamin may be phosphorylated by thiamin pyrophosphokinase, also called diphosphokinase, in an energy-dependent reaction to generate TDP. Dephosphorylation of the vitamin, however, is required before it crosses the intestinal cell's basolateral membrane to enter the blood. Thiamin is typically released from the intestinal cells into the blood within 1–2 hours to provide for distribution to tissues.

Transport, Tissue Uptake, and Storage

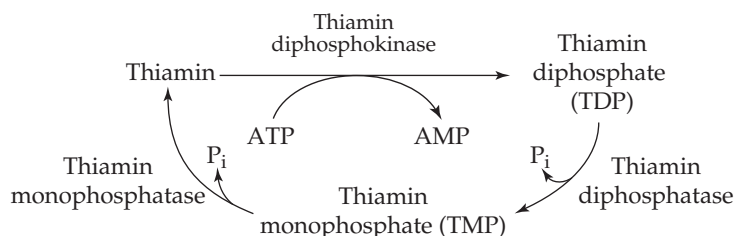
About 90% of the thiamin in the blood is present within the blood cells (mostly red and some white) as TDP (which is formed within the cells). The normal reference range for blood TDP concentrations is about 70–180 nmol/L. Smaller amounts of thiamin (either bound to albumin or free) and TMP are also found in the plasma. Blood thiamin concentrations usually range from about 2.5 to 7.5 $\mu\text{g}/\text{dL}$ and, when combined with TMP, the concentrations range from about 5 to 12 $\mu\text{g}/\text{dL}$.

Free thiamin crosses cell membranes via carrier proteins to enter cells. ThTr1, found especially in skeletal muscles, and ThTr2, found in the liver, kidneys, and heart (among others), mediate active thiamin uptake into most tissues. Organic cation transporter (OCT1 and OCT3) proteins may also actively transport free thiamin into some tissues. Within cells, a TDP carrier has been demonstrated

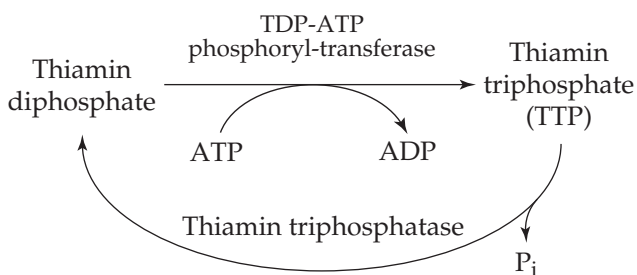
on the mitochondrial membrane and may be responsible for intracellular TDP transport.

The human body contains approximately 25–30 mg of thiamin, with small concentrations stored in the liver, skeletal muscles, heart, kidneys, and brain. Skeletal muscles are thought to contain about 40–50% of the body's thiamin. Thiamin's half-life is estimated at about 8–20 days.

Within tissues (including the liver, muscle, heart, and kidneys), thiamin is found primarily (80% of total thiamin) in a phosphorylated form as TDP. Free thiamin is found in only small quantities in the body, mostly within the central nervous system. The phosphorylation reaction requires energy and is catalyzed by thiamin pyrophosphokinase, also called diphosphokinase.



About 10% of thiamin is found in the body tissues as TTP. TTP is synthesized by action of a TDP-ATP phosphoryl transferase that phosphorylates TDP.



Hydrolysis or dephosphorylation of thiamin's phosphorylated forms occurs in tissues throughout the body. The terminal phosphate on the TTP is hydrolyzed by thiamin triphosphatase to yield TDP (shown above), and TDP can be converted to TMP by thiamin diphosphatase, which cleaves the terminal phosphate on TDP. TMP can then be converted to free thiamin by thiamin monophosphatase.

Functions and Mechanisms of Action

Thiamin, as the coenzyme TDP, is involved in energy production and nutrient metabolism as part of the *pyruvate dehydrogenase*, *α-ketoglutarate dehydrogenase*, and *branched-chain α-keto acid dehydrogenase complexes*. In peroxisomes, the coenzyme is also required for fatty acid degradation for energy production as part of *2-hydroxyacyl-CoA lyase 1*. TDP is also required for nutrient metabolism as part of *transketolase* needed for the interconversion

of phosphorylated sugars for the synthesis of *nucleotides* and some *B-vitamin coenzymes*. Thiamin also serves in a noncoenzyme capacity for nervous system function.

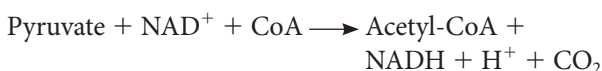
Coenzyme Roles for Nutrient Metabolism and Energy Production

The oxidative decarboxylation of pyruvate, α-ketoglutarate, and the three branched-chain amino acids (isoleucine, leucine, and valine) produce a variety of CoA metabolites and is instrumental in generating energy (ATP). Reductions or inhibition of the reactions, especially for pyruvate and α-ketoglutarate, diminish synthesis of ATP. Additionally, diminished pyruvate oxidation reduces the production of acetyl-CoA, which is vital for the synthesis of the neurotransmitter acetylcholine and for the synthesis of fatty acids, cholesterol, and other important compounds. Inhibition also results in the accumulation of pyruvate, lactate, and α-ketoglutarate. Some of TDP's specific reactions are shown as an overview in Figure 9.9 and discussed in detail hereafter.

Oxidative Decarboxylation by the Pyruvate Dehydrogenase Complex and the α-Ketoglutarate Dehydrogenase Complex

The steps that occur in the oxidative decarboxylation of pyruvate to form acetyl-CoA, shown in Figure 9.10, require a multienzyme complex known as the pyruvate dehydrogenase complex, which is bound to the mitochondrial membrane. Three enzymes make up this complex: a TDP-dependent pyruvate dehydrogenase, a lipoic acid-dependent dihydrolipoyl transacetylase, and a FAD-dependent dihydrolipoyl dehydrogenase. The roles of four vitamins—thiamin (TDP), riboflavin (FAD), niacin (NAD⁺), and pantothenic acid (CoA)—in this process are described briefly and are shown in Figures 9.10 and 9.11. ATP and Mg²⁺ are also required.

In the first reaction (Figure 9.11), the carbon-2 atom between the nitrogen and sulfur atoms in the thiazole ring of TDP ionizes to form a carbanion, which then combines with the 2-carbonyl group of pyruvate, α-ketoglutarate, and other α-keto acids, forming a covalent bond. After forming the adduct (attachment) between pyruvate and TDP, pyruvate dehydrogenase (the first enzyme of the complex) catalyzes the removal of pyruvate's COO group to form hydroxyethyl-TDP (Figure 9.10). The hydroxyethyl group is then transferred to oxidized lipoamide (which is bound to the second enzyme, dihydrolipoyl transacetylase), forming acetyl lipoamide. The acetyl lipoamide then reacts with coenzyme A to form acetyl-CoA and reduced lipoamide. Lipoamide is oxidized by the third enzyme, dihydrolipoyl dehydrogenase, which requires FAD. NAD⁺ oxidizes FADH₂. Thus, the overall reaction is:



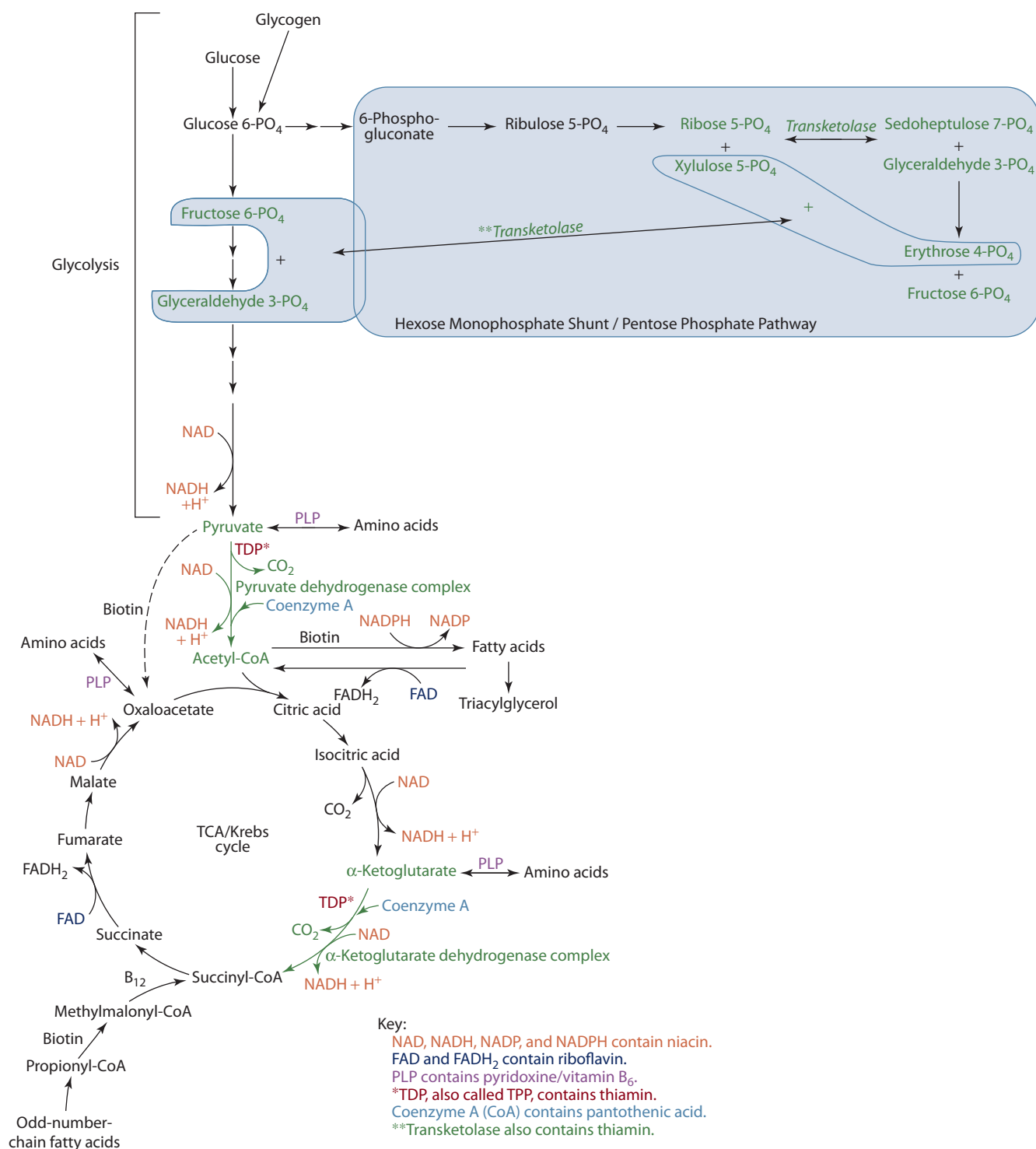
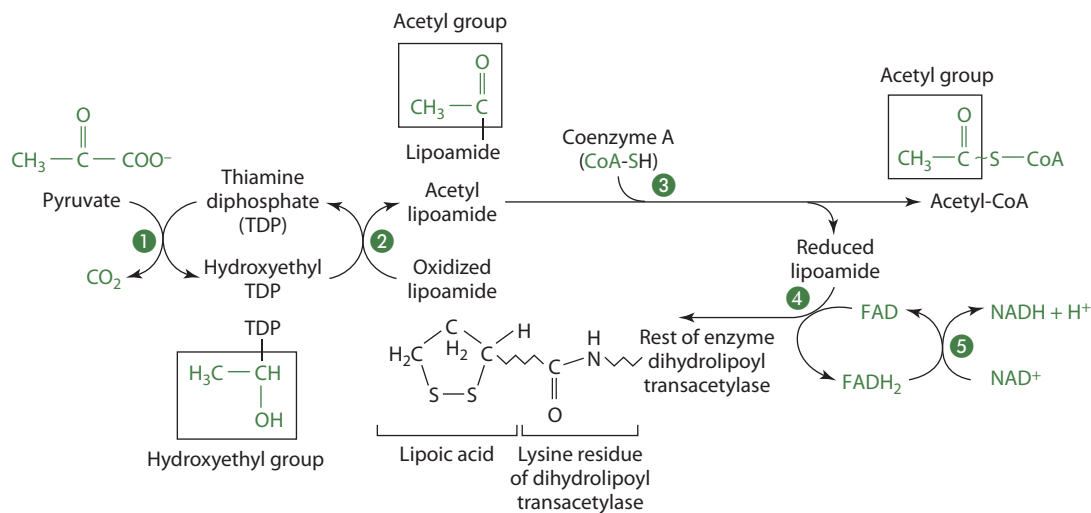


Figure 9.9 Various vitamin cofactors and their action sites in energy metabolism. The role of thiamin as TDP is shown by an asterisk.

The α-ketoglutarate dehydrogenase complex in the mitochondria decarboxylates α-ketoglutarate in a process similar to that just described for the conversion of pyruvate to acetyl-CoA. However, instead of producing acetyl-CoA, the decarboxylation of α-ketoglutarate generates the TCA/Krebs cycle intermediate succinyl-CoA.

Oxidative Decarboxylation by the Branched-chain α-Ketoacid Dehydrogenase Complex Decarboxylation of branched-chain α-keto acids, which arise from the transamination of valine, isoleucine, and leucine, is an oxidative process that is also similar to those previously described. The decarboxylation reactions generate



- 1 CO₂ is removed from pyruvate and the rest of the compound (hydroxyethyl) attaches to TDP to form hydroxyethyl TDP.
- 2 The hydroxyethyl group is transferred to oxidized lipoamide, which consists of lipoic acid attached by an amide link (CO-NH) to a lysine residue of the enzyme dihydrolipoyl transacetylase. With the transfer of the hydroxyethyl group acetyl lipoamide is generated.
- 3 Acetyl lipoamide reacts with coenzyme A (CoA-SH) to form acetyl-CoA and reduced lipoamide.
- 4 Reduced lipoamide is oxidized by the flavo (FAD)-dependent enzyme dihydrolipoyl dehydrogenase.
- 5 The reduced flavo (FADH₂) protein is oxidized by NAD⁺, which then transfers reducing equivalents to the respiratory chain.

Figure 9.10 The oxidative decarboxylation of pyruvate by the pyruvate dehydrogenase complex.

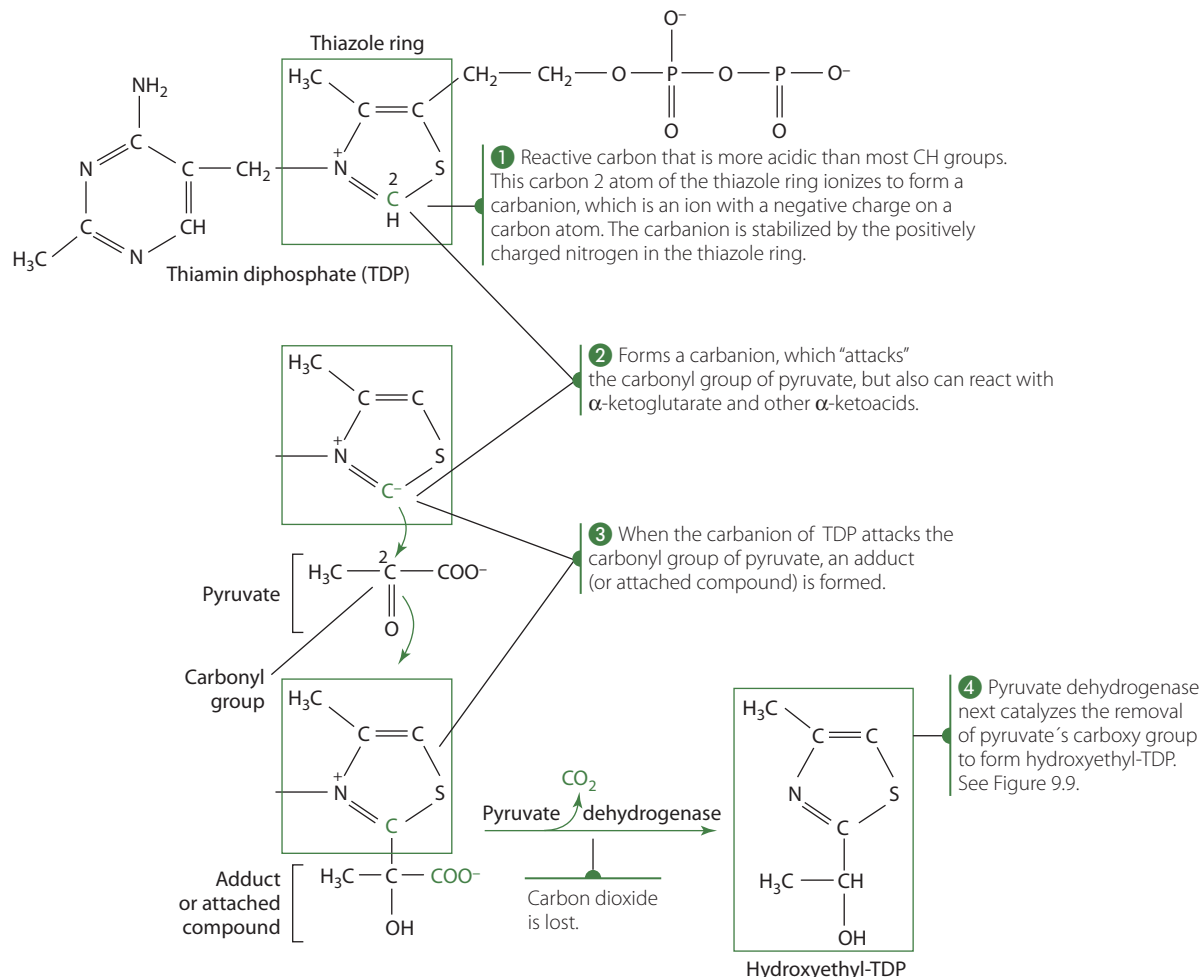


Figure 9.11 The first steps in the decarboxylation of pyruvate by thiamin diphosphate.

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isobutyryl-CoA (from valine), α -methylbutyryl-CoA (from isoleucine), and isovaleryl-CoA (from leucine) (see Figure 6.36). Failure to oxidize the α -keto acids of leucine, isoleucine, and valine (as occurs with mutations in the enzyme's one or more subunits) is characterized by the accumulation of both the branched-chain amino acids and their α -keto acids in blood and other body fluids. The condition is referred to as maple syrup urine disease and is discussed further under "Selected Pharmacological Uses/Other Roles" and in Chapter 6 in the section "Amino Acid Catabolism."

2-Hydroxyacyl-CoA Lyase 1 Activity In addition to TDP's roles in some oxidative decarboxylation reactions, TDP is used in peroxisomes where it functions as a coenzyme for 2-hydroxyacyl-CoA lyase 1 (HACL1). HACL1 participates in the α -oxidation of 3-methyl-branched fatty acids (such as phytanic acid). [Note: Phytanic acid is a branched-chain fatty acid with a methyl group (CH_3) on its beta-carbon; it is found in selected animal products, including some meat, fish, and milk.] The presence of a methyl group at the beta-carbon prevents the fatty acid from initially undergoing β -oxidation. However, once decarboxylation has occurred by α -oxidation, β -oxidation can occur. The initial steps in the α -oxidation of phytanic acid is shown in Figure 9.12. After conversion from phytanic acid to phytanoyl-CoA, the phytanoyl-CoA is next converted into 2-hydroxyphytanoyl-CoA within the peroxisome. The TDP-dependent enzyme HACL1, which also requires magnesium, catalyzes the cleavage of 2-hydroxyphytanoyl-CoA to generate formyl-CoA and pristanal (an aldehyde that has been shortened by one carbon ($n-1$) less than was originally present in the fatty acid). Pristanal is further

metabolized to pristanic acid and then to pristanoyl-CoA for entry into β -oxidation. HACL1 can also act on 2-hydroxy long-chain fatty acids in a similar manner, generating formyl-CoA and an aldehyde that has been shortened by one carbon less than the original fatty acid.

Transketolase Activity Thiamin as TDP also functions as the loosely bound prosthetic group for transketolase, a key cytosolic enzyme in the pentose phosphate pathway (hexose monophosphate shunt; see Figure 3.31). The oxidative portion of the pentose phosphate pathway generates NADPH, which is needed for fatty acid synthesis, among other roles (see the section of this chapter addressing niacin's functions). The nonoxidative portion of the pathway relies on transketolase to interconvert phosphorylated sugars containing three to seven carbons. These phosphorylated sugars play many critical roles in the body. For example, the phosphorylated sugar *ribose 5- PO_4* is used for the *synthesis of* (1) ATP, (2) *some B-vitamin coenzymes* (coenzyme (Co)A, NAD, NADP^+ , and FAD), and (3) *some nucleotides that make up DNA and RNA*. Some of the phosphorylated sugars generated in the pentose phosphate pathway are also used to produce (4) *intermediates in glycolysis*.

Two enzymes, transketolase and transaldolase, catalyze the interconversions among the phosphorylated sugars. Transketolase, which requires Mg^{2+} in addition to TDP, transfers (in reversible reactions) carbon fragments among phosphorylated ketoses (sugars containing a ketone group) and phosphorylated aldoses (sugars containing an aldehyde group). In the reaction (shown hereafter), two carbons from the ketose sedoheptulose 7- PO_4 are transferred by transketolase to the aldose glyceraldehyde

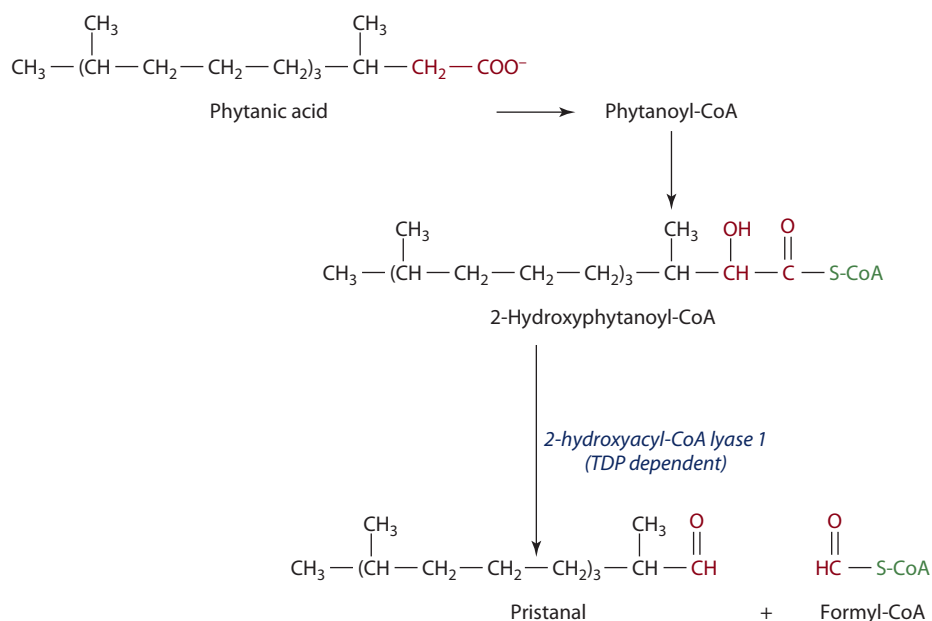
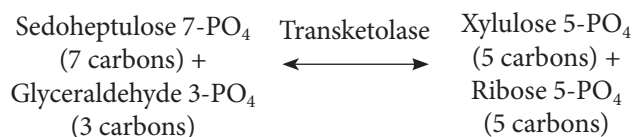
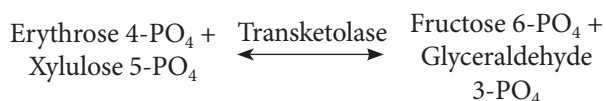


Figure 9.12 Thiamin's coenzyme role in the α -oxidation of the fatty acid phytanic acid.

3-PO₄ to form the ketose xylulose 5-PO₄ and the aldose ribose 5-PO₄.



Further interconversion among the phosphorylated sugars is catalyzed by transaldolase, which converts sedoheptulose 7-PO₄ (seven-carbon ketose) and glyceraldehyde 3-PO₄ (three-carbon aldose) to erythrose 4-PO₄ (four-carbon aldose) and fructose 6-PO₄ (six-carbon ketose). In a second reaction catalyzed by transketolase, carbon fragments are transferred such that erythrose 4-PO₄ (four-carbon aldose) and xylulose 5-PO₄ (five-carbon ketose) are used to generate fructose 6-PO₄ (six-carbon ketose) and glyceraldehyde 3-PO₄ (three-carbon aldose). The reaction can be written as:



Noncoenzyme Roles for Nervous System Functions

In addition to its coenzyme roles, thiamin is found in the central nervous system, where it is involved in multiple aspects of nervous system function. The vitamin plays both metabolic and structural roles in nervous tissue, regulating sodium channels and membrane permeability to influence nerve action potential, membrane conductance, and pre- or postsynaptic transmission. Thiamin, as TTP, also provides phosphate for the phosphorylation of regulatory proteins and synaptic proteins involved in activating chloride transport and other anion channels in nerve cells.

In the brain, as in other organs/tissues, thiamin serves as a coenzyme needed for energy production. The conversion of pyruvate to acetyl-CoA is especially noteworthy as acetyl-CoA is used not only for energy production in neurons but also serves as a precursor for the production of the neurotransmitter acetylcholine and for the production of myelin (which covers the axons of nerve cells and is needed in the conduction of nerve impulses). The article by Manzetti et al. [2] provides additional information for the interested reader on thiamin functions in the brain.

Selected Pharmacological Uses/Other Roles

Pharmacological doses of thiamin are provided to individuals diagnosed with maple syrup urine disease (MSUD), which results from genetic mutations in the branched-chain α -keto acid dehydrogenase complex. Large oral doses

(usually 100 mg or higher) of the vitamin are generally recommended for those with MSUD for several months to see if vitamin supplementation improves residual enzyme activity. Some other metabolic disorders involving altered thiamin utilization also sometimes respond to supplemental thiamin in varying doses [3,4].

Metabolism and Excretion

Thiamin in excess of tissue needs and storage capacity is excreted from the body. Thiamin is excreted mostly intact (as free thiamin) in the urine. The vitamin, however, may also be catabolized prior to excretion. Degradation of thiamin begins with cleavage of the vitamin into its pyrimidine and thiazole moieties. The two rings are then further catabolized, generating 20 or more metabolites, including, for example, thiochrome (a major metabolite sometimes used to assess thiamin status), 4-methyl thiazole 5-acetic acid, and 2-methyl 4-amino 5-pyrimidine carboxylic acid. Urinary thiamin excretion is increased with the use of high doses of loop diuretics such as furosemide (Lasix), ethacrynic acid (Edecrin), and torsemide (Demadex).

Recommended Dietary Allowance

Recommendations for thiamin intake are based on the results of studies examining urinary excretion, changes in erythrocyte transketolase activity, and thiamin intake data. The RDA for thiamin for adult men is 1.2 mg/day and for adult women is 1.1 mg/day; the requirements for men and women are 1.0 mg/day and 0.9 mg/day, respectively [5]. Differences in thiamin needs between men and women are based on differences in body size and energy needs. Thiamin recommendations with pregnancy and lactation increase to 1.4 mg/day and 1.5 mg/day, respectively [5]. The inside front cover of the book provides additional RDAs for thiamin for other age groups.

Deficiency: Beriberi

A deficiency of thiamin results in the disorder known as beriberi (*beri* means “weakness”). Signs and symptoms become apparent as body stores of the vitamin are depleted, which can occur in as little as a few weeks to about one month of consuming inadequate dietary thiamin. Use of parenteral nutrition devoid of or containing inadequate thiamin or containing excessive amounts of glucose can also cause thiamin deficiency within a few weeks. Initial symptoms of a deficiency, however, are vague and may include anorexia, fatigue, and vomiting. Because of thiamin’s roles in several pathways involved in nutrient metabolism, it is not surprising that a deficiency of the

vitamin profoundly impacts energy production as well as other body processes secondary to reductions in the production of various intermediates normally generated during nutrient metabolism.

Three forms of beriberi have been described based primarily on symptom presentation: dry (affecting primarily the nervous system), wet (affecting primarily the heart), and acute (with varied symptom presentation). Dry beriberi is characterized by peripheral neuropathy and muscle weakness and cramping, especially in the lower extremities. The neuropathy consists of symmetrical sensory and motor nerve conduction problems mostly affecting the distal parts of the limbs/extremities (i.e., the ankles, feet, wrists, and hands). These problems usually begin in the feet and progress to the calves and then the thighs. A symmetrical foot drop may be present and associated with tenderness of the calf muscles and paresthesia in the feet; in the arms, wrist drop also may occur but typically after changes have occurred in the lower extremities (Figure 9.13). There is a lack of edema that is found in wet beriberi (hence the term “dry” versus “wet”), although there is often overlap between the wet and dry forms.

Wet beriberi is characterized by more extensive cardiovascular system involvement than dry beriberi. Cardiomegaly (enlarged heart), rapid heartbeat (tachycardia), right-sided heart failure (also affecting the lungs), and peripheral edema are common manifestations.

Acute beriberi, the third form, occurs mostly in infants, especially those who are breastfed by mothers having poor thiamin status. When present in infants, the condition may also be referred to as infantile beriberi. Acute beriberi can present with symptoms similar to wet and dry beriberi, or with different symptoms. Gastrointestinal symptoms (anorexia, nausea, vomiting, and abdominal pain) may occur along with tachycardia, tachypnea, respiratory distress, and lactic acidosis. The latter results from insufficient pyruvate dehydrogenase activity (which needs thiamin for activity). (Remember, with thiamin present, pyruvate is converted to acetyl-CoA. However, without thiamin, lactic acid is produced from pyruvate, and this lactic acid accumulates, causing acidosis.) Thiamin corrects the lactic acidosis by

increasing pyruvate dehydrogenase activity and enabling more pyruvate to be decarboxylated to acetyl-CoA for entry into the TCA cycle versus being converted to lactic acid.

Treatment of beriberi depends on the severity of its symptoms and the conditions under which the deficiency occurs. In adults, a mild deficiency can be treated with oral thiamin in doses of about 10–30 mg taken three times daily for several weeks or until symptoms disappear [6]. More severe cases may require either higher oral intakes of 100–800 mg of thiamin taken in divided doses or intravenous or intramuscular administration of thiamin until symptoms disappear [6].

Alcohol Dependency and Thiamin Deficiency

Individuals with alcohol dependency are more prone to developing thiamin deficiency because of decreased food consumption (and thus thiamin intake), decreased thiamin absorption (which is impaired by alcohol), and diminished thiamin utilization (i.e., TDP formation). The chronic thiamin deficiency seen in those with alcoholism may be characterized by a neurologic disorder called Wernicke’s encephalopathy, although not everyone with alcohol dependency develops encephalopathy (the reasons for these differences have not been identified). Neurological dysfunction may occur within about 2 weeks of insufficient thiamin available to the brain [6]. Symptoms of Wernicke’s encephalopathy affecting the eyes include **ophthalmoplegia** (weakness or paralysis of the ocular muscles) and horizontal **nystagmus** (constant, involuntary eyeball movement). The other two classic symptoms include ataxia (impaired muscle control or the inability to control body movement) and altered memory status or confusion. Without treatment, additional manifestations may occur including amnesia (memory loss primarily for distant events), problems forming new memories, confabulation, and psychosis; when present the condition is referred to as Wernicke-Korsakoff syndrome or Korsakoff psychosis.

Prevention and treatment protocols for Wernicke’s encephalopathy and Wernicke-Korsakoff syndrome vary. Those at risk typically need higher than the practice guidelines of 100 mg of thiamin/day. Intravenous administration of thiamin in amounts of 200 mg or more given several times per day has been beneficial, likely enabling plasma thiamin concentrations to reach sufficient levels for transport across the blood–brain barrier [6,7]. Persistent neurological symptoms, however, may necessitate the provision of higher parenterally administered doses of the vitamin over the course of several months and usually also followed by additional oral supplementation (doses of about 100 mg taken up to three times/day) until symptoms disappear [6,7].

One manifestation seen in the thiamin deficiency disorder beriberi is neuropathy which affects primarily the distal parts of the limbs/extremities (especially the ankles, and feet) as shown in this figure.



The Reading Room/Alamy Stock Photo

Figure 9.13 Some manifestations of thiamin deficiency.

At Risk for Deficiency

While most individuals in the United States consume thiamin in recommended amounts, there are a few exceptions. Older adults may fail to ingest enough thiamin-rich foods and be at risk for deficiency. Other individuals at risk for deficiency are those with diseases/situations that impair the vitamin's absorption (i.e., some gastrointestinal tract cancers, liver disease, inflammatory bowel diseases, and bariatric gastric Roux-en-Y bypass surgery). Higher thiamin intakes are needed for those with sepsis, burns, and some cancers as well for individuals with hyperemesis (excessive vomiting as may occur during pregnancy or other situations), diabetes, and receiving dialysis for renal failure. In addition, people with congestive heart failure are at higher risk for thiamin deficiency. The higher prevalence in this population is attributable to low thiamin intakes and increased urinary thiamin losses secondary to the use of loop diuretics, especially furosemides in high (80 mg) doses or for extended periods. Treatment typically requires oral thiamin (50–100 mg) taken a few times per day. However, those with more severe heart failure may require higher oral intakes (about 300 mg) of thiamin or intravenously administered thiamin (~200 mg) for several days to weeks [7].

Toxicity

No Tolerable Upper Intake Level has been established for thiamin, and no side effects have been reported from oral intakes up to about 500 mg daily. However, thiamin (in amounts up to 100 mg) given intravenously or intramuscularly has been associated with skin irritation, headache, convulsions, cardiac arrhythmia, and anaphylactic shock, among other signs [5].

Assessment of Nutriture

Thiamin status can be assessed by measuring thiamin and/or TDP in the blood or urine and by measuring erythrocyte transketolase activity in hemolyzed whole blood. Blood thiamin concentrations less than about 2.5 µg/dL and blood TDP concentrations less than about 70 nmol/L may be suggestive of deficiency. Urinary thiamin excretion decreases with decreased thiamin status and is also correlated with intake. Urinary thiamin excretion in amounts less than ~40 µg/day or ~27 µg/g creatinine suggests thiamin deficiency. The activity of transketolase, the thiamin-dependent enzyme of the pentose phosphate pathway, is measured after the addition of thiamin to the incubation medium to assess status. An increase in transketolase activity of >25% indicates thiamin deficiency; an increase in activity of 15–25% suggests marginal status; and an increase of ~15% suggests adequate status. Transketolase

concentrations of ~120 nmol/L also have been used to indicate deficiency; concentrations of 120–150 nmol/L suggest marginal thiamin status.

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Suggested Reading

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9.3 RIBOFLAVIN (VITAMIN B₂)

In 1917 scientists discovered riboflavin (vitamin B₂), which was originally called vitamin G in the United States. Kuhn and coworkers are credited with determining its structure along with Szent-Györgyi and Wagner-Jauregg in 1933. Riboflavin consists of a flavin molecule (isoalloxazine ring) with a ribitol (sugar alcohol) side chain attached. The name *riboflavin* signifies the presence of a ribose-like side chain (ribo) and its yellow color (*flavus* means “yellow” in Latin). The structures of riboflavin and its two coenzyme derivatives, FMN (flavin mononucleotide) and FAD (flavin adenine dinucleotide), are shown in Figure 9.14.

Sources

Riboflavin is found in a wide variety of foods (Table 9.7), but especially those of animal origin. Milk and milk products such as cheeses and yogurt contribute the most to dietary riboflavin intakes, providing 0.1–0.6 mg/serving. Meat, including poultry and fish, typically provide riboflavin in amounts of 0.1–0.3 mg/serving; liver is exceptionally rich, with about 2–3 mg/3-oz serving. Fruits have little riboflavin. Refined grains are enriched with riboflavin

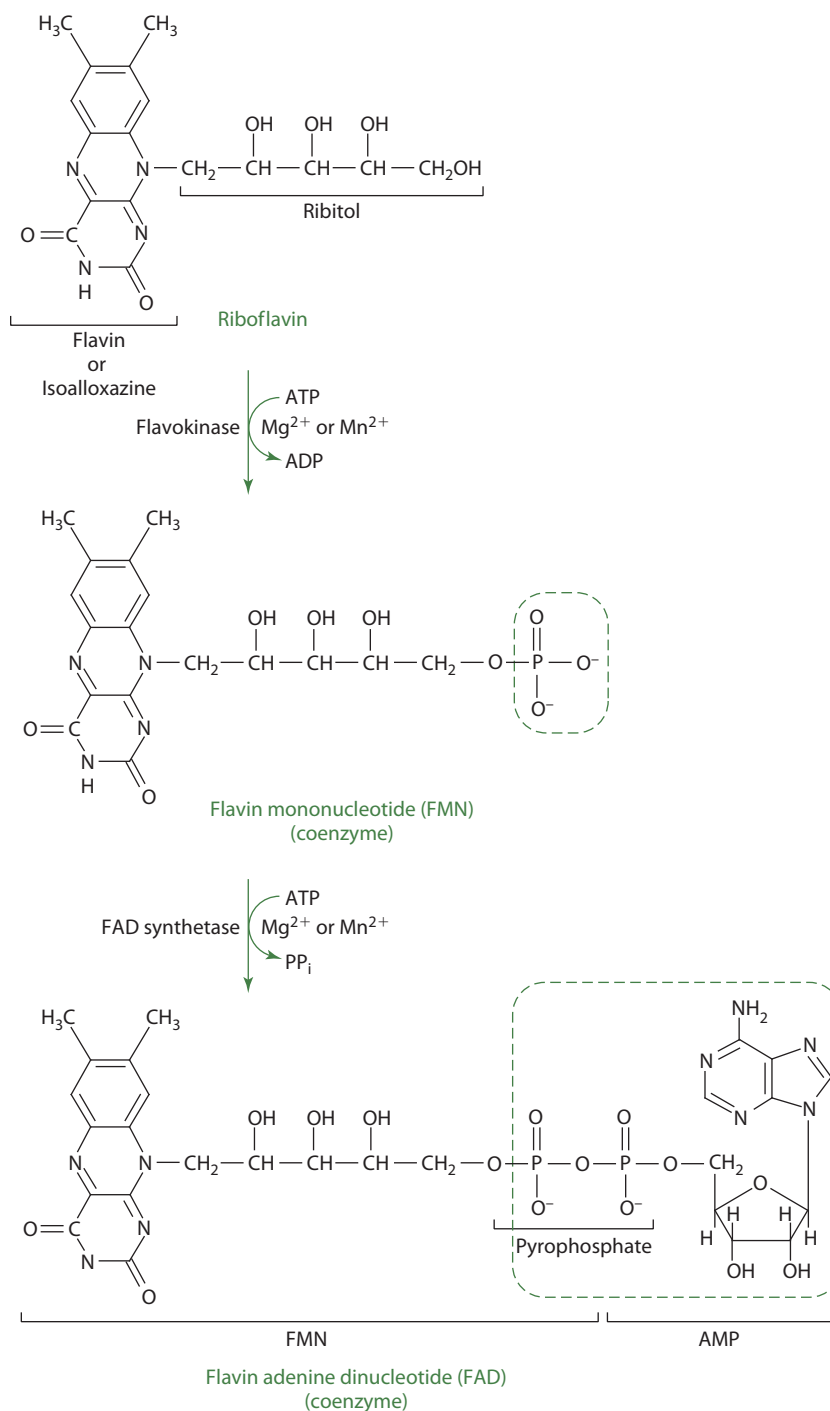


Figure 9.14 Structures of riboflavin and its coenzyme forms.

because during the milling of grains the removal of the bran and germ layers results in the loss of most (about two-thirds) of its riboflavin. Cereals may also be fortified with the vitamin.

Riboflavin in foods can be destroyed with exposure to sunlight (one reason milk is usually not sold in glass bottles); even photo (light) therapy, used to treat neonatal hyperbilirubinemia, causes riboflavin degradation and can lead to deficiency. The vitamin is fairly resistant to heat, oxidation, and acid.

Another source of riboflavin is provided by bacterial synthesis in the large intestine. Much of this microbially-produced riboflavin appears to be present in a free, absorbable form.

The form of riboflavin in food varies. Free and protein-bound riboflavin are found in milk, eggs, and enriched breads and cereals. In most other foods, the vitamin occurs as one or the other of its coenzyme derivatives FMN or FAD, although phosphorus-bound riboflavin and amino acid-bound FAD are also found in some foods.

Table 9.7 Riboflavin Content of Selected Foods*

Food (serving)	Riboflavin (mg)
Yogurt, Greek, plain, nonfat (1 c)	0.6
Milk, 2% (1 c)	0.5
Beef, steak, cooked (3 oz)	0.4
Cottage cheese (1/4 c)	0.3
Cheeses, variety (1 oz)	0.1–0.3
Meats, variety, and some fish (cod), cooked (3 oz)	0.1–0.3
Nuts, almonds (1 oz)	0.3
Mushrooms, portabella, grilled (1/2c)	0.3
Egg, cooked (1)	0.2
Quinoa, cooked (1 c)	0.2
Spinach, cooked (1/2 c)	0.2
Legumes, variety, cooked (1 c)	0.1
Rice, brown, long grain, unenriched, cooked (1/2 c)	0.1
Rice, white, enriched, cooked (1/2 c)	0.1
Bread, whole wheat (1 slice)	0.1
Bread, enriched (1 slice)	0.1

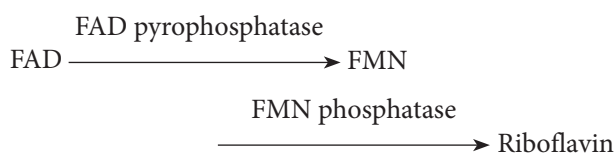
A more complete list of riboflavin-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/Riboflavin-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

In multivitamin and B-complex vitamin supplements, riboflavin is usually present as free riboflavin or bound to phosphate. The amount of the vitamin provided in the multivitamin supplements is typically about 1.7 mg while over-the-counter riboflavin supplements can be found providing doses up to about 400 mg. The Daily Value, found on food and supplement facts labels, for riboflavin is 1.3 mg.

Digestion and Absorption

The riboflavin that is found in foods attached noncovalently to proteins must be released from the protein prior to absorption; this process is accomplished by the actions of hydrochloric acid secreted within the stomach and proteases secreted by the stomach, pancreas, and small intestine. The riboflavin in foods as FAD, FMN, and riboflavin phosphate also must be released prior to absorption. FAD pyrophosphatase converts FAD to FMN, and FMN in turn is converted to free riboflavin by FMN phosphatase.



Other intestinal phosphatases, such as nucleotide diphosphatase and alkaline phosphatase, are thought to hydrolyze riboflavin from riboflavin phosphate.

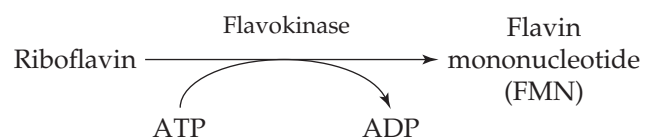
Not all bound riboflavin is hydrolyzed and available for absorption. A small amount (~7%) of FAD is covalently bound to either of two amino acids, histidine or cysteine. Thus, following consumption of foods with FAD bound to either of these amino acids, the proteins are degraded; however, the riboflavin remains bound to the histidine or cysteine residues and is unable to be used functionally in the body.

Free riboflavin is absorbed across the intestinal brush border membrane by energy-dependent riboflavin vitamin transporters (abbreviated RFVT). RFVT2 is ubiquitously present in tissues and, along with RFVT3, transports riboflavin across the brush border membrane of the small intestine. RFVT1 carries riboflavin across the basolateral membrane of the intestinal cell. In addition to carrier-mediated transport, riboflavin may be absorbed by diffusion with ingestion of pharmacological doses.

Mutations in the genes for RFVT2 and RFVT3 lead to the riboflavin transporter deficiency disorders Brown-Vialetto-Van Laere syndrome and Fazio-Londe syndrome. The conditions, which impair riboflavin absorption, are characterized by neurological degeneration, including hearing loss, sensory ataxia, progressive upper limb weakness, and optic atrophy. Riboflavin supplementation in pharmacological doses (10–80 mg/kg/day) can sometimes provide some benefit by delaying progression of some symptoms [1].

Normally, about 95% of riboflavin intake from foods is absorbed, up to a maximum of about 25–30 mg/meal at which point absorption plateaus and plasma concentrations peak [1]. Riboflavin absorption appears to be enhanced with the presence of bile, possibly through effects on intestinal motility or permeability. In contrast, alcohol impairs both riboflavin digestion and absorption.

Within the intestinal cell, riboflavin may be phosphorylated to form FMN in a reaction catalyzed by flavokinase (also called riboflavin kinase) and requiring ATP, as shown here and in Figure 9.14.



However, prior to its efflux into portal blood, FMN is dephosphorylated by a nonspecific alkaline phosphatase to generate free riboflavin.

Transport, Tissue Uptake, and Storage

Flavins are found in the plasma as riboflavin (50%), FMN (10%), and FAD (40%) and are usually bound to proteins, including albumin, fibrinogen, and globulins (principally immunoglobulins). Albumin appears to be the primary

transport protein, although riboflavin-binding proteins have been identified and may be also involved in the distribution of the vitamin to tissues. The usual reference range for plasma riboflavin is about 8–19 $\mu\text{g/L}$.

Tissues take up primarily free riboflavin from the blood via a carrier-mediated process. Riboflavin is found in small quantities in a variety of tissues. The greatest concentrations are found in the liver, kidneys, and heart. Body stores are estimated to provide enough riboflavin to meet its needs for about 2–6 weeks [2].

Following uptake into cells, riboflavin is converted to its FMN coenzyme form by flavokinase/riboflavin kinase and then to FAD by FAD synthase, as shown in Figure 9.14. Both enzymes are widely distributed in tissues, especially the liver, spleen, small intestine, kidneys, and heart. FMN is the major form (~60–95%) present in cells, followed by FAD (~5–20%). Synthesis is stimulated by several hormones including ACTH, aldosterone, and thyroid hormones, which accelerate the conversion of riboflavin into its coenzyme forms by increasing kinase activity. Synthesis is also influenced by end-product inhibition. The flavin coenzymes, once synthesized, become bound to and function as prosthetic groups for various enzymes (called flavoproteins) that are involved in oxidation-reduction reactions.

Functions and Mechanisms of Action

The flavin coenzymes can act as oxidizing agents because of their ability to accept a pair of hydrogen atoms. The isoalloxazine ring is reduced by two successive one-electron transfers with the intermediate formation of a semiquinone free radical, as shown in Figure 9.15. Reduction of the isoalloxazine ring yields the reduced forms of the flavoprotein, which can be found in FMNH_2 and FADH_2 .

Coenzyme (Flavoprotein) Roles in Nutrient Metabolism and Energy Production

Flavoproteins exhibit a wide range of redox potentials and therefore can play a variety of roles in intermediary metabolism. Different carriers transport FAD and FMN

within cells, such as from the cytosol into the mitochondria, enabling availability within organelles for coenzyme functions and also preventing degradation.

These coenzyme roles impact not only energy production as part of the *electron transport chain* (illustrated in Figures 3.27 and 3.28) but also the metabolism of nutrients.

Macronutrient Metabolism Some coenzyme roles include:

- *Oxidative decarboxylation of pyruvate* (Figure 9.9) and α -ketoglutarate where FAD serves as an intermediate electron carrier and NADH is the final reduced product.
- *Succinate dehydrogenase*, a FAD flavoprotein, that removes electrons from succinic acid to form fumarate, and that forms FADH_2 from FAD (see Figure 3.28). The electrons are then passed into the electron transport chain by coenzyme Q (see Figure 3.29).
- *Acyl-CoA dehydrogenases* requiring FAD (see Figure 5.26) for fatty acid beta-oxidation.
- *Sphinganine oxidase* requiring FAD for sphingosine synthesis.
- *L-amino oxidase*, which uses FMN in the dehydrogenation of L-amino acids to imino acids.

Antioxidant FAD is important in the regeneration of the reduced forms of glutathione and thioredoxin, which play key antioxidant roles in the body. Some examples:

- Reduction of the oxidized form of glutathione (GSSG) to its reduced form (GSH) depends on FAD-dependent *glutathione reductase*. (Note that this reaction forms the basis of one assay used to assess riboflavin status; see the “Assessment of Nutriture” section.)
- *Thioredoxin reductase*, a flavo (FAD) enzyme (also containing selenocysteine at its active site), transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds within the oxidized form of thioredoxin. The enzyme works as part of a complex

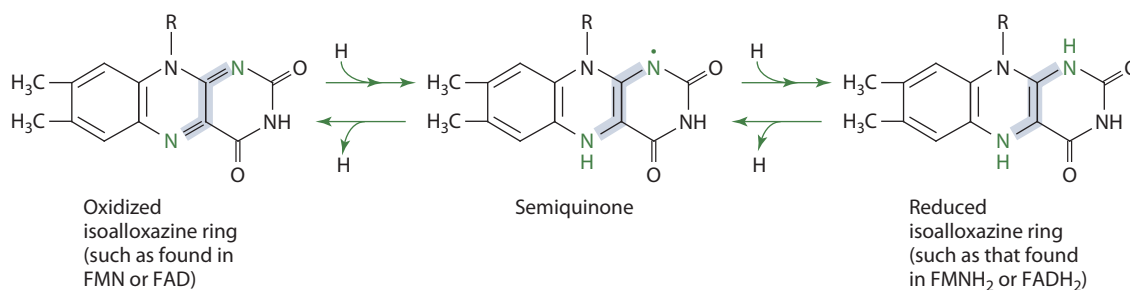
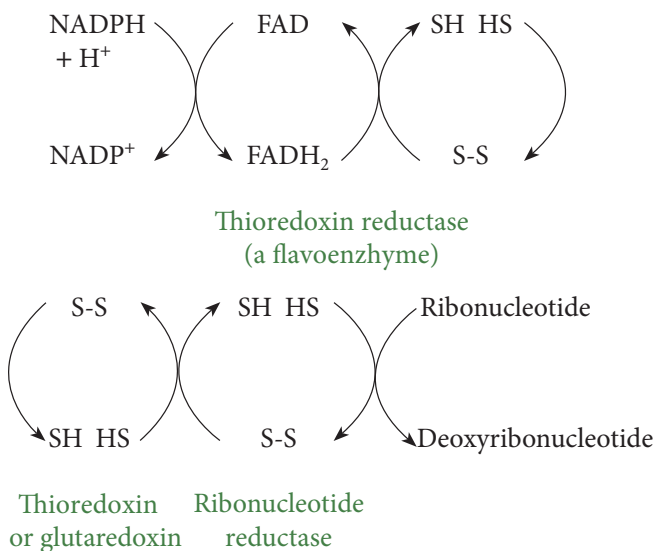


Figure 9.15 Oxidation and reduction of isoalloxazine ring.

set of reactions with ribonucleotide reductase (which contains thiol groups), as shown here:



Nucleic Acids Some roles of FAD in the nucleic acid metabolism (with the first role also involving the vitamin's use in regenerating antioxidant compounds) include:

- *Ribonucleotide reductase*, which catalyzes the conversion of ribonucleotides to deoxyribonucleotides (such as dADP, dGDP, dCDP, and dUDP; see Figure 6.28) needed for DNA synthesis. [Note that in the reaction, the sulfhydryl groups in ribonucleotide reductase become oxidized, forming a disulfide bond. Thioredoxin (or glutaredoxin, a small protein like thioredoxin) provides electrons (H), but upon donation becomes oxidized itself (containing a disulfide bond). The flavoenzyme thioredoxin reductase (or glutaredoxin reductase), which also contains sulfhydryl groups, reduces the thioredoxin (or glutaredoxin). NADPH then reduces the thioredoxin reductase (or glutaredoxin reductase) to eliminate the disulfide bond and regenerate the sulfhydryl groups.]
- As a coenzyme for an oxidase such as *xanthine oxidase* involved in purine catabolism, FAD transfers electrons directly to oxygen with the formation of hydrogen peroxide (see the section on molybdenum in Chapter 13).

Vitamins Roles of FAD in the metabolism of vitamins include:

- In vitamin B₆ metabolism (seen later in Figure 9.43), *pyridoxine phosphate oxidase* requires FMN; this enzyme is needed for the conversion of both pyridoxamine phosphate (PMP) and pyridoxine phosphate

(PNP) to pyridoxal phosphate (PLP), which is the primary coenzyme form of vitamin B₆.

- The role of FAD-dependent *aldehyde oxidase*, which is needed for (1) the catabolism of the aldehyde form of vitamin B₆ (pyridoxal) to form pyridoxic acid and for (2) the metabolism of the aldehyde form of vitamin A (retinal) to produce retinoic acid.
- As a coenzyme for methylene tetrahydrofolate reductase (MTHFR), which is needed for the synthesis of 5-methyl tetrahydrofolate (THF) from 5,10-methylene THF (the reaction is shown later in Figure 9.34). The C677T MTHFR polymorphism diminishes the ability of the vitamin to bind to the enzyme, resulting in reduced enzyme activity and increased risk of disease; this polymorphism is discussed further in the section “Folate” under the subsection “Functions and Mechanisms of Action.”
- A step in the synthesis of niacin from tryptophan that is catalyzed by kynureninase monooxygenase (see Figure 9.19).

Other FAD also has roles in the metabolism of some neurotransmitters and nitrogen-containing compounds like choline, as well as roles in the cellular processing of some proteins following their synthesis and in hydrogen peroxide production for destruction of foreign substances. Examples are:

- Some neurotransmitters (such as dopamine) and other amines (tyramine and histamine) require FAD-dependent *monoamine oxidase* for metabolism.
- In *choline catabolism*, several dehydrogenases require FAD.
- *Ero1 and sulfhydryl oxidases* are FAD dependent and help to form disulfide bonds involved in the structure or folding of selected secretory proteins. Impaired folding and subsequently impaired secretion of proteins have been observed with cellular reductions in riboflavin.
- Hydrogen peroxide production from singlet oxygen (¹O₂, which is derived from, e.g., activated white blood cells) and water via an antibody-catalyzed water oxidation pathway also appears to require riboflavin. Hydrogen peroxide assists in the destruction of foreign substances, although it is also destructive to body cells.

Selected Pharmacological Uses/Other Roles

The ingestion of riboflavin in doses of up to 400 mg is modestly effective in decreasing the frequency of migraine headaches in adults in some (but not all) studies [3]. Supplementation for at least 3 months is typically needed to determine any beneficial effects.

The autosomal recessive condition glutaric aciduria type 1 results from mutations in FAD-dependent enzyme glutaryl-CoA dehydrogenase, which converts glutaryl-CoA to glutaconyl-CoA as part of the degradation of the amino acids tryptophan and lysine. Some individuals with glutaric aciduria type 1 benefit from the ingestion of pharmacological doses of riboflavin (about 200 mg per day taken in divided doses), which enhances some residual glutaryl-CoA dehydrogenase activity and thus reduces the production of glutaric acid.

FAD is also needed as a coenzyme for several acyl-CoA dehydrogenases involved in the beta-oxidation of fatty acids in the mitochondria. A variety of mutations in these enzymes have been identified, with some mutations responsive to riboflavin supplementation. In responsive cases, riboflavin supplementation in amounts of about 25–150 mg (ingested orally in divided doses) has been needed.

Metabolism and Excretion

Riboflavin is excreted from the body to a small extent in the feces but primarily via the urine. Endogenous riboflavin appears in the feces via secretion in bile. Fecal riboflavin metabolites also arise from the catabolism of riboflavin by intestinal bacteria.

Most riboflavin is excreted from the body in the urine. With adequate intake, about 60–70% of free riboflavin (i.e., not bound to proteins) is filtered by the glomerulus and excreted intact in the urine. With less than adequate intake, carrier-mediated reabsorption of the vitamin occurs in the renal proximal tubules.

In addition to the excretion of intact riboflavin, riboflavin metabolites are also excreted in the urine. The metabolites arise (1) from tissue degradation of covalently bound flavins, (2) from degradation of the vitamin itself, and (3) from intestinal bacteria (i.e., metabolites formed in the intestinal tract by bacterial degradation of the vitamin and that were absorbed, but are subsequently excreted in the urine). The urinary metabolites present in the greatest concentrations include 7 α - and 8 α -hydroxymethyl riboflavin, 8 α -sulfonyl riboflavin, 10-hydroxyethyl flavin, and riboflavinyl peptide ester. Riboflavin bound to cysteine and histidine may also be found in the urine if absorbed in such form from the gastrointestinal tract or if generated in body cells from the degradation of flavoenzymes such as succinate dehydrogenase and monoamine oxidase [4]. Urinary riboflavin is typically found in amounts of at least 120 μ g/day or 80 μ g/g creatinine.

Unlike other vitamins, the presence of riboflavin in the urine is often visibly noticeable. Within a couple of hours following the ingestion of riboflavin (which is a fluorescent yellow compound) in a quantity such as 1.7 mg (similar to that found in a multivitamin supplement) or higher, the excretion of riboflavin can be noticed by a deepening of

the urine's color from a typical light yellow to a brighter, orangish yellow.

Recommended Dietary Allowance

The RDAs for riboflavin have been estimated through various studies examining urinary excretion of the vitamin, the relationship of dietary intake to clinical signs of deficiency, and the activity of erythrocyte glutathione reductase. Recommendations for riboflavin for adult men and women are 1.3 mg/day and 1.1 mg/day, respectively; the requirements for adult men and women are 1.1 mg and 0.9 mg, respectively [4]. With pregnancy and lactation, recommendations for daily riboflavin intake increase to 1.4 mg and 1.6 mg, respectively [4]. The inside front cover of the book provides additional RDAs for riboflavin for other age groups.

Deficiency: Ariboflavinosis

A deficiency of riboflavin, sometimes called ariboflavinosis, rarely occurs in isolation; if encountered, it is usually accompanied by other nutrient deficits. While no clear riboflavin deficiency disease has been characterized, some clinical signs of deficiency (which appear after about 3–4 months of inadequate intake) include sore throat or pharyngitis and painful lesions or vertical fissures on the outside of the lips (cheilosis) and corners of the mouth (angular stomatitis). Inflammation of the tongue (glossitis), which may also appear smooth and magenta red in color, and a red (hyperemia) and swollen (edema) mouth/oral cavity are common. These manifestations (some of which are shown in Figure 9.16) are also commonly observed in other B-vitamin deficiencies.

An inflammatory skin condition, sometimes referred to as oculo-rogenital syndrome, can also occur with riboflavin deficiency. The condition affects areas of the skin containing high concentrations of sebaceous glands, including the external ear, the nasolabial fold, eyelids, and scrotum (males). In these areas, the skin becomes reddened, scaly, greasy, and painful (seborrheic dermatitis). The eyes may be

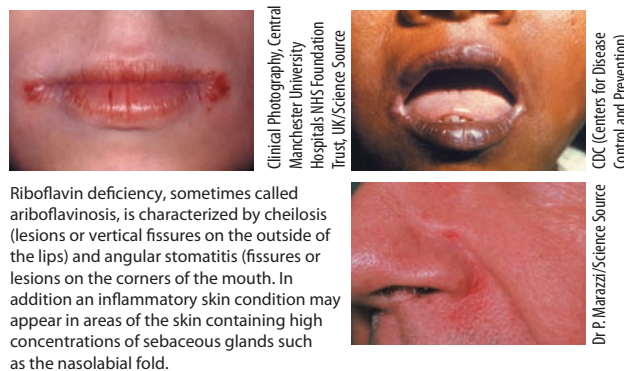


Figure 9.16 Some manifestations of a riboflavin deficiency.

affected with conjunctival infection and photophobia, and there may also be anemia and peripheral nerve dysfunction (neuropathy). Severe riboflavin deficiency may also diminish the riboflavin-dependent synthesis of the coenzyme form of vitamin B₆ and synthesis of niacin from tryptophan.

Treatment of deficiency in adults usually requires about 5–30 mg of riboflavin daily (given in divided doses) until symptoms resolve, although supplementation in amounts up to 60 mg are sometimes used. To facilitate absorption and minimize digestive tract problems, oral doses of the vitamin are best taken with food and in amounts of no more than about 25–30 mg at one time.

At Risk for Deficiency

Because of limited dietary intake and diminished absorption, people consuming excess alcohol are at risk of deficiency. The likelihood of deficiency also remains fairly common in developing countries where intakes of dairy products and meat are typically low. Moreover, newborn infants born to women with poor riboflavin status are also at risk of deficiency. And, newborns being treated with phototherapy for hyperbilirubinemia are at risk secondary to therapy-induced riboflavin destruction. Because riboflavin metabolism is altered with thyroid disease (hypothyroidism) and adrenal insufficiency, and because riboflavin excretion is enhanced with diabetes mellitus, trauma, and stress, people with these conditions are also at risk for deficiency. Tricyclic medications used to treat depression inhibit riboflavin function and may increase the likelihood of deficiency in individuals taking large doses of these medications.

Toxicity

Toxicity associated with ingestion of large oral doses of riboflavin has not been reported, and no Tolerable Upper Intake Level for riboflavin has been established [4]. Oral doses of up to 400 mg of riboflavin have been consumed in those with migraines without side effects [4].

Assessment of Nutriture

The most sensitive method for determining riboflavin nutriture is to measure the activity of the FAD-dependent enzyme erythrocyte glutathione reductase, which catalyzes the following reaction:



In this reaction, glutathione in its oxidized form is designated GSSG, and in its reduced form, GSH. In cases of a riboflavin deficiency or marginal riboflavin status, the activity of glutathione reductase is limited, and less NADPH is used to reduce the oxidized glutathione. *In vitro* enzyme activity in terms of “activity or activation

coefficients” (AC) is determined both with and without the addition of FAD to the medium. Activity coefficients represent a ratio of the enzyme’s activity with FAD to the enzyme’s activity without FAD. When the addition of FAD stimulates enzyme activity to generate an AC of 1.2–1.4, riboflavin status is considered low; an AC > 1.4 suggests riboflavin deficiency. Conversely, if FAD is added and AC is ~1.2, then riboflavin status is considered acceptable.

In addition to the use of glutathione reductase activity, the activity of an FMN-dependent enzyme, pyridoxamine phosphate oxidase, needed for interconversions of vitamin B₆ coenzyme forms, also appears to be a biomarker of riboflavin status. Urinary riboflavin excretion is also used to assess status, with urinary excretion of less than about 25 µg riboflavin/g creatinine (without recent riboflavin intake) or less than about 40 µg riboflavin per day indicative of deficiency.

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4. Food and Nutrition Board. *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic Acid, Biotin, and Choline*. Washington, DC: National Academy Press. 1998. pp. 87–122.

9.4 NIACIN (VITAMIN B₃)

Like thiamin, which was discovered through its deficiency disorder beriberi, niacin (also called vitamin B₃) was discovered through the condition called **pellagra** in humans and a similar condition called black tongue in dogs. In fact, the vitamin was once called the anti-black tongue factor because of its effect in dogs. Pellagra was especially prevalent in the southeastern United States where corn (which contains a relatively unavailable form of niacin) was a main dietary staple in the early 1900s. It was not until about 1937 that Elvehjem isolated the vitamin, which was shown then to cure both pellagra and black tongue. The vitamin was named niacin in the early 1940s. Niacin, however, is a generic term for nicotinic acid and nicotinamide (also called niacinamide), which both provide vitamin activity. Structurally, nicotinic acid is pyridine 3-carboxylic acid, whereas nicotinamide is nicotinic acid amide (Figure 9.17).

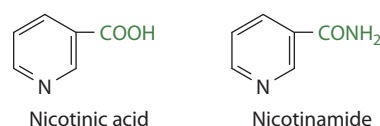


Figure 9.17 Structures of nicotinic acid and nicotinamide.

Sources

Niacin is found in several food groups, with the best sources being fish and meats, including poultry, and providing about 6–11 mg niacin/serving (Table 9.8). Beef liver is especially rich, with about 15 mg of niacin/3-oz serving. Enriched cereals and bread products, whole grains, seeds, and legumes also contain appreciable amounts (about 2–5 mg/serving) of niacin. Cereals are often fortified with the vitamin. Niacin is also found in coffee and tea and in lesser amounts in green vegetables and milk. In coffee, a compound called trigonelline is converted to niacin by heat (such as with coffee bean roasting) and acid. Niacin in foods is fairly stable; minimal losses of the vitamin result from cooking or storage.

Nicotinic acid is also produced by bacteria in the large intestine. The overall contribution of this source of the vitamin to the body's need for niacin is unclear.

In supplements, niacin is provided as both nicotinamide and nicotinic acid in varying amounts. Nicotinamide is used to fortify foods. Single-ingredient niacin supplements often provide up to about 500 mg of the vitamin. The Daily Value, found on food and supplement facts labels, for niacin is 16 mg.

Several different forms of the vitamin are present naturally in foods. In animal foods, niacin occurs mainly as

nicotinamide and its coenzyme forms, the nicotinamide nucleotides—nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Figure 9.18 shows the structures of NAD and NADP. In their oxidized forms, NAD and NADP possess a positive charge and therefore may alternatively be written NAD^+ and NADP^+ .

In plant foods, niacin is present mainly as nicotinic acid. However, in some grains, the vitamin may be bound covalently to complex carbohydrates and called niacytin, or it may be bound to small peptides and called niacinogens. These bound forms of niacin, which are only about 30% bioavailable, are found primarily in corn, but also in wheat and some other cereal products. Treatment of the cereals with bases, such as lime water, improves the availability of some bound niacin to release free nicotinic acid. Some nicotinic acid is also released from niacytin on exposure to gastric acid.

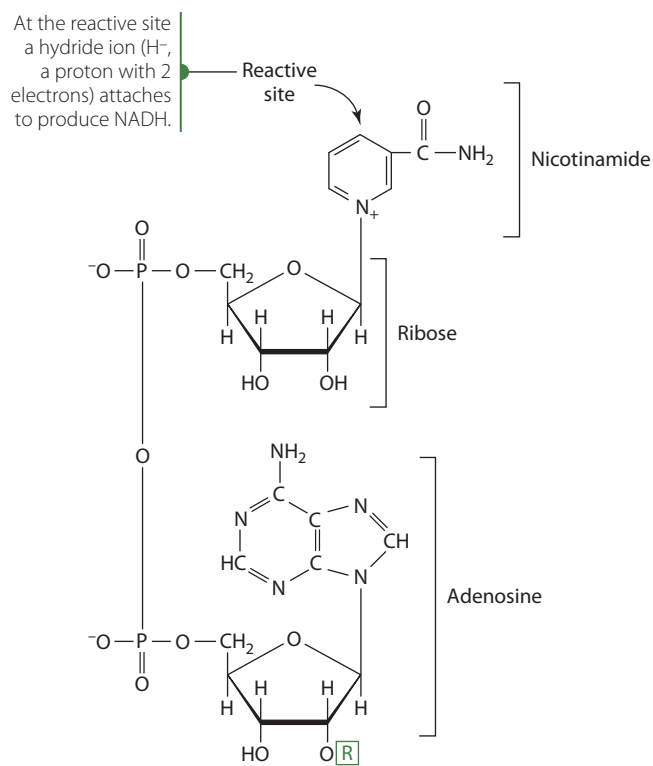
In addition to dietary sources, niacin, as NAD, can be synthesized in the body from the amino acid tryptophan. This biosynthetic pathway (sometimes called the kynurenine pathway) occurs primarily in the liver and provides an important contribution to the niacin needs of the body (see Figure 9.19). About 3% of the tryptophan that is metabolized follows the pathway, and an estimated 1 mg of niacin is produced from the ingestion of 60 mg

Table 9.8 Niacin Content of Selected Foods*

Food (serving)	Niacin (mg)
Tuna, yellow fin, cooked (3 oz)	11.3
Chicken, breast, cooked (3 oz)	10.3
Turkey, breast, cooked (3 oz)	10.0
Tuna, canned in water, drained (3 oz)	8.6
Veal, loin, cooked (3 oz)	8.5
Salmon, Atlantic, cooked (3 oz)	8.5
Swordfish, cooked (3 oz)	7.8
Beef, top sirloin, cooked (3 oz)	7.6
Halibut, cooked (3 oz)	6.1
Beef, ground, 90% lean, cooked (3 oz)	5.8
Peanut butter (2 Tbsp)	4.2
Peanuts, dry roasted (1 oz)	4.2
Spaghetti, enriched, cooked (1 c)	2.3
Sunflower seeds, dry roasted (1 oz)	2.0
Pumpkin seeds, dry roasted (1 oz)	1.3
Soymilk, unfortified (1 c)	1.3
Rice, white, enriched, cooked (1/2 c)	1.1
Lentils, cooked (1/2 c)	1.1
Bread, white, enriched (1 slice)	1.1

A more complete list of niacin-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://www.nal.usda.gov/sites/www.nal.usda.gov/files/niacin.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.



$\text{R} = \text{H}$ for NAD^+ (nicotinamide adenine dinucleotide)

$\text{R} = \text{PO}_3^{2-}$ for NADP^+ (nicotinamide adenine dinucleotide phosphate)

Figure 9.18 Structures of NAD and NADP.

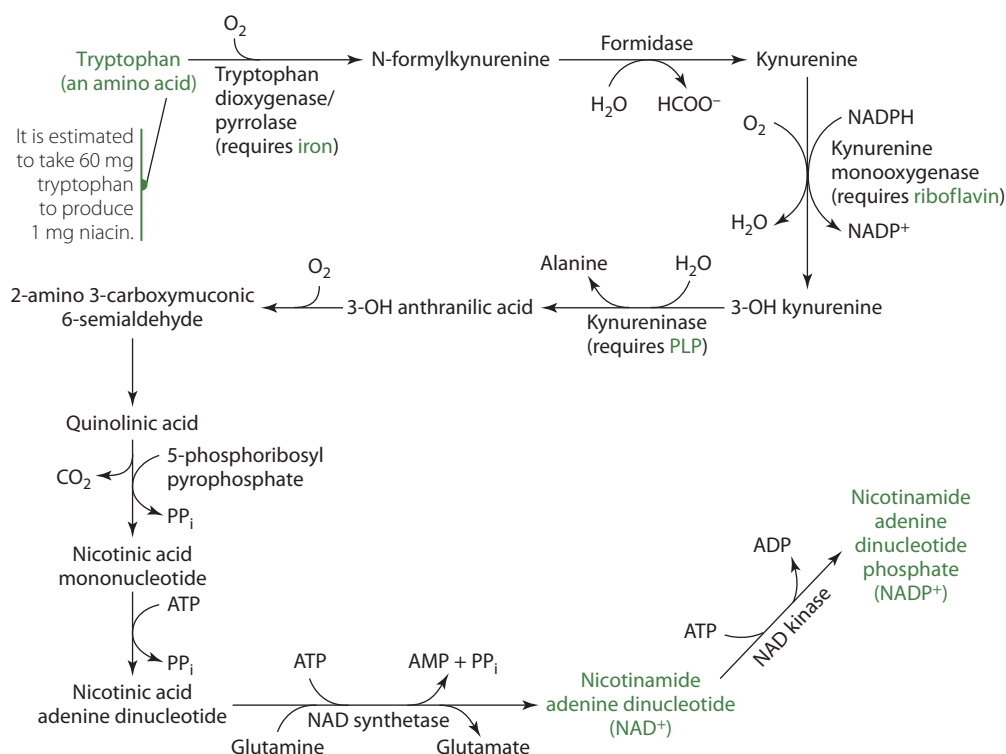


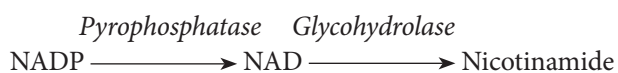
Figure 9.19 NAD⁺ and NADP⁺ synthesis from the amino acid tryptophan.

of dietary tryptophan (see the RDA section for niacin to understand how this synthesis is accounted for in niacin recommendations).

The contribution of tryptophan to niacin production is evident in those with Hartnup disease, an autosomal recessive genetic disorder. In Hartnup disease, the transporter for tryptophan is defective and thus tryptophan absorption into intestinal cells is severely reduced. It is not uncommon for individuals with this disorder to develop a niacin deficiency due to insufficient tryptophan available for niacin production; however, inadequate intakes of riboflavin (as FAD), vitamin B₆ (as PLP), and iron, which are also required in some of the reactions, can also impair niacin synthesis.

Digestion and Absorption

Digestion of NAD and NADP obtained from foods is needed for niacin's absorption. A pyrophosphatase accomplishes this digestion, enabling phosphate hydrolysis from NADP. The resulting NAD is then hydrolyzed by glycohydrolase to release free nicotinamide, which can be absorbed.



With typical dietary intakes of niacin from foods, nicotinamide and nicotinic acid are absorbed primarily in the

small intestine. The molecular nature of the carrier is still unclear, but it appears to be similar to the sodium-dependent carrier organic anion transporter (OAT)-10. When present in high concentrations (as with the ingestion of pharmacological doses), both forms of niacin are absorbed by passive diffusion in the small intestine. A sodium-independent, high-affinity transporter facilitates nicotinic acid absorption from the colon.

Transport, Tissue Uptake, and Storage

In the plasma, niacin is found primarily as nicotinamide, but also as nicotinic acid. Plasma concentrations of niacin range from about 0.5 to 8.5 μg/mL. About 15–30% of nicotinic acid in the plasma is bound to proteins. From the blood, nicotinamide and nicotinic acid move across cell membranes by simple diffusion; however, nicotinic acid transport into the kidney tubules and red blood cells appears to require a carrier, and uptake into the brain is energy dependent. No one organ stores niacin, although the liver as well as other tissues likely contain small amounts.

Nicotinamide serves as the primary precursor of NAD, which is synthesized in all tissues. Nicotinic acid may also be used to synthesize NAD, but this reaction occurs primarily in the liver. Phosphorylation of NAD by NAD kinase using ATP generates NADP. These reactions may be reversed, converting NADP to NAD and NAD to

nicotinamide, which then is available for transport to other tissues.

As NAD or NADP, the vitamin is trapped within the cell. Intracellular concentrations of NAD typically predominate over those of NADP. In the liver, excess niacin and tryptophan are converted to NAD, which is stored (but not bound to enzymes) in small amounts. NAD is found primarily in its oxidized form (NAD^+), whereas NADP is found in cells mainly in its reduced form (NADPH).

Functions and Mechanisms of Action

Over 400 enzymes, primarily dehydrogenases, require the coenzymes NAD and NADP, which act as hydrogen donors or electron acceptors and enable nutrient metabolism and energy production. Figure 9.20 demonstrates the oxidation reduction that may occur in the nicotinamide moiety of the coenzymes. In addition to its coenzyme roles, niacin functions in nonredox roles as a donor of adenosine diphosphate ribose.

Coenzyme Roles for Nutrient Metabolism and Energy Production

Although NAD and NADP are similar and undergo reversible reduction in the same way, their functions in the cell are different. The major role of NADH (the reduced form of NAD) is to transfer its electrons from metabolic intermediates in mostly catabolic pathways (such as glycolysis and the citric acid cycle) through the electron transport chain (see Figures 3.26 and 3.28), thereby producing ATP. NADPH, in contrast, acts as a reducing agent in mostly biosynthetic pathways such as fatty acid, cholesterol, and steroid hormone synthesis, as well as in oxidant defense and cytochrome P450 requiring reactions. NAD and NADP coenzymes are tightly bound to their apoenzymes and can easily transport hydrogen atoms from one part of the cell to another. Reactions in which they participate occur both in the mitochondria and in the cytosol.

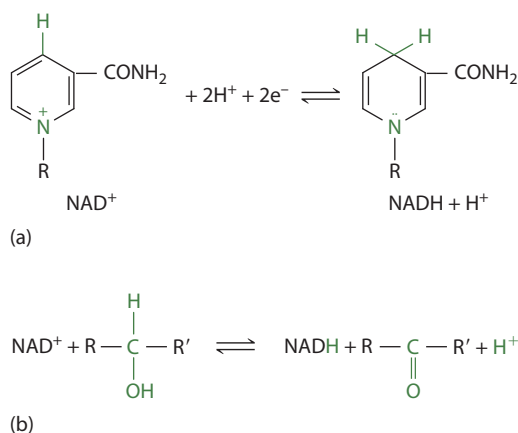


Figure 9.20 (a) The oxidation and reduction in the nicotinamide moiety. (b) The role of NAD in dehydrogenation reactions. One H of the substrate goes to NAD.

Oxidative reactions in which NAD participates and is reduced to NADH (and in turn can be transferred to the electron transport chain for ATP generation) include:

- Glycolysis (see Figure 3.20)
- Oxidative decarboxylation of pyruvate to acetyl-CoA (see Figure 9.10)
- Oxidation of acetyl-CoA in the TCA cycle (see Figure 3.21)
- β -Oxidation of fatty acids (see Figure 5.26)
- Oxidation of ethanol (see Figure 5.39).

In addition to its aforementioned roles, NAD is also required by aldehyde dehydrogenase for catabolism of vitamin B₆ as pyridoxal to its excretory product, pyridoxic acid.

NADP can be reduced to NADPH. This reaction occurs as part of the pentose phosphate pathway (see Figure 3.31) and the mitochondrial membrane malate aspartate shuttle (see Figure 3.24). The NADPH produced in these reactions is used in nutrient metabolism, including in a variety of reductive biosynthetic processes, such as:

- Fatty acid synthesis (see Figure 5.33)
- Cholesterol and steroid hormone synthesis
- Proline synthesis (see Figure 6.33)
- Deoxyribonucleotide (precursors of DNA) synthesis (see Figure 6.28)
- Glutathione, vitamin C, and thioredoxin regeneration
- Folate coenzyme synthesis (dihydrofolate [DHF], tetrahydrofolate [THF], 5-methyl THF, and 5,10-methylene THF; see Figure 9.34 later in this chapter).

Nonredox Roles with Adenosine Diphosphate (ADP) Ribose

NAD is also involved in about 50 different nonredox reactions in which adenosine diphosphate (ADP) ribose is transferred to acceptor molecules. These reactions are generally associated with cellular processes such as DNA repair, replication, and transcription; G-protein activity; chromatin structure; and intracellular calcium signaling, among others. Four ADP-ribosylation reactions are described hereafter.

Mono-ADP-Ribosyltransferase The transfer of one (mono) ADP-ribose (ARTs) from NAD to various acceptor proteins occurs by the action of mono-ADP-ribosyltransferase and forms ADP-ribosylated proteins with the release of nicotinamide. The acceptor proteins are found in the cytosol or attached to the inner cell membrane (termed *endoARTS*) and on the outside of cell membranes (termed *ectoARTS*). EctoARTS are present on the membranes of many tissues including the lungs, muscles (cardiac and skeletal), and lymph tissues, among others. An example of a substrate for ART1 is defensin, an antimicrobial

peptide important in the immune response. Other ADP-ribosylated proteins are involved, for example, with the cell cytoskeleton and in cell signaling.

Poly-ADP-Ribose Polymerases Poly ADP-ribose polymerases (PARP) transfer several (*poly* > 200) polymers of branched ADP-riboses from NAD onto various target proteins. PARP-1, the most abundant of five polymerases, binds to DNA strand breaks. This interaction leads to NAD use, poly ADP-ribose formation, and repair in DNA. Other roles of these enzymes include cell replication, differentiation, and signaling along with apoptosis. Possible related roles for niacin in cancer prevention are also under study.

ADP-Ribosylcyclases Another group of enzymes involved in ADP-ribosylation reaction is ADP-ribosylcyclases. These enzymes form cyclic ADP-ribose also using ADP-ribose. Cyclic ADP-ribose functions in some cells as a second messenger involved in control of ryanodine receptors and in mobilization of calcium from intracellular stores, especially in neurons.

Deacetylases The class of NAD-dependent deacetylases, known as sirtuins, use ADP-ribose as an acceptor as they catalyze the removal of acetyl groups (deacetylation) from target proteins containing acetylated lysine residues. The sirtuins are found in the nucleus, cytosol, and mitochondria. (Note that the acetylation of proteins, which occurs post-translationally and involves pantothenic acid, affects numerous functions including enzyme activity, cell signaling, gene expression, and interactions “or crosstalk” with other post-translationally modified proteins in nearby locations [1].) The deacetylation process involves enzymatic cleavage of NAD and the transfer of the acetyl group from the substrate protein (such as an acetylated histone) to the ADP-ribose moiety of NAD generating O-acetyl-ADP-ribose and the deacetylated substrate protein (histone). Sirtuins affect chromatin structure and also play roles in signaling pathways involved in energy metabolism in tissues, the cellular stress response, and in DNA repair, cell differentiation, and cell cycle regulation, among others. Their functions may also affect longevity, specifically delaying aging and the development of some age-related conditions through effects on chromatin and genomic stability.

Selected Pharmacological Uses/Other Roles

Pharmacological doses of both nicotinic acid and niacinamide are used in the treatment of several conditions.

Nicotinic Acid Large doses of nicotinic acid (up to 6 g/day in divided doses) are used to treat some types of hyperlipidemias (high blood lipids). Pharmacological doses of nicotinic acid significantly lower serum total cholesterol, triacylglycerols, and low-density lipoproteins

(LDLs) and increase high-density lipoproteins (HDLs). It also reduces lipoprotein (a) concentrations. Although the mechanisms of action are not fully understood, nicotinic acid may function to improve serum lipids through interactions with G-protein coupled receptors as well as interactions with various enzymes, among other means [2]. The vitamin diminishes lipolysis in adipose tissue and hepatic very low-density lipoprotein (VLDL) synthesis (via inhibition of diacylglycerol acyltransferase) and secretion and thus LDL production [2].

Despite the therapeutic benefits of nicotinic acid, some undesirable side effects are associated with its use as a drug, especially in certain forms and in doses of typically 1 g or more per day. Some of these side effects include uncomfortable redness/flushing (usually starting in the face and neck) along with burning, itching (pruritus), tingling, and headaches. The flushing, one of the most common side effects, may occur with the use of nicotinic acid in doses as low as about 30–50 mg per day. Gastrointestinal problems may include heartburn and nausea and vomiting. Liver injury, high blood uric acid concentrations (hyperuricemia), and possibly gout, along with hyperglycemia and glucose intolerance, may also occur [2]. Extended-release forms of nicotinic acid (such as Niaspan®) with fewer side effects are available; however, hepatic toxicity, headaches, and gastrointestinal distress may still result. Coingestion of nicotinic acid with aspirin or use of modified-release forms of the vitamin, such as inositol hexanicotinate, can help to reduce the flushing.

Nicotinamide While nicotinamide in large doses does not reduce blood lipids, this form of niacin is sometimes used topically to reduce inflammation associated with acne vulgaris. In addition, the vitamin has been used orally in the treatment of another skin condition, necrobiosis lipoidica, which is characterized by reddish-brown bruise-like markings most often on the lower legs. Over time the lesions become yellowish in color and atrophic plaques develop; the condition, although fairly rare, may occur in those with diabetes mellitus. Lastly, nicotinamide (500 mg doses given twice daily) reduced the rates of new nonmelanoma skin cancers (usually associated with damage due to UV radiation) and actinic keratosis in individuals who had at least two nonmelanoma skin cancers in the previous 5 years [3]. Side effects with the use of nicotinamide in doses of about 3 g/day include headache, gastrointestinal distress (heartburn, nausea), impaired glucose intolerance, and liver damage (manifested by jaundice and elevated liver enzymes).

Metabolism and Excretion

NAD and NADP undergo degradation by glycohydrolase to form ADP-ribose and nicotinamide. The released nicotinamide is then methylated and oxidized in the liver into

a variety of products that are excreted in the urine. The primary metabolites of nicotinamide are N' methyl nicotinamide (sometimes abbreviated NMN and representing ~20–30% of niacin metabolites) and N' methyl 2-pyridone 5-carboxamide (also called 2-pyridone and representing ~40–60%). Small amounts of N' methyl 4-pyridone carboxamide (called 4-pyridone) may also be present. Nicotinic acid is metabolized mainly to N' methylnicotinic acid. Little free nicotinic acid or nicotinamide is excreted (with usual physiologic niacin intake), as both compounds are actively reabsorbed from the glomerular filtrate.

Recommended Dietary Allowance

Recommendations for niacin intake include calculations of niacin derived from the amino acid tryptophan, with about 60 mg of tryptophan estimated to generate 1 mg of niacin. Total niacin thus is provided to the body as nicotinic acid and nicotinamide and from tryptophan. The term *niacin equivalent (NE)* is used to account for the provision by tryptophan.

Although recommendations are given in niacin equivalents, food composition tables report only preformed niacin. A rough estimate of niacin equivalents from a protein can be made by assuming that 10 mg of tryptophan are provided for every 1 g of high-quality (complete) protein in the diet, that is, 1 g of high-quality protein = 10 mg of tryptophan. This estimate means that an intake of 60 g of high-quality protein, for example, would provide 600 mg of tryptophan (10 mg tryptophan/1 g protein $\times$ 60 g protein = 600 mg tryptophan). Then, because it takes 60 mg of tryptophan to generate 1 mg of NE, 600 mg of tryptophan would generate 10 NEs (600 mg tryptophan $\times$ 1 mg NE/60 mg tryptophan = 10 NEs). The average U.S. diet usually contains about 900 mg of tryptophan daily, and tryptophan provides about 50% of niacin intake in the United States [4].

Information used in estimating niacin requirements and recommendations has come from several studies, including human depletion and repletion studies as well as other studies with primarily urinary metabolites of niacin serving as indicators to base requirements and recommendations. The RDAs for niacin for adult men and women are 16 mg of niacin equivalents and 14 mg of niacin equivalents/day, respectively [5]. Estimated requirements are 12 mg and 11 mg of niacin for adult men and women, respectively. With pregnancy and lactation, the RDA for niacin increases to 18 mg and 17 mg of niacin equivalents, respectively [5]. The inside front cover of the book provides additional RDAs for niacin for other age groups.

Deficiency: Pellagra

A deficiency of niacin results in the condition known as pellagra (*pelle* means “skin” and *agra* means “rough” in Italian). The four Ds—*dermatitis*, *dementia*, *diarrhea*,

and *death*—are often used as a mnemonic device for remembering its signs. The dermatitis is similar to sunburn at first and later appears hyperpigmented, rough, and cracked or scaly (Figure 9.21). The affected areas are unusual in that they are mostly those exposed to the sun, including the face and neck, and on extremities such as the back of the hands, wrists, elbows, knees, and feet. The presence of the dermatological changes on the neck is sometimes called Casal's collar or necklace. The dermatitis is suggested to arise from impaired poly(ADP-ribose) generation secondary to UV-radiation induced damage to DNA [6].

Gastrointestinal manifestations include glossitis, cheilosis, and angular stomatitis (shown in Figure 9.16) as well as nausea and vomiting and diarrhea. Inflammation of the mucosa (enteritis) is also typically present; such findings have been attributed to niacin's anti-inflammatory roles in the digestive tract [6].

The dementia or neurologic-related manifestations of pellagra (which typically occur later than other manifestations) include headache, apathy, fatigue, loss of memory, peripheral neuritis, paralysis of extremities, depression, confusion, disorientation, delusions, hallucinations, and dementia or delirium. These manifestations have been suggested to arise from disrupted generation of cyclic ADP-ribose and nicotinic acid ADP, which in turn alter calcium signaling in nervous tissues [6]. If pellagra is untreated, death occurs. Treatment of niacin deficiency requires oral intakes of about 300 mg of nicotinamide daily (usually divided into three doses of 100 mg) or about 15–20 mg of nicotinic acid for about one month.

At Risk for Deficiency

A niacin deficiency or diminished status can result from the use of several medications and from malabsorptive conditions; it is also sometimes seen in a few groups of individuals due to inadequate intake of the vitamin. The antituberculosis drug isoniazid, for example, binds with vitamin B₆ as PLP and thereby reduces PLP-dependent kynureninase activity required for niacin synthesis from



One of the manifestations of the niacin deficiency disorder Pellagra affects the skin, which becomes hyperpigmented. The changes to the skin are most apparent in areas exposed to the sun.

Figure 9.21 Some manifestations of a niacin deficiency

tryptophan. Mercaptopurine, a drug used in cancer treatment, inhibits NAD phosphorylase. Malabsorptive disorders (such as chronic diarrhea, inflammatory bowel diseases, some intestinal cancers, and Hartnup disease) may impair niacin and/or tryptophan absorption to increase the likelihood of niacin deficiency. People who consume excessive amounts of alcohol typically have poor food intakes and are at risk for niacin deficiency. Individuals with HIV and with cancer undergoing chemotherapy may have higher needs for niacin. Dietary intake of the vitamin may be insufficient among adults over 50 years of age. Supplemental niacin may be beneficial for these individuals.

Toxicity

Because of the vasodilatory effects associated with the use of supplemental niacin (see the section “Selected Pharmacological Uses/Other Roles”), a Tolerable Upper Intake Level for adults for niacin (both nicotinic acid and nicotinamide) from supplements and from fortified foods has been set at 35 mg/day [5]. Changes to this Tolerable Upper Intake Level for healthy individuals versus those being treated for hyperlipidemia or other conditions necessitating ingestion of large doses of the vitamin have been suggested [5].

Assessment of Nutriture

Several methods are employed to assess niacin status. Most methods involve measurement of one or more urinary metabolites of the vitamin. Urinary excretion of ~0.8 mg/day of N' methyl nicotinamide and of ~0.5 mg of N' methyl nicotinamide/1 g of creatinine are suggestive of poor (deficient) niacin status [7]. Marginal niacin status is suggested by urinary amounts in the range of 0.5–1.59 mg of N' methyl nicotinamide/1 g of creatinine, while levels in excess of 1.6 mg reflect adequate status [7]. This ratio, however, has been criticized as being difficult to interpret because of multiple influences on urinary creatinine excretion. It is usually employed during a period of 4–5 hours after a 50 mg test dose of nicotinamide.

Another ratio employed to assess niacin status is that of urinary N' methyl 2-pyridone 5-carboxamide (2-pyridone) to N' methyl nicotinamide (NMN). Although a ratio of ~1 is found with niacin deficiency, this ratio is not thought to be sensitive enough to detect marginal niacin intakes and may better reflect dietary protein adequacy as opposed to niacin status [7].

In addition to measurement of urinary metabolites, serum or red blood cell indicators are used to assess niacin status. NAD concentrations and the ratio of NAD to NADP (~1.0) in erythrocytes have been used. In plasma, concentrations of 2-pyridone drop below the detection

level with low niacin intakes and thus may be used as an index of niacin status [7].

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Suggested Readings

Minto C, Vecchio MG, Lamprecht M, Gregori D. Definition of a tolerable upper intake level of niacin: a systematic review and meta-analysis of the dose-dependent effects of nicotinamide and nicotinic acid supplementation. *Nutr Rev.* 2017; 75:471–90.

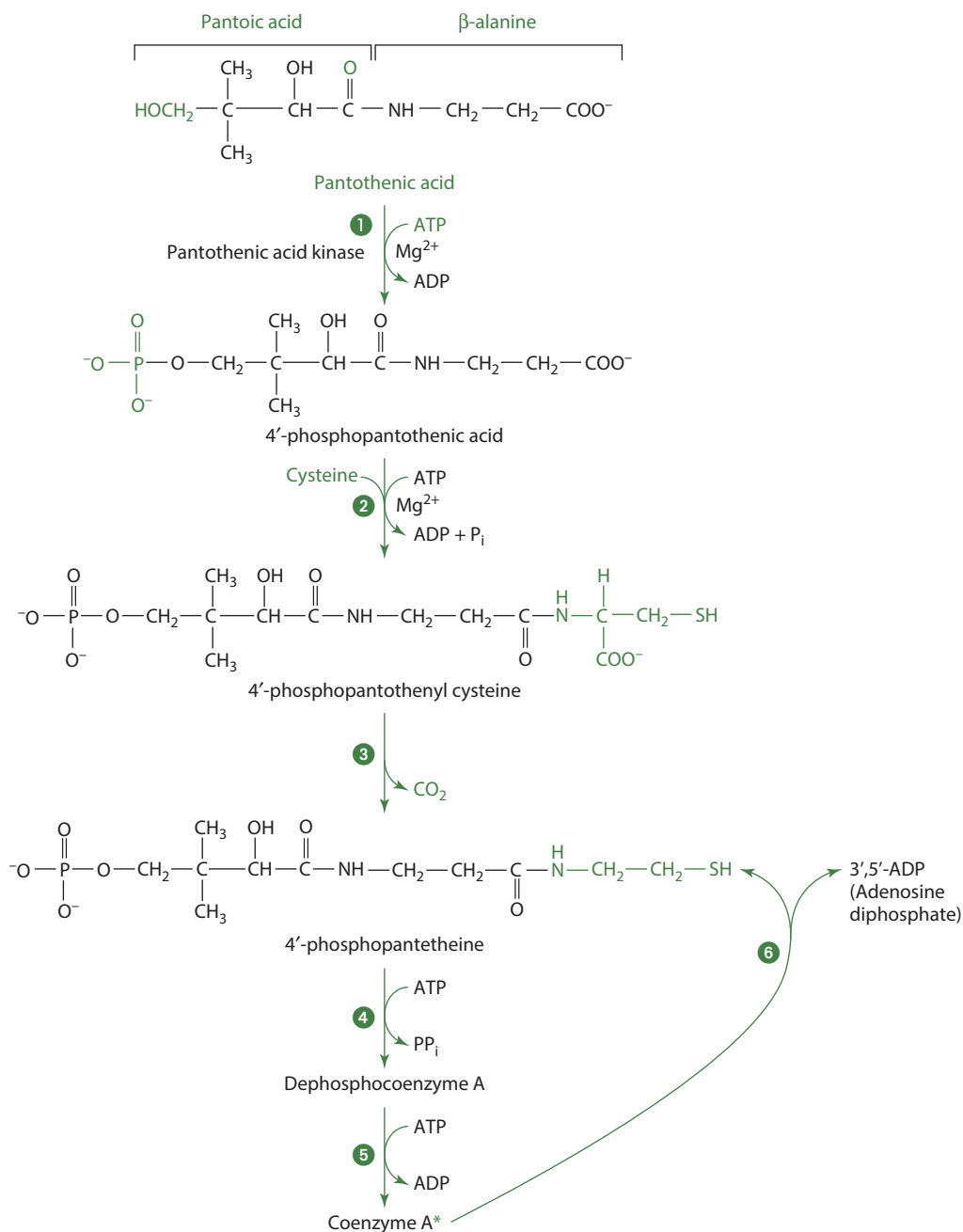
9.5 PANTOTHENIC ACID

Pantothenic acid's essentiality was not discovered until 1954, although the vitamin had been isolated in about 1931 by R. J. Williams and its structure determined in 1939. Structurally, pantothenic acid (sometimes called vitamin B₅) consists of β-alanine and pantoic acid joined by an amide linkage. The vitamin is shown at the top of Figure 9.22.

Sources

The Greek word *pantos* means “everywhere,” and the vitamin pantothenic acid, as its name implies, is found widely distributed in foods, although typically in small amounts. It is in part because the vitamin is present in virtually all plant and animal foods that deficiency is unlikely. Selected sources of the vitamin are shown in Table 9.9. Beef liver and poultry are fairly good sources, providing over 5 mg/serving. Sunflower seeds contain over 2 mg/serving. Royal jelly from bees also provides relatively large amounts (about 0.5 mg/g) of pantothenic acid.

Pantothenic acid can be destroyed with heating and freezing. It is stable when dry and in solution at a neutral pH, but destroyed in acidic and alkaline solutions. The refining of grains as well as the freezing and canning of foods decreases their pantothenic acid content by as



*Structure shown in Fig. 9.23

- 1 The synthesis begins with the rate limiting phosphorylation of pantothenic acid by pantothenic acid kinase to form 4'-phosphopantothenic acid. The reaction occurs in the cytosol.
- 2 Cysteine reacts with 4'-phosphopantothenic acid to form 4'-phosphopantothenyl cysteine. A peptide bond is formed between the carbonyl group of 4'-phosphopantothenic acid and the amino group of cysteine by the enzyme phosphopantothenylcysteine synthase. This reaction and subsequent two reactions occur in the cytosol on a protein complex with multiple catalytic sites.
- 3 A carboxyl group from the cysteine moiety is removed by phosphopantothenylcysteine decarboxylase to form 4'-phosphopantetheine. This compound is needed by acyl carrier proteins for function.
- 4 An adenylation occurs in the mitochondrial inner membrane whereby ATP reacts with 4'-phosphopantetheine; adenosine monophosphate (AMP) is attached forming dephosphocoenzyme A with the release of pyrophosphate.
- 5 Phosphorylation of the 3'-hydroxy group of dephosphocoenzyme A with ATP produces CoA. This reaction also occurs in the mitochondrial inner membrane.
- 6 CoA hydrolase

Figure 9.22 Structure of pantothenic acid and its use in the synthesis of 4'-phosphopantetheine and coenzyme A.

Table 9.9 Pantothenic Acid Content of Selected Foods*

Food (serving)	Pantothenic acid (mg)
Liver, beef or chicken, cooked (3 oz)	6.5
Sunflower seeds, dry roasted (1/4 c)	2.3
Yogurt, vanilla, nonfat (1 c)	1.3
Chicken, breast, cooked (3 oz)	1.3
Salmon, Atlantic, cooked (3 oz)	1.2
Cheese, feta (1/4 c)	1.1
Avocado (1/2)	1.0
Milk, 2% (1 c)	0.9
Egg, whole, scrambled (1)	0.7
Mushrooms, cooked (1/2 c)	0.7
Egg, cooked (1)	0.7
Lentils, cooked (1/2 c)	0.6
Beef, chuck, cooked (3 oz)	0.5
Pork, shoulder, cooked (3 oz)	0.5
Broccoli, cooked (1/2 c)	0.5
Rice, brown, cooked (1/2 c)	0.4
Potato, flesh without skin, cooked (1/2 c)	0.3
Bread, whole grain (1 slice)	0.2
Cheese, cheddar (1.5 oz)	0.2
Legumes, variety, cooked (1/2 c)	0.1–0.2

A more complete list of pantothenic acid-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at https://www.nal.usda.gov/sites/www.nal.usda.gov/files/pantothenic_acid.pdf.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

much as 75%. Most adults in the United States consume about 4–7 mg of pantothenic acid per day. Bacteria in the colon also generate pantothenic acid. However, the extent to which the microbial-produced vitamin is absorbed is unclear.

In supplements, pantothenic acid is usually found as calcium or sodium pantothenate, or as panthenol, an alcohol form of the vitamin. The amount present in multivitamin supplements is usually about 10 mg. Dosages in single-nutrient pantothenic acid supplements range from about 5 to 500 mg. The Daily Value, found on food and supplement facts labels, for pantothenic acid is 5 mg. In skin and hair products, panthenol is sometimes added as a humectant to promote moisture retention.

Digestion and Absorption

Pantothenic acid occurs in foods in free and bound forms. About 85% of the vitamin is found bound in foods as either 4'-phosphopantetheine (Figure 9.22) or coenzyme A, abbreviated CoA (shown in Figure 9.23). To free the vitamin for absorption, digestion in the small intestine is needed. More specifically, CoA is hydrolyzed by a pyrophosphatase to 4'-phosphopantetheine, which is then

dephosphorylated by a phosphatase to pantetheine. Pantetheine is subsequently converted to pantothenic acid by pantotheinase.

Pantothenic acid absorption occurs principally in the jejunum by passive diffusion when present in high concentrations and by a shared sodium-dependent multivitamin transporter (SMVT) in the proximal small intestine when present in usual concentrations. Pantothenic acid shares the SMVT with biotin and lipoic acid. Bacterially produced pantothenic acid can also be absorbed (using the SMVT) in the proximal and midtransverse sections of the colon. Panthenol, from vitamin supplements, is absorbed by diffusion and is subsequently converted to pantothenic acid within the intestinal cell. Approximately 50% (range 40–61%) of ingested pantothenic acid is absorbed [1]. However, absorption decreases to about 10% with supplement use in amounts approximately 10 times recommendations. Pantothenic acid from the intestinal cells enters portal blood for transport to the liver and other tissues.

Transport, Tissue Uptake, and Storage

Pantothenic acid is found free in the blood, primarily within the red blood cells. Blood concentrations of the vitamin usually range from about 30 to 60 µg/dL [1]. The uptake of the vitamin into red blood cells appears to occur by diffusion, while uptake into some tissues/organs requires SMVT. Pantothenic acid and 4'-phosphopantetheine are present within cells. Most pantothenic acid is used intracellularly to synthesize CoA, which is found in all tissues but in fairly high concentrations in the liver, adrenal gland, kidneys, brain, and heart. The amount of the vitamin stored in tissues is unclear.

Functions and Mechanisms of Action

Pantothenic acid is needed by cells for the synthesis of CoA and 4'-phosphopantetheine. CoA is involved extensively in nutrient metabolism and energy production as well as the transfer of acetyl and acyl groups, among other roles. 4'-phosphopantetheine is required for the activity of the acyl carrier protein (ACP), a component of the fatty acid synthase complex and for folate metabolism.

The synthesis of both 4'-phosphopantetheine and CoA from pantothenic acid is described and shown in Figure 9.22. Regulation of this pathway occurs at the first rate-limiting step catalyzed by panthothenate kinase II (PANK II), which phosphorylates pantothenic acid to produce 4'-phosphopantetheine. 4'-Phosphopantetheine acid undergoes the addition of cysteine and a decarboxylation to form 4'-phosphopantetheine. Two additional reactions requiring ATP convert 4'-phosphopantetheine to CoA. CoA can also undergo cleavage by a CoA hydrolase to regenerate 4'-phosphopantetheine. Feedback inhibition is provided by the pathway's end-product free

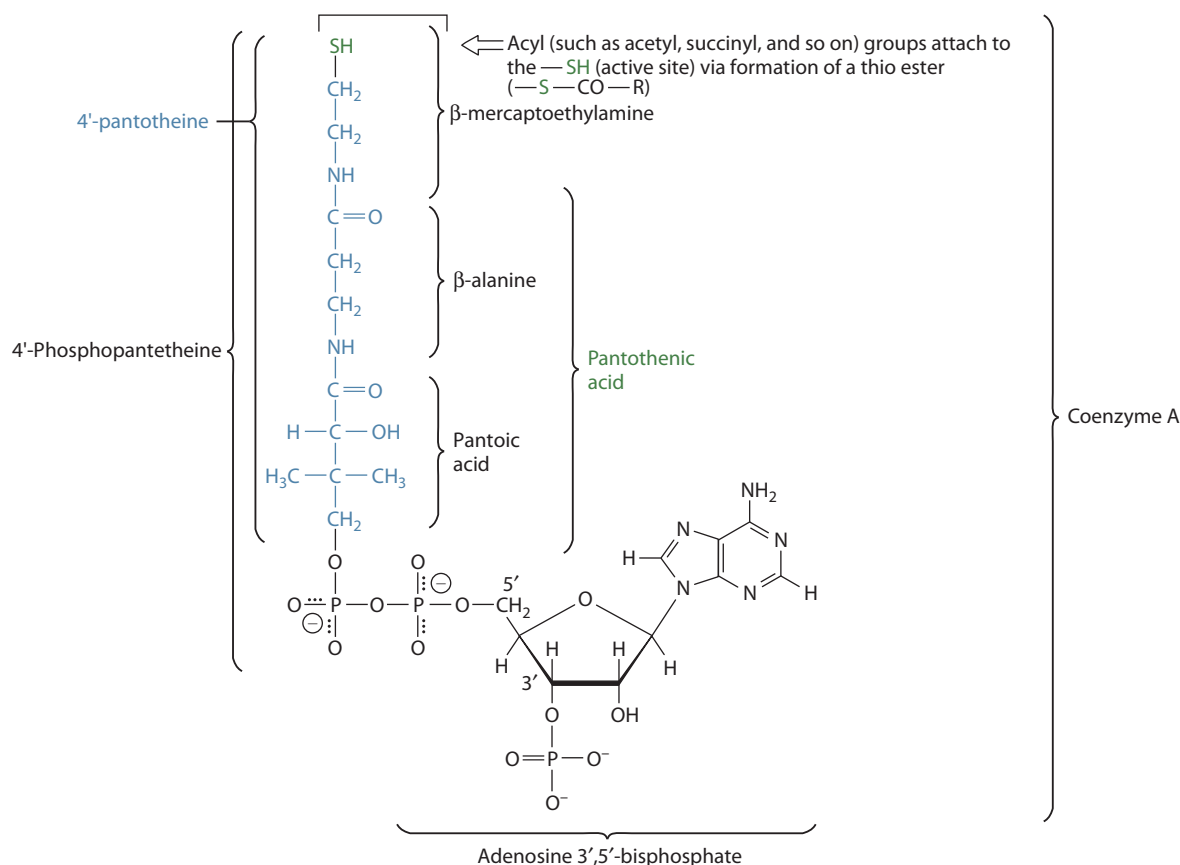


Figure 9.23 Structure of coenzyme A.

CoA, as well as by CoA thioesters (i.e., acetyl-CoA, malonyl-CoA, propionyl-CoA, and other longer-chain acyl-CoAs). Mutations in genes for pantothenate kinase are associated with a specific autosomal recessive condition known as PANK-associated neurodegeneration. The condition is characterized by dystonia (involuntary muscle contractions) and problems with speech, vision, and intellectual development.

Coenzyme A

Figure 9.23 shows the structure of CoA. Note from this figure that CoA contains several components including 4'-phosphopantetheine. It is through the sulfhydryl group (active site) in 4'-phosphopantetheine that thio ester

(—S— $\overset{\text{O}}{\parallel}$ C—R) bonds form with various carboxylic acids.

Some of these acids, which are typically 2–13 carbons in length, include:

- Acetic acid (two carbons)
- Malonic acid (three carbons)
- Propionic acid (three carbons)
- Methylmalonic acid (four carbons)
- Succinic acid (four carbons).

Most of these carboxylic acids arise in cells during metabolism. Some, like propionic acid, are also obtained by additional means. For example, propionic acid is produced from bacterial fermentation of carbohydrates in the colon and can be absorbed and contribute to the cellular supply.

CoA serves (functions) as a carrier of acetyl/acyl groups, forming thioester derivatives including acetyl-CoA, propionyl-CoA, malonyl-CoA, and succinyl-CoA, among others. It was F. Lipmann who won the Nobel prize for his work in 1957 showing that CoA facilitated biological acetylation reactions. The levels and ratios among the thioester derivatives are tightly regulated and impact a wide range of cellular metabolic activities.

Nutrient Metabolism and Energy Production CoA and its thioester derivatives are found in multiple cellular compartments and function in hundreds of metabolic reactions. It is through these reactions that pantothenic acid as CoA and its derivatives participates extensively in nutrient metabolism, including degradation reactions resulting in energy production and synthetic reactions for the production of vital compounds.

The metabolism of carbohydrate, lipids, and protein (energy-producing nutrients) relies to varying degrees on CoA. For example, a crucial reaction in nutrient

metabolism is the conversion (oxidative decarboxylation) of pyruvate (formed from the degradation of glucose as well as some amino acids) to acetyl-CoA. This acetyl-CoA, which is also formed from the catabolism of fatty acids and some amino acids, can then condense with oxaloacetate to introduce acetate for oxidation in the TCA cycle (see Figure 9.9). Acetyl-CoA is thus a common intermediate formed from the catabolism of the three energy-producing nutrients and holds a central position, intersecting both catabolic and anabolic pathways.

Pantothenic acid joins thiamin, riboflavin, and niacin in the oxidative decarboxylation of pyruvate (see Figure 9.10). These same vitamins also participate in the oxidative decarboxylation of α -ketoglutarate to succinyl-CoA, a TCA cycle intermediate and compound used with the amino acid glycine to synthesize the porphyrin ring in heme.

In lipid metabolism, CoA is important in the synthesis of cholesterol, ketone bodies, fatty acids, phospholipids, and sphingolipids. For example, in cholesterol and ketone body synthesis, acetyl-CoA and acetoacetyl-CoA react to form the key intermediate HMG-CoA (see Figure 5.37). Condensation of acetyl-CoA with activated CO_2 (involving biotin) to form malonyl-CoA represents the first step in fatty acid synthesis (see Figure 5.31).

Additionally, many of the compounds produced using CoA are involved in reactions for the synthesis of other compounds. Cholesterol, for example, once produced is used further for the synthesis of bile and steroid hormones, and sphingolipids that are generated are used further for the production of myelin, which is involved in nerve transmission.

Acetyl-CoA is also important for the production of neurotransmitters. Choline acetyltransferase catalyzes the synthesis of acetylcholine in selected (cholinergic) neurons from acetyl-CoA and choline. Insufficient availability of acetyl-CoA has been linked with neurodegenerative conditions [2,3].

The aforementioned roles of CoA and its derivatives in nutrient metabolism and energy production have been long recognized. More recently, additional roles of CoA in acetylation (acetate donated by CoA to an acceptor compound) and acylation (the donation of fatty acids by CoA thioester derivatives to an acceptor compound) are being elucidated.

Acylation, Acetylation, and Cellular Processes Pantothenic acid as part of CoA is involved in the acetylation and acylation of proteins and sugars as well as some drugs. Both acylation and acetylation of the proteins by CoA occur post-translationally. Acylation affects protein functions, activity, and location. The fatty acids most often attached to cellular proteins are myristic acid and palmitic acid. Myristoylation of proteins appears to be irreversible, while the palmitoylation of proteins can be reversed. Palmitoylation of proteins affects some

regulatory functions such as signal transduction with affected proteins including guanosine triphosphate-binding proteins and insulin receptors, among others. Acylation of cytoskeletal proteins and neuronal proteins by palmitic acid has also been demonstrated and influences cell structure and neural development.

The acetylation of cytosolic and mitochondrial (non-nuclear) proteins is extensive, with acetylation detected on almost every enzyme in the liver involved in intermediary metabolism, including glycolysis, gluconeogenesis, TCA cycle, amino acid and fatty acid metabolism, and the urea cycle [4]. A better understanding of the impact of acetylation on these enzymes is needed; however, effects on enzyme activity and structure (stability) have been documented. Acetylation of enzymes appears to have primarily an inhibitory effect on activity [4]. The means by which these proteins are acetylated are thought to be both enzymatic (such as via p300 acetyltransferase) as well as nonenzymatic. The availability of acetyl-CoA appears to “drive” or control the acetylations and are based largely on the cell’s metabolic state [4].

In addition to effects on enzymes involved with intermediary metabolism, acetyl-CoA concentrations affect the acetylation of other non-nuclear cell proteins that in turn impact a multitude of cellular events. Higher cellular acetyl-CoA concentrations, for example, have been shown to suppress autophagy through interactions with acyltransferases. In contrast, in the presence of lower acetyl-CoA concentrations, acetylation of cytosolic proteins is reduced and autophagy is induced [3]. Microtubules, made from polymerization of α - and β -tubulin dimers and that make up a part of the cell’s cytoskeleton, are also acetylated. Microtubules appear to be stabilized by acetylation and destabilized when deacetylated. The acetylation of some proteins is also thought to affect cell signaling pathways and interactions between or among post-translationally modified proteins [5,6].

In the nucleus, the acetylation of histones induces structural changes in chromatin to enhance gene expression and promote transcription. Acetylation, for example, enables interactions between transcription factors and the promoter regions of genes [5].

In addition to proteins, aminosugars, such as glucosamine and galactosamine, are also acetylated by acetyl-CoA to form N-acetyl glucosamine and N-acetyl galactosamine, respectively. These acetylated aminosugars in turn may function structurally in the cell, for example, to provide recognition sites on cell surfaces or to direct proteins for membrane functions, among other roles.

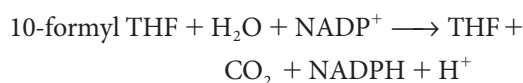
4'-Phosphopantetheine

Pantothenic acid is used in cells to produce 4'-phosphopantetheine. CoA can also be cleaved to generate 4'-phosphopantetheine (see Figure 9.22). In cells, 4'-phosphopantetheine attaches to apoproteins where it serves

as a prosthetic group and allows for biological activity. 4'-phosphopantetheine serves as a prosthetic group for acyl carrier protein and for the enzyme 10-formyl tetrahydrofolate dehydrogenase.

Acyl Carrier Protein (ACP) 4'-Phosphopantetheine attaches to apoacyl carrier protein, a small component of the fatty acid synthase complex. Phosphopantetheinyl transferase (also referred to as 4'-phosphopantetheine-apoACP transferase) carries the 4'-phosphopantetheine moiety to a serine residue in the apoacyl carrier protein. Once bound to the acyl carrier protein, it is the sulfhydryl group in the 4'-phosphopantetheine that binds and transfers acyl groups to another sulfhydryl group located in the enzyme complex. These two groups are located close to each other so that the acyl chain of the fatty acid being synthesized can be transferred between them. Thus, the 4'-phosphopantetheine component of ACP is like a “crane” to which substrates and intermediates in fatty acid synthesis “get picked up and moved” while also undergoing a progression of enzymatic modifications to extend the fatty acid chain. See Chapter 5 for a complete discussion of fatty acid synthesis including the roles of acetyl-CoA, malonyl-CoA, and acyl carrier protein.

10-Formyl Tetrahydrofolate Dehydrogenase The enzyme 10-formyl tetrahydrofolate dehydrogenase, which is required for folate metabolism, requires 4'-phosphopantetheine for enzymatic activity. The enzyme converts 10-formyl tetrahydrofolate (THF) to tetrahydrofolate (THF) and formate, and then, using NADP⁺, converts formate to CO₂, as shown here and in later in Figure 9.34.



10-formyl THF is used in the production of purines needed for DNA synthesis. A more complete discussion of folate metabolism is found later in this chapter under the vitamin “Folate.”

Metabolism and Excretion

Both CoA and 4'-phosphopantetheine are catabolized to pantothenic acid prior to excretion from the body. CoA degradation occurs in a series of reactions first catalyzed by a phosphatase to generate dephospho-CoA, and then by a pyrophosphatase to produce 4'-phosphopantetheine. The latter compound is further catabolized by additional phosphatases to pantothenic acid. Release of 4'-phosphopantetheine from acyl carrier protein is accomplished by an acyl carrier protein hydrolase; the 4'-phosphopantetheine can be reused or further degraded to pantothenic acid.

Pantothenic acid does not appear to undergo degradation prior to its excretion and is thus excreted intact

primarily in the urine. Usual urinary excretion of pantothenic acid ranges from about 1 to 8 mg/day.

Adequate Intake

The Adequate Intake (AI) recommendation for adults for pantothenic acid is 5 mg [1]. AIs for pantothenic acid of 6 mg/day and 7 mg/day are suggested for women during pregnancy and lactation, respectively [1]. The inside front cover of this book provides AIs for pantothenic acid for other age groups.

Deficiency: Burning Foot Syndrome

The pantothenic acid deficiency disorder, referred to as burning foot syndrome, is rare. The disorder has been studied with the provision of a metabolic inhibitor of the vitamin, omega methylpantothenate, and a restricted diet. The syndrome is characterized by a sensation of burning in the feet and neuritis (nerve inflammation). The condition is exacerbated by warmth and diminished with cold. Other signs and symptoms of deficiency include vomiting, fatigue, muscle weakness, arm and leg cramping, restlessness, and irritability. Although the vitamin is needed for heme synthesis, problems with hematopoiesis are not usually observed in humans with a pantothenic acid deficiency, but have been shown in some animal studies. Pantothenic acid deficiency is corrected with calcium or sodium pantothenate administration.

At Risk for Deficiency

When pantothenic acid deficiency occurs naturally, it is usually in conjunction with multiple nutrient deficiencies. Some conditions that may increase the need for the vitamin include alcoholism, diabetes mellitus, and inflammatory bowel diseases. Increased excretion of the vitamin has been observed in people with diabetes mellitus. Absorption is likely to be impaired with inflammatory bowel diseases. Intake of the vitamin is typically low in people with excessive alcohol intake.

Toxicity

Pantothenic acid toxicity has not been reported to date in humans. Intakes of about 10 g of pantothenic acid as calcium pantothenate daily for up to 6 weeks have not caused problems; however, higher intakes of 15–20 g have been associated with mild intestinal distress, including diarrhea [1].

Assessment of Nutriture

Blood pantothenic acid concentrations are thought to reflect low dietary pantothenic acid intakes; however, blood concentrations do not correlate well with changes in dietary pantothenic acid intake and status. Urinary pantothenic acid excretion is considered to be a better indicator

of status, with excretion of < 1 mg/day considered indicative of poor status.

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9.6 BIOTIN (VITAMIN B₇)

Biotin's discovery was based on research investigating the cause of what was called egg white injury. Eating raw eggs was known to result in hair loss, dermatitis, and various neuromuscular problems. Szent-Györgyi in 1931 found a substance (now called biotin) in liver that could cure and prevent the condition. It was not until about 10 years later (the early 1940s) that Kogl (from Europe) and du Vigneaud and colleagues (from the United States) determined biotin's structure. Biotin consists structurally of two rings—an ureido ring joined to a thiophene ring—with an additional valeric acid side chain (Figure 9.24). Biotin was once called vitamin H (the H refers to *haut* in German and means “skin”).

Sources

In addition to being made by bacteria inhabiting the colon, biotin is found widely distributed in foods. Food sources containing higher amounts of the vitamin include liver and eggs. Lesser amounts of the vitamin are found in other meats, canned salmon, sunflower seeds, and sweet

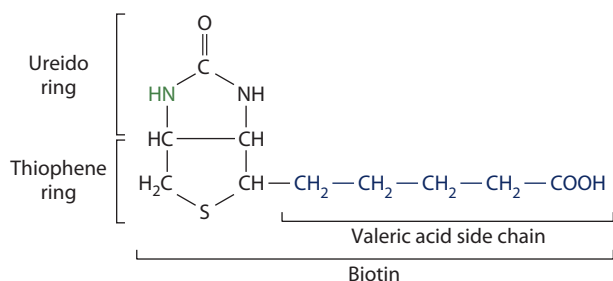


Figure 9.24 Structure of biotin.

Table 9.10 Biotin Content of Selected Foods¹

Food (serving)	Biotin (μg)
Liver, beef, cooked (3 oz)	30
Egg, cooked (1)	10
Salmon, canned (3 oz)	5
Pork chop, cooked (3 oz)	4
Ground beef, cooked (3 oz)	4
Sunflower seeds (1/4 c)	3
Sweet potato, cooked (1/2 c)	2
Almonds (1/4 c)	2

¹National Institutes of Health, Office of Dietary Supplements. Biotin. <https://ods.od.nih.gov/factsheets/Biotin-HealthProfessional/>

potatoes. Selected foods and their biotin content are shown in Table 9.10. Data on the biotin content of foods are limited when compared with that available for other vitamins.

Within foods, biotin is found free (unattached) or bound covalently to protein, usually through a lysine residue. However, in raw egg whites, a glycoprotein called avidin irreversibly binds biotin in what has been suggested as the tightest noncovalent bond found in nature. This binding to avidin in turn prevents biotin absorption; although, because avidin is heat labile (unstable with heat), eating cooked egg whites does not compromise biotin absorption.

In multivitamin and individual supplements, biotin is usually present in its free form and in amounts typically equal or several times greater than the Daily Value. The Daily Value, found on food and supplement facts labels, for biotin is 30 μg. Supplements containing biotin alone or with a few other nutrients are available, with some providing biotin in amounts up to about 10,000 μg.

Digestion, Absorption, Transport, Tissue Uptake, and Storage

Protein-bound biotin requires digestion by enzymes prior to absorption. Proteolysis by pepsin and intestinal proteases yields free biotin and/or biocytin. Biocytin consists of biotin bound to the amino acid lysine (Figure 9.25). While some biocytin may be absorbed intact by peptide carriers, most biocytin is hydrolyzed by biotinidase, which is widely present on the intestinal brush border membrane, in pancreatic and intestinal juices secreted into the small intestine, in the plasma, and in multiple cellular locations including the nucleus. Biotinidase hydrolyzes the biocytin to release free biotin and lysine. Biotinidase also cleaves covalently bound biotin from any biotinyl peptides that have been released as biotinylated proteins are degraded.

Biotinidase deficiency (first discovered in 1983) results from an autosomal recessive inborn error of metabolism. Insufficient intestinal biotinidase activity impairs the digestion of lysine-bound biotin and thus limits some biotin availability for absorption. Insufficient extraintestinal

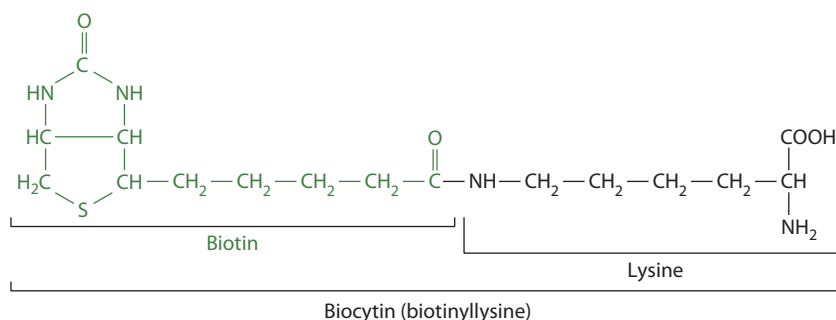


Figure 9.25 Structure of biocytin, also called biotinyllysine.

biotinidase activity hampers biotin release and thus recycling of biotin in tissues. Depending on the specific gene mutation, residual biotinidase activity varies. Some manifestations associated with the genetic disorder include lethargy, hypotonia (reduced muscle tone), seizures, and ataxia (impaired muscle control or the inability to control body movement). In addition, dermatitis (dry and erythematous, and usually present on the face) and alopecia (loss of hair from the body) may occur. Treatment requires the use of pharmacological doses of biotin taken orally; however, not all manifestations improve with supplementation.

Biotin absorption occurs by both passive diffusion and carrier-mediated transport. Biotin absorption by passive diffusion predominates with consumption of pharmacological doses of the vitamin. With physiological intake, biotin absorption is carrier mediated. This carrier, which also transports pantothenic acid and lipoic acid, is called the shared multivitamin transporter (SMVT). SMVT transcription is regulated by biotin concentrations (with high concentrations decreasing transcription) and is negatively affected by alcohol [1]. Absorption via SMVT occurs mainly in the proximal small intestine, but also in the proximal and midtransverse colon. In the colon, an accessory protein that interacts with SMVT may also be involved in the absorption of the microbial-generated biotin. Bacterially made biotin, however, cannot totally meet the body's biotin needs.

Transport of biotin across the basolateral membrane of the enterocyte for entrance into the blood is carrier mediated. Absorption of oral free biotin is typically 100%. Biotin is found in the plasma mostly (~80%) in a free state, with lesser amounts bound to plasma proteins, including albumin, α - and β -globulins, and biotinidase. Whole-blood biotin concentrations range from about 200 to 750 pg/mL. Biotin uptake into the liver, and probably other tissues, is thought to involve SMVT as well as monocarboxylate transporter (MCT) 1. Biotin is stored in small quantities mainly in the muscle, liver, and brain.

Functions and Mechanisms of Action

Biotin functions in cells as a coenzyme carrier for the transfer of “activated bicarbonate” to substrates. These reactions are vital for nutrient metabolism and energy

production. In addition, biotin functions in a noncoenzyme capacity in regulating gene expression.

Coenzyme Roles in Nutrient Metabolism and Energy Production

For coenzyme functions within cells, biotin is covalently bound to each of five apocarboxylases. The attachment of biotin (called biotinylation) to these apocarboxylases is catalyzed by holocarboxylase synthetase (HCS), which is found in the cell cytosol, mitochondria, and nucleus. The attachment of biotin by the enzyme HCS occurs in two steps:

- Biotin + ATP + HCS $\rightarrow$ Biotinyl-5'-AMP- HCS + pyrophosphate
- Biotinyl-5'-AMP-HCS + apocarboxylase $\rightarrow$ Holocarboxylase + AMP + HCS

Holocarboxylase refers to biotin attached to any of five carboxylases.

A mutation in holocarboxylase synthetase, as was first discovered in 1981, or in any of the biotin-dependent carboxylases negatively impacts nutrient metabolism. The condition is manifested by vomiting, lethargy, hypotonia (reduced muscle tone), acidosis, and seizures. Pharmacological doses (ranging from 10 mg to about 200 mg orally per day) of biotin can be used to sometimes enhance residual enzyme activity and help reduce some of the disorder's manifestations [2].

Each biotinylated carboxylase is a multisubunit enzyme to which biotin is attached by an amide linkage. Specifically, the carboxyl terminus of biotin's valeric acid side chain is linked to the epsilon amino group of a specified lysine residue in each apocarboxylase, as shown in Figure 9.26. The attachment site contains a unique amino acid sequence with the amino acid methionine present on each side of the lysine to which the vitamin attaches. The “chain” of amino acids connecting biotin and the apoenzyme is long and flexible, allowing the biotin to move from one active site of the carboxylase to another. One active site generates the carboxybiotin enzyme, and the other transfers the activated carbon dioxide (as HCO_3^-) to a reactive carbon on the substrate. Figure 9.27 illustrates the formation of the CO_2 -biotin-enzyme complex.

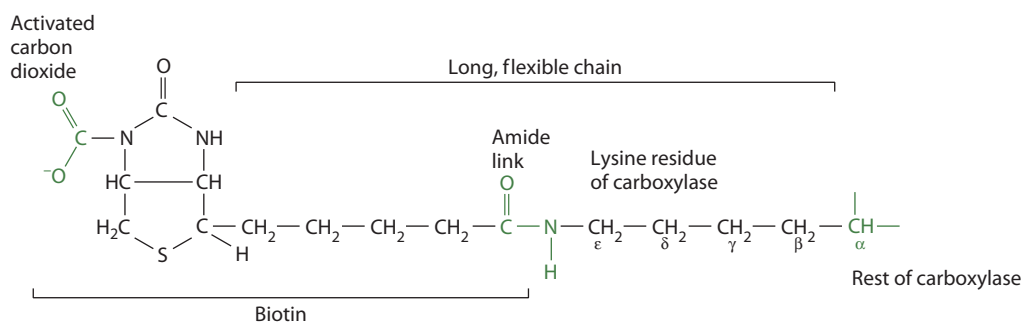


Figure 9.26 Biotin bound to the lysine residue of carboxylase and functioning as a carrier of activated CO_2 .

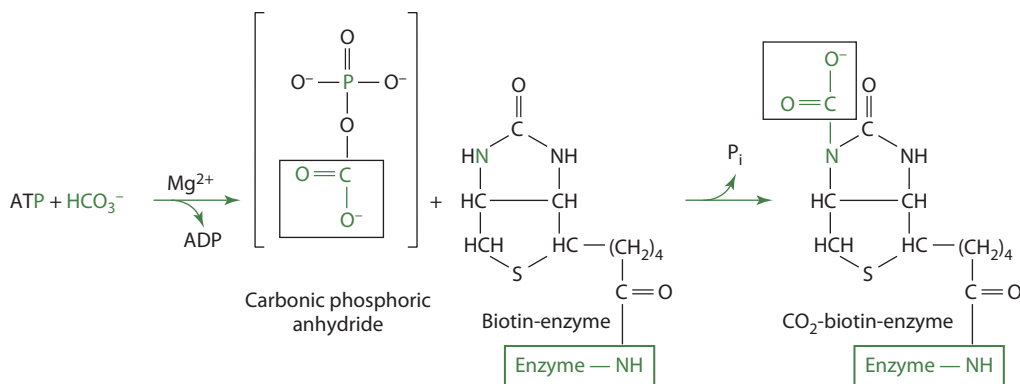


Figure 9.27 The formation of the CO_2 -biotin-enzyme complex.

The five biotinylated carboxylases (holocarboxylases), which are synthesized by holocarboxylase synthetase, are pyruvate carboxylase, acetyl-CoA carboxylase (two isoforms), propionyl-CoA carboxylase, and β -methylcrotonyl-CoA carboxylase. Table 9.11 lists these enzymes and their **carboxylation** roles in metabolism.

Pyruvate Carboxylase Pyruvate carboxylase is a particularly important enzyme because of its regulatory function and roles in gluconeogenesis and lipogenesis and in the brain neurotransmitter synthesis. Specifically, pyruvate carboxylase (a mitochondrial enzyme) catalyzes the carboxylation of pyruvate to form oxaloacetate (Figure 9.28). For its activation, pyruvate carboxylase requires the presence of acetyl-CoA as well as ATP and

Mg^{2+} . Acetyl-CoA serves as an allosteric activator, and its presence indicates the need for increased amounts of oxaloacetate. If the cell has a surplus of ATP, the oxaloacetate is then used for gluconeogenesis. However, if the cell is deficient in ATP, the oxaloacetate enters the TCA cycle on condensation with acetyl-CoA.

Acetyl-CoA Carboxylase 1 and 2 The importance of biotin in nutrient metabolism is further exemplified by its role in the initiation of fatty acid synthesis. Malonyl-CoA formation from acetyl-CoA by the regulatory and rate-limiting enzyme acetyl-CoA carboxylase 1, found in the cytosol (mainly in the liver and kidney), promotes fatty acid synthesis (see Figure 5.33). The other isoform, acetyl-CoA carboxylase 2, is found on the outer mitochondrial

Table 9.11 Biotin-Dependent Enzymes

Enzyme	Role	Significance
Pyruvate carboxylase	Converts pyruvate to oxaloacetate	Replenishes oxaloacetate for TCA cycle Necessary for gluconeogenesis
Acetyl-CoA carboxylase	Forms malonyl-CoA from acetate	Commits acetate units to fatty acid synthesis
Propionyl-CoA carboxylase	Converts propionyl-CoA to methylmalonyl-CoA	Provides mechanism for metabolism of some amino acids and odd-chain fatty acids
β -methylcrotonyl-CoA carboxylase	Converts β -methylcrotonyl-CoA to β -methylglutaconyl-CoA	Allows catabolism of leucine and certain isoprenoid compounds

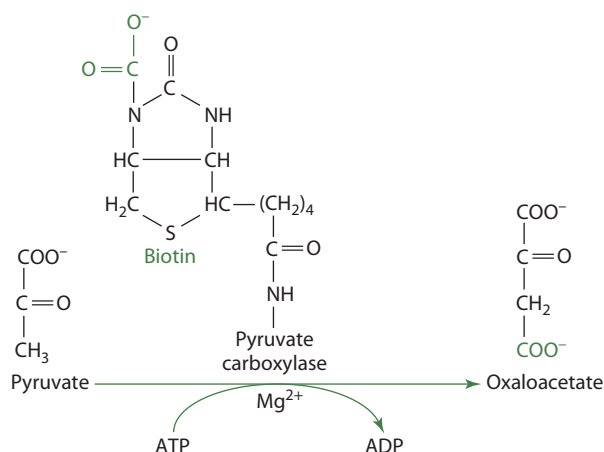


Figure 9.28 The role of biotin in the synthesis of oxaloacetate from pyruvate.

membrane, especially in muscle. This isoform also catalyzes malonyl-CoA formation from acetyl-CoA; however, within the mitochondria the malonyl-CoA serves to inhibit fatty acid uptake and thus use in beta oxidation.

Propionyl-CoA Carboxylase Propionyl-CoA carboxylase (a mitochondrial enzyme) is important for the catabolism of four amino acids, isoleucine, valine, threonine, and methionine, each of which generates propionyl-CoA. Propionyl-CoA also arises from the catabolism of odd-chain fatty acids. Propionyl-CoA carboxylase catalyzes the carboxylation of propionyl-CoA to D-methylmalonyl-CoA (Figure 9.29). Deficient or defective propionyl-CoA carboxylase activity, as occurs in the genetic disorder propionic acidemia, results in the accumulation of propionyl-CoA, which is then shifted into an alternate metabolic pathway. This alternate pathway results in increased production and urinary excretion of 3-hydroxypropionic acid (3HPA) and methylcitrate acid (MCA).

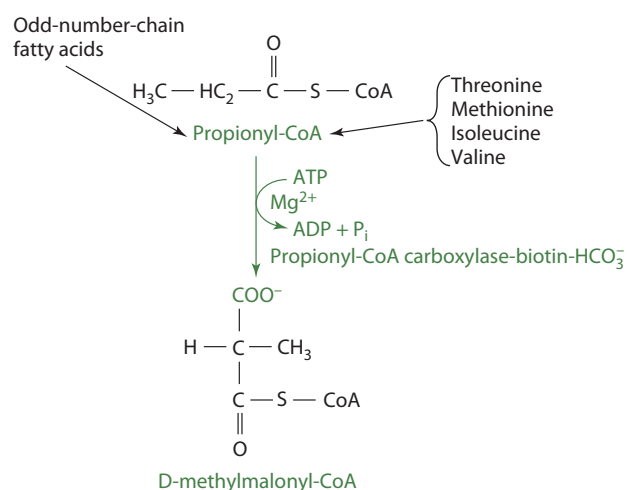


Figure 9.29 The role of biotin in the oxidation of propionyl-CoA.

β -Methylcrotonyl-CoA Carboxylase β - (or 3-) Methylcrotonyl-CoA carboxylase (a mitochondrial enzyme) is important in the degradation of the amino acid leucine. During leucine catabolism (see Figure 6.36 and Figure 9.30), β -methylcrotonyl-CoA is formed and is subsequently carboxylated by biotin-dependent β -methylcrotonyl-CoA carboxylase to form β -methylglutaconyl-CoA. The latter compound is further catabolized to generate acetoacetate and acetyl-CoA.

Deficient β -methylcrotonyl-CoA carboxylase activity, due to an inborn error of metabolism, causes the accumulation of β -methylcrotonyl-CoA, which is then shunted into an alternate metabolic pathway. This alternate pathway

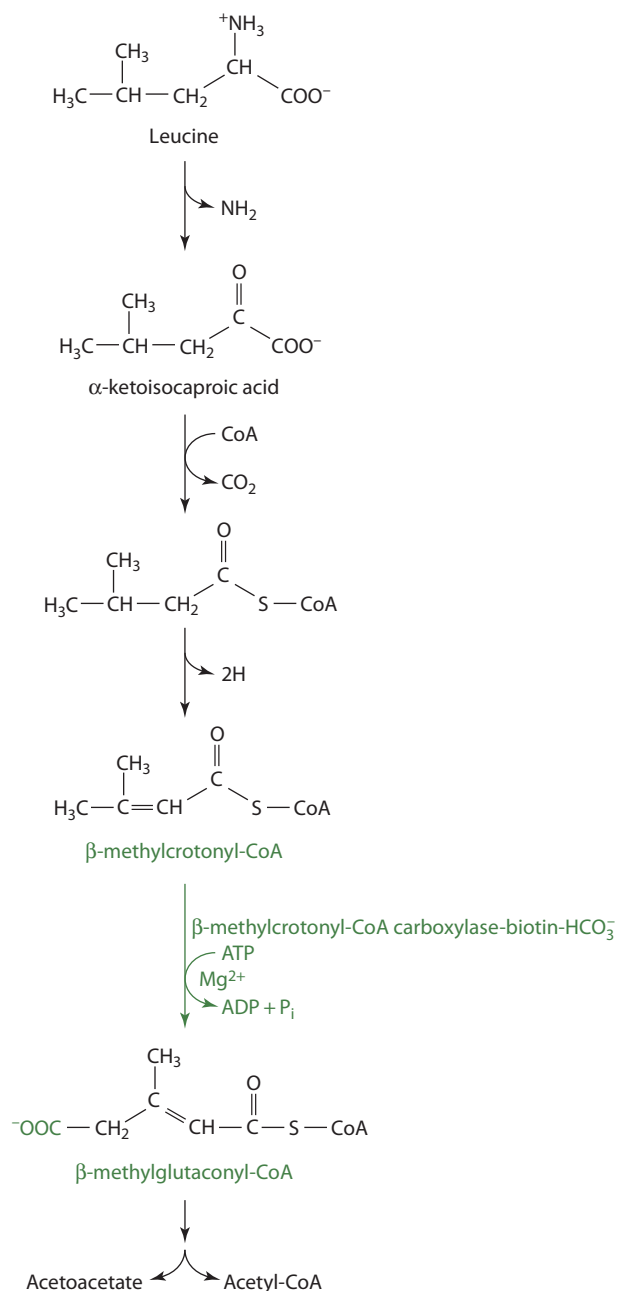


Figure 9.30 The role of biotin in leucine catabolism.

results in increased production and urinary excretion of 3-hydroxyisovaleric acid (3HIA), 3-methylcrotonylglycine (3MCG), and isovalerylglycine (IVG). Increased 3HIA and decreased biotin concentrations in the urine are considered indicative of biotin deficiency.

With the normal turnover of these holocarboxylases, the protein portion of the enzyme is degraded. The biotin initially remains attached to the lysine residue of the enzyme until it is further hydrolyzed by biotinidase which is found in the plasma and intracellularly. This free biotin can then be reused as new apocarboxylases are synthesized. This recycling of the endogenous vitamin reduces the need for dietary biotin.

Noncoenzyme Roles in Histone Modification, Gene Expression, and Cell Signaling

In addition to biotin's coenzyme roles, biotin functions in the modification (biotinylation) of histone proteins as well as in gene expression and cell signaling. Histones, of which there are five classes—H₁, H_{2A}, H_{2B}, H₃, and H₄—are small proteins that group together and are found bound to or associated with DNA. DNA base pairs are wrapped in the histones, which when tightly packed together minimize access to gene promoter sequences. Modification of the histones, however, can “open up this packing.” Histones consist of a flexible amino (also called N) terminus (often called the histone tail) and a globular domain. It is the tail section of the histones that can be covalently modified (e.g., by biotinylation, acetylation, and methylation) to affect chromatin structure, chromosomal stability, and gene regulation. Biotinylation of the histones, which is mediated by holocarboxylase synthetase, causes the histones to “uncoil” and thus creates pores through which transcription factors can enter to reach DNA and activate gene promoter sequences. Biotinylation of histones, however, appears to mainly affect chromatin condensation and not gene expression, as only a tiny fraction of histones is biotinylated [3].

More specific effects of biotin on gene expression appear to occur as the result of interactions between holocarboxylase synthetase (that is present in the nucleus and attached to chromatin) and DNA methyltransferases to facilitate histone methylation [3,4]. Holocarboxylase synthetase also influences genes through the formation of multiprotein complexes with nuclear receptor corepressor proteins; these proteins enhance the binding of histone deacetylases to chromatin. The deacetylases “pull off” histone acetylation (i.e., remove acetyl groups) from various sites to enhance gene repression [3].

Biotinyl-5'-AMP-holocarboxylase synthetase has also been shown to regulate the expression of genes needed for the utilization of biotin (i.e., as part of carboxylases and holocarboxylase synthetase). The regulation involves the sGC-PKG signal transduction pathway. Other roles

in cell signaling pathways include those involving cGMP, nuclear factors (NF)- κ B, transcription factors Sp1 and Sp3, and receptor tyrosine kinases. Moreover, studies utilizing biotin-deficient experimental conditions demonstrate changes in the transcription or enzymatic activity of multiple enzymes required for glucose metabolism including glucokinase, phosphoenolpyruvate carboxykinase, and 6-phospho-fructokinase [2]. The vitamin also impacts the cell cycle, with arrest of the cell cycle observed with biotin deficiency [3].

Selected Pharmacological Uses/Other Roles

The use of pharmacological doses of biotin has mainly been employed in the management of a handful of inborn errors of metabolism that involve biotin-dependent enzymes. Individuals with propionic acidemia, for example, are generally supplemented with oral biotin (5–10 mg per day) to determine its impact, if any, on residual enzyme activity. Because it is the alpha subunit of the enzyme propionyl-CoA carboxylase that binds biotin, supplementation with the vitamin can sometimes improve enzyme activity in those with certain mutations in the alpha subunit.

Inborn errors in the genes for both biotinidase and holocarboxylase synthetase affect the availability and use of biotin for metabolic functions. Oral biotin supplements in amounts of 5–20 mg daily are used to treat biotinidase deficiency (i.e., help improve enzyme activity); oral doses of 10–200 mg of biotin daily may be utilized for those with holocarboxylase synthetase deficiency to try to improve enzyme activity [2].

Metabolism and Excretion

Catabolism of the biotin holocarboxylases occurs by proteases and ultimately yields biocytin. The biocytin is then degraded by biotinidase to lysine and free biotin, which may be reused or excreted. Biotin metabolites are excreted from the body mainly via the urine. Only small amounts (about 18–127 nmol/day) of intact biotin (and biocytin) are found in the urine. A few urinary metabolites are formed from the oxidation of the sulfur in biotin's ring; these metabolites include biotin sulfoxide and biotin sulfone (Figure 9.31). Most of the metabolites arise from the degradation of biotin's valeric acid side chain by β -oxidation. These metabolites include bisnorbiotin and tetranorbiotin (Figure 9.31) and, to a lesser degree, derived metabolites such as bisnorbiotin methyl ketone and tetranorbiotin methyl ketone. Smoking may accelerate biotin catabolism in women.

Biotin that has been synthesized by intestinal bacteria but not absorbed is found excreted in the feces. Very little dietary biotin that has been absorbed is excreted in the feces.

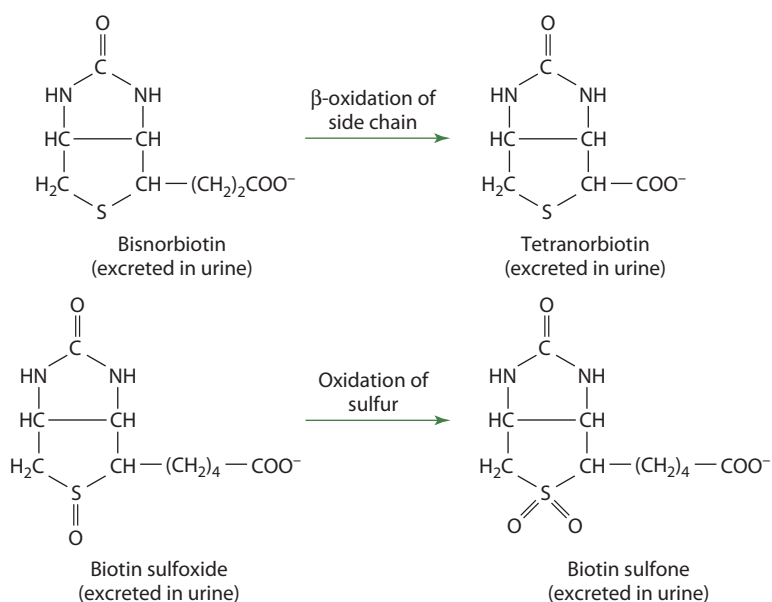


Figure 9.31 Selected metabolites from biotin degradation.

Adequate Intake

Because bacterially synthesized biotin is not sufficient to maintain normal biotin status, humans need to obtain biotin from the diet. The Adequate Intake (AI) recommendation for biotin for adults is 30 μg per day [5]. Adequate Intakes for biotin of 30 μg and 35 μg per day are suggested for women during pregnancy and lactation, respectively [5]. The inside front cover of this book provides additional AIs for biotin for other age groups.



One manifestation of a biotin deficiency is a red scaly dermatitis as shown in this photo.

Figure 9.32 Some manifestations of a biotin deficiency.

Deficiency

Biotin deficiency due to inadequate intake of the vitamin is rare. The deficiency, however, has resulted from excessive consumption of raw egg whites (which contain avidin; see the section discussing biotin sources). Some of the neurologic symptoms associated with a biotin deficiency include lethargy, paresthesia in extremities, hypotonia (reduced muscle tone), depression, and hallucinations. The most notable cutaneous symptom is a red, dry, scaly dermatitis found around the eyes, nose, mouth, and perineum (Figure 9.32). In addition, anorexia, nausea, alopecia (body hair loss), brittle nails, and muscle pain may occur. Death may result if biotin deficiency goes untreated. Therapeutic doses of up to 10 mg of biotin daily are typically used to treat deficiency in adults.

At Risk for Deficiency

Biotin deficiency or poor biotin status may be present in selected populations. People who ingest raw eggs in excess amounts are likely to develop a deficiency because avidin's binding to the biotin prevents the vitamin's absorption. Impaired biotin absorption may also occur with

gastrointestinal disorders such as inflammatory bowel disease and in chronic consumers of excessive alcohol. Biotin status has been shown to decline in some women during pregnancy and with lactation, as well as in individuals on anticonvulsant drug therapies such as phenobarbital, phenytoin, and carbamazepine [2].

Toxicity

Toxicity from oral biotin ingestion has not been reported, and no Tolerable Upper Intake Level has been established [5]. Fairly large oral doses (up to 200 mg) of biotin have been given daily, without side effects, to people with inherited disorders of biotin metabolism. Biotin supplements taken orally and use of biotin as a hair and skin conditioning agent in cosmetic-type products have been shown to be safe, but scientific studies documenting its effectiveness in treating hair and nail problems are lacking. Several conditions/situations can promote hair loss, such as with rapid weight loss; thyroid conditions; extreme stress; inadequate intakes of protein, zinc, and iron; excessive selenium

intake; and the use of certain medications, among others. Moreover, ingestion of large doses of biotin have been shown to interfere with diagnostic tests, specifically causing elevations in thyroid hormone concentrations and causing reductions in serum troponin concentrations (used in diagnosis of a heart attack).

Assessment of Nutriture

The evaluation of biotin in the blood and urine is used most often to assess biotin status. While blood biotin concentrations normally exceed 200 pg/mL, low blood biotin concentrations have not been shown to accurately reflect intake or status; they will, however, decrease (along with urinary biotin excretion) after about 2–4 weeks of consuming a biotin-deficient diet [5,6].

Decreased urinary biotin excretion ($\sim 6 \mu\text{g/day}$) and increased urinary excretion of 3-hydroxyisovaleric acid ($> 3.3 \text{ mmol/mol creatinine}$) and 3-hydroxyisovaleryl carnitine ($> 0.06 \text{ mmol/mol creatinine}$), generated from altered metabolism of β -methylcrotonyl-CoA, are sensitive and early indicators of biotin deficiency [5]. Normal 3-hydroxyisovaleric acid excretion is $\sim 0.2 \mu\text{mol/mg}$ of creatinine. Better indicators of biotin status are white blood cell biotinylated methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase [6].

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Suggested Reading

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9.7 FOLATE (VITAMIN B₉)

Folate's and vitamin B₁₂'s discoveries resulted from the search to cure the disorder megaloblastic anemia in the late 1870s and early 1880s. As with some of the other vitamins,

eating liver was shown to cure the condition. Mitchell and colleagues are credited with folate's discovery in 1941. Its chemical synthesis in a lab was reported in 1945. The word *folate* from Italian means “foliage.” The Latin word *folium* means “leaf.” The vitamin is also less commonly referred to as folacin.

Folate (also called pteroylglutamate or pteroylmonoglutamate) is composed of three parts that are all required for vitamin activity. The three parts are:

- 2-amino-4-hydroxypteridine, more commonly called pterin or pteridine, that is conjugated by a methylene group ($-\text{CH}_2-$) to
- para-aminobenzoic acid (PABA) to form pteric acid; the carboxy group of PABA is peptide bound to
- the amino group of glutamic acid (an amino acid; also called glutamate as found at physiological pH).

Although humans can synthesize each of these components, they do not have the conjugase enzyme necessary for the coupling of the pterin molecule to PABA to form pteric acid.

Folate is a general term that includes multiple forms of the vitamin. These forms include all those found in the body and naturally in foods (including tetrahydrofolate and its derivatives). It may also include the oxidized form (folic acid) found in fortified foods and most supplements. Tetrahydrofolate and folic acid are shown in Figure 9.33 in the monoglutamate form. Yet, within body cells and within foods, the glutamic acid residue of the vitamin is attached to additional (usually up to another eight) glutamic acids (also referred to as glutamic acid residues). The additional glutamic acids are linked via gamma-peptide bonds to the glutamate in the folate. When these additional glutamates are attached, this form of folate is sometimes referred as pteroylpolyglutamate.

Tetrahydrofolate (THF) and THF derivatives (Figure 9.33) are found in foods and are made in body cells. THF derivatives are formed with the additions of specific one-carbon groups on nitrogen atoms at positions 5 and 10 on THF; the one 3-carbon groups include methyl, methylene, methenyl, formyl, and formimino.

Sources

Selected food sources of folate are depicted in Table 9.12. Folate is found in significant quantities in vegetables, especially dark green leafy vegetables such as spinach. Some other food sources of the vitamin are legumes, lentils, and peas, along with some fruits and their juices. Liver (such as from beef) also provides relatively large amounts of folate, over 200 $\mu\text{g}/3\text{-oz}$ serving.

Raw foods are typically higher in folate than cooked foods because of folate losses incurred with cooking. Folate

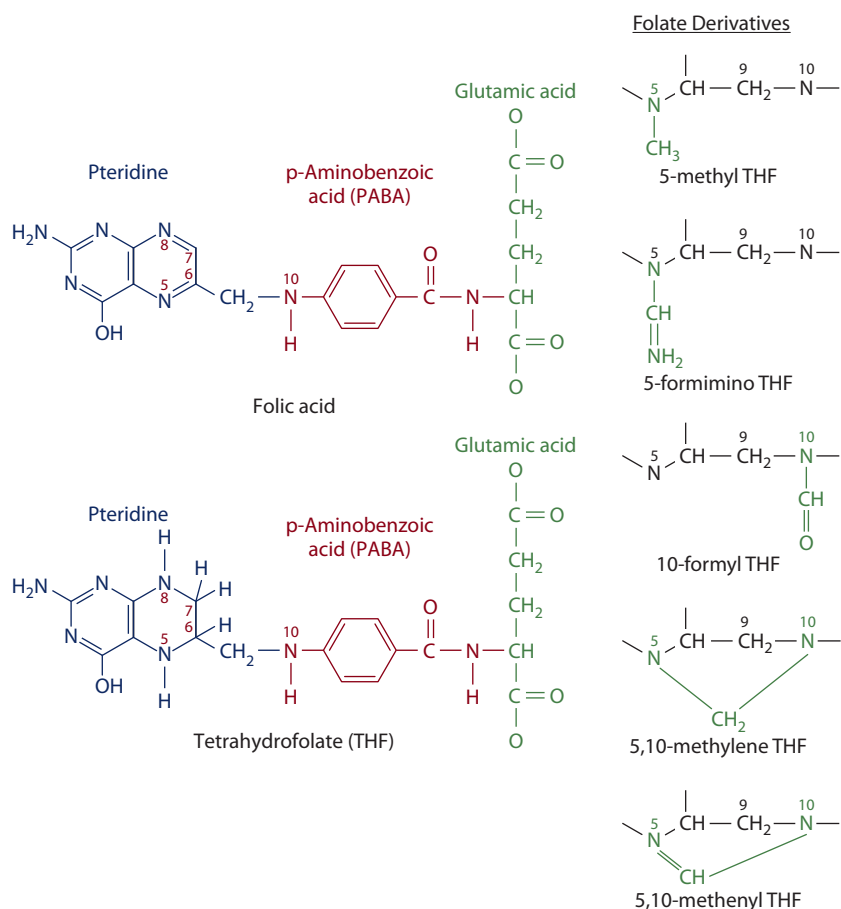


Figure 9.33 Structures of folic acid and tetrahydrofolate (THF).

Table 9.12 Folate Content of Selected Foods*

Food (serving)	Folate ($\mu\text{g DFE}$)
Lentils, cooked (1/2 c)	160
Spinach, cooked (1/2 c)	130
Asparagus, cooked (1/2 c)	127
Peas, black-eyed, cooked (1/2 c)	105
Broccoli, cooked (1/2 c)	92
Brussels sprouts, cooked (1/2 c)	78
Greens, cooked (1/2 c)	30-75
Avocado (1/2)	59
Peas, green, cooked (1/2 c)	47
Kidney beans, cooked (1/2 c)	45
Papaya, cubed (1/2 c)	27
Peanuts, roasted (1 oz)	27
Orange (1)	29
Banana (1)	24
Cantaloupe, cubed (1/4 c)	20
Egg, cooked (1)	22
Milk, 1% (1 c)	12

A more complete list of folate-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/Folate-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

is destroyed by heat, oxidation, and exposure to ultraviolet light. It is also reduced by 50–80% with certain types of food processing and preparation. Thus, consuming folate-rich foods raw or after cooking them quickly in a little water can help to minimize loss of the vitamin.

Fortification of flours, grains, and cereals with folic acid (140 μg of folic acid per 100 g of product) was initiated in 1998. These fortified cereals, breads, and grain products now represent major dietary sources of the vitamin. Enriched white bread provides 2.5–3 $\mu\text{g/slice}$, and fortified oatmeal provides 100 $\mu\text{g/cup}$. Some juices are also fortified with folic acid. Because of this fortification program, more Americans are meeting recommendations for folate intakes.

Bacteria in the colon generate folate. The overall contribution of microbial-derived folate in meeting the body's need for folate is not clear.

Naturally occurring forms of folate in foods are the THF derivatives—5-methyl THF, 5-formyl THF, and 10-formyl THF—although others may also be present. Over 75% of these folates have multiple glutamic acid residues attached.

In supplements (and in fortified foods), the vitamin is usually provided as folic acid. This form, which is very stable, also has only one glutamic acid attached to the PABA (i.e., folic acid monoglutamate). Most multi-vitamin preparations contain 400 μg of folic acid. Some

single-ingredient supplements contain up to 1,000 µg of folic acid. Also available are some single-ingredient supplements that provide the vitamin in the monoglutamate form as 5-formyl THF (denoted 5-FTHF or folinic acid) and as 5-methyl (denoted as 5-MTHF or methylfolate; it may also have an “L” preceding the name or be referred to as levomefolic acid).

The bioavailability of folate from foods varies based on multiple factors including intestinal pH, genetic variability in enzymatic activity needed for folate digestion, the food matrix, and the presence of dietary constituents such as inhibitors. The overall absorption of dietary folate is estimated at about 50%, but may range from 10 to 90%.

In contrast to folate in foods, folic acid and 5-methyl THF, as a supplement, are almost completely (100%) absorbed (especially if consumed on an empty stomach). But, when folic acid from a fortified food or supplement is consumed with natural food sources of folate, the vitamin is about 85% bioavailable [1]. Because of the difference in the efficiency of folate absorption from foods versus folic acid from supplements and fortified products, dietary folate equivalents (DFE) are used in recommendations for dietary folate intakes (see the “Recommended Dietary Allowances” subsection in this section). The vitamin’s Daily Value, found on food and supplement facts labels, is also expressed as equivalents and is 400 µg DFE.

Digestion and Absorption

Digestion is not required for folic acid obtained from fortified foods and supplements or for 5-formyl THF and 5-methyl THF in supplements because they are already present in the monoglutamate form. Folate in foods, however, is found in polyglutamate forms and must be digested to the monoglutamate form in order to be absorbed.

The digestion of the polyglutamate forms of THF and THF derivatives is performed by folate hydrolase (also referred to as glutamate carboxypeptidase) in the proximal small intestine. This zinc-dependent enzyme functions as an exopeptidase “cleaving off” each glutamate in stepwise fashion to ultimately generate a monoglutamate. Zinc deficiency and a more acidic pH (as occurs with pancreatic exocrine insufficiency and causes reductions in the bicarbonate content of pancreatic juice) diminish enzyme activity and thus folate digestion. Alcohol ingestion and inhibitors in certain foods, such as legumes, lentils, cabbage, and oranges, also diminish enzyme activity and thus impair digestion of the vitamin’s polyglutamate forms and reduce folate absorption.

Folate is absorbed into enterocytes by carrier-mediated transport and diffusion. The main carrier responsible for transporting folate (monoglutamate form), including folic acid, into intestinal cells is the proton-coupled folate transporter (PCFT). PCFT, which co-transport a proton (H^+) with folate, is found mostly in the proximal jejunum and

duodenum and requires an acidic pH of about 5.5–6. Passive diffusion also contributes to the vitamin’s absorption in the small intestine when pharmacological doses of the vitamin have been ingested. In the colon, a reduced folate carrier (RFC; also found on other tissue cell membranes) enables the absorption of bacterially produced reduced forms of folate like 5-methyl THF.

Hereditary folate malabsorption, a rare genetic disorder, results from mutations in the gene for PCFT. The condition is characterized by diarrhea, megaloblastic anemia, failure-to-thrive, and in some individuals, neurological problems. High (pharmacological) oral doses or lower intravenously administered doses of the vitamin are needed to overcome the absorptive defect.

Within the intestinal cell, glutamic acids may be added to the vitamin by folylpolyglutamate synthetase to generate polyglutamate forms; however, these are removed by gamma-glutamyl hydrolases prior to transport out of the intestinal cell. Folate crosses the intestinal cell’s basolateral membrane into portal circulation using a carrier protein known as multidrug resistant-associated protein (MRP) 3.

Transport, Tissue Uptake, and Storage

Folate is found in the blood as a monoglutamate either free (~1/3) or bound to proteins (~2/3), including albumin, α_2 macroglobulin, and a high-affinity folate-binding protein, which is thought to represent a soluble form of the cell membrane-derived folate receptor. The major form of folate found in systemic blood (and cerebral spinal fluid) is 5-methyl THF; smaller concentrations of THF, 10-formyl THF, and other THF derivatives are also present. Typical plasma folate concentrations range from about 3 to 20 µg/L. More folate is found in cerebral spinal fluid and red blood cells than the plasma. Furthermore, the folate in the red blood cells is attained during erythropoiesis since folate is not taken up by mature red blood cells. Thus, in contrast to plasma concentrations, red blood cell folate concentrations (which when adequate exceed 140 µg/L) represent an index of longer-term (2–3 months) folate status.

The uptake of folate into the liver and other tissues is carrier mediated. RFCs, which are ubiquitously expressed on cell membranes, deliver folate (especially 5-methyl THF) from systemic circulation into cells. PCFT (also found on many extraintestinal tissue cell membranes including the liver, pancreas, kidneys, and spleen, among others) as well as organic anion transporting polypeptides (OATP) B1 and B3 (found on hepatocytes) also enable tissue folate uptake. Lastly, folate receptors (FR) α , β , and γ mediate folate uptake via endocytosis into some tissues, including the brain. Mutations in the gene coding for FR α impair folate uptake across the blood–brain barrier and are associated with low cerebral spinal fluid folate concentrations and neurological disorders [2].

Within liver (and other) cells, the vitamin is found mainly as THF (~33%), as 5-methyl THF (~33%), and as 5-formyl THF and 10-formyl THF (~33%). The liver is the main site where folic acid is converted into THF. Reduction reactions convert the folic acid first to dihydrofolate (DHF) and then to THF. These reductions occur in the cytosol through the action of NADPH-dependent dihydrofolate reductase, which catalyzes the addition of four hydrogens at positions 5, 6, 7, and 8 on the substrate. Ingestion of large amounts of folic acid, however, have been shown to exceed the enzyme's capacity, resulting in transient high blood concentrations of unmetabolized folic acid [3]. Whether high serum concentrations of unmetabolized folic acid pose health risks requires further study (see also the section on toxicity).

Folate as THF and THF derivatives, upon entry into liver (and other) cells, becomes bound to typically five or six glutamate residues. Polyglutamate synthetase catalyzes the ATP-dependent additions of the glutamates, which are usually added one at a time. The addition of these glutamates traps the folate within the cell, prevents its degradation, and enables "storage." The glutamate residues can be removed by gamma-glutamyl hydrolases to allow for hepatic metabolism of the vitamin (via the folate cycle) or to allow the vitamin to be either released from the liver into blood or secreted from the liver into bile. About half of the folate that is initially absorbed is secreted, via MRP2 and breast cancer resistant protein (BCRP) carriers, into the bile where it undergoes enterohepatic recirculation and almost complete reabsorption.

Body stores of folate range from about 7 to 30 mg, with about one-half stored in the liver and lesser amounts in the kidneys, among other organs. Storage occurs in association with intracellular folate-binding proteins. The main storage forms of folate are the polyglutamate forms of THF and 5-methyl THF. These polyglutamate forms can be converted to monoglutamate forms by gamma-glutamyl hydrolase for folate use within cells or release into the blood.

The availability of folate to tissues where rapid cell division is occurring appears to be regulated when folate availability is limited. The mechanisms of this regulation are unclear but may involve changes in the rate of synthesis of polyglutamates and the release of folate monoglutamates from less metabolically active tissues to the liver, which then redistributes the folate to the actively proliferating cells.

Functions and Mechanisms of Action

THF and THF derivatives are found mostly in the cell mitochondria and cytosol and, to a lesser extent, in the nucleus. THF derivatives serve as cosubstrates, specifically carrying and transferring single- or one-carbon groups (units) in a variety of enzymatic reactions. The mitochondria contain

especially high concentrations of THF and 10-formyl-THF and lesser amounts of 5-methyl and 5-formyl THFs. The cytosolic folate pool is higher in 5-methyl THF, followed by THF, 10-formyl THF, and 5-formyl THF. The forms of folate found in the nucleus are less clear but are thought to include THF and 5,10-methylene THF (but perhaps others).

THF derivatives are involved in the metabolism of nutrients including choline/betaine and some amino acids and in the production of purines and pyrimidines. The purines and pyrimidines serve as nitrogenous bases in DNA and RNA. Moreover, because of its role as a carrier/donor of one-carbon methyl groups, folate also impacts DNA, histones, RNA, and nuclear receptors.

The THF derivatives and their one-carbon units that attach to THF at N5 and/or N10 on the pteridine ring (commonly written without the N) are shown in Figure 9.33 and illustrated hereafter along with the oxidation states.

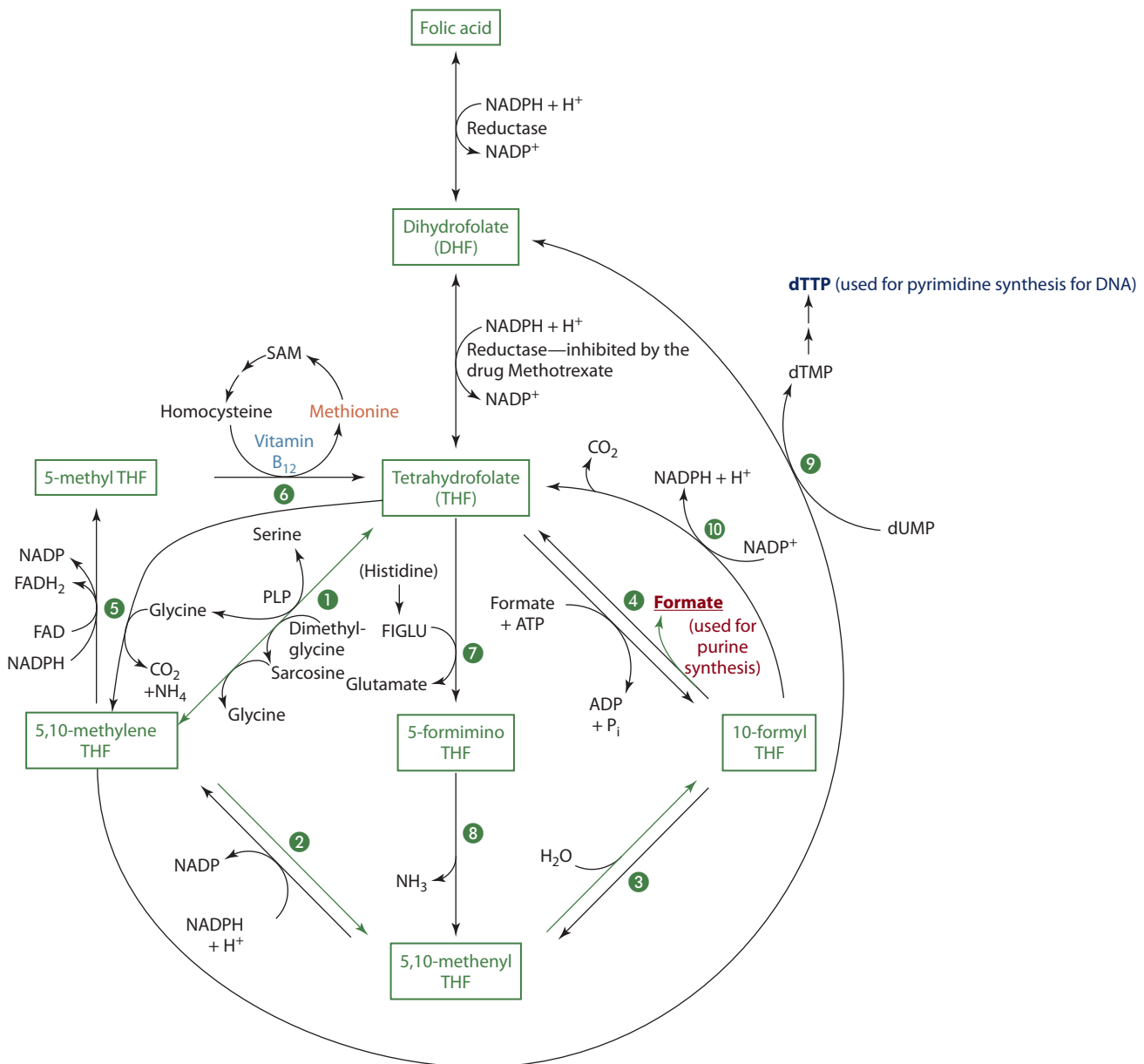
5- and 10-formyl THF	O=CH	Formate
5-formimino THF	—HC=NH—	Formate
5,10-methenyl THF	=CH—	Formate
5,10-methylene THF	—CH ₂ —	Formaldehyde
5-methyl THF	—CH ₃	Methanol

The formyl derivatives represent the most oxidized forms and, excluding THF, 5-methyl THF is the most reduced form. Table 9.13 provides an overview of some of the metabolic roles of these derivatives, which are interconvertible (as depicted in Figure 9.34), except that 5-methyl THF cannot be converted directly back to 5,10-methylene THF. Multiple enzymes are responsible for carrying out the reactions shown in Figure 9.34.

Cosubstrate Carrier of One-Carbon Units from Amino Acid and Choline/Betaine Metabolism for Purine and Pyrimidine Generation for DNA and RNA

Folate is involved in the metabolism of several amino acids including serine, glycine, histidine, and methionine and in the metabolism of choline/betaine. One-carbon units released in some of these reactions are "carried" and "donated" by THF derivatives to enable purine and pyrimidine synthesis.

Serine and Glycine Metabolism The amino acids serine and glycine (which can both cross between the mitochondria and cytosol as needed) represent major sources of one-carbon units for use in folate reactions. Serine is metabolized by the enzyme serine hydroxymethyltransferase, which requires vitamin B₆ as pyridoxal phosphate (PLP) for activity and is found in the cytosol and mitochondria (and possibly the nucleus) in all



Red arrows indicate predominant direction of the pathway in the mitochondria for formate synthesis

Enzymes involved in interconversions of coenzyme forms of THF:

- ① Serine hydroxymethyltransferase (coenzyme-PLP)—folate accepts carbon units as 5,10-methylene from serine resulting in 5, 10-methylene THF and glycine generation.
- ② Methylene THF dehydrogenase*—the generation of 5,10-methylene THF from 5,10-methenyl THF is important given the roles of 5,10-methylene THF in serine synthesis and pyrimidine synthesis.
- ③ Methenyl THF cyclohydrolase.* The production of 10-formyl THF is especially important for purine synthesis.
- ④ Formate THF ligase/synthetase. 10-formyl THF is especially important for purine synthesis.
- ⑤ Methylene-THF reductase—the generation of 5-methyl THF is essential for methionine synthesis from homocysteine.
- ⑥ Methionine synthetase (coenzyme-B₁₂)—see Figure 9.30 for details on this reaction.
- ⑦ Formiminotransferase—folate accepts a formimino group from FIGLU and facilitates the final step in histidine catabolism.
- ⑧ Cyclodeaminase.
- ⑨ Thymidylate synthetase—5,10-methylene THF provides the formaldehyde group for this reaction needed for pyrimidine synthesis.
- ⑩ 10-formyl THF dehydrogenase.

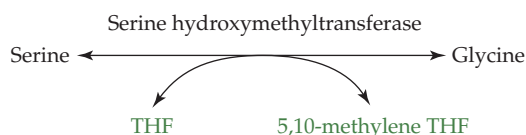
*Part of a complex called methylene THF dehydrogenase 1.

Figure 9.34 An overview of folate metabolism and the roles of tetrahydrofolate (THF) derivatives.

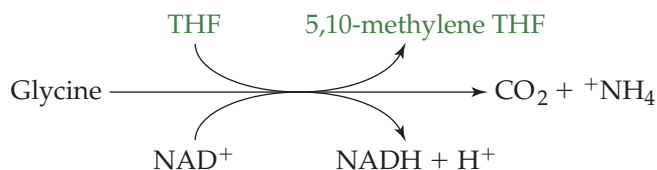
Table 9.13 Forms of Folate and Their Metabolic Roles in the Body

Folate Form	Roles
10-formyl THF	Folate transfers formate as 10-formyl THF for purine synthesis: 5-phosphoribosylglycinamide ribonucleotide (GAR) conversion to 5-phosphoribosyl formylglycinamide (FGAR) by glycinamide ribonucleotide formyltransferase and 5-phosphoribosyl 5-amino 4-imidazole carboxamide ribonucleotide (AICAR) conversion to 5-phosphoribosyl 5-formamido 4-imidazole carboxamide ribonucleotide (FAICAR) by aminoimidazolecarboxamide ribonucleotide formyltransferase
5,10-methylene THF	Folate transfers formaldehyde as 5,10-methylene for pyrimidine synthesis: Deoxyuridine monophosphate (dUMP) conversion to deoxythymidine monophosphate (dTMP) by thymidylate synthetase Folate receives formaldehyde from serine and glycine degradation: Serine conversion to glycine by serine hydroxymethyltransferase Glycine degradation by the glycine cleavage system Folate receives formaldehyde from choline degradation: As part of choline degradation, dimethylglycine and its catabolic product sarcosine are degraded to glycine by dimethylglycine dehydrogenase and sarcosine dehydrogenase, respectively
5-formimino THF	Folate receives a formimino group in histidine degradation: Formiminoglutamate (FIGLU) conversion to glutamate by formiminotransferase
5-methyl THF	Folate provides a methyl group for methionine synthesis: Homocysteine conversion to methionine by methionine synthase

tissues (especially the liver and kidneys). In this reaction that is reversible, the enzyme transfers a one-carbon unit from serine to THF to generate 5,10-methylene THF and the amino acid glycine.



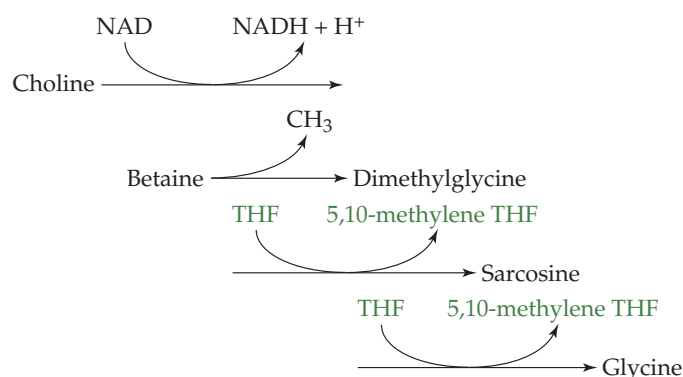
Glycine (generated from serine or protein degradation or present from the diet) can undergo degradation by the glycine cleavage system and provide to THF a one-carbon unit to generate 5,10-methylene THF along with ammonium and carbon dioxide, as shown here.



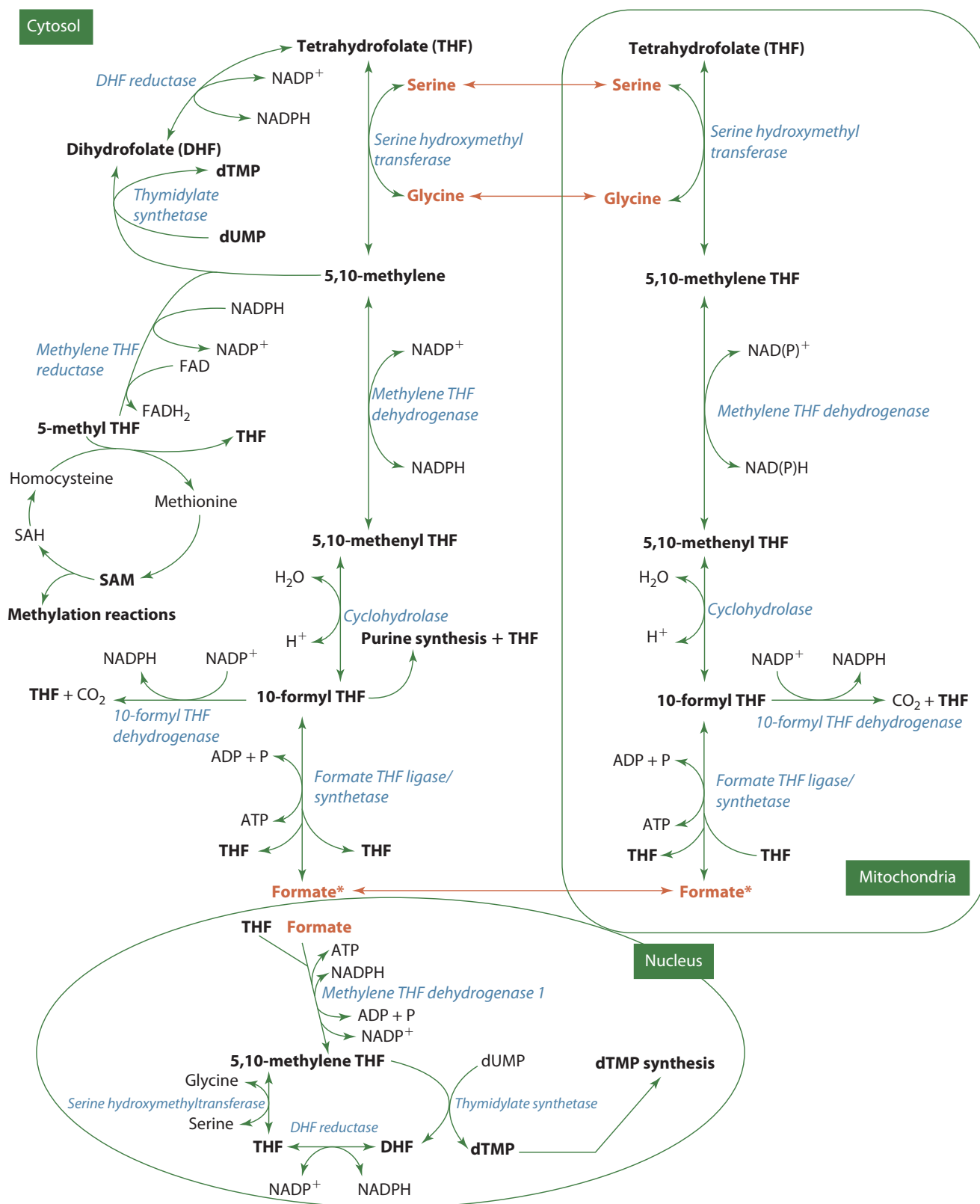
The 5,10-methylene THF (produced from serine and glycine metabolism) can be used to generate 5-methyl THF in the cytosol or can undergo conversion to 5,10-methenyl THF and subsequently to 10-formyl THF in both the cytosol and mitochondria (Figure 9.35). See more on the metabolism and use of 5,10-methylene THF under the section “Links with Purine and Pyrimidine Synthesis and DNA and RNA Production.”

Choline/Betaine Metabolism Betaine (also called trimethylglycine) is found in foods and can also be generated (primarily in the liver) from choline (see Figure 6.12 and

later Figure 9.37). (Note that choline is also made in the body and found in foods.) Betaine metabolism (which occurs in both the mitochondria and cytosol of primarily the liver and kidneys) generates dimethylglycine and a methyl group (one-carbon unit). This methyl group has several important uses, as discussed later in the section “Carrier of One-Carbon Units for Methionine and SAM Synthesis.”



The further degradation of dimethylglycine (generated from betaine) also provides a one-carbon group. In a reaction catalyzed by dimethylglycine dehydrogenase, dimethylglycine gives a one-carbon group to THF, producing 5,10-methylene THF and sarcosine (also called monomethylglycine). Sarcosine can also be further catabolized by sarcosine dehydrogenase producing glycine, with THF again functioning as the carbon acceptor and forming 5,10-methylene THF. Riboflavin as FAD is also required by this dehydrogenase. This series of reactions generates multiple 5,10-methylene THF, which can be used throughout the cell, as discussed in the next section.



*Formate also arises in cells from the α -oxidation of branched-chain fatty acids and during tryptophan and histidine catabolism

Figure 9.35 Selected aspects of folate metabolism within the cytosol, mitochondria, and nucleus.

Links with Purine and Pyrimidine Synthesis and DNA and RNA Production The 5,10-methylene THF (created from one-carbon units in the conversion of serine to glycine, from glycine catabolism, from betaine [and sarcosine] catabolism, and from histidine degradation [discussed in the next section]) is used/metabolized in the mitochondria, cytosol, and nucleus for the production of other key THF derivatives including 10-formyl THF and formate.

- Mitochondrial use of 5,10-methylene THF includes production of 10-formyl THF, which can be then used for N-formylation for the synthesis of Met-tRNA^{MET} (the initiation transfer RNA). 10-Formyl THF also undergoes hydrolysis to formate.
- Formate, which can cross back and forth across intracellular compartments, then moves into the cytosol where it reforms 10-formyl THF (Figure 9.35).
- Cytosolic use of 10-formyl THF provides the one-carbon units at positions 2 and 8 for the synthesis of adenine and guanine (see also Figures 6.29 and 6.30). These purines are required for the production of the nucleic acids DNA and RNA.
- Formate can also move into the nucleus where it is used to generate 5,10-methylene THF for use in thymidylate synthesis, as discussed next.

In pyrimidine synthesis, thymidylate synthetase uses 5,10-methylene THF *generated in the cytosol and in the nucleus* to convert (via reductive methylation) deoxyuridine monophosphate (dUMP) to thymidylate (deoxythymidine monophosphate, dTMP) and dihydrofolate (DHF) (Figures 9.34 and 9.35). The DHF that is formed can be converted into THF by dihydrofolate reductase, a NADPH-requiring enzyme. Thymidylate (after additional phosphorylations to form dTTP) is used for DNA synthesis.

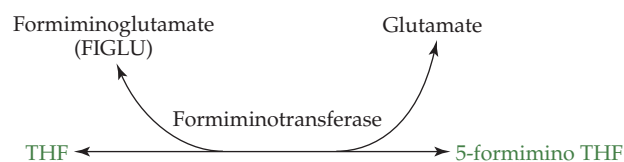
Thymidylate, however, also plays another role in the central nervous system, where it is used to produce guanosine triphosphate (GTP). GTP functions in the production of tetrahydrobiopterin (BH₄); BH₄ serves as a cofactor for several enzymes responsible for the synthesis of neurotransmitters including dopamine, serotonin, and nitric oxide. In fact, reductions in 5-methyl THF concentrations have been associated with changes in nitric oxide. Nitric oxide is a potent vasodilator and also affects endothelial function. Diminished nitric oxide concentrations in those with low folate status may be one mechanism for linking deficiency of the vitamin with increased risk of hypertension and stroke (see the section “Association with Diseases”) [4].

The involvement of THF derivatives in purine and pyrimidine synthesis makes folate essential for DNA and RNA synthesis and cell division. In fact, the folate-dependent reaction generating thymidylate is rate limiting

to DNA replication and both DNA and RNA are affected with disruptions in purine/pyrimidine ring formation secondary to folate deficits. The synthesis of cells with short lifespans, such as enterocytes and red blood cells (erythrocytes), is particularly affected if folate is inadequate (see the section on folate deficiency).

Folate's role in cell division has made it a “target” for some drug therapies. Both thymidylate synthetase and dihydrofolate reductase are especially active in cells, including tumor cells, undergoing division. The drug methotrexate is used in the treatment of some cancers, rheumatoid arthritis, and psoriasis, among other conditions. The drug serves as an antagonist, binding to dihydrofolate reductase's active site and preventing synthesis of THF and ultimately DNA and RNA needed for actively dividing cells. The enzyme thymidylate synthase is also the targeted enzyme for another drug (5-fluorouracil) used in cancer treatment. The drug binds to the enzyme, resulting in less dTMP (and dTTP) production, an accumulation of dUMP, reduced DNA synthesis (especially in rapidly dividing cells), and cell death.

Histidine Degradation The final reaction in histidine catabolism also requires THF and generates 5-formimino THF, which can be further metabolized to other THF derivatives. Histidine catabolism begins with its deamination to generate urocanic acid, which undergoes further metabolism to yield formiminoglutamate (FIGLU). The formimino group is removed from FIGLU by formiminotransferase to generate glutamate; THF receives the formimino group to yield 5-formimino THF, as shown here and in Figure 9.36.



This reaction has been used diagnostically as a basis for determining folate deficiency, although other approaches are now more often used. In the diagnostic procedure, subjects ingest an oral histidine load dose, and FIGLU excretion is measured in the urine. With a folate deficiency, FIGLU accumulates in the blood and is excreted in higher than normal concentrations in the urine. With adequate folate (THF) status, the THF is converted into 5-formimino THF and FIGLU is converted to glutamate, with little to no FIGLU appearing in the urine.

The 5-formimino THF generated in the reaction can be converted to 5,10-methenyl THF and subsequently to 5,10-methylene THF (if needed) by cyclodeaminase and methylene THF dehydrogenase, respectively (Figure 9.34). Thus, histidine, like serine, glycine, and choline/betaine, provides a source of one-carbon units.

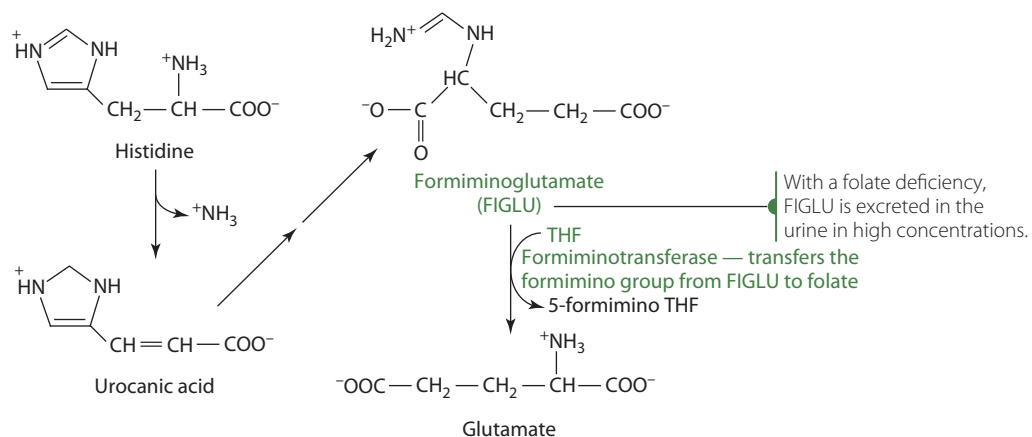
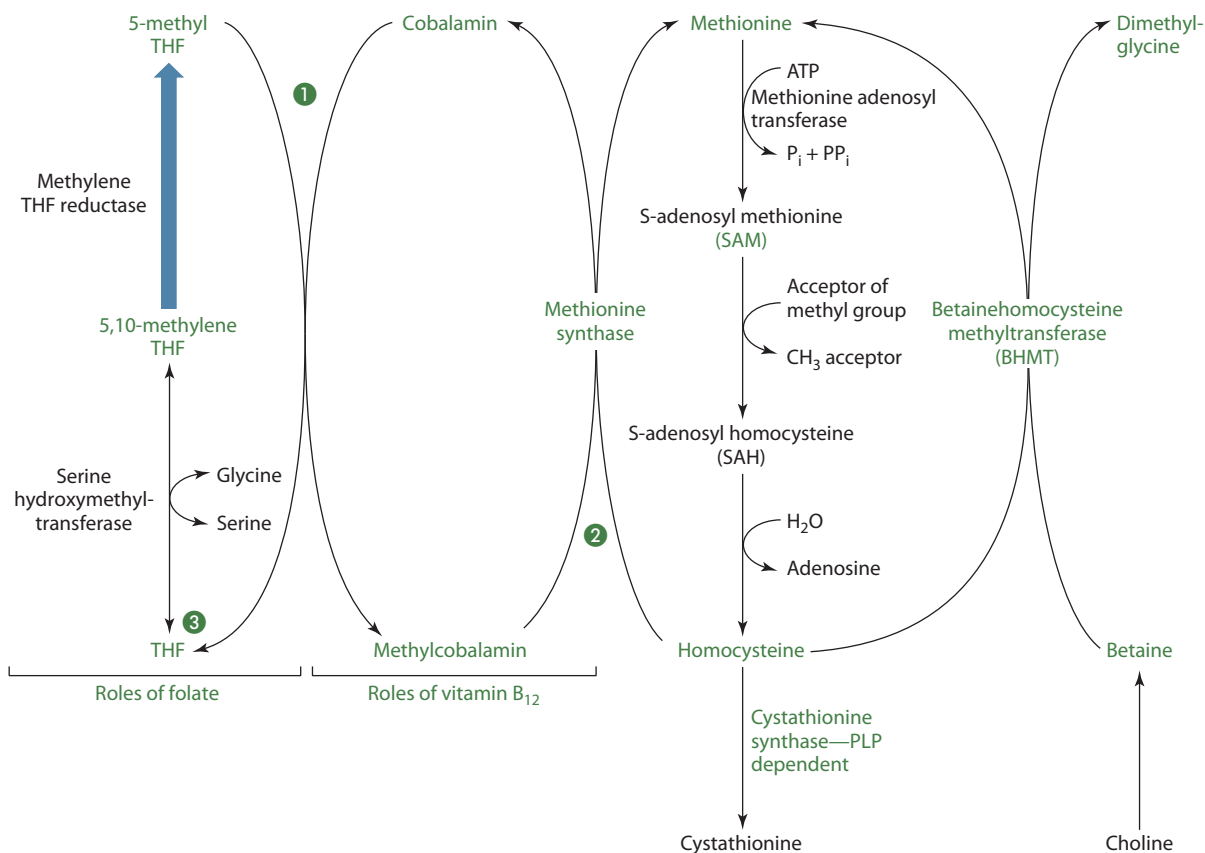


Figure 9.36 The role of folate in histidine catabolism.

Carrier of One-Carbon Units for Methionine and SAM Synthesis

Folate as 5-methyl THF serves as a carrier of one-carbon units (specifically as a methyl donor) in the regeneration of methionine from homocysteine (Figure 9.37). The

reaction, which occurs in the cytosol, requires methionine synthase (also called homocysteine methyltransferase) and cobalamin (vitamin B₁₂) as a tightly bound prosthetic group. Cobalamin, while bound to methionine synthase, picks up the methyl group from 5-methyl THF



- 1 Cobalamin, which is bound to the enzyme methionine synthase, picks up the methyl group on 5-methyl THF, forming THF and methylcobalamin.
- 2 Methylcobalamin, which is still bound to the enzyme methionine synthase, gives the methyl group to homocysteine, which then forms methionine and reforms cobalamin.
- 3 THF must be reconverted to 5-methyl THF for the reaction to proceed again. This process requires two reactions catalyzed first by serine hydroxymethyl transferase to generate 5,10-methylene THF. Second, methylene THF reductase converts 5,10-methylene THF to 5-methyl THF, which can once again donate its methyl group to cobalamin.

Figure 9.37 The resynthesis of methionine from homocysteine, showing the roles of folate and vitamin B₁₂.

to generate methylcobalamin and THF. The methylcobalamin then serves as the methyl donor, providing its methyl group to homocysteine and forming methionine (see also the vitamin B₁₂ section “Functions and Mechanisms of Action”). This production of methionine is critical since it provides the methyl group for S-adenosyl methionine (SAM) synthesis.

Another substance, however (besides folate and vitamin B₁₂), can also provide for the remethylation of homocysteine. Betaine, obtained from the diet or generated in the liver from choline degradation, provides a methyl group that can be transferred to homocysteine by the hepatic enzyme betaine homocysteine methyltransferase to generate methionine (Figure 9.37). Methionine in turn can be used to produce SAM.

SAM functions in body cells as a major methyl donor, involved in over 100 methylation reactions, including, for example, DNA, RNA, and protein (including histone) methylation, myelin maintenance, neural function, and synthesis of polyamine, carnitine, and catecholamines, among others. Methylated DNA and histones influence gene expression and gene silencing. (See Chapter 6 for additional information about SAM.)

SAM concentrations and folate availability regulate, in part, methionine metabolism, specifically affecting methionine synthesis from homocysteine and the degradation of homocysteine. SAM concentrations, which increase with increased cellular methionine concentrations, stimulate the transsulfuration pathway in which homocysteine is degraded in an irreversible reaction, to cystathionine by cystathionine synthase (Figure 9.37). Higher SAM concentrations and folate availability (as 5-methyl THF) also inhibit methylene THF reductase activity to reduce 5,10-methylene THF conversion to 5-methyl THF. In contrast, low 5-methyl THF availability decreases remethylation of homocysteine, methionine formation, and SAM formation. Insufficient SAM reduces methylation reactions in cells.

Interactions with Other Nutrients

A synergistic relationship exists between folate and vitamin B₁₂. This relationship is sometimes called the methyl-folate trap. The following sequence of events leads to the methyl-folate trap (tracing the reactions shown in Figures 9.34 and 9.37 is helpful). In the synthesis of methionine from homocysteine, the methyl group from 5-methyl THF is normally transferred to vitamin B₁₂ (cobalamin) that is attached to the enzyme methionine synthase. Without vitamin B₁₂ to accept the methyl group from 5-methyl THF, the 5-methyl THF accumulates and is “trapped” and other forms of folate, like THF, cannot be produced. Thus, the cells have folate, but not in a form that can be used for DNA synthesis. In contrast, with adequate vitamin B₁₂ status, the THF resulting from the methionine

resynthesis can be used to make the forms of folate needed for DNA synthesis, including 10-formyl THF (which is needed for purine synthesis) and 5,10-methylene THF (which is needed for thymidylate synthesis in pyrimidine metabolism).

Association with Diseases

Inadequate intake of folate and/or poor folate status as well as genetic mutations in enzymes affecting folate metabolism have been linked with several diseases and health problems. Based on some of folate’s functions, some of the effects of folate deficiency or poor folate status can be broadly categorized as

- Reductions in SAM (and methionine) resulting in hypomethylation of chromatin and corresponding effects on gene expression and genomic stability
- Impaired purine and pyrimidine synthesis resulting in diminished DNA synthesis and stability and reduced cell division rates.

Genetic Mutations: Methylene THF Reductase 677C>T

Genetic polymorphisms (variations shared by over 1% of the population) have been identified in the genes for several enzymes involved in folate metabolism; these polymorphisms alter the availability of folate and its derivatives. One of the most common polymorphisms is methylene THF reductase (MTHFR) C677T (also written 677C>T). C677T represents a point mutation in the MTHFR gene in which the cytosine “C” nucleotide (normally found) has been substituted by the thymine “T” nucleotide (abnormal) at position 677. This substitution in the DNA results in the insertion of valine (abnormal) instead of alanine (normal) in the enzyme MTHFR. This type of polymorphism is known as a single-nucleotide polymorphism (SNP), usually pronounced as “SNIP.” As a consequence of the resulting amino acid substitution in MTHFR, the ability of the FAD coenzyme to bind to MTHFR is reduced, and both MTHFR activity and stability are reduced. In heterozygous individuals (those with one normal “C” and one abnormal “T” allele), MTHFR activity is reduced up to about 30–35%, and in homozygous individuals (those with two abnormal “TT” alleles), activity is reduced up to about 60% [5].

Ramifications of this polymorphism, especially in homozygous individuals, include low serum 5-methyl THF concentrations, high serum homocysteine concentrations, hypomethylation of DNA, and increased risk of several diseases. Some of the health problems include cardiovascular diseases, hypertension, stroke, some cancers, psoriasis, neural tube defects, and neurological and psychiatric conditions, among others. The impact is greatest among individuals with low folate intake.

The prevalence of MTHFR C677T varies across ethnicities and continents, with higher frequencies generally among individuals from Northern Europe, Japan, Mexico, and Central and South America and lower among those from Africa and Asia (except Japan). The worldwide prevalence of the MTHFR 677TT genotype is estimated at 10%.

Treatment of MTHFR C677T (to improve folate status) requires the ingestion of higher amounts of dietary folate via supplementation in the form of 5-methyl THF. In addition, riboflavin supplements (1.6 mg/day) have been shown to be beneficial in reducing blood pressure in some individuals with hypertension [4].

Cancer

Unstable DNA, DNA strand breaks, and insufficient DNA repair (which disrupt the cell cycle and cell division) can occur as the result of folate deficiency or poor folate status and can increase cancer risk. These changes stem, in part, from inadequate thymidylate production, ongoing misincorporation of uracil for thymidine in DNA, and increased cellular uracil accumulation. Insufficient methylation (hypomethylation) due to reduced SAM formation secondary to folate deficiency also disrupts genomic integrity, including chromosomal stability. Aberrant methylation associated with folate deficiency may, for example, activate oncogenes or reduce tumor suppression genes to promote cancer. Folate deficiency (or poor folate status) and/or low folate intake have been associated (in some but not all studies) with an increased risk in the development (initiation) of many cancers, especially colon cancer, but also lung cancer and esophageal adenocarcinoma, among other gastrointestinal and nongastrointestinal cancers [6–8].

Cardiovascular Disease and Stroke

Folate and vitamin B₁₂ participate in the regeneration of methionine from homocysteine and vitamin B₆ is needed for the catabolism of homocysteine (Figure 9.37); low intakes of these three vitamins, especially folate, are inversely associated with plasma homocysteine concentrations. Elevated plasma homocysteine concentrations (> 11 µmol/L) are associated with premature heart disease, occlusive vascular disease, and cerebral (i.e., stroke) and peripheral vascular diseases. The mechanisms by which hyperhomocysteinemia increases disease risk are not clear, but may result from impaired endothelial function, increased vascular lesions, and/or enhanced platelet adhesiveness and clotting, among others. A 5 µmol/L increase in serum homocysteine concentrations increases the risk for heart disease by 20–30% [9]. Supplementation of folic acid alone or with vitamins B₁₂ and B₆ in people (both healthy and with heart disease) with hyperhomocysteinemia typically normalizes or reduces blood homocysteine concentrations and reduces stroke risk (in those

with low folate status), but does not consistently diminish the risk of cardiovascular events or mortality [8,9].

Cognitive Decline and Dementias

Elevations in plasma homocysteine concentrations as well as low folate status have been associated with cognitive dysfunction, Alzheimer's dementia, and other dementias. Most (but not all) supplementation trials with folate (and other B-vitamins), however, have not demonstrated significant improvements in cognitive functions [8,10,11].

Depression

Depression has also been associated with low serum and red blood cell folate concentrations, although neither is a good indicator of cerebral spinal fluid folate concentrations. Links between low folate status and inadequate response to antidepressant medications have also been demonstrated, yet findings of supplementation trials providing folic acid alone or in combination with antidepressant medications are mixed. Provision of 5-methyl THF alone or in combination with antidepressant medications has generally shown more promise than supplementation with folic acid [8,12,13].

Neural Tube Defects

Because of evidence that folic acid supplementation taken before (during the periconceptional period of pregnancy) or about the time of conception may reduce the incidence of neural tube defects, the Centers for Disease Control and Prevention (CDC) suggests 400 µg of synthetic folic acid/day for women capable of becoming pregnant. However, the mechanism(s) by which folate plays a role in the etiology of neural tube defects is unclear [14]. Consistent with this information, foods that are good sources of folate (i.e., that provide ≥ 10% of the 400 µg or at least 40 µg/serving) are permitted by the U.S. Food and Drug Administration (FDA) to make the health claim “Healthful diets with adequate folate may reduce a woman's risk of having a child with a neural tube (brain or spinal cord) defect” [15].

Metabolism and Excretion

Folate is excreted from the body in both the urine and the feces. Within the kidneys, folate-binding proteins in the brush border membrane facilitate tubular reabsorption of the vitamin to retain folate, if needed. Excess folate may be present in the urine intact or as a metabolite secondary to hepatic degradation prior to urinary excretion. Oxidative cleavage of folate is thought to occur between C9 and N10 of polyglutamate forms of the vitamin. This cleavage generates para-aminobenzoyl polyglutamate and pteridine. All but one of the glutamates is then hydrolyzed, and usually the compound is acetylated to form the major urinary metabolite N-acetyl para-aminobenzoyl glutamate.

Smaller amounts of para-aminobenzoyl glutamate are also found in the urine.

In addition to urinary losses, folate (up to about 100 μg) is secreted by the liver into the bile. MRP2 and BCRP move folate across the apical bile canicular membrane from the hepatocyte and into the bile. Most of this folate, however, is reabsorbed with enterohepatic recirculation, so fecal vitamin losses are minimal. Folate of microbial origin that has not been absorbed, however, may appear in the feces in relatively high amounts.

Recommended Dietary Allowance

The establishment of recommendations for folate intake consider its bioavailability as well as several indices of nutriture. Folate requirements are estimated at 320 μg per day [1]. Recommendations for folate are provided as dietary folate equivalents (DFE), which account for the higher bioavailability of folic acid taken as supplements or from fortified foods versus folate from foods. The RDA for adults for folate is 400 μg DFE per day and increase to 600 μg DFE and 500 μg DFE for pregnancy and lactation, respectively [1]. Calculations of DFE can be made using folate information available from food and supplement product labels as follows.

1 DFE = 1 μg food folate = 0.5 μg folic acid from a supplement taken without food (on an empty stomach) with synthetic folic acid, when ingested on an empty stomach, providing twice the folate from foods [1].

1 DFE = 1 μg food folate = 0.6 μg folic acid from a supplement or fortified food consumed with a meal or, stated alternately, 1 DFE = μg food folate + $(1.7 \times \mu\text{g}$ folic acid) with the bioavailability of folic acid supplemented in foods greater by a factor of 1.7 than folate found naturally in foods [1]. As an example, if a person consumed a vitamin containing 100 μg of folic acid along with some bread containing 50 μg of folic acid as part of a meal, the DFE = natural food folate + $(1.7 \times 150 \mu\text{g})$.

Additional RDAs for folate for other age groups are provided on the inside front cover of the book.

Deficiency: Megaloblastic Macrocytic Anemia

Folate deficiency results in megaloblastic macrocytic anemia—the release into circulation of red blood cells that are fewer than normal in number as well as larger in size and abnormally nucleated (immature). Some other signs and symptoms may include fatigue, weakness, headaches, irritability, difficulty concentrating, shortness of breath, and heart palpitations.

The deficiency is characterized initially (within about a month or two if the diet is devoid of folate) by reductions in plasma folate and then by increases in plasma homocysteine concentrations. Red blood cell folate concentrations

diminish after about 3–4 months (remember red blood cells live about 90–120 days) of low folate intake. After approximately 4–5 months, rapidly dividing cells such as those in the gastrointestinal tract and blood become megaloblastic. Mean cell volume (MCV) increases, and hypersegmentation (increased lobes) of white blood cells (neutrophils) occurs, along with decreased blood cell counts. The reduced number of and abnormal red blood cells in turn diminish the oxygen-carrying capacity of the blood and may result in shortness of breath, fatigue, and weakness. Treatment of the deficiency usually requires oral ingestion of 1–5 mg folate daily. Use of 5-methyl THF appears to be more effective than folic acid in improving red blood cell and plasma folate concentrations [16].

Megaloblastic macrocytic anemia, also called megaloblastic anemia, is relatively common in the United States. It may result from a deficiency of either folate or vitamin B₁₂, both of which disrupt DNA synthesis (replication) and thus cell division. As discussed under the functions of folate, the 10-formyl THF form of folate is needed for purine synthesis, and the 5,10-methylene THF form is needed for pyrimidine synthesis. Deficient thymidylate (pyrimidine) impairs DNA synthesis and results in cell cycle arrest in the S-phase of cell division. Effects are most evident in cells with rapid turnover such as hemopoietic cells and enterocytes. In the bone marrow, precursor red blood cells exhibit deranged DNA synthesis and defective cell maturation and division, resulting in abnormally large (macrocytic) and immature red blood cells (megaloblasts) with shortened lifespans. Over time, the megaloblasts steadily increase in number in the blood whereas the numbers of healthy red blood cells decrease in the blood, with negative effects on the blood's oxygen-carrying capacity. Figure 9.38 reviews the formation and maturation of erythrocytes.

The effects of folate deficiency on the cells of the gastrointestinal tract, which like blood cells exhibit rapid turnover, are multiple. Manifestations typically include a bright red tongue, glossitis, sores in the mouth, shortening of the villi height, and thinning of the layers of the gastrointestinal tract. The latter two changes may impair nutrient absorption and promote diarrhea.

At Risk for Deficiency

Some conditions associated with an increased need for folate include excessive alcohol ingestion (which inhibits folate digestion and thus absorption) and malabsorption disorders such as inflammatory bowel diseases. Folate malabsorption also occurs secondary to gastric bypass procedures used in the treatment of obesity. Several medications affect folate status. The diuretic furosemide—used to treat hypertension and congestive heart failure, among other conditions—decreases intestinal folate absorption. Folate deficiency has been observed in people taking diphenylhydantoin or phenytoin, anticonvulsants used

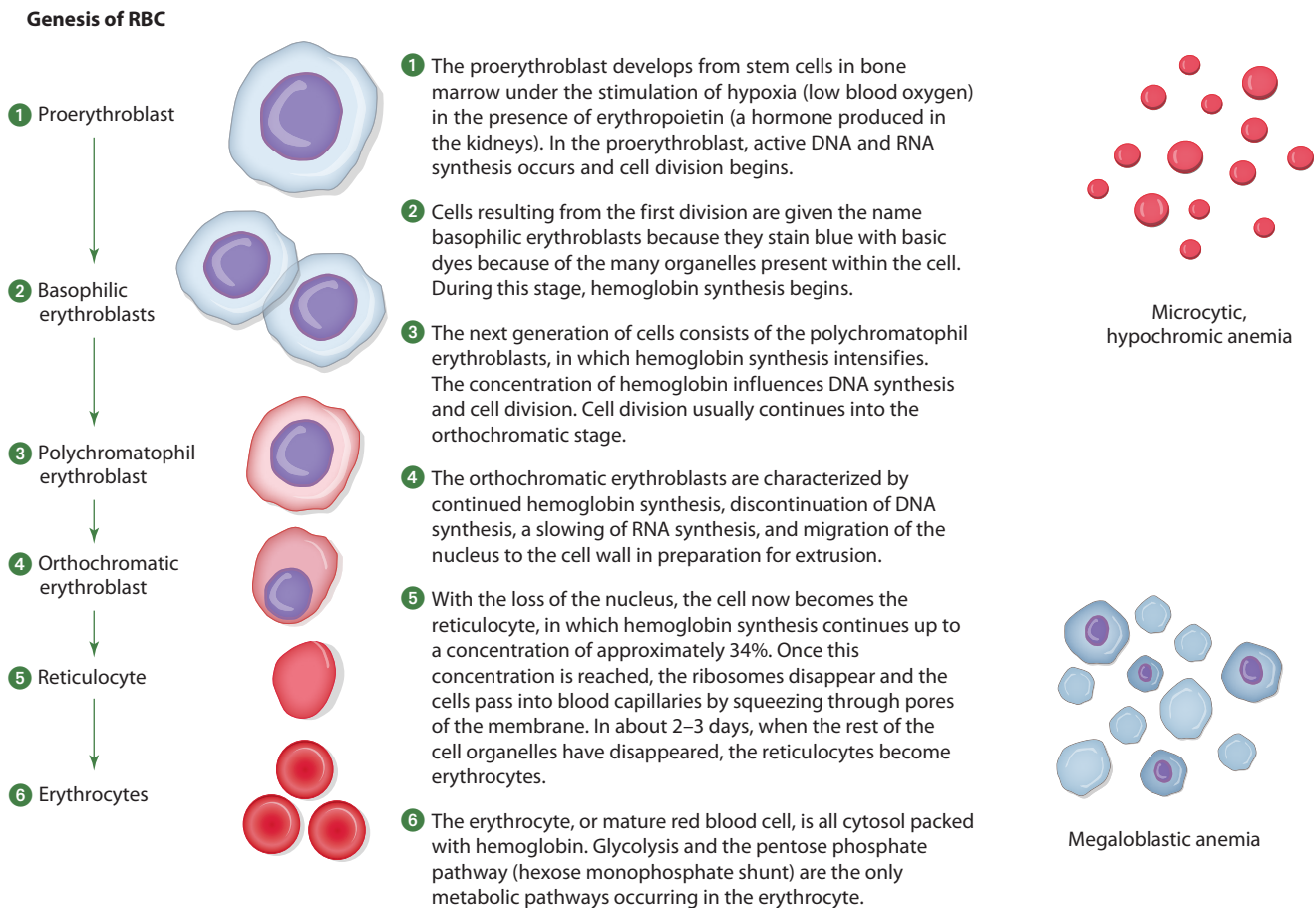


Figure 9.38 Genesis and maturation of the red blood cells (left); red blood cells characteristic of microcytic and megaloblastic anemias (right).

to treat epilepsy. Folate and phenytoin each inhibit the gastrointestinal cellular uptake of the other. Methotrexate, used to treat rheumatoid arthritis and some cancers, among other conditions, binds to dihydrofolate reductase and thus prevents THF synthesis. Other drugs, including cholestyramine (used to treat high blood cholesterol concentrations) and sulfasalazine (used to treat inflammatory bowel diseases), interact with folate and can increase the likelihood of folate deficiency.

Toxicity

A Tolerable Upper Intake Level for adults of 1,000 μg (1 mg) for synthetic folic acid in supplements or from fortified foods (not natural foods) is based on the ability of folate to mask the neurological manifestations of vitamin B_{12} deficiency [1]. Folic acid supplements can alleviate the megaloblastic anemia caused by a vitamin B_{12} deficiency, but not the neurological damage, which progresses undetected and is irreversible (see the “Deficiency” section for vitamin B_{12}).

Immediate side effects with very high (15 mg) folic acid intakes (i.e., 15 times the Tolerable Upper Intake Level) include insomnia, malaise, irritability, and gastrointestinal

distress [1,14]. The use of folic acid-containing multivitamin supplements (providing the RDA) along with usual consumption of folic acid–fortified foods has resulted in daily folic acid intakes in excess of the Tolerable Upper Intake Level in both children and older adults [17,18]. The long-term effects of these more moderately higher intakes are unknown. While meta-analyses have not supported associations between cancer and folic acid, supplemental folic acid in amounts exceeding about 2½ times recommendations has been shown to increase cancer risk and cancer mortality, especially in individuals with precancerous tumors [3,19,20]. More studies are needed to identify risks and mechanisms by which folic acid exerts its effects.

Assessment of Nutriture

Folate status is most often assessed by measuring folate concentrations in the plasma, serum, or red blood cells. Serum or plasma folate levels reflect recent dietary intake; thus, true deficiency must be interpreted through repeated measures of serum or plasma folate. Serum folate concentrations less than about 3 $\mu\text{g}/\text{L}$ typically suggest deficiency. Red blood cell folate concentrations are more reflective of folate tissue status than is serum folate and represent

vitamin status at the time the red blood cells were synthesized. Red blood cell folate concentrations less than about 140 µg/L suggest folate deficiency; however, concentrations are also lowered with a vitamin B₁₂ deficiency [1,21].

Formiminoglutamate (FIGLU) excretion may also be used to measure folate nutriture because folate as THF must be available for the formimino group to be removed from FIGLU and glutamate to be formed (see Figure 9.36). FIGLU excretion is measured in a 6-hour urine collection after ingestion of 2–5 g oral L-histidine. Normal FIGLU excretion is ~35 µM/day in folate-adequate adults, whereas with folate deficiency it rises to > 200 µM/day [14]. A deficiency of vitamin B₁₂, however, also elevates FIGLU excretion.

The deoxyuridine suppression test, another method for assessing folate status, measures the availability of folate for thymidine synthesis. In this test, the activity of thymidylate synthetase is measured in cultured lymphocytes or bone marrow cells. The reaction catalyzed by thymidylate synthetase is dependent on folate and, indirectly, on vitamin B₁₂; therefore, the change in activity elicited by adding one or the other vitamin allows the deficiency to be identified. In other words, if a person were folate deficient, adding folate—but not vitamin B₁₂—would normalize enzyme activity. Likewise, if a person were vitamin B₁₂ deficient, adding vitamin B₁₂—and not folate—would normalize thymidylate synthetase activity. In the case of a deficiency of both vitamins, enzyme activity could be normalized only by adding both vitamins [14].

A functional marker of folate and vitamin B₁₂ deficiencies is elevated plasma homocysteine concentrations. Remember, both vitamins are required for the remethylation of homocysteine to methionine and with a deficiency of either vitamin (as well as vitamin B₆), plasma homocysteine concentrations become elevated.

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Suggested Readings

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9.8 VITAMIN B₁₂ (COBALAMIN)

Vitamin B₁₂ (also called cobalamin) was the last vitamin to be discovered. It was isolated in 1948 by Smith (from England) and by Rickes and others (from the United States). Its structure was discovered by Hodgkin; however, Minot and Murphy in 1926 showed that eating large amounts of liver could help correct pernicious anemia associated with deficiency of the vitamin. It took about two decades to identify the vitamin in liver.

Vitamin B₁₂ (sometimes referred to as a corrinoid because of the corrin nucleus) consists of a macrocyclic ring made of four reduced pyrrole rings linked together (called a tetrapyrrolic corrin ring core). In the ring's center is an atom of cobalt (Co) to which is attached, at almost right angles, the nucleotide 5,6-dimethylbenzimidazole.

Also attached to the cobalt atom in vitamin B₁₂ is one of the following:

Group Attached	Resulting Compound
—CN	Cyanocobalamin
—OH	Hydroxo or hydroxycobalamin
—deoxyadenosyl	deoxy-5'-or 5'-deoxy-adenosylcobalamin
—CH ₃	Methylcobalamin

The structure of cyanocobalamin is shown in Figure 9.39. Only deoxy-5'-adenosyl-cobalamin (subsequently called adenosylcobalamin) and methylcobalamin are active as coenzymes in humans.

Sources

Dietary sources of vitamin B₁₂ come primarily from animal products (Table 9.14). Foods of plant origin do not naturally contain the vitamin. Major sources of vitamin B₁₂ are meat and meat products as well as fish and seafood (such as clams and oysters). Milk and milk products such as cheese, cottage cheese, and yogurt contain less of the vitamin than meat/meat products; however, absorption

Table 9.14 Vitamin B₁₂ Content of Selected Foods*

Food (serving)	Vitamin B ₁₂ (μg)
Clams, cooked (3 oz)	84
Oysters, cooked (3 oz)	11
Trout, rainbow, farmed, cooked (3 oz)	3.5
Salmon, Atlantic, cooked (3 oz)	2.6
Tuna fish, canned in water, drained (3 oz)	2.5
Haddock, cooked (3 oz)	1.8
Beef, sirloin, cooked (3 oz)	1.4
Milk, low fat (1 c)	1.2
Cheese, Swiss (1 oz)	0.9
Cod, cooked (3 oz)	0.9
Yogurt, low fat (1 c)	0.9
Cottage cheese (1/2 c)	0.7
Pork, loin, cooked (3 oz)	0.6
Egg, cooked (1)	0.6
Chicken, breast, cooked (3 oz)	0.3

A more complete list of vitamin B₁₂-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminB12-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

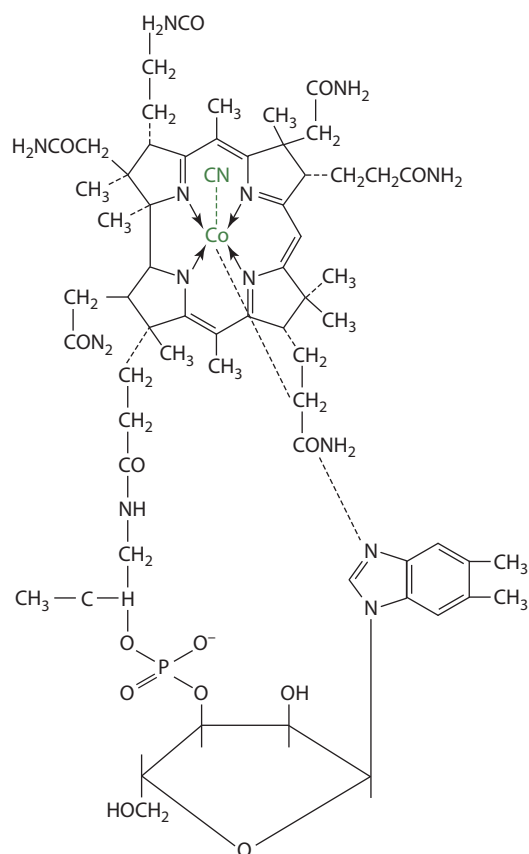


Figure 9.39 Structure of vitamin B₁₂ (as cyanocobalamin).

may be better due to the presence of binders in dairy products. Plant-derived foods, such as ready-to-eat cereals, nutritional yeast, and soy milk, are sometimes fortified with the vitamin. The Daily Value, appearing on food and supplement facts panels, for vitamin B₁₂ is 2.4 μg. Vitamin B₁₂ is fairly stable and resistant to light, heat, and oxidation.

Cyanocobalamin and hydroxocobalamin and, to a lesser extent, methylcobalamin represent the main forms of the vitamin used in multivitamin and single-ingredient supplements and to fortify foods. Orally dissolving sublingual products and nasal sprays containing vitamin B₁₂ are also available. The amount of vitamin B₁₂ in supplements ranges from a few μg up to several thousand. The cyano- and hydroxocobalamin forms are readily converted into the methylcobalamin and adenosylcobalamin forms, which are used within body cells.

Digestion and Absorption

Several steps are required for the digestion of vitamin B₁₂ to facilitate its absorption (Figure 9.40).

- 1 Ingested cobalamins (from foods) are released from the food proteins to which they are bound through the actions of pepsin and hydrochloric acid in the stomach. (Note: Vitamin B₁₂ ingested from supplements and fortified foods is not bound to proteins and thus does not require this initial hydrolysis.)

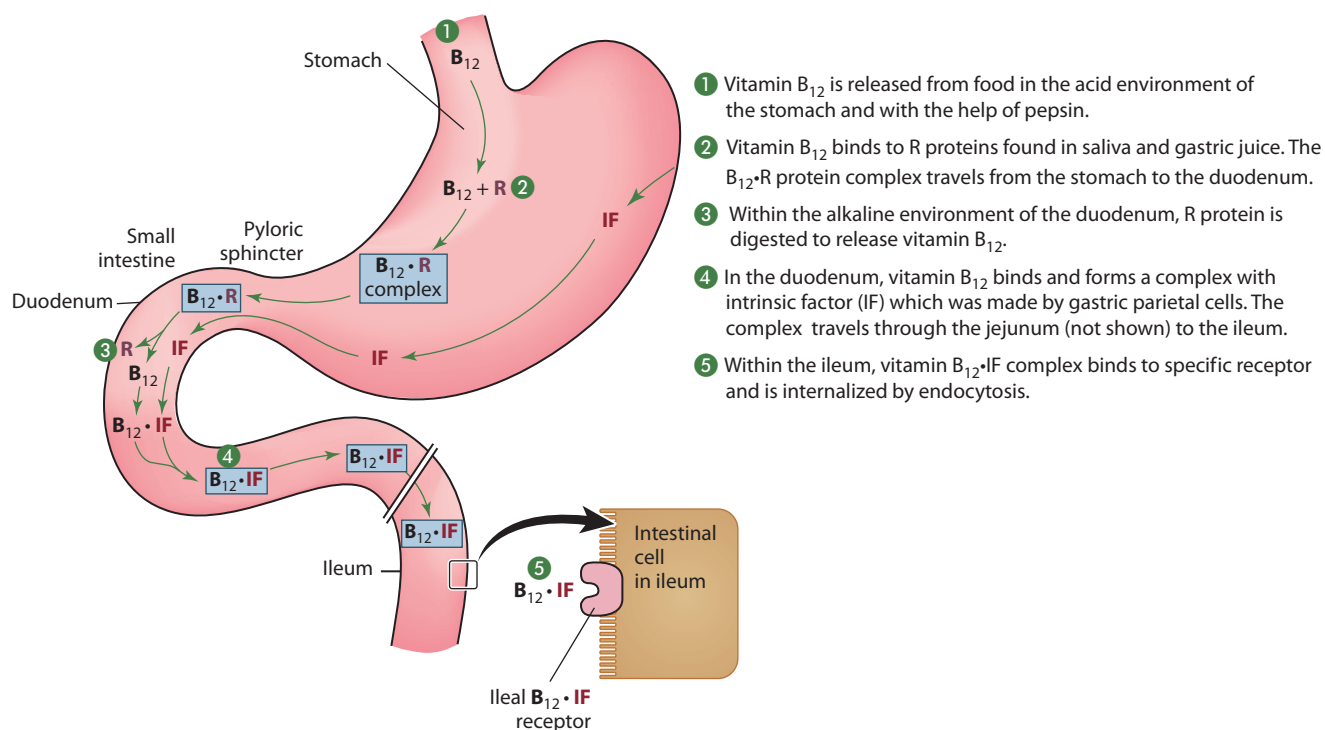


Figure 9.40 Vitamin B₁₂ absorption.

- 2 Within the stomach, the now “free” vitamin B₁₂ (all forms) binds to an R protein, also called haptocorrin or transcobalamin I. (Note: this R protein originates primarily from the saliva and is thought to protect vitamin B₁₂ from bacterial use and from potential hydrolysis within the stomach).
- 3 The R protein–vitamin B₁₂ complex next moves from the stomach into the duodenum.
- 4 Within the alkaline environment of the duodenum, the R protein is hydrolyzed by pancreatic proteases, and now free vitamin B₁₂ binds to intrinsic factor (IF), a glycoprotein that is synthesized by parietal cells in the stomach but which interacts with vitamin B₁₂ in the duodenum.
- 5 The vitamin B₁₂–IF complex moves from the duodenum to the distal ileum, where it interacts with a cubam receptor on the brush border membrane of ileal cells. The cubam receptor is formed from two proteins, cubilin and amnionless, and may also involve another protein, megalin.

Most ingested vitamin B₁₂ is absorbed as part of this vitamin B₁₂–IF receptor complex. The binding of the vitamin–IF complex to the cubam receptor triggers active endocytotic internalization. Release of the vitamin from IF and the receptor occurs within the enterocyte by the

actions of lysosomal cathepsin L. The released cubam receptor recycles back to the ileal cell membrane. The released IF is degraded within the lysosome. The released vitamin is next transported by a protein out of the lysosome and into the cytosol where it may undergo metabolism. This carrier-mediated intestinal absorption of vitamin B₁₂ is saturated with vitamin intakes of about 1.5–2.0 µg per meal.

In addition to carrier-mediated absorption, vitamin B₁₂ is also absorbed by passive diffusion; this route of absorption, however, typically occurs when pharmacological doses of vitamin B₁₂ are ingested and is limited to about 1–3% of the ingested dose. Thus, if a 1,000 µg vitamin B₁₂ dose was consumed, about 2 µg would be absorbed from IF-mediated absorption plus another 30 µg from passive diffusion (3% of 1,000 µg). Overall absorption of vitamin B₁₂ with usual intake is estimated at 50% (ranging from about 11 to 65%), with decreased absorption efficacy as intake increases [1,2]. For example, about 56% of the vitamin was absorbed with ingestion of 1 µg; the percentage dropped to 16% with the ingestion of 10 µg [2].

Vitamin B₁₂, once absorbed into the enterocyte, is transported within the cell by various intracellular proteins referred to as **chaperones**. Chaperones direct the intracellular movement of nutrients and in some cases also influence aspects of nutrient metabolism; mutations in genes coding for chaperones may interfere with both digestive

as well as absorptive processes (as discussed further under the section “Deficiency”) [2]. The extent to which the various forms of the vitamin undergo metabolism (such as the conversion of cyano- and hydroxocobalamin into the methylcobalamin and adenosylcobalamin) within intestinal cells is unclear. The vitamin moves across the ileum’s basolateral membrane by the carrier protein multidrug resistance protein (MRP) 1, although other proteins may also function in this capacity.

Transport, Tissue Uptake, and Storage

Following its absorption, vitamin B₁₂ appears in the blood in about 3–4 hours, with peak levels occurring after about 8–12 hours. Methylcobalamin comprises about 60–80% and adenosylcobalamin up to 20% of total blood cobalamin; these forms of the vitamin circulate in systemic blood bound to two proteins, transcobalamin (TC) II and haptocorrin (HC).

- TCII, which is made in many body cells including enterocytes, carries primarily newly absorbed cobalamin in a one-to-one ratio (referred to as holoTCII) in the blood. HoloTCII accounts for up to about 20% of cobalamin in the blood and has a half-life of less than 2 hours.
- Haptocorrin (synthesized by white blood cells) transports most (about 80%) of vitamin B₁₂ in the blood and has a half-life of about 10 days. It functions as a circulating storage form of the vitamin and delivers cobalamin from peripheral tissues back to the liver.

A fairly common genetic mutation in TCII results in the substitution of cytosine (C) for guanine (G) at base pair 776. This substitution, which leads to the insertion of arginine instead of proline, in turn diminishes TCII’s ability to bind and transport B₁₂ to tissues. An estimated 20% of the population is homozygous for the GG variant, which is associated with low serum vitamin B₁₂ and reduced vitamin B₁₂ availability to tissues for use.

The uptake of vitamin B₁₂ (such as adenosylcobalamin and methylcobalamin) into tissues is receptor dependent, requiring TCII receptors (also referred to as TCB1R). The TCII-cobalamin complex (i.e., holoTCII), upon binding to receptors, enters cells by endocytosis with subsequent fusion to lysosomes that provide for proteolytic degradation of TCII and release of the vitamin within the cell cytosol. As in the enterocytes, hepatocytes (and other cells) contain chaperones responsible for trafficking of the vitamin. While all of the vitamin’s chaperones (and chaperone functions) have not been identified, some chaperones include lipocalin-1-interacting membrane receptor domain-containing (LMBD) 1, adenosine triphosphate (ATP)–binding cassette subfamily D member (ABCD) 4, and cobalamin 1C (cb1C) [3]. Cb1C, along with other chaperones, facilitates the interconversions of the vitamin’s

various forms (cyanocobalamin, hydroxocobalamin, methylcobalamin, and adenosylcobalamin) in the cytosol and mitochondria. For example, these chaperones enable the vitamin to be present in the cytosol as methylcobalamin for use as a coenzyme for methionine synthase, and to be present in the mitochondria as adenosylcobalamin for use as a coenzyme for L-methylmalonyl-CoA mutase. (Note: The synthesis of adenosylcobalamin requires the vitamin’s reduction and a subsequent reaction with ATP.)

Vitamin B₁₂, unlike other water-soluble vitamins, can be stored (retained) in the body for relatively long periods of time. About 2–3 mg of the vitamin resides mainly (~50%) in the liver. The muscles store about 30% of the vitamin, with lesser amounts found in the pituitary gland, bone, kidneys, heart, brain, and spleen. Adenosylcobalamin is the main form of the vitamin in most of these storage sites (such as the liver, kidneys, and brain), but small amounts of hydroxocobalamin and methylcobalamin may be present. The amount of the vitamin available in stores in an adult is estimated to be sufficient to prevent a deficiency (if no further intake of vitamin occurs) for about 3–5 years.

Another important aspect to vitamin B₁₂ nutriture is its enterohepatic circulation, which accounts in part for the vitamin’s long biological half-life. Specifically, the vitamin is secreted from the liver into the bile, which is then released into the duodenum. Within the duodenum, the (secreted) vitamin binds to IF and is reabsorbed in the ileum as previously described for dietary vitamin B₁₂. Disruptions in ileal absorption thus diminish uptake of ingested vitamin B₁₂ as well as recirculating vitamin B₁₂.

Functions and Mechanisms of Action

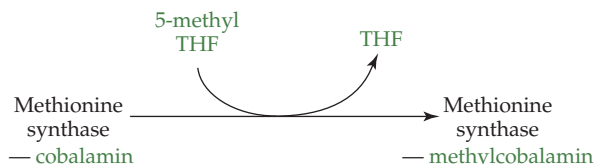
Two enzymatic reactions requiring vitamin B₁₂ have been recognized in humans. One of these reactions requires methylcobalamin as a coenzyme for methionine synthase, and the other relies on adenosylcobalamin as a coenzyme for L-methylmalonyl-CoA mutase. These reactions facilitate nutrient metabolism and energy production. Moreover, because of interactions with folate, the vitamin is involved in the synthesis of purines and pyrimidines for use in nucleic acids and in the provision of methyl groups with the generation of SAM.

Methionine Synthase

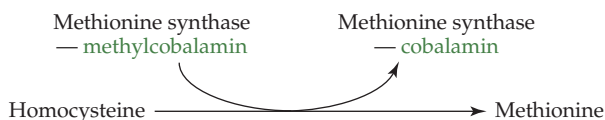
Two reactions are responsible for converting homocysteine to methionine. One uses betaine to supply the needed methyl group and requires the enzyme betaine-homocysteine methyltransferase. This enzyme, however, does not require vitamin B₁₂. The other reaction requires the methylcobalamin form of the vitamin as a coenzyme for methionine synthase (also called homocysteine methyltransferase) (see Figure 9.37). This reaction, which occurs in the cytosol, is shown hereafter as a two-step process to

facilitate an understanding of the sequential nature of the reaction.

- First, cobalamin bound as a coenzyme to methionine synthase picks up the methyl group from 5-methyl tetrahydrofolate (THF), forming methylcobalamin (while still bound to methionine synthase) and THF.



- Next, methionine synthase releases the methyl group from its bound methylcobalamin for transfer to homocysteine, producing methionine and cobalamin.



In this transfer, however, oxidation of the vitamin can occur and result in the loss of methionine synthase activity. Reactivation of enzyme is accomplished by an NADPH-dependent flavoenzyme methionine synthase reductase. Polymorphisms in the gene for this reductase (causing reduced methionine synthase activity) have been linked with an increased risk of neural tube defects in individuals with suboptimal vitamin B₁₂ status as well as in individuals with 5,10-methylene tetrahydrofolate reductase (MTHFR) mutations [4].

L-Methylmalonyl-CoA Mutase

The second of the vitamin B₁₂-dependent reactions is catalyzed by L-methylmalonyl-CoA mutase (a dimer), requiring two adenosylcobalamin molecules (one per subunit). The enzyme converts L-methylmalonyl-CoA to succinyl-CoA in the mitochondria (Figure 9.41). Succinyl-CoA provides for energy production as a TCA cycle intermediate and is used in the first step in the production of heme (heme in turn is needed for hemoglobin synthesis and as a cofactor for the activity of some enzymes).

L-methylmalonyl-CoA (the substrate for the enzyme) is made from D-methylmalonyl-CoA, which in turn is generated from propionyl-CoA. Propionyl-CoA arises from the oxidation of four amino acids (methionine, valine, isoleucine, and threonine) and from odd-chain fatty acids (found in some foods). The conversion of propionyl-CoA to D-methylmalonyl-CoA is an ATP-, Mg²⁺, and biotin-dependent reaction (previously discussed in the section on biotin; see Figure 9.29).

The reaction catalyzed by methylmalonyl-CoA mutase is used as a means of assessing vitamin B₁₂ status, as discussed further under the section “Assessment of Nutriture.”

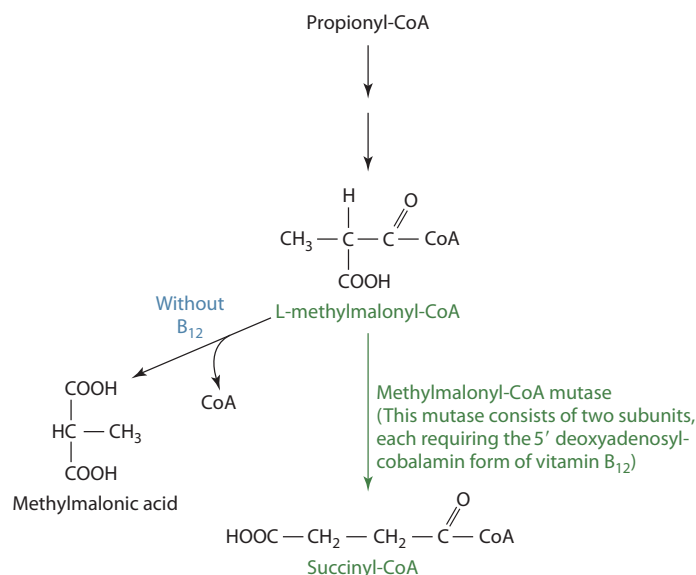


Figure 9.41 Role of vitamin B₁₂ in oxidation of L-methylmalonyl-CoA.

Moreover, some of the neurological manifestations of vitamin B₁₂ deficiency are thought to arise from alterations in metabolism secondary to insufficient methylmalonyl-CoA mutase activity, as discussed under the section “Deficiency.”

Selected Pharmacological Uses/Other Roles

Supplementation with oral vitamin B₁₂, usually in pharmaceutical amounts of 1–2 mg daily, can sometimes enhance residual methylmalonyl-CoA mutase activity in those with the genetic disorder methylmalonic acidemia. Similar pharmacological doses of the vitamin are sometimes used to overcome deficits in carrier-mediated absorption of the vitamin. Large doses of the vitamin, however, are also purported to cure a variety of other problems, including fatigue and weight gain. Unfortunately, scientific studies supporting the use of the vitamin to boost energy and enhance weight loss (among other claims) are generally lacking.

Metabolism and Excretion

Vitamin B₁₂ undergoes little to no degradation (metabolism) prior to excretion. About 0.1% (2 µg) of vitamin B₁₂ is excreted through the bile. However, most (about 75%) is reabsorbed in the ileum after binding to intrinsic factor in the proximal small intestine. About 0.25 µg of the vitamin is excreted daily in the urine. Trace dermal losses of vitamin B₁₂ may also occur.

Recommended Dietary Allowance

Recommendations for vitamin B₁₂ intake are based on estimates of the vitamin's intake and turnover and on amounts of the vitamin needed for the maintenance of

normal serum vitamin indices and hematological status. The RDA for adults for vitamin B₁₂ is 2.4 µg per day [1]. Increases of 0.2 µg and 0.4 µg per day above the RDA are suggested for women during pregnancy and lactation, respectively [1]. The requirement for the vitamin for adults is 2.0 µg/day [1]. People age 51 years and older are counseled to consume foods fortified with the vitamin or to consume vitamin B₁₂ supplements (usually between 25 and 100 µg per day) because of age-associated changes to the gastrointestinal tract that limit the absorption of food-bound forms of the vitamin [1]. The inside front cover of the book provides additional recommendations for vitamin B₁₂ intake for other age groups.

Deficiency: Megaloblastic Macrocytic Anemia and Neuropathy

Vitamin B₁₂ deficiency occurs typically in stages with reductions in vitamin concentrations and functions. Initially, serum vitamin B₁₂ concentrations diminish, although they may remain normal until vitamin stores become depleted. Next, cellular vitamin concentrations diminish, affecting the activities of both vitamin B₁₂-dependent enzymes. DNA synthesis decreases and plasma homocysteine and methylmalonic acid concentrations increase. Finally, morphological and functional changes occur in blood cells (i.e., hematological effects). Neurological impairments may also be manifested. There is also evidence that deficiency of vitamin B₁₂ may be involved in the etiology of neural tube defects in developing embryos [4].

Hematological effects of vitamin B₁₂ deficiency include hypersegmentation of neutrophils, low blood leukocyte and thrombocyte counts or pancytopenia, and megaloblastic macrocytic anemia. Fatigue and skin pallor may also be present. The anemia occurs with a deficiency of vitamin B₁₂ because of the inability of cells to use 5-methyl THF for the formation of other folate derivatives needed for DNA synthesis, including 10-formyl THF (which is needed for purine synthesis) and 5,10-methylene THF (which is needed for thymidylate synthesis in pyrimidine metabolism) (see the section “Interactions with Other Nutrients” under “Folate”). Consequently, with a vitamin B₁₂ deficiency (as with a folate deficiency), megaloblastic macrocytic anemia occurs with deranged DNA synthesis and defective cell differentiation and maturation especially evident in blood cells, which exhibit rapid turnover. Related cardiorespiratory effects such as shortness of breath and palpitations may also be present.

Neurological problems, which may be irreparable, occur in about 75–90% of individuals with vitamin B₁₂ deficiency and are not responsive to folate therapy. About 20% of individuals display neurological signs but not anemia. Neurologic problems are manifested as irritability, memory loss, disorientation, hallucinations, psychosis,

and dementia as well as clumsiness, poor coordination, numbness, paraesthesia, and/or pain in extremities (mostly in the lower limbs), abnormal gait, increased loss of coordination, loss of a sense of relative position (proprioception), loss of vibration sense or touch in the ankles and toes, swelling of myelinated fibers, and demyelination of neuron sheaths. While motor fibers can be demyelinated, the demyelination impacts generally peripheral and central nerves, with effects on selected areas of the spinal cord containing sensory fibers for vibration and position sense. Myelin (myelin sheath) is found wrapped around nerve axons and serves like an electrical insulator to assist with nerve conduction.

The exact mechanisms of the neuropathy and demyelination have not been clearly elucidated. However, some studies have linked reductions in methylmalonyl mutase activity with aspects of the neuropathy. More specifically, reductions in vitamin B₁₂-dependent methylmalonyl mutase activity result in the accumulation of methylmalonyl-CoA and methylmalonic acid, formed from hydrolysis of methylmalonyl-CoA, in body fluids. High plasma methylmalonic acid concentrations are thought to destabilize myelin or promote the formation of abnormal myelin. Deficient vitamin B₁₂ may also reduce the provision of methyl groups needed for the production of phosphatidylcholine present in Schwann cell membranes and that make up the myelin sheath.

At Risk for Deficiency

The likelihood of a vitamin B₁₂ deficiency increases with aging secondary to changes in gastrointestinal tract functions, although multiple factors increase the risk for deficiency, including the following.

- *Inadequate intake* is most likely to occur in a strict vegetarian (vegan), especially in an infant or young child with minimal stores of the vitamin.
- *An altered (too high/alkaline) gastric pH* may occur due to diminished (*hydrochlorhydria*) or absent (**achlorhydria**) hydrochloric acid production by parietal cells. Parietal cell functions are diminished with conditions such as *atrophic gastritis* (characterized by a loss and inflammation of gastric cells) and *pernicious anemia* (an autoimmune condition that affects 1–2% of the population in which the body produces antibodies that attack the gastric parietal and mucosal cells). *Medications*—H₂ blockers and proton pump inhibitors—for the treatment of ulcers and gastroesophageal reflux disease also diminish hydrochloric acid production to increase gastric pH. Such changes in pH impair vitamin B₁₂ release from food protein.
- *Parietal cell destruction causing insufficient intrinsic factor* may result from *atrophic gastritis*, *pernicious anemia*, and other conditions. Without sufficient intrinsic factor produced by the parietal cells and present in the

intestine, carrier-mediated vitamin B₁₂ absorption is impaired.

- *Altered (too low/acidic) duodenal pH* may occur with *impaired pancreatic exocrine function* (which causes insufficient release of bicarbonate) as well as with *Zollinger-Ellison syndrome*. Zollinger-Ellison syndrome occurs with the presence of a gastrin-producing tumor and results in excessive hydrochloric acid production secondary to the high gastrin (remember gastrin stimulates the parietal cells to release hydrochloric acid). The overproduction of acid with Zollinger-Ellison syndrome and the underproduction of bicarbonate with pancreatic insufficiency negatively impact the duodenum, lowering its luminal pH. The lower than normal pH in the duodenum impairs the release of vitamin B₁₂ from R protein. If the vitamin is not released from R protein in the duodenum, it cannot bind to intrinsic factor.
- *Defects in the cubam receptor*, caused by mutations in the genes for cubilin or amnionless, result in vitamin B₁₂ malabsorption.
- *Impaired intestinal integrity or function*, especially if affecting the ileum, occurs with celiac disease and Crohn's disease (among other conditions) and may decrease the absorptive surface and prevent the vitamin from binding to receptors in the ileum. Intact ileal cells are needed for receptor-mediated absorption of the vitamin-intrinsic factor complex.
- *Malabsorption syndromes* decrease not only the absorption of ingested cobalamin but also interfere with its enterohepatic circulation, thereby increasing the amount of vitamin B₁₂ required to meet body needs.
- *Resection of portions of the stomach and/or small intestine* reduces the secretions and the sites needed for the digestion and absorption of the vitamin.
- *Competition*. People with *parasitic infections* such as tapeworms may develop a vitamin B₁₂ deficiency because the parasite uses the vitamin and consequently limits the vitamin's availability to the infected person. Similarly, the prolonged use of H₂ blockers and proton pump inhibitors (used to treat ulcers and gastroesophageal reflux disease) is associated with diminished absorption of vitamin B₁₂ because of *bacterial overgrowth*. Bacterial overgrowth in the more alkaline intestinal environment occurs when the medication diminishes acid production. Furthermore, the bacteria use vitamin B₁₂ for their own growth, which limits the vitamin's availability to the individual. Treatment with antibiotics is usually needed to retard bacterial overgrowth and subsequently improve vitamin absorption and status.
- *Use of nitrous oxide* (an anesthetic agent), primarily in people who have poor vitamin B₁₂ status, may result in

deterioration of nervous system function, especially demyelination problems. Mechanisms by which nitrous oxide alters vitamin B₁₂ metabolism and induces deficiency are under investigation but may involve inactivation of methionine synthase.

- *Other*. The use of the hypoglycemic medication Metformin in the treatment of type 2 diabetes has been associated with low vitamin B₁₂ status. The exact mechanism is not clear, but the drug is thought to reduce the vitamin's absorption.

Vitamin B₁₂ deficiency due to inadequate intake of the vitamin (without neurologic symptoms) is typically treated with up to 1 mg of vitamin B₁₂ daily for the first week or so, followed by a slightly lower dose of the vitamin for about 1 or 2 months. Treating pernicious anemia or deficiency secondary to malabsorption usually requires monthly intramuscular injections of vitamin B₁₂ in amounts of 500–1,000 µg or oral ingestion of pharmacological amounts (1,000–2,000 µg) of the vitamin [1]. Vitamin B₁₂ nasal sprays are also available. Nascobal[®], for example, provides the vitamin as cyanocobalamin (500 µg/spray) in a nasal spray that is beneficial to people with malabsorptive disorders. A hematological response to supplementation may take up to 2 months; however, improvements in some parameters (such as the reticulocyte count) may be evident within about 10 days of beginning treatment. Serum methylmalonic acid concentrations may also start to diminish within the first week of supplementation.

Toxicity

Although no clear toxicity from massive doses of vitamin B₁₂ has been reported, neither has any benefit been noted from an excessive intake of the vitamin by people with adequate vitamin status [1]. No Tolerable Upper Intake Level for vitamin B₁₂ has been established [1].

Assessment of Nutriture

Vitamin B₁₂ status is assessed using several indices. Moreover, because no one “best” approach has been identified, assessment should employ the use of at least two approaches. One common approach involves the measurement of serum concentrations of the vitamin. Serum total vitamin B₁₂ concentrations include measurements of cobalamin bound to both transcobalamin II and haptocorrin. Concentrations generally less than about 200 pg/mL (based on a radioassay method) are considered deficient, while those between 200 and 300 pg/mL suggest borderline (subclinical) deficiency [5]. Yet, what is considered as “normal” serum vitamin B₁₂ concentrations vary, and usefulness of serum total vitamin concentrations has been criticized given it is only holotranscobalamin II that readily provides the vitamin to tissues.

The measurement of plasma holotranscobalamin II concentrations provides an indication of vitamin B₁₂'s availability and, under normal conditions, respond to changes in dietary intake. Holotranscobalamin II concentrations less than 25 pmol/L are indicative of deficiency, although deficiency should also be considered with concentrations between 25 and 70 pmol/L [5].

Measurement of methylmalonic acid and homocysteine are also used as functional markers to assess vitamin B₁₂ status. Normally, no or only trace amounts of methylmalonic acid are excreted in the urine; however, with vitamin B₁₂ deficiency, methylmalonic acid concentrations increase in the serum and urine. The response of serum methylmalonic acid to vitamin B₁₂ depletion and repletion is sometimes used in the diagnosis of vitamin B₁₂ deficiency and in monitoring the response to treatment. Alternately, absolute serum methylmalonic acid concentrations are assessed, with those greater than 280 nmol/L considered indicative of possible vitamin B₁₂ deficiency. Serum homocysteine concentrations also rise with deficiencies of vitamin B₁₂, but also folate and vitamin B₆. In those with adequate folate and vitamin B₆ status, serum homocysteine concentrations exceeding 20 μmol/L may suggest vitamin B₁₂ deficiency [5]. As with other indicators of vitamin status, cutoff values for homocysteine and methylmalonic acid for diagnostic purposes vary considerably.

A breath test to assess vitamin B₁₂ status has also been developed; carbon dioxide concentrations in the breath are measured following ingestion of labeled propionate. Deficiency of the vitamin is indicated by subnormal production of labeled carbon dioxide.

Other older and less frequently used tests to assess vitamin B₁₂ nutriture include the deoxyuridine suppression test, discussed previously in the "Assessment of Nutriture" subsection of the "Folate" section, and the Schilling test. The Schilling test involves orally administering radioactive vitamin B₁₂ and measuring urinary excretion of the

vitamin. Below-normal urinary excretion of the vitamin suggests impaired absorption. In lieu of the Schilling test, the presence of antibodies to intrinsic factor and/or parietal cells may be directly measured in the blood as an indicator of an autoimmune response and pernicious anemia.

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Suggested Reading

Kennedy DO. B vitamins and the brain: mechanisms, dose and efficacy – a review. *Nutrients*. 2016; 8:68–97.

9.9 VITAMIN B₆

Vitamin B₆ was isolated in 1934 and its structure confirmed in 1939. Some of the initial research was aimed at correcting dermatitis in rats. Kuhn and Szent-Györgyi are credited with isolating the vitamin (which was called pyridoxine due to its structural homology to pyridine) in 1938 that cured the dermatitis. The pyridoxal and pyridoxamine forms of the vitamin were identified in the mid-1940s.

Vitamin B₆ exists as six vitamers, shown in Figure 9.42. Pyridoxine (PN) represents the alcohol form with a hydroxymethyl group at C4 (—CH₂OH), pyridoxal (PL) the aldehyde form with an aldehyde group at C4 (—CHO),

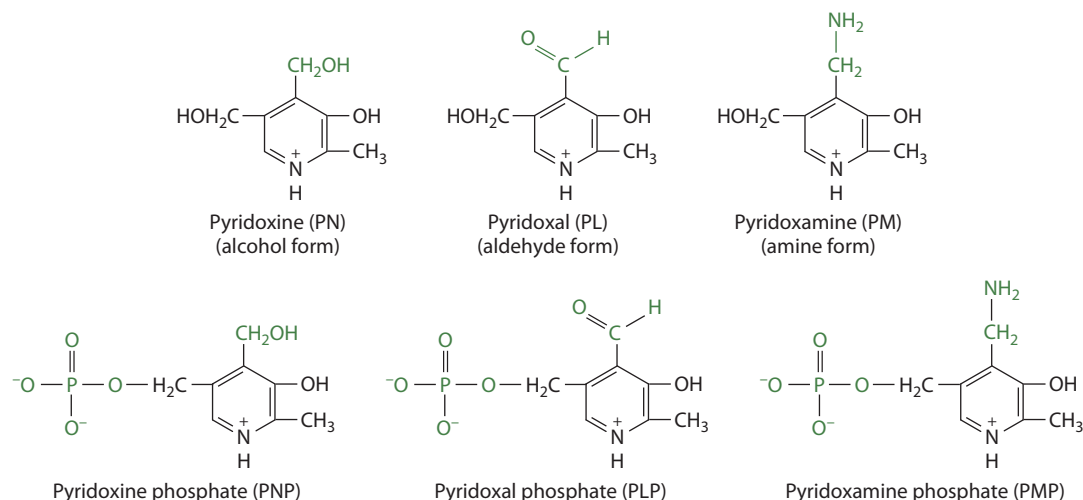


Figure 9.42 Vitamin B₆ structures.

and pyridoxamine (PM) the amine form with an amino-methyl group at C4 ($-\text{CH}_2\text{NH}_2$). Each has a 5'-phosphate derivative (i.e., phosphorylated form): pyridoxine phosphate (PNP), pyridoxal phosphate (PLP), and pyridoxamine phosphate (PMP).

Sources

All B₆ vitamers are found in food. Pyridoxine, the most stable of the compounds, and its phosphorylated form are found almost exclusively in plant foods. In some plants, the pyridoxine may be conjugated and present as a glucoside. The phosphorylated forms of pyridoxal and pyridoxamine (and lesser amounts of the unphosphorylated forms) are found primarily in animal products.

Animal products, especially beef, fish, pork, and chicken, provide about 0.3–0.9 mg of vitamin B₆ per serving. Of the plant foods, whole-grain products, some vegetables (such as potatoes), some fruits (e.g., bananas), and nuts as well as fortified cereals contribute to dietary vitamin B₆. Selected food sources of the vitamin are shown in Table 9.15.

Most multivitamins provide vitamin B₆ in amounts of about 2 mg. Dosages in single-ingredient vitamin B₆

supplements vary from about 2 to 300 mg, with some sustained-release products containing up to 500 mg. The form of vitamin B₆ in supplements and in fortification of foods is generally pyridoxine hydrochloride; the form is shown on the nutrition facts label along with the Daily Value, which for vitamin B₆ is 1.7 mg. Some single-ingredient supplements, however, also provide the vitamin as PLP alone or in combination with pyridoxine hydrochloride. The vitamin is available in liquid form as well as tablets and capsules for oral administration.

The bioavailability of vitamin B₆ from foods is influenced by the food matrix and by the extent and type of processing to which the foods are subjected. The vitamin is fairly stable with cooking; however, much of the vitamin originally present in foods can be lost if exposed to prolonged high heat (especially with sterilizing and canning). Vitamin losses from plant foods are generally less than from animal products. Loss of the vitamin may also occur with food storage as well as with milling and refining of grains.

Digestion and Absorption

For vitamin B₆ to be absorbed, the phosphorylated vitamers must be dephosphorylated. Alkaline phosphatase, a zinc-dependent enzyme found on the intestinal brush border, and other intestinal phosphatases hydrolyze the phosphate from the phosphorylated vitamers to yield free pyridoxine (PN), pyridoxal (PL), or pyridoxamine (PM).

At physiological intakes, free pyridoxine, pyridoxal, and pyridoxamine are thought to be absorbed primarily in the jejunum by passive diffusion. Carriers in the intestinal membranes have not yet been identified for vitamin B₆ transport. Absorption of some pyridoxine glucosides may also occur by passive diffusion, although glucosidase within the intestinal cell typically hydrolyzes the glucosides to free the pyridoxine. Little additional metabolism of the vitamin occurs within intestinal cells, although some pyridoxine may be phosphorylated. Most pyridoxine, pyridoxal, and pyridoxamine are released directly into portal blood. Overall absorption of vitamin B₆ is about 75%, with a range of about 61–92% [1].

Transport, Tissue Uptake, and Storage

PLP and PL are the main (75–90% of the total) forms of the vitamin found in systemic blood. Most PLP in the plasma is bound to albumin but the absolute concentration found in the plasma is very small. The normal reference range for serum/plasma PLP concentrations is about 5 (or 7.5) to 50 µg/L and for vitamin B₆ is about 8.6 to 27.2 µg/L.

The liver is the main organ that takes up (by passive diffusion) and metabolizes newly absorbed vitamin B₆. Figure 9.43 shows these reactions and other interconversions of the B₆ vitamers. Unphosphorylated forms of the vitamin are phosphorylated by a kinase using ATP

Table 9.15 Vitamin B₆ Content of Selected Foods*

Food (serving)	Vitamin B ₆ (mg)
Tuna, yellowfin, cooked (3 oz)	0.9
Chicken, breast, cooked (3 oz)	0.6
Chickpeas, canned (1/2 c)	0.6
Potato, baked (1 medium)	0.6
Salmon, sockeye, cooked (3 oz)	0.5
Banana (1)	0.4
Pork, loin, cooked (3 oz)	0.4
Beef, ground, 85% lean, cooked (3 oz)	0.3
Chicken, dark meat, cooked (3 oz)	0.3
Beef, sirloin, cooked (3 oz)	0.3
Cottage cheese, 1% low fat (1 c)	0.2
Tofu, firm (1/2 c)	0.1
Raisins, seedless (1/4 c)	0.1
Watermelon, cubed (1 c)	0.1
Spinach, cooked (1/2 c)	0.1
Broccoli, cooked (1/2 c)	0.1
Zucchini, cooked (1/2 c)	0.1
Carrots, raw, sliced (1 c)	0.1
Nuts, mixed, dry roasted (1 oz)	0.1
Seeds, sunflower (1 oz)	0.1
Bread, whole grain (1 slice)	0.1

A more complete list of vitamin B₆-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminB6-Food.pdf>.

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

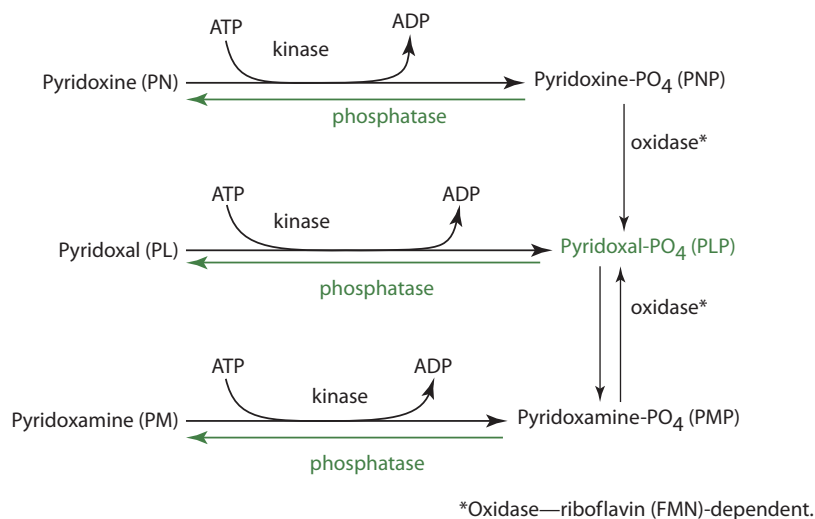


Figure 9.43 Vitamin B₆ metabolism in the liver.

within the cytosol of hepatocytes and other organs. PNP and PMP are then generally converted by the action of an FMN-dependent oxidase to the main vitamers and coenzyme form PLP. The oxidase that catalyzes this reaction is dependent upon adequate riboflavin (a coenzyme) and is found mainly in the liver and intestine and to lesser extents in the muscle, kidneys, brain, and red blood cells. These reactions that provide for the interconversions among the forms of the vitamin are sometimes called the “salvage pathway.” Mutations in genes for these enzymes result in metabolic imbalances and, in some cases, a vitamin B₆ deficiency.

PLP and PL (with possibly smaller amounts of the other vitamers) are released from the liver into the blood for transport to extrahepatic tissues. PLP, however, is hydrolyzed by a phosphatase to PL for cellular uptake from the blood. Red blood cells, for example, take up PL from the blood. An intracellular kinase subsequently converts it to PLP; the PLP then binds to hemoglobin within the cell to prevent its degradation.

The body contains about 40–185 mg of vitamin B₆. The liver stores only about 5–10% of the vitamin. Muscles represent the major (75–80%) storage site where the vitamin is found, primarily as PLP, bound to glycogen phosphorylase. Phosphorylation of the vitamin prevents its diffusion out of the cell, and the binding of the vitamin to protein prevents hydrolysis by phosphatases. Other tissues with substantial amounts of the vitamin are the brain, kidneys, and spleen; in these tissues the vitamin is also found in its phosphorylated form and is typically bound to enzymes and/or PLP-binding proteins (PLPBP) in the cytosol and mitochondria. Entry of PLP into the brain occurs similar to uptake into other tissues, that is, requiring initial release from albumin and dephosphorylation (usually by alkaline phosphatase). PL diffuses across the blood–brain barrier for uptake into the choroid plexus (a network of nerves and vessels that produce cerebrospinal fluid) and brain cells. The vitamin is found within the cerebrospinal fluid

as PLP; within the brain cells PLP is produced from PL by the action of pyridoxal kinase.

Functions and Mechanisms of Action

The PLP form of vitamin B₆ functions as a coenzyme for over 100 enzymes. The majority of the reactions are involved in nutrient (amino acid) metabolism, including the production of several neurotransmitters. In addition, PLP as a coenzyme is involved in one-carbon metabolism with folate (and thus affects nucleic acid production) as well as the synthesis of heme, sphingolipids, carnitine (and thus affecting fatty acid oxidation), and glucose (from glycogen catabolism). Vitamin B₆'s noncoenzyme role affects gene expression.

Coenzyme Roles for Nutrient Metabolism and Energy Production

As a coenzyme in reactions, PLP attaches by a Schiff base linkage to the epsilon amino group of a lysine present at the enzyme's active site. The PLP is then typically transferred to the amino group of the amino acid substrate, creating a new Schiff base link. The α , β , and γ carbons of the amino acid become more active as a result of this Schiff base link (Figure 9.44).

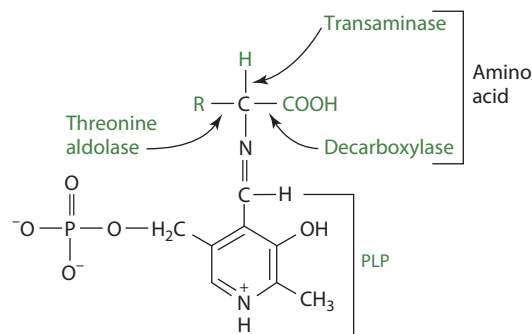


Figure 9.44 The covalent bonds of an acid that can be made labile by its binding to PLP-containing enzymes.

Some of the reactions involving amino acids that are catalyzed by PLP include transamination (which can also be catalyzed by PMP), dehydration (elimination)/deamination, decarboxylation, transulfhydration, transelenation, cleavage, racemization, and synthesis. In addition to its participation in reactions involving amino acid substrates, vitamin B₆ functions in the initial step of glycogen catabolism, a reaction important in the liver for glucose production. Each of these types of reactions is discussed briefly along with some examples related to nutrient metabolism.

Transamination Transamination reactions involve the transfer of an amino group ($^-\text{NH}_2$) from one amino acid to an α -ketoacid and, like the deamination reactions discussed next, are important for the *synthesis of nonessential amino acids* and for the use of amino acid carbon skeletons for *energy and glucose production* (e.g., gluconeogenesis).

The most common aminotransferases for which PLP or PMP serve as coenzymes are aspartic amino transferase (AST; also called glutamate oxaloacetate transaminase [GOT]) and alanine aminotransferase (ALT; also called glutamate pyruvate transaminase [GPT]) (see Figure 6.5 for the reactions catalyzed by these transaminases). Figures 9.45a and 9.45b show the two phases of transamination

and demonstrate how the coenzyme forms a Schiff base. In the first phase, the corresponding α -keto acid of the amino acid is produced along with PMP. In the second phase, the transamination cycle is completed as a new α -keto acid substrate receives the amino group from the PMP. The corresponding amino acid is generated, along with regeneration of the PLP.

Dehydration, Elimination, or Deamination PLP also participates in reactions in which an amino group is removed from a compound such as an amino acid and released as ammonia or ammonium ion. Such reactions may be called dehydration, elimination, or deamination reactions. Threonine dehydratase is an example of a PLP-dependent enzyme that is involved in such a reaction, specifically removing water and the amino group from the amino acid threonine; the reaction is shown in Figure 6.6. These types of reactions produce substrates that, for example, may be further catabolized for energy production.

Decarboxylation Decarboxylation reactions involve the removal of the carboxy (COO^-) group from an amino acid or other compound. Many of these reactions are involved in the *production of neurotransmitters* for nervous system function and other body processes. Some examples

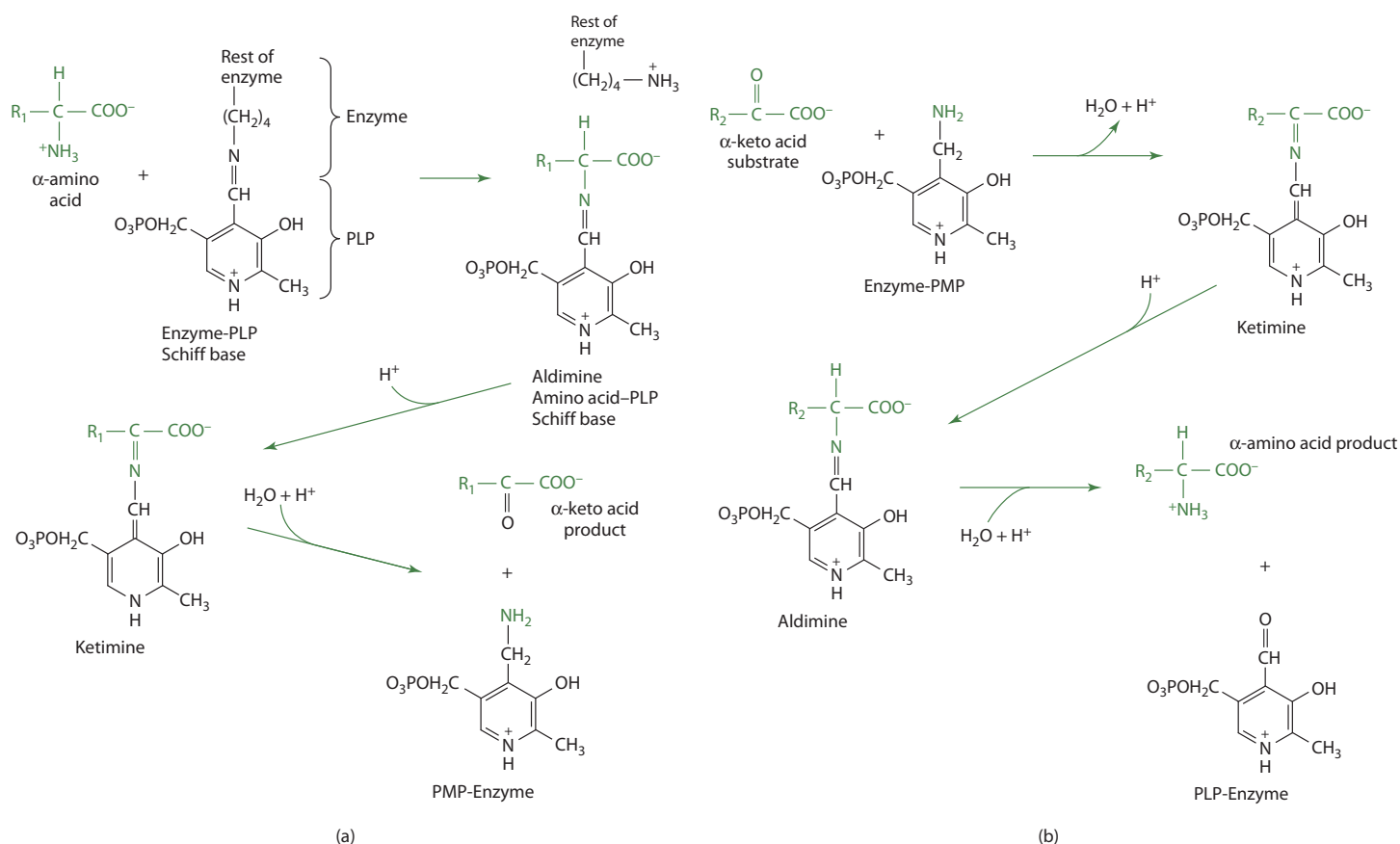


Figure 9.45 (a) The role of vitamin B₆ in transamination, phase 1. (b) The role of vitamin B₆ in transamination, phase 2.

of some common decarboxylation reactions include the synthesis of:

- **γ -Aminobutyric acid (GABA)** from the amino acid glutamate (see Figure 6.38)
- **Serotonin** from 5-hydroxytryptophan (see Figure 6.39)
- **Histamine** from the amino acid histidine (see Figure 6.14)
- **Dopamine** following decarboxylation of dihydroxyphenylalanine, which is generated from the amino acid tyrosine (see Figure 6.10)
- **Taurine**, a neuromodulatory compound, during cysteine metabolism in a vitamin B₆-dependent reaction (see Figure 6.12).

Disruption in the production of neurotransmitters from a vitamin deficiency or toxicity are thought to contribute to many of the manifestations associated with these conditions (e.g., deficiency, toxicity).

Transsulfhydration PLP is required for the activity of two enzymes, cystathionine β -synthase and cystathionine lyase, catalyzing reactions in the transsulfhydration pathway (which is part of the methionine metabolism). The two PLP-dependent enzymatic reactions facilitate homocysteine catabolism and regulation and provide for the synthesis of *cysteine*, a *nonessential* amino acid, which is required for the production of proteins, the antioxidant glutathione, and another amino acid, taurine. Moreover, hydrogen sulfide, which functions as a signaling molecule in the body, is generated from side reactions associated with the pathway (see Figure 6.12 for the transsulfhydration reactions and methionine metabolism).

Transselenation Similar to transsulfhydration, selenomethionine may be converted through the transselenation pathway to selenocysteine (see Figure 13.17). γ -Lyase, which is PLP dependent, directly cleaves the selenium from selenomethionine to generate selenide. Selenocysteine β -lyase, also PLP dependent, generates selenide from selenocysteine. Selenide is converted into selenophosphate, an important intermediate in the synthesis of the body's *selenium-dependent enzymes*, which serve in various *antioxidant roles*.

Cleavage An example of a cleavage reaction requiring PLP is the removal of the hydroxymethyl group from serine. In this reaction, PLP is the coenzyme for a transferase that transfers the hydroxymethyl group of serine to tetrahydrofolate (THF) to form glycine and another THF coenzyme (see Figure 9.34). This reaction is also important in the *production of nucleic acids*, as discussed further under the section “Other Synthetic Reactions.”

Racemization PLP is required by racemases that catalyze the interconversion of D- and L-amino acids. Although

such reactions are more prevalent in bacterial metabolism, some occur in humans.

Other Synthetic Reactions Vitamin B₆ is also necessary as a coenzyme in the first step in the *synthesis of heme* (see Figure 13.7). Heme is needed not only for hemoglobin formation but also is a component of many enzymes. PLP is required for aminolevulinic acid synthetase, which catalyzes the condensation, followed by decarboxylation, of glycine with succinyl-CoA to form aminolevulinic acid (ALA). ALA is used next to synthesize porphobilinogen (PBG), the parent pyrrole compound in porphyrin synthesis. Through a series of reactions, PBG is converted into protoporphyrin IX, which, with the addition of Fe²⁺ by ferrochelatase, forms heme. Defects in heme synthesis from a vitamin B₆ deficiency can result in microcytic anemia (a problem usually only seen in infants, but accounts for vitamin B₆ sometimes being listed along with vitamins B₁₂ and folate as being needed for hematopoiesis).

PLP functions as a cofactor for another condensation reaction necessary for *sphingolipid synthesis*. Specifically, the amino acid serine condenses with palmitoyl-CoA in a reaction catalyzed by a PLP-dependent transferase to form 3-dehydrosphinganine, a compound that serves as a precursor for sphingolipids. Sphingolipids have many roles in the body (see Chapter 5), including the production of myelin for nerve transmission.

In fatty acid metabolism, the PLP-dependent enzyme δ -6-desaturase catalyzes the synthesis of selected polyunsaturated fatty acids through desaturation of linoleic and γ -linolenic acids. Also related to lipid use in the body is vitamin B₆'s coenzyme role in the synthesis of carnitine, a nitrogen-containing compound required for *fatty acid oxidation* and thus *energy production*.

Niacin synthesis from tryptophan also requires an important PLP-dependent reaction. Specifically, kynureninase required for the conversion of 3-hydroxykynurenine to 3-hydroxyanthranilic acid requires vitamin B₆ (PLP) as a coenzyme (see Figure 9.19).

Nucleic acid production involves the vitamin B₆ (PLP)-dependent enzyme serine hydroxymethyltransferase. This enzyme transfers a one-carbon unit from serine to THF to generate 5,10-methylene THF and glycine. The 5,10-methylene THF in turn is used to synthesize thymidylate (dTMP) (pyrimidine), which is needed for DNA synthesis and to synthesize other forms of THF needed for the remethylation of homocysteine to methionine. Furthermore, in the mitochondria, the 5,10-methylene is used to produce 10-formyl THF, which upon hydrolysis generates formate for purine synthesis.

Glycogen Degradation Glycogen is catabolized by glycogen phosphorylase to form glucose-1-PO₄ (see Figure 3.18); vitamin B₆ is required for glycogen phosphorylase activity. More specifically, the vitamin is believed to

be needed for the transfer of the phosphate and/or to stabilize the compound and permit covalent bonding of the phosphate to form glucose-1-PO₄. Most vitamin B₆ found in muscle is present as PLP, which in turn is bound to glycogen phosphorylase. It is this role of the vitamin that is thought to account for the use of more than 50% of the body's vitamin B₆.

Noncoenzyme Role for Gene Expression

Although the coenzyme roles of vitamin B₆ have been more thoroughly investigated, PLP also affects gene expression. The vitamin modulates steroid hormone binding as well as transcription factor binding to regulatory regions on DNA. The vitamin affects the synthesis of albumin by interacting with DNA ligand-binding sites; such interactions suppress gene transcription and reduce albumin mRNA production [2]. Similar interactions from PLP binding to regulatory regions on DNA negatively impact mRNA production for a number of other proteins.

Selected Pharmacological Uses/Other Roles

Those possibly benefiting from pharmacological doses of vitamin B₆ include individuals with primary hyperoxaluria (type 1), which results from a mutation in the gene for the vitamin B₆-dependent enzyme alanine glyoxylate aminotransferase and increases the risk for oxalate kidney stone formation. Vitamin B₆ (in amounts up to about 20 mg/kg body weight per day) may improve residual enzyme activity (in some individuals) and reduce the production of oxalic acid that occurs secondary to the disorder.

Homocysteinuria results from mutations in the gene for cystathionine β-synthase, a PLP-dependent enzyme. The enzyme is one of many needed to oxidize the amino acid methionine. Cystathionine β-synthase activity in many individuals with homocysteinuria (close to 50%) improves to some extent with vitamin B₆ supplementation. Recommended doses vary considerably, ranging from about 100 to 500 mg given orally; however, individuals responding to supplementation should be monitored for signs of toxicity (the Tolerable Upper Intake Level for adults is 100 mg/day).

Pharmacological doses of vitamin B₆ have been shown to be of some benefit in the treatment of other inborn errors of metabolism affecting protein and enzymes involved in the vitamin's metabolism. For example, some infants born with an inherited, intractable seizure disorder also have been shown to benefit from pharmacological doses of vitamin B₆ [3].

Metabolism and Excretion

Vitamin B₆ is excreted primarily in the urine, with very little excreted in the feces. 4-Pyridoxic acid is the major urinary metabolite and results from the oxidation of pyridoxal by either NAD-dependent aldehyde dehydrogenase, found in

all tissues, or FAD-dependent aldehyde oxidases, found in the liver and kidneys. Ingesting larger doses (about 100 mg or more) of the vitamin as pyridoxine may result in urinary excretion of intact pyridoxine and 5-pyridoxic acid, and lower urinary 4-pyridoxic acid excretion.

Recommended Dietary Allowance

The RDA for vitamin B₆ for adult men age 19–50 years is 1.3 mg per day (requirement 1.1 mg) and for men age 51 years and older, 1.7 mg per day (requirement 1.4 mg) [1]. For adult women age 19–50 years, the RDA for vitamin B₆ is also 1.3 mg per day (requirement 1.1 mg), and for women age 51 years and older it is 1.5 mg daily (requirement 1.3 mg) [1]. With pregnancy and lactation, recommendations for vitamin intake increase to 1.9 mg and 2.0 mg, respectively [1]. Recommendations are based largely on the maintenance of adequate plasma vitamin concentrations [1]. Some have suggested the recommendations need to be raised [4]. The inside front cover of the book provides additional recommendations for vitamin B₆ for other age groups.

Deficiency

Vitamin B₆ deficiency is relatively rare in the United States. In the 1950s, deficiency occurred in infants because of severe heat treatment of infant milk. The heat processing resulted in a reaction between the PLP and the epsilon amino group of lysine in the milk proteins to form pyridoxyl-lysine, with little vitamin activity.

Signs and symptoms of vitamin B₆ deficiency (which can occur in as little as 2–3 weeks of insufficient intake but may take up to ~2½ months) include a **seborrheic dermatitis** rash on the face (cheeks and nasolabial fold), neck, shoulders, and buttocks areas. Weakness and fatigue, along with cheilosis, glossitis, and angular stomatitis (Figure 9.16), may also be present. Deficiency also affects the central and peripheral nervous systems. Neurological problems include depression, confusion, peripheral neuropathy, and (especially in infants) seizures and convulsions. A hypochromic, microcytic anemia (seen usually in infants) may also occur due to impaired heme synthesis. Deficiency also impairs niacin synthesis from tryptophan and inhibits the metabolism of homocysteine, which may result in hyperhomocysteinemia, a risk factor for heart disease. A vitamin B₆ deficiency is usually treated with daily oral supplements of the vitamin in amounts of 2.5–25 mg (or up to about 100 mg if needed) for a few weeks.

At Risk for Deficiency

Groups particularly at risk for vitamin B₆ deficiency are older adults, who may have a poor intake of the vitamin and may also have accelerated hydrolysis of PLP and oxidation of PL, and people who consume excessive amounts of alcohol

(alcohol can impair the conversion of pyridoxine and pyridoxamine to PLP, and the presence of acetaldehyde formed from alcohol metabolism enhances coenzyme degradation). Systemic inflammation appears to both alter tissue distribution and increase vitamin B₆ catabolism, suggesting higher needs for the vitamin in this situation. People on a variety of drug therapies may also be at risk. For example, isoniazid used to treat tuberculosis interferes with vitamin activity (the vitamin is also used to treat an isoniazid overdose). Penicillamine used to treat some autoimmune conditions and Wilson's disease inactivates the vitamin. Corticosteroids, used to suppress the immune system in some inflammatory conditions, promote vitamin excretion, and anticonvulsants, used to diminish seizures, inhibit vitamin activity. Oral contraceptive use may also result in suboptimal vitamin B₆ status, perhaps by affecting the metabolism and/or altering tissue concentrations of the vitamin. Malabsorptive conditions (such as Crohn's disease and celiac disease, among others) reduce the vitamin's absorption.

Toxicity

Pharmacological doses of vitamin B₆ have been advocated to prevent or treat a variety of states, including hyperhomocysteinemia, carpal tunnel syndrome, morning sickness, premenstrual syndrome, depression, and muscular fatigue. Although some beneficial results from megadoses of the vitamin have been noted with selected conditions, indiscriminate use of the vitamin is not without risk. Excessive pyridoxine use (>~200 mg/day but sometimes lower amounts) for usually prolonged time periods can cause sensory and peripheral neuropathy along with problems with movement. Some signs and symptoms include unsteady gait, paresthesia in the extremities, and impaired tendon reflexes. Intakes in excess of 2 g/day may cause not only paresthesia in the feet and hands but also impaired motor control or ataxia (impaired muscle control or the inability to control body movement). High intakes may also cause degeneration of neurons (dorsal root ganglia) in the spinal cord, loss of myelination, and degeneration of sensory fibers in peripheral nerves [4]. The Tolerable Upper Intake Level for vitamin B₆ is 100 mg/day for adults to minimize the development of neuropathy [1]. Vitamin B₆ supplementation, at even low doses, is not recommended for individuals with Parkinson's disease who are being treated with L-dopa (levodopa); the vitamin interferes with the effectiveness of drug therapy.

Assessment of Nutriture

Plasma PLP concentrations are thought to be the best indicator of vitamin B₆ tissue stores, with PLP concentrations of less than 5 µg/L suggestive of vitamin deficiency and

concentrations of 5–7.5 µg/L suggestive of marginal status; adequacy is indicated by plasma PLP concentrations > 7.5 µg/L [1].

Urinary vitamin B₆ (measured over several days for a period of 1–3 weeks) and urinary 4-pyridoxic acid are also commonly used to assess vitamin B₆ status. Urinary vitamin B₆ excretion of ~0.5 µM/day or ~20 µg/g creatinine and urinary 4-pyridoxic acid concentrations of ≤ 30 µg/L/day are thought to indicate deficiency [5]. Urinary 4-pyridoxic acid excretion, however, is considered to be a short-term indicator of vitamin B₆ status, and cutoff values are controversial [1].

A functional test for the vitamin measures xanthurenic acid excretion following tryptophan loading (2 g or 100 mg of tryptophan/kg body weight). Abnormally high xanthurenic acid excretion is found in vitamin B₆ deficiency because 3-hydroxykynurenine, an intermediate in tryptophan metabolism, cannot lose its alanine moiety and be converted to 3-hydroxyanthranilic acid, as should occur (see Figure 9.19). Instead, 3-hydroxykynurenine is converted to xanthurenic acid, which is excreted in the urine in greater than normal amounts—that is, > 25 mg/6 hours.

Measuring transaminase activity before and after adding vitamin B₆ is an additional technique for determining vitamin B₆ nutriture, especially longer-term vitamin status. However, because of a variety of limitations with the assays, these tests are better used as an adjunct to other tests. The erythrocyte transaminase index examines the activity of glutamic oxaloacetic transaminase (abbreviated GOT or AST) and glutamic pyruvic transaminase (abbreviated GPT or ALT) after the addition of vitamin B₆. Deficient vitamin B₆ status is suggested by GOT/AST activity of >1.85 following the addition of the vitamin and by GPT/ALT activity of >1.25 following the addition of the vitamin [5].

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Suggested Reading

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SUMMARY

The water-soluble vitamins include a group of eight B-vitamins and vitamin C. The vitamins share a number of similarities as well as differences.

- Water-soluble vitamins are present in a wide variety of foods, especially those from the dairy, fruit, vegetable, grain/grain products, and protein-rich food groups.
- Water-soluble vitamins, with the exception of vitamin B₁₂, are absorbed primarily in the proximal small intestine and are subsequently transported to tissues via the blood.
- Water-soluble vitamins are stored in relatively small quantities in tissues (with the exception of vitamin B₁₂) with an excess of the vitamins excreted primarily via the urine.
- All water-soluble vitamins participate in reactions, through varying mechanisms of action, needed for nutrient metabolism and/or energy production.
- Some water-soluble vitamins also play roles in hemato-poiesis and gene expression.
- RDAs have been established for all water-soluble vitamins with the exceptions of biotin and pantothenic acid.
- TULs have been established for niacin, folic acid, vitamin B₆, and vitamin C, with use of supplements and fortified foods typically contributing to intakes in excess of TULs.

Perspective

TYPES OF HUMAN RESEARCH STUDIES AND THEIR LIMITATIONS

Throughout the chapters of this book the findings of research studies are discussed. A brief overview of the types of research studies is provided here to facilitate the reader's understanding of different types of research design used in conducting studies and some of their limitations.

Research involving humans is called epidemiology, classically defined as the study of the determinants and distribution of disease frequency in human populations. Nutritional epidemiology may then be defined as the study of the nutritional determinants of disease.

Research may be classified as primary or secondary. *Primary* research produces original data by original investigators. The investigators design the study, collect and analyze the data, and publish the results. *Secondary* research utilizes previously generated information or data. In secondary research it is not the responsibility of the investigators to collect the data. A meta-analysis is an example of secondary research.

Research is also classified as *observational* or *experimental* and further categorized as *descriptive* or *analytical* (as shown in Table 1). In *observational* studies, the investigator does not control the way subjects are exposed to factors of interest, but simply collects information (data) and investigates relationships between exposure and outcomes. *Descriptive* research

is designed to document a condition or situation that reflects a natural course of events. Descriptive studies often provide "initial observations" that form the basis of specific hypotheses to be tested by additional research. Descriptive research can be used to identify associations among factors; however, it does not test, verify, or establish causal relationships among factors. Thus, all descriptive research is observational.

- *Cross-sectional studies* are observational studies of individuals in which measures of exposure and outcome are taken from a single point in time (sometimes described as a "snapshot" of the sample population). Cross-sectional studies provide information regarding associations between exposure and outcome, but do not allow for the determination of cause-and-effect. This type of observational descriptive study is useful in formulating hypotheses to be tested by additional research.
- *Case reports*, another example of an observational descriptive study, provide a description of a single individual exhibiting an unusual or noteworthy outcome with regard to an exposure. Case reports are limited in their application to the general population but do suggest potential areas of research.

Analytical research is designed to test hypotheses with regard to clearly defined factors and conditions. When appropriately designed and executed, analytical studies can help establish causal relationships among factors. Analytical studies may be *observational* or *experimental*.

- *Case-control studies*, an example of observational analytical research, compare persons with an established outcome or disease ("cases") with persons who do not have the disease ("controls"). Information can be obtained on many exposure variables, although the validity of this information may be limited because the data are collected retrospectively. Case-control studies are generally less expensive and can be completed more quickly than cohort studies.
- *Cohort studies*, another example of observational analytical research, involve a cohort (i.e., a group of individuals) in whom information about exposure and outcome are collected over time. Each subject enters the cohort at a defined point in time, when exposure to a suspected causative factor is initially measured. The cohort is then followed over time so that the rate of disease development and exposure can be measured concurrently (prospectively). Cohort studies can also be retrospective, in which measures of exposure are collected from the past.

In *experimental* studies, the investigator assigns an exposure (or treatment/intervention) to subjects in a controlled manner. One group serves as a control and is not exposed to any extraneous change. The other group(s), matched as possible in characteristics to the subjects of the control group, are exposed to the treatment/intervention. Experimental research designs enable the investigator

Table 1. Types of Research Studies

	Individual Subject Data	Population/Group Data
Observational		
Descriptive	Cross-sectional studies Case reports	Ecological studies
Analytical	Case-control studies Cohort studies	Ecological studies
Experimental		
Analytical	Clinical trials	Community trials

to control or manipulate one or more variables, designated as dependent or independent, in an effort to examine the relationship between the variables. The independent variable is the variable controlled or manipulated by the investigator. The dependent variable occurs as the result of the influence of the independent variable. Experimental studies more directly assess cause-and-effect relationships between exposures and outcomes.

- *Clinical trials* are an example of experimental analytical research. Experimental studies in which exposure is manipulated by the investigator so that relationships with disease

outcomes can be measured more directly. Clinical trials may be conducted on subjects who already have a disease (secondary prevention trials) or on subjects who, at the beginning of the study, are free from disease (primary prevention trials).

Studies of *populations* are designed to use summary data collected from groups of people. Exposures and outcomes are related only at the group level. Observational studies comparing populations or groups of people are referred to as *ecological studies*. These studies, which can be descriptive or analytical, use summary measures of exposure and outcome

(e.g., mean or median) to represent the population. Consequently, there are generally few data points and it is harder to disentangle multiple factors associated with exposure and outcome. Ecological studies are generally helpful for examining and proposing new hypotheses that could lead to studies of individuals from which causality may be inferred with greater confidence. Community trials, which are experimental analytical studies, are similar to experimental studies described in the preceding paragraph but include large groups of individuals typically living or working within a defined geographical area (such as a city).

FAT-SOLUBLE VITAMINS

LEARNING OBJECTIVES

- 10.1** Identify particularly good food sources of the fat-soluble vitamins.
- 10.2** Explain how the fat-soluble vitamins are digested, absorbed, transported in the blood, and stored.
- 10.3** Describe the metabolism of the fat-soluble vitamins in the small intestine, liver, and kidneys.
- 10.4** Describe the functions and mechanisms of action of the fat-soluble vitamins.
- 10.5** Identify the means by which the fat-soluble vitamins are excreted.
- 10.6** Describe recommended intakes, deficiencies, and toxicities associated with the fat-soluble vitamins.
- 10.7** Identify measures used for fat-soluble vitamin status.

THIS CHAPTER ADDRESSES EACH of the four fat-soluble vitamins—A, D, E, and K—and the carotenoids. Like the water-soluble vitamins (discussed in Chapter 9), the fat-soluble vitamins have several similar characteristics. Their absorption and transport, in contrast to those of the water-soluble vitamins, are closely associated with the absorption and transport of ingested lipids. Thus, digestion and absorption are greatest when some dietary fat is present with the fat-soluble vitamins in the digestive tract and when there is sufficient production and delivery of bile as well as pancreatic enzymes and secretions. Absorption of the fat-soluble vitamins occurs most rapidly from the duodenum and jejunum (proximal small intestine), but quantitatively, absorption appears to be greatest from the jejunum, as destruction or resection of the jejunum is often associated with fat-soluble vitamin deficiencies. Additionally, destruction or resection of the ileum disrupts the enterohepatic recirculation of bile; in such cases, the synthesis of new bile often cannot compensate for the loss of bile in the feces, causing malabsorption of fat-soluble vitamins and fat. The enterocyte also must be functional to synthesize the chylomicrons, which initially transport all fat-soluble vitamins along with fat out of the intestine and into the lymphatic system. Also, unlike water-soluble vitamins, the fat-soluble vitamins are stored in greater quantities in body tissues—mainly in the liver, adipose, and/or cell membranes, although the amount stored varies widely among the fat-soluble vitamins. Table 10.1 provides an overview of the functions, deficiency syndrome, food sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI) of each of the fat-soluble vitamins. The RDAs and AIs for all nutrients and for all age groups are provided on the inside front cover of the book.

As with the discussion of the water-soluble vitamins, each of the fat-soluble vitamins is considered (when information is available) in terms of structure, sources, absorption (also digestion where applicable), transport, tissue uptake, storage, functions and mechanisms of action, metabolism and excretion, recommended intake, deficiency, toxicity, and assessment of nutriture. Specific interrelationships with other nutrients are also noted for selected vitamins. Table 10.2 provides an overview of some of the manifestations associated with fat-soluble vitamin deficiencies. Yet, while treatment of deficiency (especially if severe) is typically best accomplished with supplements, it is consumption of diets rich in fruits, vegetables, and whole grains (and not ingestion of

Table 10.1 The Fat-Soluble Vitamins: Function, Food Sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI)

Vitamin	Biochemical or Physiological Function	Food Sources	RDA or AI
Vitamin A Retinol, retinal, and retinoic acid	Synthesis of rhodopsin; cell growth, cell differentiation; bone development; and immune function	Liver, dairy products, and fortified foods	900 μg RAE ^a
Provitamin Carotenoids	Antioxidant	Sweet potato, carrots, spinach, butternut squash, greens, broccoli, and cantaloupe	700 μg RAE ^b
Vitamin D D ₂ —ergocalciferol and D ₃ —cholecalciferol Provitamin — 7-dehydrocholesterol	Regulator of bone mineral metabolism, blood calcium homeostasis, cell differentiation, proliferation, and growth	Fatty fish and their oils and fortified foods	15–20 μg ^{c,d}
Vitamin E Tocopherols and tocotrienols	Antioxidant	Vegetable oils, nuts, and seeds	15 mg α -tocopherol ^c
Vitamin K Phylloquinones and menaquinones	Activates blood-clotting factors and proteins in bone by γ -carboxylating glutamic acid residues	Vegetables, especially leafy vegetables, and legumes	120 μg ^{a,e} 90 μg ^{b,e}

^a Adult males^b Adult females^c Both males and females^d Varies with age for adults; see text^e Adequate Intake**Table 10.2** Some Common Signs Associated with Fat-Soluble Vitamin Deficiencies

System/Sites	Common Selected Signs	Possible Vitamin Deficit
Skin	Rough, red, “bumpy” skin, keratinization of epithelium	A
Muscle	Weakness, pain	D
Skeletal bone	Excessive bone growth	A
	Inadequate bone mineralization, pain	D, K
Blood vessels/ cells	Red blood cell fragility	E
	Hemolytic anemia	E
	Prolonged blood clotting	K
Ocular Retina	Pigmented retinopathy	E
	Nyctalopia, night blindness	A
Conjunctiva	Xerosis, Bitot’s spots	A
Cornea	Xerosis, ulcerations/keratomalacia	A

supplements) that is most often associated with reduced risk of diseases [1–6].

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10.1 VITAMIN A AND CAROTENOIDS

Vitamin A was initially found to be an essential growth factor in animal foods and was called fat-soluble A. McCollum and Davis, followed by Osborne and Mendel, are credited with its discovery in about 1915. Today, the term *vitamin A* (also called preformed vitamin A or retinoids) is generally used to refer to a group of compounds that possess the biological activity of all-*trans* retinol. The retinoids are structurally similar and include retinol, retinal, retinoic acid, and retinyl ester, as well as synthetic analogues. Structurally, retinoids contain a β -ionone ring and a polyunsaturated side chain with either an alcohol group (retinol, shown in Figure 10.1a), an aldehyde group (retinal, also called retinaldehyde, Figure 10.1b), a carboxylic acid group (retinoic acid, Figure 10.1c), or an ester group (retinyl ester [Figure 10.1d], such as retinyl stearate or palmitate [Figure 10.1e]). The side chain is made up of four **isoprenoid** units with a series of conjugated double bonds. The double bonds may exist in a *trans* (as in all-*trans* retinol) or a *cis* configuration.

Carotenoids are red, orange, and yellow lipid-soluble pigments found mainly in plants. Of the over 700 carotenoids found in nature, less than about 10% are in commonly consumed foods, and only about 10 are found in

detectable concentrations in human blood and tissues. Structurally, carotenoids consist of an expanded carbon chain containing conjugated double bonds, with usually but not always an unsubstituted β -ionone ring at one or both ends of the chain. Three carotenoids, which can be converted into retinal in the body, are known as provitamin A carotenoids and include β -carotene (Figure 10.1f), α -carotene (Figure 10.1g), and β -cryptoxanthin (Figure 10.1h); these carotenoids are found most often in the all-*trans* form but can occur as *cis* isomers. Of the three, β -carotene exhibits the greatest amount of provitamin A activity. Some other important carotenoids, while not vitamin A precursors (nonprovitamin A carotenoids), include lycopene (an open-chain analog of β -carotene; Figure 10.1i) and many oxycarotenoids (also called oxygenated

carotenoids or xanthophylls), such as canthaxanthin (Figure 10.1j), lutein (Figure 10.1k), and zeaxanthin.

Sources

Preformed vitamin A (retinoids) is found primarily in selected foods of animal origin, especially liver, dairy products (including milk, cheese, and butter), eggs, and fatty fish and their oils (such as tuna, sardines, and herring) (Table 10.3). Some products, such as reduced-fat milk, margarine, and breakfast cereals, are fortified with vitamin A. In the United States, of foods containing preformed vitamin A, dairy products and fortified cereals contribute most to vitamin intake. Retinoids can undergo oxidation if exposed to varying degrees of, for example, oxygen, light, heat, and some metals.

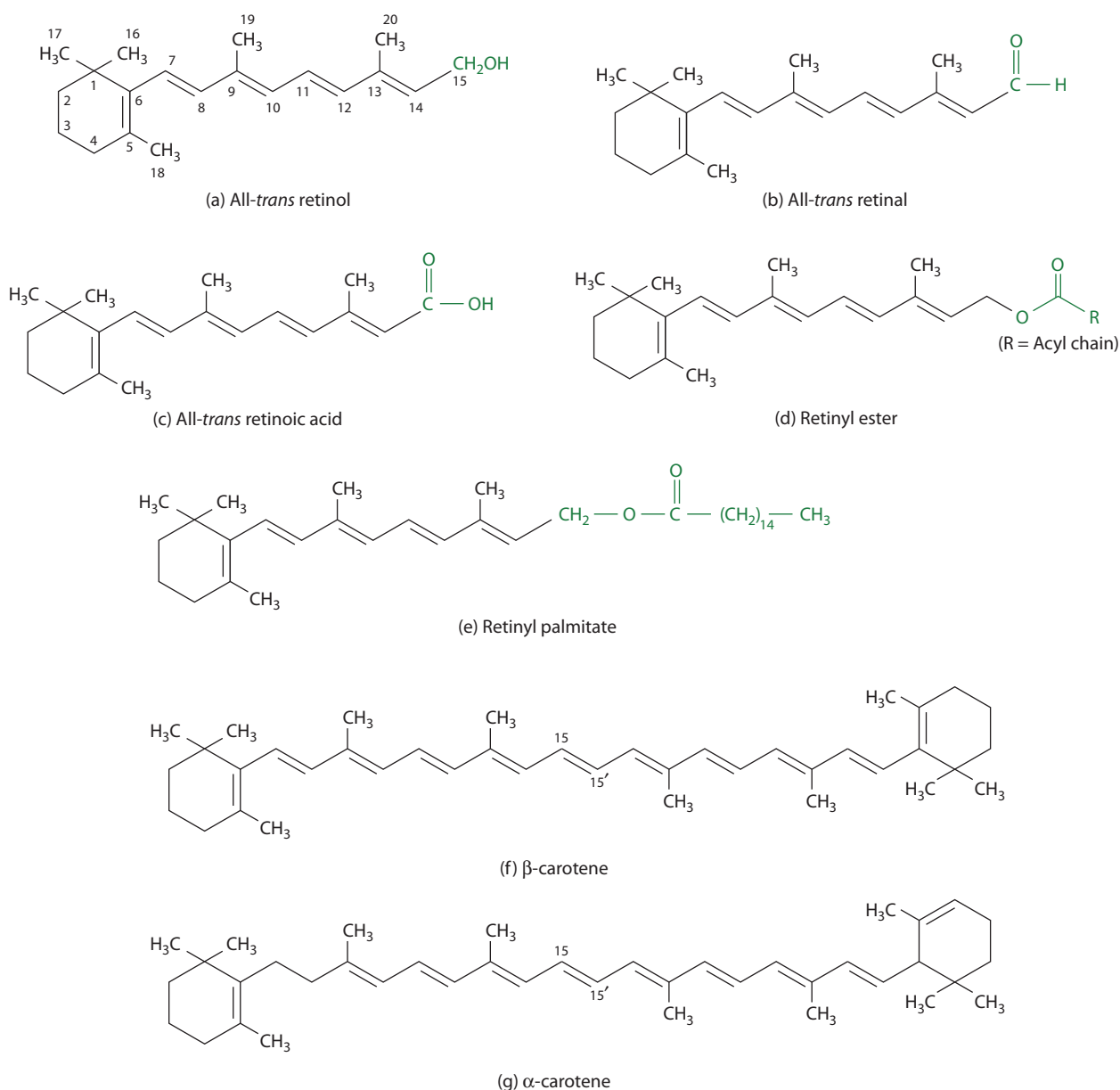


Figure 10.1 Vitamin A and carotenoid structures.

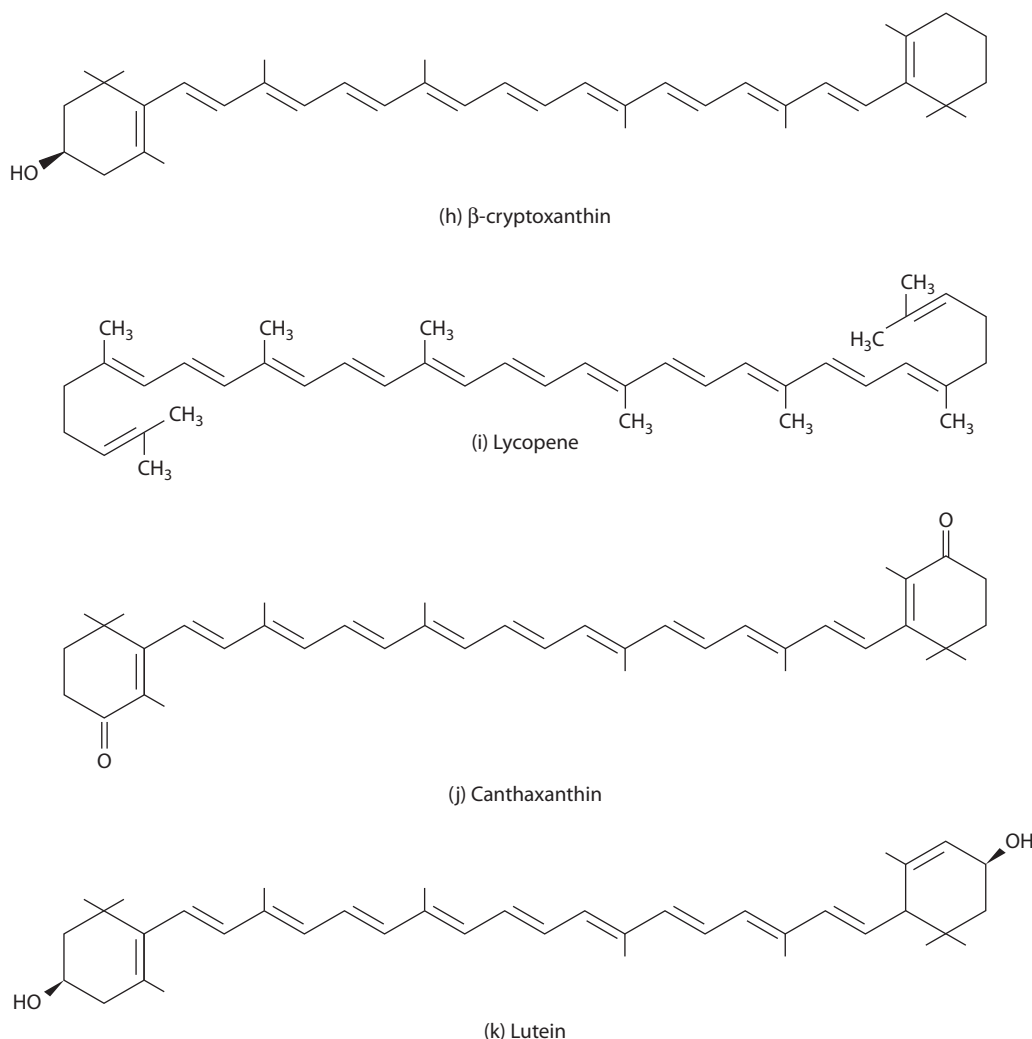


Figure 10.1 (Continued) Vitamin A and carotenoid structures.

Table 10.3 Vitamin A and β -carotene Contents of Selected Foods*

Food (serving)	Vitamin A (μ g RAE)	Food (serving)	β -carotene (mg)
Liver, beef, cooked (3 oz)	6,600	Spinach, cooked ($\frac{1}{2}$ c)	6.9
Herring (3 oz)	220	Carrots, cooked ($\frac{1}{2}$ c)	6.5
Milk, low-fat or fat-free, fortified (1 c)	150	Collards, cooked ($\frac{1}{2}$ c)	5.8
Margarine, fortified (1 Tbsp)	150	Kale, cooked ($\frac{1}{2}$ c)	5.7
Sardines (3 oz)	92	Carrots, raw (1 medium)	5.1
Salmon, cooked (3 oz)	59	Winter squash, cooked ($\frac{1}{2}$ c)	2.8
Tuna (3 oz)	55	Cantaloupe (1 c)	3.2
Cheeses (1 oz)	60–85		
Herring oil (1 Tbsp)	13.6		
Cod liver oil (1 Tbsp)	13.5		

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>. A list of vitamin A-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminA-Food.pdf>. Also see <http://lpi.oregonstate.edu/mic/dietary-factors/phytochemicals/carotenoids>.

The main form of preformed vitamin A in foods is as retinyl esters in which the vitamin is attached to a long-chain fatty acid such as palmitate (Figure 10.1e). Pharmaceutical vitamin preparations typically contain vitamin A (alone or as part of a multivitamin/mineral supplement) as all-*trans* retinyl acetate or all-*trans* retinyl palmitate, although some products may also provide the provitamin form β -carotene. The amount of preformed vitamin A in single-ingredient products varies considerably, ranging from about 750 to 3,000 μ g retinol activity equivalents. Other forms of the vitamin are available for individuals with fat malabsorptive disorders, including, for example, Aquasol A oral, a water-miscible form of the vitamin, as well as vitamin A palmitate, which can be taken sublingually.

Vitamin A information found on the labels of vitamin preparations list the amount of the vitamin using the units “ μ g retinol activity equivalents” (RAE). Foods may also list the vitamin A content on nutrition facts labels, although they are not required to do so by the U.S. Food and Drug Administration (FDA). RAEs are used to account for the

differences in retinol generated in the body from provitamin forms versus preformed vitamin A. This topic is discussed further under the section “Recommended Dietary Allowance.” When listed on the label, in addition to absolute quantities of vitamin A in the product, the vitamin is given as a percentage of the Daily Value, which for vitamin A is 900 μg RAE.

Carotenoids are synthesized and found in a wide variety of plants. The most common dietary carotenoids are β -carotene, α -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin. Generally, the carotenoids are found in significant amounts in yellow, orange, and red (brightly colored) fruits and vegetables such as carrots, watermelon, papayas, tomatoes, tomato products (ketchup, chili sauce, tomato paste, tomato juice, spaghetti sauce), squash, pink grapefruit, and pumpkins. Green vegetables also contain some carotenoids, but the pigment is masked by (green) chlorophyll. Carrots typically represent a major source of both α - and β -carotene in the American diet. Other major dietary contributors of β -carotene are listed in Table 10.3.

Alpha-carotene is found in most of the same foods as β -carotene. Some examples include pumpkin (5.8 mg/ $\frac{1}{2}$ cup), carrots (2.9 mg/ $\frac{1}{2}$ cup cooked and 2.1 mg/raw carrot), winter squash (0.7 mg/ $\frac{1}{2}$ cup cooked), plantains (0.8 mg), collard greens (0.2 mg/ $\frac{1}{2}$ cup cooked), and tomatoes (0.1 mg), among others.

Fruits, such as papaya (2.3 mg), tangerine (0.4 mg) and watermelon (0.2 mg/wedge); orange juice (0.4 mg/ $\frac{1}{2}$ cup); and pumpkin (1.8 mg/ $\frac{1}{2}$ cup), red pepper (0.6 mg), carrots (0.15 mg/ $\frac{1}{2}$ cup cooked), and yellow corn (0.1 mg/ $\frac{1}{2}$ cup cooked) contribute to dietary β -cryptoxanthin intake.

Tomatoes, along with tomato juice and sauces, are the richest sources of *lycopene*, a carotenoid that is red in color. The lycopene content of some tomato-based products is 75.4 mg/cup tomato paste, 54.4 mg/cup tomato puree, 26.4 mg/cup condensed tomato soup, 23.3 mg/cup vegetable juice, 220 mg/cup tomato juice, and 4.6 mg/cup of diced tomatoes. Other foods including watermelon (13 mg/wedge), papaya (2.3 mg), and pink grapefruit (1.7 mg/ $\frac{1}{2}$) also provide dietary lycopene.

Good sources of *zeaxanthin* and *lutein* include kale (12.8 mg/ $\frac{1}{2}$ c cooked), spinach (18.9 mg/ $\frac{1}{2}$ c cooked), turnip greens (9.7 mg/ $\frac{1}{2}$ c cooked), mustard greens (4.2 mg/ $\frac{1}{2}$ c cooked), broccoli (1 mg/ $\frac{1}{2}$ c cooked), Brussels sprouts (1.2 mg/ $\frac{1}{2}$ c cooked), and yellow corn (0.8 mg/ $\frac{1}{2}$ c cooked). Small amounts are also found in peppers (yellow), zucchini, green beans, kiwi, honeydew melon, and egg yolks. *Canthaxanthin*, a red-orange carotenoid, is found in plants as well as in fish and seafood such as sea trout and crustaceans. Meat and fish are not major sources of carotenoids, but because animals and fish feed on plants, they can accumulate some carotenoids.

Carotenoids also may be added to foods. β -Carotene and canthaxanthin, for example, are approved by the FDA for use as food color additives.

β -Carotene is sometimes included in multivitamin preparations and can be found in single-ingredient preparations in varying amounts. Other carotenoids, such as lutein, lycopene, and zeaxanthin, are also available as single-ingredient supplements in varying amounts and as part of multivitamin supplements focused on eye health.

Digestion and Absorption

Because it is bound to other food components, vitamin A requires some digestion before it can be absorbed from the small intestine. Retinol, for example, is typically bound to a fatty acid as a retinyl ester (see retinyl palmitate previously shown in Figure 10.1e) and may also be bound, like carotenoids in foods, to protein from which it must be released. Although heating plant foods weakens some complexes, such as protein-carotenoid complexes, enzymatic digestion is still required. Carotenoids and retinyl esters are initially hydrolyzed from protein by pepsin in the stomach. Because of their fat solubility, the freed (i.e., no longer bound to protein) retinyl esters and carotenoids typically coalesce, along with other lipids, to form fat globules in the stomach. These fat globules containing the vitamin are emptied into the duodenum, where bile emulsifies them (emulsification results in large fat globules being broken up into smaller droplets).

Proteolytic enzymes in the duodenum can hydrolyze any remaining protein-bound retinyl esters or carotenoids not freed in the stomach. Hydrolysis of retinyl and carotenoid esters by various hydrolases and esterases occurs at the same time that triacylglycerols, phospholipids, and cholesteryl esters are being hydrolyzed by pancreatic enzymes. Pancreatic lipase and pancreatic cholesterol ester hydrolase, secreted into the lumen of the small intestine, free the bound vitamin. Additionally, enzymes such as retinyl ester hydrolase and phospholipase on the intestinal brush border membrane can contribute to the vitamin's digestion. Pancreatic hydrolases cleave shorter-chain retinyl esters, whereas intestinal brush border hydrolases act on longer-chain retinyl esters.

Next, mixed micelles form within the lumen of the small intestine from the bile, digested lipids, retinol, and some carotenoids. The micellar solution diffuses through the lumen and unstirred water layer adjacent to the enterocytes, enabling close contact with the brush border membrane. Here the mixed micelle components dissociate, allowing for what was formerly thought as solely passive diffusion of retinol and the carotenoids along a concentration gradient into intestinal cells. However, newer studies suggest the absorption of carotenoids occurs by other mechanisms.

Carotenoids are also absorbed by a facilitated process that requires binding to scavenger receptor class B type 1 (SR-B1). These receptors/transporters are found in the small intestine, among other tissues, and enable uptake

of carotenoids and some other fat-soluble vitamins into enterocytes; these receptors also enable uptake of high-density lipoproteins into various tissues. SR-B1 availability on the brush border membrane of enterocytes is regulated via negative feedback by an intestine-specific homeobox (ISX) transcription factor. Thus, under situations of higher cellular vitamin A (retinoic acid), absorption of carotenoids by SR-B1 is decreased. This decrease in absorption occurs from increased ISX expression, which results from the actions of retinoic acid interacting (via RARs, discussed in detail under the section “Functions and Mechanisms of Action,” in the subsection “Gene Expression”) with the promoter region of the gene for ISX transcription factor. Once synthesized, the ISX transcription factor suppresses the expression of genes coding for SR-B1; it also reduces β -carotene’s conversion to retinal by suppressing the gene coding for the enzyme β -carotene-15,15’-oxygenase 1, which is responsible for the generation of retinal from β -carotene (discussed later in this section).

Absorption of vitamin A and carotenoids occurs most rapidly in the duodenum and jejunum but continues to a large extent throughout the jejunum. However, the efficiency of absorption differs between the two forms. Approximately 70–90% of dietary preformed vitamin A is absorbed as long as the meal contains some (~5–10 g or more) fat. β -carotene absorption generally ranges from about 20 to 50% but can be <5%. Multiple factors both enhance and reduce carotenoid absorption, and factors that increase the absorption of some carotenoids can decrease the absorption or bioavailability of other carotenoids. Some factors generally enhancing absorption include the presence of fat, higher carotenoid content of the food, and heat processing of the food (e.g., cooked vs. uncooked food). Differences in particle size, the food

matrix including fiber content (which, for example, can interfere with micelle formation), and the presence of excessive vitamin E, among other factors, can reduce the absorption of some carotenoids.

Metabolism of Carotenoids within the Enterocyte

Within the enterocyte (as well as the liver, adipose, lungs, and kidneys, among other organs), provitamin A carotenoids, including α - and β -carotene and β -cryptoxanthin, undergo metabolism. The extent to which these carotenoids are converted to retinoids in the enterocytes is influenced by several factors, such as an individual’s vitamin A status and the amounts and forms of the carotenoids consumed. Conversion of β -carotene to retinal is reduced with higher vitamin A (retinoic acid). The mechanism, mentioned earlier in this section, is mediated by ISX transcription factor, which binds to the gene coding for a key enzyme responsible for retinoid production from β -carotene in the enterocyte.

The synthesis of vitamin A (specifically all-*trans* retinal) from β -carotene (and other provitamin A carotenoids) is accomplished by two enzymes through either central cleavage or noncentral cleavage of retinal.

- Central cleavage of retinal (its primary metabolic fate) occurs via the enzyme β -carotene-15,15’-oxygenase 1 (BCO1), an iron-dependent cytosolic enzyme that is also found in the other tissues, including the liver, lungs, kidneys, and retina. Central cleavage of β -carotene generates two molecules of retinal (Figure 10.2), while central cleavage of β -cryptoxanthin produces one molecule each of retinal and 3-hydroxyretinal. The overall efficiency of β -carotene conversion is estimated at about 50%.
- Noncentral cleavage of provitamin carotenoids by the enzyme β -carotene-9’,10’-oxygenase 2 (BCO2) also

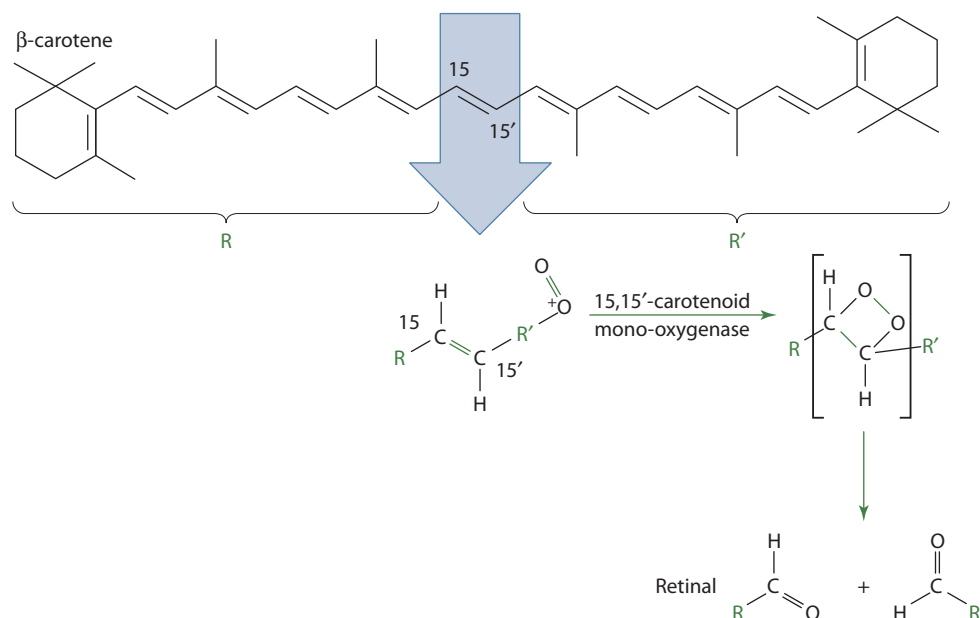


Figure 10.2 Central cleavage of β -carotene to retinal.

occurs within the enterocyte and generates retinal along with several metabolites (alcohols, aldehydes, etc.). Non-provitamin A carotenoids, such as lycopene and the xanthophylls lutein, zeaxanthin, and canthaxanthin, among others, also serve as a substrate for this enzyme, which generates several 3-hydroxy metabolites.

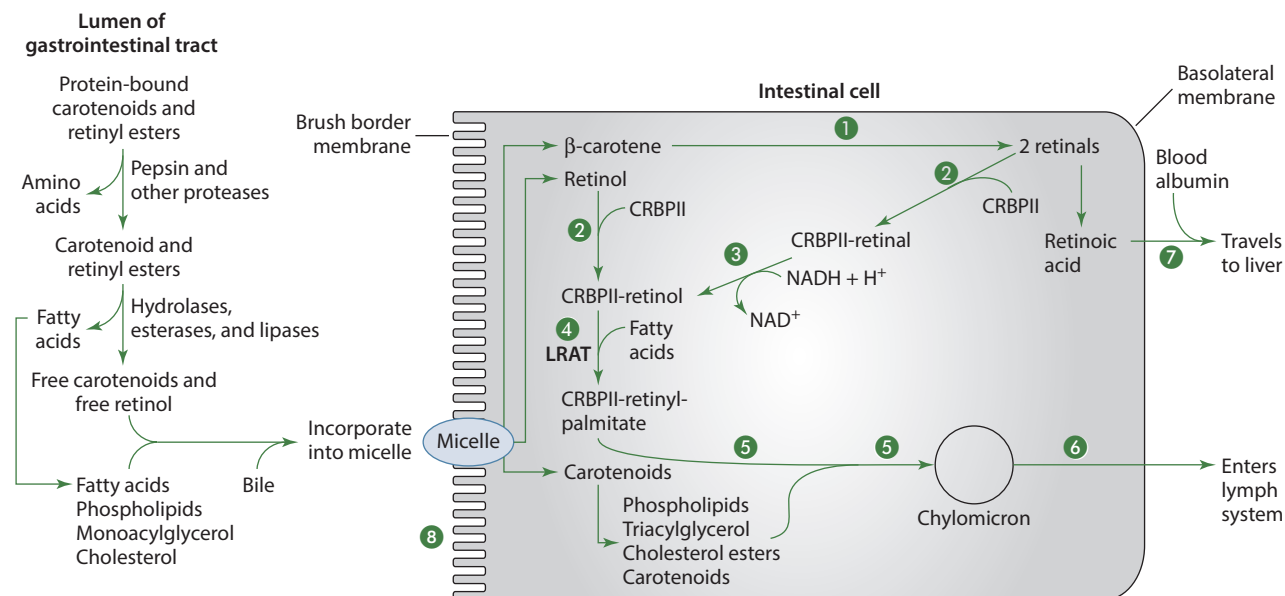
Within the enterocyte, retinal that is generated from β -carotene, α -carotene, and β -cryptoxanthin is converted subsequently to retinol by retinal/retinaldehyde reductase, an NADH-dependent enzyme (this reaction is discussed further in the next section). An estimated 12 μg of β -carotene produces about 1 μg of retinol; an estimated 24 μg of α -carotene or β -cryptoxanthin produces about 1 μg of retinol [1,2].

Metabolism of Retinoids within the Enterocyte

Within enterocytes, retinoids undergo additional metabolism (Figure 10.3). To recap, retinol present in intestinal cells arises from dietary intake or from provitamin A carotenoids that have been hydrolyzed to retinal and then reduced to retinol via reductase activity. In order to move out of the intestinal cell, retinol must

be reesterified. Two metabolic pathways are present for reesterification within the enterocyte.

- The primary pathway involves cellular retinol-binding protein (CRBP) type II, a cytosolic binding protein that helps regulate retinol use in cells. CRBP II, which binds both retinol and retinal, directs the reduction of retinal to retinol and the subsequent esterification of retinol to a fatty acid. CRBP II-bound retinol is esterified by lecithin retinol acyl transferase (LRAT) to form mainly retinyl palmitate, but also retinyl stearate, retinyl oleate, and retinyl linoleate, among others. LRAT specifically transfers sn-1 fatty acids from intracellular membrane-associated phospholipids, such as phosphatidylcholine, to retinol that is bound to CRBPs. LRAT is thought to be the main enzyme responsible for esterification in the small intestine, liver, pigment epithelium of the retina, and likely other tissues.
- The second, minor pathway for reesterification is catalyzed by acyl-CoA retinol acyl transferase (ARAT). In contrast to LRAT, the fatty acids used by ARAT to esterify retinol are those present in the enterocyte from dietary fat consumption.



- 1 β -carotene is converted into two retinal molecules. See Figure 10.3 for details of this reaction.
- 2 Cellular retinol-binding protein (CRBP) II binds to both retinol and retinal in the intestinal cell.
- 3 Retinal, while attached to CRBP II, is reduced to retinol by retinal/retinaldehyde reductase to form CRBP II-retinol.
- 4 Lecithin retinol acyl transferase (LRAT) esterifies a fatty acid (palmitic acid) onto the CRBP II-bound retinol to form CRBP II-retinyl-palmitate.
- 5 Retinyl esters are incorporated along with phospholipids, triacylglycerol, cholesterol esters, carotenoids, and apoproteins to form a chylomicron.
- 6 Chylomicrons leave the intestinal cell and enter the lymph system and ultimately the blood.
- 7 Retinoic acid can directly enter the blood, where it attaches to albumin for transport to the liver.
- 8 Not pictured is carotene absorption by scavenger receptor class B type 1 (SR-B1) on the brush border membrane

Figure 10.3 Digestion and absorption of carotenoids and vitamin A and reesterification of retinol in the intestinal cell.

Within the enterocyte, newly formed retinyl esters combine with small amounts of unesterified retinol and any unmetabolized carotenoids for incorporation into chylomicrons. These chylomicrons, which also contain cholesterol esters, phospholipid, triacylglycerols, and apoproteins, are next released across the basolateral membrane by exocytosis into lymphatic capillaries (also called lacteals) for transport via initially the lymphatic system and then the circulatory system to tissues.

However, not all vitamin A is esterified and incorporated into the chylomicron within intestinal cells. Some additional fates of the vitamin occur within the enterocyte (as well as other cells) (shown in Figure 10.4 and later in Figures 10.5 and Figure 10.10).

- Small quantities of unesterified retinol may be released from the intestine and into portal blood.
- Some retinol that is bound to CRBP II is oxidized to retinal, and subsequently to retinoic acid within intestinal cells. This retinoic acid may be used for various functions, including regulation of gene expression, or it may be released into the blood and transported bound tightly to albumin (Figure 10.3).
- Retinol may also be used within some (primarily extraintestinal) tissues to produce 11-*cis* retinal that is needed for vision and 9-*cis* retinoic acid that functions in gene expression (discussed in more detail under the section “Functions and Mechanisms of Action”).
- Some retinol may be conjugated (especially in the liver but also in the intestine) to glucuronic acid to form retinoid/retinoyl β -glucuronide. The retinoyl β -glucuronide can enter the circulation through the portal vein; however, hepatic retinoyl β -glucuronide is usually excreted in the bile. Retinoyl β -glucuronide concentrations in the plasma are typically low. In tissues, retinoyl β -glucuronide appears to function in part like retinoic acid, promoting growth and cell differentiation, but not through interactions with nuclear retinoic acid receptors.
- Retinol, along with retinal and retinoic acid, may also undergo metabolism, especially in the liver, by the cytochrome P450 system to generate a variety of oxidized metabolites.

Transport, Tissue Uptake, and Storage

Chylomicrons, containing primarily newly formed retinyl esters, unmetabolized carotenoids (including provitamin A and nonprovitamin A), cholesterol esters, phospholipids, triacylglycerols, and apoproteins, enter first into the lymphatic system and then via the thoracic duct (thereby initially bypassing the liver) into general circulation (i.e., the blood) to enable the delivery of the vitamin to extrahepatic tissues. Some of the tissues that utilize the contents of the

chylomicrons include the bone marrow, blood cells, spleen, muscle, lungs, kidneys, and adipose tissue. The lungs, kidneys, and spleen are especially thought to take up retinyl esters from the chylomicrons along with adipose tissue, which may retain about 15–20% of the body’s vitamin A.

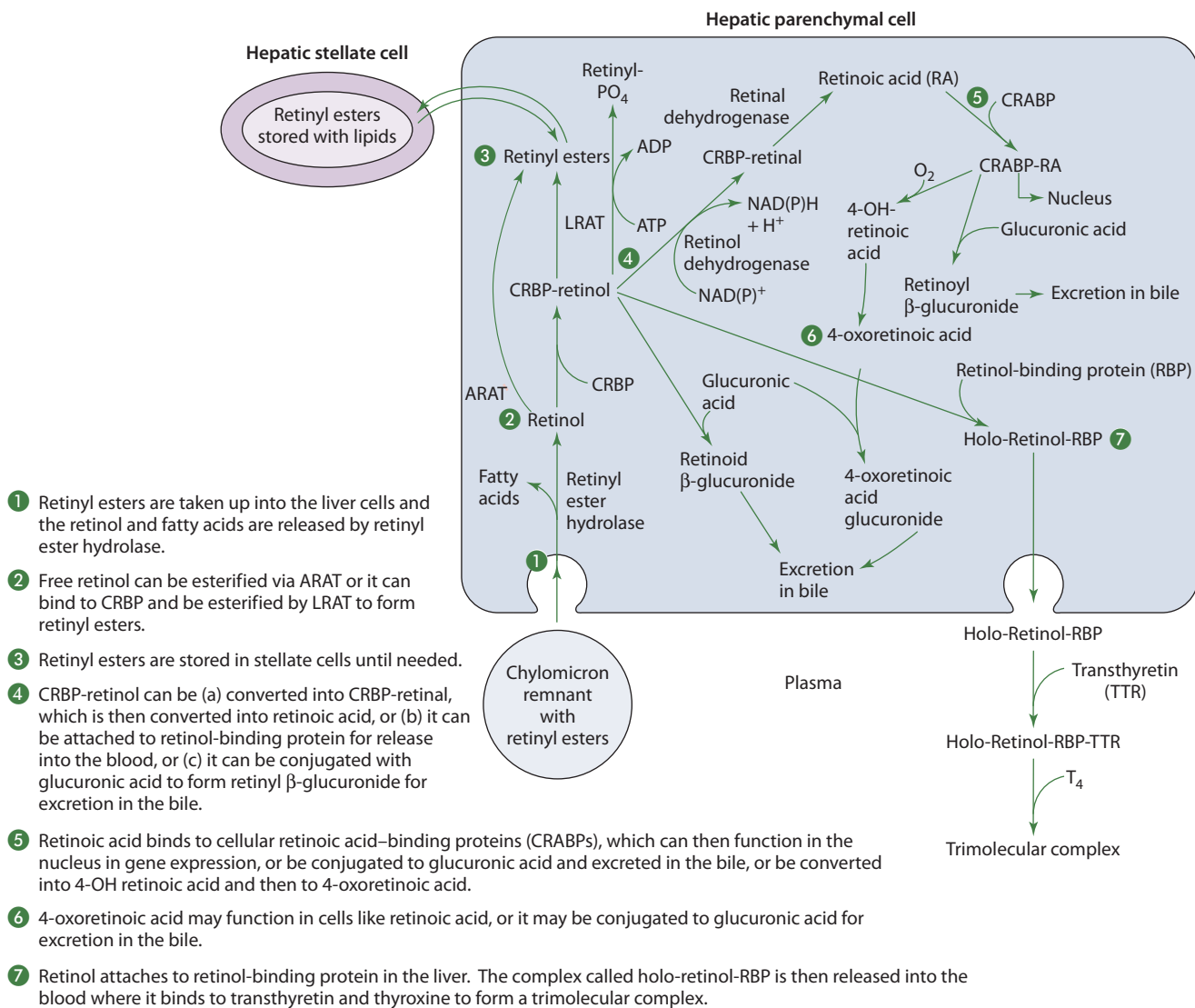
Chylomicron remnants are formed as lipids and vitamin A and other constituents are removed from the circulating chylomicrons. Retinyl esters and carotenoids not taken up by peripheral tissues are transported to the liver as part of the chylomicron remnant, which is taken up by receptor-mediated endocytosis and degraded by lysosomes. About 65–75% of chylomicron retinoids are cleared from circulation by the liver.

Vitamin A

The handling of retinyl esters that reach the liver is shown in Figure 10.5. Initially, the retinyl esters are hydrolyzed to retinol by a retinyl ester hydrolase following their uptake by the hepatic **parenchymal cells** (functional cells of an organ); the released retinol then binds to CRBP type I (CRBP1). CRBP1-bound retinol is typically directed within liver cells for conversion to retinal or retinoic acid (discussed later in the section “Functions”), for storage, or for release into the blood.

The handling of retinoic acid in cells differs from that of retinol. Retinoic acid is usually produced in small amounts from either retinal or β -carotene within cells, although small amounts may be taken up from the blood. Within the cell cytosol, the retinoic acid binds to cellular retinoic acid-binding proteins (CRABPs), which regulate cellular retinoic acid metabolism and direct its usage intracellularly. Metabolism and control of intracellular concentrations of retinoic acid occur in part through enzymes associated with cytochrome P450 family 26 (CYP26), which is found in the liver and brain, among other tissues. This system, for example, catalyzes the oxidation and glucuronidation of retinoic acid to generate polar metabolites such as 4-oxoretinoic acid (shown in Figures 10.4 and Figure 10.5). 4-Oxoretinoic acid appears to be involved, along with retinoic acid, in the synthesis of **gap junction** proteins needed for cell growth (see the “Functions and Mechanisms of Action” subsection under “Vitamin A”).

Vitamin A is stored predominately in the liver, with lesser quantities in the kidneys, lungs, and adipose tissue. Within the liver, small amounts of retinol are stored in the parenchymal cells, but most (80–95%) is stored in the liver’s perisinusoidal cells called **stellate cells** (also known as Ito cells), which constitute less than 15% of total liver cells. (Note that retinoic acid does not accumulate in appreciable amounts in the liver or other tissues.) In the stellate cells, vitamin A (retinol) is stored as retinyl esters (primarily retinyl palmitate, but also retinyl stearate, oleate, and linoleate) with lipid droplets. Hydrolases can release the retinol from its stores as needed for use, including release into the blood.



Hepatic stores of vitamin A mostly impact plasma concentrations of the vitamin when stores are very high or low. When hepatic vitamin A concentrations are less than 20 μg vitamin A/g of liver, plasma retinol concentrations usually decline. When stores are adequate but not excessive, plasma vitamin A concentrations remain fairly constant, even with a wide range of dietary intake. As hepatic concentrations of vitamin A reach $> 300 \mu\text{g/g}$ of liver, plasma concentrations rise. Hypervitaminosis A occurs with hepatic vitamin A concentrations $> 1 \mu\text{mol/g}$ liver. Toxicity occurs with hepatic vitamin A concentrations $> 10 \mu\text{mol/g}$ liver [3].

Vitamin A release from the liver and into the blood requires two proteins—retinol-binding protein (RBP) and transthyretin—that are synthesized primarily by hepatic parenchymal cells. RBP synthesis depends not only on adequate vitamin A but also on adequate zinc and protein status; RBP synthesis diminishes with dietary zinc and

protein inadequacies, causing the accumulation of vitamin A in the liver. Inflammation also impairs the release of the vitamin A–RBP complex (discussed next) from the liver.

For transport out of the liver, one molecule of retinol combines with one apoRBP to form a holo-retinol–RBP complex, also called simply holo-RBP (Figure 10.5). The retinol that attaches to apoRBP may have come from the diet or from released hepatic stores of the vitamin. If released from stores, the vitamin will be in the form of retinyl esters and will require a hydrolase to free the retinol. In the holo-retinol–RBP complex, the retinol is bound in an interior, hydrophobic region, since it is fairly insoluble in the aqueous environment of the blood. RBP in the plasma is about 85% saturated with retinol. Once the holo-retinol–RBP complex is formed, it interacts noncovalently with transthyretin, a tetrameric thyroid hormone (T_4) transport protein (also known as prealbumin) in the plasma. This interaction with transthyretin helps to stabilize the

complex and, due to its now larger size, reduces the likelihood of clearance/excretion by the kidneys.

The holo-retinol-RBP-transthyretin ternary complex represents the circulating source of vitamin A for extra-hepatic tissues. It has a half-life of about 11–15 hours in the plasma. Blood/serum concentrations of the complex remain fairly constant even when total hepatic retinol concentrations vary considerably. Normal plasma retinol concentrations range from about 30 to 86 $\mu\text{g/dL}$ (1 to 3 $\mu\text{mol/L}$).

Uptake of retinol from the holo-retinol-RBP-transthyretin complex by target tissues is mediated in a few tissues by RBP4 receptor 2 (RBPR2). Most tissues, however, take up the holo-retinol-RBP-transthyretin complex by a protein transporter called “stimulated by retinoic acid 6” (STRA6), which binds to the RBP portion of the complex to enable cellular retinol absorption. (Note that apo-RBP returns to the blood where it is either reused or taken up and degraded by the kidneys.) Retinol uptake by STRA6 also involves linkages with cytosolic LRAT for retinol esterification and CRBP1 for intracellular transport. STRA6 is found on most plasma membranes with the exception of the liver and intestines (colon). Some of the many tissues that use vitamin A from the complex include blood-brain epithelia, adipose tissue, skeletal muscle, heart, lungs, kidneys, eyes, white blood cells, and bone marrow.

Carotenoids

Carotenoids reaching the liver via chylomicron remnants also typically undergo metabolism, including storage, cleavage, and/or release into the blood for transport to other tissues. Carotenoids being released into blood are incorporated into very low-density lipoproteins (VLDLs) and can be transferred among the various lipoproteins. Within the lipoproteins, the carotenoids differ in position; some carotenoids, such as β -carotene, concentrate in the hydrophobic core, whereas others with polar groups are found partly covering the lipoprotein surface. In a fasting state, β -carotene and α -carotene are transported mainly in low-density lipoproteins (LDLs) and VLDLs, while lutein, zeaxanthin, and β -cryptoxanthin are transported mainly in LDLs and high-density lipoproteins (HDLs) with smaller amounts in VLDLs. Overall, the transport of carotenoids in the blood is accomplished mainly by LDLs (55%), followed by HDLs (31%) and VLDLs (14%) [4]. The most common carotenoids in the blood are β -carotene, α -carotene, lycopene, lutein, zeaxanthin, and β -cryptoxanthin. The half-life of the carotenoids in the blood varies with β -carotene at less than 2 weeks, lycopene at about 12 days to 1 month, and lutein and zeaxanthin at about 1–2 months [4]. Serum carotenoid concentrations are most reflective of dietary intake and not body stores.

The uptake of carotenoids into target tissues and their storage differs from that of retinol. Carotenoid uptake occurs as part of the lipoprotein and is mediated by

apoprotein receptors. Scavenger receptor class B type 1 (SR-B1) may also be involved in the uptake of some carotenoids from the lipoproteins to tissues and vice versa. Carotenoids, like vitamin A, are stored mainly in the liver, followed by adipose tissue, with lesser amounts in the kidneys, skin, and lungs; however, some tissues concentrate specific carotenoids. For example, the retina of the eye and some regions of the central nervous system are relatively rich in lutein and zeaxanthin. Moreover, levels of lutein and zeaxanthin in some tissues, including the brain, appear to be associated with the presence of glutathione S transferase (GSTP1) and a specific membrane protein, StARD3. The significance of these interactions is not clear.

Functions and Mechanisms of Action

Vitamin A

Vitamin A is recognized as being essential for vision as well as for cellular differentiation, growth, reproduction, bone development, and immune system functions. In the case of vision, it is the 11-*cis* retinal form that binds to the protein opsin. For the other biological processes, it is all-*trans* retinoic acid and 9-*cis* retinoic acid that are needed.

Vision Several parts of the eye work together to ensure vision. For example, light enters the eye through the cornea, the outermost tissue that covers the front of the eye. The muscles of the iris adjust the size of the pupil in response to the dimness or brightness of the light. The pupil becomes larger in darker lighting and becomes smaller in brighter lighting. The light passes through the lens and the vitreous humor (which shapes the eye) and hits the retina, the inner lining at the back of the eye. The retina contains specialized cells, called rods and cones, which contain photo- or light receptor pigments and mediate phototransduction (the process by which light is converted into electrical signals for the brain). Cone cells facilitate clear vision with color in bright light (day). As darkness falls or light dims, the rod cells serve as the photo- or light receptors. In other words, in brighter environments cone cells are used, and when shifting into a darker environment, the rod cells are used. Rod cells contain the photo-sensitive compound **rhodopsin**, which consists of vitamin A as 11-*cis* retinal and the protein opsin. Rhodopsin facilitates the capture and conversion of a photon into a series of signals (chemical in nature) to enable clearer vision in dimly lit environments. This process is reviewed in the next few paragraphs.

The Visual Cycle: Simplified Overview In basic terms (shown in Figure 10.6), when a flash of light hits the retina (as may occur when leaving a dark movie theater), a series of events occurs: the *cis*-retinal portion of rhodopsin is converted to *trans*-retinal, the rhodopsin molecule is cleaved, and signals are sent to the part of the brain

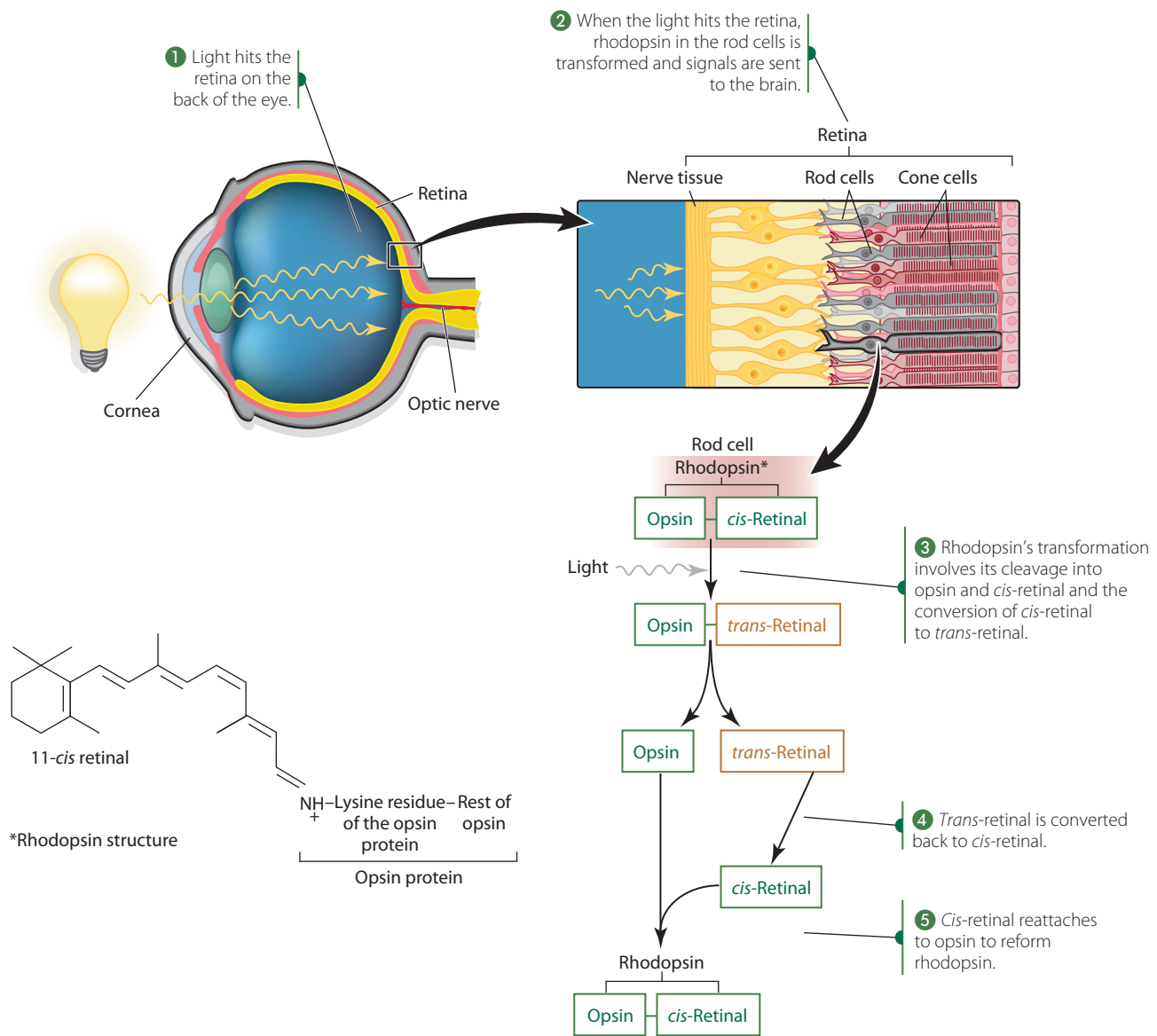


Figure 10.6 An overview of the role of vitamin A as a part of rhodopsin in vision.

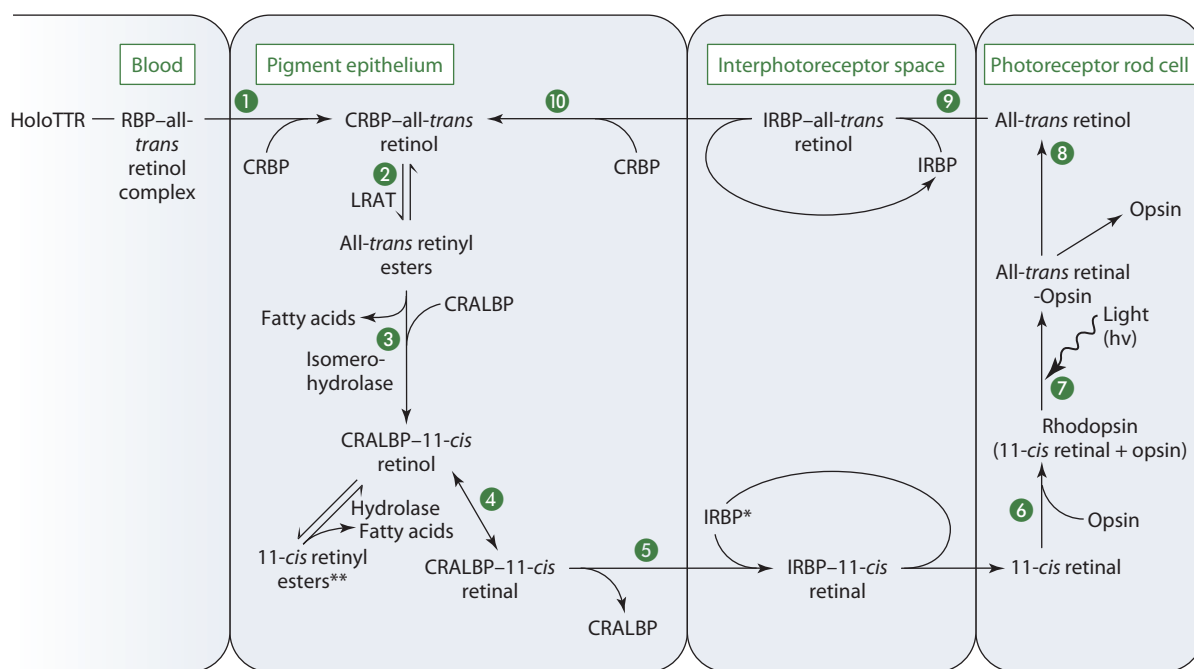
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involved with eyesight. To regain vision in the dark (e.g., reentering the dark movie theater), the rhodopsin molecule must be remade using vitamin A as *cis*-retinal. The *trans*-retinal (generated when light “hits” the eye) is transported from the photoreceptor rod cells into the pigment epithelium of the retina, where it is converted back to *cis*-retinal. The *cis*-retinal is then transported back to the rod cell, where it reattaches to opsin to form rhodopsin. Inadequate vitamin A disrupts the ability to reform rhodopsin and results in a failure of vision in the dark (dim light), a condition known as night blindness.

Vitamin A Metabolism as Part of the Visual Cycle Figure 10.7 shows the visual cycle (the formation and reformation of rhodopsin after its degradation) in more detail. This section focuses on how vitamin A is metabolized as part

of the visual cycle. Retinol is transported to the retina via the blood as part of the holo-retinol-RBP-transthyretin complex. Following the binding of the complex to receptors on the pigment epithelium (a pigmented cell layer) adjacent to the photoreceptor rod cells (Figures 10.7 and Figure 10.8), retinol enters the pigment epithelium cells and binds to CRBP. Much of the retinol is then converted by LRAT to all-*trans* retinyl esters, and some is isomerized into 11-*cis* retinyl esters. The all-*trans* retinyl esters in turn may be stored in the pigment epithelium and metabolized by an all-*trans* retinyl ester isomerohydrolase, as needed, to generate 11-*cis* retinol plus a fatty acid. Similarly, the 11-*cis* retinyl esters may be hydrolyzed, as needed, to 11-*cis* retinol and a fatty acid by retinyl ester hydrolase.

For the visual cycle to operate effectively, the oxidative conversion of 11-*cis* retinol to 11-*cis* retinal is necessary.



* IRBP: Interphotoreceptor retinol-binding protein.

** Stored until needed.

- 1 All-trans retinol moves out of the blood [where it is found as part of a complex with transthyretin (TTR) and retinol-binding protein (RBP)] and into the pigment epithelium adjacent to the rod cell. In the pigment epithelium it attaches to CRBP (cellular retinol-binding protein).
- 2 All-trans retinol is converted into all-trans retinyl esters by LRAT.
- 3 All-trans retinyl esters are converted to 11-cis retinol, which is then attached to cellular retinal-binding protein (CRALBP).
- 4 11-cis retinol is converted to 11-cis retinal while attached to CRALBP.
- 5 11-cis retinal detaches from CRALBP and attaches to interphotoreceptor retinol-binding protein (IRBP) for transport across the interphotoreceptor space and into the photoreceptor rod cell. IRBP releases the 11-cis retinal upon delivery into the photoreceptor rod cell.
- 6 11-cis retinal attaches to opsin to form rhodopsin.
- 7 Light hits the rod cell causing the cleavage of rhodopsin.
- 8 All-trans retinal is first converted to all-trans retinol before being ultimately converted back to 11-cis retinal.
- 9 All-trans retinol attaches to IRBP for transport across the interphotoreceptor space and into the pigment epithelium.
- 10 All-trans retinol is released from IRBP and attaches to CRBP in the pigment epithelium to enter the cycle again at 2.

Figure 10.7 The visual cycle.

The 11-cis retinal is next transported from the pigment epithelium into the photoreceptor rod cells (and thus across the interphotoreceptor matrix/space) by interphotoreceptor (also called interstitial) retinol-binding protein (IRBP); this protein resides within the retina's interphotoreceptor space that lies between the pigment epithelium and the photoreceptor rod cells.

Within the photoreceptor rod cells, 11-cis retinal binds as a protonated Schiff base to a lysine amino acid residue in the protein opsin (Figure 10.6) to produce the rhodopsin. Rhodopsin is embedded in disks located in the rod's outer segment, which is enclosed within a restricted compartment of the retina created by tight junctions between cells (Figure 10.8). The cells on the blood side are one layer thick and form the pigment epithelium. The "outer

limiting membrane" is formed on the vitreal side of the photoreceptor cells by specific junctions between the photoreceptor cells and the Müller cells (Figure 10.8).

Within the photoreceptor cells, the rhodopsin responds to small amounts of light. When a quantum of light (hv) hits the rhodopsin, changes occur in the vitamin A portion of the molecule such that 11-cis retinal is photoisomerized to generate all-trans retinal (Figures 10.6 and Figure 10.7). The term *bleaching* is often used to describe this event because a loss of color occurs. A chain of events triggered by the absorption of light by 11-cis retinal results in an electrical signal or impulse to and along the optic nerve to the brain. To transmit through the cell to the plasma membrane the message that light has hit the rhodopsin, a cascade of reactions involving **transducin** (a G protein),

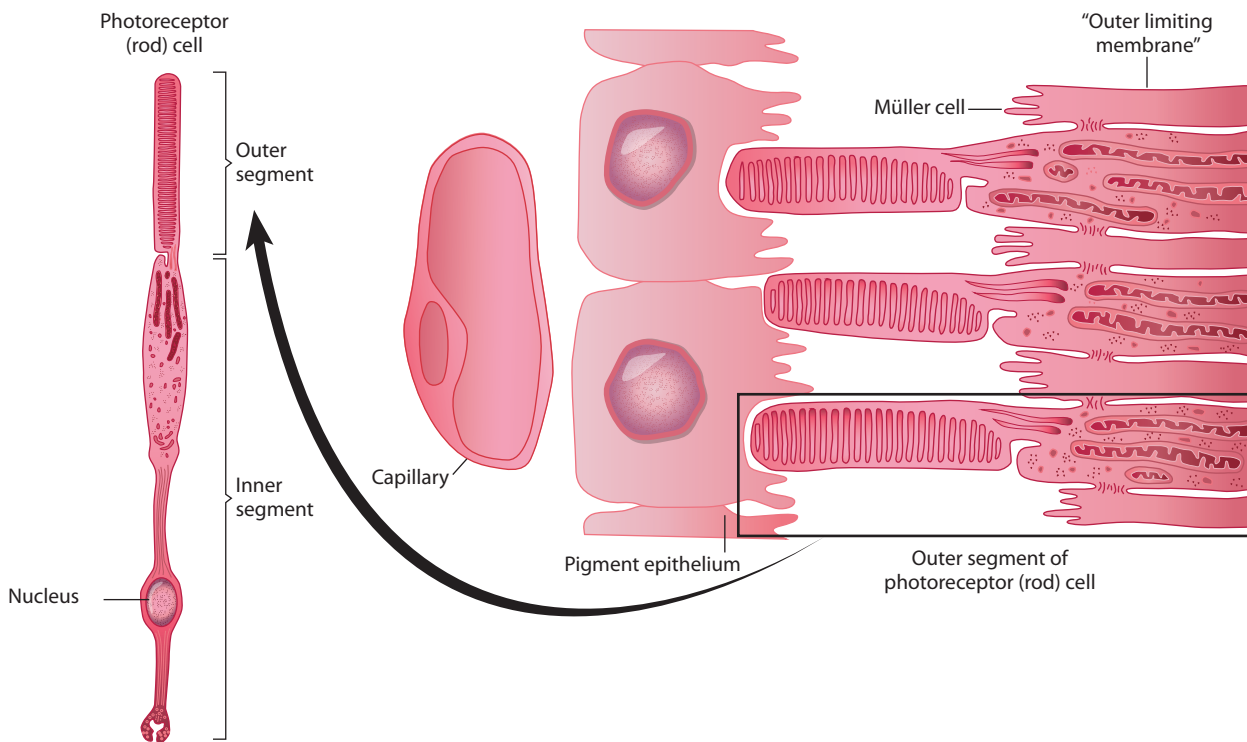


Figure 10.8 Photoreceptor (rod) cells, their structure, and their surroundings.

phosphodiesterase, and cGMP occurs. This reaction cascade causes sodium channels in the plasma membrane to be blocked and the rod cell to hyperpolarize, resulting in signals by the optic nerve leading to specific areas of the brain.

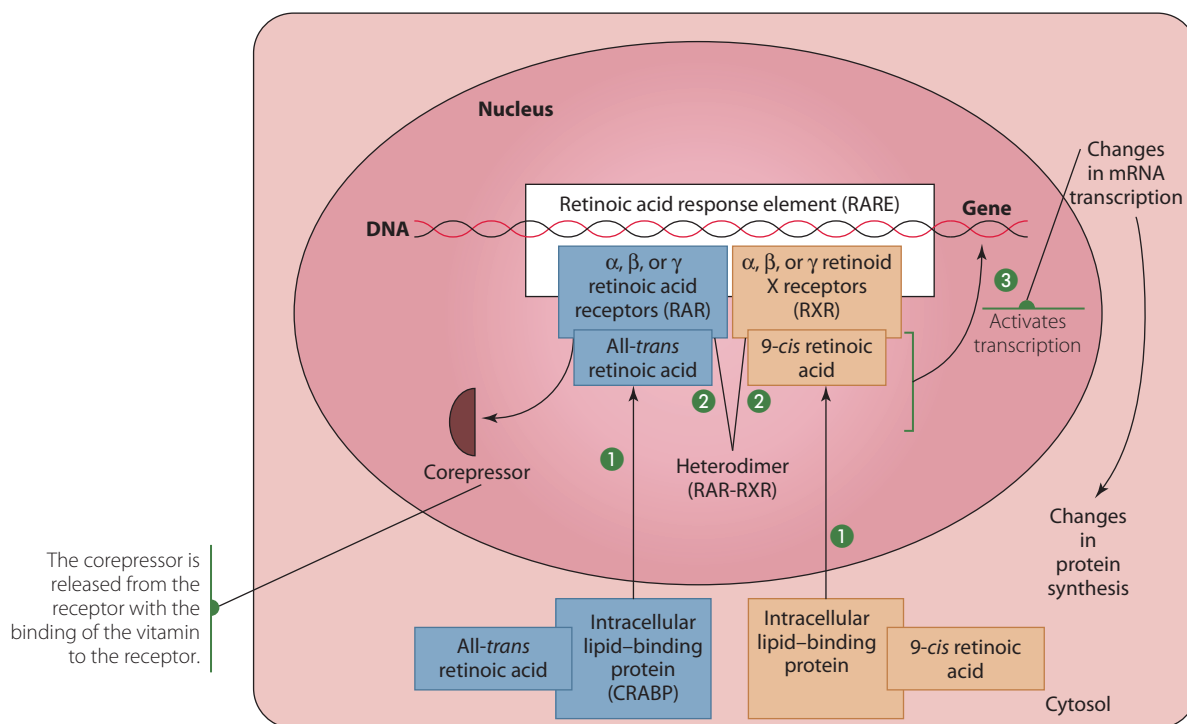
The all-*trans* retinal formed as a result of light must be converted back to 11-*cis* retinal, and the rhodopsin must be regenerated. The steps for this conversion are thought to involve hydrolysis of all-*trans* retinal from the rhodopsin, reduction of all-*trans* retinal to all-*trans* retinol, and transport of all-*trans* retinol across the interphotoreceptor matrix and into the retinal pigment epithelium by IRBP (Figure 10.7). Within the pigment epithelium, the all-*trans* retinol may be metabolized as initially described—that is, in the same manner as upon uptake into the pigment epithelium—to repeat the visual cycle.

Gene Expression Two forms of vitamin A, all-*trans* retinoic acid and the isomer 9-*cis* retinoic acid (both generated from retinol), exert genomic effects to regulate gene expression following their transport into the nucleus and their binding to specific nuclear receptors. Transport of the vitamin into the nucleus for interactions with nuclear receptors is carried out by two cytosolic intracellular lipid-binding proteins (iLBPs): cellular retinoic acid-binding protein (CRABP) II and fatty acid-binding protein (FABP) 5.

Once within the nucleus, the vitamin interacts with nuclear receptors. Nuclear receptors include retinoic acid receptors (RAR) and retinoid X receptors (RXR) (with each group made of three isotypes designated α , β , and

γ) as well as peroxisome proliferator-activated receptors (PPAR) and retinoid-related orphan receptors (ROR). All-*trans* retinoic acid binds all three RAR isotypes as well as ROR and PPAR isotype β/δ . The isomer 9-*cis* retinoic acid binds RAR as well as the three RXR isotypes. The binding of all-*trans* and 9-*cis* retinoic acid to their respective nuclear receptors results in conformational changes that alter the receptor functions (Figure 10.9).

The vitamin-receptor complex functions (i.e., activity), however, are also influenced by other factors. For example, some vitamin-receptor complexes, such as all-*trans* retinoic acid-ROR, function as a monomer, interacting with ROR response elements (RORRE) located in the promoter regions of specific genes on DNA. In contrast, other vitamin-receptor complexes must interact with another receptor complex, forming either **homodimers** or **heterodimers** before interacting with the DNA. A homodimer is formed when two of the same receptors interact, such as RXR-RXR. A heterodimer is formed between two or more different receptors, such as RXR-RAR, VDR (vitamin D receptor)-RXR, RXR-PPAR, and RXR-TR (thyroid hormone receptor). The ability of the heterodimer to enable transcription upon interacting with specific domains within the response elements occurs in part through the actions of histone acetyl transferase (note niacin and biotin also play roles in histone acetylation and deacetylation). This enzyme acetylates histone proteins, allowing the chromatin to “open” and transcription activity to proceed. In contrast, gene transcription may also be suppressed by the actions of histone deacetylase, which



- 1 All-*trans* or 9-*cis* retinoic acid moves into the nucleus of the cell bound to binding proteins.
- 2 All-*trans* retinoic acid binds to retinoic acid receptors (RAR) and 9-*cis* retinoic acid binds to retinoid X receptors (RXR).
- 3 Binding of the vitamin to the nuclear receptors and interaction with response elements on the DNA enhances the transcription of selected genes. The absence of bound vitamin A usually results in the repression of gene transcription.

Figure 10.9 Hypothesized mode of action for retinoic acid on gene expression.

removes acetyl groups from the histones and promotes chromatin condensation.

Other factors influencing vitamin–receptor complex activity and thus gene expression include the need for coactivators and/or the removal of corepressors that are attached to the DNA receptor. Typically, as vitamin A binds to a receptor, the resulting conformational change causes the release of the corepressors—and (in some cases) the recruitment of coactivators. An example of a nuclear corepressor includes silencing mediator for retinoid and thyroid hormone receptors, among others. When no vitamin A is present, the corepressors are typically found attached to the nuclear receptors and bound to the response elements on the DNA.

The genes affected by retinoic acid are mostly unknown but are thought to code for multiple proteins including enzymes, growth factors, and transcription factors, among others, and impact a wide variety of cellular processes. *Cell death*, or *apoptosis*, is an example of a cellular process regulated in part by retinoic acid. The vitamin is delivered by CRABP to the cell nucleus, where it binds to RAR and interacts with a RARE to up-regulate the expression of caspase 9 and Bcl2, two proteins involved in the apoptotic pathway (see Chapter 1 for further information on apoptosis). Another process affected in part by the genomic actions of vitamin A is *osteogenesis*, which appears to be

repressed by the ROR β . *Lipolysis* is activated by vitamin A interactions with the nuclear PPAR β/δ receptors, while *adipocyte differentiation* is inhibited by retinol, retinal, and β -carotene, among others.

Retinoic acid, typically as all-*trans* retinoic acid, may also indirectly affect gene expression by initiating events that lead to changes in gene expression. For example, all-*trans* retinoic acid as well as retinol may stimulate (via kinase activity) the phosphorylation of selected binding proteins, which (once phosphorylated and translocated to the nucleus) bind to response elements to affect gene expression (and signaling, discussed in the next section). Another nongenomic action is known as retinoylation, in which retinoic acid is attached post-translationally to selected proteins. One impact of retinoylation appears to be enhanced *cell differentiation*.

Signaling Vitamin A, as retinol, in complex with RBP and transthyretin, serves as an extracellular signal compound upon attachment to the cell membrane receptor protein STRA6. This attachment, which promotes cellular uptake of retinol, also induces phosphorylation of a specific tyrosine residue on STRA6. This phosphorylation then activates a specific signaling cascade (called signal transducers and activators of transcription [STAT]/janus kinase [JAK]–mediated signaling cascade) to affect gene

expression and various cellular processes. Certain activated STATs (several are present in cells, but only certain ones are activated by this cascade), after a series of additional reactions, form dimers that translocate to the nucleus and serve as transcription factors for the expression of genes involved in the regulation of the *insulin response* and *lipid metabolism*. STRA6 alone also functions as a surface signaling receptor that can be activated by hormones, cytokines, and growth factors.

Cell Differentiation, Proliferation, and Growth Vitamin A, as retinoic acid, is also involved in cell differentiation, the process by which an immature cell is transformed into a specific type of mature cell. Epithelial cells, found as part of our skin and in all internal body tracts, such as the respiratory, gastrointestinal, and urogenital tracts, are one example of cells that require vitamin A. Retinoic acid helps maintain both the normal structure and the functions of epithelial cells. For example, retinoic acid directs the differentiation of **keratinocytes** (immature skin cells) into mature epidermal cells through interactions with nuclear chromatin and thus changes in gene expression. With vitamin A deficiency, keratin-producing cells replace mucus-secreting cells in many body tissues, especially the eyes, skin, gastrointestinal tract, and trachea, and disrupt normal tissue properties and functions.

In addition to epithelial cells, retinoic acid is thought to regulate the proliferation and differentiation of some myeloid precursor cells in the bone marrow. Progenitor cells in the bone marrow, for example, differentiate into immature dendritic cells with adequate retinoic acid. The dendritic cells, which mature after exposure to an antigen, function to present antigens to other immune system cells, such as T-cells, to augment the body's immune response. Retinoic acid plays many additional roles involved with immune system functions, including in situations involving an inflammatory response.

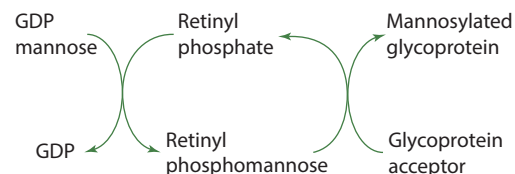
Retinoids affect a variety of other cell processes via multiple mechanisms. In hematopoietic cells, retinoic acid mediates insulin receptor substrate (IRS) 1 levels by stimulating the binding of the ubiquitin–proteasomal complex to IRS1; this attachment induces the degradation of IRS1. IRS1 regulates cell proliferation and survival in selected cells.

The vitamin's ability to impact growth appears to be cell-specific, whereby it enhances proliferation and survival in some cells, whereas, in others, it induces differentiation, cell cycle arrest, and/or apoptosis. Interactions between retinoic acid and RAR receptors, for example, can inhibit cell growth of some tumors. Alternately, retinoic acid interactions with PPAR β/δ stimulate cell growth and inhibit apoptosis.

Another possible means by which growth is influenced may involve effects on gap junctions. Retinoic acid and 4-oxoretinoic acid, generated from retinoic acid, have

been shown to increase the synthesis of specific gap junction connexin proteins by stabilizing their mRNA. Gap junctions (cell-to-cell channels formed from connexin proteins) are important for the exchange of small signaling compounds and thus for cell-to-cell communication. A lack of gap junction communication results in uncontrolled cell growth, as can occur with cancer cells. Thus, vitamin A, in preserving this communication, plays a role in the control of cell growth.

Retinoic acid also may be able to modify cell surfaces, possibly by increasing glycoprotein synthesis at the gene level or by improving the attachment of glycoproteins to cell surfaces to induce cell adhesion. The production of fibronectin, which binds to collagen and other proteins and plays roles in cell differentiation, adhesion, and growth (and thus normal tissue maintenance and wound healing), appears to be under the influence of retinoic acid. Retinol may also play a more direct role in glycoprotein synthesis. CRBP-bound retinol, after undergoing phosphorylation using ATP in the liver among other tissues, generates retinyl phosphate. Retinyl phosphate can be converted to retinyl phosphomannose (also called mannosyl retinyl phosphate) in the presence of GDP mannose. Retinyl phosphomannose can donate its mannose to glycoprotein acceptors, which then become mannosylated glycoproteins. Such changes in the glycan portion of the glycoprotein can affect differentiation of cells through their effects on cell recognition, adhesion, and cell aggregation. These reactions (which have been suggested as a coenzyme role) involving vitamin A and glycosylation are shown here.



Other Functions Vitamin A, as retinol but not as retinoic acid, is essential for *reproductive processes* in both males and females, although the mechanism(s) of its action(s) is unclear. *Bone development and maintenance* also require vitamin A; either too much or too little of the vitamin negatively impacts bone mineral density. Vitamin A is thought to be involved in the regulation of **osteoblasts** (bone-forming cells) and/or **osteoclasts** (cells participating in bone resorption). Although the mechanism of action is unclear, vitamin A deficiency results in excessive deposition of bone by osteoblasts and reduced bone degradation by osteoclasts. Excess vitamin A, in contrast, stimulates osteoclasts and inhibits osteoblasts, decreasing bone mineral density and increasing fracture risk. Vitamin A is also involved in iron metabolism (see “Nutrient Interactions”).

Several aspects of *immune system function*, both humoral and cell-mediated, are influenced by vitamin A. Retinoic acid, produced in some immune cells such as antigen-presenting cells, stimulates phagocytic activity, cytokine production, and natural killer cell activity. The vitamin's actions on immune functions may be mediated at least in part through effects on gene transcription. Depletion studies suggest that vitamin A appears to be needed for T-lymphocyte function and for antibody response to viral, parasitic, and bacterial infections. Thus, people with vitamin A deficiency may have an impaired ability to resist and fight infections and increased morbidity and mortality risk. Supplementation with vitamin A has been shown to reduce death associated with measles and diarrhea in developing countries where vitamin A deficiency is common.

Another role of vitamin A, likely mediated by effects on gene expression, cell differentiation, and growth, is in *morphogenesis/embryogenesis*. Specifically, retinoic acid is thought to act as a morphogen in embryonic developments; nuclear retinoic acid receptors have been found in different cells during different times of development in the embryo.

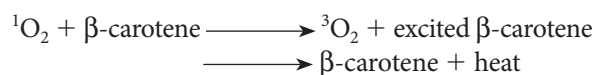
Retinoids and Cancer Because of its ability to induce differentiation and apoptosis and to inhibit proliferation, retinoids have been used as chemotherapeutic agents for treating some cancers. Some retinoids (and chemically related derivatives of retinoids) effectively promote cell cycle arrest and terminal differentiation of cancer cells. All *trans*-retinoic acid (tretinoin), for example, is used in the treatment of lymphomas, acute promyelocytic leukemia, cervical cancer, lung cancer, neuroblastoma, and glioblastoma, while 13-*cis* retinoic (isotretinoin) and other synthetic retinoids are undergoing clinical trials for the treatment of other cancers, including squamous cell skin cancer and neuroblastoma, among others. Transformations and modifications of retinoids are being tested for their antiproliferative and/or pro-differentiative activities and cytotoxic actions on various cancer cell lines.

Carotenoids

Carotenoids are present mainly in lipoproteins and in cell membranes (with some like β -carotene and lycopene in interior sections due to their hydrophobic structure, and some with polar functional groups like zeaxanthin present closer to the membrane surface). The mechanism by which the carotenoids function is linked in part to their structure, more specifically their extended system (often nine or more) of conjugated double bonds that make them soluble in lipids and capable of quenching singlet oxygen and free radicals (atoms or molecules with one or more unpaired electrons).

Antioxidant The carotenoids function as antioxidants, with the ability to react with and quench singlet oxygen and free radicals. Because of their location in membranes,

carotenoids are especially valuable in the protection of cell membranes, whereas vitamin C, which is water soluble, is more active in aqueous environments such as the cytosol of cells and the blood. **Quenching** is a process by which electronically excited molecules or atoms, such as singlet oxygen, are inactivated. Singlet molecular oxygen, also called singlet oxygen, $^1\text{O}_2$, is a very reactive and destructive compound. Singlet oxygen, like free radicals, readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. Carotenoids can react with (quench) singlet oxygen, and it is the conjugated double-bond systems within the carotenoids that permit the quenching. The singlet oxygen ($^1\text{O}_2$) transfers its excitation energy and returns to the ground state ($^3\text{O}_2$), and the carotenoid receiving the energy enters an excited state. Resonance states in the excited carotenoid allow some stabilization. Carotenoids then release the energy in the form of heat.



In addition to quenching singlet oxygen, carotenoids have the ability to react directly with free radicals (radical scavenge), especially peroxy radicals ($\text{ROO}^\bullet$), which cause lipid peroxidation. Such actions by carotenoids decrease circulating peroxide concentrations and reduce lipid peroxidation (thus helping to minimize damage to cells). Radical scavenging by carotenoids involves the donation of a hydrogen atom or an electron and results in the formation of a carotenoid radical. Electron delocalization over the carotenoid's conjugated double bond system stabilizes the carotenoid.

Although most studies have focused on β -carotene, other carotenoids have higher capacities to donate an electron or hydrogen. Of those studied, lycopene appears to have the highest capacity, followed in descending order by α -tocopherol (vitamin E), α -carotene, β -cryptoxanthin, zeaxanthin, β -carotene, and lutein. However, carotenoids can act synergistically and, in combination with vitamins (such as vitamin E), can be more effective than any one carotenoid itself. In the eyes, for example, the carotenoids lutein and zeaxanthin are important in visual protection and acuity and in shape discrimination. There is also growing evidence that suggests these carotenoids may be critical during prenatal periods for visual and brain development. Zeaxanthin and lutein exert antioxidant functions in the eyes through their abilities to absorb photons of sunlight, including blue light. These actions are thought to serve protective antioxidant functions. Additional interactions between antioxidant nutrients are discussed in the Perspective at the end of this chapter.

Other Roles Carotenoids, like retinoids, also inhibit *cell proliferation* and stimulate *cell differentiation* while

also affecting *growth and apoptosis*. A few examples are provided. β -cryptoxanthin may enhance bone anabolism via effects on osteoclast and osteoblast activities. Lycopene inhibits the growth and proliferation of various cancer cells and induces cell differentiation, and canthaxanthin and β -carotene have been shown *in vitro* to inhibit carcinogen-induced neoplastic transformation. Carotenoids, like retinoids, also enhance or induce gap junction communication. Some carotenoids, for example, up-regulate the *gene expression* of proteins, such as connexin, that enable connections (bridges) to form between cells; such actions in turn affect cell proliferation and growth.

Another means by which some carotenoids (such as lycopene and various carotenoid metabolites) affect gene expression is through nuclear factor E_2 -regulated factor 2 (Nrf2). Nrf2 is a transcription factor that is found in the cell cytosol typically in association with a specific protein. Increased cytosolic concentrations of selected carotenoids or carotenoid metabolites are thought to facilitate the uncoupling of the protein from Nrf2, which enables the Nrf2 to translocate to the nucleus and act as a transcription factor to enhance the expression of genes coding for a group of proteins known as phase II/antioxidant detoxifying enzymes. Nrf2 plays significant roles during carcinogenesis and is considered a molecular target for cancer prevention. The phase II/antioxidant detoxifying enzymes provide defense in response to oxidation and other “stresses” on cells. Although much additional research is needed, the expression of other genes such as those coding for enzymes involved in apoptosis may also be affected by the carotenoid metabolites.

Selected Pharmacological Uses/Other Roles Because of the ability of carotenoids to react with free radicals and quench singlet oxygen, carotenoids are thought to be protective against some conditions/diseases.

Eye Health Free radicals are thought to contribute to the development of *cataracts*, which affect the lens of the eye, and the development of *age-related macular degeneration*, which affects the macula in the center of the eye’s retina. Both conditions contribute to the development of blindness in older adults. As discussed under “Vitamin C” in the subsection “Selected Pharmacological Uses/Other Roles,” carotenoids such as lutein and zeaxanthin, which are found in the macula, as well as other carotenoids, such as β -carotene, can inhibit the oxidation of cell membranes and thus may be protective against eye damage. Mixed results have been documented in studies examining dietary intakes and/or plasma concentrations of carotenoids (lutein, zeaxanthin, and/or β -carotene) and risk of cataracts and macular degeneration; while inverse correlations have sometimes been reported, meta-analyses have concluded

that carotenoids as part of antioxidant supplementation do not lower the risk or slow the progression of cataracts or of age-related macular degeneration. However, one older study, the Age-Related Eye Disease Study (AREDS), a randomized, placebo-controlled 5-year clinical trial, which provided a combination of antioxidants [including vitamin C (500 mg), vitamin E (400 IU), beta-carotene (15 mg), zinc (80 mg), and copper (2 mg)], reported modest effects in slowing the progression of age-related macular degeneration [4]. No additional benefits were found in a second 5-year trial, AREDS2, which supplemented those with macular degeneration with a modification of the original formulation [adding omega-3 fatty acids (1,000 mg), lutein (10 mg), and zeaxanthin (2 mg), removing β -carotene, and reducing zinc (25 mg)] [5].

Heart Health Carotenoids have been shown *in vitro* to reduce/prevent the oxidation of LDL and cell membrane lipids. Increased blood concentrations of reactive oxygen and nitrogen species promote LDL oxidation and cell membrane lipid oxidation, factors contributing to the development of atherosclerotic plaque in blood vessels and heart disease. Supplementation with carotenoids, however, has not been shown to prevent or improve heart disease in most studies and at present, supplementation is not recommended.

Cancer Carotenoids are thought to deter or protect against cancer via several means, including regulation of cell growth and cell cycle progression, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of intercellular gap junction communication, immune modulation, and apoptosis, among others. Intervention trials using β -carotene, however, have failed to show protective effects. Moreover, two intervention trials conducted back in the 1990s providing 20 mg of β -carotene along with other antioxidants (including vitamin A) found an increased risk of lung cancer and of mortality from cancer and heart disease in a high-risk population (smokers and those with asbestos exposure) [6,7]. Studies examining relationships between β -carotene and other cancers, including prostate cancer, report mixed results. Moreover, additional studies in less high-risk populations have not found beneficial effects from β -carotene supplementation in reducing the risk of cancer or mortality from cancer. The Institute of Medicine states that “beta-carotene supplements are not advisable for the general population” [2]. Yet, while a multitude of studies do not support the use of supplements in disease prevention, many epidemiological studies have shown that people with high intakes of fruits and vegetables, which are also rich in carotenoids, have a lower incidence of many chronic diseases. Thus, individuals should be encouraged to obtain dietary carotenoids through the ingestion of a variety of fruits and vegetables.

Carotenoids and Health Claims Health claims that are approved by the FDA are targeted at carotenoid-rich foods (fruits and vegetables) as well as grain products. These claims require the food to be low in fat and a good source of dietary fiber without fortification. An example model claim is “Low-fat diets rich in fiber-containing grain products, fruits and vegetables may reduce the risk of some types of cancer, a disease associated with many factors” [8]. Another sample claim for fruits, vegetables, or grain products that are low in (saturated and total) fat and cholesterol and contain at least 0.6 g of soluble fiber per reference amount without fortification is “Diets low in saturated fat and cholesterol and rich in fruits and vegetables and grain products that contain some types of dietary fiber, particularly soluble fiber, may reduce the risk of heart disease, a disease associated with many factors” [8]. Another model claim—“Low-fat diets rich in fruits and vegetables (foods that are low in fat and may contain dietary fiber, vitamin A, or vitamin C) may reduce the risk of some types of cancer, a disease associated with many factors”—has also been approved for fruits and vegetables that are a good source of vitamin A or C or dietary fiber [8].

Interactions with Other Nutrients

Vitamin A and carotenoids interact with vitamins E and K. Excess dietary vitamin A intake interferes with vitamin K absorption. High β -carotene intake, in turn, may decrease plasma vitamin E concentrations.

Protein and zinc influence vitamin A status and transport. First, the activity of carotenoid dioxygenase, which cleaves β -carotene, is depressed by an inadequate protein intake. Second, overall vitamin A metabolism is closely related to protein and zinc status because the transport and use of the vitamin depend on two vitamin A-binding proteins and because zinc is required for protein synthesis as well as for alcohol dehydrogenase activity, which converts retinol to retinal. Impairments in the synthesis of

retinol-binding protein and transthyretin have been shown to diminish retinol mobilization from the liver.

Iron metabolism is also interrelated with both carotenoids and vitamin A. Iron is a cofactor for BCO1, the enzyme responsible for the conversion of β -carotene into retinal. Vitamin A (likely as retinoic acid) is involved in iron absorption, mobilization, and utilization. The vitamin plays a role in hematopoiesis as well as regulating the expression of several genes involved in iron utilization, including ferroportin, which is required for iron release from cells. Vitamin A deficiency is associated with decreased incorporation of iron into red blood cells and diminished mobilization of iron from stores, resulting in low red blood cell counts and low hemoglobin concentrations. Thus, vitamin A deficiency, usually chronic, can be associated with iron-deficiency anemia, and in this situation supplementation of vitamin A (as opposed to supplementation with iron) improves indices of iron status.

Metabolism and Excretion

While small amounts of vitamin A may be expired by the lungs as CO₂, the vitamin is excreted primarily in the urine and feces with the relative amounts varying based on vitamin intake. Urinary excretion of vitamin A metabolites usually accounts for up to about 60% of vitamin A excretion, and fecal excretion accounts for the remaining 40%; however, with higher intakes, fecal excretion generally exceeds urinary excretion [1]. For urinary excretion, retinol and retinoic acid are typically oxidized at the β-ionone ring and then conjugated to generate polar, water-soluble metabolites. Many of these metabolites, especially those that are short chain and acidic, are excreted by the kidneys. Oxidized products of vitamin A, containing intact chains and conjugated to glucuronic acid (such as retinoic acid glucuronide and 4-oxoretinoic acid glucuronide [Figure 10.10]) or to taurine, are generally secreted into the bile for ultimate fecal excretion. Some polar vitamin A metabolites secreted into the bile, such as 4-oxoretinoic

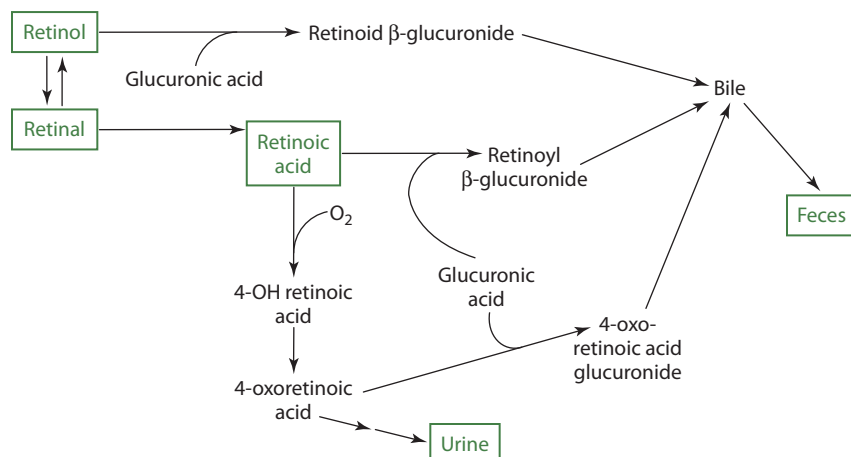


Figure 10.10 Metabolism of vitamin A, showing some excretory products that are secreted into the bile for removal from the body.

acid glucuronide, however, can be absorbed and returned to the liver through the enterohepatic circulation. This recycling helps to partially conserve the body's supply of vitamin A.

Carotenoids are metabolized, depending on the individual carotenoid, into a variety of compounds for excretion. Carotenoid metabolites are excreted primarily into the bile for fecal elimination.

Recommended Dietary Allowance

The Recommended Dietary Allowance (RDA) for vitamin A is based on the requirement plus twice the coefficient of variation (20%), with the value rounded to the nearest 100 μg . The requirements for vitamin A for adult men and women are 625 μg RAE and 500 μg RAE, respectively [1]. The requirement and RDAs for vitamin A are expressed as retinol activity equivalents (RAE) to account for differences in the biological activities of the carotenoids whereby 1 μg RAE is equivalent to:

- 1 μg retinol
- 2 μg supplemental β -carotene,
- 12 μg dietary β -carotene, and
- 24 μg dietary α -carotene or β -cryptoxanthin

The RDAs for vitamin A for adult men and women are 900 μg RAE and 700 μg RAE, respectively, if the food or supplement source is a retinoid (i.e., preformed vitamin A) [1]. The RDA of 900 μg RAE is also equivalent to

- 1,800 μg RAE supplemental β -carotene,
- 10,800 μg RAE food-derived β -carotene, and
- 21,600 μg RAE food-derived α -carotene or β -cryptoxanthin [1,9].

See the inside cover of the book for additional recommendations for women during pregnancy and lactation. An older means of reporting vitamin A used International Units (IUs). Further information about IUs is provided in the boxed feature International Units – Vitamin A.

INTERNATIONAL UNITS – VITAMIN A

INTERNATIONAL UNITS represent the quantity of a nutrient that produces a particular biologic effect, but do not account for differences in biological activities among related compounds such as occurs with the carotenoids and retinoids. Current recommendations for vitamin A are expressed as retinol activity equivalents (RAE) as

discussed under “Recommended Dietary Allowance”; the use of IUs is being phased out. Conversion factors between IUs and RAE are:

- 1 IU retinol is equivalent to 0.3 μg RAE
- 1 IU β -carotene from food is equivalent to 0.05 μg RAE

- 1 IU β -carotene from a supplement is equivalent to 0.15 μg RAE
- 1 IU α -carotene or β -cryptoxanthin is equivalent to 0.025 μg RAE.

Deficiency

Vitamin A deficiency is less common in the United States than in developing countries, where inadequate intake and deficiency occur frequently in children under 5 years of age. Vitamin A deficiency is one of the most prevalent of the vitamin deficiencies worldwide and often results in blindness and death.

Vitamin A deficiency is characterized by multiple signs and symptoms. Anorexia and retarded growth in infants and children are common. Sense of taste and smell may also be impaired. In the intestines, the changes in differentiation, the keratinization of mucosal cells, and reduction of mucous-secreting cells alter villi digestive and absorptive functions, often causing diarrhea. Vitamin A deficiency causes the skin

to become dry and scaly. It may also appear to have “goose bumps,” and there may be obstruction and enlargement of hair follicles (Figure 10.11). Some of these changes occur secondary to keratinization of epithelial cells with accompanying failure of normal differentiation.

In the eyes, the effects of vitamin A deficiency impact the retina, conjunctiva, and cornea. Initially, an inability to adapt to dim light occurs (and may be one of the first symptoms of deficiency). If not corrected, the condition progresses to an inability to see in dim light, called night blindness or nyctalopia. The problems result from impaired production of rhodopsin in the rod cells in the retina of the eye. Xerosis or **xerophthalmia** (dryness found in the conjunctiva and cornea because of inadequate mucus production) also occurs and is associated



Figure 10.11 Selected manifestations of vitamin A deficiency.

with the disappearance of goblet cells in the conjunctiva and the enlargement and keratinization of epithelial cells. This keratinization of the epithelial cells in the conjunctiva also promotes Bitot's spots (Figure 10.11). **Bitot's spots** are small, white, foamy-looking accumulations of sloughed cells and secretions. As the xerosis worsens, corneal scarring and ulcerations as well as keratomalacia (softening of the cornea) may occur; these may ultimately lead to corneal perforation and blindness. Vitamin A deficiency can also result in bone overgrowth with excessive new bone deposition and reduced bone degradation. The increased susceptibility to infections associated with vitamin A deficiency often leads to premature mortality. Plasma retinol concentrations indicative of deficiency are typically less than 20 $\mu\text{g/dL}$ (0.7 $\mu\text{mol/L}$), with concentrations less than 10 $\mu\text{g/dL}$ (0.35 $\mu\text{mol/L}$) suggestive of severe deficiency.

Treatment of vitamin A deficiency requires supplementation of the vitamin. The dosage varies widely (from 606 to 60,600 μg RAE of vitamin A) depending on the person's age and the severity of the deficiency, including the coexistence of other illnesses/conditions such as measles or pneumonia. Some of vitamin A's deficiency manifestations such as night blindness, xerosis, and Bitot's spots usually begin to improve within a few days following supplementation, but several weeks to months may be required for more complete healing.

At Risk for Deficiency

Individuals may be at risk for vitamin A deficiency due to reductions in nutrient digestion and absorption, increased needs, increased losses, or inadequate intake. Individuals with malabsorptive disorders in which steatorrhea (excessive fat in the feces or fat malabsorption) occurs require higher amounts of the vitamin (often in a water-miscible form) to meet the body's needs; fat malabsorption is most commonly found in those with conditions affecting the small intestine (ileum), pancreas, liver, or gallbladder.

People with chronic nephritis (affecting the kidneys), acute protein deficiency, intestinal parasites, or acute infections may also become vitamin A deficient and need higher vitamin A intakes. Measles infections, for example, in developing countries are associated with high mortality. Measles is thought to depress vitamin A status (which may already be low in children in developing countries due to inadequate vitamin A intake and absorption) by diminishing the vitamin's function as well as by increasing urinary vitamin A excretion. Vitamin A supplements are recommended by the World Health Organization and the United Nations Children's Fund for the routine treatment of measles in populations in which vitamin A deficiency is likely. Such supplementation has been shown to significantly reduce mortality from measles in young children.

Toxicity

The Tolerable Upper Intake Level (UL) for preformed vitamin A is 3,000 μg RAE per day [1]. Ingesting larger amounts (such as 15,000 μg) of vitamin A (even as a single dose) may result in acute hypervitaminosis A. While doses this large and the resulting acute toxicity are usually the result of the consumption of supplements, massive dietary intakes from the ingestion of liver from polar bears have been reported to produce acute toxicity [10]. Symptoms of acute hypervitaminosis A include nausea, vomiting, double or blurred vision, increased intracranial pressure, headache, dizziness (vertigo), diarrhea, skin desquamation (peeling), and poor muscle coordination.

Chronic intake (daily for months or years) of vitamin A in excess of recommended amounts can also lead to hypervitaminosis A. For example, a chronic oral intake of retinol in amounts of about four times the RDA can cause toxicity. Chronic vitamin A toxicity is manifested by a variety of maladies, including anorexia; dry, itchy skin with increased desquamation; alopecia (hair loss)

and coarsening of the hair; ataxia; bone and muscle pain; conjunctivitis and ocular pain; headache; nausea, vomiting and abdominal pain; and liver damage. Additionally, bone mineral density decreases and bone fracture risk increases. In bone, excess vitamin A promotes premature epiphyseal closures and increases cortical bone resorption and periosteal bone formation (periosteum covers bone), causing a thinning of long bones and effects on metatarsals (bones in the feet) and metacarpals (bones in the hands).

The effects of hypervitaminosis A on the liver, the primary storage site for vitamin A, are multiple. They include fat-storing cell **hyperplasia** (excessive cell proliferation) and hypertrophy, fibrogenesis, sclerosis of veins, portal hypertension, and congestion in perisinusoid cells, which leads to hepatocellular damage and cirrhosis or a cirrhosis-like hepatic disorder. Some toxic effects of excess vitamin A intake are thought to be mediated by changes in the regulation of vitamin A (retinoid) receptors in the nucleus and by effects on cell differentiation and growth, among other means.

Another mechanism by which excess vitamin A is thought to exert toxic effects is related to its transport in the blood. When vitamin A intake is in large excess, serum retinol levels rise (often above 200 $\mu\text{g/dL}$, whereas normal is 30–86 $\mu\text{g/dL}$), and retinol is no longer transported exclusively by RBP but instead is carried as retinyl esters by plasma lipoproteins to the tissues. It has been suggested that when retinol is presented to the cell membranes in a form other than in an RBP complex, the released retinol produces toxic effects [1].

Excessive intake of vitamin A in its natural form or in a synthetic form (as found in the oral acne treatment medications Accutane® and Roaccutane® [isotretinoin]) is also **teratogenic** (causes birth defects). Because of the associated risk of birth defects among infants born to women using synthetic retinoids for acne in the early months of their pregnancy, dermatologists prescribe contraceptives for patients in their childbearing years taking the drug.

Carotenoids, in contrast to vitamin A, appear to have few side effects. In fact, β -carotene is listed on the Generally Recognized as Safe (GRAS) list with the FDA for use as a dietary and nutrient supplement as well as for use as a colorant in foods, drugs, and cosmetics. The most commonly cited problem with supplemental carotenoid use is hypercarotenosis, also called carotenodermia; this problem is most often seen in people ingesting about 30 mg or more of β -carotene daily for at least 1–2 months and with plasma β -carotene concentrations in excess of about 250 $\mu\text{g/dL}$. Hypercarotenosis results in a yellow discoloration of the skin, especially in the fat pads or fatty areas of the palms of the hands and soles of the feet. The condition usually disappears once carotenoid intake is decreased. While no Tolerable Upper Intake Level has been established for β -carotene or other carotenoids [2], supplementation has

been shown to be detrimental to some populations such as smokers [6,7]. Consequently, carotenoid supplements are not advised for the general public [2]; instead, the public is encouraged to consume at least 2½ cups each of fruits and vegetables per day.

Assessment of Nutriture

Vitamin A status may be assessed in a variety of ways. To assess for night blindness, electrophysiological measurements, made by electroretinograms, directly measure the level of rhodopsin and its rate of regeneration in the eye. Other eye problems may be detected using conjunctival impression cytology, a histological method of assessment that involves examining morphological changes in the epithelial cells of the conjunctiva. A reduction in goblet cells and the derangement (enlargement and flattening) of epithelial cells in the conjunctiva suggest vitamin A deficiency.

Plasma retinol concentrations are frequently measured as a biochemical indicator of vitamin A status. Plasma retinol levels reflect status best if the person has exhausted their stores (primarily in the liver) of the vitamin, as with deficiency, or if the stores are filled to capacity, as with toxicity. Use of plasma retinol concentrations, however, also depends on the adequacy of dietary energy, protein, and zinc because of their roles in the synthesis of retinol-binding protein. Moreover, use of plasma retinol is unreliable as an indicator of vitamin A status in people with infection or inflammation, both of which depress plasma vitamin concentrations. In addition, multiple genetic polymorphisms have been identified that influence plasma/serum vitamin concentrations. Plasma retinol concentrations less than ~20 $\mu\text{g/dL}$ (0.7 $\mu\text{mol/L}$) are usually considered deficient, with concentrations less than ~10 $\mu\text{g/dL}$ (0.35 $\mu\text{mol/L}$) considered severely deficient. Plasma retinol concentrations of 20–30 $\mu\text{g/dL}$ (0.7–1.05 $\mu\text{mol/L}$) are suggestive of marginal status. Plasma retinol concentrations of 30–86 $\mu\text{g/dL}$ (1.05–3 $\mu\text{mol/L}$) are considered adequate, while concentrations above 86 $\mu\text{g/dL}$ (3 $\mu\text{mol/L}$) are thought to be excessive (toxic).

Adequacy of vitamin A stores in the liver can be assessed by the relative dose response (RDR) test or the modified relative dose response (MRDR) test. The RDR test involves measuring changes in plasma retinol concentration before and 5 hours after oral administration of retinyl esters (usually as acetate or palmitate). Blood is taken initially, vitamin A is ingested, and 5 hours later blood is taken again. Retinol concentrations of the blood are determined, and the difference in concentration is calculated and divided by the 5-hour concentration. RDR is then expressed as a percentage.

$$\frac{5\text{-hour plasma retinol} - \text{initial plasma retinol}}{5\text{-hour plasma retinol concentration}} \times 100$$

A % RDR equal to or greater than 20% suggests inadequate liver vitamin A stores [1]. The MRDR test involves measuring the ratio of 3,4 dihydroretinol to retinol in the blood after administering a single dose of 3,4 dihydroretinyl acetate. This test, unlike the RDR, requires that only one blood sample be taken, about 4–6 hours after the vitamin is ingested. An MRDR ratio at 5 hours of less than 0.04 in healthy adults indicates adequate vitamin status.

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Suggested Readings

Napoli JL. Cellular retinoid-binding proteins, CRBP, CRABP, FABP5: effects on retinoid metabolism, function and related diseases. *Pharmacol Ther*. 2017; 173:19–33.

10.2 VITAMIN D

Through the years vitamin D (also known as calciferol) has been associated with skeletal growth and strong bones. This association arose because early in the 20th century it was shown that **rickets**, a childhood disease characterized by improper bone development, could be prevented by a fat-soluble factor D in the diet or by body exposure to ultraviolet light. Emphasis was placed on the dietary factor; therefore, any compound with curative action on rickets was designated as vitamin D. E. McCollum is credited in 1922 with the vitamin's discovery.

Structurally, vitamin D is derived from a steroid and is considered to be a seco-steroid because one of its four rings is broken. Vitamin D contains three intact rings (A, C, and D) with a break in the B ring between carbons 9 and 10 (see the structure of previtamin D_3 in Figure 10.12). The two main forms of the vitamin (Figure 10.12), D_2 (also called ergocalciferol) and D_3 (also called cholecalciferol), differ in the structure of their side chains, but not in their general metabolism or functions in the body.

Sources

Vitamin D is found in foods and can be synthesized in the body's skin cells with exposure to ultraviolet sunlight.

Foods

Vitamin D is provided in foods (although relatively few in number) primarily as D_3 ; even fewer foods provide the vitamin as D_2 . The main food source of D_2 is mushrooms that have been exposed to UVB radiation or sun-dried. Shitake mushrooms ($\frac{1}{2}$ cup, cooked), for example, provide about 0.5 μg vitamin D_2 . The largest quantities of vitamin D_3 are found in fatty fish (and their oils) such as swordfish, salmon, tuna, and sardines. Small amounts of the vitamin are found in egg yolks and liver (Table 10.4). Because so few foods contain much vitamin D, in the United States selected foods—including some milk, yogurt, cheese, butter, and margarine, as well as some brands of orange juice, breads, and breakfast cereals—are fortified with the vitamin, usually as D_3 . In the United States, for example, milk and orange juice may be found fortified with 2.5 μg (100 IU) of vitamin D_3 /cup.

The vitamin D content of foods has been typically expressed as international units (IU) or μg , whereby 1 μg vitamin D = 40 IU and 1 IU = 0.025 μg vitamin D. However, nutrition and supplement facts labels now provide the vitamin D content in μg and as a percentage of the Daily Value, which is 20 μg . In foods, vitamin D is fairly stable and thus not prone to cooking, storage, or processing losses.

Most vitamin D supplements provide the vitamin as D_3 , although some still contain D_2 . Supplementation with the D_3 form, however, is more potent than the D_2 form [1]. The amount of the vitamin provided in the multivitamin supplements is typically about 10–20 μg (400–800 IU). Over-the-counter supplements providing solely vitamin D often contain up to 50 μg (2,000 IU).

Sunshine-Induced Cutaneous Vitamin D Production

In addition to obtaining vitamin D from foods, individuals can synthesize vitamin D_3 from the steroid 5,7-cholestradienol, commonly called 7-dehydrocholesterol (Figure 10.12). 7-Dehydrocholesterol, which is derived from cholesterol, is made in the skin's sebaceous glands and secreted

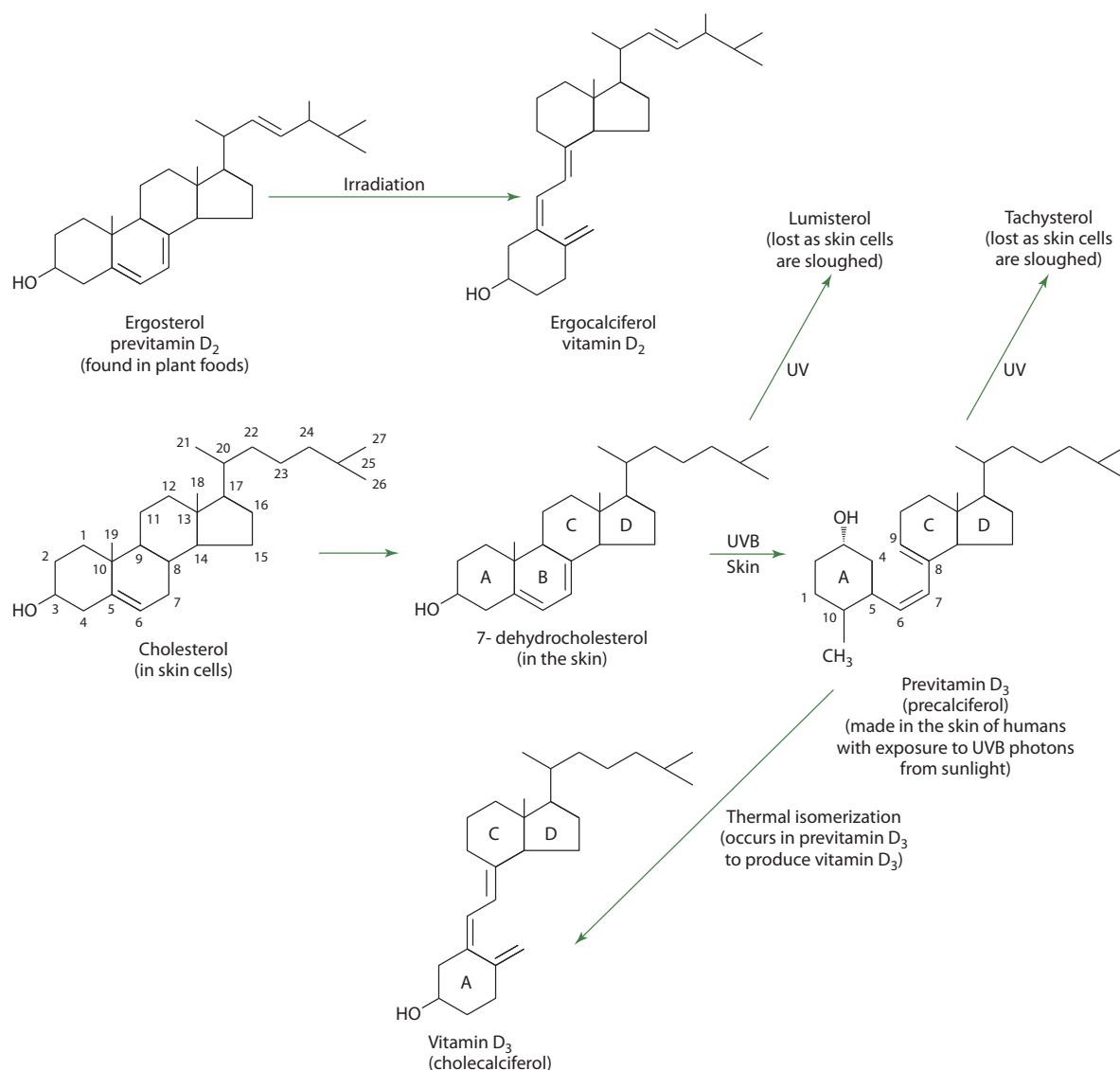


Figure 10.12 Production of vitamin D₂ (ergocalciferol) and vitamin D₃ (cholecalciferol).

Table 10.4 Vitamin D Content of Selected Foods*

Food (serving)	Vitamin D (μg)
Cod liver oil (1 Tbsp)	34
Swordfish, cooked (3 oz)	14
Salmon, sockeye, cooked (3 oz)	11
Sardines, canned (3 oz)	4.1
Tuna fish, canned (3 oz)	3.8
Herring (3 oz)	2.4
Liver, beef, cooked (3 oz)	1
Orange juice, fortified (1 c) – *amount varies with brand	3.5
Milk, low-fat or fat free, fortified (1 c)	2.5
Yogurt, fortified (1 c)	2.6
Margarine, fortified (1 Tbsp)	1.5
Egg yolk, cooked (1)	1
Cheeses (1 oz)	0.1–0.2
Butter (1 Tbsp)	0.2

*The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>. A list of vitamin D-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminD-Food.pdf>.

onto the skin's surface, where it becomes incorporated into the skin's dermis and especially the epidermis layers. The conjugated set of double bonds (five to seven) in ring B of 7-dehydrocholesterol allows the absorption of specific wavelengths of light found in the ultraviolet range. Thus, during direct exposure to sunlight (the photons do not go through glass), ultraviolet B (UVB) photons (wavelength ~285–320 nm) penetrate into the epidermis and dermis, allowing 7-dehydrocholesterol in the plasma membranes of skin cells to absorb the photons; this event causes ring B to open, forming previtamin D₃ (also called precholecalciferol). The unstable double bonds in previtamin D₃ are rearranged (a process also called thermal isomerization) over a period of several hours to a few days, resulting in generation of vitamin D₃/cholecalciferol (Figure 10.12).

If sun exposure is prolonged, excess production of vitamin D₃ in the skin is prevented through the generation of the inactive metabolites (photoisomers) lumisterol and tachysterol (Figure 10.12). Because lumisterol, tachysterol, nor previtamin D₃ has much affinity for vitamin D's main

blood transport protein (vitamin D-binding protein), these compounds are lost as the skin cells slough off.

This cutaneously produced vitamin D₃, which diffuses from the skin into the blood, represents the major source of the vitamin for many persons. A 15- to 30-minute exposure of the skin (in a fair-skinned individual wearing a bathing suit) between about 10:00 AM and 3:00 PM during the spring, summer, and fall produces about 250–500 µg of vitamin D₃, an amount that is about 100 times more than is present in fortified milk (2.5 µg). Even a 5- to 10-minute exposure of the arms and legs at midday by a fair-skinned individual can generate about 75 µg, about five times the RDA (15 µg) for adults. In those with darker skin color (higher melanin content), longer (up to six times) sun exposure time is needed to generate the vitamin than in those with lighter skin color, since melanin blocks some of the UVB rays.

The cutaneous production of vitamin D is affected by multiple factors including time of day, season, latitude, altitude, cloud cover, air pollution, skin pigmentation, sunscreen use, and age. The seasonal, time of day, and latitudinal effects are related to the zenith angle of the sun. During the winter, the morning and late-afternoon hours, and North and South of latitudes of about 40 degrees, there is an increase in the zenith angle of the sun, which results in a longer UVB photon path and fewer UVB photons reaching the surface of the earth. Such effects diminish vitamin D production in the skin. Additionally, older adults may produce up to 75% less vitamin D₃ in the skin than younger adults due to diminished 7-dehydrocholesterol content in the skin.

Absorption

Dietary vitamin D (both D₃ and D₂) requires no digestion. The vitamin is absorbed from a mixed micelle (formed within the lumen of the small intestine from digested lipids and other fat-soluble vitamins in association with fat and with the aid of bile) that diffuses through the lumen and unstirred water layer adjacent to the enterocytes, enabling close contact with the brush border membrane. From this site, the vitamin is thought to be absorbed by passive diffusion into the intestinal cell. Whether carriers are involved in vitamin's absorption (as have been identified for the other fat-soluble vitamins) is unclear. About 50% of dietary vitamin D is absorbed. Although the rate of absorption is most rapid in the duodenum and proximal jejunum, the largest amount of vitamin D is absorbed in the jejunum. The absence of a jejunum (secondary to surgical resection) increases the likelihood of vitamin D deficiency by about fivefold [2].

Within the intestinal cell, vitamin D is incorporated primarily into chylomicrons, which also contain other fat-soluble vitamins, cholesterol esters, phospholipid, triacylglycerols, and apoproteins. The chylomicrons are released by exocytosis into lymphatic capillaries/lacteals for transport

via the lymphatic system, and then, via the thoracic duct, enter into general circulation (i.e., the blood). As with vitamin A, conditions associated with fat malabsorption negatively impact the intestinal absorption of vitamin D.

Transport, Tissue Uptake, and Storage

Chylomicrons transport vitamin D that came from the diet to nonhepatic tissues throughout the body. Chylomicron remnants deliver the vitamin to the liver, which takes up the remnants by endocytosis. In contrast to dietary vitamin D, vitamin D₃ that is made in the skin slowly diffuses from the skin into dermal capillaries and is picked up for transport in the blood by a vitamin D-binding protein (VDBP).

Hepatic Uptake and Metabolism of Vitamin D to 25-Hydroxy Cholecalciferol

Vitamin D reaching the liver either by way of chylomicron remnants or by VDBP must be hydroxylated by cytochrome P450 hydroxylases to begin the generation of vitamin D's active form. These hydroxylases are collectively referred to as mixed-function oxidases (the enzymes reduce one atom of molecular oxygen to water and one to the hydroxyl group) and are abbreviated as CYP followed by numbers and letters. In the liver, 25-hydroxylase (primarily CYP27A1), which is magnesium and NADPH-dependent, functions in the mitochondria to hydroxylate vitamin D₃ at carbon 25 to form 25-hydroxy (OH) D or vitamin D, also called calcidiol or 25-OH cholecalciferol (Figure 10.13). Another 25-hydroxylase (CYP2R1) found in microsomes also hydroxylates D₃ as well as D₂. While the enzyme is largely unregulated, 25-hydroxylase is more efficient during periods of vitamin D deprivation than when normal amounts of the vitamin are available.

Serum 25-OH D Concentrations and Vitamin Storage

After its hepatic synthesis, most 25-OH D is secreted from the liver. About 85–90% of 25-OH D in the blood is bound to and transported by VDBP, about 10–15% is bound loosely to albumin, and <1% is free (unattached). The blood is the largest single pool of the vitamin, with serum 25-OH D concentrations reflective of the hydroxylation of dietary vitamin D and cutaneously produced vitamin D. 25-OH D in the blood has a half-life of about 2–3 weeks.

With high intakes of the vitamin, more vitamin D is stored (most nonhydroxylated) in the body's adipose tissue. Release of the vitamin from adipose tissue, however, occurs slowly. Individuals with greater than normal amounts of body fat (as may be found in those who are overweight or obese) appear to store more of the vitamin in adipose tissue than those with less body fat. In addition to the blood and adipose tissue, small amounts of the vitamin, both as nonhydroxylated vitamin D and as 25-OH D, are found in the muscle.

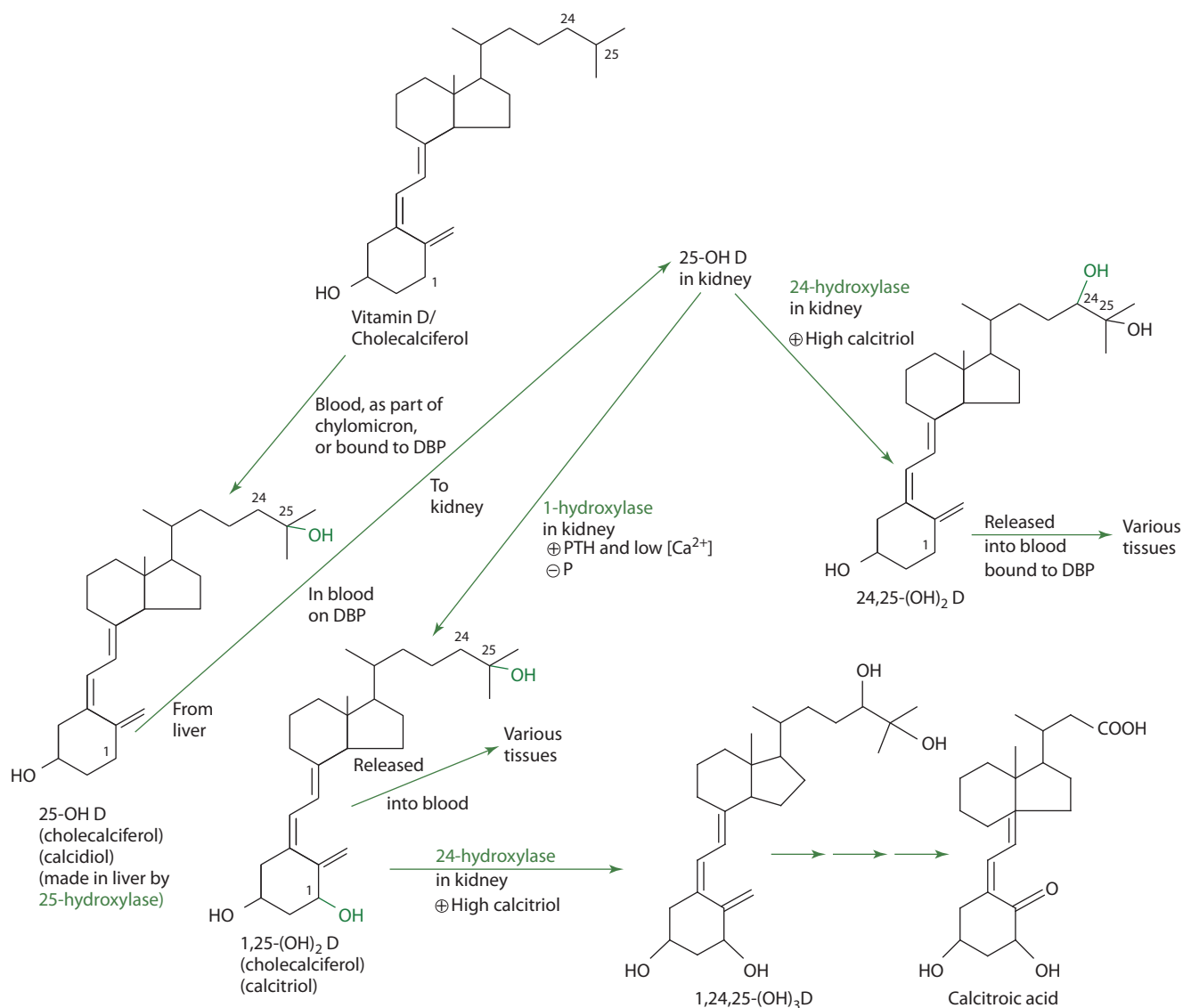


Figure 10.13 Hydroxylations of vitamin D.

Measurement of serum 25-OH D concentrations typically includes the three forms of 25-OH D (i.e., free and that bound to VDBP and to albumin); free and albumin-bound levels of the vitamin can also be calculated. Serum 25-OH D concentrations are commonly used as an indicator of vitamin D status. Serum 25-OH D concentrations between about 30 and 40 ng/mL (75 and 100 nmol/L) are generally thought to be sufficient to maintain healthy bone and muscle. The optimal serum 25-OH D concentration is unclear; recommendations range from about 30 to 60 ng/mL, with concentrations less than 20 ng/mL (50 nmol/L) generally indicative of deficiency. (Note: To convert ng/mL to nmol/L, multiply by 2.496.)

Factors influencing serum 25-OH D While dietary vitamin D intake, sunlight exposure, and liver function impact circulating serum 25-OH D concentrations, concentrations

are also affected by factors influencing VDBP and albumin. Infections, inflammation, and trauma, for example, reduce production of both proteins, and thus reduce serum 25-OH D concentrations. Concentrations of VDBP and albumin, which are both produced in the liver, are also reduced with liver disease and with renal disorders, such as nephrotic syndrome, secondary to increased urinary losses of the proteins. VDBP production is increased by estrogen and is thus lowered with menopause. Polymorphisms in genes coding for VDBP, as well as for enzymes needed for vitamin D activation, also influence serum 25-OH D concentrations. Several gene variants for VDBP have been identified. Moreover, VDBP production differs across various races. The polymorphisms result in differences in both serum 25-OH D and VDBP concentrations among different groups, with, for example, lower concentrations found in African Americans than in Caucasians. Because

of the multiple factors affecting serum 25-OH D concentrations, its usefulness as an indicator of vitamin D remains controversial.

Renal Metabolism of 25-OH Vitamin D to 1,25-(OH)₂ Vitamin D

From the blood, 25-OH D–VDBP is taken up by the kidneys and filtered by the glomerulus, a process that is increased by parathyroid hormone (PTH). Specifically, 25-OH D–VDBP binds and forms a complex with a cubulin-megalin membrane receptor on the plasma membrane of proximal tubule cells. The complex is internalized by endocytosis into the cells, where the 25-OH D is released and is then hydroxylated by 1-hydroxylase at position 1 to form the vitamin's active form, 1,25-(OH)₂ (vitamin) D (also called calcitriol or 1,25 dihydroxycholecalciferol; Figure 10.13). The 1-hydroxylase (CYP27B1), a magnesium and NADPH-dependent mitochondrial enzyme, is expressed in the highest concentrations in the kidneys. Genetic mutations in the gene for CYP27B1 result in the vitamin D deficiency condition rickets; the condition must be treated with 1,25-(OH)₂ D (1 µg /day) as opposed to cholecalciferol / D₃, which represents the usual form of the vitamin in supplements [3].

Control of Calcitriol Synthesis The renal synthesis of calcitriol is tightly regulated primarily by two hormones, PTH and fibroblast-like growth factor (FGF) 23. The hormones are in turn influenced largely by blood calcium and phosphorus concentrations. PTH (secreted primarily when serum calcium concentrations are low but also to a lesser extent when serum magnesium concentrations are low) stimulates the synthesis of the 1-hydroxylase, and thus the synthesis of calcitriol. In contrast, FGF23, which is secreted mainly by osteocytes, reduces 1-hydroxylase expression (to reduce calcitriol production) and stimulates 24-hydroxylase to promote the synthesis of 24,25-(OH)₂ D (which represents a step in the vitamin's degradation). High serum concentrations of calcium (hypercalcemia) and phosphorus (hyperphosphatemia) inhibit calcitriol synthesis. Additionally, 1,25-(OH)₂ D (i.e., calcitriol), the 1-hydroxylase's end product, inhibits the 1-hydroxylase production by binding to a vitamin D response element (VDRE) on the promoter region of the 1-hydroxylase gene (thereby decreasing the vitamin's activation); calcitriol also stimulates the production of another mixed-function oxidase, 24-hydroxylase. The 24-hydroxylase (CYP24A1), which is present in most body cells, generates 24,25-(OH)₂ D from the hydroxylation of 25-OH D, and 1,24,25-(OH)₃ D from hydroxylation of 1,25-(OH)₂ D (Figure 10.13); these 24-hydroxylation reactions represent steps in the vitamin's inactivation. These metabolites may be further oxidized in the kidneys to generate a variety of excretory products.

Vitamin D₂, although less frequently consumed, is metabolized in the body to calcitriol by the same

25-hydroxylase and 1-hydroxylase as vitamin D₃. The metabolism of D₂, however, produces additional metabolites not generated by D₃ and is less efficient in calcitriol production than D₃ [1].

Plasma Calcitriol Concentrations The calcitriol that is made in the kidneys is released into the blood, where it binds to VDBP for transport to tissues; however, like 25-OH D, some calcitriol may also be present in the blood free (unbound). VDBP's binding to calcitriol appears to be loose to facilitate the vitamin's release to tissues; this is in contrast to the tight binding of 25-OH D to VDBP. Calcitriol in the blood has a half-life of about 2–6 hours; normal plasma calcitriol concentrations range from about 20 to 40 pg/mL, considerably lower than plasma 25-OH D concentrations.

Target Tissues and Extrarenal Calcitriol Production

Vitamin D's target tissues are extensive, with receptors for the vitamin found, for example, in the intestine, bone, kidneys, heart, muscle, pancreas (β-cells), brain, skin, colon, prostate, breast, hematopoietic system, central nervous system, and immune system. Moreover, while the liver displays significant 25-hydroxylase activity, this enzyme is also found in other organs, suggesting the vitamin may be hydroxylated within nonhepatic tissues for intracellular (local) use. Similarly, while the kidneys are thought to be the main source of plasma 1,25-(OH)₂ D for tissue use, it is clear that many other tissues possess 1-hydroxylase (CYP27B1) and can use 25-OH D from the plasma to make their own calcitriol, provided that enough 25-OH D is available in the blood. CYP27B1 is present, for example, in macrophages and other immune system cells, in skin cells such as keratinocytes, among other tissues.

The mechanism(s) by which 25-OH D and calcitriol enter tissues for use is not fully understood. The looser binding of calcitriol to VDBP and the lower affinity of the vitamin for albumin are thought to enable better dissociation and allow for the vitamin's diffusion into tissues for use [4]. Another possibility includes interactions with vitamin D–VDBP and a membrane transporter to facilitate cellular uptake of the vitamin such as occurs in the kidneys. However, the extent to which other tissues can take up protein-bound forms of vitamin D via such a complex is not clear [4].

Functions and Mechanisms of Action

Calcitriol has several functions; its most widely recognized role is in serum calcium homeostasis with effects on the kidneys, small intestine, and bone. Additional roles include serum phosphorus homeostasis; cell differentiation, proliferation, and growth; and muscle structure and function. The mechanisms by which calcitriol performs these functions may be divided into two categories—nongenomic

and genomic—although the details of these mechanisms have not been clearly elucidated. This section first describes some of the possible nongenomic, and then the genomic, mechanisms of action of the vitamin and then discusses the functions of vitamin D.

Mechanisms of Action

Many *nongenomic actions* of calcitriol are mediated by the activation of signal transduction pathways (also called intracellular signaling) linked to cell membranes. Calcitriol binding to cell membrane receptors in selected tissues (especially intestine, parathyroid, liver, and pancreatic β -cells) triggers a series of events through signal transduction pathways to evoke relatively rapid (seconds to minutes) cellular responses. One such vitamin membrane receptor that has been identified and linked with these nongenomic actions of vitamin D is known as membrane-associated rapid-response steroid-binding (MARRS) protein. The many actions initiated from these intracellular signaling pathways include increased calcium uptake, increased intracellular calcium concentration, and/or transcellular calcium flux in cells such as enterocytes, osteoblasts, adipocytes, and skeletal muscle. These cellular events are thought to be mediated primarily by the generation of second messengers and other compounds such as mitogen-activated protein (MAP) kinase, protein kinase C, cyclic AMP, tyrosine kinase, phospholipase C, diacylglycerol, inositol phosphate, and arachidonic acid. More details of vitamin D-mediated nongenomic intestinal cell calcium uptake are discussed in the section “Calcitriol and the Intestine.”

Calcitriol also exerts its functions through *genomic mechanisms* of action (and thus regulates gene expression in target cells). Calcitriol’s genomic mechanism of action is similar generally to that described for retinoic acid. Vitamin D, as calcitriol, moves from the cytosol into the nucleus, where it (behaving like a hormone) binds to and induces conformational changes to nuclear vitamin D receptors (VDRs; Figure 10.14). Nuclear VDRs have been

found in over 30 organs, including the bone, intestine, kidneys, lungs, muscle, and skin. These nuclear VDRs are part of a so-called superfamily of receptors that also includes receptors for retinoic acid and thyroid and steroid hormones. The conformational changes in the VDRs as well as perhaps phosphorylation enables dimerization of the VDR with another receptor (commonly retinoid X receptor, or RXR), forming a heterodimer.

Interactions between the complex (calcitriol–VDR–RXR) and specific DNA nucleic acid sequences called vitamin D response elements (abbreviated VDRE) that are found in the promoter regions of target genes in turn affect gene expression. However, as with vitamin A, once the calcitriol–VDR–RXR is bound to the DNA, additional comodulatory (either coactivator or corepressor) proteins are often required. Two significant coactivators include steroid receptor coactivators (SRC-1, SRC-2, and SRC-3) and vitamin D–receptor interacting protein (DRIP). These coactivators in turn enable (often via recruitment of other proteins such as histone acetyl transferases or histone methyltransferases) alterations in chromatin structure (such as destabilization of histone–DNA interactions) to facilitate transcription. DRIP functions to link the VDRE and the initiation complex (including RNA polymerase II, among other proteins), which is needed to facilitate transcription. Examples of corepressors include silencing mediator for retinoid or thyroid-hormone receptors (SMRT) and nuclear receptor corepressor (NCoR). While most interactions between calcitriol and genes are thought to involve interactions with RXR, the vitamin may directly inhibit gene expression via VDR alone or via transcription factors. Over 200 genes are thought to have VDRE and to be directly or indirectly influenced by calcitriol. Through these genomic interactions, vitamin D exhibits multiple biological actions, with many of the effects influencing body calcium and its functions.

One of the most investigated roles of calcitriol relates to serum calcium homeostasis. In fact, in addition to its genomic interactions with VDRE on genes, calcitriol also

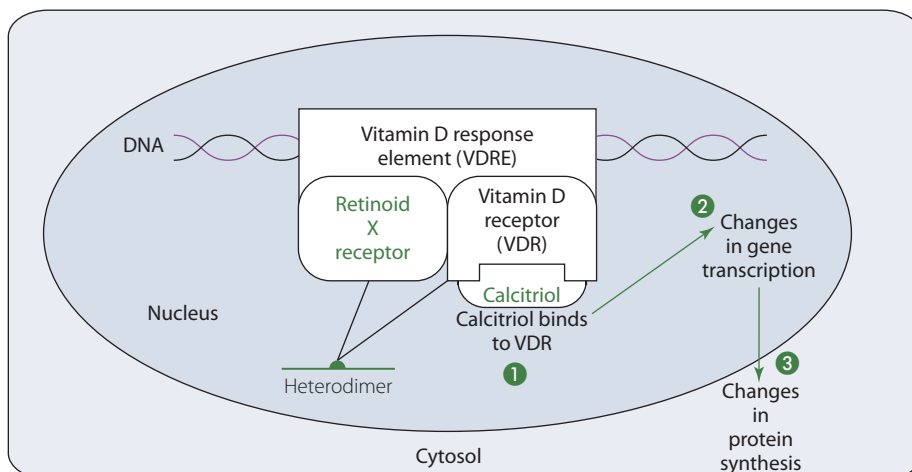


Figure 10.14 Proposed role of calcitriol bound to VDR on DNA in gene expression.

appears to exert its effects through direct interactions with messenger (m)RNA to enhance or inhibit translation of selected proteins. The proteins are typically, but not exclusively, involved in calcium homeostasis and/or functions and include, for example, osteocalcin, osteopontin, 24-hydroxylase (CYP24), the epithelial transient receptor potential cation channel vanilloid-type subfamily member 6 (TRPV6), calbindin, and Ca^{2+} -ATPase. Vitamin D's role in calcium homeostasis is discussed first, followed by calcitriol's effects on phosphorus homeostasis; on cell differentiation, proliferation, and growth; and on muscle. Finally, some of calcitriol's other roles in the body are discussed.

Increasing Serum Calcium

Calcitriol, with PTH, functions to raise serum calcium concentrations into the normal range, about 8.5–10.5 mg/dL (2.12–2.62 mmol/L). As transient decreases in serum calcium or hypocalcemia (low serum calcium concentrations, i.e., < 8.5 mg/dL) occurs, activation of calcium-sensing receptors triggers the secretion of PTH from the parathyroid gland and into the blood. (Low serum concentrations of 25-OH D [especially less than about 30 ng/mL] and magnesium also promote PTH secretion; PTH concentrations are thought to plateau with serum 25-OH

D concentrations between about 30 and 40 ng/mL.) The PTH travels to the bone (discussed later) and to the kidneys; in the kidneys it stimulates 1-hydroxylase to convert 25-OH D to calcitriol.

Calcitriol, once synthesized, functions within the kidneys. The vitamin is also released into the blood bound to VDBP and then acts alone (or with PTH) on its other target tissues (the intestine and bone), causing serum calcium concentrations to rise to within the normal range. The effects of calcitriol on its target tissues—kidneys, intestine, and bone—are discussed next and are shown in Figure 10.15.

Calcitriol and the Kidneys Calcitriol induces calcium reabsorption from the glomerular filtrate into the distal tubules of the kidneys. More specifically, calcitriol is transported into the nucleus of renal cells, where it exerts genomic effects, interacting with nuclear VDRs to directly regulate specific genes encoding for proteins involved in calcium reabsorption, including transient receptor potential vanilloid 5 (TRPV5), calbindin D28k, sodium/calcium exchanger 1 (NCX1), and plasma membrane calcium pump 1 (PMCP1). As the result of these interactions, selective DNA transcriptions occur that result in the biosynthesis of new mRNA molecules and

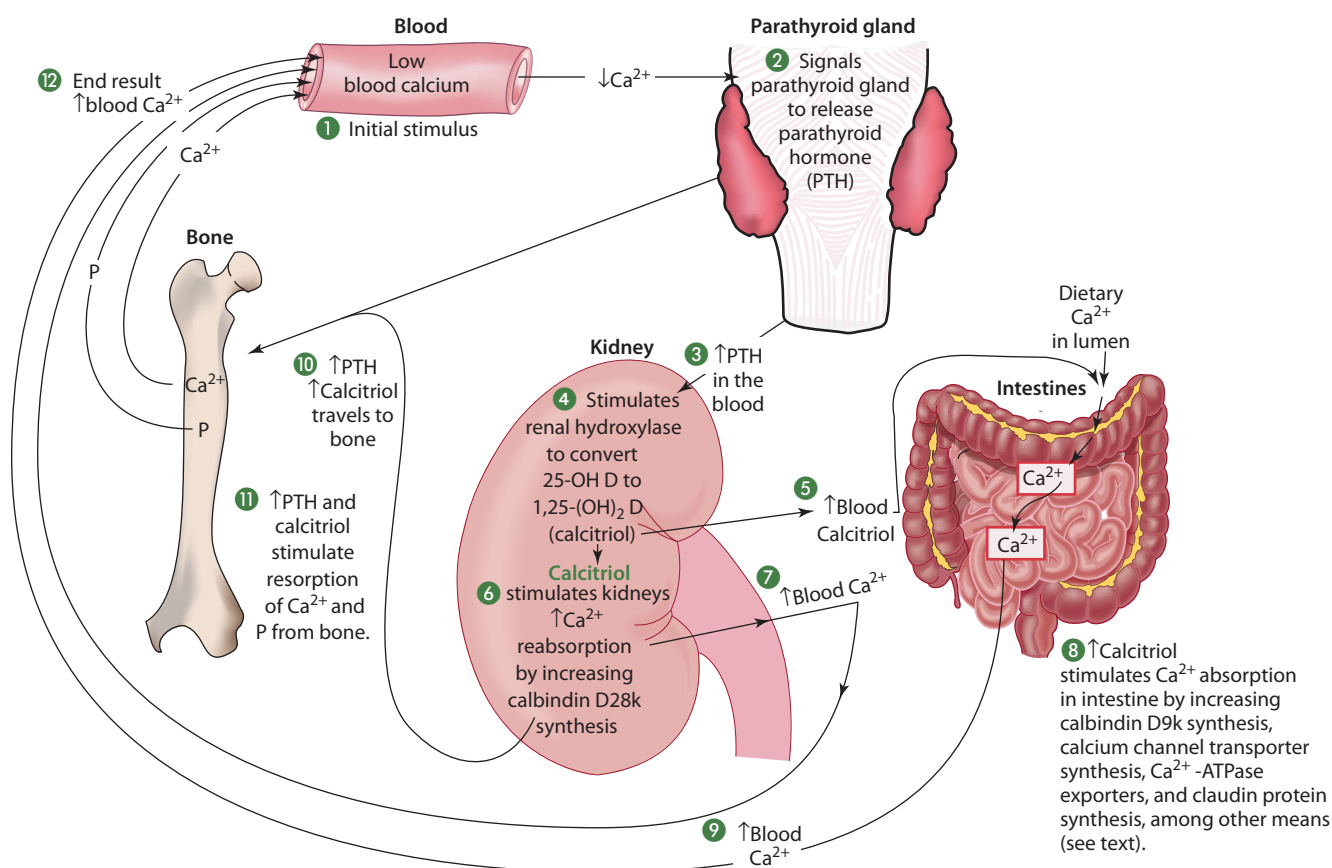


Figure 10.15 Calcitriol, 1,25-(OH)₂ D, synthesis and actions with parathyroid hormone (PTH).

ultimately proteins. The specific roles of these proteins in the kidneys are listed below.

- TRPV5, a calcium channel protein, enables calcium reabsorption from the filtrate across the apical membrane in the distal tubule.
- Calbindin D28k transports calcium within the cytosol to the plasma membrane.
- NCX1 and PMCP1 enable calcium release across the basolateral membrane.

These actions serve to increase the reabsorption of calcium back into the blood, and thus to raise serum calcium concentrations to within a normal range.

Calcitriol and the Intestine The primary function of calcitriol in the intestine is to increase the absorption of calcium (as well as phosphorus, as discussed later). Serum 25-OH D concentrations of 30–40 ng/mL appear to maximize calcium absorption, which is normally about 30% (of ingested calcium). However, with a vitamin D deficiency only about 10–15% of ingested calcium is absorbed. The vitamin is believed to act through genomic and nongenomic mechanisms to exert its effects.

With respect to genomic effects on intestinal cell calcium absorption, calcitriol is transported into the enterocyte and carried into the nucleus, where it interacts with nuclear VDRs to directly regulate specific genes encoding for proteins involved in calcium uptake and transport. As occurred in the kidneys, these proteins act at the brush border, in the cytosol, and at the basolateral membrane of the intestinal cells, especially in the duodenum and jejunum, to promote calcium absorption. The actions are similar to those described in the kidney but involve different proteins.

- At the brush border membrane of the enterocyte, calcitriol enhances the absorption of calcium by increasing transient receptor potential vanilloid 6 (TRPV6), a calcium channel transporter.
- Within the enterocytes, the vitamin enhances cellular calcium transport by its genomic effects, specifically interacting with VDREs associated with the gene for a calcium-binding protein called calbindin D9k, a smaller form of the protein calbindin D28k that is found in the kidneys. Calbindin D9k, which binds two calcium atoms, transports over 90% of calcium through the cytosol of enterocytes.
- Finally, vitamin D enhances the extrusion of calcium across the basolateral membrane of the enterocyte and into the plasma by enhancing the synthesis of Ca^{2+} -ATPase pumps, including plasma membrane calcium ATPase (PMCA1b), among others.

Vitamin D also exerts control over paracellular calcium absorption; paracellular meaning “occurring between the

enterocytes.” The vitamin enhances the expression of genes that code for a specific group of transmembrane proteins, called claudins, found in the tight junctions between cells, including those of the intestine. Calcium absorption through tight junctions involves channel aquaporin 8 and a cell adhesion protein, cadherin 17, which requires claudins 2 and 12 to function. The synthesis of claudins 2 and 12 is activated by vitamin D.

Calcitriol and the Bone Elevated serum PTH concentrations, along with calcitriol, direct the mobilization of calcium out of bone to raise serum calcium concentrations to within a normal range (Figure 10.14). More specifically:

- Calcitriol interacts within the nucleus of mature osteoblasts to induce the expression of a cytokine called receptor activator of $\text{NF}\kappa\text{B}$ ligand (RANKL).
- The RANKL released from the osteoblasts then interacts with the receptor protein RANK, found on the cell surface of immature osteoclast precursors (also called preosteoclasts), to stimulate (via signal transduction) the differentiation, production, and maturation of the osteoclasts.
- The mature osteoclasts in turn release hydrochloric acid, alkaline phosphatase, collagenase, and other hydrolytic enzymes and substances, which dissolve and catabolize (eat away at) the bone matrix (especially along the outer surface of the bone). The net effect of these actions is an increase in serum calcium (and phosphorus) concentrations at the expense of the bone.
- PTH also works via a second (and faster mechanism) in bone by stimulating the activity of calcium pumps to move calcium from its small presence in bone fluid (located between the bone membrane and adjacent bone) across the membrane and into the plasma. Calcium lost from the bone fluid is replaced with calcium present on the bone surface.

In addition to promoting bone resorption to increase calcium availability, vitamin D also inhibits the mineralization of the bone matrix. Calcitriol accomplishes this function by increasing the expression of the genes that code for osteopontin and for several enzymes that enable pyrophosphate (PPi) production. Both of these (pyrophosphates and osteopontin) inhibit bone mineralization. The vitamin also impacts the synthesis of other proteins including osteocalcin and matrix Gla protein found in bone and other connective tissues.

Indirect Effects Although calcitriol is not thought to be directly involved in decreasing serum calcium concentrations should they rise above normal (hypercalcemia), calcitriol is indirectly involved through effects on PTH. Elevations in the serum concentrations of both calcitriol and ionized serum calcium down-regulate

fibroblast growth factor 23 (FGF 23) and decrease PTH production through long and short feedback loops. The long feedback loop is indirect, resulting from elevated serum ionized calcium's inhibitory effect on the parathyroid gland's secretion of PTH. The short feedback loop is direct: calcitriol decreases the transcription of the gene for parathyroid hormone by interacting with the VDR bound to VDRE for the gene for PTH.

In addition, with higher serum calcium concentrations, calcitonin (a hormone produced by parafollicular endocrine clear [C] cells of the thyroid gland) is released. Calcitonin blocks calcium mobilization from bones by inhibiting osteoclast activity. In addition, it inhibits the tubular resorption of calcium in the kidneys, leading to increased urinary calcium excretion. Furthermore, but in contrast to their detrimental effects when present in high concentrations, calcitriol and PTH, when present in usual concentrations and with sufficiently available calcium and phosphorus, promote bone anabolism and mineralization.

Phosphorus Homeostasis

Calcitriol affects phosphorus homeostasis. In the intestine, calcitriol is thought to increase the activity of brush border alkaline phosphatase, which hydrolyzes phosphate ester bonds to free the food-bound phosphorus and thus enable its absorption in the small intestine. Calcitriol also up-regulates the number of carriers responsible for the sodium-dependent absorption of phosphorus at the brush border membrane of intestinal cells (especially of the jejunum and ileum). In bone, calcitriol promotes the resorption of phosphorus out of bone and into the blood. (See Chapter 11's section "Phosphorus Homeostasis" for a more complete discussion of the subject.)

Cell Differentiation, Proliferation, and Growth

The local presence of the calcitriol within many non-calcium-regulating tissues is thought to help maintain normal cell growth, promote terminal differentiation, and inhibit cell proliferation. Some examples of cells affected by vitamin D include premyeloid white blood cells and stem cells, which differentiate into macrophages and monocytes in the presence of adequate calcitriol. Calcitriol also induces in the bone marrow cell differentiation of stem cell monocytes, which become mature osteoclasts. Vitamin D's ability to stimulate skin epidermal cell differentiation while inhibiting proliferation has been applied in the treatment of psoriasis (a disorder marked by enhanced proliferation and failed differentiation of keratinocytes). Specifically, calcitriol binds to VDRs in keratinocytes to alter the expression of regulatory proteins that serve to inhibit proliferation of the epidermis and induce normal differentiation in those with psoriasis.

Other mechanism(s) by which vitamin D affects aspects of cell differentiation, proliferation, and growth are not clear but are likely related to effects on genes coding for

various regulatory factors, including ones that control cell growth (along with invasiveness/metastases and angiogenesis/blood vessel growth) and cell death. Calcitriol, for example, slows cell cycle progression by inhibiting key regulators of the transition from the gap (G) 1 phase to the synthesis (S) phase of the cell cycle. The vitamin also affects cell growth by altering concentrations of regulatory proteins involved in apoptosis such as caspases and bcl-2. These actions, among others, of vitamin D have contributed to numerous studies investigating possible anticancer roles and benefits from vitamin D supplementation.

Muscle Function

Muscle dysfunction (myopathy) is well documented in individuals with vitamin D deficiency. The most commonly reported problems include muscle weakness and pain, difficulty rising from a squatting or sitting position, difficulty walking (especially up stairs), and increased falls. Biopsies of muscle in those with vitamin D deficiency show atrophy of primarily type II (fast-twitch) muscle fibers (note that it is these fibers that are utilized to prevent falls). Vitamin D supplementation, in those with deficiency, in turn increases the type II muscle fiber area and diameter, as well as the synthesis of muscle cytoskeletal proteins such as calmodulin needed for muscle contraction.

In muscle, calcitriol is thought to function through genomic mechanisms to enhance calcium uptake and thus intracellular calcium concentrations. Specifically, the vitamin is thought to increase the transcription of genes for calcium ATPase pumps (that actively transport calcium into the sarcoplasmic reticulum and sarcolemma) and for voltage-sensitive calcium channels. Such changes in intracellular calcium concentrations are needed for contraction and relaxation of muscle. Calcitriol is also thought to modulate muscle contractility through nongenomic mechanisms. For example, through binding to cell surface receptors, calcitriol activates second-messenger pathways to rapidly increase intracellular calcium concentrations by promoting calcium release from intracellular stores. Additionally, through second-messenger pathways, calcitriol enhances myogenesis, cell proliferation, differentiation, and apoptosis. Optimal muscle function is thought to require serum 25-OH D concentrations of at least 30 ng/mL.

Immune System Function

Calcitriol profoundly impacts multiple aspects of the immune system (both innate and acquired) and thus helps to protect the body against infection by microorganisms, including bacteria, viruses, and parasites. Almost all cells of the immune system, including, for example, activated T- and B-cells, antigen-presenting dendritic cells, monocytes, macrophages, and cytotoxic T-cells, express VDRs and many produce calcitriol. In addition, the production of many proteins involved in immune system function by

these cells, such as cytokines, defensins, and antimicrobial peptides like cathelicidin by macrophages, are affected by the genomic actions of vitamin D. Vitamin D generally appears to up-regulate the production of many anti-inflammatory cytokines and down-regulate the synthesis of several proinflammatory cytokines.

Other Roles

Vitamin D has also been linked with a variety of other roles in the body. Some of these roles in the pancreas include protection of pancreatic β -cells (via decreasing apoptosis) and modulation of insulin synthesis (via interacting with VDRE in the promoter region of the gene for insulin). Insulin secretion also appears to be affected secondary to vitamin D–induced changes in calcium concentrations.

Cardioprotective functions of the vitamin are thought to involve control of genes influencing heart and blood vessel functions. Deficiency of the vitamin, for example, is associated with up-regulation of the renin-angiotensin-aldosterone system, hypertrophy of vascular smooth muscle cells, and left-ventricular hypertrophy, which negatively impact heart function [5]. Vitamin D also impacts blood pressure, promoting endothelial cell–dependent vasodilation; it also affects arterial pressure and myocardial contractility via elevations in PTH and stimulates calcium uptake by cardiac muscle cells to enable contractility [5]. Thyroid-stimulating hormone secretion from the pituitary gland is also stimulated by calcitriol with subsequent stimulation of thyroid hormone production.

Interactions with Other Nutrients

Interrelationships among vitamin D, calcium and phosphorus have been discussed previously. Vitamin D also exerts small beneficial effects on magnesium absorption.

Metabolism and Excretion

Calcitriol hydroxylation at carbon 24 by a 24-hydroxylase generates the trihydroxy metabolite 1,24,25-(OH)₃ D (see Figure 10.13), which may be further oxidized to 1,25-(OH)₂ 24-oxo D. Subsequent reactions, including side-chain cleavage, yield the major end-product calcitroic acid (see Figure 10.13). Other vitamin D metabolites are also formed after additional reactions involving hydroxylation, oxidation, and conjugation usually with glucuronic acid. Calcitroic acid and vitamin D metabolites are excreted primarily through the bile in the feces. Little (<30%) of the vitamin is excreted via the urine.

Recommended Dietary Allowance

An RDA for vitamin D, which was published for the first time in 2010 [6], assumes minimal sun exposure and suggests an intake of 15 μ g of vitamin D for children (age 1 year and older), adolescents, and adults, including

women who are pregnant or lactating. Recommended intakes increase to 20 μ g of vitamin D for adults older than 70 years of age. Requirements are estimated at 10 μ g for these age groups.

Sufficient amounts of vitamin D are thought to be obtainable for many individuals (depending on latitude) with exposure to sunlight for about 15 minutes for individuals with lighter-skin pigmentation (longer times in the sunlight are needed for those with darker skin) at midday a few times per week. Such exposure is thought to be able to raise serum 25-OH D concentrations to 45 ng/mL and to reduce more deaths than would occur from skin cancers [7].

Deficiency

Vitamin D deficiency is widespread across the United States and the world. Vitamin D deficiency most notably affects the bones, but its effects on the bones of infants and children differ from those of adults. In infants and children, vitamin D deficiency results in rickets, while in adults the condition results in osteomalacia.

Rickets

Rickets is characterized primarily by reductions in the mineralization of the bone's epiphyseal (also called growth) plates, resulting in various deformities. Signs of rickets appear at about 6 months of age in affected infants. More specifically, without enough vitamin D, the epiphyseal cartilage continues to grow and enlarge (widen) but without sufficient replacement by bone matrix and minerals. The widening of the epiphyseal plates is especially visible at the ends of long bones (i.e., wrists, ankles, and knees) and at costochondral junctions (referred to as rachitic rosary and resembling beading at the junction of the ribs and cartilage). In addition, because little mineral is present in the skeleton, the long bones of the legs bow (Figure 10.16) and knees knock as weight-bearing



Bowing of the legs secondary to weight bearing and inadequate bone mineralization

Figure 10.16 Selected manifestations of vitamin D deficiency.

activities such as standing and walking begin. The spine may also become curved, and pelvic and thoracic deformities occur. Frontal bossing (a prominent protruding forehead), widening of the fontanelles (the soft membranous space between the cranial bones where ossification is not yet complete), and softening of the fontanelle borders appear sometime between about 6 and 14 months of age. Other manifestations may include bone pain in the legs or back, delayed age of standing or walking, frequent falling, delayed growth, neuromuscular irritability, and hypocalcemic seizures. Biochemical indicators of deficiency may include elevated total or bone alkaline phosphatase and PTH concentrations, low 24-hour urinary calcium excretion, and low serum calcium and/or phosphorus and 25-OH D concentrations. Radiography of long bones at the knees and wrists shows widening of the growth plates and fraying of the margin of the metaphysis, indicating impaired mineralization.

Osteomalacia

In adults and older children, insufficient vitamin D leads to **osteomalacia** (*malacia* means “softening” and *osteo* refers to bone). Osteomalacia results from the failure to mineralize (or reduced mineralization of) already formed bone with closed growth plates (i.e., unlike in infants and young children, the epiphyseal plates are closed and enough mineral in the bone is present to prevent deformities with weight-bearing activities in adults and older children). Insufficient vitamin D over time induces secondary hyperparathyroidism, which increases bone turnover and bone mineral loss. Bone resorption is characterized by increased urinary excretion of bone collagen by-products such as hydroxyproline, N-telopeptide, pyridinoline, and deoxypyridinoline. Additionally, as new bone is produced by osteoblasts (remember bone is constantly being remodeled), it does not get mineralized sufficiently. Thus, over time, the amount of nonmineralized bone exceeds mineralized bone. The progressive demineralization results in bone pain (characterized as throbbing or aching), especially at the joints (such as the shoulders), and increased risk of fractures. Muscle weakness and pain and gait instability may also be present. Biochemical indicators of deficiency are usually the same as those seen in rickets. Radiographic findings may include low bone density and skeletal pseudofractures and/or nontraumatic fractures (fragility).

Vitamin D in doses of 20–50 µg daily for 2–3 months are recommended for adults with mild deficiency or at risk for deficiency. A severe deficiency of vitamin D is often treated with high loading doses of the vitamin—such as 1,250 µg given once per week for 6–12 weeks followed by lower doses (20–50 µg) of the vitamin taken daily. Obese individuals may require higher doses (two to three times) of the vitamin to prevent and treat deficiency than nonobese individuals. Intakes of up to about 50 µg vitamin D are recommended for those with

malabsorption conditions and those on medications affecting vitamin D metabolism. However, for those with liver and/or renal dysfunction who cannot generate the 25-OH D or 1,25-(OH)₂ D forms of the vitamin, supplementation directly with 25-OH D or 1,25-(OH)₂ D is necessary. Supplementation with 20 µg 25-OH D has also been shown to effectively raise serum 25-OH D concentrations to a greater degree than supplementation with D₃.

Individuals being treated for deficiency should be monitored. Typically, serum 25-OH D concentrations (along with other biochemical indicators associated with rickets and osteomalacia) should be rechecked after about 3 months of supplementation. Some radiological healing may be evident after 1 month, but complete healing usually requires a longer time frame.

At Risk for Deficiency

While exposure of skin (without sunscreen) to sunlight can maintain adequate vitamin D nutrition for most of the world's population, people with insufficient sun exposure and those with certain diseases or conditions may be at risk for vitamin D deficiency. Those at risk are listed hereafter.

- Older adults represent a population group at risk due to insufficient vitamin D intake, low sunlight exposure, age-associated 7-dehydrocholesterol content reductions in the skin, and reduced renal 1-hydroxylase activity in response to PTH.
- Impaired vitamin D absorption may occur in disorders characterized by fat malabsorption, such as Crohn's disease, celiac disease, cystic fibrosis, pancreatitis, and liver disease as well as with some bariatric surgical procedures used to treat obesity.
- Disorders affecting the parathyroid, liver, or kidneys impair synthesis of the active form of the vitamin.
- People on anticonvulsant drug therapy may develop an impaired response to vitamin D secondary to increased hepatic metabolism of the vitamin to inactive forms and subsequent reductions in calcium absorption.
- Infants may be at risk for deficiency because human milk is low in vitamin D and exposure to sunlight is typically minimal; recommendations for the prevention of vitamin D deficiency for exclusively or partially breastfed infants are 10 µg vitamin D daily, an amount that is equal to the RDA.

Associations with Diseases

Low vitamin D intake and/or status has been associated with increased risks of hypertension, cardiovascular disease, type 2 diabetes, certain cancers, falls, fractures, infections, inflammation, and some autoimmune disorders such as rheumatoid arthritis, Crohn's disease, multiple sclerosis, and type 1 diabetes mellitus, among other conditions.

However, not all studies have found such relationships. Given the multiple roles of the vitamin in the body and the large number of factors influencing serum 25-OH D concentrations, these inconsistencies are expected. Meta-analyses of the randomized controlled studies evaluating the effects of vitamin D on the prevention or treatment of these conditions, however, have failed to document consistent benefits from vitamin D supplementation. A common exception has been among groups of individuals with vitamin D deficiency, whereby supplementation with vitamin D has fairly consistently reduced some health risks and/or symptom severity.

Toxicity

The Tolerable Upper Intake Level for vitamin D has been set at 100 μg for children age 9 years and older, adolescents, and adults [6]. However, while vitamin D is one of the vitamins most likely to cause overt toxicity, many believe this upper level is too low and could perhaps be doubled.

Unlike oral ingestion of the vitamin, excessive sun exposure does not result in overproduction of endogenous vitamin D₃. Cutaneous vitamin D production reaches a maximum of about 500 μg , and extensive whole-body ultraviolet light irradiation generally raises serum 25-OH D concentrations to about 40–80 ng/mL [8].

A safe (healthy) upper limit for serum 25-OH D concentrations is unclear, although serum concentrations in excess of 150 ng/mL are generally agreed as being diagnostic of toxicity and concentrations between 80 and 150 ng/mL suggestive of possible toxicity [6]. In relation to health, a U-shaped relationship has been suggested, with detrimental consequences associated with both low and high concentrations of 25-OH D.

Manifestations of toxicity in adults include high serum ionized calcium (greater than about 5.7 mg/dL), high serum total calcium (about 11–16 or higher mg/dL), high serum phosphorus (greater than about 5 mg/dL), and hypercalciuria. The high serum calcium concentrations lead to calcinosis, that is, calcification of soft tissues including organs such as the kidneys, heart, and lungs, along with blood vessels. The calcification damages the blood vessels and tissues, resulting in manifestations such as hypertension, headache, renal dysfunction (characterized by polyuria, polydipsia, azotemia, and possibly nephrolithiasis and renal failure), heart damage, and, in some cases, death.

In the 1950s an epidemic of “idiopathic hypercalcemia” among English infants was traced to a daily intake of vitamin D between 50 and 75 μg . Symptoms of toxicity in the infants included anorexia, nausea, vomiting, hypertension, renal insufficiency, and failure to thrive. Because so many foods are now fortified with vitamin D and many over-the-counter products also contain the vitamin, the

consumer needs to consider all sources of the vitamin to ensure intake is not excessive.

Assessment of Nutriture

Serum 25-OH D concentrations are most often used to assess vitamin D status, with concentrations in the range of 21–29 ng/mL constituting vitamin D insufficiency, those less than 20 ng/mL (50 nmol/L) generally indicative of deficiency, and those less than about 10 or 12 ng/mL (25 or 30 nmol/L, respectively) representing severe vitamin D deficiency. Toxicity is considered when serum 25-OH D concentrations exceed about 150 ng/mL. However, the measurement and diagnostic use of total serum 25-OH D (vs. free and/or albumin-bound 25-OH D [which is likely more available to tissues for use] or vs. other indices) as an indicator of vitamin D status remains an area of investigation.

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10.3 VITAMIN E

Vitamin E encompasses eight compounds (vitamers). Each of these eight compounds contains a phenolic functional group on a chromanol/chromane ring (sometimes called the head of the molecule) and an attached phytyl side chain (sometimes called the **phytyl tail** of the molecule). The eight compounds (Figure 10.17) are usually divided into two classes:

- The tocopherols, which have saturated side chains with 16 carbons
- The tocotrienols (also called trienols), which have unsaturated side chains with 16 carbons.

Each class is composed of four vitamers that differ in the number and location of methyl groups on the

chromanol ring. Vitamers in both classes are designated as α , β , γ , or δ . Only α -tocopherol has biologic activity and can meet the body's need (requirement) for the vitamin, as the body cannot interconvert the vitamers.

Structurally, tocopherols and tocotrienols contain three chiral centers with a configuration of “R” at three positions. R and S are used to designate stereoisomers of asymmetrical molecules such as vitamin E. The naturally occurring and biologically active form of α -tocopherol exists as only one stereoisomer, RRR α -tocopherol. In this RRR form, the R-configuration is at carbon 2 of the chromanol ring and carbons 4 and 8 of the side chain.

The term *tocopherol* is derived from the Greek word *tokos*, which means “childbirth,” and *phero*, which means “to bear or bring forth.” This terminology is based on the vitamin's discovery by H. Evans and K. Bishop in 1922, when they found that rats could not reproduce when given a diet of rancid lard. Wheat germ oil provided the needed vitamin; the oil was later purified, and the vitamin was extracted and named vitamin E (following D, which had been previously discovered). The chemical structure of the vitamin was identified in 1938.

Sources

Vitamin E, in its various forms, is found primarily in plant foods, especially nuts, foods made from nuts (e.g., nut butters), seeds, and the oils from plants. As seen in Table 10.5,

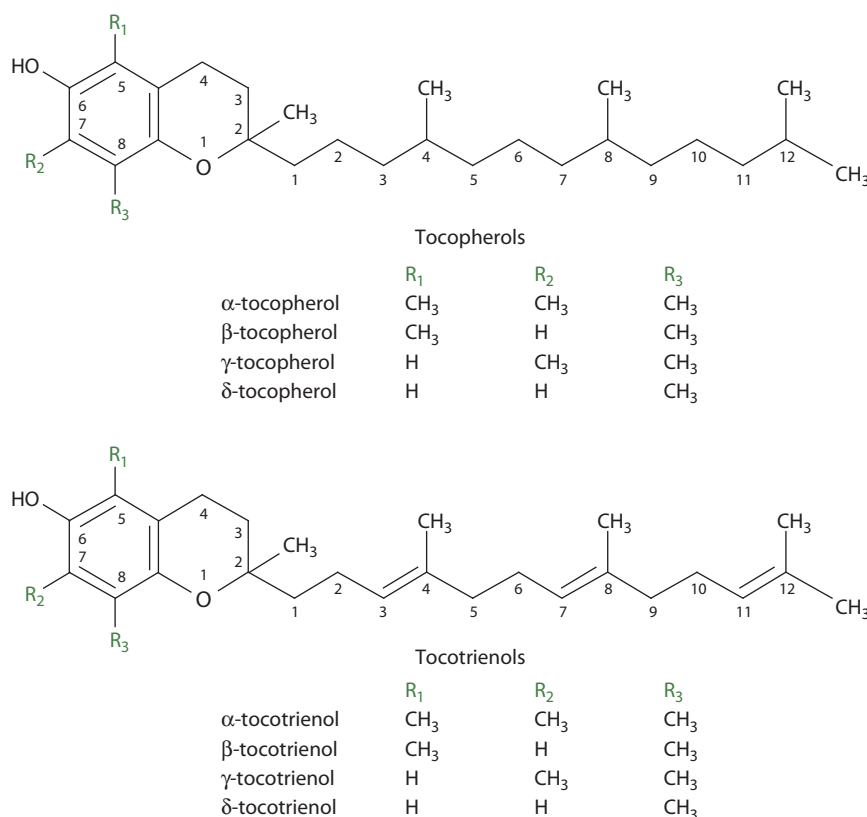


Figure 10.17 The structures of the various forms of the tocopherols and tocotrienols.

Table 10.5 Vitamin E Content of Selected Foods*

Food (serving)	Vitamin E (mg)
Wheat germ oil (1 Tbsp)	20.3
Sunflower oil (1 Tbsp)	5.6
Safflower oil (1 Tbsp)	4.6
Canola oil (1 Tbsp)	2.4
Corn oil (1 Tbsp)	1.9
Soybean oil (1 Tbsp)	1.1
Almonds, dry roasted (1 oz)	7.0
Hazelnuts (1 oz)	4.3
Peanut butter (2 Tbsp)	2.9
Peanuts, dry roasted (1 oz)	2.2
Cashews (1 oz)	0.3
Sunflower seeds (1 oz)	7.4
Margarine (1 Tbsp)	1.6
Mayonnaise (1 Tbsp)	0.7
Broccoli, cooked (½ c)	1.1
Collards, cooked (½ c)	0.8
Carrots, raw (½ c)	0.4
Avocado, raw (½ c)	1.5
Kiwifruit (1 medium)	1.1
Tomato (1 medium)	0.7
Bread, whole wheat (1 slice)	0.8
Oatmeal, instant (1 packet)	0.2

*The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>. A list of vitamin E-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitaminE-Food.pdf>.

which provides the α -tocopherol content of some of these plant foods, wheat germ oil provides significant quantities of vitamin E; lesser (but still relatively high) amounts are also present in other oils. Some oils, including soybean, canola, and corn, contain less α -tocopherol and more δ -tocopherol. Foods (especially full-fat varieties) made from vegetable oils, such as salad dressings, mayonnaise, and margarine, also represent major sources of α -tocopherol. Other plant sources providing relatively small amounts of vitamin E include whole-grain cereals and breads and some fruits and vegetables. The green (chloroplast) portions, such as the leaves of plants, contain more α -tocopherol, while the other portions of the plant provide some γ -, δ -, and β -tocopherols. In foods of animal origin, vitamin E, primarily α -tocopherol, is found concentrated in fatty tissues. Higher-fat ground beef (i.e., 20% fat/80% lean) provides about 0.4 mg of α -tocopherol/3-oz cooked portion. Smaller amounts of the vitamin are found in cheese, eggs, and seafood. Animal products, however, when compared to plants, represent an inferior source of vitamin E.

Tocotrienols are found in small quantities in seeds, legumes especially soybeans, palm and coconut oils, cocoa

butter, and cereal grains, especially the bran and germ fractions of barley, rice, rye, and oats. Palm oil, one of the more saturated oils from a plant source, is also one of the richest natural sources of tocotrienols, with 70% of its vitamin E as tocotrienols and 30% as tocopherols. Rice bran oil is also a good source of δ -tocotrienol.

While the FDA does not require vitamin E to be listed on food labels unless the product has been fortified with the vitamin, the vitamin E content, when present on the label, is given in mg as well as a percentage of the Daily Value, which for vitamin E is 15 mg. Supplements vary in amount and form of vitamin E. Most single-ingredient supplements provide vitamin E in amounts ranging from about 15 to 450 mg. Multivitamin preparations usually provide about 15 mg.

The form of vitamin E in supplements may be natural or chemically synthesized (synthetic); the product label should indicate the form. Naturally occurring vitamin E is found as the stereoisomer RRR α -tocopherol (usually labeled as d- α -tocopherol). Synthetic forms of α -tocopherol provide a mixture of the vitamin's eight possible stereoisomers, including four R-forms (RRR, RSR, RRS, and RSS) and four S-forms (SRR, SSR, SRS, and SSS); these products are usually labeled as DL- or dl- α -tocopherol or as all-racemic (all-rac) α -tocopherol. Supplements containing the stereoisomer mixtures are not as active (about 50% less on an equal-weight basis) as the naturally occurring RRR d- α -tocopherol. Thus, 1 mg vitamin E/RRR d- α -tocopherol is equivalent to 2 mg *all rac-* or *dl-alpha*-tocopherol.

Vitamin E in supplements and fortified foods may be in the form of an ester. Examples of esterified forms of the vitamin include dl- α -tocopheryl acetate and dl- α -tocopheryl succinate. Esterification helps to protect the vitamin from oxidation and thus prolong the shelf-life of the product; unesterified vitamin E is easily oxidized and relatively unstable.

Vitamin E, usually as vitamin E acetate, is also found in many creams designed for topical use. While concentrations of the vitamin in these products vary considerably, credible scientific studies have not supported the use of these creams in the prevention of scarring (a frequently encountered claim).

Vitamin E, like other fat-soluble vitamins, is susceptible to destruction during food preparation and storage. The processing of some foods also significantly reduces their vitamin E content; for example, the germ is removed in the milling of wheat to make white flour, and thus substantial amounts of the vitamin are lost. Tocopherols are also oxidized with lengthy exposure to air. In addition, exposure of the vitamin to light and heat can also lead to increased destruction. Thus, for example, the roasting of nuts reduces their vitamin E content.

Digestion and Absorption

Whereas the tocopherols are found free in foods, the tocotrienols are found esterified and must be hydrolyzed before absorption. Similarly, synthetic ester forms of the tocopherols, such as tocopheryl acetate or succinate, must be digested before absorption. Pancreatic esterase and especially duodenal mucosal esterase (also called carboxyl ester hydroxylase) function in the lumen or at the brush border membrane of enterocytes to hydrolyze tocotrienols and synthetic ester α -tocopherols so they can be absorbed.

Vitamin E is absorbed primarily in the jejunum by passive diffusion; bile salts are required for emulsification, solubilization, and subsequent formation of a mixed micelle. This micelle diffuses through the unstirred water layer adjacent to the brush border membrane of the enterocyte. The vitamin is then believed to passively diffuse into cells, although uptake may also occur via one or more transporters including scavenger receptor class B type 1 (SR-B1), cluster of differentiation (CD36), and Niemann-Pick Cholesterol 1-like 1 (NPC1L1) [1]. Simultaneous digestion and absorption of dietary lipids with vitamin E improves the vitamin's absorption, which ranges from about 20 to 70%. Higher intake of the vitamin appears to reduce its absorption. Little is known about tocotrienol absorption; however, the involvement of the NPC1L1 transporter in δ -tocotrienol absorption has been suggested.

Transport, Tissue Uptake, and Storage

Absorbed tocopherols and tocotrienols, present in the enterocytes, are incorporated (without esterification) into chylomicrons along with other fat-soluble vitamins, cholesterol esters, phospholipids, triacylglycerols, and apoproteins. Intracellular protein(s) needed to “traffick”/carry α -tocopherol and other forms of the vitamin are thought to include sec 14p-like proteins (SEC14L2-4) and tocopherol-associated protein (TAP1-3). The chylomicrons are released by exocytosis into lymphatic capillaries/lacteals for transport via the lymphatic system, and then, via the thoracic duct, enter into general circulation (i.e., the blood). As with vitamins A and D, conditions associated with fat malabsorption negatively impact the intestinal absorption of vitamin E.

Hepatic uptake of vitamin E occurs following the delivery of the tocopherols and tocotrienols via chylomicron remnants. The liver metabolizes the various forms of vitamin E, but only RRR α -tocopherol is incorporated into nascent VLDLs. An α -tocopherol transfer protein (TTP), made in the liver, carries out this transfer while also preventing the vitamin's oxidation. An ATP-binding cassette A1 (ABCA1) transporter may also facilitate the transfer and release of the α -tocopherol-rich VLDL from the liver and into the blood. A deficiency or absence of TTP caused by gene

mutations leads to a vitamin E deficiency. Additionally, conditions affecting the liver, such as nonalcoholic fatty liver disease and metabolic syndrome, among others, may disrupt the “trafficking” of the vitamin and affect its use. It is because of the specificity of TTP that other forms of the vitamin are not resecreted into the circulation.

α -Tocopherol is the primary form of vitamin E found in the blood; concentrations of other forms of vitamin E in the plasma are considerably lower. In the blood, the vitamin (tocopherols and tocotrienols) is exchanged among the various lipoproteins, which maintain plasma concentrations and distribute the vitamin to tissues. The exchange of the vitamin among the lipoproteins, primarily HDLs and LDLs, is mediated by a phospholipid transfer protein. LDLs, which possess the highest concentrations of vitamin E, are thought to contain about five to nine α -tocopherol molecules per LDL. The half-life of RRR α -tocopherol in the plasma is about 48 hours. Plasma α -tocopherol (bound within lipoproteins) concentrations usually range from about 5 to 20 mg/L.

Tocopherol uptake into cells occurs in association with the uptake of lipoproteins. Thus, vitamin E is taken up (1) as receptor-mediated uptake of LDLs occurs, (2) through lipoprotein lipase-mediated hydrolysis of chylomicrons and VLDLs, (3) through HDL-mediated nutrient delivery, and (4) possibly by other mechanisms. A phospholipid transfer protein (PLTP) may facilitate vitamin E transfer from the lipoproteins to the cell membranes. Uptake of the vitamin across the blood–brain barrier is thought to occur as HDLs are taken up via SR-B1 receptors. ApoE lipoproteins, generated by astrocytes, are thought to take up the vitamin E and transport it throughout the central nervous system.

Within the cytosol as well as other locations (including the nucleus) of cells, α -tocopherol is thought to bind to similar proteins, as indicated in the intestinal cells, for intracellular transport/trafficking (e.g., TAP1, TAP2, and TAP3 and SEC14L2, SEC14L3, and SEC14L4). ABCA1 transporter is also thought to be involved in trafficking and efflux of the vitamin from cells; this protein is also known to transport cholesterol and phospholipids.

Within cells, vitamin E is primarily incorporated in the phospholipid bilayer of membranes including the plasma, mitochondrial, and endoplasmic reticulum (among others). Vitamin E's chromanol group is likely directed toward the membrane surface (near the phosphate region of the phospholipid), and its phytyl tail is directed toward the hydrocarbon region. The presence of the vitamin within membranes influences membrane properties including fluidity, permeability, stability, and shape (curvature). Through its presence in specific microdomains of membranes, vitamin E also indirectly affects the activity of protein receptors and enzymes involved in signal transduction [2]. The vitamin has several other functions, as discussed in the next section.

There is no one storage organ for vitamin E. The largest amount (over 90%) of the vitamin is concentrated in an unesterified form in fat droplets in adipose tissue. The concentration of vitamin E in adipose tissue increases linearly with absorbed dietary vitamin E; however, the release of the vitamin from adipose tissue is slow (similar to vitamin D), even during periods of low vitamin E intake. Other tissues that take up smaller amounts of vitamin E include the liver, lungs, heart, muscle, adrenal glands, spleen, and brain. Unlike the adipose tissue, the vitamin E concentration in these tissues remains relatively constant or increases only at a slow rate with increased ingestion of the vitamin. During times in which vitamin E intake is low, the liver, skeletal muscle, and plasma serve as available sources of the vitamin.

Functions and Mechanisms of Action

The principal function of vitamin E (both tocopherols and tocotrienols) is as an antioxidant. It is in this capacity that the vitamin maintains the integrity of membranes and lipoproteins, protecting them (and other components) from oxidation. The mechanism by which vitamin E protects the membranes from destruction is through its ability to prevent the oxidation (peroxidation) of unsaturated fatty acids contained in the phospholipids of the membranes, including the cell's plasma membrane and organelle membranes (mitochondrial, endoplasmic reticulum, among others). Tissues with cell membranes especially susceptible to oxidation include the lungs, brain, and erythrocytes. Erythrocyte membranes, for example, are vulnerable because they are high in polyunsaturated fatty acids and are exposed to high concentrations of oxygen. In addition to the vitamin's antioxidant functions, *in vitro* studies provide evidence of many additional possible roles for the vitamin, including signal transduction and gene expression. Each of these roles are discussed hereafter.

Antioxidant Roles

As an antioxidant, vitamin E can destroy singlet oxygen and can stop reactions involving free radicals (sometimes called free-radical termination or chain-breaking). This section addresses each of these aspects of vitamin E function.

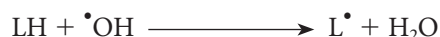
Singlet Molecular Oxygen Destruction Singlet molecular oxygen, also called singlet oxygen, $^1\text{O}_2$, is a very reactive and destructive compound that may be formed in the body from lipid peroxidation of membranes, transfer of energy from light (photochemical reactions), the respiratory (oxidative) burst occurring in neutrophils (enzymatic reactions), and from dismutation of superoxides (spontaneous reaction). Singlet oxygen readily reacts with organic molecules such as protein, lipids, and DNA and thus can damage cellular components unless removed. As discussed earlier in this chapter in the section

on the antioxidant functions of carotenoids, quenching is a process by which electronically excited molecules, such as singlet oxygen, are inactivated. Specifically, physical quenching occurs when the singlet excited oxygen is deactivated without light emission and generally involves electron energy transfer. Like carotenoids, vitamin E has oxygen-quenching abilities. The ability of vitamin E to physically quench singlet oxygen is related to the free hydroxyl group in position 6 of vitamin E's chromane ring (Figure 10.17). Yet, all tocopherols are not equal in their quenching abilities: α -tocopherol was found to be as or more effective in the quenching of singlet oxygen than β -tocopherol and γ -tocopherol, followed in descending order by δ -tocopherol. Similarly, among the tocotrienols, δ -tocotrienol is more effective in free-radical elimination than β -tocotrienol, followed in descending order by γ -tocotrienol and α -tocotrienol.

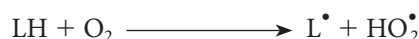
Free-Radical Termination The structure of vitamin E, specifically the phenolic hydroxyl group, provides hydrogen ions to terminate the actions of free radicals and thus limit their destruction (lipid peroxidation) of cell membranes and other cell components. Reactions involving free radicals often occur in three phases: initiation, propagation (ongoing generation), and termination, with the last involving vitamin E. A description of the three phases, including the reactions occurring in each, is presented next.

Initiation typically begins with a free radical, such as, for example, a superoxide anion (O_2^-), hydroxyl radical ($\cdot\text{OH}$), peroxy radical ($\text{ROO}\cdot$), or hydroperoxyl ($\text{HO}_2\cdot$) radical. Free radicals are highly reactive and rapidly take electrons from surrounding organic molecules. If the organic molecule is a polyunsaturated fatty acid (PUFA) present in the phospholipid portion of the cell membrane, the membrane is damaged. In fact, membrane lipid peroxidation is thought to represent a primary event in oxidative cellular damage. Specifically, hydrogen atoms from the methylene groups ($-\text{CH}_2-$) found between double bonds in polyunsaturated fatty acids ($-\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}-$) are primary targets for proton abstraction by radicals. Examples of initiation reactions are:

- The reaction between lipid compounds (LH) such as PUFA and free hydroxyl radicals ($\cdot\text{OH}$) leads to the formation of a lipid carbon-centered or alkyl radical ($\text{L}\cdot$) and water, as shown here and in Figure 10.18:



- Alternately, lipid compounds (LH) can react with molecular oxygen (O_2) to generate lipid carbon-centered or alkyl radicals and the hydroperoxyl radical $\text{HO}_2\cdot$, as follows:



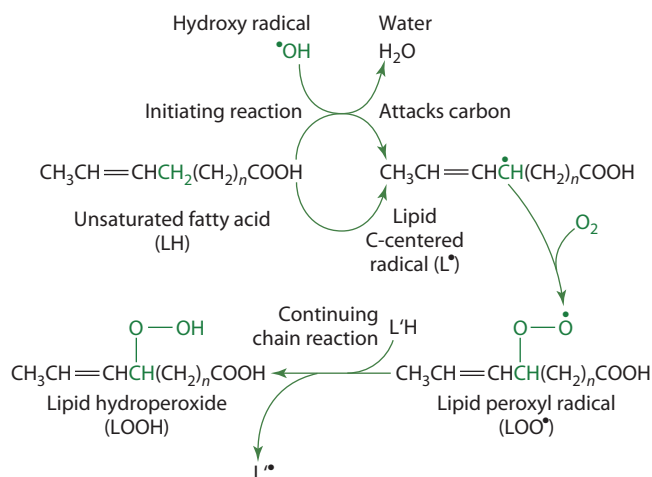


Figure 10.18 Initiating and chain reactions caused by hydroxy free-radical attack on unsaturated fatty acid.

Once lipid carbon-centered or alkyl radicals are formed, they may react to form additional radicals in propagation reactions. Propagation is the second step in the process of lipid peroxidation.

- Lipid carbon-centered or alkyl radicals can react with molecular oxygen in a propagation reaction to form a lipid peroxy radical $\text{LOO}^\bullet$ and promote peroxidation, as shown here and in Figure 10.18:



Lipid peroxy radicals ($\text{LOO}^\bullet$), once formed, can abstract a hydrogen atom from other organic compounds including more polyunsaturated fatty acids ($\text{L}'\text{H}$) in membranes or in lipoproteins to generate lipid hydroperoxides (LOOH) and a chain reaction with the $\text{L}'^\bullet$, as shown here and in Figure 10.18:



Termination of chain reactions (also referred to as free-radical scavenging) is the final step. Without termination, one initiating event could result in the generation of thousands of lipid peroxides and massive cellular damage.

Vitamin E located in or near membrane surfaces can react with peroxy radicals ($\text{LOO}^\bullet$) before they interact with fatty acids in cell membranes or other cell components. Thus, vitamin E terminates chain-propagation reactions. Specifically, vitamin E (EH , reduced state), because of the reactivity of the phenolic hydrogen on its carbon 6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, provides a hydrogen for the reduction of lipid peroxy radicals, as shown here:



Vitamin E (EH) also provides a hydrogen for the reduction of lipid carbon-centered radicals:



In these reactions, vitamin E, after hydrogen donation, becomes oxidized. $\text{E}^\bullet$ represents oxidized vitamin E (also called an α -tocopherol radical or a tocopheroxyl radical). This tocopheroxyl radical can react with another peroxy radical to form an inactive product such as tocopherylquinone. Alternately, the tocopheroxyl radical that is generated may be reduced; such a reaction is important since it enables the vitamin's reuse.

Regeneration of the reduced form of vitamin E (Figure 10.19) requires reducing agents, such as other vitamins (e.g., vitamin C), reduced glutathione (GSH), NADPH, ubiquinol, or dihydrolipoic acid; see also the Perspective at the end of this chapter. Vitamin E is only one line of defense against oxidative tissue damage and, consequently, the vitamin likely functions better in the presence of other antioxidants.

Signal Transduction

Vitamin E and long-chain metabolites of the vitamin [hydroxychromanol (α -13' OH) and α -13'-carboxychromanol (α -13' COOH); Figure 10.20] affect signal transduction by binding directly to enzymes and transport proteins involved in signal transduction. For example, vitamin E binds to and inhibits the activity of protein kinase C (PKC), a serine/tyrosine kinase that functions in

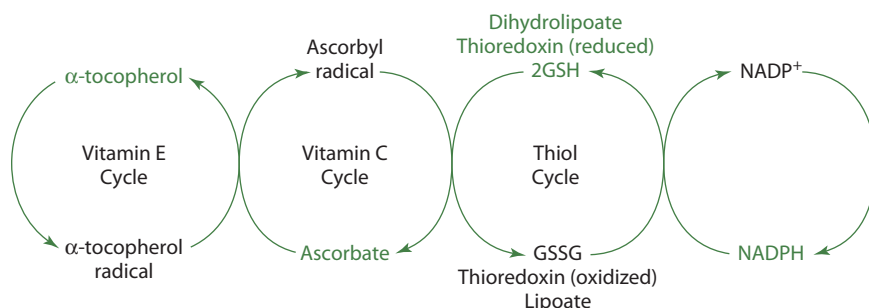


Figure 10.19 The regeneration of vitamin E (α -tocopherol).

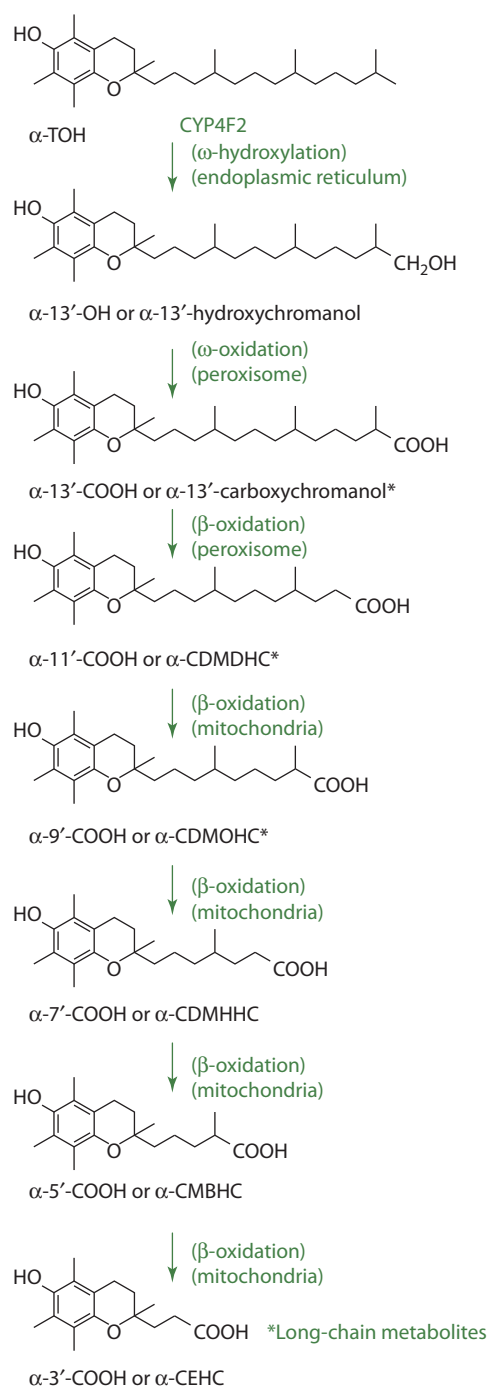


Figure 10.20 Vitamin E metabolism.

the transduction of signals from G protein–coupled tyrosine kinase receptors and nonreceptor tyrosine kinases to the nucleus via the hydrolysis of phospholipids. This inhibition serves one of many means by which the vitamin exhibits anti-inflammatory effects as well as protective effects against lipid deposition in blood vessels and platelet aggregation [3].

The vitamin, along with its long-chain metabolites, also inhibits phospholipase A2 (PLA2); 5-, 12-, and 15-lipoxygenases (LOX); and cyclooxygenase 1 and 2 (COX1, COX2). Phospholipase A2 catalyzes the release

of arachidonic acid from glycerol's second carbon within plasma membrane phospholipids. Arachidonic acid is then metabolized to generate a number of bioactive inflammatory prostaglandins, thromboxanes, and interleukins, among other compounds. Inhibition of PLA2 by vitamin E as well as inhibition of 5-lipoxygenase by long-chain vitamin E metabolites helps reduce inflammation and suppress the release of inflammatory cytokines. It is perhaps noteworthy that long-chain vitamin E metabolites are typically found at sites of inflammation in the body [4]. The vitamin also appears to help diminish platelet aggregation (and thus blood clot formation).

Gene Expression

Vitamin E influences the activities of several transcription factors, including, for example, peroxisome proliferator-activated receptor γ (PPAR γ), nuclear factor erythroid-derived 2-like 2 (NRF2), nuclear factor kappa β (NF κ β), retinoic acid-related orphan receptor α (ROR α), hypoxia-inducible factor α (HIF α), estrogen receptor β (ER β), and steroid and xenobiotic receptor (SXR) [3]. Changes in the activities of these transcription factors by vitamin E in turn impact gene expression and several cellular processes. Some of these cellular processes involve steroidogenesis (including cholesterol synthesis), lipid uptake (via scavenger receptors CD36 and SR-B1, among others), vitamin E use (TTP), antioxidant defense, the cell cycle, inflammation, cell adhesion, and blood coagulation, among other activities [2]. A more specific example includes vitamin E's interactions with SXR, which affects the transcription of genes coding for (1) selected cytochrome P450 enzymes involved in vitamin E's metabolism and (2) CD36 involved in the vitamin's cellular uptake. Changes in the CD36 expression in turn also affect the uptake of oxidized LDLs, among other lipids.

Vitamin E also impacts specific micro (mi)RNAs, a group of noncoding RNAs that bind to untranslated regions on mRNA to inhibit translation of the mRNA into protein. One miRNA is thought to be able to interact with hundreds of different target mRNAs and thus inhibit multiple genes and silence entire metabolic pathways.

Immune System Function

Vitamin E, which can be found embedded in the plasma membranes of immune system cells, modulates numerous immune functions. Its impact on the immune system relates to many previously discussed roles of the vitamin including effects on cell membranes, gene expression, signal transduction, and antioxidant defense, which in turn impact the cell cycle and inflammation, among other activities. The vitamin appears to largely impact T-cell function, although humoral immunity is also affected. Improvements in immune system function in older adults with increased vitamin E intakes have been suggested as one reason for increasing recommended intakes for this population group.

Selected Pharmacological Uses/Other Roles

Vitamin E's antioxidant and anti-inflammatory properties form a logical basis for its likely benefits in reducing risk of and/or treatment of *heart disease* as well as *cancer*. However, consistent, convincing beneficial effects have not been demonstrated in the prevention and/or treatment of either heart disease or cancer with vitamin E supplementation. Moreover, a few studies found an increased mortality and increased risk of prostate cancer with vitamin E supplementation. Thus, the use of vitamin E supplements is not recommended at present for cancer or heart disease prevention or treatment.

Because free-radical-induced damage contributes to the development of *cataracts* and *age-related macular degeneration*, and because vitamin E functions as an antioxidant, it is also logical that vitamin E (like vitamin C and β -carotene) may help in prevention or treatment. As discussed under the section “Carotenoids,” “Eye Health,” with the exception of a multisupplement trial (The Age-Related Eye Disease Study), which provided vitamin E (400 IU), along with vitamin C (500 mg), β -carotene (15 mg), zinc (80 mg), and copper (2 mg), and found only modest effects in slowing the progression of age-related macular degeneration [5], most other studies and meta-analyses of studies providing vitamin E alone or with other antioxidants have not reported benefits on eye health from supplementation.

Vitamin E supplementation has also been suggested to diminish oxidation in individuals with conditions characterized by increased metabolic stress and thus lipid peroxidation such as iron toxicity, diabetes, metabolic syndrome, and nonalcoholic fatty liver disease, among others. By maintaining plasma cell membranes, vitamin E has also been suggested to enhance cellular glucose uptake (which is especially important for those with diabetes). Additionally, vitamin E's antioxidant function has been linked to improved bone health and reduced fracture risk. Further studies are needed to evaluate these possible roles.

In *neurodegenerative diseases* such as Alzheimer's disease, protein aggregates typically accumulate and are coupled with the loss of specific neuronal cell populations. Because oxidative stress (free radicals) is thought to be linked (at least in part) with the development of some neurodegenerative conditions, provision of antioxidants such as vitamin E is theorized to be beneficial. Yet, as with other conditions, consistent benefits of vitamin E supplementation in older adults with cognitive decline, individuals with Alzheimer's disease, as well as those with other neurodegenerative diseases (such as Parkinson's disease and other forms of dementia) have not been documented. Benefits from topical vitamin E used to prevent scar formation have also not been documented in scientific studies; however, vitamin E creams, usually containing α -tocopherol acetate, may improve skin dryness.

Tocotrienols

Research focusing on tocotrienols is expanding. Tocotrienols, especially δ -tocotrienol, function in cell signaling and as antioxidants, like other forms of vitamin E. Additionally, the tocotrienols exhibit cholesterol-lowering abilities by suppressing the activity of the rate-limiting enzyme 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase in cholesterol synthesis. This form of the vitamin also inhibits platelet aggregation and monocyte adhesion as well as exhibits anti-inflammatory properties. Other studies suggest that tocotrienols also exhibit antiproliferative, antiapoptotic, and antiosteoporotic properties with implications in the prevention of cancer, diabetes, osteoporosis, and neurodegenerative conditions. Further research should help to more clearly elucidate these possible functions and their mechanisms of action.

Interactions with Other Nutrients

Because the antioxidant functions of vitamin E in the body are closely tied to those of selenium-dependent glutathione peroxidase (an enzyme that converts lipid peroxides into lipid alcohols), an interrelationship exists between vitamin E and selenium. The actions of both nutrients are complementary, and higher concentrations of one nutrient can reduce the effects of lower concentrations of the other nutrient. Similarly, some of vitamin C's functions also complement vitamin E, and vitamin C can regenerate vitamin E following its oxidation.

A relationship between vitamin E and dietary polyunsaturated fatty acids has been suggested because the requirement for the vitamin increases or decreases as the degree of unsaturation of fatty acids in body tissues rises or falls; body tissue lipids, in turn, are influenced by dietary lipid intake. However, foods high in polyunsaturated fatty acids also tend to be relatively good sources of vitamin E.

A high intake of vitamin E can interfere with other fat-soluble vitamins. For example, vitamin E inhibits the absorption and metabolism of both β -carotene and vitamin K, including the conversion of phyloquinone to menaquinone [6]. Vitamin E, when present in high concentrations, may also increase the risk for bleeding secondary to interference in vitamin K's role in blood clotting.

Metabolism and Excretion

Higher concentrations of α -tocopherol or any of the other forms of vitamin E within the liver increase the vitamin's degradation, a process requiring several cytochrome P450 enzymes. Hepatic metabolism of vitamin E (all forms) begins with an omega (ω)-hydroxylation reaction of the vitamin's phytol side chain. The initial product, a long-chain metabolite, is hydroxychromanol (α -13'OH). The hydroxychromanol next undergoes further metabolism in the peroxisome to form another long-chain metabolite

known as α -13'-carboxychromanol (α -13'COOH). These two long-chain metabolites, although only present in tiny (nanomolar) concentrations, have been found to exhibit regulatory functions (discussed in the section "Functions").

Degradation of the long-chain vitamin E metabolites occurs in a series of reactions analogous to β -oxidation of fatty acids. The reactions occur initially in the peroxisome and later in the mitochondria and effectively truncate the vitamin's phytyl side chain (Figure 10.20). The end products include several medium- and short-chain metabolites called carboxyethyl-hydroxychromans (CEHC). Prior to urinary or fecal excretion, these carboxyethyl hydroxychromans are usually conjugated to glucuronic acid or sulfate. Urinary excretory products of the vitamin also include α -tocopheronic acid and α -tocopheronolactone conjugated to either glucuronic acid or sulfate.

Recommended Dietary Allowance

The recommendations for vitamin E are based on intake of the natural (labeled D- or d-) RRR α -tocopherol form and the synthetic all-rac stereoisomeric forms (RSR, RRS, and RSS) of α -tocopherol (labeled as dl and used in fortified foods and vitamin supplements) [6]. However, because these synthetic forms of the vitamin are only about 50% (by weight) as active as the same amount of the natural form, 1 mg d- α -tocopherol is equal to 2 mg *all rac*- (dl-) α -tocopherol. The boxed feature, International Units – Vitamin E, shows the conversions between international units, which were often used prior to 2020, and the natural and synthetic forms of the vitamin.

The RDA for vitamin E for adult men and women (including during pregnancy for women) is 15 mg of naturally occurring RRR α -tocopherol [6]. During lactation, recommendations are slightly higher, at 19 mg α -tocopherol for women [6]. The RDA for vitamin E for adults is based on the vitamin E requirement plus twice the coefficient of variation, rounded to the nearest mg [6]; however, the approach and specific methodology used to determine the vitamin's requirements and subsequent recommendations have been questioned [7]. Higher recommended vitamin E intakes for older adults have also been suggested in scientific literature to improve immune system function [7].

Deficiency

A deficiency of vitamin E resulting in clinical manifestations in humans is rare. However, individuals with mutations in the gene coding for the α -tocopherol transfer protein (TTP) develop a vitamin E deficiency; the condition is called ataxia with isolated vitamin E deficiency (AVED). As indicated by the name, a primary characteristic is ataxia (uncoordinated muscle movements or poor muscle coordination). The neuromuscular impairments are thought to result in part from degeneration of neurons and associated effects on muscle. Other manifestations may include peripheral neuropathy (exhibited by pain or numbness in extremities), loss of vibratory sense, skeletal muscle pain (myopathy), and weakness.

Vitamin E deficiency also negatively impacts cell membranes, which is not unexpected given the vitamin's role in maintaining the membrane integrity. The effects are apparent, especially in red blood cells, which become fragile and lyse (break), causing hemolytic anemia; such effects are often seen prior to neuromuscular manifestations. In the skin, ceroid pigments often accumulate and appear as visible brown spots. Plasma/serum α -tocopherol concentrations less than 5 mg/L are generally suggestive of deficiency.

At Risk for Deficiency

A few population groups are at risk for deficiency, including premature infants (who typically exhibit impaired fat utilization secondary to prematurity) and individuals with fat malabsorption disorders such as cystic fibrosis (characterized by pancreatic lipase deficiency) and hepatobiliary system disorders, particularly chronic cholestasis (characterized by decreased bile production). Individuals with specific genetic disorders affecting some lipoproteins—for example, individuals with abetalipoproteinemia—may develop a vitamin E deficiency because of a lack of microsomal transfer protein needed to assemble or secrete lipoproteins containing apolipoprotein B. Treatment of vitamin E deficiency in such circumstances necessitates the ingestion of large (5–10 g or about 100 mg/kg body weight) doses of the vitamin, whereas individuals with fat malabsorption disorders typically require water-soluble/miscible forms of the vitamin.

INTERNATIONAL UNITS – VITAMIN E

INTERNATIONAL UNITS (IUs) represent the quantity of a nutrient that produces a particular biologic effect, but do not account for differences in biological activities

among related compounds. While the use of IUs is being phased out, conversion information among the natural versus synthetic forms of vitamin E is as follows:

1 IU = 0.67 mg α -tocopherol if the vitamin E is from natural sources
1 IU = 0.45 mg α -tocopherol if the vitamin E is synthetic.

Inadequate dietary vitamin E intake is common among Americans, with over two-thirds of adults not meeting recommendations and in some cases requirements for the vitamin [6]. It is especially likely that people following a low-fat diet limit intake of foods that are major sources of vitamin E [6].

People with oxidative stress and/or inflammatory conditions (such as smokers or those with metabolic syndrome) may have higher requirements for vitamin E, but specific recommendations for these population groups have not been made.

Toxicity

Vitamin E appears to be one of the least toxic of the fat-soluble vitamins, although mild gastrointestinal problems (nausea, diarrhea, flatulence) may occur in some individuals with vitamin E intakes greater than 200 mg. However, it is because of an increased tendency for bleeding/impaired blood coagulation (due to antiplatelet effects and/or abnormal blood clotting) that a Tolerable Upper Intake Level of 1,000 mg of α -tocopherol for adults has been established [6]. This recommendation for an upper level of intake includes any form of supplemental α -tocopherol [6]. In addition to increased bleeding, higher intakes of the vitamin (1,000 mg or greater) have also been associated with moderate gastrointestinal distress; possible increased severity of respiratory infections; and occasional reports of muscle weakness, fatigue, and double vision.

Assessment of Nutriture

Evaluation of vitamin E status relies primarily on blood analyses. Plasma concentrations are responsive to dietary intake under deficiency and toxicity situations, with concentrations <5 mg/L suggesting deficiency and concentrations exceeding about 20 mg/L reflecting possible toxicity. A crude estimate of vitamin E status can also be obtained from an erythrocyte hemolysis test that compares the amount of hemoglobin released by red blood cells during incubation with dilute hydrogen peroxide *vs* the amount released during distilled water incubation. The result is expressed as a percentage, with $>20\%$ indicating deficiency and generally associated with plasma α -tocopherol concentrations below 5 mg/L; however, variables other than vitamin E status influence *in vitro* hemolysis. Other functional assessments include the measurements of expired ethane or pentane and indicators of lipid peroxidation.

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10.4 VITAMIN K

Vitamin K was named after the Danish word *koagulation*, which means “coagulation.” In the 1920s, H. Dam discovered that chicks fed a low-fat and cholesterol-free diet became hemorrhagic (i.e., they bled excessively) and that their blood took a long time to clot. The missing vitamin called K that corrected the problem was identified in about 1935. Dam (along with Doisy) was recognized with a Nobel prize in medicine in 1941 for the discovery.

Compounds with vitamin K activity have a 2-methyl 1,4-naphthoquinone ring with a substitution at position 3. The naturally occurring forms of vitamin K are phyloquinone (vitamin K₁), which has a phytyl group at position 3 of the ring (2-methyl 3-phytyl 1,4-naphthoquinone), and menaquinone (vitamin K₂), which has an unsaturated multiprenyl group at position 3. There are several menaquinones based on the number of isoprenoid groups. Menaquinones (abbreviated MK) are designated as MK-*n*, with *n* indicating the number of isoprenoid units in the side chain. One menaquinone, MK-4 (also called menatetrenone), is unique in that it can be synthesized in the body from phyloquinone; other menaquinones cannot be produced in the body from phyloquinone. Figure 10.21 depicts the structures of phyloquinone and menaquinone-7 (MK-7).

Sources

Dietary vitamin K is provided mostly as phyloquinone from the ingestion of plant foods. The richest and the main dietary sources of phyloquinones include leafy green vegetables, especially collard greens, spinach, turnip greens, swiss chard, some salad greens, and broccoli. Oils (especially soybean, canola/rapeseed, and olive) and margarine from plants represent other major sources of the vitamin. Smaller amounts of phyloquinone are found in some

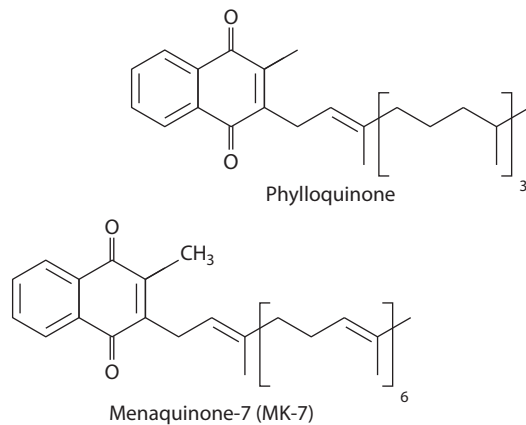


Figure 10.21 Structures of vitamin K.

fruits, among a few other foods. Phylloquinones from oils are thought to be better absorbed than those from plant foods, with estimates of absorption from vegetables ranging from 15 to 20%. Table 10.6 provides information on the vitamin K content of selected foods.

The vitamin K content of foods is not required to be listed on food labels by the FDA unless the food has been fortified with the vitamin. When present on food labels (and on supplement labels), the quantity of the vitamin is listed in μg as well as a percentage of the Daily Value, which for vitamin K is 120 μg . The vitamin is destroyed with excessive exposure to light and heat.

Menaquinones, primarily MK-6 through MK-11, are synthesized by a variety of facultative and obligate anaerobic bacteria that reside in the body's intestines. Examples of menaquinone-producing obligate anaerobes include *Bacteroides*, *Bacillus fragilis*, *Eubacterium*, *Propionibacterium*, and *Arachnia*; the facultative anaerobe *Escherichia coli* also generate menaquinone. Bacterial synthesis of vitamin K, however, is not sufficient to meet the body's needs for the vitamin [1].

In addition to bacterial provision of menaquinones, some foods contribute MK-4 and MK-7. MK-4 is present in relatively small amounts in many animal products such as salmon, milk, eggs, beef, chicken, and cheeses (Table 10.6). MK-7 is found in fermented foods, cultured vegetables, and dairy products including yogurt, milk and cheeses. A Japanese dish called natto, which is made from fermented soybeans, is particularly rich in MK-7 (Table 10.6). Foods fermented with bacteria such as some cheeses also provide some MK-8 and MK-9. These longer-chain menaquinones (with about seven or more units) tend to be better absorbed than MK-4.

Single-ingredient supplements of vitamin K are available as phylloquinone and as menaquinones, primarily MK-4 and MK-7. Water-soluble forms of the vitamin are also manufactured for people with fat malabsorptive disorders. Multivitamin/mineral preparations usually provide the vitamin as phylloquinone; however, the vitamin is often not included in supplements or, if included,

Table 10.6 Vitamin K (Phylloquinone and Menaquinone) Content of Selected Foods

Food (serving)	Phylloquinone (μg)	Food (serving)	Menaquinone (μg)
Collards, frozen, cooked ($\frac{1}{2}$ c)	520	Natto (3 oz)	850 (MK-7)
Turnip greens, frozen, cooked ($\frac{1}{2}$ c)	426	Chicken breast, rotisserie (3 oz)	13 (MK-4)
Spinach, raw (1 c)	145	Ground beef, broiled (3 oz)	6 (MK-4)
Kale, raw (1 c)	113	Ham, cooked (3 oz)	6 (MK-4)
Broccoli, cooked ($\frac{1}{2}$ c)	110	Egg, hard boiled (1)	4 (MK-4)
Pumpkin, canned ($\frac{1}{2}$ c)	20	Salmon, cooked (3 oz)	0.3 (MK-4)
Okra, raw ($\frac{1}{2}$ c)	16	Shrimp, cooked (3 oz)	0.3 (MK-4)
Lettuce, iceberg, raw (1 c)	14	Cheese, cheddar (1.5 oz)	4 (MK-4)
Vegetable juice ($\frac{3}{4}$ c)	10	Cheese, mozzarella (1.5 oz)	2 (MK-4)
Carrots, raw (1 medium)	8	Milk, 2% (1 c)	1 (MK-4)
Soybean oil (1 Tbsp)	25		
Canola oil (1 Tbsp)	10		
Olive oil (1 Tbsp)	8		
Soybeans, roasted ($\frac{1}{2}$ c)	43		
Edamame, frozen, prepared ($\frac{1}{2}$ c)	21		
Pine nuts (1 oz)	15		
Cashews, dry roasted (1 oz)	10		
Nuts, mixed, dry roasted (1 oz)	4		
Blueberries ($\frac{1}{2}$ c)	14		
Grapes ($\frac{1}{2}$ c)	11		

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>. A list of vitamin K-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/VitK-Phylloquinone-Food.pdf> and <https://ods.od.nih.gov/pubs/usdandb/VitK-Menaquinone-Food.pdf>.

usually contains no more than about 120 μg . In contrast, the amount of phylloquinone and menaquinone present in single-ingredient supplements ranges widely from about 5 μg to several milligrams.

Absorption

Phylloquinone requires no digestion and is absorbed from the proximal small intestine, particularly from the jejunum. Like the other fat-soluble vitamins, both phylloquinone and dietary MK-4 and -7 are incorporated into a mixed micelle within the lumen of the small intestine along with bile, digested lipids, and other fat-soluble vitamins. The micellar solution diffuses through the lumen and unstirred water layer adjacent to the enterocytes, enabling close contact with the brush border membrane. Here the mixed micelle components dissociate, allowing for what was once believed as solely passive diffusion of the vitamin along a concentration

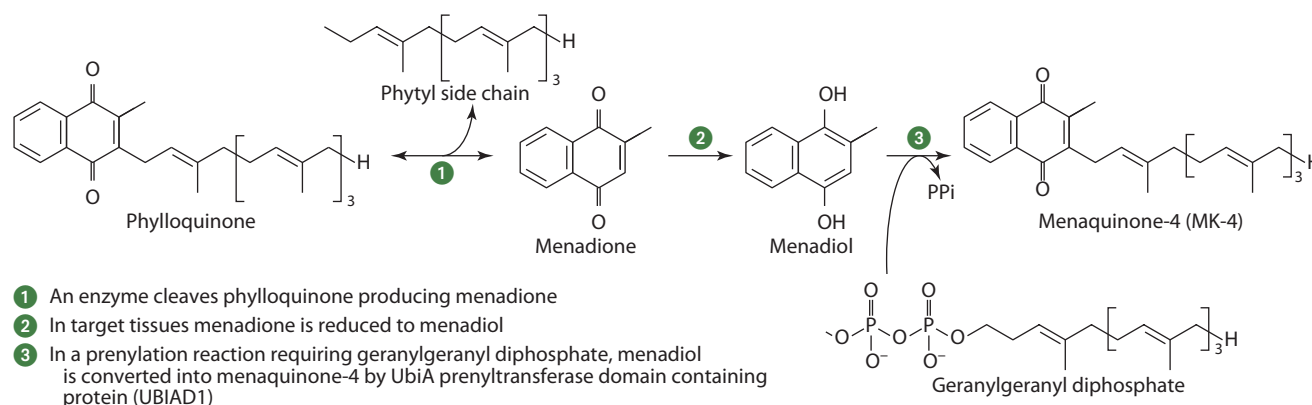


Figure 10.22 Phylloquinone metabolism and the generation of menaquinone-4.

gradient into intestinal cells. However, intestinal cell uptake of phylloquinone may also or instead occur via scavenger receptor class B type 1 (SR-B1), cluster of differentiation (CD36), and Niemann-Pick Cholesterol 1-like 1 (NPC1L1) [2]. As with the other fat-soluble vitamins, absorption of both phylloquinone and MK-4 and -7 is enhanced by the presence of dietary fats, bile salts, and pancreatic enzymes and juices and is negatively impacted by fat malabsorption disorders that are associated with impaired secretion of bile and/or pancreatic enzymes and juices.

Menaquinones that are synthesized by bacteria in the lower digestive tract are also absorbed, likely by passive diffusion, from the ileum and colon. However, the ability to absorb and use the bacterially produced vitamin varies considerably among individuals and has been difficult to determine accurately [1].

Transport, Tissue Uptake, and Storage

Within the enterocytes, vitamin K is incorporated into chylomicrons that carry the vitamin into the lymphatic and then the circulatory system for delivery of the vitamin to tissues. Chylomicron remnants deliver any vitamin K not taken up by tissues to the liver. From the liver, the vitamin is incorporated into VLDLs for secretion into the blood and transport to extrahepatic tissues.

In addition to incorporation into chylomicrons within the intestinal cells, phylloquinone may also be partially metabolized. This metabolism involves the removal of the vitamin's phytyl tail/side chain by a cleaving enzyme and results in the formation of menadione (Figure 10.22). The menadione then enters the lymphatic system as part of the chylomicron with subsequent entry into the blood where it is taken up by target tissues.

Within nonhepatic target tissues, menadione generated from phylloquinone undergoes further metabolism to MK-4. In the first step, menadione is reduced to menadiol. Next, menadiol undergoes prenylation to form MK-4 in a reaction catalyzed by UbiA prenyltransferase domain-containing protein 1 (UBIAD1) and requiring geranylgeranyl diphosphate.

It has been estimated that 5–25% of ingested phylloquinone is converted into MK-4 [2].

Phylloquinone is the main circulating form of the vitamin in the blood, although small amounts of some menaquinones may also be present. The vitamin is rapidly taken up for use by tissues, usually within about 24 hours of its appearance in the blood [3]. Normal plasma phylloquinone concentrations range from about 0.2 to 3.2 ng/mL (0.5 to 6.4 nmol/L).

Phylloquinone is stored primarily in cell membranes in several tissues, including the lungs, kidneys, bone marrow, pancreas, heart, brain, and adrenal glands. MK-4 is found throughout the body with higher amounts in the pancreas, kidneys, salivary glands, brain, and bone. The liver rapidly metabolizes vitamin K but retains only about 10% of the vitamin. Longer-chain menaquinones are converted to MK-4. Hepatic concentrations of phylloquinone range from about 2 to 20 ng/g of liver; hepatic concentrations of menaquinones (mainly MK-6, MK-7, MK-10, and MK-11) are about 10 times higher [3]. The body's pool of vitamin K, estimated at 50–100 µg, is low among the fat-soluble vitamins and turnover of the pool is fairly rapid at about 1.5 days [4].

Functions and Mechanisms of Action

Vitamin K is necessary for the post-translational carboxylation of specific glutamic acid (glutamyl) residues in proteins to form γ -carboxyglutamic acid (Gla) residues. The formation of Gla residues is necessary for blood clotting (hemostasis) and bone mineralization, which is discussed in further detail next along with a few lesser-characterized vitamin K-dependent proteins, which extend the vitamin's impact to processes such as phagocytosis, cell proliferation, cell adhesion, migration, apoptosis, and gene expression.

Blood Clotting

The vitamin K-dependent post-translational carboxylation of glutamic acid residues forms γ -carboxyglutamic acid on several proteins required for the coagulation of blood. The four most well-studied vitamin K-dependent blood-clotting proteins, also called factors and followed by

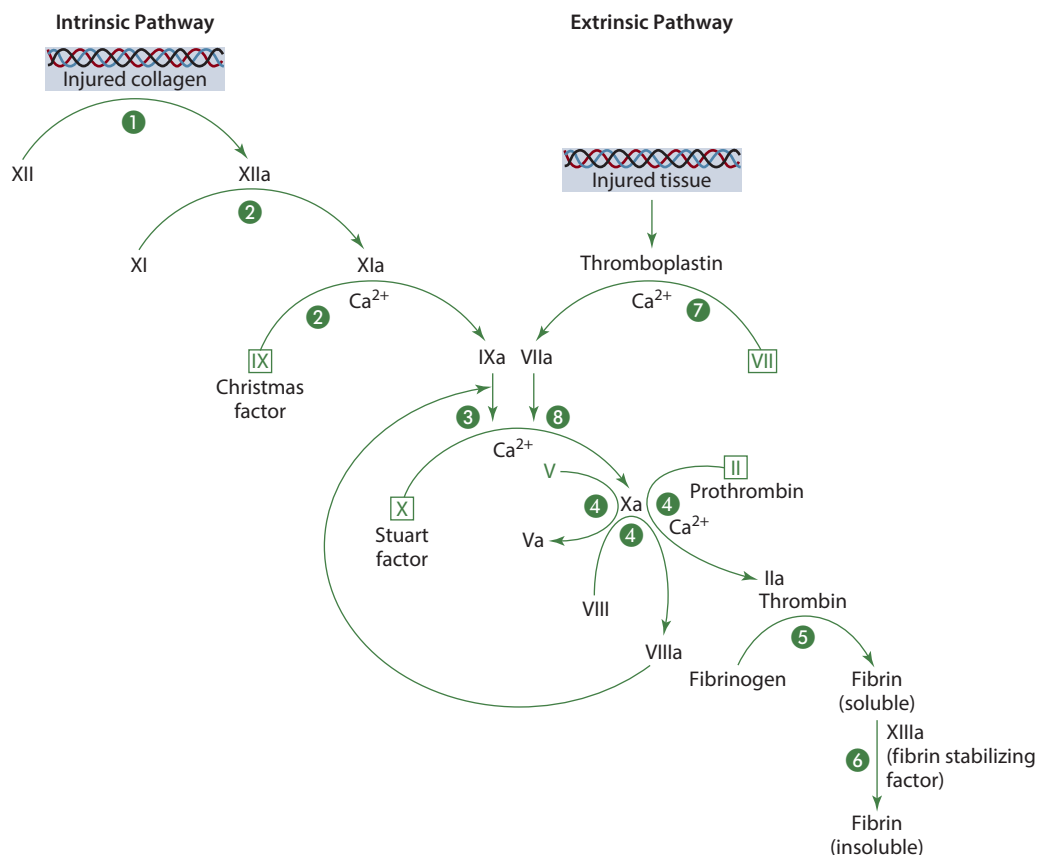
a Roman numeral, are factors II (also called prothrombin), VII, IX, and X. In addition, proteins C, S, Z, and M, also involved in blood clotting, require vitamin K for carboxylation.

Overview of Blood Clotting For blood to clot, fibrinogen, a soluble protein, must be converted to fibrin, an insoluble fiber network. Two pathways, intrinsic and extrinsic, lead to clot formation, as shown in Figure 10.23 and briefly described here.

In the intrinsic pathway, the coagulation process is initiated by the adsorption of factor XII, which circulates in the blood, onto a substance such as collagen, which becomes exposed with tissue injury. Upon contact, factor

XII becomes *activated*, as indicated by the letter *a* next to the factor.

- Through a series of reactions in the intrinsic pathway and the extrinsic pathway (which is activated by the release of thromboplastin by injured tissue), vitamin K–dependent factor X becomes activated, creating Xa, by factors IXa and VIIa, respectively.
- Factor Xa in turn activates vitamin K–dependent factor II (prothrombin) to produce IIa (thrombin).
- Thrombin catalyzes the proteolysis of fibrinogen to yield fibrin.
- Fibrin molecules aggregate to form a meshlike polymer, which then undergoes cross-linking by fibrin stabilizing



- 1 Initial step: Factor XII adsorbs onto a substance such as collagen, which becomes exposed with injury to a tissue. Upon contact, factor XII becomes activated (denoted by an "a").
- 2 Factor XIa, which becomes activated by XIIa, activates factor IX, a vitamin K–dependent protein.
- 3 Factor IXa activates factor X, another vitamin K–dependent protein.
- 4 Factor Xa converts factor II, prothrombin (vitamin K–dependent) into thrombin.
- 5 Thrombin alters fibrinogen to produce fibrin for clot formation.
- 6 Factor XIIIa, fibrin stabilizing factor, creates insoluble fibrin for final clot formation.
- 7 In the extrinsic pathway, factor VII, a vitamin K–dependent protein, becomes activated in the presence of thromboplastin released by injured tissue.
- 8 Factor VIIa works with factor IXa to activate factor X.

Figure 10.23 An overview of blood clotting. Vitamin K–dependent proteins are boxed.

factor to form an insoluble fibrin clot and stop bleeding (hemorrhage).

Other blood-clotting proteins—designated C, S, Z, and M—have also been identified as vitamin K–dependent carboxylated proteins. The function of protein M is unknown, but the other three proteins inhibit the blood-clotting process and thus exhibit anticoagulant functions. Protein Z inhibits factor Xa. Protein C inactivates factors VIIIa and Va and, along with protein S, enhances fibrinolysis to disrupt the clotting process. Protein S may also be involved in the regulation of osteoclast activity.

The Role of Vitamin K in Carboxylation of Glutamic Acid Residues Found on Some Blood Clotting Proteins

This section uses prothrombin as a “model” to more thoroughly describe the carboxylation process. Proteins like prothrombin require a vitamin K–dependent enzyme for the carboxylation of glutamic acid residues residing in the N-terminal. Once carboxylated, this glutamic acid (and the protein) is referred to as γ -carboxyglutamic acid (Gla), as shown in Figure 10.24. The carboxylation is required for the protein to become functional. The enzyme responsible for the γ -carboxylation, vitamin K–dependent γ -glutamyl carboxylase, is associated with rough endoplasmic reticulum (where vitamin K–dependent proteins are carboxylated), primarily in the liver. The liver is also where the hemostatic factors are synthesized. The enzyme, however, is found in all human tissues. This widespread occurrence of γ -glutamyl carboxylase suggests that the need for carboxylated proteins that can bind calcium is broad.

Gla residues are synthesized post-translationally. Gla residues on the blood-clotting proteins function to bind calcium. The calcium then mediates the binding of Gla proteins to negatively charged phospholipids on membrane surfaces of blood platelets and endothelial cells at the site of injury. This adsorption is essential in hemostasis.

The Vitamin K Cycle The participation of vitamin K in the carboxylation of glutamic acid residues in proteins necessitates some additional reactions, called the vitamin K cycle, to ensure the availability of the vitamin in the form needed for Gla production. More specifically,

the γ -glutamyl carboxylase enzyme requires vitamin K in its reduced form, referred to as vitamin K dihydroquinone or KH_2 . However, as γ -glutamyl carboxylase carboxylates glutamic acid residues in selected proteins, vitamin K dihydroquinone gets converted to vitamin K 2,3-epoxide. This epoxide form of the vitamin must be re-converted back to the vitamin K dihydroquinone form for further function. The cycle includes three forms of the vitamin, as a dihydroquinone, a 2,3 epoxide, and a quinone. No ATP is required in the cycle. Energy for the carboxylation is derived from the oxidation of vitamin K dihydroquinone by oxygen in its conversion to vitamin K 2,3-epoxide. The steps of the vitamin K cycle, in which vitamin K is converted to its reduced form and functions in the carboxylation process, are shown in Figure 10.25 and are reviewed briefly here.

- The carboxylation of glutamic acid residues in vitamin K–dependent proteins is catalyzed by γ -glutamyl carboxylase, which requires vitamin K dihydroquinone for activity. During the reaction, the dihydroquinone form of vitamin K is converted to vitamin K 2,3-epoxide. A two-step reaction next converts the epoxide back to the reduced form.
- Vitamin K epoxide reductase complex 1 (VKOR / VKORC1) catalyzes the conversion of vitamin K 2,3-epoxide to vitamin K quinone in a reaction requiring dithiol (RSH-HSR).
- Vitamin K quinone is next converted back to vitamin K dihydroquinone in a reaction also catalyzed by VKOR. A second epoxide reductase that requires NAD(P)H may also catalyze the conversion of vitamin K quinone to dihydroquinone; however, the dithiol-dependent quinone reductase appears to be the main physiological pathway for regenerating KH_2 .

Anticoagulants Anticoagulants may be prescribed to some people at risk for thrombotic events (e.g., a heart attack). Some anticoagulants (such as Warfarin, Coumadin, and Jantoven) function to antagonize the synthesis of vitamin K by interfering with the activity of the reductases that convert vitamin K 2,3-epoxide to vitamin K quinone and that convert vitamin K quinone to vitamin K dihydroquinone

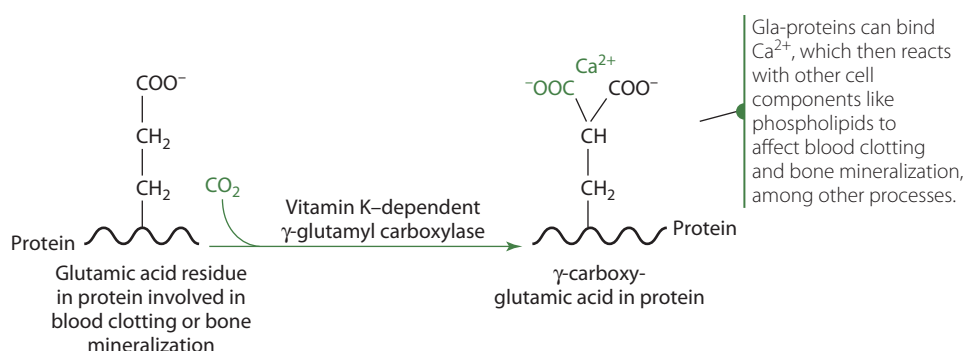


Figure 10.24 Production of γ -carboxyglutamic acid (Gla) via vitamin K–dependent carboxylation.

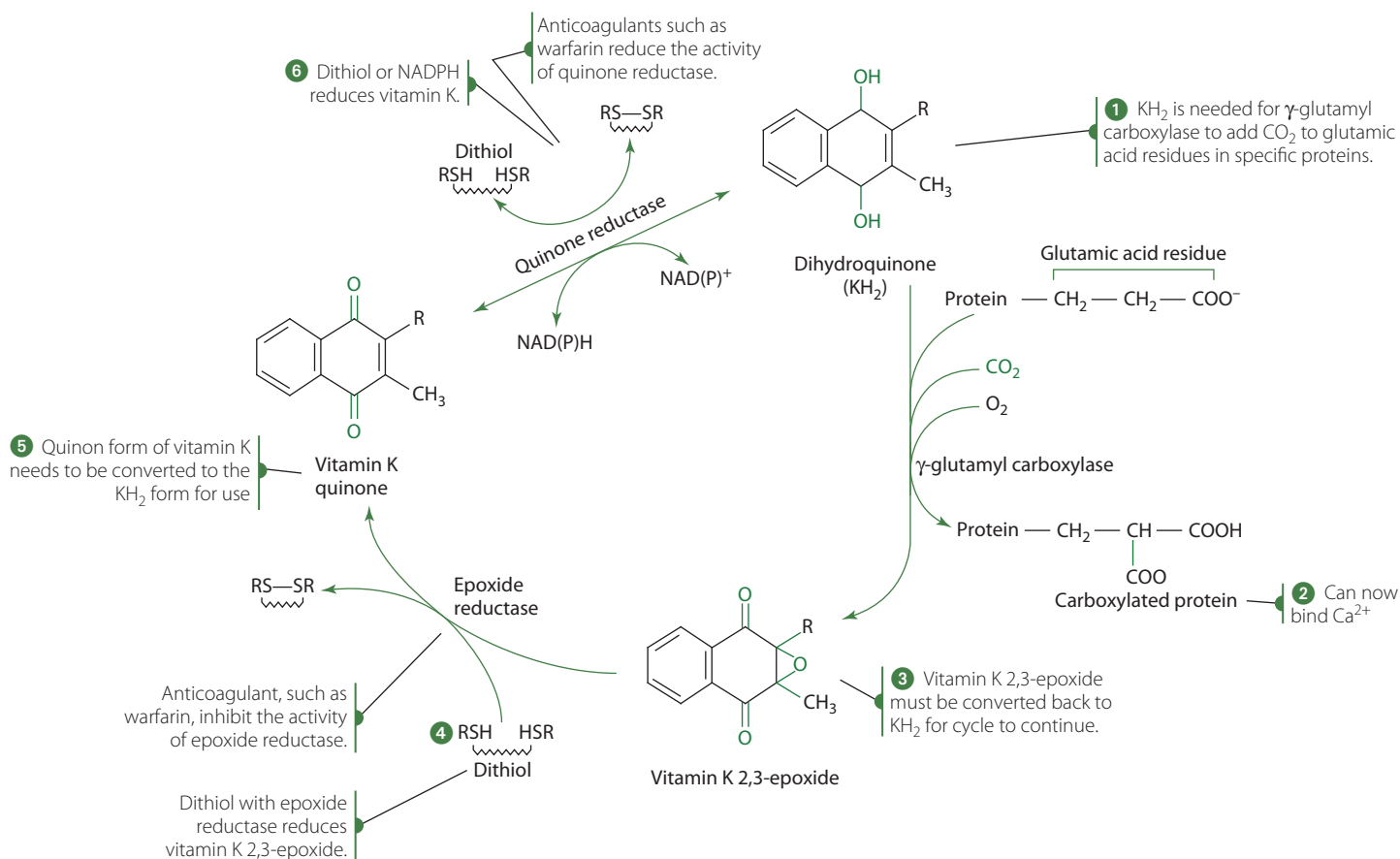


Figure 10.25 The vitamin K cycle.

(Figure 10.25). VKOR activity is more sensitive to (affected by) the drug than the NAD(P)H-dependent reductase. The goal of drug therapy is to prolong bleeding time and thus reduce blood clotting. The effectiveness of the medication, however, can be overridden with the ingestion of foods high in vitamin K, such as obtained from about a pound of broccoli daily [5]. Thus, people who are taking anticoagulant medications are instructed to maintain a consistent, yet adequate intake of vitamin K, but also to avoid consumption of large quantities of foods rich (about 700–1,500 μg or more of phyloquinone) in vitamin K at a single meal. Vitamin K can also serve as an antidote for warfarin, if the drug is present in higher than acceptable concentrations in the blood.

Bone Mineralization

Bones take up vitamin K from circulating lipoproteins in a receptor-dependent manner. Within the bone, the vitamin is used for carboxylation reactions similar to those described in the section “Blood Clotting.” The main vitamin K-dependent protein in bone, cartilage, and dentine is osteocalcin (also sometimes called bone Gla protein). In bone, osteocalcin is secreted primarily by osteoblasts during extracellular matrix (protein) formation. Following vitamin K-dependent carboxylation, osteocalcin’s three Gla residues facilitate the binding of calcium ions to the

hydroxyapatite lattice in the extracellular matrix of bone. Plasma osteocalcin is sometimes used as an index of bone formation (discussed further under the sections addressing deficiency and assessment). Another highly present Gla protein found in bone is osteonectin, a phosphoprotein that binds to both calcium and collagen. Other Gla proteins that are present in bone to facilitate mineralization include fibronectin, matrix Gla protein, osteopontin, and bone sialoprotein-1.

Bone Health Associations between serum vitamin K and bone mineral density or fracture risk as well as knee osteoarthritis are numerous in the scientific literature. However, evidence showing a protective influence of supplemental vitamin K (about 45 mg of MK-4 and from about 500 to 5,000 μg of phyloquinone/day) on bone health remains equivocal, and data at present are not thought to be sufficient to endorse vitamin K supplementation to prevent bone loss or fractures [3,6,7]. Yet, in Japan and some Asian countries, 45 mg MK-4 is recommended in the treatment of osteoporosis.

Other Roles for Vitamin K-Dependent Protein

Several other vitamin K-dependent proteins (listed hereafter) have been identified, but many of their roles remain unclear. Some of these proteins are found in bone, but many are found in tissues throughout the body.

Moreover, many undergo carboxylation in extrahepatic tissues.

- Growth arrest–specific protein (Gas) 6 is found in a variety of tissues, including smooth muscle, endothelial cells, natural killer cells, macrophages, and the heart, kidneys, and bone marrow, among others. The protein is thought to be involved with phagocytosis as well as cell proliferation, adhesion, migration, and apoptosis.
- Periostin is expressed in bone and other collagen-rich tissues but is also situationally expressed in other tissues. The protein appears to bind integrins to affect cell adhesion and migration; it may also be involved in extracellular bone matrix formation.
- Transmembrane Gla proteins (TMG) are found in multiple extrahepatic tissues and may function as cell surface receptors.
- Proline-rich Gla proteins (PRG) appear as integral membrane proteins in several tissues.
- Gla-rich protein (GRP), which contains more Gla residues than other identified Gla proteins (hence its name) appears to have anti-inflammatory as well as bone mineralization functions.
- Osteocalcin, in addition to its role in bone, appears to modulate and may inhibit vascular calcification as well as modulate insulin secretion, tissue sensitivity to insulin, and glucose utilization.
- Matrix Gla protein, containing five Gla residues, is expressed in fibroblasts (collagen-producing cells in connective tissues), chondrocytes (in cartilage), vascular smooth muscle, and endothelial cells, among others. Its most investigated role is in preventing calcium deposition in blood vessels and other soft tissues. In fact, a lack of matrix Gla protein is associated with extensive arterial calcification. However, the presence of matrix Gla protein mRNA in a variety of tissues, including the brain, heart, kidneys, liver, lungs, and spleen, suggests broader roles for the protein.

Roles for Menaquinones

Investigations of the roles of menaquinones in the body show involvement in gene expression through actions as a ligand with orphan nuclear steroid and xenobiotic receptor (SXR). Some of the genes targeted by SXR-MK-4 are those coding for proteins involved in drug metabolism, vitamin D metabolism (including genes coding for 24-hydroxylase and 25-hydroxylase), and osteoblast activity.

Interactions with Other Nutrients

Vitamins A and E are known to antagonize vitamin K; excesses of both interfere with vitamin K absorption. Vitamin E's antagonistic effects also include possible interference with vitamin K metabolism. Specifically, vitamin E

is thought to inhibit metabolism of phyloquinone and to increase hepatic oxidation and excretion of all forms of vitamin K [8].

Metabolism and Excretion

Phylloquinone is rapidly and almost completely metabolized, producing a variety of metabolites (many uncharacterized) before being excreted. The metabolism usually involves stepwise oxidation of the phytyl side chain at position 3 with subsequent conjugation. Most of phylloquinone's metabolites are conjugated with glucuronic acid for excretion primarily in the feces by way of the bile; however, some metabolites are also excreted in the urine. Menaquinone degradation is similar to phylloquinone; the isoprenoid side chain is shortened and the metabolites, such as menadiol, are then conjugated with primarily glucuronic acid for fecal excretion or with phosphate and sulfate for excretion in the feces and urine.

Adequate Intake

Lack of data has hampered the efforts to estimate requirements for vitamin K [1]. Adequate Intake (AI) recommendations are set at 120 and 90 $\mu\text{g}/\text{day}$ for adult males and females (including those who are pregnant or lactating), respectively. Bacterial-generated menaquinones cannot maintain adequate vitamin K status [1]. Metabolic studies suggest that the current vitamin K recommendations, which are based on the vitamin's need for coagulation function, may be inadequate to maximize carboxylation of proteins needed for bone health [6,7].

Deficiency

While a deficiency of vitamin K is relatively rare in healthy adults, a subclinical vitamin K deficiency is more likely and has been induced with the daily provision of about 10 μg of phylloquinone. Biochemically, a deficiency is characterized by serum vitamin K concentrations less than about 0.2 ng/mL (which drop below normal after about 2 weeks of inadequate intake) and serum undercarboxylated osteocalcin concentrations exceeding or equal to 4.0 ng/mL. Undercarboxylated prothrombin concentrations may also rise significantly and prothrombin time is elevated (discussed further under "Assessment").

A severe vitamin K deficiency is associated with bleeding (hemorrhage), and deficiency of the vitamin is called vitamin K–deficiency bleeding (VKDB). The undercarboxylated blood-clotting factors cannot effectively bind calcium and interact with cell membrane phospholipids exposed on tissue injury, an interaction necessary for thrombin generation and clot formation.

Treatment of vitamin K deficiency in adults is accomplished with the provision of the metabolite/precursor menadiol in oral dosages of 5–10 mg/day. Injections of menadiol

may also be used. An oral form of vitamin K (phytonadione) is also used in doses ranging from about 2.5 to 5 mg/day. Phytonadione can also be injected under the skin.

At Risk for Deficiency

Infants are at high risk for vitamin K deficiency bleeding, and the condition was once referred to as hemorrhagic disease of the newborn. Newborns are particularly at risk because (1) breast milk is low in vitamin K, (2) their vitamin K stores are low because inadequate amounts cross the placenta, and (3) their intestinal tract is not yet populated by vitamin K–synthesizing bacteria. Because of the risk of bleeding, an intramuscular injection of 0.5–1 mg of phyloquinone shortly after birth is recommended for all infants [1]. A deficiency in infants is characterized typically by bleeding within the gastrointestinal tract, nasal cavity, and skin, among other sites including the brain. Intracranial bleeding can result in neurologic damage and death. The condition may appear in newborns within the first week of life, especially if the mother was prescribed medications that interfere with vitamin K utilization during pregnancy, or may develop later in infancy. This latter form more commonly appears in exclusively breastfed infants or in those with severe malabsorptive disorders.

Other population groups that are at risk for a vitamin K deficiency are older adults, those being treated chronically with antibiotics, and those with severe gastrointestinal malabsorptive disorders. Older adults tend to consume inadequate amounts of the vitamin; however, the problem of inadequate intake may be more widespread in the United States, where intakes of the vitamin are often less than 80 µg daily [1]. People consuming vitamin K–poor diets and on prolonged sulfonamides and broad-spectrum antibiotic drug therapy are at risk for vitamin K deficiency owing to the coupled effects of low dietary intake and antibiotic-induced destruction of gastrointestinal tract bacteria that manufacture and contribute a source of vitamin K. Reductions of over 70% in bacterial-produced vitamin K can occur with the use of broad-spectrum antibiotics. Individuals with fat malabsorptive disorders such as cystic fibrosis, obstructive jaundice, Crohn's disease, chronic pancreatitis, and liver disease are also at risk of deficiency since the vitamin is best absorbed with fat. Water-miscible forms of the vitamin are available for those with fat-malabsorptive conditions.

Toxicity

Ingestion of large amounts of phyloquinone and menaquinone has not been shown to cause toxicity. No Tolerable Upper Intake Level for vitamin K has been established [1].

Assessment of Nutriture

Multiple biomarkers are generally used to assess vitamin K status since no single index or biomarker clearly indicates deficiency and adequacy. Plasma or serum concentrations

of phyloquinone reflect recent (within about 24 hours) intake of the vitamin; concentrations less than about 0.2 ng/mL are associated with deficiency.

Whole-blood clotting times and prothrombin (or other blood-clotting proteins) time are used to identify potential deficiency of vitamin K. Prothrombin time, which measures the time required for a fibrin clot to form following the addition of calcium and other substances to citrated plasma, is normally between about 11 and 14 seconds; times greater than 25 seconds are associated with major bleeding and may indicate possible vitamin K deficiency. This test, however, is relatively insensitive because plasma prothrombin concentrations must usually decrease considerably (sometimes 50% or more) before any effects on prothrombin time are observed.

Another fairly sensitive means of assessing vitamin K status is to measure the percentage of undercarboxylated vitamin K–dependent proteins, such as prothrombin or osteocalcin, or the ratio of under- to fully carboxylated proteins. Vitamin K deficiency results in the secretion of under- or partially carboxylated proteins into the blood from either the liver (as with prothrombin) or the bone (as with osteocalcin). However, one protein (such as prothrombin) may be 100% carboxylated, whereas another protein (such as osteocalcin) is often found to be up to only 40% carboxylated; the physiological significance of these differences is not clear at present.

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Suggested Reading

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SUMMARY

The fat-soluble vitamins include a group of four vitamins, and they share a number of similarities as well as differences.

- While fat-soluble vitamins are present in a wide variety of foods, vitamins A and D are found in just a few animal-based foods and the vitamins E and K and the carotenoids are present in higher amounts in mostly plant-based foods. Also unique is the ability to synthesize vitamin D in the skin with exposure to ultraviolet light from the sun.
- Fat-soluble vitamins are absorbed by similar means, requiring the presence of some fat and bile for the formation of initially micelles and then chylomicrons for transport and distribution to body tissues.
- Fat-soluble vitamins are stored in relatively larger quantities than water-soluble vitamins and are found in higher concentrations in the liver, adipose tissue, and cell membranes.
- Fat-soluble vitamins serve a variety of roles in the body, including vision; gene expression; cell differentiation, proliferation, and growth; antioxidant defense; bone mineralization; calcium and phosphorus homeostasis; signal transduction; immune system function; and blood coagulation, to name a few.
- RDAs and ULs have been established for all fat-soluble vitamins with the exception of vitamin K.

Perspective

ANTIOXIDANT NUTRIENTS, REACTIVE SPECIES, AND DISEASE

Although different sections in several chapters of this book have discussed nutrients with antioxidant functions, nowhere within those chapters is this information brought together to provide a more comprehensive review of how these individual nutrients function together to protect the body from destructive radicals and nonradical species. That is the purpose of this Perspective, which first reviews free-radical chemistry and formation, followed by a discussion of the damage caused by these species, and finally an explanation of how the antioxidants function together to eliminate radical and nonradical species.

FREE-RADICAL CHEMISTRY AND FORMATION

Back in probably one of your first chemistry courses, you learned about atoms. It is here that a brief review of free-radical chemistry begins. Atoms contain protons and neutrons, which are found in the nucleus. You may remember that the atomic weight of an element is a function of its number of protons and neutrons, whereas the atomic number represents solely the number of protons. Atoms also have electrons, which revolve in orbitals (also called shells) around the nucleus. An atomic orbital holds a maximum of two electrons, which are generally found in pairs in the orbitals. The term *free radical* represents an atom, molecule, or molecular fragment that is capable of free existence (although in most cases for very short time periods) and has one or more unpaired electrons. The unpaired electron is found alone in the outer orbital and is usually denoted by a superscript dot. The imbalance in electrons in the orbitals results in most cases in the high reactivity of the free radicals and their pro-oxidant activities.

Free radicals are most often derived from oxygen and nitrogen, along with a few arising from sulfur and carbon. The free

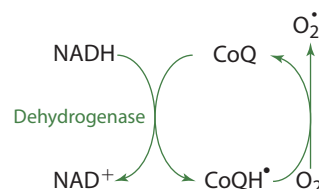
radicals are classified based on origin with those derived from oxygen (or oxygen-centered) called reactive oxygen species (ROS), those derived from nitrogen (or nitrogen-centered) called reactive nitrogen species (RNS), those derived from sulfur (or sulfur-centered) called reactive sulfur species (RSS), and those derived from carbon (or carbon-centered) called reactive carbon species (RCS). The term *species*, however, includes not only free radicals but also nonradicals, and the term *reactive* is most appropriately used when comparing different radicals because reactivity with other compounds is relative. Table 1 provides a partial listing of some common reactive oxygen and nitrogen species.

Sources of Free Radicals and Nonradicals

Reactive species can be formed in the body due to exposure to external/environmental substances and from internal/endogenous reactions or processes as listed in Table 2. Endogenous reactive species are generated daily through both enzymatic and nonenzymatic reactions at multiple sites in the body. Once produced, these reactive species exert effects within the cell and sometimes the surrounding area, generally within a radius of about 30 angstroms.

A major generator of reactive oxygen species occurs in the mitochondria of cells

as part of the electron transport/respiratory chain, especially at complex I and complex III (see Figures 3.28 and 3.30). Shown hereafter is superoxide radical production as a result of autoxidation reactions and the leaking of electrons from the electron transport chain onto oxygen (i.e., a one-electron reduction of oxygen to generate the superoxide radical). This leaking of electrons onto oxygen occurs during the passage of electrons from the ubisemiquinone form of coenzyme Q ($\text{CoQH}^\bullet$) to CoQ (also called ubiquinone), as shown here:



Other enzymatic reactions occurring in the mitochondria as well as in the endoplasmic reticulum and peroxisomes also contribute to the generation of reactive species in the body (see Table 2). Some reactions both produce and remove reactive species such as those catalyzed by superoxide dismutase. In the endoplasmic reticulum, the heme-containing cytochrome P450 enzyme system (which consists of a reductase that transfers electrons from NADPH, and a second unit that

Table 1 Some Reactive Oxygen and Nitrogen Species

Reactive Oxygen Species		Reactive Nitrogen Species	
Oxygen-Containing Radicals	Oxygen-Containing Nonradicals	Nitrogen-Containing Radicals	Nitrogen-Containing Nonradicals
Superoxide $\text{O}_2^\bullet$	Hydrogen peroxide H_2O_2	Nitric oxide $\text{NO}^\bullet$	Nitrous acid HNO_2
Hydroxyl $^\bullet\text{OH}$	Singlet oxygen $^1\text{O}_2$	Nitrogen dioxide $^\bullet\text{NO}_2$	Peroxynitrite ONOO^-
Hydroperoxyl $\text{HO}_2^\bullet$	Hypochlorous acid HOCl		Nitrosyl anion NO^-
Alkoxyl $\text{RO}^\bullet$ / Lipid alkoxyl $\text{LO}^\bullet$	Ozone O_3		Peroxynitrous acid ONOOH
Peroxyl $\text{ROO}^\bullet$ / Lipid peroxyl $\text{LOO}^\bullet$			Dinitrogen trioxide N_2O_3

Table 2 Sources Promoting Reactive Species Production in the Body

Environmental Sources	Endogenous Sources
Smog/ozone	Oxidative phosphorylation reactions of the electron transport /respiratory chain
Industrial chemicals such as pesticides or solvents	Reactions catalyzed by mitochondrial enzymes such as monoamine oxidase, α -ketoglutarate dehydrogenase, glycerol phosphate dehydrogenase, and manganese-dependent superoxide dismutase
Foreign pathogens (bacteria, viruses, parasites)	Reactions catalyzed by endoplasmic reticulum cytochrome P450 enzymes, prostaglandin synthesis cyclooxygenases, thiol oxidases, and copper-dependent amine/diamine oxidases
Ultraviolet light/radiation	Reactions catalyzed by some peroxisomal enzymes such as xanthine oxidase, urate oxidase, acyl-CoA oxidases, α -hydroxy oxidase, and D-aspartate and D-amino acid oxidases
X-rays	Cytosolic and extracellular reactions catalyzed by zinc- and copper-dependent superoxide dismutase, among others
Some drugs/medications	Phagocytic reactions as part of immune defense in neutrophils, macrophages, monocytes, and eosinophils
Environmental pollutants such as automobile exhaust	

binds molecular oxygen and the substrate being hydroxylated) is a major contributor of reactive species. This system is needed for the metabolism of fatty acids, steroids, and therapeutic drugs and in the conversion of nonpolar compounds to polar compounds for elimination (fecal or urinary). In addition to those listed in Table 2, reactive oxygen species are generated in larger amounts with tissue injury or damage, especially if involving ischemia (inadequate blood flow and, thus, oxygen supply). Possible reasons for increased ischemia-induced reactive species production include (1) increased neutrophil activation by compounds released by the damaged tissues, (2) disruption of the electron transport chain, and (3) secondary production associated with the generation of xanthine oxidase, especially if the ischemia affects the intestine or endothelial cells of blood vessels. Moreover, the reactions that produce reactive species as well as some of the reactions needed to eliminate reactive species can lead to the production of other reactive species. Thus, radicals can breed more radicals (as seen in several of the reactions shown in this Perspective and in Figure 1).

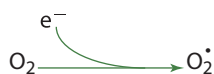
Reactive Oxygen Species

The generation of reactive oxygen species (ROS) starts with the uptake of oxygen. Up to about 4% of the body's oxygen use is thought to result in the production of ROS. The generated nonradicals are often fairly stable, with half-lives lasting seconds

to minutes, while the radicals that are produced are quite unstable, with half-lives of microseconds, nanoseconds, or milliseconds.

Superoxide Radicals ($O_2^{\cdot-}$ or $O_2^{-\cdot}$)

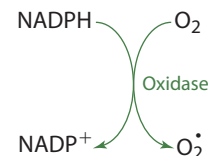
The superoxide radical is an oxygen-centered radical (e.g., the unpaired electron resides on the oxygen). The addition of an electron to molecular oxygen (O_2) (which is itself a biradical because it has two unpaired electrons residing in separate orbitals and that cannot form a pair) results in one unpaired electron.



Superoxide radicals are formed when molecular oxygen reacts with different compounds. Some of these include: (1) reactions involving the production of the catecholamines epinephrine and dopamine, (2) some reactions involving folate as tetrahydrofolate, (3) cytochrome P450 catalyzed reactions, (4) some reactions of the electron transport chain, and (5) some reactions needed in immune system function.

Activated white blood cells (such as macrophages, monocytes, and neutrophils), for example, use large amounts of oxygen and generate substantial quantities (up to 10^{-11} M) of superoxide radicals as foreign substances are being taken up for destruction via phagocytosis. (Note: This extensive

oxygen-requiring process by which white blood cells destroy organisms is sometimes called the *respiratory* or *oxidative burst*.) Superoxide radical production in these cells is thought to begin with the action of NADPH oxidase, which reduces oxygen, as shown here:

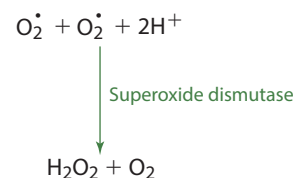


Superoxide radicals, once generated, can go to produce other reactive species, such as hydrogen peroxide, hydroperoxyl radicals, and the **peroxynitrite** anion. Fortunately, superoxides are not lipid soluble and thus do not diffuse too far away from their site of production.

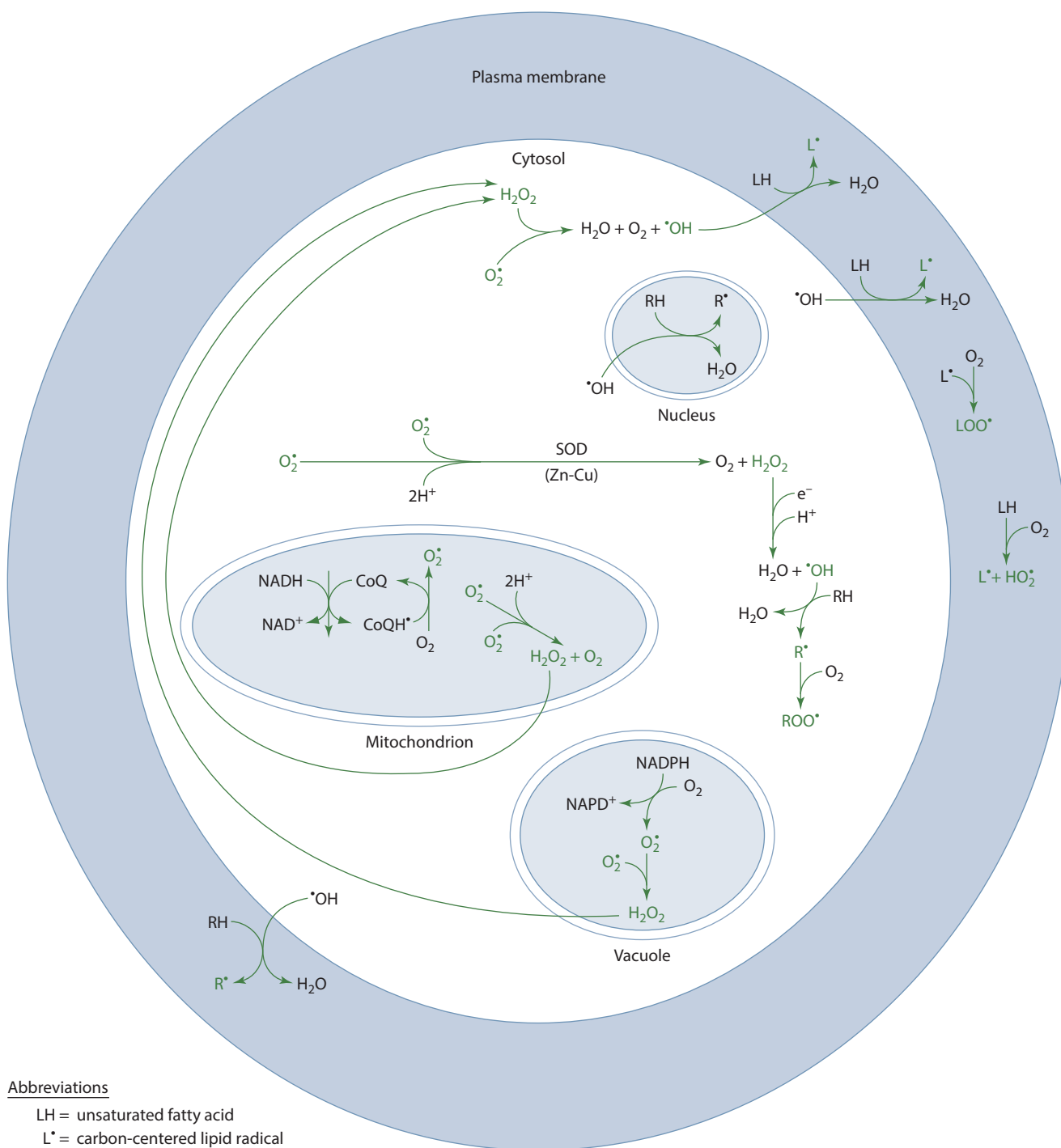
Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is a reactive oxygen species but is not a radical because it does not have any unpaired electrons. Hydrogen peroxide is generated in the body through the actions of several enzymes including (1) acyl CoA oxidases involved in the degradation of very long-chain fatty acids, (2) thiol oxidase involved in the transfer of electrons from dithiols to molecular oxygen, (3) superoxide dismutase, and (4) amine oxidases, as well as during the elimination of some reactive species. A few of these reactions are highlighted hereafter.

Superoxide dismutase (SOD) (which is found extracellularly, intracellularly in the cytosol where it depends on zinc and copper for activity, and in the mitochondria where it requires manganese) removes superoxide radicals, as shown here:



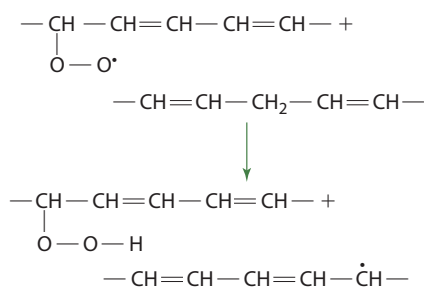
Amine oxidases (which are copper dependent and involved in neurotransmitter synthesis and the conversion of a variety of amines to aldehydes) also generate hydrogen peroxide. The general reaction catalyzed by an amine oxidase, found in the blood and body tissues, is as follows:



Abbreviations

- LH = unsaturated fatty acid
- $L^{\bullet}$ = carbon-centered lipid radical
- RH = organic compound (e.g., amino acid and nucleic acid)
- $O_2^{\bullet-}$ = superoxide radical
- $\bullet OH$ = hydroxy radical
- $R^{\bullet}$ = carbon-centered nonlipid radical
- H_2O_2 = hydrogen peroxide
- $ROO^{\bullet}$ = nonlipid peroxy radical
- $LOO^{\bullet}$ = peroxy radical
- $HO_2^{\bullet}$ = hydroperoxyl radical

Figure 1 Generation of reactive species.



Should lipid peroxides, also known as peroxidized fatty acids, come in contact with free iron, amplification results with alkoxy ($\text{LO}^\bullet$) and peroxy ($\text{LOO}^\bullet$) radicals also generated, as shown in these two reactions:

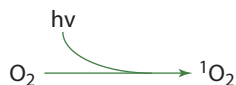


Like peroxy radicals, the alkoxy radical can in turn initiate chain reactions with other polyunsaturated fatty acids in membranes, as follows:



Singlet Molecular Oxygen ($^1\text{O}_2$)

Singlet molecular oxygen, also called singlet oxygen, possesses higher energy and is more reactive than ground-state oxygen, which typically exists in triplet ($^3\text{O}_2$) rather than in singlet ($^1\text{O}_2$) form. Specifically, in singlet oxygen, the peripheral electron in the oxygen structure is excited to an orbital above the one it normally occupies. This excited form of oxygen can be generated from lipid peroxidation of membranes by enzymatic reactions, such as that occurring between hydrogen peroxide and hypochlorous acid in the respiratory burst in phagocytic white blood cells (i.e., $\text{H}_2\text{O}_2 + \text{HOCl} \rightarrow ^1\text{O}_2 + \text{H}_2\text{O} + \text{HCl}$). Singlet oxygen is also generated in body cells during reactions catalyzed by various enzymes including dioxigenases and lipoxygenases, the dismutation of superoxides (spontaneous reaction), and through the transfer of energy from light (photochemical reactions), as shown here:



Singlet oxygen, being a reactive oxygen species (although not a free radical), can

damage DNA as well as other cellular components and tissues unless it is removed.

Reactive Nitrogen Species

Reactive nitrogen species (RNS) include the two free radicals, nitric oxide (also called nitrogen monoxide) and nitrogen dioxide, as well as many nonradicals listed in Table 1. Two nitrogen-centered radicals as well as one non-radical peroxynitrite are discussed hereafter.

Nitric Oxide/Nitrogen Monoxide ($\text{NO}^\bullet$)

Nitric oxide is generated in the body from the amino acid arginine and molecular oxygen in a NADPH-dependent reaction catalyzed by nitric oxide synthase, which has three different isoforms (neuronal, endothelial, and inducible). Nitric oxide, once generated, easily diffuses through the cytosol and plasma membrane due to its solubility in both aqueous and lipid environments. In spite of its lifespan of just a few seconds, nitric oxide affects numerous, diverse physiological processes in the body. Some of these physiological processes include blood pressure regulation, neurotransmission, immune regulation, and smooth muscle relaxation. Nitric oxide's role as a vasorelaxant is applied in medicine. Nitroglycerin, for example taken for ischemic chest pain (angina), generates nitric oxide in the body, which relaxes coronary blood vessels and increases blood (and thus oxygen) flow to the heart.

Nitric oxide, however, can cause damage. The compound can react (1) with oxygen to form another radical nitrogen dioxide, (2) with a superoxide radical to form peroxynitrite (a nonradical reactive species), and (3), in a reaction shown here, with thiols (RSH) to form nitrosothiol: $\text{NO}^\bullet + \text{RSH} \rightarrow \text{RSNO} + \text{O}_2 + \text{H}^+$. The **nitrosothiol** (RSNO) that is produced is harmful in that it may attack other compounds unless it is terminated by combining with another thiol ($\text{R}'\text{SH}$) to produce $\text{RSH} + \text{R}'\text{SNO}$ or $\text{RSSR}' + \text{HNO}$.

Nitrogen Dioxide ($\text{NO}_2^\bullet$)

Nitrogen dioxide is a fairly potent free radical formed when nitric oxide reacts with oxygen ($\text{NO} + \text{O}_2 \rightarrow \text{NO}_2^\bullet$). The radical can coact with carbonate (CO_3^{2-}) radicals to produce nitrated compounds and can react with a superoxide radical to generate another nonradical **peroxynitrate** ($\text{O}_2\text{NNOO}^\bullet$).

Nitrogen dioxide is particularly destructive to unsaturated fatty acids, abstracting

a hydrogen atom and inducing isomerization of *cis*-double bonds in unsaturated fatty acids by a reversible addition reaction. These actions damage the lipids and, if the lipids are part of a cell membrane, damage the membrane. Nitrogen dioxide is also destructive to amino acids within proteins (primarily tyrosine, tryptophan, and cysteine), forming nitrated molecules, which disrupt the protein and normal cellular processes.

Peroxynitrite Anion (ONOO^-)

Peroxynitrite, an anion (not a free radical), is formed by the reaction between nitric oxide and a superoxide radical. It is a strong oxidizing agent. Once produced (often at the site of tissue injury), it quickly and directly attacks amino acids (such as cysteine, methionine, and tyrosine in proteins), causing protein **nitrosylation** and extensive functional damage to the protein. It also attacks polyunsaturated fatty acids and DNA. Moreover, peroxynitrite may also react with carbon dioxide to produce the reactive species carbonate and nitrogen dioxide.

DAMAGE DUE TO REACTIVE SPECIES

Once formed, reactive species attack by taking electrons from cell constituents. Free radicals damage nucleic acids in DNA and RNA. Damage includes modifications to purine and pyrimidine bases in the DNA that may result in mutations in genes as well as disruption to the deoxyribose-phosphate backbone, which may induce strand breaks. Free-radical attacks vary, with some radicals, such as hydroxyl radicals, inducing changes in all DNA bases versus other radicals only damaging specific bases. RNA is thought to be more susceptible to damage than DNA given its locations in cells and the existence of fewer repair mechanisms.

Free radicals also take electrons from proteins (especially amino acids such as tyrosine, tryptophan, proline, histidine, or arginine and those with sulfhydryl groups, such as cysteine). These attacks may break the peptide bonds in the protein backbone or disrupt or fragment the protein structure. Oxidative damage to proteins may cause cross-linking between amino acids or aggregation, resulting in changes in the secondary or tertiary structures. Such events may even lead to premature degradation of the protein.

Free radicals also take electrons from polyunsaturated fatty acids in cell membranes or in the membranes of intracellular organelles, such as the nucleus, mitochondria, or endoplasmic reticulum. Free-radical attack on polyunsaturated fatty acids present in the phospholipid portion of the cell membranes can lead to degradation of the membrane. Extensive damage in a red blood cell, for example, may cause hemolysis of the membrane and thus the cell. Aqueous peroxy and peroxynitrite radicals may induce oxidation of LDLs.

BENEFITS OF FREE RADICALS

The production of free radicals when present in low to moderate concentrations can, however, be of benefit. The main benefit is in their abilities to heighten the immune/inflammatory response, helping to defend against pathogenic microorganisms. In addition, some reactive species play beneficial roles in cell signaling pathways and in redox regulation and in the “detoxification” of some medications and compounds. The abilities of some radicals to react with and remove other radicals but also generate more radicals in the process can be viewed as both beneficial and detrimental.

ANTIOXIDANTS AND THEIR FUNCTIONS

Overproduction of reactive oxygen and nitrogen species and their attack on DNA, proteins, and polyunsaturated fatty acids have been implicated as a cause of or contributor to a variety of conditions and diseases. Antioxidants function as electron donors or *redox agents*, helping to (1) prevent free-radical formation, (2) terminate or eliminate formed free radicals, and (3) regenerate antioxidants.

Ideally, antioxidants suppress or prevent the formation of free radicals before any damage has occurred. Many enzymes (sometimes with cosubstrates) function in this capacity, including catalase, glutathione peroxidase, and phospholipid hydroperoxide glutathione reductase. Glutathione, a cosubstrate in some reactions involving glutathione peroxidase, for example, plays a vital role as a reducing agent by providing hydrogen ions from its sulfhydryl group (SH). In the absence of prevention, antioxidants can terminate the actions of reactive species such as through disrupting chain-propagation and scavenging. Vitamin E, for example, acts to terminate chain propagation in unsaturated lipids by reacting with carbon-centered

radicals. Several antioxidants can also scavenge radicals. Some scavenge radicals best in aqueous locations such as vitamin C, uric acid and thiols, while others work in both areas such as some thiols, carotenoids, and ubiquinol, or in primarily lipophilic areas such as vitamin E and some carotenoids. Lastly, antioxidants function to regenerate other antioxidants (discussed toward the end of the Perspective). Table 3 lists some antioxidants and their actions, and Figure 2 shows some of the reactions by which reactive species are destroyed. This information is also reviewed in the next section.

Superoxide Radical Elimination: Vitamin C and Superoxide Dismutase

Superoxide radicals can be eliminated through the actions of vitamin C and the enzyme superoxide dismutase. Vitamin C (present under physiological conditions as ascorbate AH^-), being water soluble and hydrophilic, is found in aqueous regions, such as the blood and the cytosol of cells. The vitamin provides electrons to reduce the superoxide radical and forms hydrogen peroxide, as shown here:

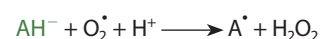
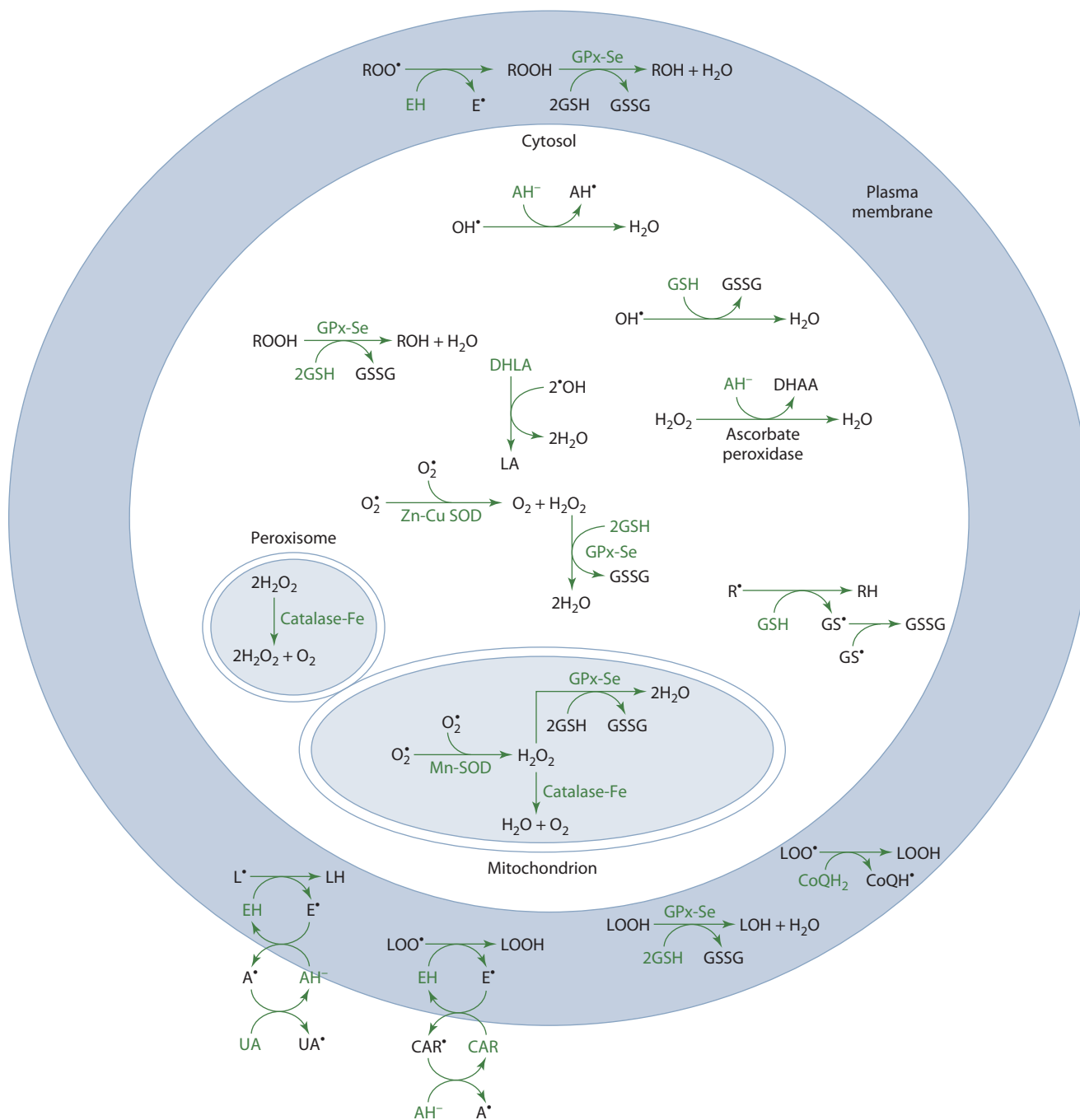


Table 3 Antioxidants and Some of Their Actions

Category	Antioxidant	Reactive Species Substrates
Enzymes	Superoxide dismutase – copper-zinc dependent (cytosol, extracellular) and manganese-dependent (mitochondria) in multiple tissues including blood vessels	Superoxide radicals
	Catalase – iron dependent (peroxisomes, cytosol, mitochondria) in multiple tissues, especially phagocytes	Hydrogen peroxide
	Glutathione peroxidase – selenium dependent (cytosol, mitochondria, extracellular) in multiple tissues; uses glutathione	Lipid hydroperoxides and hydrogen peroxide
	Myeloperoxidase – iron dependent, mostly in activated white blood cells (released from granules into vacuoles that contain the engulfed foreign substances)	Hydrogen peroxide
Thiols, characterized by presence of sulfhydryl groups (R-SH) found in aqueous and lipophilic areas	Glutathione – (cytosol, mitochondria, plasma) in multiple tissues Thioredoxin – part of thioredoxin reductase (selenium-dependent flavo/FAD enzyme) system, ubiquitous in the body Dihydrolipoic acid – in multiple tissues	Hydroxy radicals, lipid peroxide radicals, singlet oxygen, hydrogen peroxide
Vitamins	E (membranes – plasma, endoplasmic reticulum and mitochondria, lipoproteins) – ubiquitous in the body	Carbon-centered radicals, peroxy radicals, singlet oxygen, hydroxy radicals
	Carotenoids (membranes, lipoproteins) – ubiquitous in the body	Singlet oxygen, peroxy radicals, multiple others
	C (blood and cytosol, aqueous environments) – ubiquitous in the body	Superoxide radicals, hydrogen peroxide, hydroxy radicals, alkoxyl radicals, peroxy radicals, singlet oxygen, lipid hydroperoxides
Other	Ubiquinol (mitochondria), ubiquitous in the body	Hydroxy radicals, peroxy radicals
	Uric acid (aqueous) – in blood	Hydroxy radicals, singlet oxygen, peroxynitrite, transition metals
	Metallothionein (cysteine-rich protein) in multiple cells	Hydroxy radicals



Abbreviations

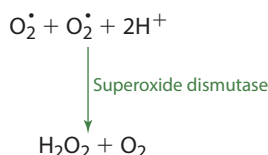
EH = vitamin E
AH₂ = vitamin C
SOD = superoxide dismutase
GSH = reduced glutathione
GSSG = oxidized glutathione
O₂[•] = superoxide radical
LOO[•] = peroxy radical
LH = unsaturated fatty acid

$L^{\bullet}$ = carbon-centered lipid radical
 RH = organic nonlipid compound
 $R^{\bullet}$ = carbon-centered nonlipid radical
 H_2O_2 = hydrogen peroxide
 $ROO^{\bullet}$ = nonlipid peroxy radical
 ROOH = nonlipid peroxides
 LOOH = lipid peroxides
 DHLA = dihydrolipoic acid
 LA = lipoic acid

A[•] = ascorbyl radical
AH⁻ = ascorbate
DHAA = dihydroascorbic acid
UA = uric acid
UA[•] = uric acid radical

Figure 2 The interactions among selected antioxidant nutrients to prevent cell

Superoxide radicals may also be eliminated by the action of the enzyme superoxide dismutase (SOD), which works considerably faster than vitamin C to inactivate superoxide radicals. The enzyme eliminates superoxide radicals but generates hydrogen peroxide, as shown here:



Hydrogen Peroxide Elimination: Vitamin C, Glutathione Peroxidase, and Catalase

Hydrogen peroxide disposal occurs through several means in cells and tissues. In a reaction catalyzed by ascorbate peroxidase, vitamin C (as $\text{AH}^{\bullet}$) provides the needed electrons to convert hydrogen peroxide into water and dehydroascorbic acid.

Glutathione peroxidase, using sulfhydryl groups (SH) from the thiol glutathione (GSH), reduces hydrogen peroxide to water. However, in the reaction, a glutathione (glutathionyl) radical ($\text{GS}^{\bullet}$) is formed and must react with another radical (creating a disulfide bond between the two now-oxidized glutathione molecules). The oxidized glutathione molecule is designated as GSSG or GS-SG.



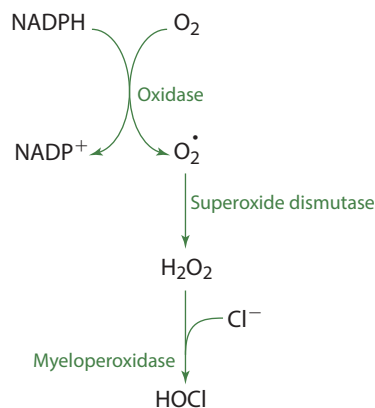
Catalase is another key enzyme in hydrogen peroxide removal, as shown in this reaction:



In fact, catalase and glutathione peroxidase represent the primary means for preventing the accumulation of H_2O_2 in cells. Glutathione peroxidase, however, because of its dual (mitochondrial and cytosolic) cellular locations and its greater activity at

lower hydrogen peroxide concentrations, is thought to be more active than catalase in cellular hydrogen peroxide removal.

A third enzyme, myeloperoxidase, found mostly within activated white blood cells, uses hydrogen peroxide produced from superoxide radicals to generate a potent toxic acid, hypochlorous acid (HOCl).

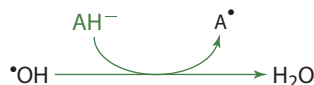


Hypochlorous acid, along with other potent compounds, helps to destroy the cell membrane of the bacteria to promote death (lysis) of the foreign substance.

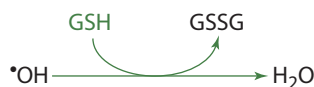
Hydroxyl Radicals Elimination: Vitamin C, Thiols, Ubiquinol, and Uric Acid

Vitamin C as well as thiols (including glutathione and dihydrolipoic acid), ubiquinol, and possibly other substances such as uric acid and metallothionein defend against hydroxyl radicals. Some of the reactions are provided hereafter.

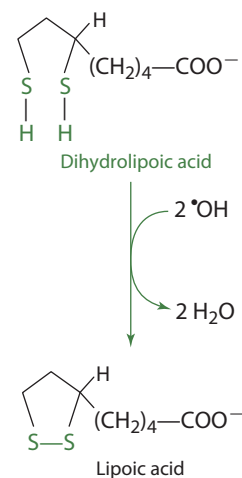
Vitamin C rapidly and effectively reacts with hydroxyl radicals in the blood and cytosol of cells before they can initiate oxidative damage.



Glutathione also reacts directly with hydroxyl radicals in aqueous or lipid environments, as shown here:



Dihydrolipoic acid functions in a similar manner, as shown here:



Dihydrolipoic acid is generated in the body from lipoic acid (also called thioctic acid), which is obtained from the diet or produced in the body. The lipoic acid is reduced in cells to dihydrolipoic acid by the actions of dihydrolipamide dehydrogenase, glutathione reductase, or thioredoxin reductase.

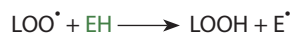
Carbon-Centered Peroxyl and Alkoxy Radicals and Peroxide Elimination: Vitamin E, Carotenoids, Ubiquinol, Vitamin C, Thiols, Uric Acid, and Glutathione Peroxidase

The elimination of carbon-centered and peroxyl radicals as well as lipid peroxides and some reactive nitrogen species is mediated by multiple antioxidants. Carotenoids work to eliminate reactive species by radical addition, electron transfer, and hydrogen abstraction. Vitamin E, being lipid soluble and located near or in membranes, effectively reacts with many radicals, especially carbon-centered radicals and those that initiate peroxidation, such as peroxyl radicals. Specifically, vitamin E donates its phenolic hydrogen on the carbon 6 hydroxyl group. Vitamin E's chromanol ring then stabilizes the unpaired electron.

- Vitamin E (EH) terminates carbon-centered radicals (as shown below) before they abstract further hydrogens from other polyunsaturated fatty acids:

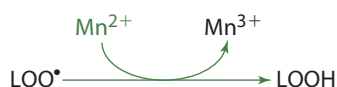


- Vitamin E (EH) prevents peroxidation of polyunsaturated fatty acids by reacting with peroxy radicals (LOO[•]), as illustrated in this reaction:

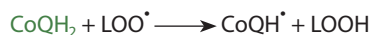


Thus, vitamin E (as well as carotenoids) terminates chain-propagation reactions.

Some transition metals, such as manganese, may be able to scavenge peroxy radicals, as shown here:



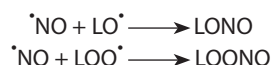
In addition to the aforementioned nutrients, ubiquinol (CoQH₂) the reduced form of coenzyme Q also acts as a potent antioxidant. Ubiquinol is a small molecule that transports electrons and ultimately generates ATP in the electron transport chain. However, ubiquinol has been also found in small quantities in lipoproteins, where it is thought to be used before vitamin E in the termination of peroxy radicals (as shown hereafter) and thus helpful in preventing LDL oxidation.



Vitamin C effectively scavenges alkoxyl (RO[•]) and peroxy (ROO[•]) radicals, producing hydroxy acid (ROH) and peroxide (ROOH), respectively, as shown here:



Nitric oxide (NO[•]) can also act as an antioxidant to terminate lipid alkoxyl (LO[•]) and peroxy (LOO[•]) radicals, as shown here:



Most of the resulting LOONO homolyzes to produce nitrogen dioxide, NO₂[•], and an alkoxyl radical, LO[•]; and then recombines to produce alkyl nitrates (LONO₂); however, a small percentage remains as free radicals. Additionally, uric acid removes peroxynitrite (ONOO[•]) before it causes protein nitrosation.

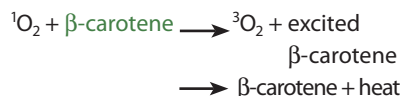
Although eliminating peroxy radicals (LOO[•]) is helpful, the often simultaneous generation of lipid peroxides/peroxidized fatty acids (LOOH) can cause problems if the peroxidized fatty acids are within hydrophobic regions of cell membranes. The problems occur because peroxidized fatty acids are polar compounds, and the polarized peroxidized fatty acids, once liberated from the phospholipid in the membrane by the actions of phospholipase A₂, destroy the normal architecture of the cell as they migrate from the nonpolar region where they are generated.

Thiols and the selenium-dependent enzyme glutathione peroxidase help to eliminate lipid peroxides. Thiols, like dihydrolipoic acid, glutathione and thioredoxin, act in both aqueous and lipid environments as antioxidants by providing reducing equivalents (hydrogen ions). Glutathione peroxidase uses glutathione in its reduced form (GSH) and catalyzes the conversion of the peroxides (LOOH) to hydroxy acids (LOH) and water as follows:



Singlet Oxygen Elimination: Carotenoids, Vitamin C, Uric Acid, and Thiols

Carotenoids as well as vitamin C, uric acid, and thiols quench singlet oxygen. (See “Vitamin A and Carotenoids” for a discussion of quenching). Shown hereafter is the removal of singlet oxygen by β-carotene with the release of energy in the form of heat (and which prevents the need for carotenoid regeneration).



REGENERATION OF ANTIOXIDANTS

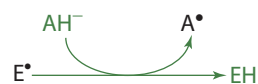
When antioxidants provide reducing equivalents, the antioxidants may also become oxidized during the process. Regenerating the antioxidants is important for further defense against reactive species. Without the recycling of antioxidants, the body

would quickly succumb to oxidative stress. It has been estimated that fewer than nine vitamin E molecules exist for every 1,000–2,000 unsaturated fatty acids in cell membrane phospholipids or lipoprotein molecules. Thus, regenerating or recycling oxidized vitamins is critical to prevent massive damage.

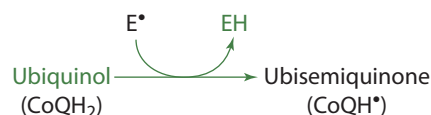
Redox potential (or reduction potential) is used to predict some reactions between radicals and antioxidants. Substances that have been oxidized can abstract an electron (hydrogen) from a reduced substance with a lower redox potential; or, stated in reverse, a reduced substance (with its specific redox potential) can donate an electron (hydrogen) to an oxidized substance with a more positive reduction potential. The carotenoids, for example, have higher redox potentials than vitamins C and E. Consequently, the carotenoids readily take an electron (hydrogen) from either of these two vitamins. The following section discusses the recycling of some antioxidants.

Vitamin E Regeneration: Vitamin C, Ubiquinol, and Thiols

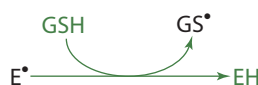
The regeneration of vitamin E is thought to initially require the migration of the vitamin to the membrane surface. At the cell surface, several compounds regenerate vitamin E. Vitamin C (AH[•]) can regenerate α-tocopherol from its radical form (E[•]), but it too will then need to be regenerated.



Ubiquinol (CoQH₂) also recycles vitamin E, as shown here:

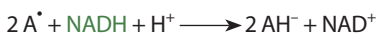


Reduced glutathione (GSH) may donate its hydrogen atom to reform vitamin E, as follows:

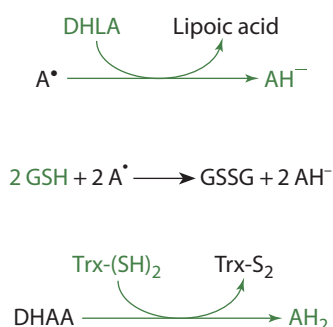


Vitamin C Regeneration: Niacin, Thiols, and Uric Acid

Several vitamins and compounds function to regenerate some (but not all) vitamin C. Niacin, in its coenzyme form NADH, provides for the regeneration of vitamin C in a reaction catalyzed by semidehydroascorbic acid reductase, as follows:



Shown next are some of the recycling efforts contributed by the thiols, including dihydrolipoic acid (DHLA) and glutathione (GSH), which act on the ascorbyl radical to regenerate ascorbate, as well as thioredoxin, which recycle dehydroascorbic acid (DHAA) to ascorbic acid.

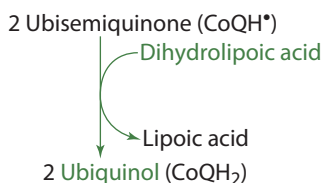


An interaction between two vitamin C radicals also permits the regeneration of vitamin C, as follows:

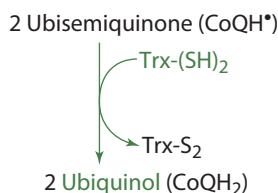


Ubiquinol Regeneration: Thiols

Ubisemiquinone (CoQH•) that is produced during the regeneration of vitamin E by ubiquinol (CoQH₂) can be converted back to ubiquinol using dihydrolipoic acid, as shown here:

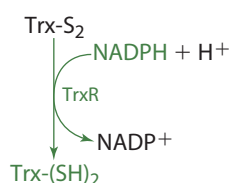


or by the thioredoxin-thioredoxin reductase (Trx) system, as shown here:

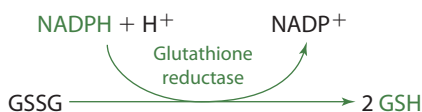


Thiol Regeneration: Niacin, Glutathione Reductase, and Other Thiols

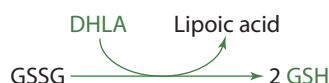
Niacin as NADPH provides reducing equivalents to regenerate thioredoxin in a reaction catalyzed by thioredoxin reductase (TrxR), as shown here:



Glutathione reductase, a flavoprotein that requires FAD as a cofactor, regenerates oxidized glutathione (GSSG) with niacin as NADPH providing the reducing equivalents, as shown here:



Lastly, thiols can help to regenerate other thiols. Dihydrolipoic acid (DHLA), for example, is thought to be able to regenerate glutathione, as shown here:



ANTIOXIDANTS AND DISEASE

Oxidative or nitrosative stress resulting from the overproduction of reactive species leads to an imbalance within the body's defense system and is thought to

contribute to aging and the development of several diseases and conditions, including some cancers, heart disease, stroke, cataracts, diabetes mellitus complications, rheumatoid arthritis, neurodegenerative disorders, and ischemia-reperfusion injury, among others. Nutrients *in vitro* often demonstrate specific functions or abilities, such as inhibiting lipoprotein cholesterol oxidation, inhibiting cell proliferation or transformation, or modulating the immune system, that are thought to provide protection against the development of disease. Despite the promising results of many *in vitro* studies, the results of supplementation trials *in vivo* to prevent or treat disease are not consistent, and sometimes have shown that vitamin supplementation may be detrimental to health. Similarly, although many studies have shown that people who either consume diets rich in foods (especially fruits and vegetables) that contain antioxidant nutrients or who have high plasma antioxidant nutrient concentrations have a reduced risk of many diseases or conditions, other studies do not support such associations. New supplementation trials are being conducted, as are *in vitro* and *in vivo* studies, to better elucidate the roles and effects of antioxidants. As these studies continue to clarify the roles of the antioxidant nutrients, scientists and other health professionals will continue to reevaluate current recommendations and perhaps develop new guidelines defining optimal levels of nutrients to prevent diseases. However, enjoying a diet rich in a variety of fruits, vegetables, teas, herbs, spices, and whole grains is always encouraged to help prevent disease and maintain health.

Suggested Reading

Liguori I, Russo G, Curcio F. et al. Oxidative stress, aging, and diseases. *Clin Interv Aging*. 2018; 13:757–72.

MAJOR MINERALS

LEARNING OBJECTIVES

- 11.1** Identify particularly good food sources of calcium, phosphorus, and magnesium.
- 11.2** Describe the processes by which calcium, phosphorus, and magnesium are absorbed and factors influencing their absorption.
- 11.3** Describe the functions/roles of calcium, phosphorus, and magnesium in the body.
- 11.4** Identify the means by which calcium, phosphorus, and magnesium levels are regulated in the body.
- 11.5** Describe recommended intakes, deficiencies, and toxicities associated with calcium, phosphorus, and magnesium.
- 11.6** Identify measures used to assess calcium, phosphorus, and magnesium status.

THE IMPORTANCE OF MINERALS IN NUTRITION and metabolism cannot be overstated, despite the fact that they constitute only about 4% of total body weight. Their functions are many and varied. They provide for normal cellular activity, determine the osmotic properties of body fluids, impart hardness to bones and teeth, regulate vital processes, and function as obligatory cofactors in metalloenzymes, among several other roles.

Minerals can be defined as inorganic elements that come from the earth (water, soil, and plants). Minerals are classified as major (also called macro) or trace (also called micro), although the trace minerals are sometimes further divided into an ultratrace category. The major minerals, in contrast to the trace and ultratrace minerals, are found in greater abundance in the body and are required by adults in amounts greater than 100 mg/day. Trace minerals are required by adults in amounts of about 1–100 mg/day, and ultratrace elements are required by adults in amounts less than 1 mg/day. Minerals are referred to as **ions** when they carry a charge, as occurs with a loss or gain of electrons. Minerals that lose electrons have a positive charge and are called cations, while those that gain electrons have a negative charge and are called anions.

The major minerals include calcium, phosphorus, magnesium, sodium, potassium, and chloride, shown on the periodic table in Figure 11.1. Because of their role in maintaining electrolyte balance in body fluids, sodium, chloride, and potassium are discussed in association with water in Chapter 12. Sulfur is not discussed in the chapters addressing minerals because it does not function independently in the body as a nutrient. Sulfur is found in the body (about 175 g) as part of the structures of vitamins (thiamin and biotin) and of sulfur-containing amino acids (methionine, cysteine, and taurine) and lipoic acid, among other compounds. Because of its presence in amino acids, sulfur is commonly found in protein-containing foods and within body proteins, especially those in skin, hair, and nails.

Table 11.1 provides an overview of the major minerals, including information on general functions, approximate body content, deficiency, food sources, and recommended intakes. Note from Table 11.1 the difference in body content between the major and trace minerals, with the major mineral content of the body ranging from ~28 to 1,200 g and the trace mineral content ranging from <1 mg to ~4 g. In considering the body's mineral content, keep in mind that 1 pound equals about 454 g and an ounce about 28.4 g.

when fortified with calcium, provide similar amounts of calcium as found in cow's milk. However, not all plant-based “milks” are fortified with calcium.

Additional foods containing in excess of about 200 mg calcium per serving include canned salmon and sardines (with bones). Tofu and collard greens also provide over 100 mg calcium per serving. Smaller amounts of calcium can be found in other vegetables—such as turnip greens, broccoli, cauliflower, and kale—as well as nuts and legumes and legume products, as shown in Table 11.2. Other significant food sources of calcium include those fortified with the mineral, such as some breads and fruit juices. The Daily Value for calcium, which is used on food and supplement facts labels, is 1,300 mg.

In contrast to the aforementioned foods, meats and grains (not fortified) are relatively poor sources of calcium. In addition, some vegetables, such as spinach, rhubarb, and Swiss chard, are also poor sources because they contain large amounts of oxalic acid, which binds calcium

and inhibits its absorption, as discussed in the “Factors Influencing Absorption” section.

Calcium is found in multivitamin/mineral supplements as well as in supplements alone or with vitamin D and sometimes with magnesium. The forms of calcium in these supplements (shown on the product label) vary, with the most common forms being calcium carbonate and calcium citrate. Other less-common forms that may be used to fortify foods or found in supplements include calcium acetate, calcium lactate, calcium gluconate, calcium monophosphate, tricalcium phosphate, calcium amino acid chelates, and calcium citrate malate. Of the two widely available forms, calcium citrate is beneficial for those with limited gastric acid production (as may occur in older individuals) and does not need to be ingested with food (although it can be). Calcium carbonate, common in many supplements as well as in antacid medications like Tums®, is a relatively inexpensive form but is better absorbed when consumed with food and its use can be associated with gastrointestinal side effects such as gastric or intestinal pain, constipation, gas, and/or bloating.

The amount of elemental calcium provided by supplements ranges from about 200 to 750 mg per tablet. Because the percentage of calcium by weight in different supplements varies, different amounts of supplements must be consumed to obtain a specific amount of calcium. For example, to obtain 500 mg of calcium from calcium carbonate, 1.26 g (40% calcium by weight) would need to be ingested, whereas a person would need to ingest 5.49 g of calcium gluconate (which is 9% calcium by weight), 3.53 g of calcium lactate (which is 14% calcium by weight), 2.37 g of calcium citrate (which is 21% calcium by weight), or 2.16 g of calcium acetate (which is 23% calcium by weight). Alternately expressed, supplements containing 1,000 mg (1 g) of calcium carbonate by weight provide 400 mg of elemental calcium while those containing 1,000 mg (1 g) of calcium citrate by weight provide 210 mg of elemental calcium.

Calcium-containing supplements are found in the marketplace as pills (which can be quite large), chewable products (gummies, tablets, soft-chews, other), powders, and liquids. Sources of calcium used for supplements, however, should also be considered. For example, calcium carbonate from fossilized oyster shell or dolomite may be contaminated with aluminum and lead, and thus should not be used. Bone meal preparations may also contain lead and should be avoided.

Digestion, Absorption, and Transport

For calcium to be absorbed and used by body tissues, it first must be released from food constituents.

Table 11.2 Calcium Content of Selected Foods*

Food (serving)	Calcium (mg)
Milk, varying fat content (1 c)	275–300
Almond milk, fortified (1 c)	300–450
Soy milk, fortified (1 c)	300
Tofu (soybean curd) (½ c)	125–227
Yogurt, plain, low fat (6 oz)	311
Cottage cheese, low fat (1 c)	138
Cheeses, variety (1 oz)	100–225
Salmon, canned, with bones (3 oz)	190
Sardines, canned, with bones (3 oz)	325
Orange juice, fortified (1 c)	250–350
Collard greens, cooked (½ c)	175
Turnip greens, cooked (½ c)	99
Kale, cooked (½ c)	47
Cauliflower, cooked (½ c)	40
Broccoli, cooked (½ c)	21
Navy beans, cooked (1 c)	126
Edamame, shelled, cooked (½ c)	90
Pinto beans, cooked (1 c)	79
Kidney beans, cooked (1 c)	64
Chickpeas/garbanzo beans (½ c)	77
Almonds (1 oz)	75
Walnuts (1 oz)	28
Pecans (1 oz)	20
Peanuts (1 oz)	15

*The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

A more complete list of calcium-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/Calcium-Food.pdf>.

Digestion

Calcium is present in foods and dietary supplements as relatively insoluble salts. It takes about 1 hour at an acidic pH (as occurs in the stomach) for calcium to be solubilized (to exist as free Ca^{2+}) from most calcium salts. Solubilization does not necessarily ensure better absorption, however, because free calcium can bind to other dietary constituents, limiting its bioavailability, as discussed in the “Factors Influencing Absorption” section.

Absorption

Calcium absorption occurs in the small intestine via two major mechanisms: (1) carrier-mediated, transcellular transport and (2) paracellular diffusion. The transcellular transport system for calcium operates primarily in the duodenum and proximal jejunum, is saturable (at low to moderate calcium intakes), requires energy and a carrier, and is regulated by calcitriol (the active form of vitamin D). At usual calcium intakes, up to about 400–500 mg/meal and daily intakes of about 1,000 mg, this carrier-mediated transport system accounts for about 50–60% of total calcium absorption in the small intestine. Absorption of calcium using this system can be divided into three phases.

- The first phase involves a channel protein carrier called transient receptor potential (TRP) vanilloid (V)6 (abbreviated TRPV6 and also called calcium transporter 1 or CaT1) to get the calcium across the brush border membrane of the enterocyte. Synthesis of this TRPV6 transporter is enhanced by calcitriol and estrogen; its expression declines with age (which accounts in part for the higher calcium recommendations for older adults).
- Next, a binding protein called calbindin D9k, which binds two calcium ions, transports the mineral across the cytosol to the basolateral membrane of the enterocyte. Synthesis of calbindin is enhanced by calcitriol and, like TRPV6, calbindin expression declines with age.
- Third, following release by calbindin, calcium extrusion occurs across the enterocyte's basolateral membrane and into the blood. The extrusion process involves primarily a high-affinity (limited-capacity) Ca^{2+} -ATPase pump (also referred to as PMCA1b) and, to a lesser extent, a low-affinity $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (referred to as NCX1). NCX1 exchanges three Na^{+} ions for one Ca^{2+} ; PMCA1b pumps Ca^{2+} out at the expense of ATP. Calcitriol enhances the synthesis of both pumps.

The second major route for calcium absorption occurs paracellularly (i.e., between cells), rather than through/across them. Paracellular absorption of calcium occurs throughout the small intestine, but mostly in the jejunum and ileum. This absorption process is mediated by solvent drag or as the result of different gradients.

- Solvent drag is induced by sodium efflux (via $\text{Na}^{+}/\text{K}^{+}$ -ATPase), which results in hyperosmotic conditions in the paracellular space. This hyperosmotic environment induces the diffusion of water in a direction that is from the lumen toward the plasma. As the water moves, there is also the movement/“drag” of ions such as calcium.
- A high calcium concentration gradient between the lumen side of intestinal cells and basolateral side of intestinal cells/plasma also increases permeability between enterocytes to promote paracellular calcium absorption.

Several factors affect the tight junctions between enterocytes, and thus influence paracellular absorption. (1) Increases in intracellular calcium ion concentrations are thought to partially mediate the paracellular absorptive process through a series of reactions that have not been well characterized. (2) Another influential factor is the presence of specific membrane-spanning proteins in the enterocyte cell membrane. Claudins 2, 12, and 15 (membrane-spanning proteins) appear to selectively enhance paracellular calcium absorption. Claudin-2, for example, forms charge-selective pores (through the presence of negatively charged aspartic acid residues in its extracellular domain), which extend into the paracellular space. The protrusion forms a tight-junction pore (i.e., an opening between cells) that is permeable to not only calcium, but also magnesium and other solutes. (3) Calcitriol also plays a role; it is thought to enhance the expression of genes that code for selected transmembrane proteins responsible for altering the structure of the tight junctions to increase calcium permeability. Thus, in situations with higher calcium concentrations, increased claudin-2, and adequate calcitriol, paracellular calcium absorption into the body is enhanced.

While the overwhelming majority of calcium is absorbed in the small intestine as described above, small amounts, about 4–10% (or ~8 mg), of dietary calcium may be absorbed by the colon. Calcium becomes available for absorption in the colon as bacteria degrade fermentable fibers to which calcium is bound.

Overall, calcium absorption averages about 25–30% in adults. Calcium absorption from calcium supplements (providing 250 mg of calcium) varies from about 27 to 39%, depending on the specific calcium salt used in the supplement and, in some cases, whether it is consumed under fasting conditions or with food. The amount of calcium ingested affects absorption, with generally greater calcium absorption occurring when provided in a dose of 500 mg or less. Individuals requiring supplemental calcium in amounts greater than 600 mg elemental calcium should divide the dose.

Factors Influencing Absorption Several factors, including stage of life, influence calcium absorption. Infants, children, adolescents, and women during pregnancy and lactation exhibit greater calcium absorption than other age

groups. (Note that calcium requirements are also increased in these groups.) Infants, for example, can absorb over 75% of calcium (from breast milk) and young children may absorb up to about 60% of dietary calcium, while young adults may absorb up to about 30%. With aging, calcium absorption diminishes to about 15–25% in middle-age adults and to sometimes lower percentages in older-age adults. This decline may also be associated in part with age-related decreases in both gastric acid production and calcitriol production and in estrogen in women.

Dietary factors also affect calcium absorption (Table 11.3). The ingestion of food and the presence of lactose and sugar alcohols are thought to improve calcium solubility and thus absorption, although the effect of lactose is thought to be more pronounced in infants than in adults. Higher protein intakes also enhance calcium absorption. While the exact mechanism is unclear, peptides generated from the digestion of protein may facilitate paracellular calcium absorption by promoting solvent drag. This higher calcium absorption associated with protein ingestion compensates for any corresponding increase in urinary calcium excretion; thus, higher protein intakes have no overall effect on calcium balance as long as calcium intake is adequate.

Calcium absorption is also diminished by dietary components. Reductions in intestinal calcium absorption can result from diets high in oxalic acid, phytic acid, fiber, and divalent cations and in medical conditions associated with fat malabsorption and reduced gastric acid production.

- Oxalic acid (oxalate [$\text{C}_2\text{O}_4^{2-}$] is the salt-forming ion of oxalic acid) (Figure 11.2) when present in the digestive tract with calcium can reduce calcium absorption to

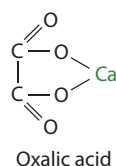
< 5%. Oxalate chelates ionized calcium, has a very low solubility (<0.1 mmol/L; optimal solubility is thought to range from about 0.1 to 10.0 mmol/L), and increases fecal calcium excretion. Oxalic acid is found in a variety of vegetables (e.g., spinach, rhubarb, Swiss chard, beets, celery, eggplant, greens, okra, squash), fruits (e.g., currants, strawberries, blackberries, blueberries, gooseberries), nuts (pecans, peanuts), and beverages (tea, Ovaltine, cocoa), among other foods.

- Phytic acid (phytate or inositol hexaphosphate; shown later in Figure 11.8) and dietary fiber, found in whole-grain breads and wheat bran cereals, seeds, nuts, legumes, and soy isolates, also inhibit intestinal calcium absorption, but not to the same extent as oxalic acid. Specifically, phytate binds calcium and decreases its availability, especially when present in a phytate:calcium molar ratio >0.2. However, most individuals in the United States do not consume enough phytic acid or dietary fiber to profoundly affect calcium absorption. Moreover, calcium that is bound to fiber is typically released and absorbed in the colon with fermentation of fiber.
- Divalent cations, especially magnesium and zinc, when present in high concentrations in the digestive tract, can inhibit calcium absorption. The inhibition occurs especially when the diet is low in calcium and contains an excess of magnesium or zinc (usually when taken in the form of a supplement).
- Unabsorbed dietary fatty acids found in significant quantities in the gastrointestinal tract can interfere with calcium absorption by forming insoluble calcium “soaps” (calcium–fatty acid complexes) in the lumen of the small intestine. These calcium-containing soaps cannot be absorbed and are excreted in the feces. The presence of relatively large amounts of unabsorbed fatty acids in the feces is referred to as steatorrhea (usually characterized by the presence of greater than about 7 g of fecal fat per day). Steatorrhea is common in several gastrointestinal tract disorders, such as inflammatory bowel diseases, as well as with liver disease (cirrhosis) and pancreatic disorders, such as pancreatitis and cystic fibrosis.

In addition to the aforementioned dietary factors, calcium absorption is also affected by other gastrointestinal situations and moderated by other means. Diminished gastric acid (as may occur with aging and the use of some medications like proton pump inhibitors for the treatment of gastroesophageal reflux disease or ulcers) may reduce calcium absorption. Insufficient gastric acid may limit the solubilization of some calcium salts in the stomach and thereby reduce its intestinal absorption. Some low-molecular-weight forms of calcium, however, may not need to be solubilized before being absorbed, especially through paracellular routes. Calcium absorption may be under the influence of calcium-sensing receptors, which are present

Table 11.3 Interactions between Calcium and Selected Nutrients/Substances

Nutrients/Substances Enhancing Calcium Absorption	Nutrients/Substances Inhibiting Calcium Absorption
Vitamin D	Oxalic acid
Sugars and sugar alcohols	Phytic acid
Protein	Fiber
	Excessive divalent cations (Zn and Mg)
	Unabsorbed fatty acids
Nutrients Enhancing Urinary Calcium Excretion	Nutrients Whose Absorption May Be Inhibited by Excessive Calcium
Sodium	Phosphorus
Protein	Iron
Caffeine	Fatty acids



Oxalic acid

Figure 11.2 The binding of calcium by oxalic acid.

on the brush border membrane of enterocytes among other nearby sites. These sensors monitor free ionized calcium concentrations within the lumen and paracellular spaces and are thought to impact calcium absorption in an undefined manner. In addition, two hormones, fibroblast growth factor (FGF) 23 and stanniocalcin, negatively influence calcium absorption. FGF23 specifically appears to inhibit vitamin D-mediated actions on transcellular calcium absorption; these actions may be part of a negative feedback loop to control calcium absorption.

Transport

Calcium is transported in the blood in three forms. Some calcium (~40–45%) is bound to proteins, mainly albumin but also prealbumin and globulins. Some calcium (up to ~10%) is complexed with anions such as sulfate, phosphate, citrate, and/or bicarbonate and about 45–50% of calcium is found free (also referred to as ionized) in the blood. Protein-bound calcium is in equilibrium with ionized calcium. Thus, as ionized calcium is taken out of the

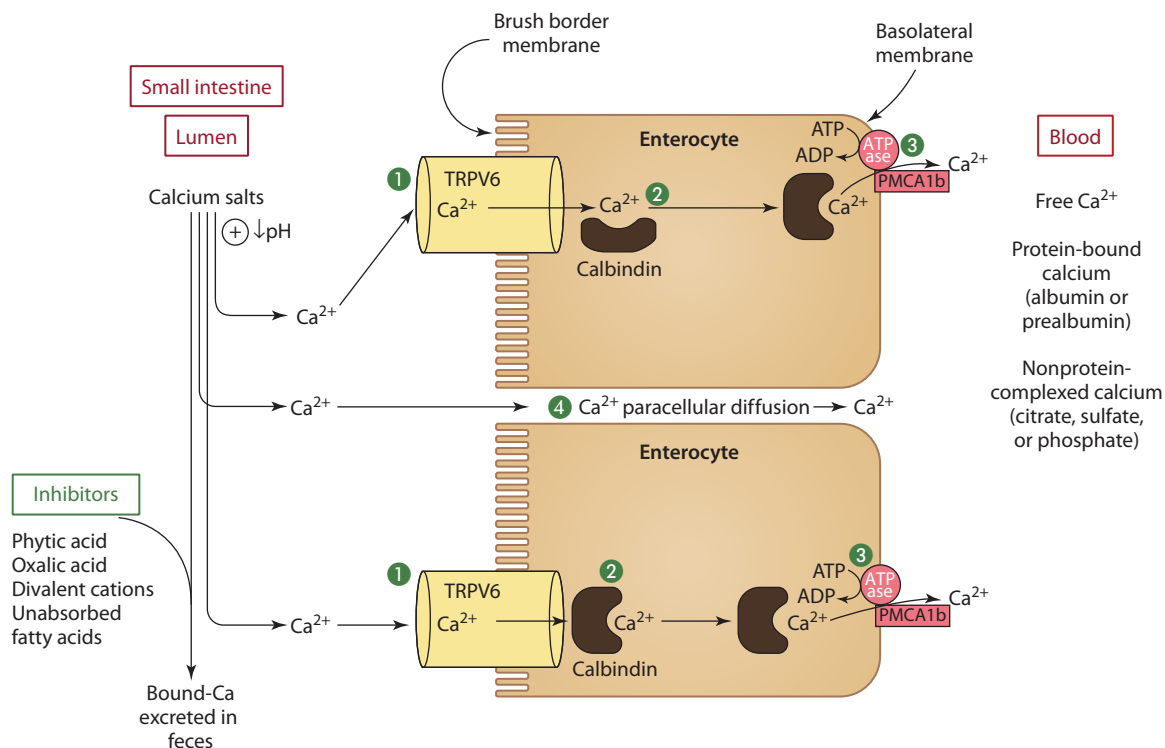
blood and enters tissues/cells, some calcium in the blood that is bound to blood proteins is released to replenish the free, ionized calcium pool. Another protein, Fet-A, which is synthesized and released from the liver into blood, is also involved with calcium in the blood under some conditions. Fet-A binds circulating calcium and phosphate ions, helping to prevent calcification of soft tissues. Figure 11.3 provides an overview of calcium digestion, absorption, and transport.

Regulation and Homeostasis

Calcium concentrations are tightly controlled both intracellularly and extracellularly. Extracellular regulation is discussed first, followed by intracellular regulation.

Extracellular Calcium Concentration Regulation

Calcium concentrations are tightly maintained in the plasma/serum (i.e., extracellular fluid). Total serum calcium concentrations normally range from about



- 1 Ca^{2+} crosses the brush border membrane of the enterocyte through a calcium channel TRPV6.
- 2 Ca^{2+} binds to calbindin D, which carries the calcium across the cytosol of the enterocyte.
- 3 Ca^{2+} -ATPase (PMCA1b) or a $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger (NCX1) pump calcium across the basolateral membrane for entrance into the blood.
- 4 Some Ca^{2+} is absorbed between cells, typically with high Ca^{2+} concentrations in the lumen.

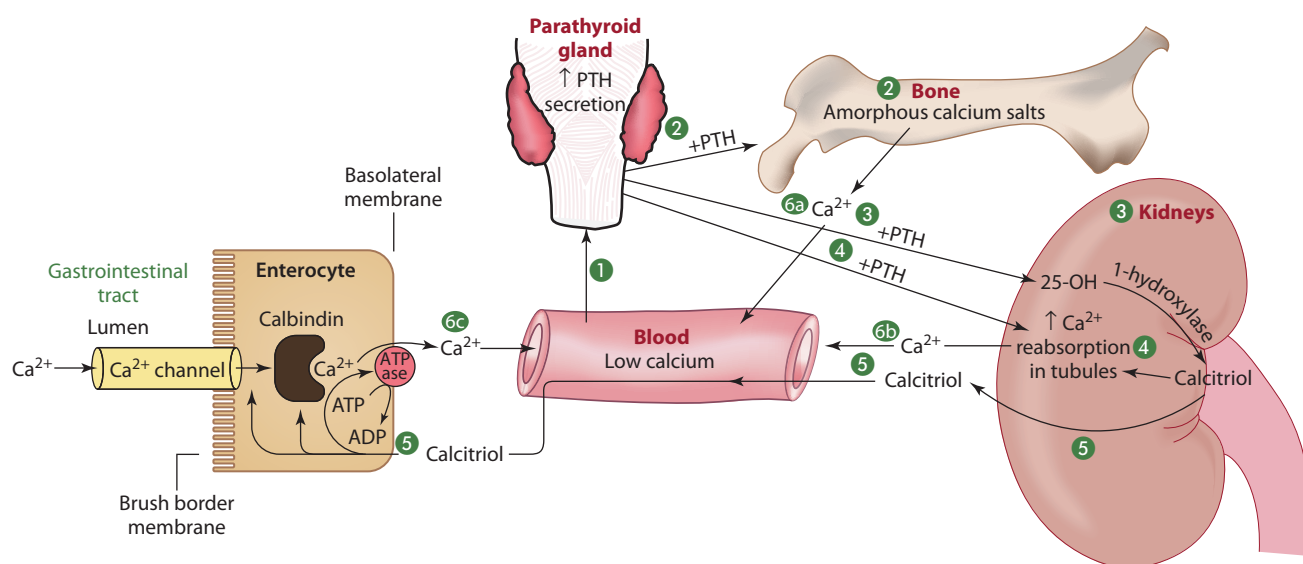
Figure 11.3 Calcium digestion, absorption, and transport.

8.5 to 10.5 mg/dL, and ionized serum calcium concentrations range from about 4.6 to 5.2 mg/dL. Three main hormones are involved in calcium homeostasis: PTH, calcitriol, and calcitonin. PTH is released by the chief cells of the parathyroid gland in response to primarily low serum calcium concentrations. Calcitriol is the active form of vitamin D in the body; the kidneys synthesize and release this hormonal form of the vitamin into circulation. Calcitonin is synthesized by the parafollicular (also called C) cells of the thyroid gland and is released primarily when serum calcium concentrations rise toward/above the upper end of the usual range. The actions of each of these hormones are discussed further in the next several paragraphs as part of an overview of calcium regulation and is shown later in Figure 11.4.

Ionized calcium is removed from the blood with secretion into the digestive tract, excretion in the urine, and uptake by tissues. Calcium-sensing receptors (CaSR) found on the parathyroid gland (as well as on renal cells and enterocytes, among others) monitor blood calcium concentrations. CaSR detect a lowering (fluctuation) of the serum concentration of calcium (and to a lesser extent to calcitriol and other minerals like magnesium and phosphorus) and within minutes the parathyroid gland releases

PTH into the blood. PTH responds to this decrease and functions (along with calcitriol) to increase serum calcium concentrations through the following actions.

- PTH increases serum calcium concentrations through interactions with the kidneys. In the kidneys, PTH stimulates the transcription of 1-hydroxylase to promote the synthesis of calcitriol from 25-OH vitamin D. Calcitriol production results in increased renal reabsorption of filtered calcium by interacting with nuclear vitamin D receptors to induce the transcription of the gene that codes for proteins (TRPV5, calbindin D28k, and extrusion pumps) needed for renal calcium reabsorption (also discussed under “Excretion”).
- PTH increases serum calcium concentrations through interactions with the bone. Specifically, PTH interacts with receptors on osteoblasts (bone-building cells). Osteoblasts perform several functions, including some that are regulated by calcitriol; one such function is stimulating the production of osteoclasts (bone-degrading cells). Osteoclasts in turn promote the release of calcium (resorption) from the bone into the blood, enabling an increase in serum calcium concentrations (see also an overview of bone under “Bone Mineralization”).



- 1 Low blood calcium signals the parathyroid gland to release parathyroid hormone (PTH) into the blood.
- 2 PTH binds to bone cell receptors and triggers the resorption or breakdown of bone mineral for the release of calcium into the blood.
- 3 PTH acts on the kidneys to synthesize the active form of vitamin D, calcitriol.
- 4 PTH and calcitriol promote the reabsorption of calcium from the kidneys and into the blood.
- 5 Calcitriol leaves the kidneys and travels to the intestine, where it promotes calcium absorption across the brush border membrane, its transport in the cell cytosol, and egress into the blood.
- 6 Calcium enters the blood (a) after release from bone, (b) after release from kidneys, and (c) after absorption from intestinal cells.

Figure 11.4 An overview of blood calcium regulation by parathyroid hormone (PTH) and calcitriol (also called $1,25(\text{OH})_2$ vitamin D) in response to low blood calcium concentrations.

- Calcitriol acts in the small intestine to enhance transcellular calcium absorption. As previously discussed, calcitriol enhances intestinal calcium absorption interacting with nuclear vitamin D receptors to induce the transcription of the gene that codes for proteins (TRPV6, calbindin D9k, and extrusion pumps) needed for intestinal absorption.
- Calcitriol also enhances paracellular calcium absorption in the intestine through its effects on genes coding for the synthesis of specific claudin proteins. Calcitriol may also exert effects on calcium through binding to specific membrane-associated rapid response steroid-binding (MARRS) protein receptors on the basolateral membrane and subsequent activation of intracellular second messengers (such as phospholipase A2 and protein kinase C).

The net effect of these actions by PTH and calcitriol is to increase serum calcium concentrations into the normal range. Once the serum concentrations of calcium are appropriate, the secretion and actions of PTH and calcitriol are suppressed. Suppression of PTH results from (1) interactions between calcium and CaSR on the parathyroid gland signal via second messengers and (2) calcitriol through interactions with nuclear receptors in the parathyroid gland. Calcitriol production is reduced by (1) diminished PTH and (2) interactions between calcium and CaSR on kidney cells. Reductions in serum calcium concentrations result from lower calcitriol production, which

- diminish renal calcium reabsorption leading to increased urinary calcium excretion and
- reduce intestinal calcium absorption.

Elevations in serum calcium also trigger calcitonin release from the thyroid gland. Calcitonin suppresses PTH production and release and inhibits osteoclast activity. Table 11.4 summarizes the actions of parathyroid hormone, vitamin D, and calcitonin and Figure 11.4 depicts an overview of blood calcium regulation.

Intracellular Calcium Concentration Regulation

Low free Ca^{2+} concentrations (100 nmol, or approximately 0.0001 times the concentration in the extracellular fluid) are tightly maintained within the cytosol of cells in its

Table 11.4 A Summary of the Effects of Parathyroid Hormone (PTH), Calcitriol, and Calcitonin on Calcium Homeostasis

	PTH	Calcitriol	Calcitonin
Serum calcium	↑	↑	↓
Bone calcium	↓	*	↑
Renal calcium reabsorption	↑	↑	↓
Intestinal calcium absorption	↑	↑	No effect

*Works with PTH

normal resting state. However, for calcium to carry out many of its functions, it must gain entry into and be present in higher concentrations in the cell cytosol. Then, as the cell is restored to its resting state, the excess calcium must be removed from the cytosol.

Calcium gains entry into the cytosol of cells from (1) extracellular sites through cell membrane channels (such as voltage-dependent slow channels, ligand-gated channels, or stretch-activated channels) as well as from (2) intracellular sites such as the endoplasmic (or sarcoplasmic) reticulum and the mitochondria, among other organelles. The efflux of organelle-sequestered calcium into the cytosol typically requires a Ca^{2+} -ATPase pump or release channel (such as the **ryanodine receptor** of the sarcoplasmic reticulum in skeletal muscle), shown later in Figure 11.7.

Once calcium has gained entry into the cell cytosol to perform its functions, the cell's normally low resting calcium concentration must be reestablished (i.e., calcium must be exported from the cell cytosol). This removal from the cytosol can be accomplished by moving the calcium out of the cell or by sequestering the calcium in an organelle.

- The main system responsible for moving calcium out of cells is the $\text{Ca}^{2+}/3\text{Na}^{+}$ exchanger, which exhibits a low affinity but high capacity for calcium. A $\text{Ca}^{2+}/2\text{H}^{+}$ (proton)-ATPase pump, which exhibits high affinity but a low capacity for calcium, also exports calcium, extruding it in exchange for two hydrogen ions (protons); this pump, however, is thought to account for only minor adjustments in cellular calcium concentrations. (Some of the ATPases responsible for maintaining calcium concentrations are shown later in Figure 11.7.)
- Restoration of resting cytosolic concentrations of calcium can also be completed through calcium sequestration (storage/restorage) in cellular organelles such as the mitochondria, endoplasmic (or sarcoplasmic) reticulum, nucleus, and vesicles. This function is performed by Ca^{2+} -ATPase, which pumps Ca^{2+} from the cytosol into the organelles for storage until needed in the cell cytosol again, either for function or to maintain cytosolic concentrations in the event that concentrations drop below resting levels. Within organelles, calcium binds to proteins (such as calsequestrin in the sarcoplasmic reticulum) or complexes with phosphate (as in the mitochondrion). Calcium-binding proteins function as an important buffer within cells to prevent excessive free calcium.

Functions and Mechanisms of Action

Calcium plays several vital roles in the body, including bone (skeleton) mineralization, muscle contraction, blood clot formation, signal transduction (mediating signaling from activated cell membrane receptors) to enable neurotransmission, and enzyme activation, among other roles.

Bone Mineralization

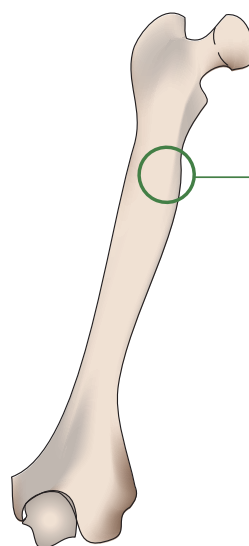
Calcium's most widely known function is in the mineralization of teeth and bone. In teeth, calcium, along with phosphate, carbonate, and magnesium, is found in the outer enamel surface. Calcium and other minerals are found throughout bone, with about 50–70% of the weight of bones consisting of minerals.

Bone tissue can be divided into two main types: cortical and trabecular. Most bones possess a dense outer layer of cortical bone that surrounds trabecular bone. Some bones, such as the sternum, ribs, vertebrae, and pelvis in adults, also contain a cavity for bone marrow. Some characteristics of cortical and trabecular bone include the following:

Cortical Bone	Trabecular Bone
Is compact or dense	Has a spongy appearance
Represents about 75–80% of total bone in the body	Represents about 20–25% of total bone in the body
Consists of layers of mineralized proteins	Consists of an interconnected system of mineralized proteins
Is found mainly on the surfaces of all bones and the shafts/central region (diaphysis) of long bones of the limbs (i.e., between the ends of bones); it covers/protects trabecular bone and bone marrow	Is found in relatively high concentrations in the axial skeleton (vertebrae and pelvic region) and the ends (epiphyses) of long bones such as the femur near the hip and knee joints
Is less metabolically active and has a lower turnover rate than trabecular bone.	Is more metabolically active and has a higher turnover rate than cortical bone

Because of its higher turnover rate, trabecular bone can be more rapidly depleted of calcium, with a poor calcium intake, than cortical bone. Consequently, trabecular bone is more susceptible to osteoporosis, as discussed in the Perspective at the end of this chapter.

Bone, shown in Figure 11.5, is made up of an organic matrix (consisting of proteins and ground substance), water, lipids, and minerals. The minerals contribute to bone rigidity and strength, although such properties are also a function of bone mass, the material properties comprising the bone, and bone architecture, among other factors.



Bone Composition
Organic matrix 20–40%
Minerals 50–70%
Water 5–10%
Lipids <3%

Figure 11.5 The composition of bone.

An examination of the mineral weight of bones shows that calcium comprises about 37–40% and phosphorus about 50–58%. Smaller amounts of magnesium are present along with even smaller amounts of other minerals such as sodium, potassium, fluoride, and strontium. Some of these minerals plus hydroxyl groups make up hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, a crystal latticelike substance found bound to proteins and ground substance in bone. Carbonate is also found in bone, comprising about 2–8% of the weight of bones. This carbonate is usually associated with calcium, potassium, and sodium, but the overall amount present in bone varies, especially with changes in systemic acid–base balance. The process of mineralization is provided in the boxed feature, An Overview of Bone. Minerals found in bone are mainly in the form of small hydroxyapatite crystals. Whether some amorphous and/or other forms of calcium, phosphorus and magnesium [such as $\text{Ca}_3(\text{PO}_4)_2$ (tricalcium phosphate), $\text{Mg}_3(\text{PO}_4)_2$ (trimagnesium phosphate), $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (brushite), $\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$ (octacalcium phosphate), and $\text{Ca}_{18}\text{Mg}_2[\text{HPO}_4]_2[\text{PO}_4]_{12}$ (whitlockite)] are present and/or are converted into hydroxyapatite are unclear and a matter of debate in the scientific literature.

AN OVERVIEW OF BONE

THE MINERALS IN BONE are embedded in proteins and ground substance, which together form the bone matrix or scaffolding (also called the extracellular matrix). It is the protein matrix that provides bone's shape and three-dimensional structure.

Osteoblasts, discussed later in this section, synthesize and release proteins and ground substances to form the bone matrix. Proteins in bone include primarily type I collagen (about 85–90% of proteins), along with tiny amounts of a few other types of

collagen. Noncollagenous proteins represent about 10–15% of total bone proteins. Many of these noncollagenous proteins contain the amino acid glutamic acid, which has been carboxylated (a COO^- group has been added) in the gamma

(Continued)

position by vitamin K–dependent enzymes. These glutamic acid carboxylated proteins are referred to as Gla proteins. Examples of some Gla proteins found in bone are osteocalcin (also called bone Gla protein), fibronectin, matrix Gla protein, osteonectin, osteopontin, and bone sialoprotein-1 (BSP1). The Gla proteins are important in mineralization, facilitating interactions between the bone proteins and minerals and regulating, in part, mineral deposition. For example, osteonectin, a phosphoprotein and one of the most abundant of the GLA proteins in bone, binds both calcium and collagen. Osteocalcin (another major Gla protein) and matrix Gla protein bind calcium and hydroxyapatite. Osteopontin and bone sialoprotein-1 bind to hydroxyapatite. In fact, bone sialoprotein-1's multiple Gla residues are thought to bind numerous (close to 100) calcium ions to in essence dock the protein to the hydroxyapatite.

In addition to collagens and GLA proteins, the bone matrix is made up of ground substance, which consists mostly of glycoproteins and proteoglycans. Glycoproteins contain proteins covalently bound to typically short carbohydrate chains. Proteoglycans are similar to glycoproteins but typically are larger, consisting of a core protein covalently conjugated to one or more glycosaminoglycans (made up of long chains of repeating disaccharides). It is the glycosaminoglycan portion of the proteoglycan that interacts with matrix proteins to enhance bone strength and resilience. Hyaluronic acid and chondroitin sulfate are two glycosaminoglycans associated with bone and cartilage.

Proteins and ground substance are synthesized by bone cells. Among the main types of bone cells (osteoblasts, osteocytes, and osteoclasts), osteoblasts, which originate from the bone marrow, are bone-building cells with about a 3-month lifespan. Under the influence of PTH, calcitriol, and estrogen, among other hormones, osteoblasts secrete collagen and other proteins as well as ground substance to form the bone's extracellular matrix. Osteoblasts also release matrix vesicles, which “bud” from the osteoblast's cell membrane. These now extracellular matrix vesicles attach to the underlying bone matrix and provide a site for the uptake and concentration of calcium and phosphate and formation

of hydroxyapatite. Within the matrix vesicle, which also contains a nuclear core made up of proteins, enzymes, and acidic phospholipids, the calcium and phosphorus accumulate and once the solubility product reaches sufficient levels, precipitation of hydroxyapatite crystals occurs. Microvesicles may also be exocytosed from the matrix vesicles and provide sites for additional mineral deposition and further crystal development (growth in size and aggregation). Mature hydroxyapatite crystals are about 3 nm thick, 50 nm long, and 25 nm wide [1]. Over time, other substances may attach to the surface of the crystals, which are deposited along the protein matrix. Bone mineralization can be further modified by a variety of enzymes, such as alkaline phosphatase, pyrophosphatase, phosphodiesterase 1, and phosphoprotein kinases, and other substances/proteins. Some enhance whereas others inhibit the mineralization process.

As the osteoblasts secrete the proteins and ground substance and mineralization occurs, the osteoblasts become embedded in the matrix. With further embedding, some osteoblasts undergo apoptosis and others undergo differentiation and morphological changes to become either osteocytes or lining cells. Osteocytes—that is, osteoblasts that have been incorporated into bone matrix—represent over 90% of all the cells in bone where they monitor and maintain the integrity of the surrounding bone (including new bone matrix formation and mineralization). These cells, for example, produce substances (such as sclerostin) that inhibit the actions of osteoblasts and substances (such as transforming growth factor β), which interfere with the actions of osteoclasts. Lining cells, which have a relatively flat shape, form a membrane (called the periosteum) that covers the bone surface. Lining cells regulate the flux of minerals into and out of bone. They are also involved to a lesser extent in bone remodeling, functioning to degrade the nonmineralized surface layer at specific sites to form bone-remodeling compartments where the osteoclasts will function.

Osteoclasts, another type of bone cell, are large, multinucleated (with about 2–10 nuclei) cells that resorb (break down or degrade) previously made bone. Osteoclasts arise from the actions of osteoblasts, which produce two proteins: macrophage

colony-stimulating factor (MCSF) and receptor activator of nuclear factor κ -B ligand (RANKL). RANKL initiates osteoclastogenesis. RANKL, upon binding to RANK receptors found on osteoclast precursor cells (and in the presence of MCSF), stimulates precursor cell proliferation and differentiation into osteoclasts as they relocate to areas of bone targeted for resorption. At the designated sites, a sealing zone is formed that restricts bone resorption activities to an exposed area beneath the osteoclasts. Several phosphorylation cascades lead to the expression of genes that enable the osteoclasts to synthesize and release proteases, acids, and other substances that resorb bone. Bone resorption is facilitated by the release of hydrochloric acid (via vacuolar ATPase proton pumps) and carbonic anhydrase into selected regions within the bone. Other acids, including citric and lactic acids, are also released to dissolve the amorphous mineral complexes. Lysosomes in osteoclasts release enzymes such as cathepsin K, matrix metalloproteinases, and hydrolases that break down the bone protein matrix. Remnants from the resorption are released into extracellular fluid.

Osteoclasts respond to PTH, calcitriol, and calcitonin, among other hormones and signaling compounds. The activities of osteoclasts, for example, are inhibited by several substances such as by transforming growth factor β released by osteocytes and by osteoprotegerin, a cytokine whose gene expression is down-regulated by a calcitriol–VDR–RXR heterodimer. Osteoclasts play an important role in increasing serum calcium concentrations to a normal level in times of inadequate calcium intake but also contribute to bone fragility and osteoporosis if not balanced by adequate bone formation by osteoblasts.

Bones are constantly undergoing remodeling. In children and adolescents, skeletal turnover occurs such that formation of bone exceeds resorption of bone. Skeletal turnover continues into adulthood, with peak bone mass occurring in early adulthood. During the fifth decade, bone mass begins to decline. Although the need for calcium in bone modeling is continuous, its greatest benefits in promoting the formation of a sturdy skeletal mass occur during linear bone growth and the years immediately following.

Other Roles

The small amount (1%) of body calcium that is not associated with bone (nonosseous) is responsible for a variety of functions in the body. The calcium needed for these functions is taken out of the blood (extracellular sites) or taken from sites within cell organelles where it has been stored (intracellular sites). Magnesium (discussed later in the chapter) often opposes calcium, including its release from intracellular sites, its flux within the cytosol, and its cellular actions. It is the ionized calcium that is needed, which means that the numerous regulatory functions of calcium are performed by <0.5% of the total body calcium.

Most of calcium's nonosseous roles are involved with regulation of cellular events and metabolic processes, and often require interactions with one or more proteins, such as calmodulin, a cytosolic calcium-binding protein operative in most body cells. Calmodulin consists of two similar globular lobes (each with two Ca^{2+} -binding sites) joined by a long helix. The binding of calcium activates calmodulin by changing its conformation (Figure 11.6); this conformational change allows the protein to stimulate or interact with a variety of enzymes. A few of calcium's many roles in the body are listed hereafter.

- Blood clotting requires calcium. Calcium interacts with cell membrane phospholipids and with Gla residues of specific blood-clotting proteins (see Chapter 10) to facilitate clot formation.
- Skeletal muscle contraction necessitates calcium. Increased intracellular calcium concentrations, which are typically achieved by calcium secretion (from calcium release channels in the membranes of intracellular storage sites such as the sarcoplasmic reticulum) allow released calcium to bind to troponin C, which has four calcium binding sites. The binding of calcium to troponin C results in a conformational change in the protein

and alters interactions with other proteins to enable an interaction between actin and myosin, resulting in muscle contraction. Once the plasma membrane repolarizes, calcium is pumped back into cisternae of the sarcoplasmic reticulum via a Ca^{2+} -ATPase and bound to calsequestrin, and myosin and actin can no longer interact to sustain muscle contraction.

- Visceral smooth muscle contraction requires increased intracellular calcium concentrations (however, the calcium influx into the cytosol of visceral smooth muscle cells comes from the extracellular fluid via gated channels rather than from intracellular organelles as with skeletal muscle). Calcium binding to calmodulin triggers activation of myosin light-chain kinase, which phosphorylates the light chain of myosin. Myosin light chain phosphorylation permits crosslinking between myosin and actin to facilitate smooth muscle contraction. Reductions in intracellular calcium inactivate myosin light-chain kinase and dephosphorylation of myosin light chain facilitates relaxation.
- Calcium promotes vasorelaxing and membrane-stabilizing effects on the smooth muscle cells, possibly through interactions with the central and peripheral sympathetic nervous systems. These, among other, actions of calcium may account for its inverse association in some studies with blood pressure.
- Membrane permeability is affected by calcium, which binds to both membrane proteins and phospholipids. Changes in membrane fluidity occur with calcium-protein interactions that affect protein cross-linking. Enhanced membrane rigidity and electrical resistance may result from calcium's interaction with membrane phospholipids.
- Calcium is involved in nerve transmission whereby neurotransmitters interact with receptors on nerve cells to open ion channels, especially calcium and sodium channels. Ion channels (porelike protein structures in membranes) are involved in the generation of action potentials in nerve cells. Calcium, for example, can enter the nerve ending and trigger the release of acetylcholine. The acetylcholine in turn diffuses to and binds to receptors to trigger another series of events, ultimately including depolarization to generate an action potential.
- Calcium, alone or with interactions with calmodulin, acts as a messenger within cells, activating enzymes to thus modulate numerous body processes. Calcineurin, a protein phosphatase, for example, modulates cellular responses including neuronal functions, ion channel maintenance, and T-cell activation, among others. Phosphorylase kinase, in response to hormones and nerve stimulation via calcium and cAMP, phosphorylates (and thereby activates) glycogen phosphorylase (the enzyme responsible for glycogenolysis, i.e., degrading glycogen to glucose-1- PO_4).

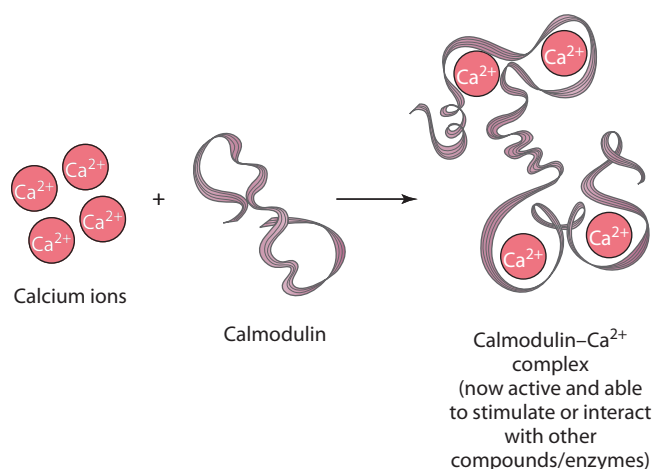


Figure 11.6 Schematic representation of the structural change that occurs in calmodulin following the binding to calcium (Ca^{2+}) ions.

Some of the many enzymes that may be affected either directly by increased free cytosolic Ca^{2+} or through increases in protein-bound Ca^{2+} are listed in Table 11.5. Figure 11.7 shows some of calcium's intracellular actions and mechanisms by which cytosolic calcium concentrations are maintained.

Table 11.5 Selected Enzymes Regulated by Calcium and/or Calmodulin

Adenylate cyclase	Myosin kinase
Ca-dependent protein kinase	NAD kinase
Ca/Mg-ATPase	Nitric oxide synthase
Ca/phospholipid-dependent protein kinase	Phosphodiesterase
Calcineurin	Phospholipase A_2
Cyclic nucleotide phosphodiesterase	Phosphorylase kinase
Glycerol-3-phosphate dehydrogenase	Pyruvate carboxylase
Glycogen synthase	Pyruvate dehydrogenase
Guanylate cyclase	Pyruvate kinase

Interactions with Other Nutrients

Nutrients or substances that inhibit or enhance calcium absorption were discussed in the section on calcium absorption. Additional interactions (also listed in Table 11.3) occur between calcium and phosphorus, iron, and fatty acids and can reduce the availability of these nutrients

Ingestion of large amounts of calcium along with phosphorus-containing foods inhibits phosphorus absorption. The use of calcium supplements (about 2–3 g/day, or in a calcium-to-phosphorus ratio $>3:1$) to inhibit intestinal phosphorus absorption has been employed in the medical management of hyperphosphatemia (high blood phosphorus concentrations) secondary to kidney failure. This strategy, however, is used less often than in the past due to its propensity to increase calcium phosphate deposition in soft tissues.

Calcium from dietary sources as well as from supplements (especially in doses of 800 mg or more) transiently decreases iron absorption. Calcium acts by promoting the

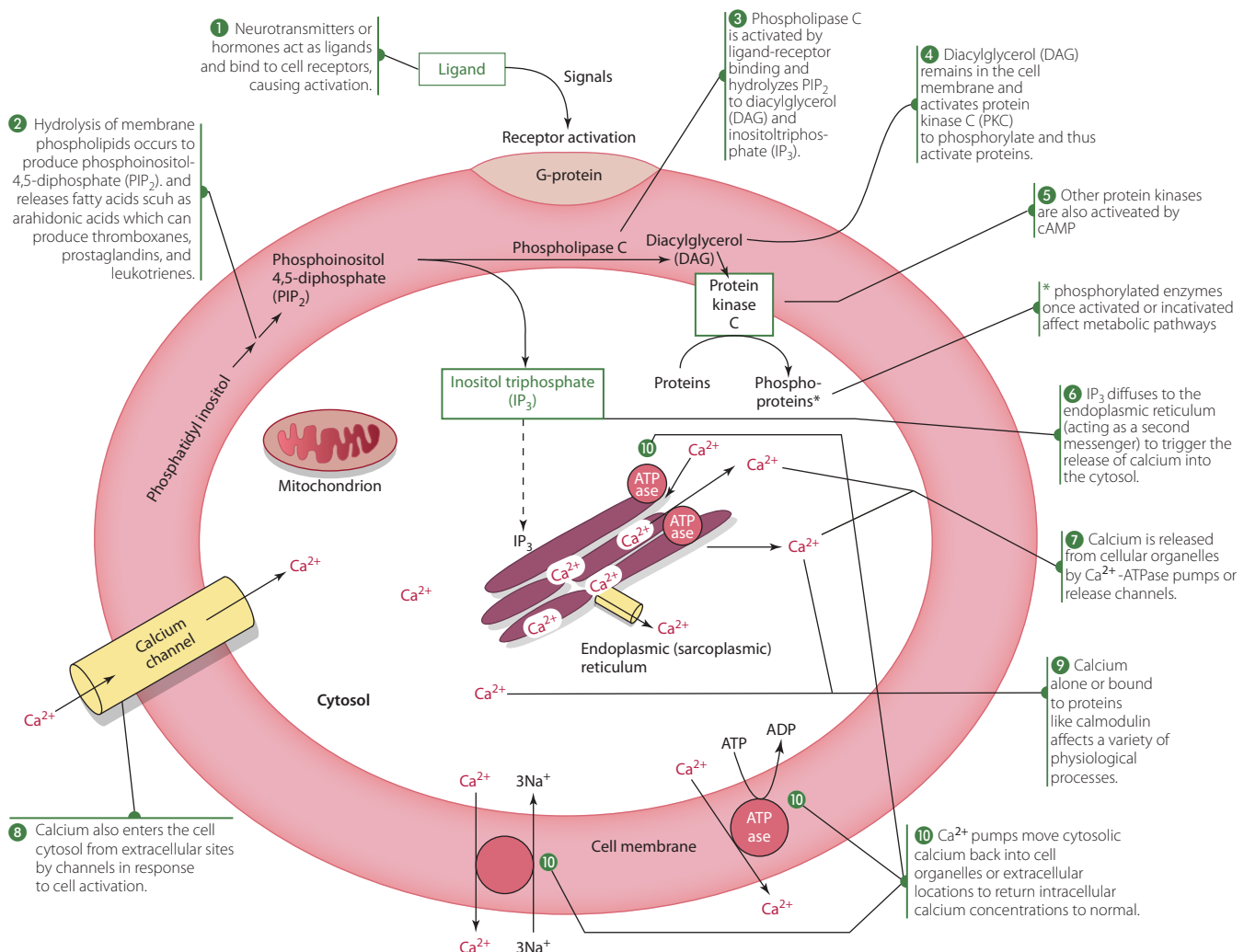


Figure 11.7 Some of calcium's intracellular actions and mechanisms by which cytosolic calcium concentrations are maintained.

temporary relocation of the iron transporter ferroportin from the enterocyte's basolateral membrane to the cytosol. Additional inhibitory effects of calcium on iron absorption may occur through actions on DMT1 (which transports nonheme iron across the brush border membrane) and/or alter membrane fluidity. These interactions are not thought to be of such magnitude to cause iron deficiency; however, for those being treated for iron deficiency, iron supplements should not be consumed with milk or calcium supplements (to help maximize iron absorption).

Calcium diminishes the absorption of fatty acids and (when consumed in gram amounts) can inhibit the reabsorption of bile in the ileum. These changes alter bile's fatty acid and the bile acid profile and, in turn, may favorably affect the colonic environment and reduce colon cancer risk.

Excretion

Calcium losses from the body occur via the feces and urine, with lesser amounts (up to about 25 mg/day) lost through insensible routes (dermal, hair, nails). Extreme sweating, however, can increase calcium losses about six-fold. Fecal losses of calcium from endogenous sources (the sloughing of mucosal cells and calcium that is not reabsorbed from digestive juices—saliva, gastric juice, pancreatic juice, and bile) range from about 45 to 200 mg/day. Caffeine ingestion has been shown to increase calcium secretion into the gut; however, whether the secreted calcium is reabsorbed and the extent of the secretion have not been determined. A systemic review, however, found caffeine intakes of up to 400 mg/day did not negatively affect bone health or cardiovascular health [2].

The kidneys are critical in maintaining calcium homeostasis. The kidneys filter ionized calcium and calcium that is bound to anions (but not protein-bound calcium). Of this filtered calcium, about 98% is reabsorbed, resulting in urinary excretion of about 100–240 mg calcium daily. In the proximal convoluted tubule, most (about 60–70% of the 98%) of the filtered calcium is reabsorbed passively (by diffusion and solvent drag) and parallels sodium and water reabsorption, with perhaps a small amount absorbed by active transport. In the thick ascending limb of the loop of Henle, another 20% of the 98% of filtered calcium is reabsorbed primarily passively, with possibly a small amount reabsorbed by active transport. The distal convoluted tubule reabsorbs about 5–10% of calcium; reabsorption occurs strictly by active transport in this region and serves as the major regulator of calcium balance in the body.

Active calcium reabsorption in the kidneys is similar to that described in the small intestine and involves three phases.

- Calcium crosses the renal brush border (apical) cell membrane through a membrane transporter called transient receptor potential calcium channel vanilloid (TRPV) 5.
- Calcium then attaches to an intracellular cytosolic calcium binding protein, calbindin D28k, for transport to the cell's basolateral membrane.
- Calcium extrusion across the basolateral membrane is accomplished by a Na^+ - Ca^{2+} exchanger (NCX1) and a Ca^{2+} -ATPase (PMCA1b).

Several factors influence urinary calcium excretion. Hormones, including PTH and calcitriol, both stimulate calcium reabsorption to reduce urinary calcium excretion. Serum calcium concentrations also play a role, with hypercalcemia increasing urinary calcium excretion via increases in filtered calcium by the glomeruli and decreases in tubular calcium reabsorption. Hypocalcemia has the opposite effects. In addition, calcium handling in some segments of the kidneys are controlled by sensors. For example, in the thick ascending limb of the loop of Henle, CaSR present in the basolateral membrane respond to serum calcium concentrations by altering tight junction calcium permeability via changes in claudin expression. High serum calcium concentrations typically suppress ion movement through various paracellular channels to reestablish serum calcium homeostasis. Medical conditions, such as acid–base imbalances among others, and medications, such as diuretics, also impact the handling of calcium by the kidneys. For example, in the ascending loop of Henle, loop diuretics inhibit NKCC2, a transporter of sodium and chloride, an action that also decreases calcium reabsorption. Thiazide diuretics, which target the distal tubule, have the opposite effect, promoting calcium reabsorption.

Urinary calcium losses are also increased by some dietary factors; however, these are not thought to negatively impact health for most individuals, especially when consuming an adequate diet. The most commonly cited nutrients affecting urinary calcium excretion include protein, caffeine, and sodium. However, while protein's stimulatory effects on urinary calcium excretion are well documented, they are offset by protein's ability to enhance intestinal calcium absorption and decrease endogenous calcium secretion into the gastrointestinal tract. Moreover, while caffeine consumption in amounts up to 400 mg/day (one to four cups of coffee) transiently (1–3 hours) increases urinary calcium excretion by up to about 10 mg, such caffeine intakes have not been associated with adverse effects on bone [2]. Sodium intake in excess of needs leads to increased urinary sodium; however, the effects are most pronounced when coupled with low calcium intakes. Excretion of excess urinary sodium in amounts of, for example, about 1,000 mg is associated with urinary calcium losses of about 25 mg. The mechanism for the interaction between the nutrients is unclear but may result from competition between the minerals (in the presence of high sodium and low calcium, but not high calcium) for renal reabsorption in the proximal tubule and thick ascending loop of Henle. Such findings emphasize

the importance of consuming a diet that provides adequate dietary calcium. Potential links with osteoporosis are discussed in the Perspective at the end of this chapter.

Recommended Dietary Allowance

The Recommended Dietary Allowance (RDA) for calcium is 1,000 mg daily for adult men age 19–70 years and women age 19–50 years, including pregnant and lactating women [3]. For females age 51 years and older and males age 71 years and older, the recommendations for calcium intake are slightly higher at 1,200 mg/day. The inside covers of this book provide additional recommendations for calcium for other age groups.

The U.S. Food and Drug Administration (FDA) has approved several health claims related to calcium. One example claim is “Regular exercise and a healthy diet with enough calcium help teen and young adult white and Asian women maintain good bone health and may reduce the risk of osteoporosis later in life” [4]. Another claim added to the aforementioned statement on foods providing 40% or more of the Daily Value states, “Adequate calcium intake is important, but daily intakes above about 2,000 mg are not likely to provide any additional benefit” [4]. Foods citing these claims should not provide more phosphorus than calcium on a weight-for-weight basis [4].

Deficiency

Infants and children with insufficient intake of calcium, and usually also a codeficiency of vitamin D, develop rickets. Rickets is characterized by defective mineralization of the bone matrix and growth plate (the growth plate, also called epiphyseal plate, is found at the ends of long bones and is where growth occurs in bone until skeletal maturity is reached, i.e., before epiphyseal closure). The resulting weak and misshaped bone is especially noticeable in the legs, which curve or bow outward (seen when the infant begins to stand and bear weight) and in the ribs at the costochondral junction (see Chapter 10 under the section “Vitamin D” in the subsection “Deficiency”).

Inadequate calcium intake in adults (who, in contrast to infants and children, already have formed bones) can lead to osteopenia (reduced bone mineral density) and ultimately osteoporosis (more severe reduction in bone mineral density). The effects are mediated by chronic elevations in serum PTH (termed *secondary hyperparathyroidism*) occurring in response to chronic insufficient vitamin D and dietary and serum calcium concentrations. The higher serum PTH promotes bone resorption to correct the low serum calcium concentrations. Thus, over time, bones lose more and more calcium. In addition, the bone matrix is typically progressively lost, and osteopenia and ultimately osteoporosis develop. Osteoporosis increases bone fragility and fracture risk. Unfortunately,

much of the U.S. population, particularly females over 12 years of age, fails to consume the recommended amount of calcium and many are also deficient in vitamin D. Osteoporosis and diet are discussed further in the Perspective at the end of the chapter.

While serum calcium concentrations are tightly regulated, as previously discussed, conditions that reduce PTH secretion and/or are associated with impaired vitamin D status or other medical issues can result in declines in serum calcium below the lower end of the reference range (**hypocalcemia**). Serum calcium concentrations below about 7 mg/dL cause increased neuromuscular excitability, whereby an action potential is triggered with a smaller than usually needed stimulus. The neuronal excitability is accompanied by intermittent contractions of muscle that fail to relax; muscles of the hands and feet (extremities) are usually most affected. Paresthesia (numbness or tingling, especially in the hands and feet) may also be present. **Tetany** is characterized by continuous severe muscle spasms and can cause respiratory dysfunction and death. Some clinical signs include a positive Chvostek sign (spasm of facial muscles elicited by tapping the facial nerve near the parotid gland) and Trousseau phenomenon/sign (spasmodic contractions of muscles of the hand initiated by pressure on the brachial artery). Treatment of tetany and hypocalcemia generally requires intravenous calcium administration, usually as calcium chloride or gluconate. Mild-to-moderate reductions in serum calcium concentrations may respond to oral calcium supplements in those with adequate gastrointestinal tract function.

At Risk for Deficiency

Inadequate calcium intake, poor calcium absorption, excessive calcium losses, or a combination of these factors contribute to calcium deficiency. Factors reducing calcium absorption have been discussed in an earlier section. In clinical practice, problems are seen primarily in individuals with conditions causing fat malabsorption (such as exocrine pancreatic conditions, cirrhosis, and intestinal conditions). Such conditions reduce calcium absorption not only directly from fatty acids–calcium soap formation, but also secondary to diminished fat-soluble vitamin absorption, including vitamin D. Decreased gastrointestinal transit time, as may occur with diarrhea, diminishes intestinal calcium absorption. Use of some diuretics increases urinary calcium excretion. Immobilization (such as from bedrest)—for example, associated with trauma/injury—also promotes calcium loss from bone and increases urinary calcium losses. Individuals at risk for calcium deficits should be provided with information on foods rich in calcium and approaches to increase dietary calcium intake. If dietary intake from calcium-rich foods is not possible, information on supplemental calcium should be provided to meet recommendations.

Calcium Intake and Disease Risk

Inadequate dietary calcium intakes have been associated with increased risks of several conditions such as colon cancer, type 2 diabetes mellitus, obesity, and osteoporosis. Several of calcium's functions in the body provide plausible means by which calcium is thought to exert beneficial effects and reduce disease risk. However, while data demonstrating positive associations between calcium use and disease risk reduction are deemed promising, the data are considered insufficient to recommend the routine use of a calcium supplement to reduce or prevent colon cancer, type 2 diabetes, or obesity. The Perspective at the end of this chapter addresses the topic of osteoporosis.

Toxicity

A Tolerable Upper Intake Level of 2,500 mg of calcium has been recommended for adults 19–50 years of age [3]. The recommendation decreases to 2,000 mg of calcium for those age 51 years and older [3]. This upper level was set based on the results of some studies showing positive associations between use of supplemental calcium and risk of kidney stones. High calcium intakes from dietary sources have not been typically associated with higher risk of stone formation, except in those with idiopathic **hypercalciuria** and fat malabsorption disorders.

Idiopathic hypercalciuria is a medical condition characterized by high urinary calcium levels (>4 mg/kg body weight per day or about 250–300 mg/day) without a known cause (i.e., there is an absence of disease causing the hypercalciuria). Individuals with this condition who consume excessive amounts of calcium are at increased risk of developing calcium-containing kidney stones. However, dietary calcium restriction is not recommended.

An increased risk of developing kidney stones also occurs in individuals who have fat malabsorption syndromes (such as those with chronic pancreatitis, cystic fibrosis, Crohn's disease, or liver failure). Fat malabsorption enhances intestinal oxalic acid absorption and calcium oxalate stone formation in the kidneys. The associated events include (1) increased intestinal oxalic acid absorption because the fatty acids (present in large amounts in the small intestine due to malabsorption) bind to ingested dietary calcium and form an insoluble complex that is excreted in the feces. Note that under usual conditions (without fat malabsorption), dietary calcium binds to oxalic acid in the intestine and reduces oxalic acid absorption. (2) In the absence/reduction of available dietary calcium, the absorbed oxalic acid increases in the blood and travels to the kidneys for excretion. (3) The presence of the increased concentrations of oxalates in the kidneys is thought to contribute to calcium-oxalate stone formation. However, multiple other factors, including for example inadequate fluid consumption, also contribute to stone development in susceptible individuals.

Calcium-alkali syndrome (once called milk-alkali syndrome) has been documented in those consuming excessive quantities of calcium (formerly in the form of milk but more commonly now as antacids or supplements containing calcium carbonate and sodium bicarbonate). The condition is characterized initially by hypercalcemia and metabolic alkalosis. The excessive calcium intake (especially more than 4 g/day) promotes hypercalcemia. The hypercalcemia decreases PTH secretion. Retention of bicarbonate also occurs and causes a systemic alkalosis. Hypercalcemia and systemic alkalosis may result in lethargy, anorexia, nausea, vomiting, and heart arrhythmias. Hypercalcemia may also be observed with some cancers (especially those affecting the bone), immobility, and adrenal insufficiency, as well as from conditions affecting the parathyroid gland. Symptoms of hypercalcemia vary depending on the etiology and severity, but may include bradycardia, ventricular arrhythmias, coma, and death.

Calcium ingestion, usually when taken as a supplement, has been linked with increased risks of cardiovascular disease, ischemic heart disease, myocardial infarction (i.e., heart attack), and cardiovascular mortality. It has been suggested that acute increases in serum calcium concentrations that result for a few hours following the ingestion of large intakes of calcium may “overwhelm” the usual mechanisms that regulate serum calcium concentrations. The resulting periods of hypercalcemia in turn have been suggested to trigger changes (such as calcification of blood vessels, increased vascular resistance, and thrombogenesis, among other events) that increase cardiovascular disease risk. More recent evaluations of the data, however, suggest that a calcium intake (from foods and supplements) that is below the Tolerable Upper Intake Level (2,000–2,500 mg/day) is not associated with increased cardiovascular disease risk [5–7].

Assessment of Nutriture

No routine biochemical method is available to directly assess calcium status. Serum calcium is so exquisitely regulated that it indicates little about either calcium status or intake. Abnormal serum concentrations of calcium, however, can occur with various diseases, especially renal failure, some cancers, and disorders affecting the parathyroid or thyroid glands, among others. Changes in blood pH also affect the concentrations of free versus bound calcium. For example, as blood pH increases (becomes more alkaline), protein-bound calcium increases and ionized calcium decreases in the blood. Conversely, with acidosis or a lower blood pH, serum ionized calcium increases and protein-bound calcium decreases. Changes in blood proteins also impact serum calcium concentrations. For example, in the presence of normal serum albumin concentrations, the ratio between bound calcium and ionized calcium remains constant. However, when serum protein

concentrations are depressed, corrections are needed to adjust for the corresponding decrease that occurs in the protein-bound fraction of calcium. For each 1 g/dL decrease in serum albumin, total serum calcium decreases 0.8 mg/dL. The following equations can be used for estimating calcium:

- Protein-bound serum calcium (mg/dL) = $0.44 + [0.76 \times \text{albumin (g/dL)}]$
- Total serum calcium (mg/dL) = $[0.8 \times (\text{normal albumin} - \text{actual albumin})] + \text{measured calcium (mg/dL)}$

Because the majority of calcium is found in bone, it is common to assess bone mineral density, especially in those at risk for osteoporosis. Assessment of bone is accomplished by several methods.

- Dual-energy X-ray absorptiometry (abbreviated DEXA or DXA), a widely used method, involves scanning specific sites at two different energy levels using an X-ray tube. Radiation exposure is low, and the procedure is relatively quick.
- Computerized tomography (CT) scans measure variances in tissue density. X-rays are taken as the person is held in a scanner. Radiation pulses are emitted, collected, and processed to reconstruct the image and calculate bone density. This method, however, is less precise and accurate than DEXA.
- Single-photon absorptiometry exposes a portion of a limb, usually the radius (forearm) or os calcis (heel), to radiation. The quantity of bone mineral is inversely proportional to the amount of photon energy transmitted from the bone, as measured by a scintillation counter.

More information on osteoporosis is provided in the Perspective at the end of this chapter.

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11.2 PHOSPHORUS

Among the minerals, phosphorus is second only to calcium in abundance in the body. The human body contains about 500–850 g of phosphorus, representing about 0.8–1.4% of body weight. Of total body phosphorus, about 85% is in the skeleton, 1% is in extracellular fluids, and the remaining 14% is associated with soft tissues.

Sources

Phosphorus is widely distributed in foods. The best food sources include protein-rich foods like meat, poultry, fish, eggs, milk, and milk products. Milk, for example, contributes typically over 200 mg phosphorus per 1 cup serving. Similarly, some meats, poultry, and fish provide 150–250 mg phosphorus/3-oz serving. Table 11.6 lists the phosphorus content of selected foods.

Table 11.6 Phosphorus Content of Selected Foods*

Food (serving)	Phosphorus (mg)
Milk and yogurt, various types (1 c)	185–350
Cheeses, various types (1 oz)	85–155
Egg, poached (1)	98
Chicken, thigh, meat only, roasted (3 oz)	267
Chicken, breast, meat only, roasted (3 oz)	200
Beef, loin, top sirloin, grilled (3 oz)	197
Beef, ground, 75% lean, 25% fat, broiled (3 oz)	161
Tuna, bluefin, cooked, dry heat (3 oz)	277
Halibut, cooked, dry heat (3 oz)	244
Salmon, Atlantic, farmed, cooked (3 oz)	214
Mixed nuts, oil roasted (1 oz)	123
Walnuts, dry roasted (1 oz)	98
Pecans, dry roasted (1 oz)	79
Peanut butter (2 Tbsp)	114
Legumes, cooked (½ c)	230–250
Lentils, cooked (½ c)	178
Peas, green, cooked (½ c)	94
Cauliflower, cooked (½ c)	20
Bread, whole wheat (1 sl)	57
Cola-type soft drinks (12 oz)	25–40
Tea and coffee (1 c)	< 10

*The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

A more complete list of phosphorus-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://www.nal.usda.gov/sites/www.nal.usda.gov/files/phosphorus.pdf>.

Another source contributing what may be significant amounts of dietary phosphorus is food additives, found especially in processed foods. Examples of some common phosphorus-containing additives are phosphoric acid along with inorganic phosphate salts such as sodium phosphate, sodium polyphosphate, and dicalcium phosphate. These additives are found in a variety of processed foods including frozen foods, dry food mixes, bread and baked goods, canned vegetables, condiments, sauces, and cola-type soft drinks, among others. They are added, for example, to enhance a food's color, provide pH stability, contribute to product leavening, and maintain moisture content, among many other roles. The amount of phosphorus provided by additives varies, but they may contribute as much as 1,000 mg per day [1,2]. Manufacturers are not required to include the amount of phosphorus provided in foods on the nutrition facts panel unless the food has been enriched or fortified with phosphorus. The use of phosphorus-containing additives in a food does not qualify as enrichment or fortification.

Phosphate-containing supplements, including, for example, K-Phos Neutral[®] and Phospha 250 Neutral[®], are available commercially, although supplements are usually only needed for individuals with specific medical conditions. Supplements provide phosphorus as a salt, usually disodium phosphate and dipotassium phosphate. The amount of phosphorus provided in single-ingredient supplements is generally higher than that found in multivitamin/minerals supplements. The latter usually contains about 100 mg phosphorus. The Daily Value, which is found on the food and supplement facts labels, for phosphorus is 1,250 mg.

Phosphorus in foods occurs in organic and inorganic forms. In its organic form, phosphorus is bound to proteins, sugars, and lipids. The relative amounts of inorganic and organic phosphorus vary within foods and the diet. For example, meats contain phosphorus that is largely bound to organic compounds; milk has about one-third of the phosphorus as inorganic phosphates, and the remaining two-thirds bound to organic nutrients. Over 80% of the phosphorus in grains as well as some in legumes is found as part of phytic acid. The bioavailability of phosphorus from phytic acid is limited (see “Factors Influencing Absorption”).

Digestion, Absorption, and Transport

Regardless of its dietary form, most phosphorus is absorbed from the gastrointestinal tract as free inorganic phosphate ions. Thus, bound phosphorus in foods must be digested enzymatically to release free inorganic phosphate to enable absorption.

Digestion

Several enzymes help to release bound phosphorus from foods. Phospholipase C, a zinc-dependent enzyme, for example, hydrolyzes the glycerophosphate bond in phospholipids. Alkaline phosphatase, another zinc-dependent enzyme whose activity is stimulated by calcitriol, functions

at the brush border membrane of the enterocyte to free phosphate from some, but not all (such as phytic acid), bound forms.

Absorption

Phosphorus absorption occurs throughout the small intestine, but primarily in the jejunum. About 50–80% of dietary phosphorus is absorbed, with absorption from animal products at the upper end of the range and that from plant foods, especially phytic acid-containing foods, at the lower end. About 70%, or perhaps more, of inorganic phosphates in foods as additives is absorbed.

Phosphorus absorption occurs by two processes: (1) saturable, carrier-mediated, active transport and (2) passive diffusion. The absorption of most phosphorus from the diet occurs by passive diffusion that is likely paracellular. Carrier-mediated transport across the enterocyte's brush border membrane contributes to absorption primarily when phosphorus intake is low. The carrier is a sodium-phosphate, Na^+ -Pi, cotransporter (NaPi2b, also referred to as Npt2b), which transports three sodium ions for each phosphate (as either H_2PO_4^- or HPO_4^{2-}). Phosphate transporters, PiT1 and PiT2, are responsible for intracellular phosphate transport within enterocytes (among other cells). Transport of phosphate across the enterocyte's basolateral membrane is thought to occur by facilitated diffusion.

Factors Influencing Absorption Several factors influence phosphorus absorption. Calcitriol increases the number of NaPi2b carriers on the enterocyte's brush border membrane, but its involvement may vary in different regions of the intestine and the extent to which absorption is enhanced is relatively small when compared with calcitriol's effects on calcium absorption. Calcitriol production is increased in response to PTH and decreased in response to fibroblast growth factor (FGF).

Phosphorus bioavailability is low from certain foods, especially grains (like wheat, corn, and rice) and legumes, which contain phosphate groups that are part of phytic acid (Figure 11.8). The bioavailability of phosphorus from phytic acid is relatively poor (less than about 50%) because humans do not produce phytase, a phosphate esterase that liberates phosphate from phytic acid. (Note: Yeasts in breads possess phytase that hydrolyzes phytates to yield some phosphorus available for absorption.) Moreover, when phytic acid-containing foods are consumed with foods rich in Ca^{2+} or Zn^{2+} , the phytic acid forms a

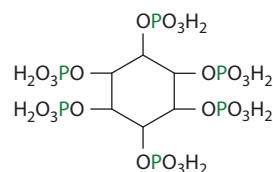


Figure 11.8 Phytic acid (phytate).

cation–phytic acid complex that prevents these nutrients from being absorbed. Soaking legumes in water that is slightly acidic may partially reduce the phytic acid content.

Phosphorus absorption is reduced with the ingestion of large amounts of calcium (as calcium carbonate or acetate), magnesium (as magnesium hydroxide or carbonate), iron (as ferric citrate and sucroferrous oxyhydroxide), and aluminum (as aluminum hydroxide). Ingestion of aluminum hydroxide (3 g) with a meal, for example, can reduce phosphorus absorption in half, from a usual 70% to about 35%. Aluminum, magnesium, and calcium are common components of antacids. Extended-release niacin, when ingested in amounts of at least 2 g daily (as may be done to treat hypercholesterolemia), also reduces phosphorus absorption. High doses of niacin in this form appear to down-regulate the number of NaPi2b transporters needed for carrier-mediated phosphate absorption.

Transport

Phosphate is quickly absorbed from the intestine, appearing in the blood within about an hour after ingestion in animal studies. Phosphate is found in the blood in several inorganic forms. Most (about 55%) is present as HPO_4^{2-} due to its greater solubility in blood than H_2PO_4^- and the trivalent anion PO_4^{3-} (which is present in trace amounts). Up to about 35% of inorganic phosphate, mainly HPO_4^{2-} , is found complexed with calcium, magnesium, or sodium as salts in the blood. About 10–20% is bound to proteins (i.e., organic phosphate) in the blood. Figure 11.9 provides an overview of phosphorus digestion, absorption, and transport.

Plasma inorganic phosphate concentrations usually range from about 2.5 to 4.5 mg/dL. Circulating plasma phosphate is in equilibrium with skeletal and cellular inorganic phosphate as well as with organic phosphates formed in intermediary metabolism. Uptake of phosphate into cells is thought to occur passively (driven by the chemical gradient), but the exact mechanism is not clear.

Regulation and Homeostasis

While not maintained within as narrow a range as serum calcium, plasma/serum phosphate concentrations are maintained through changes in phosphate absorption by the intestine, excretion by the kidneys, and movement into and out of sites in bone and other cells. Three hormones influencing these processes (as well as each other) include fibroblast growth factor 23, parathyroid hormone (PTH), and calcitriol. Fibroblast growth factor (FGF) 23, secreted mainly by osteoblasts, plays the primary role. FGF23 is released with increased serum phosphate concentrations. FGF23 diminishes calcitriol synthesis in the kidneys. FGF23 functions to lower serum phosphate by:

- Increasing urinary phosphate excretion via down-regulating the numbers of NaPi2a and NaPi2c transporters needed in the kidneys for phosphate reabsorption.
- Diminishing intestinal phosphate absorption by down-regulating the numbers of NaPi2b transporters. These effects on the intestine, however, are mediated primarily

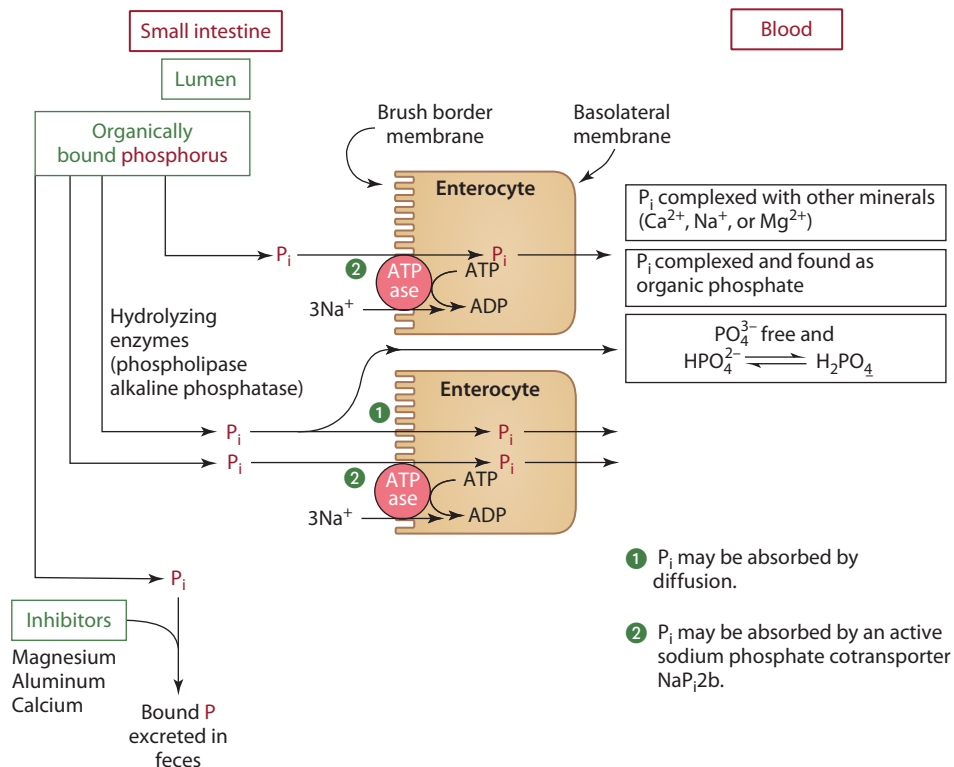


Figure 11.9 Phosphorus digestion, absorption, and transport.

by reductions in serum calcitriol (reduced 1α -hydroxylase activity and thus calcitriol synthesis and increased 24-hydroxylase activity and thus calcitriol catabolism) secondary to FGF23.

PTH also reduces the expression of NaPi2a and NaPi2c transporters needed in the kidneys for phosphate reabsorption. While PTH synthesis and secretion are mainly governed by changes in serum calcium, increased serum calcitriol, and to a lesser extent FGF23, inhibit PTH release.

Should serum phosphorus concentrations decrease, FGF23 secretion by osteocytes is reduced. The responsible mechanism is not yet established. With lower FGF23, calcitriol synthesis increases, leading to increased intestinal phosphorus absorption. Production of transporters needed for renal phosphate reabsorption are also increased due to lower FGF23 and to calcitriol-induced suppression of PTH. Thus, with the lower serum phosphate concentrations, most phosphate filtered by the kidneys is reabsorbed. The means by which bone resorption, if involved, may increase serum phosphate have not been clearly delineated. Table 11.7 summarizes the effects of FGF23, PTH, and calcitriol on serum phosphate, renal phosphate reabsorption, and intestinal phosphate absorption.

Functions and Mechanisms of Action

Phosphorus, which is found in all cells of the body, has many functions and is a component of several biologically important compounds. While most body phosphate is found within bone and used in mineralization, much of the remaining phosphate is found within cell membranes as part of phospholipids and within cells where it serves as the cell's major anion. In soft tissues, phosphate is found as phosphate esters, attached to proteins (phosphoproteins), or as free ions. It is also a structural component of many important compounds, some of which are shown in Figure 11.10 and described hereafter.

Bone Mineralization

Phosphate, like calcium and magnesium, is important in bone (skeletal) tissue, which in itself accounts for 85% of body phosphorus. In bone, phosphorus is found primarily as hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, which is laid down on collagen in the process of bone formation. In amorphous bone,

the ratio of calcium to phosphorus is about 1.3:1, similar to extracellular fluid; however, in crystalline bone, the ratio is about 1.5 to 2.0:1. Close to 200 mg of phosphate is moved into and out of bone daily. The deposition of phosphate in bone is dependent upon its concentration in extracellular fluid; this in turn influences hydroxyapatite formation. Resorption of phosphate from bone is dependent upon the activities of several enzymes, which dissolve the hydroxyapatite and perhaps other amorphous forms of the mineral. The released phosphate passes via canaliculi to the extracellular fluid. See the subsection “An Overview of Bone” under the section “Calcium” for further information on bone mineralization.

Nucleotide/Nucleoside Phosphates

Structural Roles Phosphate is an important component of the nucleic acids DNA and RNA (Figures 11.10a and b), alternating with pentose sugars to form the linear backbone of these molecules.

Energy Storage and Transfer Phosphate is of vital importance in the intermediary metabolism of the energy nutrients in the form of high-energy phosphate bonds, such as those in adenosine triphosphate (ATP) (Figure 11.10c). The energy released as the phosphate bond of ATP is broken provides for numerous cellular functions (e.g., active transport pumps for nutrient absorption and for maintenance of ion concentrations, muscle cell contraction). In addition to its presence in ATP, phosphate is found in creatine phosphate (also called phosphocreatine; Figure 11.10d). Creatine phosphate, synthesized in muscle from ATP and creatine, provides and replenishes energy to muscles as needed (e.g., during exercise) by transferring its PO_4 to ADP using creatine kinase.

Another nucleoside triphosphate, uridine triphosphate (UTP), activates substances in intermediary metabolism. For example, UTP hydrolysis enables the coupling of uridine monophosphate (UMP) and glucose-1-phosphate to form uridine diphosphate (UDP)-glucose. UDP-glucose is critical for the synthesis of glycogen.

Intracellular Second-Messenger/Signaling Compounds

Phosphate as part of cyclic adenosine monophosphate (cAMP) (Figure 11.10e), which is derived from ATP, functions as a second messenger to affect cellular metabolism. cAMP, which acts within cells by activating selected protein kinases, is generated in response to the binding of certain hormones to cell receptors. Another phosphate-containing second messenger is cyclic guanosine monophosphate (cGMP), which activates protein kinases. Inositol triphosphate (IP_3 ; Figure 11.10f) also functions as a second messenger to trigger intracellular calcium release from cellular organelles. Its actions are mediated by protein kinases. Some roles of protein kinases as they function in enzyme activation are discussed next.

Table 11.7 A Summary of the Effects of Fibroblast Growth Factor (FGF) 23, Parathyroid Hormone (PTH), and Calcitriol on Phosphorus Homeostasis

	FGF23	Calcitriol	PTH
Serum phosphate	↓	↑	Unclear
Renal phosphate reabsorption	↓	↑	↓
Intestinal phosphate absorption	↓*	↑	↑

*Primarily due to effects of calcitriol

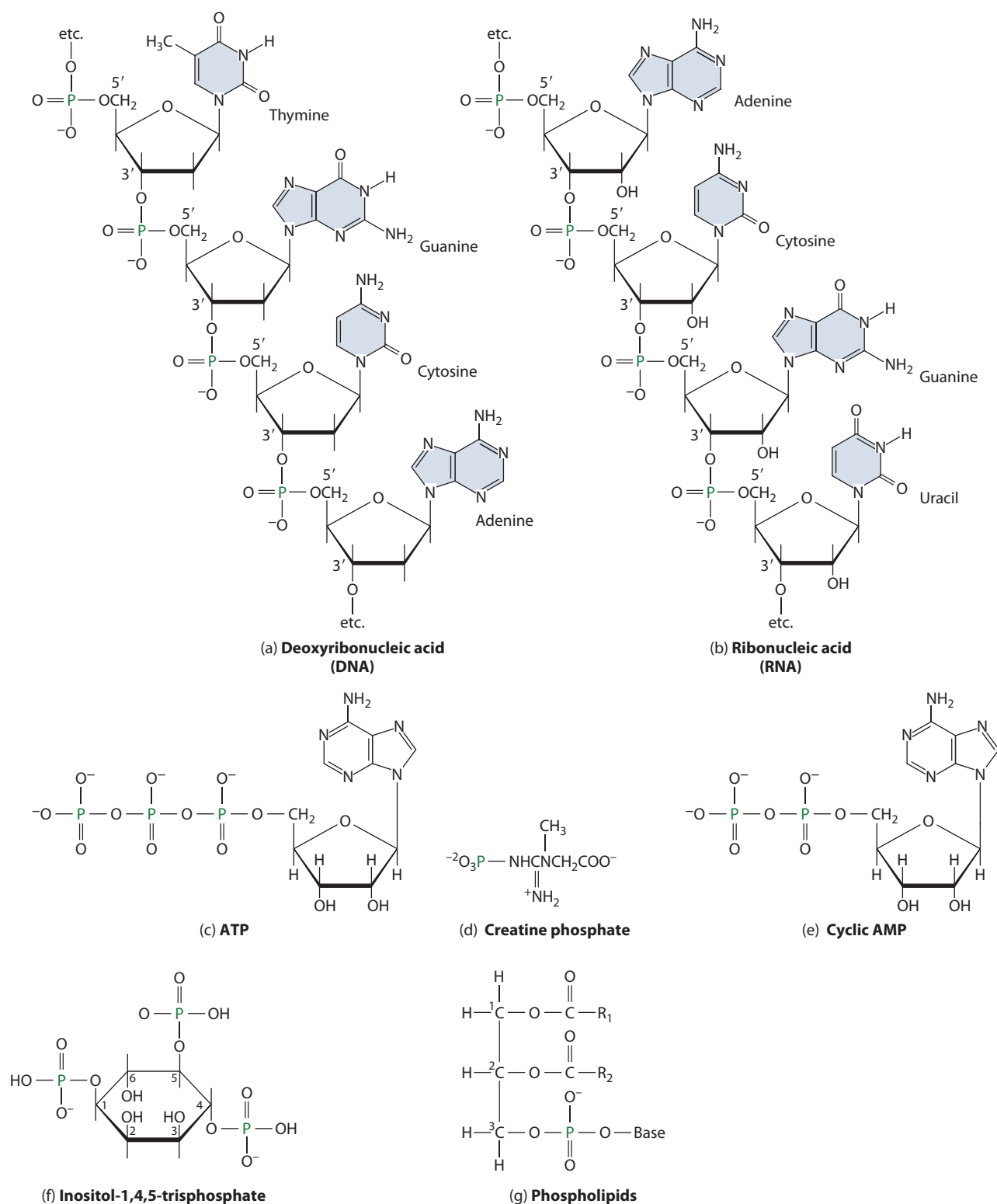


Figure 11.10 Some examples of important phosphorus-containing compounds in the body.

Phosphoproteins and Phosphorylated Forms of Vitamins

Phosphorus is also of vital importance in intermediary metabolism of the energy nutrients through the phosphorylation of different substrates in the body. Protein kinases

activated by cAMP, a phosphate-containing second messenger, phosphorylate specific target proteins within the cell, thereby changing cellular activities. Many enzymatic activities, for example, are controlled by alternating phosphorylation or dephosphorylation. An example of the role

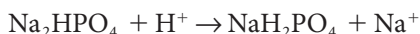
of phosphorylation and dephosphorylation of enzymes can be found in the discussion of glycogen degradation (see Chapter 3). In addition to phosphorylating proteins, phosphorus is needed for the actions of some vitamins, including thiamin and vitamin B₆. The active coenzyme forms of both of these vitamins—thiamin as thiamin diphosphate and vitamin B₆ as pyridoxal phosphate, pyridoxamine phosphate, and pyridoxine phosphate—require phosphorus.

Phospholipids

Cell membranes are made up, in part, of lipids, including phospholipids, which (as their name implies) contain phosphorus. Phospholipids, with their polar and nonpolar regions, are important to the bilayer structure of cell membranes. Each phospholipid contains a glycerol backbone with two fatty acyl chains attached at carbons 1 and 2; attached at glycerol's carbon 3 is a phosphorus-containing base (Figure 11.10g). The bases include choline (forming phosphatidylcholine), inositol (forming phosphatidylinositol), serine (forming phosphatidylserine), and ethanolamine (forming phosphatidylethanolamine). See Chapter 5 for more information on phospholipids.

Acid–Base Balance

Phosphate also functions in acid–base balance. Within cells, phosphate serves as an intracellular buffer. Within the kidneys, for example, filtered phosphate reacts with secreted hydrogen ions, releasing sodium ions in the process, as shown here:



This action removes free hydrogen ions and therefore increases pH. The following reaction also increases pH: $\text{HPO}_4^{2-} + \text{H}^+ \rightarrow \text{H}_2\text{PO}_4^-$. These reactions may be reversed to lower pH.

Oxygen Availability

Phosphate is involved indirectly in oxygen delivery. In red blood cells, the synthesis of 2,3-diphosphoglycerate (2,3-DPG), which regulates oxygen release from hemoglobin to tissues, requires phosphorus. Decreased 2,3-diphosphoglycerate associated with phosphorus deficiency can diminish release of oxygen from hemoglobin to tissues.

Excretion

Phosphate losses from the body occur via the feces and urine. Fecal losses of endogenous phosphate, in amounts usually up to about 300 mg, result from the sloughing of mucosal cells and phosphorus that is not reabsorbed from digestive juices—saliva, gastric juice, pancreatic juice, and bile. Urinary excretion is the primary means of eliminating excess phosphate and maintaining phosphate homeostasis.

Phosphate that is not bound to proteins in the blood is filtered by the glomerulus. The proximal tubule actively reabsorbs about 75–85% of this filtered phosphate; the distal convoluted tubule may reabsorb smaller amounts, up to approximately 10%, of phosphate. Up to about 15% of filtered phosphate is excreted in the urine. Urinary phosphate excretion in adults ranges from about 170 to 1,600 mg/day.

Reabsorption of filtered phosphate [$\text{Na}^+ - \text{H}_2\text{PO}_4^-$ or HPO_4^{2-}] in the proximal tubule is accomplished largely by two energy-dependent sodium-phosphate cotransporters, NaPi2a and NaPi2c, and possibly by a type 3 transporter (PiT2), which transports solely phosphate. These carriers are found on the brush border membrane of proximal tubule cells; changes (increases or decreases) in the numbers of NaPi2a and NaPi2c carriers enable corresponding changes in urinary phosphate excretion. NaPi2c appears to take hours to days to respond, whereas NaPi2a exhibits a relatively quick response to dietary or hormonal factors and is thought to play a major role in phosphate reabsorption. Transport of phosphate across the basolateral membrane for release into extracellular fluid is thought to involve other protein carriers.

Dietary intake and plasma concentrations of phosphate influence its renal handling. With low phosphorus intake and thus lower plasma phosphate concentrations, most filtered phosphate is reabsorbed. In contrast, because these NaPi carriers have a finite capacity to reabsorb phosphate (referred to as the **tubular maximum**), if the amount of phosphate filtered is greater than the tubular maximum, more phosphate will be excreted. Thus, a high phosphorus intake and corresponding higher serum phosphate concentration promotes urinary phosphate excretion.

Multiple factors, including several hormones, influence urinary phosphate levels. Reabsorption of phosphate by the kidneys is enhanced (urinary excretion reduced) by various peptides/hormones, such as insulin-like growth factor 1 (IGF-1), thyroid hormone, and growth hormone. In contrast, FGF23 and PTH promote urinary phosphate excretion by diminishing the numbers of NaPi transporters on the proximal tubule brush border membrane. The actions of PTH, however, occur within minutes whereas the actions of FGF23 take hours to days. Urinary phosphate excretion is also increased with metabolic acidosis and decreased with metabolic alkalosis.

Recommended Dietary Allowance

The RDA for phosphorus is 700 mg/day for males and females (including those who are pregnant or lactating) age 19 years and older [2]. An estimated requirement (580 mg/day) for phosphorus was determined based on the relationship between dietary phosphorus intake and plasma phosphorus concentrations as well as a known efficiency of intestinal absorption [2]. A coefficient of variation of 10% was added to the requirement and rounded

to establish this recommended intake. The inside covers of the book provide the RDAs for phosphorus for other age groups.

Deficiency

Phosphorus deficiency due to dietary inadequacy is rare. The condition usually accompanies other medical problems and is characterized biochemically by hypophosphatemia (a serum phosphorus concentration that is less than the lower end of the normal range of about 2.5 mg/dL). While individuals with moderate phosphorus deficiency, indicated by serum phosphorus concentrations between about 2 and 2.5 mg/dL, can be asymptomatic, as the deficit worsens, manifestations become apparent.

As serum phosphate concentrations drop below about 1.5 mg/dL, anorexia and confusion may occur along with muscle tissue damage (rhabdomyolysis). Bone is also impacted, especially with chronic deficits in phosphorus. Rickets occurs in infants and children and results from an inadequate mineralization of the bone matrix and growth plate (see “Deficiency” in the previous section of this chapter and the “Deficiency” subsection under “Vitamin D” in Chapter 10). In adults, phosphorus deficiency promotes osteomalacia characterized by bones that are soft due to inadequate mineralization of the bone matrix. (Typically, with osteomalacia, the proportion of the mineral content of the bone is reduced but not the protein matrix of the bone; see also “Deficiency” under “Vitamin D.”)

A severe phosphorus deficiency is manifested biochemically by serum phosphorus concentrations less than about 1.0 mg/dL. It is associated with reduced oxygen transport and delivery, reduced cardiac output, arrhythmias, decreased diaphragmatic contractility, respiratory failure, skeletal muscle and cardiac myopathy, and neurological problems (ataxia and paresthesia), as well as possible death.

Treatment of deficiency, if mild to moderate, may be corrected via diet or supplements. Increasing intake of phosphorus-rich foods is usually sufficient to correct mild phosphorus deficiencies, but supplements (usually as potassium phosphate) may be needed in situations characterized by more significant reductions in serum phosphorus concentrations. Oral phosphate supplementation, however, may be associated with diarrhea. Repletion of severe deficiency requires intravenous administration of phosphorus, usually as sodium or potassium phosphate.

At Risk for Deficiency

While phosphorus deficiency is rare, some individuals are at higher risk. Premature infants are at higher risk because of their higher needs for phosphorus and the insufficient amount found in human milk. Additionally, people who are malnourished and are being refeed enterally through a tube or parenterally (intravenously) without being given

additional phosphorus can exhibit hypophosphatemia as part of a condition called refeeding syndrome. Individuals with diabetes being treated with insulin for diabetic ketoacidosis may also exhibit hypophosphatemia (if not given added phosphorus); insulin administration promotes the uptake of both glucose and phosphate out of the blood and into cells. Critical illness and chronic alcoholic consumption may also be associated with hypophosphatemia. Situations characterized by prolonged elevations in serum PTH (secondary hyperparathyroidism such as occurs with chronic inadequacies in dietary calcium and/or vitamin D) with normal renal function may contribute to bone mineral deficits. Genetic disorders resulting in phosphorus deficiency include X-linked hypophosphatemia and hypophosphatemic rickets (also called Dent’s syndrome). These gene mutations decrease phosphate reabsorption in the kidneys and thus cause excessive urinary phosphate loss.

Toxicity

While toxicity from phosphorus is rare, a Tolerable Upper Intake Level of 4 g of phosphorus has been recommended for those age 9–70 years [2]. After age 70 years, the tolerable level drops to 3 g of phosphorus daily; this decrease is associated with an increased likelihood of impaired renal function that often occurs with aging and reduces urinary phosphate excretion [2]. For pregnant and lactating women, the Tolerable Upper Intake Levels are 3.5 g and 4 g, respectively [2].

Toxicity from phosphorus occurs most commonly in individuals with impaired renal function, especially when the glomerular filtration rate decreases below about 25 mL/minute. The resulting high serum phosphate concentration (**hyperphosphatemia**) promotes the formation and deposition of Ca-PO_4 crystals in the body’s soft tissues including subcutaneous, blood vessels, and nervous tissue. The risk of Ca-PO_4 precipitation and crystal formation increases when the calculated product of the serum calcium concentration multiplied by the serum phosphate concentration is greater than about 55 or 60 mg/dL. Other conditions increasing the risk for hyperphosphatemia include immobility, acidosis, vitamin D toxicity, and hypoparathyroidism. Chronic hyperphosphatemia (greater than about 5.5 mg/dL) associated with renal failure is treated with medications to bind dietary phosphorus and a phosphorus-restricted diet. In other cases, the underlying cause(s) of the elevated serum phosphorus concentrations must be addressed.

Hyperphosphatemia and plasma phosphate concentrations at the upper end of the normal physiologic range have been linked with cardiovascular disease, left-ventricular hypertrophy (a risk factor for heart disease), atrial fibrillation, and mortality. Several mechanisms have been theorized including effects of high serum FGF23

on the left-ventricular mass and on vascular calcification. Additional studies are needed to better delineate the mechanism(s) of action of excess phosphorus on health and whether consumption of diets providing lower phosphorus intakes have any impact on cardiovascular-related outcomes.

Assessment of Nutriture

Serum concentrations and urinary excretion of phosphorus are most often assessed to examine mineral status; however, their specificity and sensitivity are low. Serum phosphate concentrations, for example, can be maintained at the expense of tissues.

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Suggested Readings

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 Peacock M. Phosphate metabolism in health and disease. *Calcif Tissue Int*. 2020; doi: 10.1007/s00223-020-00686-3

11.3 MAGNESIUM

Of the major minerals, magnesium ranks sixth ($\text{Ca}^{2+} > \text{P} > \text{K}^+ > \text{Na}^+$ and $\text{Cl}^- > \text{Mg}^{2+}$) in overall abundance in the body, but intracellularly the cation is second only to potassium. The human body contains about 21–28 g of magnesium (close to 1% of body weight), of which approximately 50–60% is located in bone, another 39–49% in soft tissues (of which about 25% is found in skeletal muscle), and about 1% in extracellular fluids.

Sources

Magnesium is found in a wide variety of foods. Foods particularly high in magnesium include nuts, seeds, and whole-grain cereals (especially oats and barley) (Table 11.8). Food processing such as refining whole wheat, which removes the germ and outer bran layers, substantially reduces its magnesium content (by over 75%). Green leafy vegetables also provide significant amounts of magnesium; it is the chlorophyll that is found in the green leafy vegetables that contains magnesium. Legumes, lentils, spices, seafood, and dairy products also contribute to dietary magnesium. Tap water (which is “hard”) may also add small amounts of the mineral (up to about 50–60 mg magnesium/liter) to the diet (vs. soft water that is high in sodium).

Table 11.8 Magnesium Content of Selected Foods*

Food (serving)	Magnesium (mg)
Halibut, cooked (3 oz)	90
Cod, cooked (3 oz)	36
Oysters, eastern, cooked (3 oz)	30
Clams, mixed species, cooked (3 oz)	15
Legumes, cooked (½ c)	35–60
Lentils, cooked (½ c)	35
Sunflower seeds, dry roasted (¼ c)	43
Almonds, dry roasted (1 oz)	80
Cashews, dry roasted (1 oz)	74
Mixed nuts, oil roasted (1 oz)	65
Walnuts, dry roasted (1 oz)	42
Peanut butter (2 Tbsp)	50
Edamame, shelled, cooked (½ c)	50
Spinach, cooked (½ c)	78
Peas, green, cooked (½ c)	23
Broccoli, cooked (½ c)	12
Avocado, raw (½ c)	22
Banana (1)	30
Potato, baked, with skin (1)	50
Rice, white, cooked (½ c)	10
Rice, brown, cooked (½ c)	42
Oatmeal, cooked (½ c)	25
Bread, whole wheat (1 sl)	25
Milk and yogurt (1 c)	25–40
Chocolate, varying types (2 oz)	36–51
Blackstrap molasses (1 Tbsp)	43
Coffee, espresso (2 oz)	48
Cocoa, hot chocolate, instant (6 oz)	25

*FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>.

A more complete list of magnesium-containing foods from USDA Standard Reference Release 28 is also available via the National Institutes of Health, Office of Dietary Supplements at <https://ods.od.nih.gov/pubs/usdandb/Magnesium-Food.pdf>.

Another source of magnesium for some individuals may be derived from medications. The laxative Phillips’ Milk of Magnesia®, for example, contains magnesium hydroxide and provides 500 mg elemental magnesium per tablespoon. Some antacids—such as Gaviscon®, Gelusil®, Maalox®, Mylanta®, and Rolaid®—contain magnesium hydroxide and/or magnesium carbonate and provide about 50–500 mg elemental magnesium/dose. While ingestion of these medications in lower doses provides a source of magnesium, ingestion of higher doses acts as a cathartic, promoting defecation and often diarrhea, which reduces magnesium absorption (see the “Toxicity” section).

Multivitamin/mineral supplements contain elemental magnesium in amounts of about 100 mg. Single-nutrient supplements vary in magnesium content, ranging from about 25 to 300 mg of elemental magnesium/tablet. The Daily Value for magnesium, used on food and supplement

facts labels, is 420 mg. The forms of magnesium found in supplements are generally as a magnesium salt—such as magnesium sulfate (MgSO_4 , or Epsom salts), magnesium chloride (MgCl_2), and magnesium oxide (MgO), but numerous other forms are available including magnesium citrate, magnesium lactate, magnesium acetate, magnesium gluconate, magnesium glycinate, magnesium malate, magnesium thionate, and magnesium hydroxide. Absorption of magnesium from supplements varies, with absorption from magnesium oxide and magnesium sulfate generally less than that from other forms. Absorption may also be better from effervescent tablets than from capsules. To maximize absorption, magnesium supplements should not be taken at the same time as other mineral supplements, such as iron and zinc.

Single-nutrient forms of magnesium are available for oral consumption as tablets, capsules (including sustained-release forms), powders, and liquids. In addition to these, magnesium-containing oils and lotions are sold for topical use. However, while further studies are needed, the limited studies published to date do not support transdermal magnesium absorption [1].

Digestion, Absorption, and Transport

Magnesium in foods does not require digestion from food components prior to absorption. The processes by which magnesium is absorbed in the small intestine are somewhat similar to those described for calcium.

Absorption

Magnesium absorption occurs throughout the small intestine; however, the colon also absorbs magnesium, especially if disease has interfered with magnesium absorption in the small intestine. Magnesium is absorbed in the small intestine through (1) carrier-mediated transcellular transport and (2) paracellular diffusion. Carrier-mediated absorption is saturable and requires energy and transient receptor potential (TRP) melastatin divalent cation-permeable channel protein (abbreviated TRPM6). This protein is found on the brush border membrane of enterocytes and is inhibited by high cytosolic magnesium concentrations. Thus, absorption decreases with increased intracellular magnesium concentrations. This carrier system operates mostly in the distal small intestine (lower jejunum and ileum) and is responsible for up to about 30% of magnesium absorption with adequate magnesium intakes. With lower magnesium intakes, the percentage increases.

Paracellular diffusion of magnesium occurs throughout the small intestine and increases as intraluminal magnesium concentrations increase. The majority (at least 80%) of dietary magnesium is thought to be absorbed by this passive, concentration-dependent route, especially when magnesium intakes are high. The paracellular absorption of magnesium is mediated,

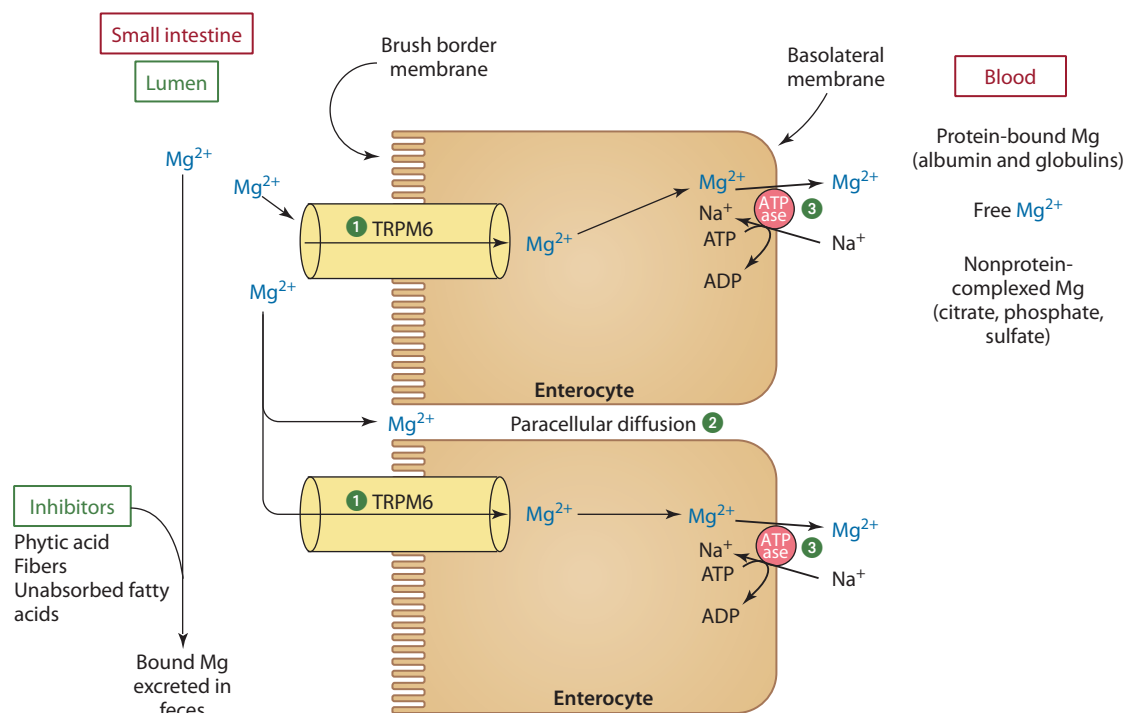
like calcium, by solvent drag or as the result of different gradients.

- Solvent drag is induced by sodium efflux (via Na^+/K^+ -ATPase), which results in hyperosmotic conditions in the paracellular space. This hyperosmotic environment induces the diffusion of water in a direction that is from the lumen toward the plasma. As the water moves, there is also the movement/“drag” of ions such as magnesium and calcium.
- A high magnesium concentration gradient between the lumen side of intestinal cells and basolateral side of intestinal cells/plasma also increases permeability between enterocytes to promote paracellular magnesium absorption.

The tight junctions through which the magnesium traverses are regulated by the presence of several transmembrane proteins. Calcitriol may enhance paracellular magnesium absorption by increasing the expression of genes that code for transmembrane proteins called claudins. Claudin-2, for example, forms charged selective pores that facilitate permeability and thus paracellular intestinal absorption of magnesium, as well as calcium, and other solutes.

Overall, about 25–75% of dietary magnesium is typically absorbed from the intestine. Absorption declines to less than 30% as magnesium intake increases above about 550 mg. Conversely, magnesium absorption may increase above 60% (up to ~75%) when magnesium intake is low (less than about 40 mg). Efflux of magnesium from cells is thought to occur by a $\text{Na}^+/\text{Mg}^{2+}$ antiport system that depends on a Na^+/K^+ -ATPase to sustain the sodium gradient. Figure 11.11 illustrates magnesium absorption and transport.

Factors Influencing Absorption Magnesium absorption is influenced by several dietary factors (Table 11.9). Vitamin D, in pharmacological doses, and protein, in some but not all studies, may increase magnesium absorption and/or its retention. Carbohydrates, such as fructose and oligosaccharides, may also increase magnesium absorption to a small extent. In contrast, absorption is decreased in the presence of high quantities of unabsorbed fatty acids, which bind to magnesium to form soaps. These magnesium–fatty acid soaps are excreted in the feces. Minerals such as phosphorus may also bind to magnesium and form a complex, $\text{Mg}_3(\text{PO}_4)_2$, within the gastrointestinal tract to render each other unavailable for absorption. The inhibition is most apparent when concentrations of magnesium are low and those of phosphorus are high. (The opposite is also true, as discussed in the section “Interactions with Other Nutrients.” Additionally, high dietary intakes of phytic acid and some dietary fibers may reduce magnesium absorption, but these effects are thought to be minimal with usual intakes in the United States.



- 1 Mg^{2+} crosses the brush border membrane of the enterocyte through a magnesium channel, TRPM6.
- 2 Mg^{2+} also may be absorbed between cells; this transport is influenced by the electron chemical gradient and solvent drag.
- 3 Mg^{2+} is pumped out of the cell across the basolateral membrane by a sodium-dependent ATPase.

Figure 11.11 Magnesium absorption and transport.

Table 11.9 Substances/Nutrients Affecting Intestinal Magnesium Absorption

Substances Enhancing Absorption	Substances Inhibiting Absorption
Vitamin D	Phytic acid
Protein	Fiber
Carbohydrates—fructose and oligosaccharides	Unabsorbed fatty acids

Transport

In the plasma/serum, most magnesium (50–60%) is found free in its ionic form as Mg^{2+} , about 20–30% is bound to protein (mostly albumin with lesser amounts bound to globulins), and about 5–15% is complexed with citrate, phosphate, sulfate, or other negatively charged anions. Like calcium, it is ionized magnesium that is physiologically active and bound magnesium is released as ionized magnesium is taken up by cells/tissues for use. Also, like calcium, plasma magnesium concentrations are maintained at the expense of bone, which provides an exchangeable magnesium pool that is able to maintain plasma concentrations. The normal reference range for plasma magnesium is about 1.8–2.3 mg/dL. However, while this reference range is frequently used in clinical practice to guide nutrition support, what is considered “normal” may actually be “low.”

Regulation and Homeostasis

Maintenance of body magnesium depends on gastrointestinal absorption and renal excretion; however, it is primarily the kidneys that control (increase or decrease) urinary losses of magnesium to maintain homeostasis. While the exact regulatory mechanisms are unclear, fluctuations in plasma magnesium concentrations and hormones, such as PTH, influence the process. More specifically, PTH increases intestinal magnesium absorption, diminishes urinary magnesium excretion (promoting magnesium reabsorption in the loop of Henle and distal convoluted tubule), and enhances bone magnesium resorption, thereby raising body and plasma magnesium concentrations. Higher serum magnesium concentrations suppress PTH secretion, whereas lower concentrations enhance PTH secretion. Increased PTH concentrations have similar effects on serum calcium, while on phosphorus only increases in intestinal absorption occur.

Free intracellular concentrations of magnesium are also rigidly maintained—at about 0.2–1.0 mmol/L—by altering membrane uptake, intracellular storage in organelles, and cellular flux. Most intracellular magnesium is bound to nucleic acids, ATP, proteins, and phospholipids. Several magnesium

transporters assist in maintaining intracellular concentrations of the mineral; some of these transporters include TRPM7 (in the heart, adipose tissue, and bone), TRPM6 (in kidneys and intestine), MagT1 (in epithelial cells) and MgT2, NIPA Mg^{2+} transporter, and SLC41 $\text{Na}^+/\text{Mg}^{2+}$ exchanger. MgT1 and 2 appear to control magnesium transport within some cellular organelles. TRPM7 is an active ion channel that transports both magnesium and calcium; it is regulated by intracellular magnesium concentrations. TRPM6 regulates magnesium transport in the small intestine and kidneys with effects on total body magnesium concentrations. The $\text{Na}^+/\text{Mg}^{2+}$ exchanger, which is ubiquitously expressed, is responsible for cellular magnesium efflux.

Functions and Mechanisms of Action

Magnesium plays a wide range of roles in the body, including functions in bone mineralization, enzymatic reactions, oxidative phosphorylation, nucleic acid synthesis, platelet activity, hormone receptor binding and signal transmission, cell membrane ion transfer, and calcium regulation, among others. Within cells, magnesium is thus associated with phospholipids as part of cell membranes (plasma, endoplasmic reticulum, and mitochondrial), as well as with proteins and nucleic acids. A few of these roles are discussed hereafter.

Bone Mineralization

Most (about 50–60%) magnesium in the body is found in bone. Although the precise roles are unclear, magnesium is thought to induce hydroxyapatite crystal formation and stabilize formed crystals. About 30% of bone's magnesium is found on the surface and serves as a reservoir to maintain plasma magnesium concentrations. Amorphous forms of magnesium associated with bone, although a subject of debate, may include, for example, $\text{Mg}(\text{OH})_2$ and $\text{Mg}_3(\text{PO}_4)_2$.

Enzymatic Reactions

Magnesium within cells is associated with numerous proteins including enzymes. In fact, magnesium is required for over 300 different enzymatic reactions either as a structural cofactor to stabilize the enzyme or as an allosteric activator of enzyme activity. Additionally, intracellular magnesium is found linked to the oxygen atoms of the phosphate groups of ATP and ADP, and assists in the transfer of a phosphate group. Figure 11.12 depicts magnesium as a ligand for the phosphate groups. Protein kinases transfer the γ -phosphate of magnesium ATP to a substrate. Listed hereafter are some of magnesium's enzymatic functions, including roles in glucose, fat, protein, vitamin, and nucleic acid metabolism:

- Glycolysis: hexokinase, glucokinase, and phosphofructokinase
- TCA cycle: oxidative decarboxylation
- Pentose phosphate pathway (hexose monophosphate shunt): transketolase reaction

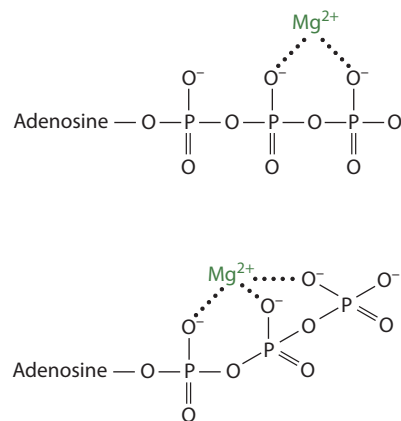


Figure 11.12 Modes by which Mg^{2+} provides stability to ATP.

- Creatine phosphate formation: creatine kinase
- β -Oxidation: initiation by thiokinase (acyl-CoA synthetase)
- Activities of alkaline phosphatase and pyrophosphatase
- Amino acid activation and protein synthesis (e.g., with ribosomal aggregation and binding messenger RNA to ribosome subunits)
- Hydroxylation of vitamin D in the 25-position; this hydroxylation is the first of two reactions needed to produce the active form of vitamin D
- Pyrimidine and purine synthesis (for DNA and RNA synthesis)
- DNA replication/synthesis and degradation, as well as the physical integrity of the DNA helix and conformation of nucleic acids
- RNA transcription.

Other Roles

Magnesium also affects several other physiological processes, including:

- Blood clotting, reducing platelet aggregation and thus thrombosis formation
- Hormone receptor binding and activation of second-messenger signaling. Magnesium bound to nucleotides such as GTP, following activation of membrane receptors, is involved in the activation of adenylate cyclase, which leads to cAMP production and its second-messenger intracellular signaling effects. Magnesium thus mediates, in part, the effects of numerous hormones, including parathyroid hormone.
- Ion channel regulation, especially potassium and calcium channels, where intracellular magnesium plays a role in the flux of these ions across cell membranes to affect cardiac and smooth muscle contractility, normal heart rhythm, nerve impulse conduction and neuromuscular conduction, vasomotor tone, and normal blood pressure

- Antagonism of intracellular calcium, whereby following cell stimulation, magnesium inhibits the calcium release (from its intracellular stores in organelles) and stimulates calcium reuptake (by Ca^{2+} -ATPase pump into the sarcoplasmic reticulum) to decrease cytosolic Ca^{2+} concentrations. Magnesium may mimic or displace calcium from calcium-binding sites or compete with calcium for nonspecific binding sites to inhibit skeletal and smooth muscle contraction.
- Insulin production, release, and action/signaling, with magnesium promoting cellular glucose uptake and utilization.

Interactions with Other Nutrients

Magnesium interacts with phosphorus and inhibits its absorption. As magnesium intake increases, phosphorus absorption decreases. The two minerals are thought to precipitate as $\text{Mg}_3(\text{PO}_4)_2$. Ingestion of large doses of magnesium (600 mg), for example, reduce phosphorus absorption by almost 50%.

An interrelationship also exists between magnesium and potassium. Magnesium influences the balance between extracellular and intracellular potassium, but its mechanism of action is unclear. Magnesium depletion is associated with increased potassium efflux from cells (i.e., cellular potassium depletion) and subsequent renal potassium excretion. When magnesium and potassium deficiencies coexist, as may occur with some diuretic drug therapies, magnesium infusions—but not potassium infusions—normalize cell potassium.

Excretion

Magnesium is excreted from the body via the feces, sweat, and kidneys, with the kidneys serving as the primary means of elimination of excess body magnesium. Fecal magnesium concentrations represent unabsorbed magnesium and a small amount of endogenous magnesium. About 25–50 mg of magnesium from endogenous sources is usually excreted daily in the feces [2]. Magnesium is lost from the skin into sweat in amounts estimated at up to approximately 15 mg/day [2].

Of the filtered magnesium (which includes magnesium in the blood that is not bound to proteins) going through the kidneys, about 95–97% is reabsorbed and 3–5% is excreted in the urine. Urinary magnesium excretion ranges from about 80 to 185 mg/day. Of the reabsorbed magnesium, about 10–30% is reabsorbed by passive (paracellular) diffusion in the proximal convoluted tubule, 40–60% is reabsorbed in the thick ascending limb of the loop of Henle likely by passive (paracellular) diffusion, and the remaining 5–10% is reabsorbed actively in the distal convoluted tubule. The active reabsorption of magnesium in the distal convoluted tubule requires the presence of the

transporter TRPM6 on the brush border membrane of the tubular cell. Extrusion of the magnesium across the basolateral membrane requires a $\text{Mg}^{2+}/\text{Na}^+$ exchanger.

Renal excretion of magnesium is affected by dietary magnesium intakes and plasma concentrations. For example, lower urinary magnesium (< 80 mg/day) occurs with lower plasma magnesium and low magnesium intake (< 250 mg/day); urinary magnesium rises to about 80–160 mg/day with higher plasma magnesium concentrations and dietary magnesium intakes > 250 mg/day [3]. These changes in renal magnesium excretion are mediated by multiple mechanisms. For example, in the thick ascending limb of the loop of Henle and distal convoluted, sensing receptors (that are present in the basolateral membrane) respond to serum magnesium concentrations by altering tight junction magnesium permeability. The alterations occur via changes in the expression of several membrane proteins, including various claudins, that affect the tight junctions. Mutations in the genes for several membrane proteins, such as claudin-16, have been shown to contribute to the development of magnesium deficiency. Activation of these sensors, however, also brings about other changes such as reducing the kidney's response to PTH (which normally increases magnesium reabsorption) to increase urinary magnesium. Generally, high serum magnesium concentrations suppress ion movement through the paracellular channels to reduce reabsorption and reestablish homeostasis.

Recommended Dietary Allowance

The RDA for magnesium varies in adults; among those 19–30 years of age, males need 400 mg and females need 310 mg magnesium per day, and among those 31 years and older, males need 420 mg and females need 320 mg magnesium daily [2]. Pregnant women age 19–30 years should ingest 350 mg daily, and those age 31–50 years should consume 360 mg of magnesium [2]. During lactation, women age 19–30 years should ingest 310 mg daily, and those age 31–50 years should consume 320 mg of magnesium [2]. The inside covers of the book provide the RDAs for magnesium for other age groups.

Deficiency

Magnesium deficiency, which is traditionally diagnosed based on total plasma/serum magnesium concentrations, is relatively common among hospitalized individuals. It may also be underestimated or the diagnosis delayed among some within the general population due to the use of an elevated serum reference range, the inability to detect small reductions in tissue magnesium, and the lack of symptoms (i.e., asymptomatic) in the initial stages. Presenting signs and symptoms, if present as plasma magnesium concentrations begin to decline below normal, are

usually vague and include fatigue, lethargy, and muscle weakness along with anorexia, nausea, and vomiting.

Metabolic effects occurring with hypomagnesemia can include decreased serum concentrations of PTH, calcium, potassium, and calcitriol. The hypocalcemia likely results from reduced PTH levels and inhibited cellular calcium release caused by the hypomagnesemia. The **hypokalemia** (low blood potassium) likely results from altered cellular transport systems (such as impaired Na^+/K^+ -ATPase) that maintain the potassium gradient and from increased urinary potassium losses. Calcitriol synthesis may be altered by decreases in PTH secretion and/or renal resistance to PTH.

Symptoms of a magnesium deficiency become more pronounced as plasma concentrations decrease below about 1.2 mg/dL; these symptoms are also more likely if the decrease occurs more rapidly versus declining gradually. Hypomagnesemia (and accompanying drops in serum potassium and calcium concentrations) alter nerve conduction and promote a state of neuromuscular hyperexcitability.

- Neuromuscular symptoms include muscle weakness, tremors, muscle fasciculations (brief spontaneous contractions of a small number of muscle fibers), paresthesia, hyperreflexia, and myoclonic jerks.
- Some cardiovascular symptoms associated with magnesium deficiency (and accompanying drops in serum potassium and calcium concentrations) include EKG changes, vasospasms, higher blood pressure, and arrhythmias—rapid heart rate (tachycardia; ventricular tachycardia), skipped heartbeats, or irregular heartbeat (fibrillation; ventricular fibrillation, premature ventricular beats).
- Some central nervous system symptoms are headache, nystagmus (rapid involuntary eye movement), irritability, restlessness, disorientation, mental confusion, personality changes, psychosis, hallucinations, and seizures.

Treatment of deficiency depends on the severity of depletion and the underlying cause(s). Correction of mild deficits characterized by plasma magnesium concentrations between about 1.1 and 1.4 mg/dL is usually accomplished with increased intake of magnesium-rich foods, but supplements in amounts ranging from about 300 to 600 mg/day may also be needed. To minimize the risk of diarrhea that can occur with oral supplementation, the total daily magnesium dose being provided as a supplement should be divided into at least three smaller quantities to be taken throughout the day (e.g., one 300 mg magnesium per day dose can be divided into three 100 mg doses). Intravenous provision of magnesium (as magnesium sulfate or chloride) is usually needed if plasma concentrations decrease below about 1.0 mg/dL, but may also be needed due to the gastrointestinal side effects associated with oral supplementation. Corrections of hypokalemia and hypocalcemia may also be needed following repletion with magnesium.

At Risk for Deficiency

Several factors can contribute to the development of a magnesium deficiency. First is inadequate intake, which is common among most adults in the United States. Excess alcohol use is also associated with inadequate magnesium intake and increased urinary magnesium excretion. Deficiency is also more likely to occur secondary to other conditions such as malabsorptive disorders (bariatric surgery, ileostomy, Crohn's disease, pancreatic insufficiency, and liver failure, among others), which reduce magnesium absorption and/or promote increased magnesium losses through vomiting or diarrhea/steatorrhea.

Many medications promote hypomagnesemia such as cyclosporine and amphotericin B, as well as proton pump inhibitors. Chronic loop and thiazide diuretic use increases urinary magnesium losses; increased renal magnesium excretion also results with the use of some chemotherapy drugs like cisplatin.

Parathyroid and thyroid gland conditions also alter magnesium. Burns may result in excessive dermal magnesium losses. Intracellular shifts in the mineral, such as occurs with refeeding syndrome, myocardial infarction, and diabetic ketoacidosis, among other conditions, also affect plasma concentrations of magnesium. Additionally, uncontrolled diabetes and metabolic syndrome along with excessive alcohol consumption increase urinary magnesium excretion. In individuals with uncontrolled diabetes, urinary magnesium increases secondary to the osmotic effects induced by high urinary glucose concentrations.

Magnesium Intake and Disease Risk

Chronic latent (without symptoms) magnesium deficiency, low serum magnesium, and/or low magnesium intakes have been associated with a number of conditions, including hypertension, cardiac arrhythmias, cardiovascular disease, type 2 diabetes, and migraines, among others. Typically, individuals in the highest quartiles for dietary magnesium intake and/or plasma magnesium concentration (within the normal physiologic range) exhibit lower disease risk than those in the lowest quartile. Yet, while these associations are not unexpected given the expansive roles of magnesium in the body, systematic reviews and meta-analyses examining the effects of supplementation trials with magnesium in varying amounts in individuals with these conditions have not consistently shown significant reductions in disease-associated outcome measures. Consequently, in the absence of a magnesium deficiency, magnesium supplementation has not been generally recommended in the management or treatment of hypertension, cardiac arrhythmias, cardiovascular disease, and type 2 diabetes. Magnesium (in amounts of usually 600 mg/day as magnesium citrate) has been suggested as being “probably effective” (levels B and C) in the prophylaxis of

migraines [4]. Consumption of magnesium-rich foods is deemed a healthful approach to help reduce disease risk and meet the nutrient's RDA. An exception is the use of grams doses (>1 g) of magnesium (given intravenously) in the treatment of preeclampsia and eclampsia.

Toxicity

A Tolerable Upper Intake Level of 350 mg magnesium from *nonfood sources* has been recommended for people age 9 years and older (including during pregnancy and lactation) [2]. An excessive intake of magnesium from food sources has not been shown to cause toxicity in healthy individuals since the kidneys can normally excrete magnesium fairly rapidly (thus preventing significant increases in plasma concentrations). However, consumption of excessive magnesium from nonfood sources, including supplements and some medications (such as some laxatives and antacids), can produce side effects. Individuals most at-risk of magnesium toxicity are those with impaired renal function.

Magnesium ingestion in doses above the Tolerable Upper Intake Level, such as from 3–5 g of magnesium salts ($\text{Mg}(\text{OH})_2$ and MgSO_4), results in a cathartic effect, characterized predominantly by diarrhea, and sometimes along with nausea, abdominal cramping, and possible dehydration. This property of magnesium is utilized in some laxative medications which include relatively high amounts of magnesium. However, the ingestion of large doses (in excess of recommendations) of some magnesium-containing antacids can produce similar effects.

Acute magnesium toxicity results in high serum/plasma magnesium concentrations (hypermagnesemia) and can cause (even with lower doses) nausea and vomiting. Ingestion of magnesium (or intravenous administration) resulting in plasma magnesium concentrations of about 4.9–7.3 mg/dL are associated with neuromuscular effects (including diminished and eventual loss of deep tendon reflexes), along with cardiopulmonary systems effects (including hypotension, apnea, and EKG changes). Manifestations appearing with plasma magnesium concentrations of about 9–12 mg/dL include flushing, double vision, muscle weakness, and slurred speech. Muscular paralysis and cardiac and/or respiratory failure (death) are likely if plasma magnesium concentrations exceed about 15 mg/dL. Intravenous administration of calcium chloride or gluconate and dialysis in those with renal failure or diuretics in those with adequate renal function may be employed to help reduce the hypermagnesemia.

Assessment of Nutriture

Assessment of magnesium status is difficult because extracellular magnesium represents only about 1% of total body magnesium and appears to be regulated. Despite

low sensitivity and specificity (e.g., normal plasma/serum levels may persist despite severe intracellular deficits), plasma/serum magnesium concentrations continue to be routinely measured to assess magnesium status, with “below-normal” plasma/serum magnesium concentrations ($<$ about 1.8 mg/dL) suggesting intracellular magnesium deficits. However, because what is considered “normal” using the serum/plasma reference range may actually be “low,” magnesium deficits may exist in those with plasma magnesium concentrations closer to the upper end of the normal range. Erythrocyte (rbc) magnesium concentrations are higher and decrease more slowly than plasma/serum concentrations with magnesium deficiency; whether or not they provide a better indication of body stores than plasma is debatable. Normal magnesium concentrations in red blood cells range from about 4.2 to 6.8 mg/dL, although, as with plasma, the upper end of the range ($>$ 6.0 mg/dL) may better represent “normal” and concentrations $<$ 6.0 mg/dL may be low [5]. Use of a serum magnesium to calcium quotient to assess magnesium status is under study. The quotient is thought to be a more sensitive indicator of magnesium status and/or turnover [5,6]

Measurement of renal magnesium excretion alone or as part of a magnesium retention test (loading test) is also sometimes used to assess subclinical magnesium deficiency. Twenty-four-hour urinary magnesium excretion values $<$ 80 mg are indicative of deficiency. For the load test, renal magnesium excretion is measured before and after the administration of the magnesium load (given orally or intravenously) to examine changes in excretion. Normally, greater than about 80% of an intravenous magnesium load is excreted within 24 hours; however, with deficiency, more is retained and less is excreted. When given intravenously, retention of $>$ 27% of the magnesium load is considered indicative of deficiency.

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SUMMARY

Calcium, phosphorus, and magnesium are three of the six minerals that are considered the body's "major" minerals. They share many similarities in terms of food sources, absorption, transport, functions, and excretion.

- Calcium and phosphorus are both found in relatively high amounts in dairy products.
- Calcium and magnesium are both absorbed using similar mechanisms, and absorption is influenced by similar dietary components. These minerals also interact to inhibit each other's absorption when present in excessive or imbalanced concentrations.
- Calcium and magnesium transport in the blood occurs in both free and bound forms, and serum concentrations of all three minerals along with overall balance within the body are regulated to varying degrees by some similar hormones.
- All three minerals are vital for bone mineralization, although calcium and magnesium have many added roles that impact a wide range of body and cellular processes such as muscle and nerve function. Phosphorus contributes further as part of DNA, RNA, phospholipids, energy storage and transfer compounds, and active forms of some B-vitamins and second-messenger compounds, to name a few.
- The kidneys primarily serve to regulate the balance of all three minerals, with imbalances apparent in those especially with chronic renal failure.
- Deficiencies of all three nutrients negatively impact bone structure and/or function (among other body processes), although dietary inadequacies and risk of deficits are more common with calcium and magnesium than phosphorus.
- Tolerable Upper Intake Levels are established for all three nutrients, although for magnesium the recommended level is based on intake from supplements.

Perspective

OSTEOPOROSIS AND DIET

One in every two women and one in every four men over the age of 50 years in the United States will suffer a fracture because of osteoporosis sometime during their lives [1]. Of the fractures occurring in women, most (over 70%) occur in those 65 years and older. Treating the fracture, however, does not necessarily restore health. About 24% of people 50 years of age and older with hip fractures attributable to osteoporosis die within 1 year of sustaining the fracture [1]. About 15% of people who have had an osteoporosis-induced hip fracture cannot walk unassisted across a room 6 months post-fracture. Another 25% move into nursing homes [1]. An estimated 54 million Americans (over 80% of whom are women) age 50 years and older have osteoporosis or low bone mass, and this number is expected to reach 64.4 million by 2030 [1]. About 9 million osteoporosis-induced fractures occur each year worldwide [1].

Osteoporosis is a systemic skeletal disease characterized by decreased bone strength associated with the deterioration of the microarchitecture of bone tissue (bone quality) and low bone mineral density, as shown in Figure 1. In osteoporosis (as in osteopenia, which occurs prior to the development of osteoporosis), the ratio of the bone's mineral content to the bone's protein matrix content is within the normal range but reduced; this is in contrast to osteomalacia (associated with mainly vitamin D deficiency) in which the proportion of the mineral in bone is reduced in relation to the protein matrix in bone. Osteoporosis results in brittle, porous, fragile bones at

increased risk for fracture. Fragility fractures are fractures that occur in the absence of major trauma or from injury that would not normally be sufficient to cause fractures in normal bones. They usually affect the spine, ribs, hip, pelvis, and/or wrist.

Bone turnover occurs throughout life. Yet, after about age 30–35 years, bone resorption (breakdown) exceeds bone formation. This bone resorption, including mineral loss, occurs at a rate of up to ~10% per decade in both men and women. However, during the first 5–10 years after menopause, the rate of loss accelerates considerably in women. Women, for example, may lose nearly 200 mg of calcium per day in the first several years after the start of menopause [2]. The menopause-related decline in estrogen production and the generally smaller body and bone mass of women contributes to the higher prevalence of osteoporosis in women than in men.

Osteoporosis affects both cortical and trabecular bone. Trabecular bone, however, which has a higher turnover rate (~25%/year), is affected to a greater extent than is cortical bone (with a 4%/year turnover rate). Cortical (or compact) bone is found mostly in the shaft/diaphysis of long bones of the limbs but also on the outer walls of all bones. Trabecular (or cancellous) bone is the honeycomb or lattice-type bone found in the vertebrae of the spine, the pelvis (hip area), and the ends of long bones (such as at the wrist). Thus, sites containing trabecular bone—the vertebral bodies (~95% trabecular bone), the femoral neck in the pelvis (~45% trabecular bone), and the distal radius and proximal humerus (~5% trabecular bone)—are the principal sites affected with osteoporosis (Figure 2). In addition, teeth may become loose or fall out because of loss of some trabecular bone in the jaw.

Osteoporosis that affects the vertebrae is associated with loss in height, vertebral pain, and rounding of the shoulders (**kyphosis**, a hunchback-type curvature of the spine, also called **dowager's hump**). The kyphosis in turn reduces the space in the chest and abdominal cavity, resulting in decreased lung capacity and thus shortness of breath, abdominal pain, reduced appetite, and premature satiety. Osteoporosis-induced vertebral fractures as well as hip fractures also typically limit activities of daily living and mobility. The limited mobility may be associated with excessive bedrest, which further weakens muscles and bones, and predisposes the person to falling and suffering further fractures. Excessive bedrest also increases the person's risk for pressure sores, also called decubitus ulcers and blood clots.

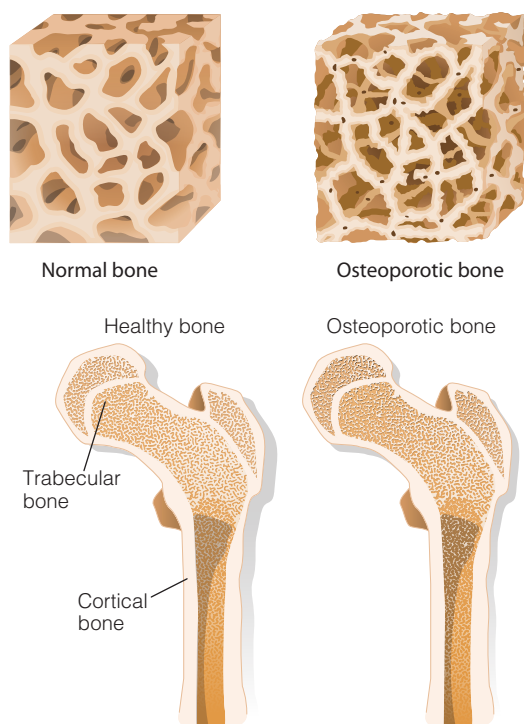


Figure 1 Normal bone/osteoporotic bone.

DIAGNOSIS AND RISK ASSESSMENT

The diagnosis of osteoporosis is based on measurement of bone mineral density, primarily by dual-energy X-ray absorptiometry (DXA or DEXA). DEXA scans use X-rays at two energy levels to assess bone mineral content and bone area; bone mineral density is then calculated using software by dividing bone mineral content by bone area in various regions of interest. Peripheral DEXA scans typically measure the extremities, including the heel, wrist, or finger. Axial (central) DEXA scans focus on the spine/vertebrae and hip. Bone mineral density thus represents the average concentration of minerals per unit area, or how tightly the bone mineral tissue is compressed in a given area (usually the lumbar spine, femoral neck, or proximal femur). It does not, however, account for bone thickness, which is often greater in individuals of higher body weight. (Quantitative computed tomography, which assesses bone density in three dimensions, may provide a better approach.)

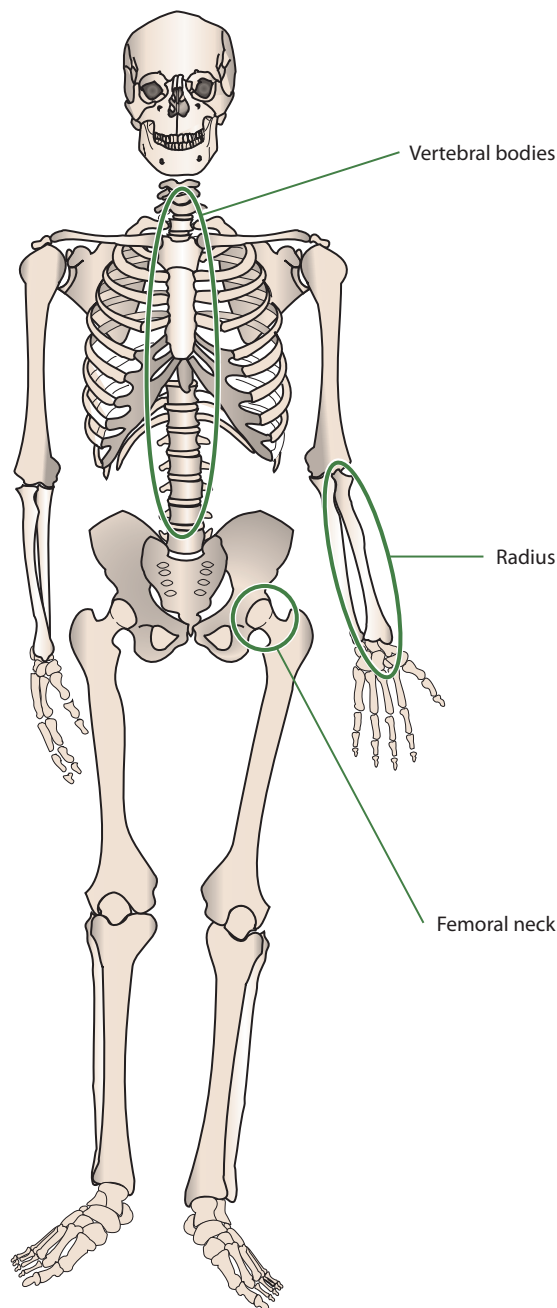


Figure 2 Major sites affected by osteoporosis.

Bone density is reported for comparison purposes as a *T* score or *Z* score; both represent units of standard deviation. *T* scores represent the number of standard deviations away from the mean bone density of gender- and race-matched young (usually 25- to 45-year-old) adults. Data from young adults gathered as part of the National Health and Nutrition Examination Survey (NHANES) III are commonly used as the reference population. A *T* score equal to 0 (to -0.99) means that the person's bone mineral density is at the mean for young adults (considered to represent peak bone mass) of the same gender and race.

- Osteoporosis is diagnosed with a *T* score greater than 2.5 standard deviations below the bone mineral density of young adults. Alternately, osteoporosis may be diagnosed with the presence of a fragility fracture or a vertebral compression fracture.

- Osteopenia is diagnosed with a *T* score of -1 to -2.49 .

For every 1 standard deviation below the mean, the risk of fracture doubles. Thus, those with osteoporosis are at considerable risk for fracture, and those with osteopenia are at risk for fracture as well as for the development of osteoporosis. *Z* scores are similar to *T* scores but represent the number of standard deviations from the mean bone density of age-matched as well as gender- and race-matched people. Thus, for older people, *T* scores will likely be lower than *Z* scores. *Z* scores are used mostly in children and adolescents.

If bone mineral density information cannot be attained, markers of bone formation and turnover/resorption may be of some prognostic value for fractures [3]. Markers of bone formation typically used include serum procollagen type 1 N propeptide, and those of bone turnover/resorption include serum C-terminal cross-linking telopeptide of type I collagen and urinary concentrations of bone collagen by-products such as hydroxyproline, N-telopeptide, pyridinoline, and deoxypyridinoline.

The Fracture Risk Assessment Tool (FRAX), developed by the World Health Organization, is a risk assessment tool that is used to examine an individual's risk for fracture. Factors considered as part of the tool include age, gender, height, weight, previous fracture, family history of (parent) hip fracture, use of glucocorticoids, rheumatoid arthritis, secondary osteoporosis, and lifestyle factors including current smoking and alcohol consumption of three or more units/day, with or without femoral neck bone mineral density. FRAX estimates 10-year probability of hip and major osteoporotic fractures using a computer-based algorithm. The FRAX assessment, however, does not account for some factors where there may be a dose-response (higher exposure, higher risk) such as the extent/dose of glucocorticoid used or cigarettes smoked or alcohol consumed, which may modify overall risk.

RISK FACTORS (NOT RELATED TO DIET)

Individuals most at risk of low bone mineral density and osteoporosis are *female*, have a *family history of the disorder (genetics)*, are *Caucasian or Asian*, have a *small frame size or low body mass index* ($< 20 \text{ kg/m}^2$), have an *early menopause* or periods of *estrogen deficiency*, and are of *advanced age* (> 65 years). People using selected *medications* such as glucocorticoids/corticosteroids (use > 3 months), thyroid hormones (taken in excess), and antiepileptic drugs are also at increased risk for bone loss and thus osteoporosis. In addition, inadequate weight-bearing physical activity/sedentary lifestyle along with some diet-related factors negatively impact bone mineral density and increase the risk for the development of osteoporosis.

DIET-RELATED RISK FACTORS

The primary nutritional factors affecting bone mineral density and fracture risk, and thus osteoporosis, are inadequate intakes of calcium and vitamin D. Other nutrients and dietary components that may play lesser roles include sodium, protein, acid-load, and potassium, as well as caffeine and alcohol.

Calcium and Vitamin D

Consumption of both calcium and vitamin D in adequate amounts is critical for the attainment of the full genetic expression of peak skeletal mass that occurs sometime in early adulthood. Moreover, it is the attainment of dense bones during these early years that

can serve in later years to possibly modulate bone loss and provide better protection against the development of weakened, osteoporotic bones. Habitual (years) inadequate dietary calcium intakes are associated with reductions in skeletal mass and contribute to osteoporotic fractures. The effects of calcium supplementation on bone mineral density in adults are less clear, with study findings differing by factors such as assessed skeletal site, usual/habitual calcium intake, and in women (often the main group under study) duration of menopause [4].

Many (but not all) studies have found that supplementation with calcium alone (usually 1,000–1,200 mg) or in combination with vitamin D (usually 10–25 μg / 400–1,000 IU) reduces the loss of bone mineral density, especially in the spine and sometimes in the hip, in postmenopausal women. Calcium supplementation provided to women during the first 5 years after menopause, however, does not always produce similar results, especially in preventing trabecular bone loss. Calcium supplementation generally appears to be more effective in maintaining bone density/reducing bone loss when provided to postmenopausal women who regularly consume inadequate dietary calcium. Yet, the duration of this preservation/maintenance of bone mineral density by supplementation is another area of uncertainty. Moreover, while supplementation may reduce bone loss in some individuals at some sites, significant reductions in fracture risk have not been consistently demonstrated, and the effectiveness of supplementation in fracture prevention remains controversial. Benefits in reducing fracture risk, shown in limited studies, include those providing supplemental calcium (total intake 1,200 mg or more) in combination with vitamin D (total intake 20 μg /800 IU or more) versus calcium alone. Furthermore, significant findings were primarily reported in analyses that considered compliance/adherence with supplement use and baseline/usual intake of the nutrients [4].

The U.S. Preventive Services Task Force (USPSTF) concluded that evidence is *insufficient* to recommend calcium and/or vitamin D supplementation for the prevention of fractures in community-dwelling asymptomatic men and postmenopausal women without a history of osteoporotic fractures, a diagnosis of osteoporosis or vitamin D deficiency, or increased risk for falls [5]. Conclusions from an expert consensus meeting of the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases and the International Osteoporosis Foundation include:

- Calcium supplementation alone, without vitamin D, for fracture risk reduction is not supported by the scientific literature [6]
- Calcium and vitamin D supplementation results in modest fracture risk reductions in some sites; however, routine supplementation as a population health strategy is not strongly supported [6]
- Calcium and vitamin D supplementation is generally appropriate for individuals with high risk of calcium and vitamin D insufficiencies [6].

Thus, individuals thought to most benefit (i.e., exhibit greater reductions in bone loss and fracture risk) from increased calcium and vitamin D intakes (by foods and/or supplements) are those with inadequate (low) intakes or with deficiency [5].

The National Academy of Medicine (formerly the Institute of Medicine) recommends a daily calcium intake of 1,000 mg/day for men age 19–70 years and women age 19–50 years, and a calcium intake of 1,200 mg/day for men age 71 and older and women age 51 years and older [7]. Recommendations for vitamin D intake are 15 μg for adults through age 70 years, and 20 μg /day for adults 70 years and older. The recommendations from the National Osteoporosis Foundation for calcium mirror those from the National Academy of Medicine, and for vitamin D suggest 20–25 μg (800–1,000 IU) for adults 50 years and older [8]. These recommendations will likely change as the gaps in current knowledge are filled with additional intervention studies.

Sodium

Increased dietary sodium intake in excess of needs increases urinary sodium excretion. Excretion of an excess of sodium is associated with increases in urinary calcium excretion mainly when calcium intakes are low. Excretion of excess sodium via the urine in amounts of about 1,000 mg is associated with urinary calcium losses of about 25 mg. Few studies link sodium and osteoporosis. A small study including 40 postmenopausal women, divided into two groups based on baseline sodium intake ($<$ or $>$ 3,400 mg/day) and put on a 6-month sodium-restricted (2,000 mg) diet, found significant reductions in urinary calcium and sodium excretion and a bone turnover biomarker only in those with higher (vs. lower) baseline sodium intakes [9]. While such findings suggest possible benefits to the skeleton from reductions in dietary sodium in those with high intakes, not all studies show such links. A larger study (over 69,000) of post-menopausal women found no associations of sodium intakes (including intakes well above and below sodium's TUL of 2,300 mg) with changes in bone mineral density measured at various skeletal sites or with changes in fracture risks from baseline to 3 or 6 years [10].

Protein and Acid Load

An adequate *protein* intake is necessary for bone health providing amino acids for the production of bone proteins for maintenance and growth. Short-term studies show high-protein diets lead to favorable changes in hormones (such as insulin-like growth hormone 1, which stimulates bone formation, and reduced PTH, which retards bone resorption); such changes are thought to exert beneficial effects on bone. While some studies have found that higher protein intakes (without changing intakes of other nutrients) increase urinary calcium, the rise is also associated with increased calcium absorption and counterbalances the losses of calcium in the urine. Moreover, the origin of the urinary calcium appears to be diet and not bone [11]. Other studies providing protein in amounts up to three times the RDA have shown no negative impacts on calcium absorption or excretion or on bone turnover biomarkers [12,13]. These findings and reviews of similar studies suggest no detrimental effects from high-protein diets on calcium homeostasis or bone health [11–13]. And, systematic reviews with meta-analyses and reviews of other **observational studies** have demonstrated higher (at or above the RDA) versus lower dietary protein intakes are associated with reductions in hip fracture risk provided dietary calcium intake is adequate [14]. Dietary protein intake has also been positively associated with bone mineral density [14].

Current dietary protein recommendations for older adults to maintain muscle and bone health range from about 1 to 1.2 g or higher (depending on other medical conditions)/kg body weight (see “Recommended Protein and Amino Acid Intakes” in Chapter 6). Inadequate protein intakes are thought to represent a greater detriment to bone health than intakes in excess of recommendations [14]. However, coupled with the need for higher protein consumption by many adults is the need for consumption of adequate amounts of calcium and vitamin D (as previously discussed) as well as adequate amounts of fruits and vegetables (as discussed in later paragraphs within this section of the Perspective).

The ingestion of protein-rich foods (such as meat, fish, eggs, and cheese and, to a lesser extent, grain products), versus from the ingestion of other food groups, has been suggested in some literature to negatively affect bone health due to the production of a higher acid load. Most of this generated acid is thought to arise from the oxidation of the sulfur-containing amino acids (such as methionine and cysteine) in the proteins, although acids also arise in the body during normal cellular metabolism throughout the day. Net acid production, buffering, and excretion of generated acids and bases are reflected in the tightly regulated blood pH level, which ranges from about 7.35 to 7.45 [15]. The blood may become mildly acidic if acid production exceeds the body's ability to buffer and/or remove the acids (via the lungs and kidneys; see Chapter 12's section “Acid–Base Balance”). The more acidic blood pH is proposed to impact bone, impairing osteoblast function and enhancing osteoclast activity. However, whether the acid load generated from protein ingestion is large enough to cause a metabolic acidosis that induces bone demineralization (contributing to osteoporosis) is unclear. Also, a subject of debate is the effectiveness of alkali ingestion (from net base-producing foods or alkalinizing salts) on neutralizing an acid load.

The urinary loss of calcium associated with the excretion of acidic urine has been estimated at 66 mg calcium/day and, when extrapolated, corresponds to a loss of 24 g/year, or a 480 g (which is about half of the skeleton's calcium content) over 20 years [16]. However, these and other estimates assume losses are solely from bone, which has not been clearly documented [15]. In fact, while studies investigating changes in calcium balance due to alterations in dietary protein (quantity or type) report significant linear relationships between urinary calcium and urinary net acid excretion, no changes in calcium balance and no relationship between change in net acid excretion and change in bone resorption markers have been documented [15,17]. Other studies have demonstrated negative effects on bone mineral density by dietary acid load, but only when calcium intake is inadequate [11,18]. A consensus paper, endorsed by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases and the International Osteoporosis Foundation, reported no direct evidence of altered bone strength, fragility fractures, or osteoporosis progression with an acid load from a balanced diet origin [15].

Buffers are present in the blood and body cells and are also provided by the diet, especially those containing sufficient *potassium* and *magnesium* and organic anions like citrate. Such nutrients and anions, found in fruits and vegetables as well as in supplements like potassium citrate or bicarbonate, favorably affect acid–base balance,

bone mineral density, and bone metabolism. Diets rich in potassium, fruits, and vegetables or diets supplemented with potassium bicarbonate or citrate enhance renal retention of calcium and improve calcium balance. Reviews of studies providing “alkali therapy” in the form of potassium bicarbonate or citrate or bicarbonate-rich mineral waters report reduced markers of bone resorption in short-term studies [15]. Limited findings from a few longer-term investigations with limited subject diversity suggest reductions in fracture risk associated with “alkali therapy.” See [11,14,15] for further information on the subject of protein and acid load and bone health. Meta-analyses along with further studies are needed to evaluate possible benefits, if any, from “alkali” therapies. Consumption of diets rich in fruits and vegetables, however, can provide needed potassium and “buffers” important for not only bone health but overall reductions in the risks of cancer, hypertension, and many other chronic diseases. Assuming the value of alkali therapies, its use has been suggested to be of possible benefit to those consuming high net acid loads who also have diminishing renal function and renal acid excretory function and lowering buffer capacity [15].

Other Nutrients

A review of nutrients and their functions in the body provides a list of other vitamins and minerals with roles in bone development. Two such vitamins include C and K, needed for the synthesis and function of several bone proteins. Remember, for example, vitamin C is needed for the synthesis of hydroxylysine and hydroxyproline, which contribute to triple helix formation of collagen, one of the main proteins in bone. Vitamin K is needed for the formation of Gla proteins in bone; the Gla proteins are important for bone strength and mineralization. Positive correlations between intakes of both vitamins C and K and bone mineral density have been demonstrated in some studies. Similarly, some (but not all) studies have shown higher intakes of these nutrients are associated with reduced fractures, but supplementation trials have not consistently shown improved bone mineral density or prevented fractures. Magnesium, like calcium and phosphorus, is present in relatively large quantities in bone. However, some (but not all) studies show associations between serum magnesium/magnesium intake and bone including density and fracture risk. Missing from the literature are randomized controlled trials evaluating the effects of magnesium on bone mineral density and fracture risk. Thus, at present, magnesium nor vitamins C and K are considered part of “standard” recommendations for osteoporosis prevention or treatment [19].

Other Dietary Constituents

Two other dietary components often considered when addressing bone health are caffeine and alcohol. Caffeine affects calcium balance and has been weakly associated in some studies with the development of osteoporosis. Caffeine (in amounts of 100–400 mg, which is about one to four cups of coffee) reduces the renal reabsorption of calcium, which leads to a temporary (~1- to 3-hour) increase in urinary calcium losses (about 10 mg), but has no net effect on daily (24-hour) urinary calcium excretion. Caffeine ingestion has also been shown to increase calcium secretion into the gut; however, whether the secreted calcium is reabsorbed and the extent of the secretion have not been determined. In relation to bone, caffeine

intake has been positively associated with fracture risk and/or bone density in women in some, but not all, studies. A systemic review demonstrated that caffeine intakes of up to 400 mg/day did not negatively affect bone health [20].

Most studies examining associations between alcohol intake and bone mineral density and/or fracture risk date back to the 1990s and early 2000s. Findings linking consumption of alcohol in moderation with bone mineral content and fracture risk are conflicting. However, higher alcohol intakes among postmenopausal women have been more strongly associated with lower bone mineral density (hip and total) and increased fracture risk [19]. The FRAX tool considers alcohol consumption of three or more units/day as part of the assessment, with one unit varying from 8 to 10 g of alcohol depending on the country. Generally, one unit equates to about 4 oz wine, 1 oz distilled spirits, and 10 oz beer. Clearly, more thorough studies are needed to better characterize the relationship of alcohol consumption (especially consumed in low or moderate amounts) on bone health. However, given associations between alcohol consumption and cancer risk, it is not unwise to suggest that if alcohol is consumed, limiting consumption to not more than 1 drink/day for women and not more than 2 drinks/day for men may be beneficial [19].

SUMMARY

Maintenance of bone health is clearly multifactorial. A person's genetic makeup cannot be changed, nor can the physiological changes accompanying aging be reversed. People usually can choose, however, a lifestyle that includes good nutrition (i.e., eating a variety of foods and getting recommended intakes of all nutrients) and regular weight-bearing exercise. Clearly, consuming recommended intakes of calcium and vitamin D is important and should be done in combination with consumption of dietary protein in amounts closer to 1.0 g (or higher depending on other health conditions)/kg body weight. Reductions in dietary sodium consumption if excessive are also worthwhile, and should be coupled with efforts to increase the ingestion of magnesium-rich and potassium-rich foods, especially fruits and vegetables, which are also rich in buffers. Intakes of fruits and vegetables and whole grains (along with other food groups) in amounts closer to those recommended by the Dietary Approaches to Stop Hypertension (DASH) diet is one approach from a food group perspective that may be beneficial for bone health. Clearly, preventing osteoporosis is a much better goal than managing its treatment, and such prevention should begin in childhood.

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WATER AND ELECTROLYTES

LEARNING OBJECTIVES

- 12.1** Identify the functions of water in the body.
- 12.2** Describe the distribution of water among body compartments.
- 12.3** Describe the means by which water is lost from the body and absorbed from the gastrointestinal tract into the body.
- 12.4** Explain how the body maintains water and sodium balance.
- 12.5** Identify particularly good food sources of sodium, potassium, and chloride.
- 12.6** Describe the processes by which sodium, potassium, and chloride are absorbed.
- 12.7** Describe the functions/roles of sodium, potassium, and chloride in the body.
- 12.8** Describe recommended intakes, deficiencies, and toxicities associated with sodium, potassium, and chloride.
- 12.9** Explain how the body maintains acid-base balance.

WATER ACCOUNTS FOR ABOUT 60% of an adult's total body weight, making it the most abundant constituent of the human body. Water is vital for life and must be maintained in appropriate amounts and locations in the body. Three minerals—sodium, potassium, and chloride—substantially influence water distribution and movement. Information on these minerals and their roles in fluid balance and acid–base balance follows discussions of water functions, body water content and distribution, and water losses, sources, absorption, needs, and regulation.

12.1 WATER FUNCTIONS

Water plays many critical roles in the body. Some of these functions include:

- *Chemical reactions.* Water is needed for chemical reactions such as those involved in nutrient catabolism.
- *Body temperature regulation.* Water has a high specific heat and thus does not readily change temperature as heat is released during normal cellular metabolism; additionally, sweat (consisting largely of water) that is generated facilitates heat removal and body temperature regulation as it evaporates from the skin.
- *Lubrication and protection.* Water is a necessary component of several secretions such as mucus, synovial fluid, and spinal fluid, among others; these secretions facilitate body processes as well as cushion and protect tissues.
- *Solvent and transport medium.* Water in body fluids contains numerous dissolved substances (solutes); examples of body fluids containing the solutes

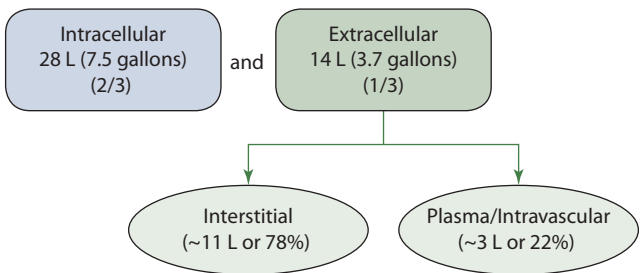
include the blood and urine, along with gastrointestinal tract secretions such as saliva, pancreatic juice, and bile, among others.

- *Maintenance of blood volume.* Water, as a large component of the blood, helps to maintain blood pressure and sustain cardiovascular system function.
- *Acid–base (pH) balance.* Water is needed for reactions involving buffers responsible for maintaining pH balance such as $\text{H}_2\text{O} + \text{CO}_2 \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+$.

12.3 BODY WATER CONTENT AND DISTRIBUTION

While body water contributes typically over one-half (range ~40–80%) of a person’s body weight, the amount varies based on age and body size and composition. Body water, for example, decreases both with age and body size. It is also affected by body composition. The body’s organ mass varies relatively little in water content, and thus does not have substantial effects on body water. For example, the bone contains about 25% water, the liver and kidneys are about 85% water, and the brain and muscle mass are about 75% water. The most significant contribution to variations in body water is the amount of fat mass. Fat mass has only about 10% water. Thus, a person with a large fat mass of, for example, 50% fat, may have 40% body water, whereas a person with lower body fat at 6% may have 70% water. It is also because males generally have a higher percentage of muscle mass and lower percentage of fat mass than females that they also have a higher percentage of body water.

Total body water can be theoretically compartmentalized into two major reservoirs: the intracellular compartment, which includes water enclosed within cell membranes, and the extracellular compartment, which includes water external to (outside of) cell membranes. The content of primarily sodium in the extracellular compartment affects its volume, with increases in total body sodium in this compartment increasing its fluid volume. The approximate distribution of the 42 L (or about 11.2 gallons) of total body water in a 70-kg (154-lb) man is:



The extracellular compartment primarily includes interstitial fluids and the plasma (also called intravascular fluid), but also fluids found in two smaller compartments, the lymph and transcellular spaces (not pictured).

- The interstitial fluid (ranging from about 10.5 to 11.2 L) directly bathes the cells and provides the medium for the passage of nutrients and metabolic products back and forth between the plasma and cells.
- The plasma/intravascular fluid (ranging from about 2.8 to 3.5 L) represents the fluid portion of the blood. Cell membranes separate intracellular fluid from interstitial fluid, and blood vessel walls separate the interstitial fluid from the plasma.
- The lymph is fluid that is being redirected (recirculated back) to the blood plasma from interstitial spaces.
- Transcellular fluid (a water-containing and sometimes viscous fluid) is found sequestered in small quantities in various cavities or spaces such as the pericardial, pleural, peritoneal, gastrointestinal tract, and synovial spaces or cavities and the aqueous humor of the eyes, among other areas.

Fluid compartment volumes for a 70-kg man are summarized in Table 12.1.

Distributed throughout the fluid compartments of the body are *electrolytes*, including both anions and cations (Table 12.2). Electrolytes are distributed in such a way that within a given compartment—the blood plasma, for example—electrical neutrality is always maintained, with the anion concentration balanced by the cation concentration. The cationic electrolytes include sodium, potassium, calcium, and magnesium (Ca^{2+} and Mg^{2+} are discussed in Chapter 11). These cations are electrically balanced by the anions, which include chloride, bicarbonate, and negatively charged proteins, along with relatively lower concentrations of organic acids, phosphate, and sulfate.

Table 12.1 Body Fluid Compartments

	Approximate Percentage of Body Weight	Approximate Percentage of Total Body Water	Approximate Volume (L) in 70-kg Man
Total body water	60	—	42
Extracellular water	20	33	14
Plasma	5	8	3
Interstitial fluid	15	25	11
Intracellular water	40	67	28

Table 12.2 Electrolyte Composition of Body Fluids

	Plasma (mEq/L)	Interstitial Fluid (mEq/L H ₂ O)	Intracellular Fluid (mEq/L H ₂ O)
Cations	153	153	195
Na ⁺	142	145	10
K ⁺	4	4	156
Ca ²⁺	5	2–3	3.2
Mg ²⁺	2	1–2	26
Anions	153	153	195
Cl [−]	103	116	2
HCO ₃ [−]	28	31	8
Protein	17	—	55
Others	5	6	130
Osmolality (mOsm/kg H ₂ O)	280–295	~300	~300

12.3 WATER LOSSES, SOURCES, AND ABSORPTION

Water is lost from the body each day primarily through the urine, with lesser amounts excreted in the feces. Additionally, losses occur via insensible (not normally noticeable) routes—evaporation from the respiratory tract and non-sweat diffusion from the skin. Urinary losses of water, the largest component, usually range from about 1 to 2 L/day. Fecal losses are usually small, less than about 200 mL/day (in the absence of diarrhea). Another 350–400 mL of water is lost daily from the respiratory tract as part of insensible losses. Insensible nonsweat losses of water from the skin are typically small, but sensible dermal losses of water as sweat (which serves as a means of eliminating heat that has been generated in the body) vary considerably depending on the environment and level of physical activity. Thus, total water losses from skin may range from as little as about 100 mL (up to about 300 mL insensible only) to well over 1 L (with extensive sweating).

Beverages and foods are the major sources of water to the body and are needed to replace its losses. Water from the ingestion of beverages usually contributes about 75–80% of intake and water from foods the remaining 20–25%. The water content of foods, however, varies tremendously with, for example, nuts and seeds containing very little water to some fruits and vegetables at over 95% water. A third, but minor, source of water to the body is referred to as metabolic water. Metabolic water is the water formed as part of some cellular biochemical reactions that occur in the body. Daily metabolic water generation totals about 200–300 mL. Estimates of metabolic water produced in

the body based on individual nutrient oxidation of 100 g each of fat, carbohydrate, and protein are about 100 mL, 55 mL, and 41 mL of generated water, respectively.

In addition to water present in the digestive tract after the ingestion of foods and beverages, a large amount of water (about 7 L) is released into the gastrointestinal tract each day as part of secretions (i.e., saliva, gastric juice, pancreatic juice, bile, intestinal juice, etc.). Just about all (over 98%) of this water, as well as that which is consumed, is absorbed from the intestines.

The majority (about 85%) of water is absorbed in the small intestine, primarily in the jejunum and the ileum, and the remainder is absorbed from the colon, especially the ascending and transverse regions. Of the ~1–1.5 L of water entering the colon each day, about 90–95% is absorbed, with only about 50–200 mL excreted daily as part of the feces. Absorption of water from the digestive tract is passive and occurs transcellularly and paracellularly; absorption is strongly influenced by osmolality. Thus, sodium absorption enhances water absorption, and the two main systems responsible for most of sodium's absorption also promote the absorption of water.

12.4 RECOMMENDED WATER INTAKE

Recommendations for water are published as an Adequate Intake (AI) with large variations in needs based not only on age and gender but also on the environment, level of physical activity, and rate of metabolism, among other factors. The AI recommendation for adult females is 2.7 L (about 91 oz) and for adult males is 3.7 L (about 125 oz) and includes water that can be obtained both from eating foods and from drinking beverages. Thus, assuming 25% of the water is derived from foods and 75% from beverages, beverage consumption by an adult female should total at least 8 cups and by an adult male at least 12 cups each day. Large variations in individual needs, however, mean that some individuals require more and some individuals need less than these recommendations. Other approaches used to estimate fluid needs for adults are based on energy intake, with 1 mL of water recommended for each 1 kcal ingested. Recommendations based on body weight suggest an intake of about 25–40 mL of water per kg body weight, depending on age and gender. Physically active adults may need to consume fluid intakes in excess of 3 L/day. Needed water may be consumed (derived) from the ingestion of foods and a number of beverages such as water, milk, fruit juices, broth, soft drinks, coffee, and tea, among others. Healthier beverage choices would be those that are lower in simple sugars and fat.

12.5 WATER (FLUID) AND SODIUM BALANCE

Water moves among the various body compartments but in a regulated manner that is strongly affected by the presence of sodium in the extracellular fluid (ECF) compartment. Thus, water and sodium balance are interrelated. Water movement across different spaces is affected by variations in pressure as listed hereafter.

- Water moves between the extracellular compartment's interstitial space (crossing the cell plasma membrane) and the intracellular space. The water movement between these compartments is regulated by *osmotic pressure*.
- Water also moves across the capillary blood vessel walls that separate the plasma and interstitial space. Differences in *hydrostatic pressure* (fluid or capillary blood pressure) and *colloidal osmotic pressure* govern this movement.

The balancing of these different pressures and the regulation of ECF volume and ECF osmolarity enable water (fluid) balance maintenance.

Osmotic Pressure

Osmotic pressure affects water passage across the cell plasma membrane. The osmolarity of the ECF is regulated to reduce deleterious osmotic movement of water

between the intracellular and extracellular compartments. In the body, water moves from areas where solute concentrations are low to areas where solute concentrations are high. **Osmosis** refers to the movement of water across a semipermeable membrane in response to differences in solute concentrations across the membrane. In the body, cell plasma membranes are selectively permeable and allow the passage of water in and out of cells; differences in osmotic pressure associated with solute concentrations influence this water movement, which occurs via osmosis.

The nature and distribution of solutes, especially sodium, potassium, and chloride, differ considerably between the intracellular and ECF compartments and play important roles in maintaining osmolarity and water distribution. Osmolarity can be thought of as the solute (particle) concentration of a fluid. As shown in Table 12.2 and Figure 12.1, within cells (intracellular), potassium represents the major cation (only small amounts of sodium are found) and phosphate is present as the major anion along with smaller amounts of protein anions. Potassium maintains primarily the osmotic balance of the intracellular fluid compartment. The ECF, in contrast to the intracellular fluid, contains sodium as the major cation (not potassium) and contains chloride (not phosphate) as its major anion. The presence of the sodium in the ECF contributes most to its osmolarity and ECF volume. The intracellular fluid and ECF are electrically balanced, and it is the sodium-potassium ATPase pumps in the cell membranes that maintain the appropriate

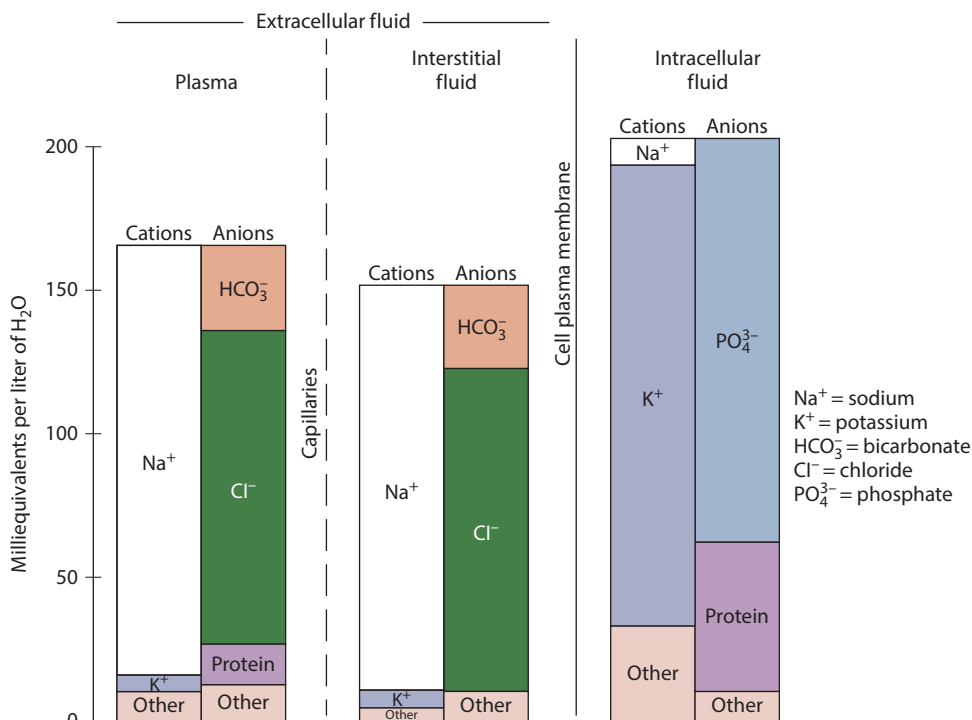


Figure 12.1 Electrolytes in the plasma, interstitial, and intracellular compartments of the body.

intracellular potassium and extracellular sodium concentrations. Other transporters and channels also facilitate the movement of solutes across the membrane to maintain electrical neutrality.

A comparison between the electrolyte composition of plasma and the interstitial fluid (which both make up the ECF) shows few differences (Figure 12.1). In both, it is sodium that is the major cation with only small amounts of potassium present and the anions include mostly chloride and bicarbonate. The main difference between the plasma and interstitial fluid is the presence of protein anions in the plasma but not in the interstitial fluid.

Disruptions in osmotic pressure affect fluid balance between the extracellular and intracellular compartments. Usually it is changes in ECF (and not intracellular fluid) osmolarity that result in osmotic pressure changes and water movement between compartments. Gains of *water without solutes* in the ECF compartment (such as the plasma) decrease ECF osmolarity (making it hypotonic) and are associated with overhydration and cell expansion/swelling (including cerebral edema). An excessive overconsumption of water, for example, dilutes the plasma, resulting in **hyponatremia** (low serum sodium less than 135 mEq/L), and reduces the plasma's approximate 0.9% salt. Conversely, losses of *water without solutes* from the ECF compartment increase serum sodium (hypernatremia) and ECF osmolarity (making it hypertonic) and result in dehydration. Cells in turn may shrink in this hypertonic environment.

Gains and losses of *fluid along with solutes (salts)* from the ECF compartment cause isotonic imbalances (in spite of the gains/losses, the fluids are still similar in composition to normal ECF). Hypervolemia (volume overload) results with gains in salt and water into the plasma, and hypovolemia (volume depletion) occurs with losses of both. Hypovolemia, for example, may occur with excessive vomiting and diarrhea, among other conditions. While a discussion of fluid and electrolyte disturbances is beyond the scope of this chapter, an understanding of the distribution of water and the role of solutes enables an understanding of how infusions (administered to correct deficits, for example) distribute in the body.

- *Water containing no salts*, for example, if administered would distribute across both extracellular fluid and intracellular fluid compartments. Thus, an intravenous administration of 1,000 mL of such fluid would result in 667 mL of water entering cells (i.e., intracellular 2/3) and 333 mL of water in the extracellular (i.e., 1/3) compartments. The 333 mL of water would further distribute with about 73 mL (22% of the 333 mL) remaining in the plasma and 260 mL of water (78% of the 333 mL) entering the interstitial space.
- The intravenous infusion of 1,000 mL of *normal saline (containing solutes, 0.9% sodium chloride)*, in comparison, distributes only into the extracellular compartments—780 mL interstitial and 220 mL intravascular.

Hydrostatic (Fluid/Capillary) Pressure

Hydrostatic (fluid/capillary) pressure affects water movement within the two main ECF compartments, between the interstitial fluid and the plasma. Just as water moves from areas of lower solute concentration to higher solute concentration (discussed in the previous section), water also moves from areas of higher pressure to areas of lower pressure. This is referred to as hydrostatic (fluid/capillary) pressure. Water distribution across the capillary endothelial surface is controlled by the balance of forces that tend to move water from the plasma into the interstitial fluid (filtration forces) and forces that move water from the interstitial fluid into the plasma (reabsorption forces). The major filtration force in the capillaries is hydrostatic pressure (P_{pl}), which is about 25 mm Hg and caused by the pumping of the heart. Weaker filtration forces include both interstitial fluid colloid osmotic pressure (Π_{isf}) at 5 mm Hg (note that this force is weak because of negligible protein present in the interstitial fluid) and interstitial fluid hydrostatic pressure (P_{isf}), which is negative at -6 mm Hg. The major reabsorption force countering the filtration force is the plasma osmotic pressure (Π_{pl}), which is approximately 28 mm Hg. The net result of these four forces at the arteriolar end of the capillaries can be described by Starling's equation:

$$\text{Filtration pressure} = (P_{pl} + \Pi_{isf}) - (\Pi_{pl} + P_{isf})$$

or after substituting the average values, we have

$$\begin{aligned}\text{Filtration pressure} &= (25 + 5) - (28 + [-6]) \\ &= (25 + 5) - (28 - 6) \\ &= (30) - (22) \\ &= 8 \text{ mm Hg}\end{aligned}$$

This positive filtration pressure indicates that a net filtration of water from the plasma into the interstitial fluid occurs at the arteriolar end of the capillaries. When filtration pressure is negative, a net reabsorption of water from the interstitial fluid into the plasma occurs. This situation exists at the venule end of the capillaries, where P_{pl} is substantially reduced while the concentration of plasma protein, and therefore Π_{pl} , correspondingly increases. The net effect of these forces on the water distribution between plasma and interstitial fluid along the course of the capillary is shown in Figure 12.2.

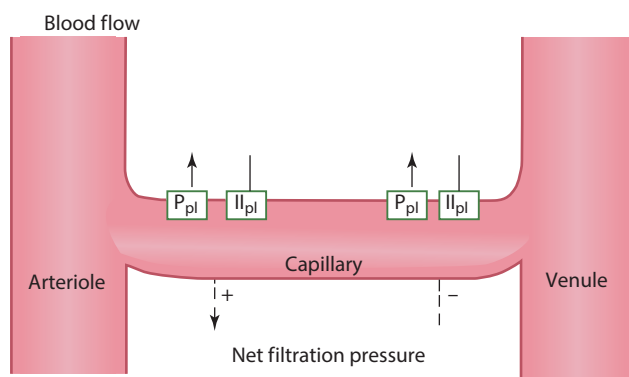


Figure 12.2 Starling's hypothesis of water distribution between plasma and interstitial fluid compartments. The relative magnitudes of the pressures, P_{pl} (plasma hydrostatic pressure) and I_{pl} (plasma osmotic pressure), are represented by the thickness of their respective arrows. There is a positive net filtration pressure at the arteriole end of the capillary and a negative net filtration pressure at the venule end.

Source: *Clinical Chemistry: Theory, Analysis, and Correlation*, 2nd ed., Kleinman, L.I., Lorenz, J.M., "Physiology and Pathophysiology of Body Water and Electrolytes," p. 373. Copyright © Elsevier 1989.

Colloidal Osmotic Pressure

Colloidal osmotic pressure, like hydrostatic pressure, affects water movement across the capillary walls (endothelium) that separates the plasma and the interstitial fluid. Because proteins are too large to pass through the capillary endothelium, the concentration of proteins is much higher in the plasma than in the interstitial fluid; this confers on the plasma a relatively high osmotic pressure and a water-attracting property. Proteins and other macromolecules that are too large to traverse the capillary endothelium are sometimes called **colloids**, and the osmotic pressure attributed to them is appropriately termed the *colloidal osmotic pressure*.

Extracellular Fluid Volume and Osmolarity and Hormonal Controls

ECF volume, like osmolarity, is also critical in the control of fluid balance. Sustaining ECF volume is additionally vital to blood pressure and cardiovascular system functions. To maintain plasma volume and keep blood pressure from dropping should ECF osmolarity increase or hypovolemia occur, water must move into (increase in) the plasma. Conversely, in the event of decreased ECF osmolarity, hypervolemia, and elevated blood pressure, water needs to move out of the plasma. Shifts in ECF volume and osmolarity serve as one of many triggers for the release of hormones responsible in part for correcting imbalances.

Several hormones including vasopressin, the renin-angiotensin-aldosterone system, and natriuretic peptides influence renal function, and in some cases the cardiovascular system, among others, to correct imbalances in the body's fluid and sodium levels. Sodium balance is mainly regulated by the hormone aldosterone (which is part of the renin-angiotensin-aldosterone system), and water

balance is mostly controlled by the hormone vasopressin. Thirst, however, also contributes to water balance when deficits occur. The sensation of thirst is triggered by the thirst center in the hypothalamus; this center is located near the osmoreceptors (which monitor ECF osmolarity, discussed in the next section) and close to the cells that secrete vasopressin.

Vasopressin

Changes in blood osmolarity are monitored by various receptors in the body, especially those found in the hypothalamus. The osmolarity of the blood ranges normally from about 280 to 295 mOsm/L (its **osmolality** is similar at about 280–295 mOsm/kg of water). (**Osmoles** [osm] represent the number of moles of each particle in solution.) The hypothalamus responds to slight increases in ECF osmolarity and signals the release of vasopressin, also called arginine vasopressin or antidiuretic hormone (ADH). It is osmoreceptor neurons present in the anterior hypothalamus that detect increasing ECF osmolarity and trigger vasopressin release. Vasopressin release is also stimulated to a lesser extent (1) by reductions in plasma volume, which are monitored by receptors in the left atrium of the heart, and (2) by angiotensin II (the role of angiotensin II is addressed under the section "Renin-Angiotensin-Aldosterone System"). Vasopressin's effects on the body (see Figure 12.3) include:

- Stimulation of the reabsorption of water in the kidneys, which reduces urine output and increases ECF volume
- Stimulation of thirst, which serves to increase fluid consumption and increase ECF volume
- Stimulation of vasoconstriction of the arterioles, which increases blood pressure.

In the kidneys, vasopressin's effects are confined to the distal tubule and collecting duct, which absorb about 20% of water independent of sodium and chloride. (The hormone does not affect passive water reabsorption occurring in the proximal tubule and loop of Henle; about 80% of water is reabsorbed from these regions and is associated with sodium and chloride reabsorption.) Vasopressin acts by binding to hormone-binding sites called V_2 receptors; the binding stimulates the production of cAMP and the movement of specific aquaporins (AQP-2) from intracellular vesicles to the cell surface. These aquaporins are water channels and facilitate water movement from the filtrate across the tubule cell and into the peritubular capillaries. The absorbed water "dilutes" the ECF and reduces the osmotic pressure. The urine that is excreted under conditions of elevated vasopressin is low in volume and concentrated.

Under conditions of hypervolemia (increased ECF volume), decreased ECF osmolarity, and higher blood pressure, vasopressin is not released and the aquaporin

water channels are endocytosed back into the cell cytosol. Consequently, the distal tubule and collecting duct reabsorb little water. Thus, in this situation, most of the 20% of the water does not get reabsorbed and is excreted as urine. The urine is now higher in volume and less concentrated

than when vasopressin concentrations were elevated. (Note the reader may want to read the brief discussion of kidney physiology provided in the boxed feature before continuing onto the sections addressing the effects of other hormones on fluid and sodium balance.)

THE KIDNEYS: A BRIEF REVIEW

THE KIDNEYS ARE RESPONSIBLE for eliminating metabolic wastes, regulating pH, and regulating the body's electrolyte and water content to maintain homeostasis; the latter is accomplished through adjustments in urine volume and osmolarity. The functional unit of the kidney is the nephron. Each kidney contains about 1–1.5 million nephrons. Each nephron in turn consists of tubular and vascular components. The tubular components of the nephron are the Bowman's capsule (a capillary network), proximal (convoluted) tubule, loop of Henle, distal (convoluted) tubule, and collecting duct. The main vascular components include the glomerulus, the afferent and efferent arterioles, and the peritubular capillaries.

The glomerulus is a tuft of about 50 capillaries that filters water and solutes from the blood as it passes through this region of the nephron. About 20% of the plasma entering the glomerulus is filtered. Glomerular filtration occurs at a rate of about 125–130 mL/minute. It is the afferent arterioles that transport blood into the glomerulus and it is the efferent arterioles that carry blood out of the glomerulus. The 80% of the plasma not entering the glomerulus flows through the efferent arterioles. The efferent arterioles branch into another network of capillaries called the peritubular capillaries. These capillaries surround the tubules and function in the exchange of substances; they can selectively reabsorb into the blood substances

that were filtered (and present in the fluid in the lumen of the tubules) and thus serve as a salvage mechanism. These capillaries may also secrete certain substances from the blood through the tubules and into the filtrate. The peritubular capillaries coalesce to drain into the renal vein.

The Bowman's capsule, which surrounds the glomerulus, collects the filtrate, that is, the fluid and substances that were filtered from the blood. The filtrate next moves into the proximal convoluted tubule and progressively through the loop of Henle (both its thin descending limb and its thick ascending limb), the distal tubule, and the collecting duct (Figure 12.3). Collecting ducts gather and direct the filtrate

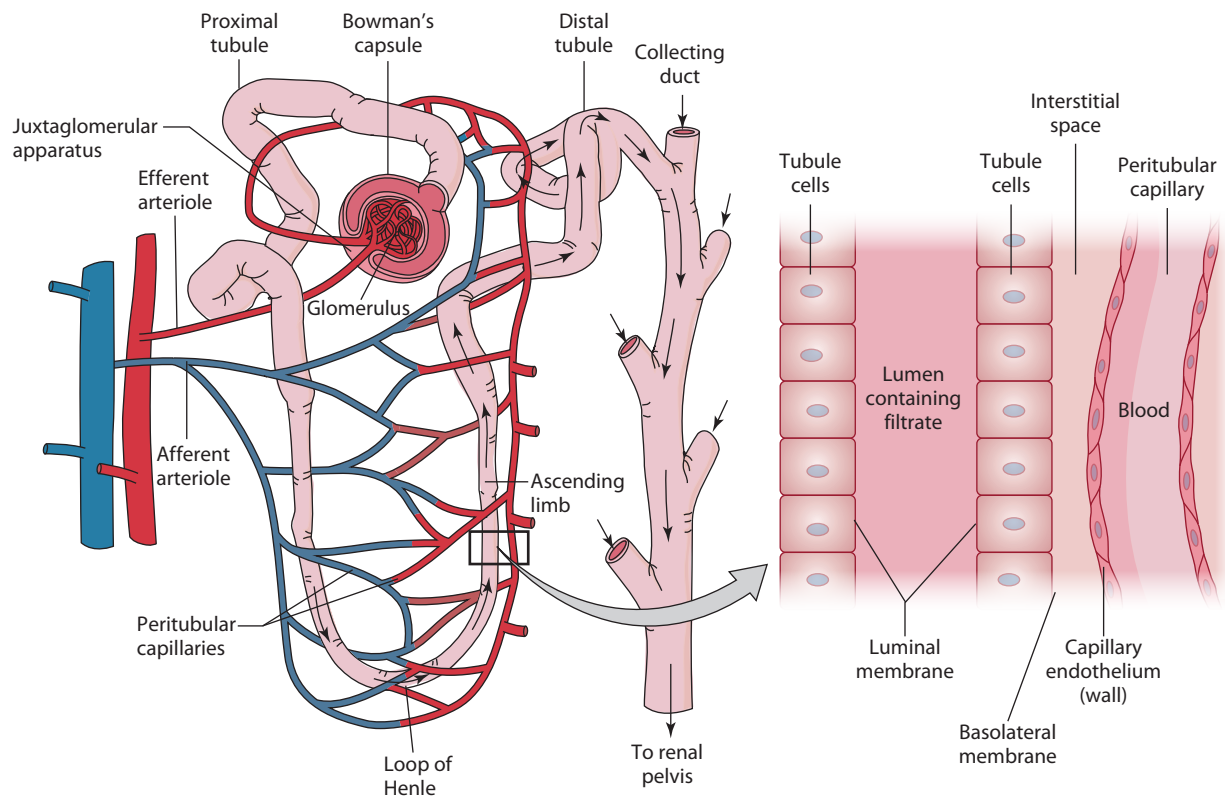


Figure 12.3 A nephron.

Source: Cengage Learning Inc. Reproduced by permission. www.cengage.com/permissions.

(Continued)

from the distal tubules of many nephrons into the ureter, a “tube” that leads the urine to the bladder. The cells making up the walls of the nephron and that surround the tubular lumen are only a single layer thick; the basolateral side of these cells is surrounded by interstitial fluid and the peritubular capillaries (Figure 12.3).

Each segment of the nephron is functionally distinct in its permeability to water and the solutes present in the filtrate. The ultimate composition of the urine depends on three processes, filtration, reabsorption, and secretion, which occur as the filtrate moves through the nephron.

- Filtration occurs as blood passes through the glomerular capillaries; these capillaries differ from other capillaries in the body in that the hydrostatic pressure within them is approximately three times greater than in other capillaries. They also have larger pores. These characteristics enable the glomerular capillaries to act as a filter, removing most everything from the blood except blood cells and proteins that exceed a molecular weight of about 50,000 daltons. The entire plasma volume (of an adult) is filtered over 60 times each day. The formed filtrate, which amounts to

over 185 L (or nearly 49 gallons) per day, contains water and other substances, including electrolytes, glucose, amino acids, and metabolic waste products.

- Reabsorption (passive and active) of selected substances from the filtrate and into the blood occurs via the peritubular capillaries; this reabsorption takes place as the filtrate passes through the tubular components of the nephron. Of the 185 L of filtrate formed daily, about 183.5 L is reabsorbed and 1.5 L is excreted as urine. This difference in the volumes means that only about 1% of the filtrate is excreted as urine, and the remaining 99% is reabsorbed into the blood. The proximal tubule reabsorbs most nutrients including about 90% (or more) of sodium, chloride, potassium, glucose, amino acids, bicarbonate, phosphate, and water. Additionally, reabsorption of water, sodium, chloride, potassium, calcium, magnesium, and bicarbonate, among other substances, occurs in the loop of Henle, distal tubule, and collecting duct. Active transport, either primary or secondary, is responsible for most of the nutrient reabsorption. As the nutrient carriers become saturated, transport maximums are reached for

some nutrients. This situation occurs most often when nutrient concentrations in the blood are high (as with hyperglycemia in those with diabetes) and can result in increased urinary excretion of the nutrient (in this case glucose).

- Secretion allows for the selective movement of substances from the blood in the peritubular capillaries and ultimately into the filtrate. This process enables the excretion of selected substances that were not a part of the 20% of the plasma that was originally filtered through the glomerulus. The ability of the tubules to secrete nutrients/substances such as potassium, hydrogen ions, creatinine, and various organic cations and anions is important in maintaining homeostasis. Secretion occurs throughout the nephron, but mostly in the proximal tubule, distal tubule, and collecting duct.

Fluid and solutes (both filtered and not reabsorbed, and secreted) remaining as filtrate in the collecting duct enter the renal pelvis and then the ureter for excretion as urine. For a more complete understanding of renal anatomy and physiology, the reader is suggested to consult a physiology text.

Renin-Angiotensin-Aldosterone System and Sodium Balance

The major regulatory factor controlling sodium (and chloride) balance in the body is the renin-angiotensin-aldosterone system (RAAS). This system enhances sodium and chloride reabsorption, with water (fluid) balance also affected since water reabsorption follows the reabsorption of the electrolytes. The RAAS is active when ECF volume and blood pressure are low and is initiated by the actions of renin.

Renin Renin, a hormone with enzymatic functions, is secreted by granular cells in the juxtaglomerular apparatus (near or adjoining the glomerulus) of the kidneys. The granular cells are innervated by the sympathetic nervous system and are stimulated to release renin by increased circulating catecholamines and when blood pressure and plasma fluid volume are reduced/low. The resulting decreased renal perfusion pressure is sensed by distention receptors and baroreceptors within the juxtaglomerular apparatus. Renin secretion into the blood starts a series of reactions that initially involves hydrolysis of angiotensinogen

and ultimately results in the release of aldosterone from the adrenal cortex. This sequence of events and its effects are illustrated in Figure 12.4.

Angiotensinogen and Angiotensin Renin functions to hydrolyze angiotensinogen, an inactive protein synthesized and released by the liver into the blood. Hydrolysis of angiotensinogen generates angiotensin I, another inactive decapeptide. Angiotensin I is subsequently hydrolyzed by angiotensin-converting enzyme (ACE), which is present in the capillaries of the lungs. Cleavage of angiotensin I produces angiotensin II, an active octapeptide.

Angiotensin II performs several functions affecting sodium and water balance and blood pressure. Angiotensin II:

- Serves as a potent vasoconstrictor of systemic arteries, increasing peripheral resistance and blood pressure. In fact, one group of blood pressure medications, known as ACE inhibitors, is used to treat hypertension and functions by inhibiting ACE and thus reducing the conversion of angiotensin I to angiotensin II.

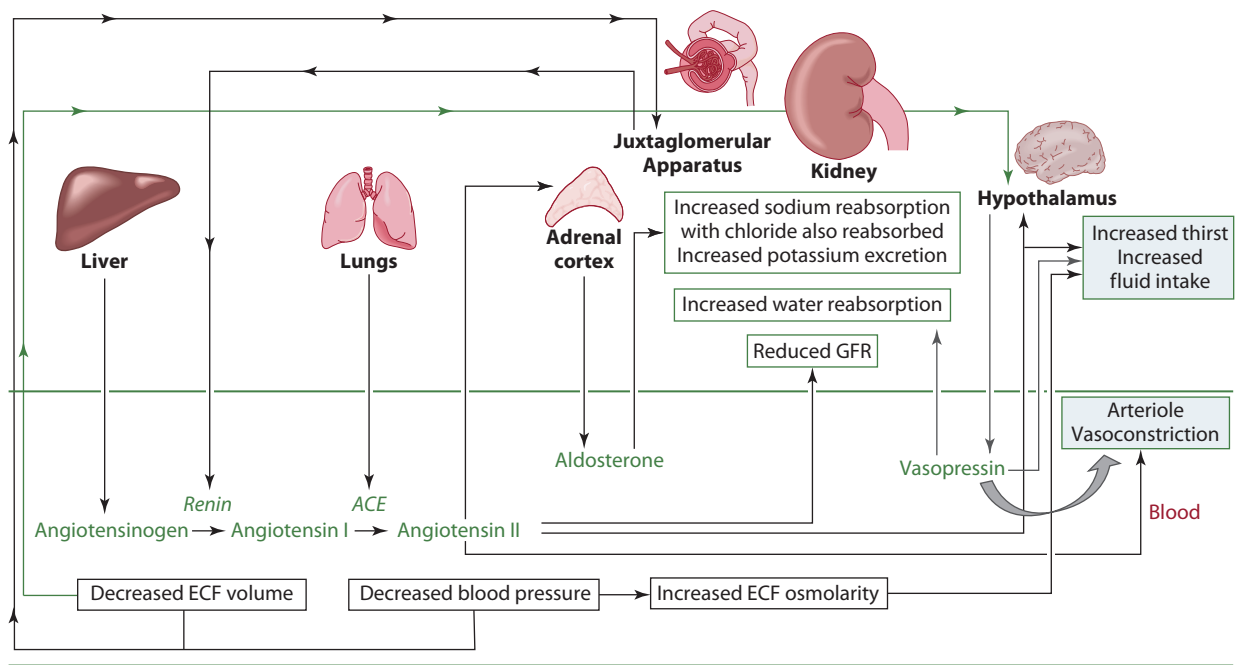


Figure 12.4 The effects of vasopressin and the renin-angiotensin-aldosterone system on water and sodium balance. Abbreviations: ACE, angiotensin converting enzyme; GFR, glomerular filtration rate.

- Increases blood pressure by stimulating the hypothalamic thirst center to increase thirst (which increases water intake and thus plasma volume)
- Stimulates vasopressin release (which increases water reabsorption by the kidneys and thus increases plasma volume) and thirst
- Reduces the glomerular filtration rate and therefore filtered sodium
- Interacts with receptors on the adrenal cortex, leading to the release of the hormone aldosterone.

Aldosterone release is also increased by angiotensin III, which is produced from angiotensin II after the hydrolytic removal of an aspartic acid residue by a plasma aminopeptidase. Angiotensin III, however, is present in considerably lower concentrations than that of angiotensin II and is thus thought to have lesser effects on maintaining fluid and sodium balance.

Aldosterone In addition to being stimulated by angiotensin, aldosterone release is stimulated by decreased concentrations of natriuretic peptides (discussed in the next section), by increased plasma potassium, and by decreased plasma sodium concentrations. Aldosterone promotes the reabsorption of sodium and the excretion of potassium in the distal tubule; chloride is passively reabsorbed with the sodium. Water reabsorption follows the sodium and chloride. More specifically, aldosterone exerts its functions by promoting the insertion of more Na^+ channels into the luminal membrane and more Na^+ – K^+ pumps into the basolateral membrane. These actions

increase the reabsorption and thus retention of salt and water. It also promotes an increase in blood volume, blood pressure, and urinary potassium excretion and hence reverses the conditions that initially triggered renin release.

The RAAS is less active once blood volume and blood pressure have been returned to normal or if elevated. Under these conditions, renin secretion is inhibited, and the sequence of activations diminished to thus reduce aldosterone secretion and reduce the reabsorption of Na^+ in the distal and collecting tubules. The sodium (and chloride) is consequently excreted in the urine. Although aldosterone regulates only about 8% of filtered sodium, the reduction in sodium reabsorption with low aldosterone levels can account for a large daily loss of sodium because of the large amount of fluid filtered by the glomerulus and the typically large consumption of dietary salt. The kidney's ability to respond to changes in the RAAS and adjust salt excretion and retention throughout the day help the body maintain sodium and fluid balance.

Natriuretic Peptides

Opposing the RAAS and promoting the loss of sodium and the lowering of blood volume and pressure are atrial natriuretic peptide (ANP), a peptide hormone synthesized in atrial cells of the heart, and B-type natriuretic peptide (BNP), a peptide synthesized by myocytes in the ventricles of the heart. Both peptides are released into circulation when the heart muscle is stretched by an expansion of ECF (plasma) volume that occurs with sodium and water retention and increased arterial blood pressure. The peptides

function to increase glomerular pressure and filtration rate, which increase the amount of sodium and water excreted (**natriuresis** and diuresis, respectively) and, in turn, decrease the plasma volume and lower blood pressure. The natriuretic peptides also reduce renin and aldosterone release and promote the dilation of blood vessels to reduce peripheral vascular resistance and thus blood pressure.

12.6 SODIUM

The body’s total sodium content is estimated at about 105 g. The majority (60–70%) of this sodium is found in ECF. About 30–40% of body sodium is located on the surface of bone, and a much lower level (less than ~10%) is found intracellularly in mostly nerve and muscle tissues. Sodium constitutes about 93% of the cations in the body fluids, making it by far the most abundant member of this family (shown in Table 12.2). An overview of sodium, along with potassium and chloride, functions, body content, food sources, deficiency symptoms, and recommendations for intake are provided in Table 12.3.

Sources

The major source (over 75%) of the sodium in the diet of Americans is salt (in the form of sodium chloride) that has been added to processed foods. Some foods contributing to dietary sodium in the United States include cold cuts/cured meats, soups, pizza, mixed dishes (with pasta and/or meat), and condiments, among other foods. Some examples of the sodium contents of these foods are provided hereafter; however, because different brands of the same foods often vary in sodium content, it is best to read food labels carefully.

- Smoked, processed, or cured deli meats (such as luncheon meats, ham, corned beef, hot dogs) can contain up to 1,000 mg of sodium in a 2- to 3-oz serving.

- Cheese, especially processed cheese, provides between 25 and 450 mg sodium/1 oz slice; swiss and ricotta are typically lower (< 100 mg) in sodium, whereas parmesan, asiago, American, and blue cheeses have sodium contents toward the higher end of the provided range.
- Soups may contain up to about 800 mg sodium/cup.
- Pickles and pickled foods such as some canned fish and sauerkraut are high in sodium, with, for example, over 700 mg sodium/medium pickle and 400–500 mg sodium/½ cup sauerkraut.
- Condiments, such as ketchup, barbecue sauce, mustard, mayonnaise, cocktail sauce, and horseradish sauce, among others, range from about 150 mg sodium/tablespoon to over 1,000 mg sodium/tablespoon (in, for example, soy sauce).
- Spices and packaged spice mixes, such as taco and Cajun seasonings, can contribute substantially to the sodium content of a meal with some spice mixes containing over 1,300 mg/teaspoon.
- Tomato-based sauces and juices can also be high in sodium, with some containing over 400 mg/quarter cup.
- Canned vegetables typically provide up to about 200–300 mg sodium/½ cup serving.
- Instant pasta and rice dishes are exceptionally high in sodium, often providing over 700 mg of sodium per half-cup, as prepared.
- Prepackaged frozen dinners can contribute up to and sometimes over 1,400 mg sodium/entrée.
- Snack-type foods, such as crackers and chips, vary in sodium content from minimal to over 1,000 mg/serving.

Water supplies up to about 10% of sodium intake for some individuals. Naturally occurring food sources of sodium such as milk and yogurt, meat, eggs, vegetables,

Table 12.3 Electrolytes: Functions, Body Content, Deficiency Symptoms, Food Sources, and Adequate Intakes (AIs)

Mineral	Selected Physiological Functions	Approximate Body Content	Deficiency Symptoms	Selected Food Sources	AI
Sodium	Water, pH, and electrolyte regulation; nerve transmission, muscle contraction	105 g	Anorexia, nausea, muscle atrophy, poor growth, weight loss	Table salt, processed and snack foods, cured meats, seafoods, condiments, milk, cheese, and bread	1,500 mg, 19–50 years
Potassium	Water, electrolyte, and pH balances; cell membrane polarization	130 g	Muscular weakness, cardiac arrhythmias, paralysis	Fruits, vegetables, legumes, nuts, and dairy products	3,400 mg males and 2,600 mg females, 19+ years
Chloride	Fluid and pH balance, component of gastric hydrochloric acid	105 g	Weakness, lethargy, hypokalemia, metabolic acidosis	Table salt, seafood, meat, and eggs	2,300 mg, 19–50 years

and fruits furnish about another 10% of consumed sodium.

- Milk, for example, contains about 120 mg of sodium per cup and yogurts up to about 160 mg per cup.
- Meats, poultry, and fish (not processed) provide about 25–30 mg sodium per ounce.
- Breads contain about 110–175 mg sodium/slice, although quick breads (muffins, biscuits) may contain >300 mg sodium/serving.
- Fresh vegetables provide typically less than 40 mg sodium per half-cup, although celery is an exception, containing about 50 mg of sodium per half-cup.
- Fresh (as well as canned and frozen) fruits are low in sodium, containing < 5 mg per half-cup serving.

Salt, as sodium chloride, added to foods from the use of a salt shaker during cooking and at the table provides roughly 15% of total sodium intake. Sodium comprises 40% by weight of salt. One teaspoon of salt provides 2,300 mg (2.3 g) or 100 mmol of sodium. Note that sodium obtained from the use of table salt (which is usually derived and refined from salt mines) does not differ from sodium found, for example, in sea salt; sea salt, however, may contain additional minerals such as iodine, potassium, magnesium, and zinc, among others.

Food labels provide information on its sodium content. Terms such as *free*, *very low*, *low*, *reduced*, or *light* in conjunction with sodium on food labels are associated with specific amounts of sodium per serving. For example:

- “Free” means less than 5 mg of sodium per serving,

- “Very low” means less than 35 mg per serving,
- “Low” means less than 140 mg of sodium per serving,
- “Reduced” or “less” indicates at least 25% less sodium per serving than the appropriate reference food
- “Light” may be used if the food is low in calories and fat and the sodium content has been reduced by at least 50%.

The Daily Value for sodium used on food labels is 2,300 mg. Health claims for sodium used on food labels (approved by the U.S. Food and Drug Administration [FDA]) can state “Diets low in sodium may reduce the risk of high blood pressure, a disease associated with many factors” as well as “Development of hypertension or high blood pressure depends on many factors. [This product] can be part of a low-sodium and low-salt diet that might reduce the risk of hypertension or high blood pressure” [1].

Estimates of sodium intake by adults in the United States greatly exceed recommendations. Intakes typically range from approximately 3,000 to 5,000 mg sodium/day with a mean of about 3,400 mg. In addition to sodium obtained from ingested foods, the secretions of the digestive tract also contain appreciable amounts of sodium among other electrolytes. The sodium content of bile (about 145 mEq/L) and intestinal and pancreatic secretions (both containing about 140 mEq/L) are greatest with lesser amounts in gastric and colonic secretions (both about 60 mEq/L). Much of this sodium is absorbed. See the boxed feature for information on milliequivalents (mEq).

ELECTROLYTES: CALCULATING MILLIEQUIVALENTS (mEq)

WHILE THIS TEXT USES primarily milligrams (or grams) in discussing quantities of electrolytes, milliequivalents (mEq) are also sometimes used. To better understand this term and calculations used to derive mEq, it is helpful to initially consult the periodic table of elements. This table provides information on the mass (in grams) of one mole (abbreviated mol) of the various elements. Calcium (one mole), for example, has an atomic mass of 40.1 g, and magnesium has an atomic mass of 24.3 g. The atomic masses for sodium, potassium, and chloride are 29 g/mol, 39.1 g/mol, and 35.4 g/mol, respectively.

The term “equivalent” (and “milliequivalent” with the prefix milli – denoting a factor of one thousandth) represents an “amount”. Use of mEq is most relevant in situations in which solutes are dissolved in a solvent (such as blood) since the dispersion is dependent on the valence of the solute; however, mEq are also sometimes used in other situations, such as to express quantities in food or recommendations for intake. The unit for measuring solutes is a (milli)mole. Milliequivalents of electrolytes are determined by dividing the number of millimoles (mmol) of the ion by its valence (electrical charge).

For Na^+ , K^+ and Cl^- , since the valence is one, mmol and mEq are the same (i.e., 1 mmol = 1 mEq). Thus, 1 mEq or 1 mmol = 23 mg of sodium; 1 mEq or 1 mmol = 39.1 mg of potassium, and 1 mEq or 1 mmol = 35.4 mg of chloride. For calcium (Ca^{2+}) and magnesium (Mg^{2+}), each with a valence of 2, 1 mEq = mmol divided 2 (which can also be expressed as multiplying by mmol by 0.5). Thus, 1 mEq of calcium = $40.1/2 = 20.05$ and 1 mEq of magnesium = $24.3/2 = 12.15$. Numerous websites are available to facilitate with these and other calculations including interconverting mEq/L, mmol/L, and mg/dL.

Absorption and Transport

About 95–100% of sodium is absorbed from the small intestine and colon. The main mechanisms enabling sodium absorption (shown and described in Figure 12.5) are (1) a nutrient-coupled Na^+ cotransporter, which functions throughout the small intestine but especially the jejunum; (2) an electroneutral Na^+ and Cl^- cotransport exchange transporter, which is active in the small intestine (jejunum > ileum) and the colon; and (3) an electrogenic system (involving a Na^+ channel), which is a lesser-used system and operates principally in distal regions of the colon.

Once absorbed into the body, sodium is transported freely in the blood. The serum's sodium concentration is maintained within a fairly narrow range, 135–145 mEq (mmol)/L. Serum sodium concentrations are indicative of fluid balance, with low concentrations (hyponatremia) reflecting excessive fluid in the plasma and high concentrations (hypernatremia) indicative of fluid loss. Electrolyte as well as fluid balance is regulated by several hormones, including vasopressin, angiotensin II, aldosterone, and natriuretic hormones, as discussed previously under the section “Water (Fluid) and Sodium Balance.”

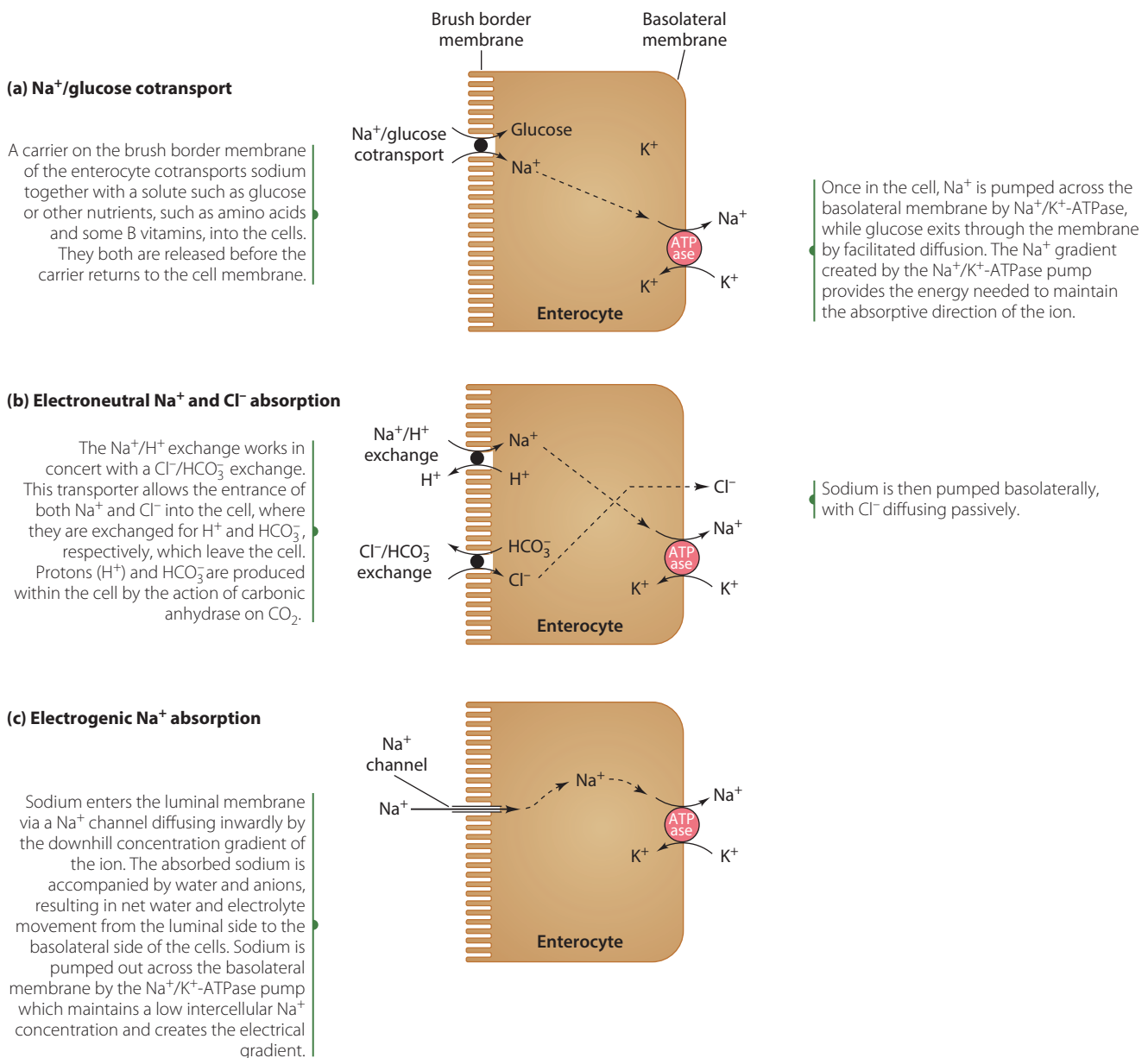


Figure 12.5 Intestinal sodium absorption.

Functions and Interactions with Other Nutrients

Within the body, sodium plays important roles in the maintenance of osmotic pressure for fluid balance. It also works with potassium and calcium in nerve transmission/impulse conduction and muscle contraction. In nerve transmission and muscle contraction, sodium functions as part of the Na^+/K^+ -ATPase pump found in the plasma membrane of cells. With the exchange of sodium for potassium and the hydrolysis of ATP, an electrochemical potential gradient generates nerve impulse conduction.

In addition to sodium's interrelationships with potassium and chloride, it has been recognized for several decades that increased dietary sodium intake is associated with increased urinary calcium excretion. Chapter 11's Perspective on Osteoporosis addresses sodium and bone health.

Excretion

Because nearly all ingested sodium is absorbed and dietary sodium intake is generally high, much larger amounts enter the body than are required. The kidneys provide the primary means of excreting excess sodium and reabsorbs sodium as needed. The proximal tubule reabsorbs up to about 67% of filtered sodium (and chloride) by paracellular and transcellular mechanisms. The loop of Henle reabsorbs an additional 25–30% via a $\text{Na}^+-\text{K}^+-2\text{Cl}^-$ cotransporter. Sodium reabsorption in the distal tubule and collecting ducts is under hormonal control. Aldosterone specifically increases sodium reabsorption by inserting additional Na^+ channels into the luminal membrane, which improves passive reabsorption from the lumen into the renal cells, and by inserting Na^+/K^+ -ATPase pumps into the basolateral membrane, which improves the active transport of the sodium from the renal cells into the plasma. In the absence of RAAS stimulation as occurs with higher sodium, ECF volume, and blood pressure, sodium is not reabsorbed and is excreted in the urine. Additionally, natriuretic peptides promote urinary sodium excretion.

In addition to urine losses, sodium is excreted in small amounts (usually less than about 125 mg) in the feces (except in the case of diarrhea or other intestinal illnesses in which losses can be much higher). Sodium losses via the skin are variable. Under conditions of moderate temperature and low levels of exercise, sodium losses in sweat are small. However, while the sodium content of sweat is ~ 50 mEq/L, large amounts of sweat can be generated under conditions of high temperature or sustained vigorous exercise and contribute to significant sodium losses.

Recommendations, Deficiency, Toxicity, and Assessment of Nutriture

The recommended intake for sodium, provided as an Adequate Intake (AI), is 1,500 mg (65 mmol) (equal to 3.8 g of salt) for adults [2]. Additional recommendations for sodium for other age groups are provided on the inside front cover of the book. The minimum amount of sodium needed to replace losses (with no sweat and maximal adaptation) is estimated at about 180 mg (8 mmol); however, this amount is not thought to represent the sodium requirement [3].

Evidence was considered “insufficient” (due to the lack of a toxicological indicator specific to excessive sodium intake) to establish a Tolerable Upper Intake Level (UL) for sodium [2]. However, the Chronic Disease Risk Reduction (CDRR) recommendation for sodium (the first nutrient with a CDRR recommendation) is 2,300 mg (100 mmol) for those age 14 years and older. The recommendation is based on the beneficial effects of reducing sodium intake (in those with and without high blood pressure) on the risks of cardiovascular disease and hypertension (and elevated blood pressure) [2]. Chronic disease risk is expected to be reduced in those who decrease their sodium intake to the CDRR value [2]. The CDRR recommendations for sodium for children age 1–3 years is 1,200 mg, age 4–8 years is 1,500 mg, and age 9–14 years is 1,800 mg. In addition to its relationship with blood pressure, excessive sodium consumption is also independently associated with an increased risk of stroke and cardiovascular disease including increased left-ventricular mass [4].

Other health-oriented organizations provide recommendations similar to the AI and CDRR for sodium intake. For example, the American Heart Association recommends 1,500 mg of sodium/day. The World Health Organization recommends a daily sodium intake of less than 2,000 mg. The *Dietary Guidelines for Americans* endorse consuming less than 2,300 mg of sodium/day.

A dietary deficiency of sodium does not normally occur because of the abundance of the mineral across a broad spectrum of foods. However, excessive sweating, involving a loss of more than about 3% of total body weight, may result in sodium deficiency. Symptoms include muscle cramps, nausea, vomiting, dizziness, shock, and coma.

Sodium is measured routinely in clinical laboratories. A 24-hour urinary sodium excretion level is most often used as a reflection of sodium intake. Urinary sodium in amounts less than about 5–10 mmol/L suggests sodium depletion. Collection of multiple 24-hour urine samples is best for assessment of sodium intake because intake typically varies greatly from day to day. Plasma sodium concentrations provide an indication of hydration status.

12.7 POTASSIUM

Potassium is the major intracellular cation, with about 98% of the body's potassium found within cells and only about 2% present extracellularly. Potassium constitutes up to about 0.19% of total body weight, or up to about 130 g of potassium in a 70-kg human.

Sources

Potassium is fairly widespread in foods but is especially abundant in fruits and vegetables, which provide the mineral along with anions like phosphate and citrate. Potassium is also found in milk and yogurt (i.e., dairy products), legumes and lentils, and meats, fish, and poultry, as shown in Table 12.4. It is also found in coconut water in amounts close to 500 mg per 1-cup serving.

Potassium may also be added to some processed foods (but not in the large quantities as found with sodium). Potassium, usually as potassium chloride, is a major constituent of many salt substitutes. Although the exact potassium content varies considerably, some salt substitutes contain over 600 mg potassium per $\frac{1}{4}$ teaspoon.

Multivitamin/mineral supplements may or may not contain potassium. If included, potassium is present in amounts up to a maximum of about 99 mg. Individual-nutrient potassium supplements are available in normal and extended-release tablets and capsules as well as dissolvable tablets, elixirs, and powders for suspension. The form is provided as a salt, usually potassium gluconate, citrate, phosphate, or bicarbonate. Use of higher-dose supplements is recommended only to correct deficiency under medical supervision. Because potassium supplements can be irritating to the gastrointestinal tract, smaller divided doses, for example taken two to three times daily, are often better tolerated. Additionally, consumption of enteric-coated forms of the mineral with meals may help reduce intestinal side effects. The Daily Value for potassium, found on food and supplement facts labels, is 4,700 mg.

Absorption, Secretion, and Transport

About 85–90% of ingested potassium is typically absorbed. Absorption occurs throughout the small intestine and to a lesser extent in the colon. Depending on

Table 12.4 Selected Food Sources of Potassium

Foods with > 400 mg	Foods with ~250–400 mg	Foods with ~100–250 mg
Tomato juice, canned, 1 cup	Tomato sauce, canned, $\frac{1}{2}$ cup	Yogurt, Greek, nonfat, plain, 6 oz
Tomato paste, canned, $\frac{1}{4}$ cup	Tomato puree, canned, $\frac{1}{4}$ cup	Tuna, canned water, drained, 3 oz
Prune juice, canned, 1 cup	Tomato, raw, 1 medium	Egg, cooked, 1 large
Carrot juice, canned, 1 cup	Prunes, dried, $\frac{1}{4}$ cup	Broccoli, cooked, $\frac{1}{2}$ cup
Orange juice, fresh, 1 cup	Raisins, $\frac{1}{4}$ cup	Asparagus, cooked, $\frac{1}{2}$ cup
Apricots, dried, $\frac{1}{4}$ cup	Avocado, $\frac{1}{2}$ cup	Green pepper, cooked, $\frac{1}{2}$ cup
Figs, dried, 5	Honeydew melon, 1 cup	Green beans, cooked, $\frac{1}{2}$ cup
Banana, 1 medium	Grapes, 1 cup	Eggplant, cooked, $\frac{1}{2}$ cup
Cantaloupe, cubed, 1 cup	Mango, 1 medium	Apple, 1 medium
Salmon, Atlantic, wild, cooked, 3 oz	Peaches, 1 cup	Kiwi, 1 medium
Clams, canned, 3 oz	Papaya, 1 cup	Grapefruit, $\frac{1}{2}$
Tuna, yellowfin, cooked, 3 oz	Milk, 1%, 1 cup	Blueberries, $\frac{1}{2}$ cup
Halibut, cooked, 3 oz	Yogurt, fruit, nonfat, 6 oz	Strawberries, $\frac{1}{2}$ cup
Potato, sweet and white, baked	Milk, soy, 1 cup	Plum, 1 small
Squash, acorn, cooked, 1 cup	Salmon, Atlantic, farmed, cooked, 3 oz	Peanut butter, 2 Tbsp
Legumes, cooked, $\frac{1}{2}$ cup (white, lima, soy, northern, pink)	Chicken, breast, cooked, 3 oz	Rice, brown and white, cooked, 1 cup
	Beef, top sirloin, cooked, 3 oz	Bread, whole wheat, 1 slice
	Spinach, raw, 2 cups	
	Spinach, cooked, $\frac{1}{2}$ cup	
	Lentils, cooked, $\frac{1}{2}$ cup	
	Legumes, cooked, $\frac{1}{2}$ cup (pinto, kidney, navy)	

* The United States Department of Agriculture (USDA's) FoodData Central publishes extensive information on nutrient contents of foods. See <https://fdc.nal.usda.gov>. Another source providing a list of potassium-containing foods is available via the National Institutes of Health, Office of Dietary Supplements at <https://www.nal.usda.gov/sites/www.nal.usda.gov/files/potassium.pdf>.

concentration in the lumen, potassium is absorbed by passive diffusion or actively by a K^+/H^+ -ATPase. This pump exchanges intracellular H^+ for luminal K^+ . Alternatively, potassium may be absorbed across the brush border membrane by potassium channels that also serve as secretory pathways in the colon. To enter the blood, the potassium accumulated in the enterocyte diffuses across the basolateral membrane through a potassium channel.

Plasma potassium concentrations are maintained within a narrow range of about 3.5–5.0 mEq (mmol)/L. This ECF concentration is regulated primarily through hormonal and renal functions. The large, rapid rises in plasma potassium concentrations that could routinely occur after eating potassium-rich foods are managed hormonally to a large extent by the action of insulin, which promotes active potassium uptake by hepatic and muscle cells. High intracellular potassium concentrations are maintained by Na^+/K^+ -ATPase pumps. The presence of dietary potassium in the digestive tract is also thought to signal, by an unclear mechanism, the kidneys, which in turn respond by increasing urinary potassium excretion. During the periods between meals, muscles release potassium back into the plasma, renal potassium reabsorption increases, and renal potassium secretion decreases to ensure plasma potassium concentrations remain within the normal range. Conditions disrupting renal (as with renal failure) and hormonal functions can have profound effects on plasma potassium concentrations and body functions.

Functions and Interactions with Other Nutrients

Potassium's intracellular to extracellular ratio is needed for its function in the maintenance of the cell's resting membrane potential. The mineral influences depolarization as well as contractility of excitable tissues, especially of smooth, skeletal, and cardiac muscle and nerve tissue. Potassium's role as the main cation within cells is also central to its function in water balance (discussed earlier in the chapter) and its role in maintaining acid–base balance (discussed at the end of this chapter). Potassium also plays a role in cellular metabolism, where it is needed, along with magnesium, for the activity of pyruvate kinase, which converts phosphoenolpyruvate to pyruvate during glycolysis and is coupled with substrate-level phosphorylation (ATP production). Potassium is thought to be needed to enhance the binding of substrates at the enzyme's active site.

Potassium, like sodium, interacts with calcium and affects the urinary excretion of calcium. However, its effect is opposite to that of sodium; whereas sodium increases calcium excretion, potassium decreases it.

Excretion

Potassium is excreted from the body mainly via the kidneys, with typically only small amounts excreted in the feces (about 195–390 mg) and in the sweat (except under extreme physical activity). However, considerably larger amounts of potassium are lost in the feces with excessive diarrhea or intestinal drainage (as with inflammatory bowel disease or an ileostomy, among other conditions); the increased losses of potassium associated with diarrhea are often secondary, in part, to high aldosterone secretion (which is released to reduce urinary water and sodium losses, but which promotes potassium loss).

Most potassium in the glomerular filtrate is actively reabsorbed in the proximal tubule; this reabsorption is unregulated. In the distal and collecting tubules, potassium can be actively secreted into the filtrate or reabsorbed as needed. Reabsorption of potassium in these regions is mediated by increased H^+/K^+ -ATPase activity. Urinary potassium excretion is reduced when potassium needs to be conserved, as with lower intakes (or with high losses from diarrhea, vomiting, etc.) and lower plasma concentrations. Most potassium that is excreted in the urine entered the filtrate via secretion associated with increased Na^+/K^+ -ATPase activity, increased tubular flow, and increased aldosterone. Urinary potassium rises with higher potassium intakes and thus higher plasma concentrations in the peritubular capillaries. Aldosterone release (which as discussed earlier is stimulated by increased plasma potassium concentrations along with decreased plasma sodium, increased angiotensin II, and decreased natriuretic peptides) enhances sodium reabsorption and stimulates potassium secretion. Changes in pH also influence potassium excretion, with acidosis reducing excretion and alkalosis increasing potassium excretion.

Recommendations, Deficiency, Toxicity, and Assessment of Nutriture

The Adequate Intake (AI) recommendations for potassium are 2,600 mg for adult females and 3,400 mg for adult males per day; these recommendations, which are based on median intakes from national surveys, represent a reduction from the previous AI for potassium of 4,700 mg [2]. Recommendations for potassium for other population groups are provided on the inside front cover of the book. Potassium intakes in the United States by adult females and males are about 2,300 mg and 3,000 mg, respectively, and below recommended intakes [5]. No CDRR recommendation for potassium was established due to insufficient evidence to characterize the effect of potassium on disease risk; however, the lack of a CDRR is not reported to indicate that potassium does not affect chronic diseases [5].

While dietary intake of potassium by many Americans is less than the AI, potassium deficiency most often results from situations (such as excessive vomiting and diarrhea) causing profound losses of fluids and electrolytes, like potassium, with corresponding reductions in the plasma's potassium concentration (referred to as **hypokalemia**). Additionally, a magnesium deficiency, which promotes potassium excretion, and the use of some medications such as thiazide and loop diuretics (used to treat high blood pressure) and glucocorticoids (used to suppress inflammation, for example) increase urinary potassium excretion and may cause deficiency. Increased urinary potassium losses can also result from various nephropathies. Furthermore, reductions in plasma potassium may occur from increased distribution of potassium into cells. This latter situation sometimes occurs as part of refeeding syndrome, a condition that can develop when insufficient potassium is provided in the nutritional treatment of those with malnutrition.

Some manifestations of hypokalemia (especially when potassium concentrations drop below about 3.0 mEq/L) include muscle weakness, lethargy, ascending paralysis, cardiac arrhythmias, and possibly death. The reduction in potassium leads to changes in resting membrane potential and excitability, with lower plasma potassium concentrations associated with membrane hyperpolarization and reduced tissue excitability. Treatment usually requires potassium supplementation as potassium phosphate, bicarbonate, or citrate.

Insufficient dietary potassium intakes, especially when coupled with high dietary sodium intakes, have been linked with increased risks for hypertension and stroke (see this chapter's Perspective). Because of these well-documented associations, the FDA has approved the health claim "Diets containing foods that are a good source of potassium and low in sodium may reduce the risk of high blood pressure and stroke" [3]. To use this claim, a food must contain at least 10% Daily Value of potassium; must contain ≤ 140 mg of sodium, ≤ 3 g of total fat, ≤ 1 g of saturated fat, and ≤ 20 mg of cholesterol; and must provide $\leq 15\%$ of energy (kcal) from saturated fat [3]. For food labeling purposes, to be considered a "rich," "excellent," or "high" source of potassium (or any other nutrient), a food must contain 20% or more of the Daily Value; to be a "good" source of potassium, the food must contain 10–19% of the Daily Value [3]. Inverse associations between dietary potassium intake and risk of kidney stones have also been reported; however, more research is required to determine if supplemental potassium is effective in reducing risk [6].

While no Tolerable Upper Intake Level has been established for potassium from foods, the use of potassium supplements should only be under the supervision of medical personnel due to risk of **hyperkalemia**, a high blood/plasma potassium concentration. While hyperkalemia is usually associated with renal failure (since it is the kidneys

that are responsible for eliminating excess potassium from the body), the use of potassium supplements in extremely large doses may also cause such problems if renal function is reduced. Hyperkalemia, like hypokalemia, impacts resting membrane potential and tissue excitability, specifically reducing both. Heart function is negatively affected (as with hypokalemia), resulting in many types of arrhythmias and possible death. Some additional manifestations of hyperkalemia (especially when concentrations exceed about 5.5 mEq/L) include muscle weakness, twitching and cramping, and ascending paralysis.

Potassium status is typically assessed based on plasma/serum potassium concentrations, although plasma concentrations do not always correlate with tissue potassium concentrations. Both depletion and excess potassium in the blood can be lethal.

12.8 CHLORIDE

Chloride is the most abundant anion (at 88%) in the ECF; about 12% is found intracellularly. Chloride's negative charge neutralizes the positive charge of the Na^+ with which it is usually associated. In this respect, it is of great importance in maintaining electrolyte balance. Total body chloride content is similar to that of sodium, representing about 0.15% of body weight, or about 105 g in a 70-kg human.

Sources

Nearly all the chloride consumed in the diet is associated with sodium in the form of sodium chloride, or salt. Salt, which is about 60% chloride, is abundant in a large number of foods, particularly in snack items and processed foods. Chloride is also found in eggs, fresh meats, and seafood. The average adult consumes an estimated 2,000–8,000 mg (2–8 g) of chloride each day. The Daily Value for chloride, used on supplement and nutrition labels, is 2,300 mg.

In addition to chloride obtained from food ingestion, the secretions of the digestive tract also contain appreciable amounts of chloride. The chloride content of gastric juice is greatest (about 130 mEq/L); the chloride content of bile and intestinal secretions is each about 100 mEq/L, with lesser amounts in pancreatic secretions and colonic secretions (about 75 mEq/L and 40 mEq/L, respectively). Much of this chloride is absorbed.

Absorption, Secretion, and Transport

Chloride is almost completely absorbed in the intestines. Its absorption closely follows that of sodium for the establishment and maintenance of electrical neutrality and osmotic balance. In the Na^+ /glucose (nutrient) cotransport system (described in the section on sodium), chloride

follows the actively absorbed Na^+ . The absorbed Na^+ creates an electrical gradient that provides the drive for the accompanying inward diffusion of Cl^- between cells. In the electroneutral Na^+/Cl^- cotransport absorption system, chloride is absorbed in exchange for bicarbonate as sodium is absorbed in exchange for H^+ . In the electrogenic Na^+ absorption mechanism, chloride follows the absorbed sodium passively. Thus, regardless of the absorptive mechanism, basically wherever sodium goes, chloride is not far behind to maintain neutrality and osmotic balance.

Secretory mechanisms for the electrolytes, especially chloride, potassium, and bicarbonate, are also found throughout the gastrointestinal tract. One mechanism for intestinal chloride secretion is active and electrogenic (Figure 12.6). Intestinal cells take up chloride from the blood across the basolateral membrane by way of a $\text{Na}^+/\text{K}^+/\text{Cl}^-$ cotransport pathway. Chloride accumulating in the enterocyte exits via a Cl^- channel across the brush border membrane into the lumen; potassium channels on the basolateral membrane allow the potassium present in the enterocyte to reenter the blood.

Intestinal cells (especially those in the crypt) also secrete chloride into the lumen via a cyclic AMP-dependent chloride channel called the cystic fibrosis transmembrane conductance regulator (CFTR). In this pathway, activation of adenylyl cyclase and production of cyclic AMP stimulate the CFTR, which secretes the chloride through a channel into the lumen. Mutations in the *CFTR* gene result in the disorder cystic fibrosis. However, dysfunction of this channel may also occur secondary to the presence of bacterial toxins, such as the cholera toxin. Toxins, which activate adenylyl cyclase and elevate cAMP, cause these channels to continuously release chloride accompanied by sodium and water (fluid) into the lumen, resulting in severe diarrhea and ultimately dehydration. Other channels also enable chloride secretion. Secretion of chloride in some tissues may be calcium-induced and occur via calcium-activated Cl^- conductance and by ligand-gated anion channels, among other means. Cl^- channels in colonic cells also

provide for chloride transport. The secretion of chloride may be accompanied by sodium secretion (both paracellularly and transcellularly) and water movement via osmosis.

Functions

Chloride has multiple functions in addition to its role along with sodium in maintaining fluid balance. The formation of gastric hydrochloric acid requires chloride, which is secreted along with protons from the parietal cells of the stomach. Chloride is released by white blood cells during phagocytosis to assist in the destruction of foreign substances. Also, chloride acts as the exchange anion for HCO_3^- in red blood cells. This process, sometimes called the *chloride shift* and shown later in Figure 12.7, allows the transport of tissue-derived CO_2 back to the lungs in the form of plasma HCO_3^- .

Excretion

Chloride excretion occurs through three routes—the gastrointestinal tract, the skin, and the kidneys—with losses through each route closely reflecting those of sodium. Excretion of chloride via the feces is normally minimal, approximately 35–70 mg/day. Losses through the skin are similar quantitatively to sodium losses, that is, normally quite small except in cases of high temperature and vigorous exercise. The major route of chloride excretion is through the kidneys, where it is primarily regulated indirectly with sodium. Sodium reabsorption promotes paracellular chloride reabsorption, although some chloride is also reabsorbed by active transcellular mechanisms. About two-thirds of chloride reabsorption occurs in the proximal tubule and another 20–25% occurs by both passive transport and active sodium-coupled transport in the loop of Henle. The remaining chloride is reabsorbed in the distal tubule and collecting ducts via sodium-coupled transport. Chloride secretion can also occur (with sodium) as needed to maintain ECF.

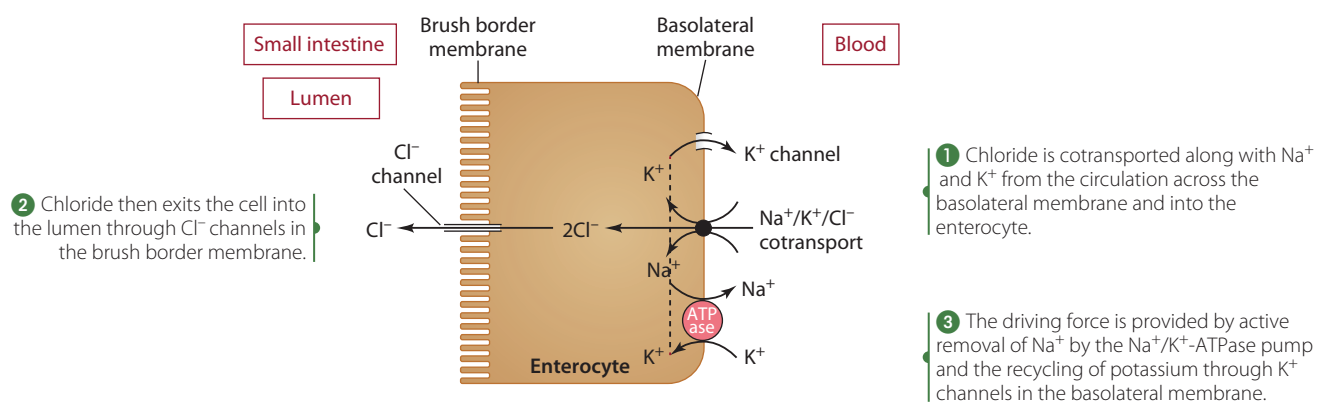


Figure 12.6 One mechanism for intestinal chloride secretion.

Recommendations, Deficiency, Toxicity, and Assessment of Nutriture

The Adequate Intake recommendation for chloride for adults is 2,300 mg (65 mmol) per day [2]. Additional recommendations for chloride for other age groups are provided on the inside front cover of the book. A Tolerable Upper Intake Level for chloride is 3,600 mg (100 mmol) [2].

Dietary deficiency of chloride does not occur under normal conditions. As is the case for the other electrolytes, imbalances and deficiency arise chiefly through gastrointestinal tract disturbances such as severe diarrhea and vomiting. The condition results in weakness, lethargy, and metabolic acidosis. Convulsions may also occur with chloride deficiency.

Chloride status is assessed through evaluation of its concentration in the serum, which usually ranges from about 101 to 111 mEq/L. However, like that of other serum solutes, chloride concentration depends on the plasma volume. It is possible, for example, for total body chloride to be diminished but plasma chloride concentrations to appear normal or even be elevated (if body water was lost with the chloride loss).

12.9 ACID–BASE BALANCE: CONTROL OF HYDROGEN ION CONCENTRATION

The maintenance of the hydrogen ion concentration in body fluids within a narrow range is vital to normal physiological function. In fact, acid–base (pH) regulation is one of the most important aspects of homeostasis since even slight deviations from normal can cause marked alterations in enzyme-catalyzed reaction rates, cellular uptake and regulation of metabolites and minerals, and oxygen uptake and release from hemoglobin. Because the body regulates acid–base balance in conjunction with fluid–electrolyte balance, it is addressed in this chapter.

The degree of acidity or alkalinity of fluids is determined by its concentration of protons (H^+). The **hydrogen ion** concentration in body fluids is generally quite low, regulated at approximately 4×10^{-8} mol/L, with a range of a low of about 1.0×10^{-8} mol/L to a high of about 1.0×10^{-7} mol/L; values outside this range are not compatible with life.

Because expressing H^+ in terms of its actual concentration is awkward, the concept of pH, which is the negative logarithm of the H^+ concentration, was devised to simplify the expression. It enables concentrations to be expressed as whole numbers rather than as negative exponential values: $pH = -\log[H^+]$, with bracketed values symbolizing concentrations. Throughout this discussion, this designation

is used to signify concentrations of substances other than protons. The pH of ECF, in which the H^+ concentration may be assumed to be approximately 4×10^{-8} mol/L, can therefore be calculated as follows:

$$\begin{aligned} pH &= -\log(4 \times 10^{-8}) \\ \text{or} \quad pH &= \log(1/(4 \times 10^{-8})) \\ (\text{dividing}) \quad pH &= \log(0.25 \times 10^8) \\ pH &= \log 0.25 + \log 10^8 \\ (\text{taking logs}) \quad pH &= -0.602 + 8 \\ pH &= 7.4 \end{aligned}$$

As the molar concentration of H^+ becomes smaller and the value of the negative exponent of 10 becomes larger, the pH correspondingly increases. High acidity is associated with high H^+ concentration and low pH; the term *acidosis* refers to a rise in extracellular (principally plasma) H^+ concentration beyond the normal range. Low acidity is associated with low H^+ concentration and high pH and, when referring to the plasma, is often called *alkalosis*.

An acid, as it relates to fluid acid–base regulation, may be defined as a substance capable of releasing protons (H^+). The metabolism of the macronutrients continuously generates organic acids such as lactic and pyruvic acids (see Chapter 3) and acetoacetic acid and β -hydroxybutyric acid (see Chapter 5), which must be neutralized. Acidic salts of sulfuric and phosphoric acids are also generated metabolically from sulfur- and phosphorus-containing substances, respectively. Additionally, carbon dioxide, the product of the complete oxidation of energy nutrients, is itself indirectly acidic because, on combination with H_2O , it forms carbonic acid (H_2CO_3). To guard against such fluctuations in pH,

- chemical buffer systems operate in the blood and in cells,
- the lungs respond through changes in respiration, and
- the kidneys respond through changes in substrate excretion and reabsorption.

In addition to these systems, the bones serve as a “bank,” accepting and releasing cations and anions to facilitate balance.

The chemical buffer systems in the blood and cells are the quickest to respond to changes in pH, working typically in less than a minute to remove H^+ from the environment or to release H^+ into the environment as needed to reestablish the appropriate pH. The actions of the lungs, which serve to eliminate or retain carbon dioxide, CO_2 (and therefore carbonic acid, H_2CO_3), usually take minutes to hours to respond. The actions of the kidneys, which function in the secretion of H^+ , the reabsorption and

regeneration of bicarbonate (HCO_3^-), and the production of ammonia enabling ammonium ion formation, are the slowest, requiring hours to days, to respond.

Chemical Buffer Systems

A buffer is a substance that reversibly binds protons. In the body, buffers function to resist changes in pH despite the addition of acids or bases to the environment. An explanation of buffers is presented in the boxed feature. A discussion of the body's chemical buffer systems follows.

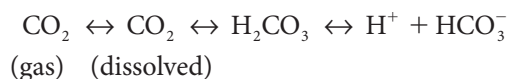
The Body's Chemical Buffers

Several physiologically important chemical buffers maintain the narrow pH range of ECF at approximately 7.35–7.45. One such buffer is the bicarbonate (HCO_3^-)–carbonic acid (H_2CO_3) system. Additional buffers that help to maintain acid–base (pH) balance include hemoglobin, other proteins present in cells as well as in ECF, and the phosphate system found mainly in cells. Additionally, potassium (K^+) plays a role through ion exchange with H^+ between compartments.

Bicarbonate–Carbonic Acid The bicarbonate–carbonic acid buffer system is composed of the weak acid carbonic acid (H_2CO_3) and its salt or conjugate base, the bicarbonate ion (HCO_3^-). The carbonic acid dissociates reversibly into H^+ and HCO_3^- , as shown here:



The buffering capacity of this reaction arises from the fact that either added protons or added hydroxide ions will be neutralized by corresponding shifts in the equilibrium. The H_2CO_3 can be formed both from the acidification of HCO_3^- (i.e., $\text{H}^+ + \text{HCO}_3^-$) and from the reaction of dissolved CO_2 with water (i.e., $\text{CO}_2 + \text{H}_2\text{O}$). The lungs regulate the CO_2 and the kidneys control HCO_3^- . The overall reaction involving carbon dioxide, carbonic acid, and bicarbonate ion (shown later in Figures 12.7 and Figure 12.8) is:



Normally, the ratio of the concentration of HCO_3^- to H_2CO_3 in plasma is 20:1, and the apparent pK_a value for

PRINCIPLES OF BUFFERS

BUFFERS EXIST AS PAIRS, consisting usually of a weak acid (represented as HA) and its conjugate base (A^-). The conjugate base is the residual portion of the acid following the release of the proton (H^+). The conjugate base of a weak acid is basic because it tends to attract a proton and to regenerate the acid. The dissociation of a weak acid and the reunion of its conjugate base and proton comprise an equilibrium system:



The equilibrium expression for this reaction, called the acid dissociation constant (K_a), is represented as

$$K_a = \frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]}$$

The equation can be rearranged to

$$[\text{H}^+] = \frac{K_a[\text{HA}]}{[\text{A}^-]}$$

Taking the negative logarithm of both sides of the equation gives

$$-\log [\text{H}^+] = -\log K_a - \log \frac{[\text{HA}]}{[\text{A}^-]}$$

These values become

$$\text{pH} = \text{pK}_a + \log \frac{[\text{A}^-]}{[\text{HA}]}$$

This equation, referred to as the Henderson-Hasselbalch equation, shows how a buffer system composed of a weak acid and its conjugate base resists changes in pH if a strong acid or base is added to the system. For example, if the molar concentrations of the conjugate base and the acid are equal, then the ratio of $[\text{A}^-]$ to $[\text{HA}]$ is 1.0, and the logarithm of this ratio is 0, making the pH of the system equal to the pK_a of the acid.

The pK_a , which is the negative logarithm of the acid dissociation constant (K_a), of any weak acid is a constant for that particular acid and simply reflects its strength (i.e., its tendency to release a proton). If a strong acid or a strong base is added to this system, the ratio of $[\text{A}^-]$ to $[\text{HA}]$

changes and therefore the pH changes, but only slightly. Suppose, for example, that both the conjugate base and the free acid are present at 0.1 mol/L concentrations and suppose also that the pK_a of the acid is 7.0. As shown previously, if the ratio is 0.1:0.1, the pH is 7.0. The addition of enough hydrochloric acid (a strong acid) to the buffer in the example to make its final concentration 0.05 mol/L shifts the equilibrium to the left (to make HA). The 0.05 mol/L of H^+ (from the fully dissociated HCl) will combine with an equal molar amount of A^- to form HA. The new $[\text{A}^-]$ concentration therefore becomes 0.05 mol/L ($0.1 - 0.05$) and $[\text{HA}]$ is 0.15 mol/L ($0.1 + 0.05$). The logarithm of this new ratio (0.05:0.15, or 0.33) is -0.48 . Inserting this value into the Henderson-Hasselbalch equation, we can see that the pH decreases by only 0.48. In other words, the pH decreased from 7.0 to 6.52 by making the system 0.05 mol/L hydrochloric acid. In contrast, this same concentration of HCl in an unbuffered, aqueous solution would produce an acid pH between 1.0 and 2.0.

H_2CO_3 is 6.1. Using the Henderson-Hasselbalch equation, we can also show how a normal plasma pH of 7.4 results from these values:

$$\begin{aligned}\text{pH} &= \text{pK}_a + \log \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \\ &= 6.1 + \log \frac{20}{1} \\ &= 6.1 + 1.3 \\ \text{pH} &= 7.4\end{aligned}$$

Alterations in the 20:1 ratio of $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$ change the pH, as discussed later in the sections on the respiratory and renal regulatory systems.

Proteins and Hemoglobin Proteins are found both within the blood and within cells. As **amphoteric** substances (substances that possess both acidic and basic groups), proteins are capable of neutralizing acids or bases due to the presence of carboxylic acid ($\text{R}-\text{COOH}$) and amino ($\text{R}-\text{NH}_3^+$) groups, which dissociate as:

1. $\text{R}-\text{COOH} \leftrightarrow \text{R}-\text{COO}^- + \text{H}^+$
2. $\text{R}-\text{NH}_3^+ \leftrightarrow \text{R}-\text{NH}_2 + \text{H}^+$

At physiological pH, the carboxylic acid is largely dissociated into its conjugate base ($\text{R}-\text{COO}^-$) and a proton (H^+), so the equilibrium is shifted strongly to the right. At that same pH, however, the amino group, being much weaker as an acid (a stronger base), is only weakly

dissociated, and its equilibrium greatly favors to the left. If protons, in the form of a strong acid, are added to a protein solution, they are neutralized by reaction 1 because their presence will cause a shift in the equilibrium toward the undissociated acid (right to left). Strong bases, as contributors of hydroxide (OH^-) ions, will likewise be neutralized because, as they react with the protons to form water, the equilibrium of reaction 2 shifts to the right to restore the protons that were neutralized.

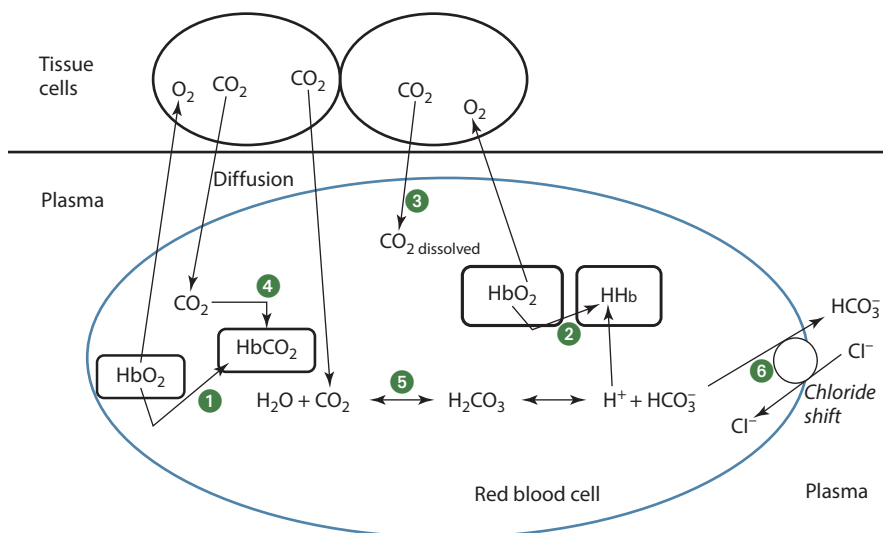
The protein hemoglobin (Hb) serves as an important physiological buffer because of its high concentration in red blood cells and its ability to bind H^+ , forming HHb, a weak acid. Hemoglobin's binding of H^+ is shown and described further in Figure 12.7.

Phosphate The phosphate buffer system operates mainly within cells (only small amounts are present in ECF). The system consists of dibasic phosphate, HPO_4^{2-} , and monobasic phosphate, H_2PO_4^- , complexed with sodium as Na_2HPO_4 and NaH_2PO_4 , respectively. In the kidneys, filtered HPO_4^{2-} reacts with H^+ in the filtrate as:



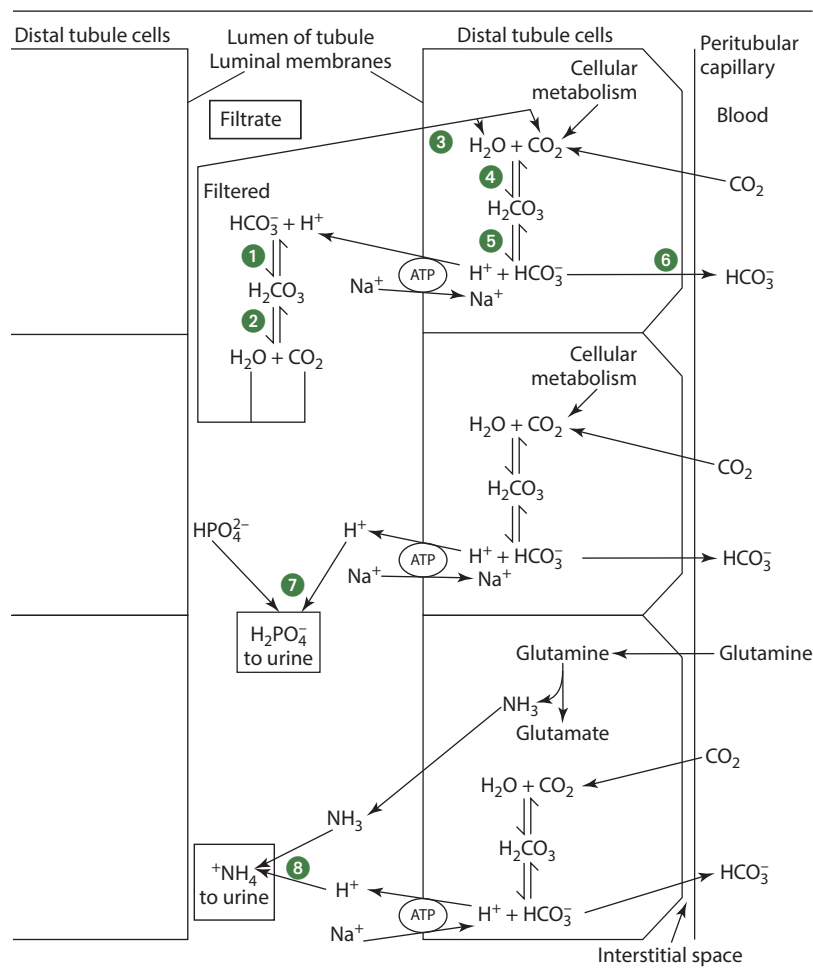
The H_2PO_4^- (NaH_2PO_4) is then excreted in the urine, and thus enables the removal of “bound H^+ ” from the body. (See also Figure 12.8.)

Potassium Potassium helps maintain acid–base balance as part of a cellular ion exchange system. The electrolyte shifts in and out of cells in exchange for H^+ as needed



In red blood cells (rbc), as Hb releases oxygen (O_2) to tissues, it can react ① with carbon dioxide (CO_2) and ② with hydrogen ions (H^+) and serve as a buffer. The CO_2 in the rbc enters via diffusion from tissues and may ③ remain dissolved, or ④ bind to Hb, or ⑤ react with water forming carbonic acid (H_2CO_3). The latter then dissociates into H^+ and HCO_3^- . The HCO_3^- is then ⑥ released into the plasma in exchange for chloride (Cl^-) in a process referred to as the chloride shift. The bicarbonate–carbonic acid buffer system, like Hb, is important for acid–base balance in cells and the blood.

Figure 12.7 Red blood cell hemoglobin (Hb) as a buffer for hydrogen ions and the release of the buffer bicarbonate (HCO_3^-).



Filtered bicarbonate, HCO_3^- , is conserved by its ability to react with secreted H^+ in the tubule lumen ① through a reaction catalyzed by carbonic anhydrase (found on the luminal membrane), to form carbonic acid, H_2CO_3 .

② The carbonic acid dissociates in the filtrate into H_2O and CO_2 .

③ Both H_2O and CO_2 diffuse into the tubule cell.

④ The H_2O and CO_2 in the tubule cell form carbonic acid, H_2CO_3 , through the action of intracellular carbonic anhydrase.

⑤ The carbonic acid, H_2CO_3 , then dissociates in the tubule to form “new” bicarbonate, HCO_3^- .

⑥ The bicarbonate next enters the peritubular capillary blood, which may occur in exchange with chloride.

⑦ HPO_4^{2-} which is present in the filtrate when plasma phosphate concentrations are elevated can also react with secreted H^+ to form monobasic phosphate, H_2PO_4^- ; this compound is excreted in the urine.

⑧ Ammonia, which is generated in the tubule cells by the glutamine catabolism, enters the filtrate where it can also react with secreted H^+ to form an ammonium ion, $^+\text{NH}_4$. The ammonium ion is subsequently excreted in the urine.

Figure 12.8 Acid–base balance by the kidneys.

based on blood pH. With acidosis (blood pH <7.35), K^+ moves out of cells and into the plasma and H^+ moves out of the plasma and into cells. The movement reduces the blood H^+ concentration, and thus increases its pH. Conversely, with alkalosis (blood pH >7.45), K^+ moves into cells and H^+ moves out of cells into the plasma. This movement increases the blood H^+ concentration, and thus decreases its pH. The resulting changes in serum potassium

concentrations in these circumstances, however, can be life-threatening if too substantial.

Respiratory Regulation

When the chemical buffer systems are not sufficiently managing acid–base balance, the lungs can respond within minutes, acting as a regulator of the $\text{HCO}_3^-/\text{H}_2\text{CO}_3$ system

by retaining or exhaling CO_2 . Increases in respiration are triggered by both rises in blood H^+ and rises in CO_2 concentrations. Increases in blood H^+ trigger an increase in respiration, and thus the exhalation of more CO_2 (i.e., more CO_2 -generated H^+ if one thinks of CO_2 as acid/ H_2CO_3 -forming). Breathing rate is restored (slowed) as more CO_2 is exhaled and less is present in the blood. Similarly, if plasma levels of CO_2 rise, perhaps because of accelerated metabolism, more H_2CO_3 is formed. This reaction, in turn, causes a fall in pH as the acid dissociates to release protons. The elevated CO_2 itself, as well as the resulting increase in H^+ concentration, is detected by the respiratory center of the brain, resulting in an increase in the respiratory rate. This hyperventilation increases CO_2 loss through the lungs substantially and therefore decreases the amount of H_2CO_3 . This mechanism increases the ratio of HCO_3^- to H_2CO_3 by reducing H_2CO_3 , thus elevating the pH to a normal value. Conversely, if plasma pH rises for any reason (because of either an increase in HCO_3^- or a decrease in H_2CO_3), the respiratory center is signaled accordingly and causes a slowing of the respiration rate. As CO_2 then accumulates, the H_2CO_3 concentration rises and the pH decreases.

Renal Regulation

In contrast to the more rapid actions of the chemical buffer systems and the lungs, the kidneys take hours or days to effect change in pH. They act mainly by controlling the secretion of H^+ , by conserving or producing HCO_3^- , and by synthesizing and excreting ammonia, which reacts with H^+ to form ammonium ions for excretion. Filtered phosphate also facilitates H^+ excretion.

With consumption of a normal diet, about 50–100 mEq of H^+ ions are generated daily and must be removed from the body to prevent a progressive metabolic acidosis. Filtered H^+ ions are not reabsorbed. The proximal, distal, and collecting tubules also actively secrete H^+ , removing it from the blood in the peritubular capillaries and into the filtrate. The H^+ s in the filtrate are used initially to form carbonic acid needed for bicarbonate conservation (as described later in this section). Any additional H^+ get excreted in the urine typically in association with ammonia or phosphates. The pH of the urine is usually around 6, with a range from about 4.5 to 6.5.

The presence of substances in the filtrate to combine with the H^+ enables the excretion of more H^+ and

minimizes direct effects on urine pH. Two substances functioning in this capacity (“neutralizing or buffering” the H^+) include dibasic phosphate (HPO_4^{2-} , discussed in the section on phosphate buffers) and ammonia (NH_3) (Figure 12.8). In the renal tubule cells, ammonia, which is generated from glutamine degradation by glutaminase, diffuses into the tubular lumen of the collecting ducts where it combines with H^+ to form ammonium ions ($^+\text{NH}_4$). The ammonium ions remain in the filtrate and are excreted primarily as chloride salts in the urine.

Bicarbonate is critical for acid–base balance. Renal conservation (production) of bicarbonate occurs by an indirect process (shown in Figure 12.8) because of bicarbonate’s relatively large size and charge. Briefly, in the filtrate, H^+ and HCO_3^- form H_2CO_3 by the action of carbonic anhydrase. The H_2CO_3 then disassociates into CO_2 and H_2O . The CO_2 and H_2O diffuse into the tubular cell, where carbonic anhydrase enables H_2CO_3 formation. The relatively high tubular cell pH allows the dissociation of the H_2CO_3 into HCO_3^- and H^+ , after which the bicarbonate can reenter peritubular capillary blood and the H^+ can be actively secreted by the Na^+/H^+ carrier back into the filtrate. The net result enables H^+ secretion and bicarbonate “reabsorption,” even though it is not the same bicarbonate ion.

Like respiratory regulation, renal regulation of pH is directed at maintaining a normal ratio of $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$. In alkalosis, for example, in which the plasma ratio of $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$ increases as the pH rises above 7.4, a net increase occurs in the excretion of bicarbonate. This increase occurs because the high extracellular HCO_3^- concentration increases its filtration, while the relatively low concentration of H_2CO_3 decreases the secretion of H^+ . Therefore, the fine balance between HCO_3^- and H^+ that normally exists in the tubules no longer is in effect. Also, because no HCO_3^- ions can be reabsorbed without first reacting with H^+ (Figure 12.8), all the excess HCO_3^- passes into the urine, neutralized by sodium ions or other cations. In effect, therefore, HCO_3^- is removed from the ECF, restoring the normal ratio of HCO_3^- to H_2CO_3 and pH. In acidosis, the ratio of plasma $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$ decreases, meaning that the rate of H^+ secretion rises to a level far greater than the rate of HCO_3^- filtration into the tubules. As a result, most of the filtered HCO_3^- is converted to H_2CO_3 and reabsorbed as CO_2 (Figure 12.8), while the excess H^+ is excreted in the urine. As a consequence, the ECF ratio of $[\text{HCO}_3^-]$ to $[\text{H}_2\text{CO}_3]$ increases, as does the pH.

SUMMARY

Maintaining body fluids and electrolytes is vitally important for health and nutrition.

- Intracellular fluid provides the environment for myriad metabolic reactions that take place in cells.
- The interstitial fluid compartment of the ECF mass allows nutrients to migrate into cells from the bloodstream and metabolic waste products from the cells to return to the bloodstream.

These fluids contain the electrolytes, dissolved minerals that have important physiological functions. Electrolyte concentrations and their intracellular and extracellular distribution must be precisely regulated.

- The mechanism for achieving this regulation is exerted largely through the kidneys, which also enable homeostatic maintenance of fluid volume.

Fluid volume control by the kidneys is mostly hormone mediated.

- Vasopressin, produced in the hypothalamus, stimulates the tubular reabsorption of water from the glomerular filtrate.
- Aldosterone, a product of the adrenal cortex, increases the reabsorption of sodium, which indirectly stimulates vasopressin release through the resulting rise in ECF osmotic pressure.

Thirst centers in the brain, which respond to fluctuations in blood volume or ECF osmolality, are also important regulators of fluid balance by their influence on the amount of fluid intake.

Sodium, potassium, and chloride are freely filtered by the glomerulus but are selectively conserved by tubular reabsorption through active transport systems.

Normal physiological functions depend on the body's fluid and acid–base balance.

- The plasma is well buffered against changes in pH, primarily by proteins and by the bicarbonate–carbonic acid system.
- Restoration of normal pH is accomplished through compensatory mechanisms of the kidneys and lungs, which function to maintain a normal ratio of bicarbonate to carbonic acid.
- The bicarbonate concentration is under the control of the kidneys, which can either conserve the ion by reabsorbing it to a greater extent or increase its excretion.
- The lungs control carbon dioxide and thus carbonic acid concentrations with hyperventilation “blowing off” carbon dioxide to increase pH and hypoventilation (or a slowing of respiration) retaining carbon dioxide and therefore the carbonic acid level to decrease pH.

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Perspective

MACROMINERALS AND HYPERTENSION

Hypertension (high blood pressure), diagnosed when systolic and/or diastolic blood pressure values are $\geq 130/80$ mm Hg, respectively, affects close to 30% of adults in the United States. The causes of hypertension are often unknown (and designated as essential hypertension); in other cases, the condition results from disorders affecting the kidneys, endocrine, or neurologic system (and designated as secondary hypertension). Yet, no matter whether it is essential or secondary, hypertension is a major risk factor for stroke as well as for cardiovascular disease. The risk for heart disease increases progressively as blood pressure increases above 115/75 mm Hg [1,2]. In adults, a 2 mm Hg reduction in blood pressure can reduce mortality secondary to stroke by 6% and secondary to heart disease by 4% [3].

Although some risk factors for hypertension are not controllable (e.g., genetic predisposition, race, aging), others can be modified by a person's commitment to lifestyle changes that include diet modifications. Yet, because hypertension is a heterogeneous disease with a variety of precipitating factors, dietary modifications work for some but not all individuals with hypertension. This Perspective focuses on some dietary interventions for the treatment and prevention of hypertension in adults.

SODIUM

Sodium (as salt—sodium chloride) was one of the first nutrients directly linked to hypertension. A plethora of studies has been conducted over several decades examining salt and/or sodium intake and blood pressure across and within population groups. While study designs, time periods, and subjects included and excluded have varied, as have the means used to assess sodium intake (analysis of diet or urinary sodium excretion), overall evidence typically shows dietary sodium

consumption is directly related to blood pressure [1]. It is also linked to risks for stroke and cardiovascular disease. Studies indicate relatively consistently that reductions in sodium intake are associated with the lowering of systolic blood pressure in normotensive individuals (about -2 to -3 mm Hg), and to a greater extent in those with hypertension (about -5 mm Hg) [1]. Some adults (as many as 30–50%) exhibit a more pronounced direct blood pressure response to sodium ingestion than others; these individuals (considered to be “sensitive to sodium”) frequently include those who are obese, African American, have diabetes, are older than 65 years of age, and have higher blood pressure.

The current American College of Cardiology/American Heart Association Guidelines for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults recommend a sodium intake (for both the prevention and treatment of hypertension) of 1,500 mg per day (1,500 mg is also the AI for sodium) [1,2,4]. The CDDR for sodium is 2,300 mg [4]; individuals consuming diets in excess of 2,300 mg sodium are encouraged to reduce intakes to at least 2,300 mg and, ideally, to 1,500 mg. Individuals with hypertension and taking hypertensive medications also benefit from sodium reduction.

The Dietary Approaches to Stop Hypertension (DASH) sodium trials provided some of the most convincing evidence that dietary changes could positively impact blood pressure. More specifically, the studies, which compared the effects of the DASH diet with three levels of sodium intake (3.3 g, 2.4 g, and 1.5 g) with the effects of a control diet, showed that additional blood pressure reduction could be achieved through reduction in dietary sodium to 1.5 g/day [5,6]. The DASH diet is discussed in more detail later in the Perspective. Further details on foods high in sodium are provided in this chapter's

section on sources of sodium. Food labels provide valuable information to help consumers identify the sodium content of foods, although changing dietary behaviors typically takes more time and further education.

POTASSIUM

While deficits in potassium intake augment the effects of excess sodium in the pathogenesis of hypertension, the relationship between potassium intake and blood pressure has also been shown to be independent of sodium. Higher potassium intakes typically reduce blood pressure, especially in those with hypertension, those ingesting large amounts of sodium, and those who are salt sensitive [1]. Blood pressure reductions of about 4–5 mm Hg have been reported in those with hypertension ingesting supplemental potassium (about 1,380 mg); ingestion of similar amounts of potassium by normotensive individuals has also resulted in small (about 2 mm Hg) reductions in blood pressure [1]. Even greater reductions in blood pressure with potassium supplementation have been reported in individuals who were also consuming larger amounts of sodium [1].

The Adequate Intake recommendations for potassium are 2,600 mg for adult females and 3,400 mg for adult males per day [4]. The current Guidelines for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults recommend potassium intakes of 3,000–5,000 mg per day [1].

While most studies investigating the effects of potassium on blood pressure provided potassium as supplements (typically potassium chloride), ingestion of potassium from foods along with reductions in dietary sodium is also effective in reducing blood pressure. Following the DASH diet plan (discussed in more detail later in the Perspective) or a DASH-type diet that is rich in fruits

and vegetables, and that includes other potassium-rich foods such as legumes, nuts, seeds, and low-fat dairy products, may enable individuals to improve their dietary potassium intake and reduce blood pressure.

OTHER DIETARY AND LIFESTYLE FACTORS

In addition to reducing the intake of sodium and increasing the intake of potassium, the current Guidelines for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults also include recommendations on alcohol consumption and weight loss for those who are overweight or obese [1]. These recommendations along with information on the DASH diet plan are addressed hereafter.

Alcohol

A direct dose-dependent relationship has been demonstrated between blood pressure and alcohol consumption (especially with ingestion of six or more drinks per day) [1]. Significant reductions in both systolic and diastolic blood pressure result from restricting alcohol intake in those consuming three or more drinks per day [1]. Recommendations to lower blood pressure suggest moderation of alcohol intake (among those who drink); moderation is defined as <1 drink/day for women and <2 drinks/day for men [1]. A drink is defined as 12 oz beer, 5 oz wine (12% alcohol), or 1.5 oz of 80-proof distilled spirits.

Weight Loss

A direct relationship has been established in numerous studies between hypertension and excessive body weight (overweight and obesity). Moreover, weight loss is associated with reductions in blood pressure (about 1 mm Hg per kg body weight lost) and is thus encouraged for those who are overweight or obese and who have hypertension or an elevated blood pressure [1]. Increased physical activity combined with energy (kcal) restriction are recommended to promote weight loss in individuals with hypertension.

Lifestyle

Some lifestyle approaches have also been shown to be of benefit in the prevention and treatment of hypertension. The

DASH trials found that low-fat diets rich in fruits, vegetables, and low-fat dairy products were more effective in reducing blood pressure than a control diet low in fruits and vegetables and with an average fat content of ~36% of kcal. The DASH diet (2000 kcal) plan includes 8–10 servings of fruits and vegetables, 6–8 servings of grains/grain products, 2–3 servings of low-fat dairy products, and limits meat, fish, and poultry to <6 oz daily; red meat, fats, and sugar-sweetened foods and beverages are also limited. Consumption of beans, nuts, and seeds is recommended, with an intake of up to 5 servings/week. The sodium content of the initial DASH diet was about 3.3 g, but lowering dietary sodium further (to 1.5 g) was shown to provide greater benefits. Foods included in the diet plan are rich in potassium as well as magnesium and calcium, with intake of all three nutrients (among others) meeting or exceeding recommendations depending on exact food choices [7]. The inclusion of foods rich in calcium and magnesium is important since insufficient intakes of both nutrients have been linked with higher blood pressure. However, because the results of supplementation trials with these minerals on blood pressure have been inconsistent (with no or only very small reductions in blood pressure), the use of calcium or magnesium supplements in the prevention and treatment of hypertension is not currently recommended. Nonetheless, adults with hypertension should be encouraged to consume foods rich in both nutrients and the DASH diet plan facilitates this objective.

In summary, while the effects of a multitude of nutrients, foods, and diet plans on blood pressure have been examined, study findings to date have not warranted inclusion in current guidelines. Thus, at present, the guidelines for the prevention and treatment of hypertension recommend for adults with hypertension (or with an elevated blood pressure): (1) weight loss for those who are overweight or obese, (2) a 25% reduction in current sodium intake and optimal at less than 1,500 mg, (3) a potassium-rich diet (with supplement use as needed), (4) alcohol restriction to two drinks for men and one drink for women/day, and (5) the DASH diet [1]. Physical activity, including aerobic and resistance,

is also encouraged for both hypertension and cardiovascular disease prevention and treatment [1].

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Web Sites

www.nlm.nih.gov/medlineplus/highbloodpressure.html
www.cdc.gov/nchs/fastats/hypertension.htm

ESSENTIAL TRACE AND ULTRATRACE MINERALS

LEARNING OBJECTIVES

- 13.1** Identify particularly good food sources of the essential minerals.
- 13.2** Explain how the essential minerals are digested, absorbed, transported in the blood, and stored.
- 13.3** Describe the metabolism of the essential minerals in the small intestine, liver, and kidneys.
- 13.4** Describe the functions and mechanisms of action of the essential minerals.
- 13.5** Identify the means by which essential minerals are excreted.
- 13.6** Describe recommended intakes, deficiencies, and toxicities associated with the essential minerals.
- 13.7** Explain how essential mineral status is assessed.

THE TRACE MINERALS OR TRACE ELEMENTS (also called microminerals) initially gained the description “trace” because their concentrations in tissue were not easily quantified by early analytical methods. Today, however, trace minerals can be analyzed by a variety of techniques. Trace minerals or elements are now typically defined as minerals that are needed by the body in small amounts, that is, less than 100 mg/day. However, some of the trace elements may be categorized as ultratrace if required in amounts of less than 1 mg/day.

Figure 13.1 shows the periodic table, highlighting some of the essential trace and ultratrace elements. This chapter discusses the sources, digestion, absorption, transport, storage, functions, interactions with other nutrients, excretion, recommended intakes, deficiency, toxicity, and assessment of nutriture for the trace minerals iron, zinc, and copper and the ultratrace minerals selenium, iodine, and molybdenum, for which Recommended Dietary Allowances have been established by the Food and Nutrition Board. In addition, these same topics are covered for the trace mineral manganese and the ultratrace mineral chromium, for which Adequate Intakes have been established by the Food and Nutrition Board.

Very little is known about the need for many other trace and ultratrace elements, including nickel, silicon, vanadium, arsenic, and boron; therefore, no recommendations for intake exist. Chapter 14 addresses these elements, as well as fluoride, a nonessential element with established Adequate Intakes.

Table 13.1 provides an overview of the minerals addressed in the chapter, including information on the approximate mineral quantities in the body, which range from <1 mg to about 4 g. Keeping in mind that an ounce weighs about 28.4 g, contrast these amounts with the body’s concentrations of the major minerals, which range from about 35 to 1,200 g.

13.1 IRON

The human body contains about 2–4 g of iron, or ~38 mg iron/kg body weight for women and ~50 mg iron/kg body weight for men. Over 65% (~1.3–2.6 g) of body iron is found in hemoglobin, up to about 10% (~0.2–0.4 g) is found as

Table 13.1 Approximate Body Content, Selected Functions, Deficiency Symptoms, Food Sources, and Recommended Dietary Allowance (RDA) or Adequate Intake (AI) for the Essential Trace and Ultratrace Minerals

Mineral	Approximate Body Content	Selected Physiological Roles	Selected Enzyme Cofactor Roles	Selected Deficiency Symptoms	Selected Food Sources	RDA or AI* (Adults)
Chromium	4–6 mg	Possibly potentiates insulin signaling		Glucose intolerance, glucose and lipid metabolism abnormalities	Grape juice, broccoli, meats, cereals, Brewer's yeast	35 µg* male; 25 µg* female
Copper	50–150 mg	Iron use; synthesis of collagen, pigment, neurotransmitters	Superoxide dismutase, lysyl oxidase, cytochrome c oxidase, dopamine monooxygenase, amine oxidase, peptidylglycine α-amidating monooxygenase	Anemia, neutropenia, bone and blood vessel abnormalities, impaired immune function	Liver, shellfish, nuts, seeds, legumes, meat, fish	900 µg
Iodine	15–20 mg	Thyroid hormone synthesis		Enlarged thyroid gland (goiter)	Iodized salt, saltwater seafood, seaweed, dairy products, liver, eggs, legumes	150 µg
Iron	2–4 g	O ₂ transport and use; amino acid metabolism; antioxidant; carnitine, collagen, and thyroid hormone synthesis	Catalase, cytochromes, myeloperoxidase, thyroperoxidase, lysine and proline dioxygenases, phosphoenolpyruvate (PEP) carboxykinase	Anemia, fatigue, impaired work performance, decreased resistance to infection	Liver, meat, molasses, clams, oysters, nuts, legumes, green leafy vegetables, dried fruits, enriched/whole grains	8 mg male; 18 mg female
Manganese	10–20 mg	Brain and CNS function, collagen, bone, growth, urea synthesis, and glucose and lipid metabolism	Glucosyltransferase, arginase, pyruvate carboxylase, PEP carboxykinase, superoxide dismutase, glutamine synthetase	In animals, possibly humans: impaired growth, skeletal abnormalities, impaired CNS function	Wheat bran, legumes, hazelnuts, blueberries, pineapple, shellfish, poultry, meat, nuts	2.3 mg* male; 1.8 mg* female
Molybdenum	2 mg	Metabolism of purines, pyrimidines, pteridines, aldehydes; oxidation	Xanthine oxidoreductase, aldehyde oxidase, sulfite oxidase, amidoxime reductase	Hypermethioninemia, urinary xanthine, sulfite excretion, urinary sulfate and urate excretion	Legumes, meat, poultry, fish, grains	45 µg
Selenium	20 mg	Protection against hydrogen peroxide and free radicals, thyroid hormone production	Glutathione peroxidase, 5'-deiodinase, thioredoxin reductase, selenoprotein P, selenophosphate synthesis, among other selenoproteins	Myalgia, cardiac myopathy, poor growth, abnormal sulfur metabolism	Oysters, tuna, meat, poultry, fish, cereals, Brazil nuts	55 µg
Zinc	1.5–3.0 g	Nutrient metabolism, collagen formation, alcohol detoxification, carbon dioxide elimination, sexual maturation, cell replication and growth	DNA-RNA polymerase, carbonic anhydrase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, deoxythymidine kinase, superoxide dismutase	Poor wound healing, subnormal growth, anorexia, abnormal taste/smell; impaired reproductive system development	Oysters, beef, liver, poultry, whole grains, legumes	11 mg male; 8 mg female

*Indicates Adequate Intake values.

Table 13.2 Iron Content of Select Foods*

Select Foods/Food Group	Iron (mg)
Meat and poultry	
Liver, beef (3 oz)	5.6
Beef, top sirloin (3 oz)	2.0
Beef, ground (3 oz)	2.1
Chicken, dark meat (3 oz)	1.1
Chicken, white meat (3 oz)	0.9
Pork, ground (3 oz)	1.1
Seafood	
Clams, mixed species (3 oz)	2.4
Oysters, Eastern (3 oz)	7.8
Cod, Atlantic (3 oz)	0.4
Salmon, Atlantic (3 oz)	0.9
Grouper (3 oz)	1.0
Tuna, light, canned in water (3 oz)	1.3
Legumes, variety (½ cup)	1.3–3.3
Vegetables	
Spinach (½ cup)	3.2
Peas, green (½ cup)	1.2
Collards (other greens) (1 cup)	2.2
Broccoli (1 cup)	1.1
Potato, baked with skin (1 medium)	1.9
Fruits	
Raisins, seedless (¼ cup)	0.7
Apricots, dried (¼ cup)	0.9
Peaches, dried (¼ cup)	1.6
Raspberries (½ cup)	0.4
Blueberries (½ cup)	0.2
Apple (1 medium)	0.2
Tomato (1 medium)	0.3
Nuts, variety (1 oz)	0.8–1.9
Grains	
Pasta, enriched (1 cup)	1.6
Quinoa (½ cup)	1.4
Rice, brown, enriched (1 cup)	1.0
Rice, white, enriched (1 cup)	1.9
Bread, whole wheat (1 slice)	0.7
Bread, white, enriched (1 slice)	1.1
Other	
Milk, 1% fat (1 cup)	0.1
Egg, poached (1 large)	0.9
Cheese, variety (1 oz)	0.04–0.2

* Data represent cooked foods, except for fruits, nuts, and milk.

Source: U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov>

iron administered as a chelate has not been shown to be absorbed better than iron given as ferrous sulfate or ferrous ascorbate. Use of iron supplements is often associated

with gastrointestinal (GI) problems including abdominal pain, nausea, and constipation. Iron dextrans can also be administered intravenously to correct iron deficiency if oral supplementation is not effective or contraindicated. Multivitamin/mineral supplements generally contain similar forms of iron as those found in iron supplements, but the iron content of most multinutrient supplements is lower, ranging from about 8 to 18 mg. The Daily Value for iron provided on food and supplement labels is 18 mg.

Digestion, Absorption, Transport, and Storage

Figure 13.3 provides an overview of iron digestion, absorption, and transport, as well as some of iron's fates in the enterocyte.

Heme Iron Digestion and Absorption

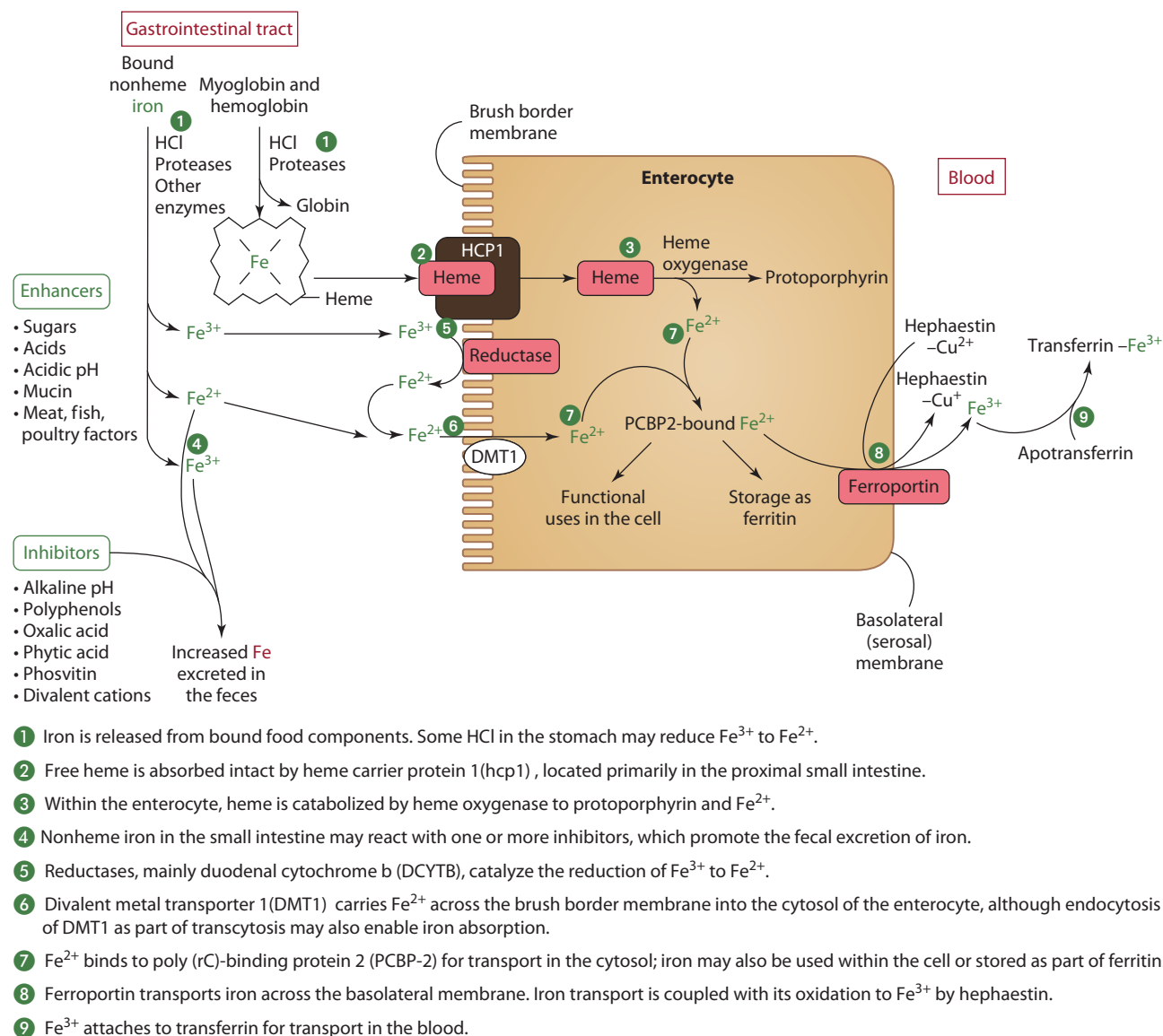
Heme iron must be hydrolyzed from the globin portion of hemoglobin and myoglobin before absorption. This digestion is accomplished by proteases in both the stomach and the small intestine and results in the release of heme from the globin. Heme, containing the iron bound to the porphyrin ring (also called a metalloporphyrin), remains soluble in the presence of the degradation products (amino acids and peptides) of globin and is readily absorbed intact in the duodenum and proximal jejunum. A heme carrier protein (HCP1) is known to transport the heme across the brush border membrane. Another protein, called heme responsive gene-1 (HRG-1), may also transport heme across the membrane, but precise mechanisms of heme absorption are incompletely understood. About 25% (range 15–35%) of heme iron from foods is absorbed.

Within the enterocyte, heme's porphyrin ring is hydrolyzed by heme oxygenase into ferrous iron and proto-porphyrin. The released iron is thought to associate with cytosolic proteins for intracellular transport. The iron is either used by the enterocyte, stored as ferritin, excreted with the sloughing of the enterocytes, or transported out of the enterocyte for use by other body tissues.

Nonheme Iron Digestion and Absorption

Nonheme iron is typically bound to components of food and must be freed (hydrolyzed) in the GI tract to be absorbed. Hydrochloric acid in the stomach and proteases in the stomach and small intestine aid in the release of nonheme iron, mostly as Fe^{3+} , from some food components. These actions in the stomach also result in the reduction of some, but not all, ferric iron (Fe^{3+}) to the ferrous state (Fe^{2+}). Ferrous iron remains fairly soluble in both the acidic environment of the stomach and the more alkaline environment of the small intestine.

Ferric iron that passes from the stomach into the small intestine mixes with alkaline juices secreted into the



intestine from the pancreas. In this more alkaline environment, some ferric iron may complex to produce ferric hydroxide $[\text{Fe}(\text{OH})_3]$, a relatively insoluble compound that tends to aggregate and precipitate, making the iron less available for absorption. Alternately, ferric iron may undergo reduction to a ferrous state by one or more reductases found primarily on the brush border membrane of duodenal enterocytes. The main ferric iron reductase is *duodenal cytochrome b* (DCYTB), although enzymes of the STEAP protein family also catalyze Fe^{3+} reduction to Fe^{2+} [1]. Cytosolic ascorbate (vitamin C) is the primary electron donor facilitating the reduction reaction. Nonheme iron must be reduced to Fe^{2+} to be transported into the enterocyte.

The main transporter for ferrous iron in the brush border membrane of intestinal cells is divalent metal transporter 1 (DMT1), although other transporters of metal

cations are likely present along the GI tract. DMT1 is found primarily in the duodenum and transports, in addition to iron, other minerals such as zinc, manganese, copper, nickel, and lead. Mineral transport via DMT1 is coupled with H^+ transport (symport) into the enterocyte. DMT1 operates at acidic pH (about 5.5). Synthesis of DMT1 is affected by iron status, with increased transporter synthesis associated with the presence of low cellular iron, and decreased expression of DMT1 associated with increased enterocyte iron concentrations. Hypoxia (low blood oxygen) also triggers an increase in DMT1 gene expression via the production of specific transcription factors (hypoxia-inducible factor 2α).

The mechanism by which ferric iron is absorbed is not clearly delineated and likely plays only a minor role in total iron uptake. A membrane protein called integrin may facilitate ferric iron absorption across the enterocyte's

brush border membrane. Integrin is thought to exist as part of the paraferitin complex, which includes an intracellular iron transport protein, mobilferrin, and a flavin-dependent ferrireductase.

Factors Influencing Iron Absorption

Several food components consumed with iron can either inhibit or enhance its absorption. Inhibitors generally act by binding nonheme iron in insoluble complexes, preventing its absorption. Enhancers of iron absorption act by reducing the more reactive Fe^{3+} to Fe^{2+} or by binding nonheme iron in bioavailable complexes, thus preventing its binding to inhibitor compounds.

The binding of nonheme iron to dietary compounds is called chelation. **Chelators** are small organic compounds that form a complex with iron and other metal ions. (In this context, the metal ion is sometimes referred to as a *ligand* because its binding to a chelate causes a change in metabolism.) If the iron–chelate complex maintains solubility and the iron is loosely bonded, the iron can typically be released at the enterocyte and absorption is enhanced. However, if the iron is strongly bonded to the chelate and insoluble, iron is not absorbed and the iron–chelate complex is excreted in the feces.

Enhancers of Nonheme Iron Absorption Some dietary factors known to enhance nonheme iron absorption include:

- Sugars, especially fructose and sorbitol
- Acids, such as ascorbic, citric, lactic, and tartaric
- Meat, poultry, and fish or their digestion products
- Mucin.

Sugars, such as fructose and sorbitol, are thought to form chelates or serve as ligands for iron. Ascorbic acid (vitamin C), along with citric, lactic, and tartaric acids, for example, acts as a reducing agent and forms a chelate with nonheme ferric iron at an acidic pH. Meat, poultry, and fish factors that enhance nonheme iron absorption have not been clearly identified, although digestion products from animal tissues high in the contractile proteins actin and myosin and cysteine-rich peptides have been shown to promote iron absorption [2].

The amount of iron available for absorption can be estimated from the quantity of vitamin C and meat, fish, or poultry that is ingested with the nonheme iron source, assuming ~500 mg body iron stores. Seventy-five units of ascorbic acid or meat, fish, or poultry (MFP) factor (one unit = 1.3 g of raw or 1 g of cooked meat, fish, or poultry or 1 mg of ascorbic acid) has been shown to maximize iron absorption when consumed with the iron source [3]. Units in excess of 75 seem to have no further benefit. The absence of enhancing factors predicts a nonheme iron absorption of only 2–3%, but 75 units of these factors can

increase absorption of nonheme iron to 8% (some suggest up to 20% if the person is also iron deficient) [4].

Mucin, an endogenously synthesized chelator, is a small protein made in both gastric and intestinal cells. Gastric mucin (sometimes called gastroferrin) is released into the lumen of the GI tract, although some mucin is also found on the brush border membrane of intestinal cells. Mucin binds multiple ferric iron atoms (as well as possibly zinc and chromium) at an acid pH and maintains ferric iron solubility in the alkaline pH of the small intestine to enhance iron absorption. Histidine, ascorbic acid, and fructose, other chelators of iron, are thought to donate the iron to mucin in the small intestine. In addition to the aforementioned compounds, a more acidic environmental pH such as that found in the upper duodenum favors iron absorption.

Inhibitors of Nonheme Iron Absorption Many dietary factors inhibit iron absorption, including:

- Polyphenols such as tannin derivatives of gallic acid, chlorogenic acids, monomeric flavinoids, and polyphenolic polymerization products (found in tea and coffee)
- Oxalic acid (found in spinach, chard, berries, chocolate, and tea, among other sources)
- Phytic acid, also referred to as phytate, inositol hexaphosphate, or polyphosphate (found in whole grains, legumes, lentils, nuts, and seeds)
- Phosvitin, a protein containing phosphorylated serine residues (found in egg yolks)
- Divalent cations such as calcium, zinc, and manganese.

Polyphenols or polyphenolic compounds, when consumed with a source of nonheme iron, can reduce iron absorption by over 50%. Coffee consumption, with or just after a meal, may reduce iron absorption by 40%.

Phytic and oxalic acids complex with iron, as well as zinc, copper, and other minerals. The phytic acid–mineral and oxalic acid–mineral complexes are insoluble and poorly absorbed. Fermentation of bread dough reduces the phytic acid content and improves the absorption of some minerals, but, in general, mineral absorption is better without the presence of phytic or oxalic acids. (Figure 13.11, in the section on zinc, shows the structures of both phytic acid and oxalic acid.) Individuals in the United States consuming primarily a plant-based, high phytic acid diet may not absorb sufficient iron and be at increased risk for deficiency.

Some minerals reduce iron absorption. Calcium in amounts of 300–600 mg and in many forms (such as calcium phosphate, calcium citrate, calcium carbonate, and calcium chloride and in milk), when given with up to 18 mg of iron as ferrous sulfate or when incorporated into food, substantially decreases iron (both heme and nonheme) absorption. The inhibitory effect, however, appears to be of short duration, and adaptation results such that iron

status is not negatively impacted [5]. Specifically, calcium transiently (for about 4 hours) inhibits iron absorption by causing the iron transporter ferroportin to relocate temporarily from the enterocyte's basolateral membrane to the cytosol. (See the next section for a discussion on the role of ferroportin.) DMT1 availability and changes in membrane fluidity may also play roles in reducing iron absorption [5].

While it is generally accepted that calcium inhibits iron absorption, the role of zinc and manganese is less certain. Zinc and manganese interact with nonheme iron and may negatively affect iron absorption, possibly by competing for DMT1 transporters, if significant amounts of zinc and manganese are present in the GI tract. On the other hand, some evidence suggests that zinc supplementation stimulates the production of DMT1 and ferroportin [6]. The mineral interactions that occur with typical ingestion of the nutrients from food sources requires much more research.

Other intraluminal factors inhibitory to iron absorption include rapid transit time, malabsorption syndromes, achylia (absence of digestive juices), and excess alkalization (i.e., increased pH) of the GI tract. The alkalization of the gastrointestinal tract is frequently associated with medications used to treat heartburn, gastroesophageal reflux disease (GERD), and ulcers. The main classes of medication are:

- H_2 receptor blockers, such as Axid (nizatidine), Tagamet (cimetidine), and Pepcid (famotidine)
- Proton pump inhibitors, such as Prevacid (lansoprazole), Prilosec (omeprazole), and Nexium (esomeprazole).

A more alkaline environment in the GI tract may also occur with aging due to age-related reductions in gastric acid production.

Overall absorption of iron from the U.S. diet is estimated at about 14–18%, but a person's iron status also affects iron absorption. Absorption may range from about 10% (for persons with normal iron status) up to about 35% (for persons who are iron deficient). In other words, iron absorption can rise to 3–6 mg daily when the body has low iron stores and can fall to 0.5 mg or less daily when iron stores are high. Additional information on the regulation of iron absorption follows the section about intestinal cell iron use. A review of algorithms used to predict iron availability reports that the effects of iron status on iron absorption can be estimated based on selected biochemical indicators of iron status [7]. Absorption of about 1–2 mg of iron each day is needed to balance out the body's usual daily iron losses.

Intestinal Cell Iron Use

The preceding sections have reviewed digestion and absorption, including factors inhibiting and enhancing iron absorption into the enterocyte. Following absorption across the enterocyte's brush border membrane, iron enters the cytosol of the cell. Free iron is also liberated

by heme hydrolysis. Because of the potential for free iron to initiate oxidative damage, little iron is thought to exist unbound within the cytosol of the enterocyte (or any cell).

Cytosolic ferrous iron attaches to proteins and possibly other substances for transport within the cell. One identified protein, poly (rC)–binding protein 2 (PCBP2), binds up to three iron atoms with high affinity and delivers iron to various sites within the enterocyte, depending on the metabolic status of the cell [8]. Ferrous iron may be delivered to ferritin for storage; to the mitochondria for uptake by mitoferrins; or to other proteins and enzymes that require iron for proper function. In addition to PCBP2, possible cytosolic carriers include amino acids such as cysteine and histidine and organic anions such as phosphate, citrate, and carboxylate.

As depicted in Figure 13.3, once in the enterocyte, iron is either:

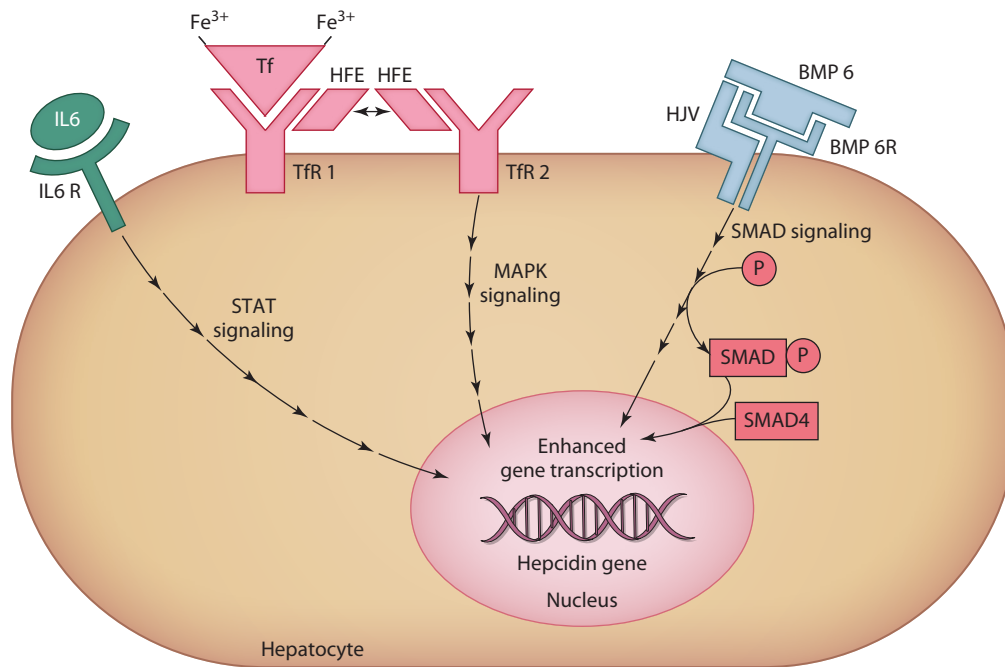
- used by the intestinal cell in a functional capacity (see “Functions and Mechanisms of Action”),
- stored in ferritin, or
- transported through the cytosol and across the basolateral membrane into the blood for delivery to other tissues.

Iron that is not needed for functional uses may be incorporated into (apo)ferritin in the intestinal cell for short-term storage. Stored iron may be released over the next few days should it be needed by the enterocyte or other nonintestinal cells. If not needed, the iron remains attached to ferritin and is excreted when the short-lived (3 days) enterocytes are sloughed off into the lumen of the GI tract.

Iron transport across the enterocyte's basolateral membrane requires the iron to bind to the membrane transport protein ferroportin. This transport of iron is also coupled with iron's oxidation to Fe^{3+} by a copper-containing protein called hephaestin (or, in the absence of hephaestin, by the copper-containing protein ceruloplasmin). Hephaestin is found on the enterocyte's basolateral membrane near ferroportin. The role of copper as part of hephaestin and ceruloplasmin in the oxidation of iron is crucial to iron metabolism. In fact, copper deficiency results in decreased ceruloplasmin and hephaestin, iron accumulation in cells, and reduced iron transport to tissues. The copper-dependent oxidation of iron to its ferric state allows the transport of iron in the blood as part of the protein transferrin. Specifically, transferrin carries one or two ferric iron atoms (becoming monoferric transferrin or diferric transferrin, respectively) for delivery to body tissues.

Regulation of Iron Absorption

Iron absorption is regulated by the levels of body iron and cellular iron. Some of these regulatory mechanisms are reviewed hereafter and are shown later in Figure 13.4.



*See text for abbreviations.

Figure 13.4 Overview of some pathways stimulating hepcidin synthesis.

Body Iron Regulation The main regulator of iron absorption and body iron metabolism is a small protein called hepcidin. This protein is released primarily from the liver when iron stores are high. Hepcidin functions to reduce intestinal iron absorption and to reduce cellular iron release. Specifically, hepcidin is released from the liver and travels in the blood attached primarily to α -2-macroglobulin. Hepcidin targets ferroportin on the basolateral cell membranes of enterocytes and on the cell membranes of macrophages. Hepcidin binds ferroportin, resulting in internalization and degradation (via ubiquitin and lysosomes) of both the hepcidin and the ferroportin. With the hepcidin-induced loss of ferroportin from the cell membranes, iron cannot be transported out of enterocytes or out of macrophages and thus cannot be released into the blood for use by other tissues. Consequently, increased hepcidin concentrations result in increased enterocyte and macrophage iron concentrations. In the case of the enterocyte, the availability of newly absorbed iron to the body is decreased. Conversely, when little iron is present, low levels of hepcidin are produced, and ferroportin concentrations on enterocyte and macrophage cell membranes remain sufficient for iron export out of the cells. This situation enables iron release into the blood for distribution and use by body tissues.

The liver recognizes the body's iron status based on transferrin saturation or more specifically by the binding of diferric transferrin to the transferrin receptor 1 (TfR1) on its cell membranes. This binding in turn releases a high-iron (HFE) protein that is normally attached to hepatocyte TfR1; this released HFE protein then binds to TfR2,

another type of transferrin receptor present on hepatocyte membranes (Figure 13.4). The HFE–TfR2 complex is thought to serve as a “sensor” or “signal” of the body's iron status and in turn stimulates hepcidin synthesis at the transcription level through mitogen-activated protein kinase (MAPK) intracellular signaling. In contrast, in the presence of low plasma iron (i.e., reduced transferrin saturation), TfR1 remains attached to HFE and prevents its interaction with TfR2. Without the intracellular signaling cascade, hepcidin transcription is repressed.

Another pathway, referred to as BMP6/SMAD, also affects hepcidin synthesis and is shown in Figure 13.4. BMP (bone morphogenic protein) 6 is secreted from liver cells as its iron content increases. In this pathway, BMP6 binds to hepatic membrane receptors for BMP6 and hemojuvelin (HJV); hemojuvelin serves as a coreceptor and is required for BMP6 receptor signaling for hepcin production. The formed complex promotes phosphorylation of several SMAD (an abbreviation for small mothers against decapentaplegic) proteins, and upon subsequent binding of SMAD4, the complex translocates into the nucleus and interacts with response elements in the promoter region of the gene for hepcidin. This interaction enhances hepcidin gene transcription. In contrast, with low cellular iron, BMP6 is not released from the liver and signaling through SMAD is reduced.

Defects in any of the genes coding for proteins that are involved in the signaling pathways modulate hepcidin synthesis, and thus impact iron status. Transmembrane serine protease s6 (TMPRSS6; also called matriptase-2), for example, degrades hemojuvelin and thus reduces

hepcidin synthesis. An absence of hepcidin (e.g., due to a genetic defect) enables the sustained presence of ferroportin on cell membranes and thus continued iron absorption/uptake, cellular accumulation, and toxicity (see the “Toxicity” section). In contrast, mutations in the *matriptase-2* gene cause overproduction of hepcidin and result in iron-refractory iron-deficiency anemia. Iron deficiency is refractory when, after about 4–6 weeks of treatment with iron, hematological parameters fail to rise (normally, hemoglobin concentrations should rise at least 1 g with iron therapy).

Hepcidin and Inflammation/Infection Other triggers of hepcidin synthesis include inflammation and infection. In fact, under such conditions, macrophages and neutrophils also produce hepcidin. As discussed in the Chapter 6 Perspective, macrophages detect pathogen-associated, as well as damage-associated, molecular patterns (PAMPs and DAMPs). These products of damaged and necrotic cells interact with pattern-recognition receptors, such as Toll-like receptors, among others, on macrophages (and other immune system cells) and promote the production and secretion of cytokines such as tumor necrosis factor α and interleukin (IL)6. IL6, upon binding to IL6 receptors on hepatocytes, triggers “Janus kinase (JAK)” and “signal transducer and activator of transcription” (STAT) 3 signaling. STAT, following phosphorylation, translocates into the nucleus and interacts with STAT sites in the promoter region of the hepcidin gene to enhance transcription. Because hepcidin inhibits iron release from enterocytes as well as from macrophages, these cells accumulate iron. If the infection or inflammation is prolonged, serum/blood iron concentrations may drop (hypoferremia), creating a condition commonly referred to as the anemia of chronic disease. Other factors, such as tumor necrosis factor α , also contribute to reductions in iron absorption and reduce serum iron concentrations, especially during the initial stages of inflammation.

Tissue Oxygen and Erythropoietic Activity In contrast to inflammation and infection, conditions such as hypoxia decrease hepcidin synthesis. Hypoxia is detected initially by *hypoxia inducible factor* in the kidneys and stimulates erythropoietin release. Erythropoietin, in turn, stimulates hematopoiesis in the bone marrow. While the mechanism(s) has/have not been clearly elucidated, hypoxia inducible factor appears to increase the hepatic expression of *matriptase-2*, which disrupts the signaling needed for hepcidin gene transcription. Without hepcidin, iron is released from intestinal cells and from macrophages and used functionally in erythroblasts for the synthesis of heme.

Cellular Iron Regulation In addition to hepcidin’s role in the regulation of iron absorption and body iron utilization,

the iron content of a cell affects iron absorption/uptake and utilization. The regulation involves cytosolic iron regulatory proteins (IRPs; also called binding proteins), which interact with mRNA to alter the translation of proteins needed for the body’s use of iron (Figure 13.5).

When little cellular iron is present, IRPs exist as 3Fe-4S iron sulfur clusters (ISC) and function as binding proteins. IRPs bind to iron response elements (IREs), which are stem loop structures of about 30 nucleotides located in specific 3’ and 5’ untranslated regions of mature mRNAs. These IREs are present in the mRNA for several proteins involved with iron, including transferrin receptors, ferritin, cytochrome *b* reductase, ferroportin, and DMT1, among others. In low-iron situations, IRPs bind to an IRE in the 5’ untranslated region of ferritin mRNA and act as a repressor to inhibit ribosome assembly and translation. Thus, less ferritin is made in cells when the cellular iron content is low. From a physiological standpoint, this inhibition makes sense because ferritin stores iron, and not much ferritin would be needed if the cell’s iron content was low. Simultaneously, with low cellular iron, the translation of mRNAs for ferroportin, transferrin receptors, duodenal cytochrome *b* reductase, and DMT1 is enhanced with IRPs binding to specific IREs in the 3’ untranslated region of mRNA for these proteins. These actions increase the synthesis of proteins, which enhance iron absorption by enterocytes and iron uptake by extraintestinal cells including erythroblasts. From a physiological standpoint, this too makes sense since under conditions of low iron, such changes facilitate iron absorption/uptake and utilization.

Under the opposite conditions, in which enterocytes and other cells have relatively high iron contents, IRPs contain 4Fe-4S clusters, are unable to bind to IREs, and exhibit aconitase (a TCA cycle enzyme) activity. In the case of ferritin, without interactions between IRPs and IREs in the mRNA for ferritin, the ferritin mRNA undergoes translation and more ferritin is made. Without interactions between IRPs and IREs in the mRNAs for ferroportin, transferrin receptors, duodenal cytochrome *b* reductase, and DMT1, the translation of these mRNAs is reduced and less iron is absorbed into enterocytes and taken up by extraintestinal cells.

Further regulation occurs under conditions of hypoxia. Reductions in oxygen tension within enterocytes increase iron absorption. The increase is mediated by the production of a transcription factor called hypoxia inducible factor 2 α , which enhances the translation of mRNA for both DMT1 and ferroportin and promotes iron absorption.

Transport

Iron in its oxidized ferric state is transported in the blood attached to the protein transferrin. Transferrin, a glycoprotein made primarily in the liver, has two binding sites for minerals, one near its carboxy (C)–terminal end and the other near its amino (N)–terminal end. Both binding sites

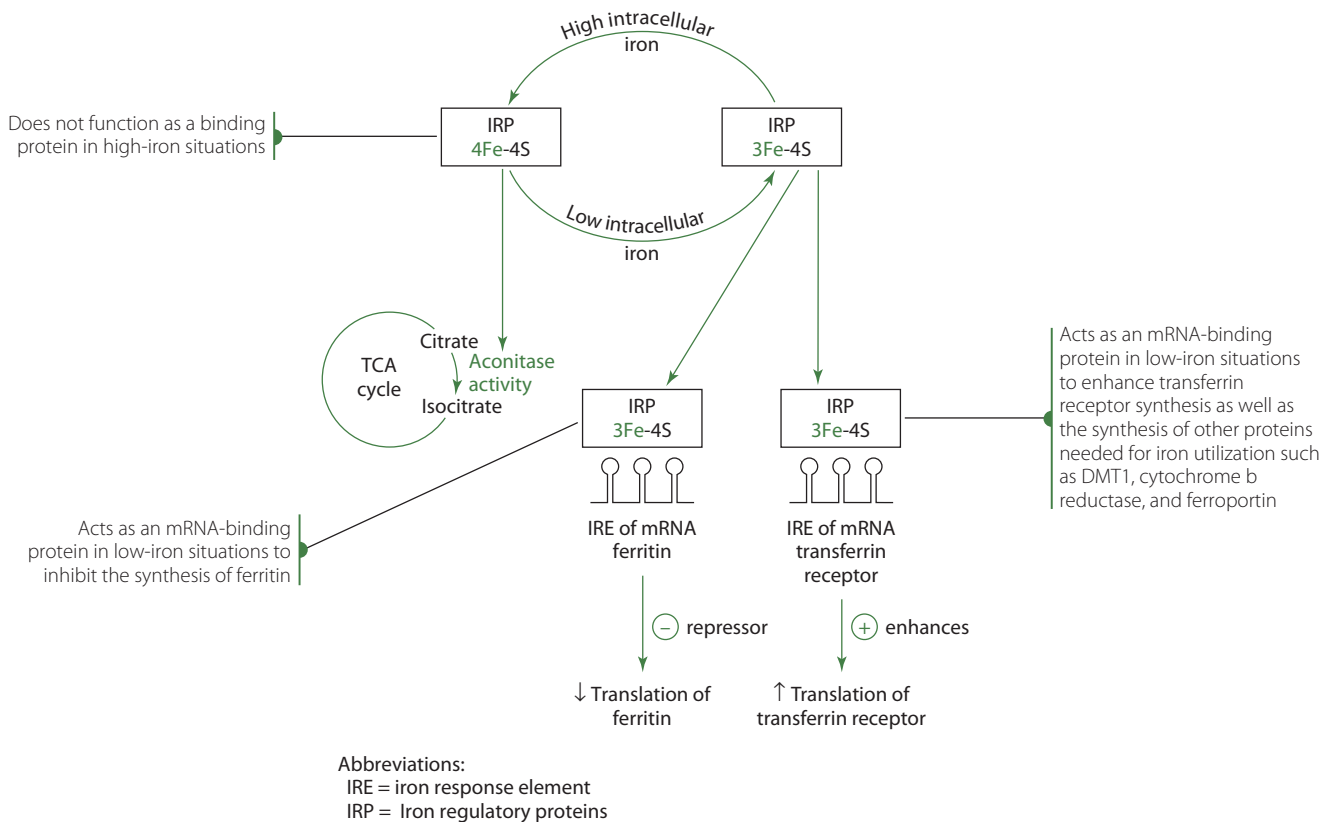


Figure 13.5 The influence of intracellular iron on the translation of ferritin mRNA and transferrin receptor mRNA.

have a high affinity for ferric iron, but the one near the amino (N)-terminal end also binds other minerals, such as chromium, followed in descending order by copper, manganese, cadmium, zinc, and nickel. The binding of ferric iron to transferrin requires the presence of an anion, usually bicarbonate, at each binding site. The plasma iron pool typically contains about 3–4 mg of iron bound to transferrin. Transferrin is typically one-third (33%, range about 30–40%) saturated with ferric iron and nontransferrin-bound iron (NTBI) is usually undetectable in the blood under normal physiological conditions. (*Note:* If all of transferrin's binding sites were occupied [e.g., as in a toxicity situation], then the transferrin would be fully [100%] saturated; it is also under toxicity situations that NTBI increases.)

The role of proteins in the transport as well as storage of iron is important because of iron's redox activity. The binding of iron by proteins serves as a protective mechanism. When iron is left unbound, its redox activity can lead to the generation of harmful free radicals. Free ferrous iron (Fe^{2+}), for example, readily reacts with hydrogen peroxide (H_2O_2) in what is known as the Fenton reaction: $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH}^- + \cdot\text{OH}$. This reaction generates a hydroxyl anion and a free hydroxyl radical ($\cdot\text{OH}$), which is extremely reactive and damaging to cells. In addition, the binding of iron by protein is important to ensure

that bacteria that may be present in the body, as with an infection, are unable to use the iron for their own (bacterial) growth. Free iron—but not protein-bound iron—is readily used by bacteria for proliferation and growth. Bacteria cannot multiply without nutrients, such as iron, acquired from the host. Thus, keeping iron attached to proteins in the body diminishes bacterial replication.

Transferrin binds and transports not only newly absorbed dietary iron that has crossed the basolateral membrane of the enterocyte, but also iron that has been released following the degradation of iron-containing compounds in the body. In fact, the overwhelming majority of iron (about 20–25 mg) entering the plasma for distribution by transferrin is contributed from hemoglobin degradation. The transferrin iron pool turns over five to eight times each day. Transferrin has a half-life of about 7–10 days.

Cellular Iron Uptake The amount of iron taken up by tissues depends in part on transferrin's saturation level and the presence of transferrin receptors on cell membranes. For example, iron delivery is greater from diferric transferrin (transferrin containing two bound iron atoms) than from monoferric transferrin (transferrin containing only one bound iron atom). The mono- and diferric transferrin bind to transferrin receptors (TfRs) on

cell membranes. Most cell membranes contain a form of transferrin receptors abbreviated TfR1; liver and intestinal cells, however, also contain an isoform known as TfR2, which preferentially binds diferric transferrin. Transferrin receptors consist of two subunits; each subunit binds one transferrin molecule.

Once the transferrin molecule with its bound iron attaches to the transferrin receptor, a complex is formed, as shown in Figure 13.6. The transferrin receptor–transferrin complex is next internalized by endocytosis and forms a vesicle (also called an endosome) within the cell's cytosol. Next, in an ATP-dependent process, protons are pumped into the endosome and reduce the pH from about 7.4 to about 5.5. In the presence of the acidic pH and possibly other factors, ferric iron atoms are released from the transferrin molecule. The apotransferrin then returns to the plasma and the receptor returns to the membrane. Use of the released ferric iron requires reduction and transport across the endosomal membrane; however, in some cells such as reticulocytes, the endosomes appear to provide the iron directly to the mitochondria to enable heme synthesis. In other cells, reduction of ferric iron to a ferrous state is thought to be accomplished by the ferrireductase activity of STEAP3. DMT1 transports the ferrous iron out of

the endosome and into the cytosol. Iron released from the endosome is typically transported to other sites for use, although it may be stored in ferritin.

The number of transferrin receptors on cell membranes increases or decreases depending on intracellular iron concentrations. In other words, intracellular iron affects the genetic expression of transferrin receptors. As discussed in the previous section, in a low-cellular-iron situation, IRPs interact with the IRE and stabilize the transferrin receptor mRNA. This stabilized transferrin receptor mRNA exhibits a longer half-life, and consequently more transferrin receptors are synthesized. Once made, these transferrin receptors become embedded in the cell's plasma membrane to promote cellular iron uptake. Thus, in conditions of low cellular iron, transferrin receptor synthesis is increased. If the intracellular iron concentration is adequate or relatively high, fewer transferrin receptors are translated and less iron is brought into the cell. Thus, transferrin receptor expression indicates the cell's need for iron uptake.

Storage

Iron not needed in a functional capacity is stored in three main sites: the liver, bone marrow, and spleen. Transferrin delivers iron to these sites, especially the liver, which stores

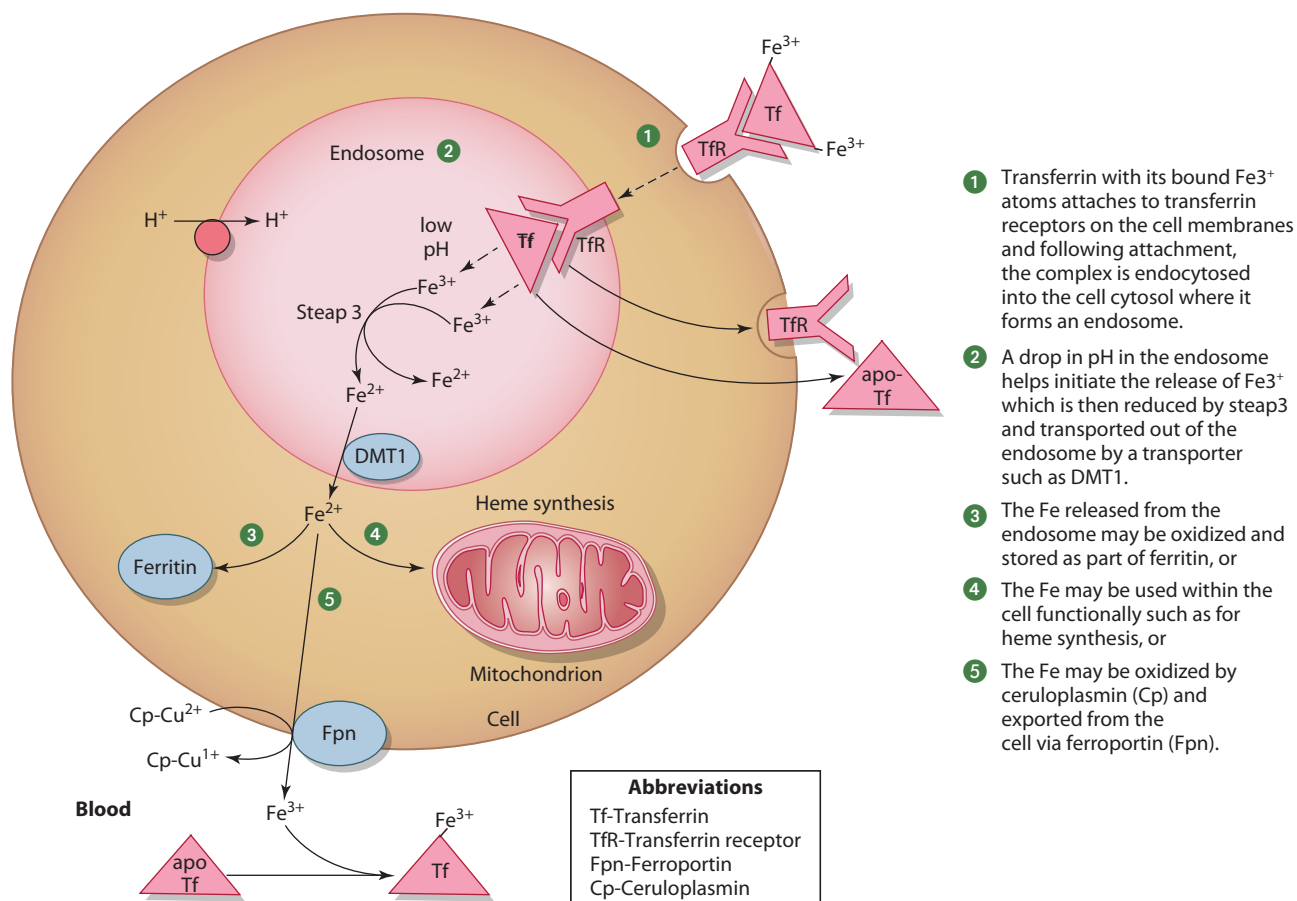
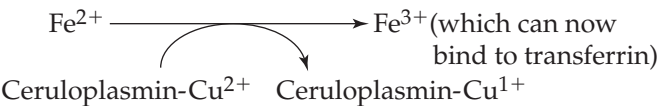


Figure 13.6 Overview of cellular iron uptake.

about 60% of the body's iron in both reticuloendothelial (RE) cells and hepatocytes. The remaining 40% is found in reticuloendothelial cells within the spleen and bone marrow (and possibly between muscle fibers). Most of the stored iron is derived from phagocytosis of red blood cells and subsequent degradation of the hemoglobin within those cells. Stored iron may be released for subsequent cellular use during times of increased needs; the released iron requires ferroportin for transport across the cell membrane and ceruloplasmin for oxidation to its ferric state and thus binding to transferrin.



Ferritin is the primary storage form of iron in cells; the role of iron in the control of ferritin synthesis was previously described in the “Regulation of Iron Absorption” section. The protein is initially synthesized as apoferritin, containing no iron atoms; it has a hollow spherelike shape and is composed of 24 protein subunits. Once iron enters apoferritin, the protein is referred to as ferritin. Ferritin's subunits are classified based on molecular mass as heavy (H) or light (L), and the proportions of H and L subunits within ferritin vary among tissues. The L form, for example, predominates in the liver and spleen and takes up iron rather slowly, compared with the H form, which is found in higher amounts in the heart and red blood cells. Iron enters apoferritin through channels or pores. The pores serve as the site of the oxidation of the ferrous iron and generate ferric oxyhydroxide crystals ($4\text{Fe}^{2+} + \text{O}_2 + 6\text{H}_2\text{O} \rightarrow 4\text{FeOOH} + 8\text{H}^+$) and ferrihydrite ($5\text{Fe}_2\text{O}_3 + 9\text{H}_2\text{O}$); molecular oxygen functions as the electron acceptor. Ferric oxyhydroxide and ferrihydrite are deposited in the interior of the protein shell. As many as 4,500 iron atoms can be stored in ferritin; however, the protein more commonly stores about 800–1,500 atoms.

Ferritin is constantly being degraded and resynthesized, providing an available intracellular iron pool. Equilibration occurs between tissue ferritin and serum ferritin. Thus, serum ferritin is used as an index of body iron stores: 1 ng of ferritin/mL serum equals ~10 mg of body iron stores. Normal serum ferritin concentrations (for adults) typically range from about 18 to 300 ng/mL; however, ferritin concentrations increase secondary to inflammation and infection/illness and under these conditions do not reflect iron stores. In other words, serum ferritin concentrations may be elevated or within the normal range in the blood, despite an individual's having little iron stores. Methods of assessing iron status are described further in the “Assessment of Nutriture” section.

Hemosiderin is another iron storage protein. Hemosiderin is thought to be a degradation product of ferritin, representing, for example, aggregated ferritin or a deposit

of degraded apoferritin and coalesced iron atoms. The content of iron in hemosiderin may be as high as 50%. The ratio of ferritin to hemosiderin in the liver varies according to the level of iron stored in the organ, with ferritin predominating at lower iron concentrations, and hemosiderin predominating at higher concentrations (iron overload). Although iron in hemosiderin can be labilized to supply free iron, iron is released at a slower rate from hemosiderin than from ferritin. The release of iron from its stores involves both proteosomal and lysosomal-mediated degradation mechanisms. Lysosomal-mediated degradation requires nuclear receptor coactivator 4 (NCOA4), which binds and transports the ferritin to the lysosomes. The released iron is then used within the cell or exported via ferroportin (and oxidized by ceruloplasmin) for transport in the blood by transferrin (see Figure 13.8).

Functions and Mechanisms of Action

Iron functions in the body as part of several proteins, including the dozens of enzymes for which it serves as a cofactor. These iron-dependent proteins are involved in diverse body processes (Table 13.3), as described in this section. Moreover, the iron in the proteins is found in different forms. For example, in many proteins, iron is present as part of heme, while in others, iron is found in a cluster with sulfur (2Fe-2S, 4Fe-4S, or 3Fe-4S), by itself as a single atom, or as part of a bridge with oxygen. Heme proteins represent the largest group and include hemoglobin, myoglobin, and cytochromes, as well as some enzymes. Iron-sulfur proteins also include several enzymes with diverse roles that participate in electron transport, the TCA cycle, and heme synthesis. Proteins that contain single iron atoms are mostly mono- and dioxygenase enzymes, and the one iron–oxygen bridge protein is found in the enzyme ribonucleotide reductase.

Table 13.3 Select Functions of Iron

Hemoglobin and myoglobin
Electron transport: ATP production
Amino acid metabolism: phenylalanine, tyrosine, tryptophan, and arginine
Niacin synthesis
Carnitine synthesis
Procollagen synthesis
Nitric oxide synthesis
Antioxidant: protection
Destruction of bacteria, viruses, and microbes
Thyroid hormone synthesis
Sulfite oxidation
DNA purine base catabolism
Carbohydrate metabolism
DNA synthesis

Hemoglobin and Myoglobin: Oxygen Delivery

The essentiality of iron is due in part to its presence in heme, which functions as a prosthetic group for many proteins. The atom of iron in the center of the heme molecule enables oxygen transport to tissues (hemoglobin); transitional storage of oxygen in tissues, particularly muscle (myoglobin); and transport of electrons through the respiratory chain (cytochromes).

Hemoglobin is synthesized in red blood cells and carries about 98.5% of the total oxygen found in the blood. Hemoglobin, a conjugated tetrameric protein, consists of four heme groups and globin, which is made up of four polypeptide chains (see Figure 6.20). Each polypeptide chain is associated with one of the heme molecules. Heme is an iron-containing derivative of porphyrin. Porphyrins, in turn, are cyclic compounds made up of four pyrrole rings joined together by methenyl bridges. Nitrogen atoms in each of the four pyrrole rings bind to the iron atom, and these bonds hold the iron atom in the plane of the porphyrin ring. The iron atom in the center of the heme has two remaining coordinate bonds available for binding. One is with an amino acid (often the nitrogen atom of histidine) of the protein to which the heme is attached. For example, in hemoglobin, the iron in the heme binds to the nitrogen of an amino acid in the protein globin; heme is found in a hydrophobic pocket of the protein. The sixth and last coordinate bond in heme proteins that bind oxygen—namely, hemoglobin and myoglobin—is positioned between the iron and oxygen. The oxygen is held quite loosely so that transfer to tissues can be rapid. In heme proteins that do not bind oxygen, the sixth coordinate bond is with atoms of amino acid groups in the protein (such as an enzyme) with which the heme group is associated. Heme synthesis accounts for the largest use of functional iron in the body. In fact, each red blood cell is thought to contain millions of hemoglobin molecules, and all the red blood cells in the body together contain about two-thirds of total body iron. Any one red blood cell may carry up to a billion oxygen atoms.

To synthesize heme for hemoglobin, iron is needed in the erythropoietic cells in the bone marrow. These cells possess transferrin receptors on their plasma membranes; transferrin delivers the iron for heme synthesis to the erythropoietic cells. Mitoferrin transports the iron (following cellular transferrin uptake and degradation and iron release from the endosome) across the mitochondrial membrane, and a mitochondrial matrix chaperone, frataxin, is thought to further transport the iron within the mitochondria, the site where heme synthesis begins. Alternately, degradation of old red blood cells within the bone marrow can directly provide the necessary iron for hemoglobin synthesis without the use of transferrin (see the “Turnover” section).

Briefly, the synthesis of heme (shown in Figure 13.7) occurs as follows:

- Glycine and succinyl-CoA condense to form Δ (also called 5)-amino-levulinic acid (ALA) in the cell's mitochondria. The reaction is catalyzed by Δ -aminolevulinic acid synthase, a vitamin B₆-dependent enzyme that is inhibited by the final end product (heme) and whose synthesis is enhanced by an IRP interaction with the IRE in the mRNA for the enzyme when the cell contains ample iron.
- Next, ALA enters the cytosol, where a zinc-dependent dehydratase catalyzes the condensation of two ALA molecules to form porphobilinogen. This enzyme is sensitive to lead, which binds to its sulfhydryl groups to inactivate the enzyme.
- Next, in a series of cytosolic reactions involving a deaminase, a synthase, and a decarboxylase, four porphobilinogens condense to form a tetrapyrrole that cyclizes. Side chains are modified, and coproporphyrinogen III is formed and enters the mitochondria through ATP-binding cassette B6.
- Coproporphyrinogen is converted in the mitochondria to protoporphyrinogen.
- Protoporphyrinogen is oxidized to form protoporphyrin IX.
- Last, an iron (Fe^{2+}) atom is inserted into protoporphyrin IX to yield heme. The insertion of iron into the heme is catalyzed by ferrochelatase, a 2Fe-2S cluster mitochondrial protein. The transcription of the ferrochelatase gene appears to be regulated by iron. Globin chains are added to the heme after it moves out of the mitochondria and into the cytosol.

Unlike the tetrameric hemoglobin, myoglobin consists of a single hemoprotein chain. Myoglobin, which is found in the cytosol of the muscle cells, facilitates the diffusion rate of dioxygen from hemoglobin in capillary red blood cells to the cytosol and mitochondria of muscle cells.

Cytochromes and Other Enzymes Involved in Electron Transport: ATP Production

Heme-containing cytochromes in the electron transport chain, such as cytochromes *b* and *c*, pass along single electrons. The transfer of electrons along the chain is made possible by the change in the oxidation state of iron. In the reduced cytochromes, the iron atom is in the ferrous state. The iron atom of the reduced cytochrome becomes oxidized to the ferric state when a single electron is transferred to the next cytochrome. The iron atom of the cytochrome receiving the electron then becomes reduced. Nonheme iron-sulfur enzymes involved in electron transport include NADH dehydrogenase, succinate dehydrogenase, and ubiquinone-cytochrome *c* reductase.

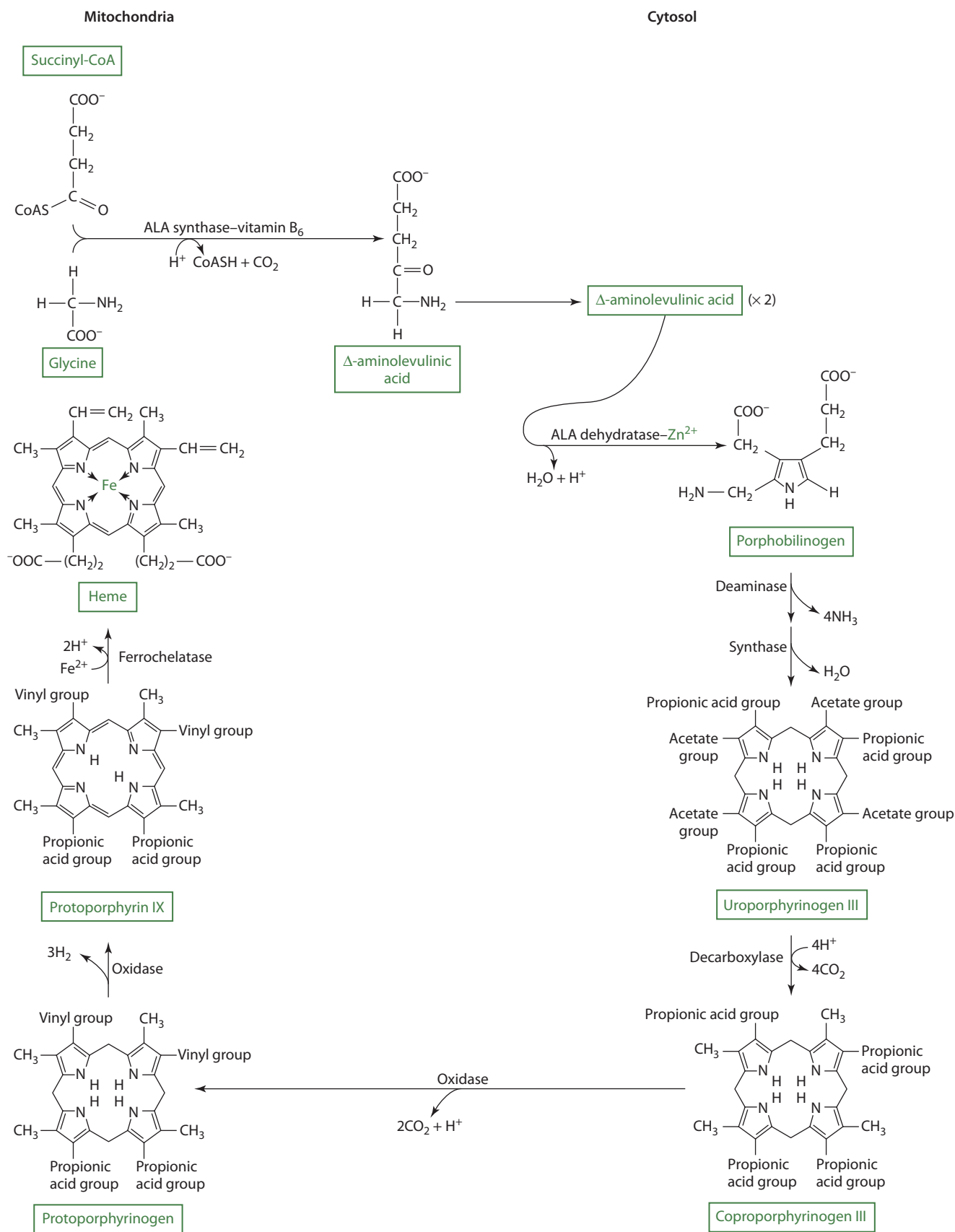


Figure 13.7 Heme biosynthesis. Vinyl group: $\text{CH}=\text{CH}_2$; propionic acid group: $(\text{CH}_2)_2\text{COO}^-$; acetate group: CH_2COO^- .

See Chapter 3 for a more thorough discussion of electron transport. Whether iron is carrying oxygen or transporting electrons, its essentiality in energy transformation is without question.

Monooxygenases and Dioxygenases: Amino Acid Metabolism, Carnitine Synthesis, and Procollagen Synthesis

Many additional enzymes involved in a variety of processes besides energy production also require iron. Some monooxygenases, which insert one of two oxygen atoms into a substrate, require a single iron atom to function. Similarly, many dioxygenases, which catalyze the insertion of two oxygen atoms into a substrate, also require iron. Some examples include:

- Phenylalanine monooxygenase (needed for amino acid metabolism)
- Tyrosine monooxygenase (needed for amino acid metabolism)
- Tryptophan monooxygenase (needed for amino acid metabolism)
- Tryptophan dioxygenase (needed for amino acid metabolism)
- Parahydroxyphenylpyruvate hydroxylase and homogentisate dioxygenase (needed for amino acid metabolism)
- Trimethyllysine dioxygenase and 4-butyrobetaine dioxygenase (needed for carnitine synthesis)
- Lysine dioxygenase and proline dioxygenase (needed for procollagen synthesis)
- Nitric oxide synthase (needed for amino acid metabolism and involved in signal transduction).

Specifically, the monooxygenases insert an oxygen atom into the aromatic amino acids phenylalanine, tyrosine, and tryptophan, respectively, and use cosubstrates to furnish the hydrogen atoms that reduce the second oxygen atom to water (see Figures 6.10 and 6.11). Tetrahydrobiopterin is frequently used as a cosubstrate, and during the reactions tetrahydrobiopterin is oxidized to dihydrobiopterin. The reactions catalyzed by phenylalanine, tyrosine, and tryptophan monooxygenases are important for amino acid metabolism. In other words, the use of these three amino acids for functions other than protein synthesis (including catecholamine, serotonin, and melatonin synthesis) depends on iron-dependent enzymes.

Heme-containing tryptophan dioxygenase (also called a pyrrolase) converts the amino acid tryptophan to N-formylkynurenine (see Figure 6.11), representing the first step of tryptophan catabolism for the generation of energy and the B-vitamin niacin. Iron deficiency reduces niacin synthesis from tryptophan.

In tyrosine catabolism for energy production (see Figure 6.10), two reactions require iron-dependent enzymes. The conversion of p-hydroxyphenyl-pyruvate to homogentisate (homogentisic acid) requires the non-heme iron-dependent enzyme p-hydroxyphenylpyruvate hydroxylase and vitamin C. Next, homogentisate dioxygenase, a single-atom iron-dependent enzyme, converts homogentisate to (4-) maleylacetoacetate. Defects in this enzyme result in the genetic disorder alkaptonuria, which is characterized by high concentrations of homogentisic acid in the urine. When this urine is excreted and the homogentisic acid is exposed to air, the compound turns a very dark color, causing the urine to appear almost black. In those with alkaptonuria, the homogentisic acid also accumulates in joints, causing arthritis.

Two of the four steps required for carnitine synthesis involve iron-dependent dioxygenases. Recall that carnitine is an important nitrogen-containing compound necessary for the transport of long-chain fatty acids into the mitochondria for oxidation. The first step in carnitine synthesis (see Figure 6.23), in which trimethyl lysine is converted to 3-OH trimethyl lysine, requires a single iron-containing trimethyl lysine dioxygenase, and the final step, in which 4-butyrobetaine is converted to carnitine, requires 4-butyrobetaine dioxygenase, another single iron-containing enzyme.

Hydroxylation reactions for procollagen synthesis are shown in Figure 9.4. Both lysine and proline dioxygenases contain single iron atoms. These reactions are important for the synthesis of the protein collagen, a component of bone, cartilage, skin, and blood vessels, among other body structures.

Two isoforms of nitric oxide synthase, a dioxygenase needed for the synthesis of nitric oxide from the amino acid arginine, contain heme iron. Nitric oxide is a potent biological effector molecule that is involved in a variety of physiological processes including regulation of blood pressure (relaxation of vascular smooth muscle) and intestinal motility, inhibition of platelet aggregation, macrophage function, and signal transduction, to name a few.

Peroxidases: Antioxidant Roles and Thyroid Hormone Synthesis

Other important reactions required to protect the body also involve iron-containing enzymes.

- Catalase, with four heme groups, converts hydrogen peroxide to water and molecular oxygen: $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Catalase is one of the major antioxidant enzymes of the body and thus helps prevent cellular damage that can be induced by hydrogen peroxide (see the Perspective in Chapter 10).
- Myeloperoxidase (also called chloroperoxidase), another heme-containing enzyme, is found in the

plasma as well as in neutrophils (white blood cells). During phagocytosis, myeloperoxidase is released into the phagocytic vesicle within the neutrophil. The phagocytic vesicle contains a variety of destructive compounds, including hydrogen peroxide (H_2O_2), free hydroxyl radicals ($\cdot\text{OH}$), and other ions such as chloride (Cl^-). Myeloperoxidase catalyzes the following reaction: $\text{H}_2\text{O}_2 + \text{Cl}^- \rightarrow \text{H}_2\text{O} + \text{OCl}^-$. The OCl^- (hypochlorite) formed in the reaction is a strong cytotoxic oxidant responsible for the destruction of foreign substances, such as bacteria. The activity of myeloperoxidase may be impaired with iron deficiency, resulting in increased susceptibility to or severity of infection.

- Peroxidases are also important in producing the thyroid hormones T_3 and T_4 . Thyroperoxidase, also called thyroid peroxidase, is a heme-dependent enzyme necessary for organification of iodide (a process in which two iodides are added to tyrosine residues on thyroglobulin). This same enzyme also conjugates the thyroglobulins to form the thyroid hormones (see the “Functions and Mechanisms of Action” section in the “Iodine” portion of this chapter). Iron deficiency decreases thyroperoxidase activity, resulting in decreased T_3 and T_4 synthesis [9].

Oxidoreductases

Some oxidoreductases that are iron- (and also molybdenum-) dependent include:

- Aldehyde oxidase, which converts aldehydes (RCOH) to alcohols (RCOOH)
- Sulfite oxidase, an iron- and sulfur-containing enzyme, converts sulfite (SO_3) to sulfate (SO_4)
- Xanthine oxidoreductase, an iron-sulfur cluster enzyme, metabolizes hypoxanthine generated from DNA purine base catabolism to uric acid.

These reactions are discussed in detail in the “Functions and Mechanisms of Action” section for molybdenum.

Other Iron-Containing Enzymes: Carbohydrate Metabolism, DNA Synthesis, and Lipid, Steroid, and Drug Metabolism

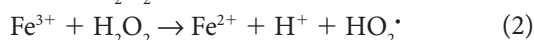
Two enzymes involved in carbohydrate oxidation require iron as a cofactor. In glycolysis, the flavoenzyme glycerol phosphate dehydrogenase has a nonheme iron component. In addition, phosphoenolpyruvate (PEP) carboxykinase, important in gluconeogenesis, requires iron for its functioning.

Another iron-dependent enzyme involved in DNA synthesis, and thus cell replication, is ribonucleotide reductase, which converts ribonucleotides into deoxyribonucleotides. This enzyme contains nonheme iron as part of a bridge with oxygen ($\text{Fe}^{3+}-\text{O}_2-\text{Fe}^{3+}$).

A few additional heme iron-containing cytochromes include cytochrome *b5* involved in lipid metabolism, and the cytochrome P450 family involved in drug metabolism and steroid hormone synthesis.

Iron as a Pro-oxidant

Intracellular iron is normally bound to proteins or a constituent of heme. However, in a state of iron excess, more free iron becomes available to catalyze free-radical reactions. Hydrogen peroxide (H_2O_2), a natural product of cellular metabolism, is a reactant in each of these reactions:



As a pro-oxidant, free ferrous iron in reaction (1) reacts with H_2O_2 to produce ferric iron, a hydroxide ion, and a hydroxyl radical ($\cdot\text{OH}$). This nonenzymatic reaction is known as the Fenton reaction. In reaction (2), ferric iron reacts with H_2O_2 to produce ferrous iron, a proton, and a hydroperoxyl radical ($\text{HO}_2\cdot$). At physiological pH, the $\text{HO}_2\cdot$ is quickly converted to the superoxide radical ($\text{O}_2\cdot$). In reaction (3), known as the Haber-Weiss reaction, $\text{O}_2\cdot$ reacts with H_2O_2 to generate molecular oxygen, a hydroxide ion, and a hydroxyl radical.

Both ferrous and ferric iron participate as pro-oxidants in cells when present in excess. And while each of the free radicals produced in these reactions may cause cellular damage, the hydroxyl radical ($\cdot\text{OH}$) is by far the most reactive oxygen species. Molecules most susceptible to attack by $\cdot\text{OH}$ are polyunsaturated fatty acids of membrane phospholipids, amino acid residues, and DNA. Therefore, avoiding iron excess is an important strategy to minimize cellular damage. Health experts caution that providing iron supplementation to individuals who are iron replete may have adverse effects, particularly in infants and young children, who may experience decreased growth and impaired cognitive and motor development [10].

Turnover

The amount of dietary iron absorbed each day—about 0.06% of the total body iron content—does not meet the daily iron needs of the body. Therefore, avid conservation and constant recycling (turnover) of iron ensures an adequate supply for its various roles in the body.

Most iron that enters the plasma for distribution or redistribution by transferrin results from hemoglobin and ferritin (also hemosiderin) degradation, a process that occurs primarily in the liver, spleen, and bone marrow. (See the section on “Storage” for ferritin degradation.) Briefly, the initial step in hemoglobin degradation involves the uptake of old (senescent) and damaged red blood cells, which normally circulate in the blood for about 120 days. Degradation via

phagocytosis occurs primarily by macrophages present in the spleen; however, reticuloendothelial cells/macrophages in bone marrow and Kupffer cells (macrophages) in the liver also degrade the red blood cells.

Once the old red blood cells are engulfed, an erythrophagosome is formed and degradation follows fusion with lysosomes. The heme portion of the hemoglobin molecule is carried by the heme-responsive gene-1 protein (HRG-1) across the phagolysosomal membrane into the cytosol where it is catabolized by heme oxygenase to release iron and protoporphyrin. Protoporphyrin is subsequently degraded to biliverdin, which is then converted into bilirubin; the bilirubin is secreted into the bile for excretion from the body. With this heme degradation, about 20–25 mg of iron per day is released for reuse. Ferroportin, the same protein responsible for iron efflux from intestinal cells, enables the transport of iron out of the macrophages and reticuloendothelial cells. Specifically, ferroportin facilitates the transport of iron into vesicles, from which it is subsequently secreted into the blood. Oxidation of the iron by ceruloplasmin is also required as the iron is transported out of the cell by ferroportin; as stated previously, this oxidation of iron enables its transport in the blood by transferrin.

The release of iron from the reticuloendothelial cells and macrophages is facilitated by low hepcidin concentrations. Iron released from these cells may be reused, for example, for erythropoiesis, for incorporation into iron-dependent enzymes, or the iron may be deposited for storage. In situations with increased hepcidin (as occurs with increased body iron as well as with inflammation and infection), ferroportin is degraded, and iron is retained within cells.

Most red blood cells are degraded in the reticuloendothelial system within the spleen, liver, or bone marrow, although some (up to ~10%) red blood cell lysis occurs within the blood. Two proteins synthesized in the liver, haptoglobin and hemopexin, remove any released free hemoglobin and heme, respectively, from the blood. Haptoglobin forms complexes with free hemoglobin, and hemopexin forms a complex with free heme in the blood. The proteins then deliver the iron-containing compounds to the liver, where further degradation occurs to enable reuse of the iron. Macrophages, however, also bind haptoglobin–hemoglobin complexes and hemopexin, and thus scavenge iron under conditions of hemolysis and tissue damage. With significant hemolysis, the quantity of iron passing through the plasma can expand to six to eight times the normal amount. In contrast, should erythropoiesis decline dramatically, as occurs on descent from high altitudes, the quantity of iron in the plasma pool may decrease to as little as one-third of normal. Figure 13.8 represents schematically the internal iron exchange in the body.

Interactions with Other Nutrients

You have read that iron and ascorbic acid interact, with vitamin C enhancing nonheme iron absorption and maintaining iron in the appropriate valence state for enzyme function. The potential also exists for vitamin C–induced release of ferric iron from intracellular ferritin, with subsequent reduction of iron to the ferrous form. Whether such reactions result in Fenton reactions and production of reactive oxygen species is unclear.

An interaction also occurs between iron and copper, with a copper deficiency resulting in iron-deficiency anemia.

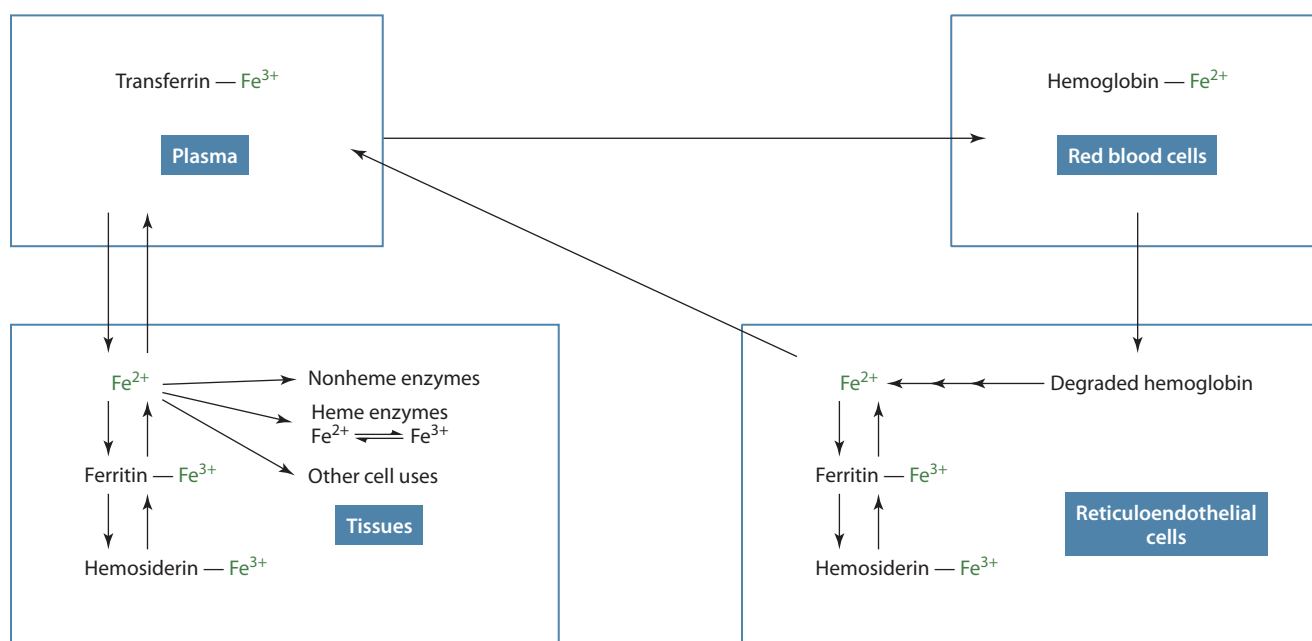


Figure 13.8 Internal iron exchange.

In the 1920s, studies revealed that iron supplements were unable to cure anemia in rats; however, ashed foodstuffs containing copper replenished blood hemoglobin concentrations [11]. Specifically, copper is needed for the ferroxidase activity of hephaestin and ceruloplasmin, which in turn enable iron to be mobilized and used for hemoglobin synthesis.

Another interaction involves iron and zinc and may occur if the two minerals are ingested together in the absence of food and if iron is present as nonheme iron in a ratio with zinc of 2 (or higher) to 1 [6]. While the interaction is not likely to occur with consumption of foods, care should be taken to avoid simultaneous coingestion of these minerals in supplement forms, as perhaps done when treating a deficiency.

Vitamin A and iron are known to interact. Reduced vitamin A status causes iron accumulation in the spleen and liver. Inadequate vitamin A status is also associated with altered red blood cell morphology and decreased plasma iron and blood hemoglobin and hematocrit. The interaction between the nutrients appears to be mediated at least in part through erythropoietin, a hormone made in the kidneys that stimulates erythropoiesis (red blood cell production). Specifically, vitamin A as retinoic acid binds to a response element in the erythropoietin gene and stimulates its transcription. Thus, with insufficient vitamin A, the erythropoietin gene is not transcribed adequately, red blood cell synthesis is diminished, and iron remains in stores. Supplementation of vitamin A in people with poor vitamin A and iron status increases erythropoietin synthesis and increases iron release from stores to provide the iron that is needed for erythropoiesis [12]. Another possible means by which vitamin A may influence iron is through interactions between retinoic acid and the transferrin receptor gene.

Iron and lead also interact. Lead inhibits the activity of Δ -aminolevulinic acid dehydratase, which is required for heme synthesis (see Figure 13.7). Lead also inhibits the activity of ferrochelatase, the enzyme that incorporates iron into heme. Thus, lead poisoning is associated with iron-deficiency anemia secondary to decreased hemoglobin production. In addition, increased absorption of lead occurs with iron deficiency and could be problematic for children, who are often iron deficient and may have increased exposure to lead. The mechanism by which lead absorption is enhanced in situations of iron deficiency is unknown, but it may involve increased lead uptake through competition for the enterocyte transporter DMT1.

Excretion

Total daily iron losses for an adult male are ~0.9–1.2 mg/day. Iron losses for postmenopausal women are a bit lower, ~0.7–0.9 mg/day because of women's smaller surface area

[13]. Most (0.6 mg) iron losses occur through the GI tract. Of this 0.6 mg, about 0.45 mg is lost through minute (~1 mL) blood loss (which occurs even in healthy people), and another 0.15 mg through losses in bile and desquamated mucosal cells. Skin losses of ~0.2–0.3 mg of iron occur with desquamation of surface cells from the skin. Finally, a very small amount, about 0.08 mg, is excreted in the urine. Losses of iron, however, may be greater in people with gastrointestinal ulcers or intestinal parasites, or with hemorrhage induced by surgery or injury.

In contrast to postmenopausal women and men, total iron losses in premenopausal women are higher, estimated at ~1.3–1.4 mg/day because of iron loss in menses. The average loss of blood during a menstrual cycle is ~35 mL, with an upper limit of ~80 mL. The iron content of blood is ~0.5 mg/mL, which translates into a loss of nearly 17.5 mg of iron per period. Averaged over a month, iron loss attributable to menses is ~0.4–0.5 mg/day; in some women, however, iron loss during menses alone may exceed 1.4 mg/day. Balancing iron losses from the body with iron absorption is important to health. Iron deficiency remains one of the most common nutritional deficiencies worldwide.

Recommended Dietary Allowance

For adult men, the requirement and Recommended Dietary Allowance (RDA) for iron are 6 mg/day and 8 mg/day, respectively. For postmenopausal women, the requirement and RDA for iron are 5 mg/day and 8 mg/day, respectively [13]. Because of the greater losses associated with menses, premenopausal women require 8.1 mg of iron/day; the recommended intake is 18 mg/day [13]. During pregnancy, though no menstrual losses occur, iron is needed for the fetus, for expanding blood volume, and for tissue and storage such that the RDA for iron is 27 mg/day. The RDA for iron is 9 mg/day during lactation [13]. The inside front cover of the book provides additional RDAs for iron for other age groups.

Deficiency

Iron deficiency occurs most often due to inadequate iron intake. Population groups most at risk for iron deficiency are:

- Infants and young children (6 months to about 4 years) because of the low iron content of milk and other preferred foods, rapid growth rate, and insufficient body reserves of iron to meet needs beyond about 6 months
- Adolescents in their early growth spurt because of rapid growth and the needs of expanding red blood cell mass
- Females during childbearing years because of menstrual iron losses

- Pregnant women because of their expanding blood volume, the demands of the fetus and placenta, and blood losses that are incurred in childbirth.

In addition, many nonpregnant females in their child-bearing years fall short of the RDA for iron because of restricted energy (caloric) intake and inadequate consumption of iron-rich foods. Furthermore, individuals with renal disease develop iron-deficiency anemia because of an impaired ability to synthesize red blood cells due to reductions in erythropoietin synthesis by the diseased kidney.

The need for iron may also be increased secondary to other iron losses or impaired iron absorption. Other conditions associated with increased iron losses include hemorrhage, hemodialysis (a form of renal replacement therapy that results in the premature destruction of some red blood cells), decreased GI transit time (faster than normal) associated with diarrhea, and infection with parasites, such as hookworm. Gastrointestinal tract conditions as well as surgical procedures (such as Roux-en-Y gastric bypass) associated with damage to or resection of the duodenum and proximal jejunum diminish iron absorption and frequently cause anemia. Impaired iron absorption may also occur with protein-energy malnutrition, renal

disease, achlorhydria (the absence of hydrochloric acid in gastric juice), and prolonged use of medications that increase the pH of the digestive tract, such as antacids and proton pump inhibitors used in the treatment of heartburn, GERD, and ulcers. Individuals who rely on a plant-based diet, in which the iron may be less bioavailable, are also more likely to develop iron deficiency.

Figure 13.9 depicts the gradual depletion of the body's iron content. Iron deficiency is associated with suboptimal health and, if not treated, usually progresses to iron-deficiency anemia. Iron-deficiency anemia impairs the oxygen-carrying capacity of the blood. Signs and symptoms of iron deficiency, more commonly seen in children, include pallor, listlessness, behavioral disturbances, impaired performance in some cognitive tasks, some irreversible impairment of learning ability, and short attention span. In adults, work performance and productivity are commonly impaired with iron deficiency. Further details about iron deficiency with and without anemia as it relates to changes that occur in indices of iron status are covered in the "Assessment of Nutriture" section.

The treatment of iron-deficiency anemia usually requires the use of iron supplements providing 30–65 mg (and sometimes as high as 120 mg) of iron. The initial effects of oral iron supplements on red blood cell counts

	Normal	Early Negative Iron Balance	Iron Depletion	Iron-Deficient Erythropoiesis	Iron-Deficiency Anemia
Iron stores	Full	Partial	Empty	Empty	Empty
Circulating iron	High	Normal	Low	Very Low	Very Low
Erythron iron*	High	Normal	Low	Very Low	Very Low
Reticuloendothelial marrow iron	2–3 ⁺	1 ⁺	0–1 ⁺	0	0
Transferrin iron-binding capacity (µg/dL)	330±30	330–360	360	390	410
Plasma ferritin (µg/L)	100±60	<25	20	10	<10
Iron absorption (%)	5–10	10–15	10–15	10–20	10–20
Plasma iron (µg/dL)	115±50	<120	115	<60	<40
Transferrin saturation (%)	35±15	30	30	<15	<15
Sideroblasts (%)	40–60	40–60	40–60	<10	<10
Erythrocyte protoporphyrin (µg/dL)	30	30	30	100	200
Erythrocytes	Normal	Normal	Normal	Normal	Microcytic Hypochromic
Serum transferrin receptors	Normal	Normal–high	High	Very high	Very high
Ferritin iron	Normal	Normal–low	Low	Very low	Very low

* Iron within circulating erythrocytes and their precursors.

Figure 13.9 Sequential changes in iron status associated with iron depletion.

Source: Adapted from Herbert V, Recommended dietary intakes (RDI) of iron in humans. *Am J Clin Nutr.* 1987; 45:679–86.

and hemoglobin concentrations take about 2 weeks. Iron therapy to increase body stores of iron may be needed for 3 months to 1 year. Side effects associated with the use of iron supplements frequently include constipation, dark stools, nausea, and/or stomach pain. Parenteral (intravenous) iron therapy is available as iron dextran, sodium ferric gluconate, and iron sucrose should oral supplements not be tolerated or not adequately improve iron status.

Supplemental iron is also recommended for specific population groups due to higher risks of deficiency. For example, pregnant women are typically advised to take a prenatal supplement providing iron (usually at least 30 mg) and, if iron deficient, a supplement providing higher amounts of iron is typically advised. In addition, the use of an iron-fortified formula may be recommended for infants less than 1 year of age who are being primarily breastfed. Iron may also be recommended for breastfed infants who are not consuming iron-fortified cereals by about 4–6 months of age. Excessive iron through dietary supplements in infants and young children can result in adverse effects, so caution is warranted [10].

Toxicity

The Tolerable Upper Intake Level for iron for adults is 45 mg/day [13]. Acute iron toxicity, which can be lethal, is observed mostly with accidental iron overload, as may occur with the ingestion of excessive numbers of iron pills or iron-containing multivitamin/mineral supplements. With acute toxicity, the excessive presence of an overload of iron atoms is thought to exceed the transport carrying capacity of transferrin. The unbound iron in turn promotes free radical production, damaging the GI tract and other tissues. Manifestations of acute toxicity initially include abdominal pain, vomiting, and diarrhea. The stool may become darker secondary to the presence of iron; however, iron-induced damage to the digestive tract mucosa also causes hemorrhage, resulting in blood in the emesis and feces. Excessive blood loss may result in hypovolemic shock. Some additional manifestations that may develop include acidosis, coma, and liver failure.

Chronic iron toxicity is generally associated with the genetic disorder hemochromatosis, a condition most often seen in Caucasian males and that becomes evident around 20 years of age. An estimated 50 per 10,000 people in the United States are homozygous for the disorder. Hemochromatosis is characterized by increased (at least two times normal) iron absorption. Mutations in one of several genes that result in diminished hepcidin synthesis cause the condition and prevent the body from accurately sensing iron stores and down-regulating intestinal iron absorption. For example, in the C282Y mutation in the HFE protein, tyrosine is substituted for cysteine because of a single base change; this alteration inhibits HFE's ability to stimulate

the synthesis of hepcidin, which tells the intestinal cells to down-regulate iron absorption. Similarly, the H63D mutation also reduces hepcidin synthesis, whereas a mutation (Q248H) in ferroportin promotes hemochromatosis due to continued ferroportin expression.

Although several mutations can cause hemochromatosis, in most people with the condition, iron absorption generally continues unregulated despite high iron stores. The absorbed iron is progressively deposited throughout the body (including within joints and tissues, especially the liver, heart, and pancreas), causing extensive organ damage and ultimately organ failure. Iron deposition in the liver, for example, leads to cirrhosis, usually by about 50 years of age. Heterozygotes for the condition do not develop as severe organ dysfunction but exhibit abnormal iron status. For example, if untreated, serum ferritin concentrations in hemochromatosis continue to rise in the blood and may exceed 1,000 µg/L (normal: < 300 µg/L).

Treatment of hemochromatosis requires frequent phlebotomy (removal of blood), usually the weekly removal of about 1 unit (~400–500 mL), which contains about 200–250 mg of iron. In addition, deferoxamine may be given. Deferoxamine works by chelating (binding to) iron in the body and increasing urinary iron excretion. Treatment of hemochromatosis usually continues as described until serum ferritin concentrations are about 50–150 µg/L and transferrin saturation is less than 45% [14]. Once these levels are achieved, the frequency with which the person undergoes phlebotomy can be diminished.

Other people at particularly high risk for iron overload are those with iron-loading anemias, thalassemia, and sideroblastic anemia. The elevated erythropoiesis in the bone marrow in people so affected causes increased iron absorption.

Assessment of Nutriture

Iron deficiency is commonly diagnosed using markers of anemia, specifically low blood hemoglobin concentration and low hematocrit (the volume percentage of red blood cells in the blood). Both measurements reflect diminished production of red blood cells, which depends on the synthesis of iron-containing heme. It is important to note that anemia develops only in the latter stages of iron deficiency, and that other indicators—such as decreased plasma ferritin—are more sensitive to early stages of iron deficiency. However, determination of hemoglobin and hematocrit are relatively easy and widely accepted methods (Figure 13.10).

In the first stages of iron deficiency, iron stores in the liver, spleen, and bone marrow are diminished. Although iron stores can be aspirated and measured from bone marrow, the routine test involves measurement of plasma (or serum) ferritin. Decreases in plasma ferritin concentration

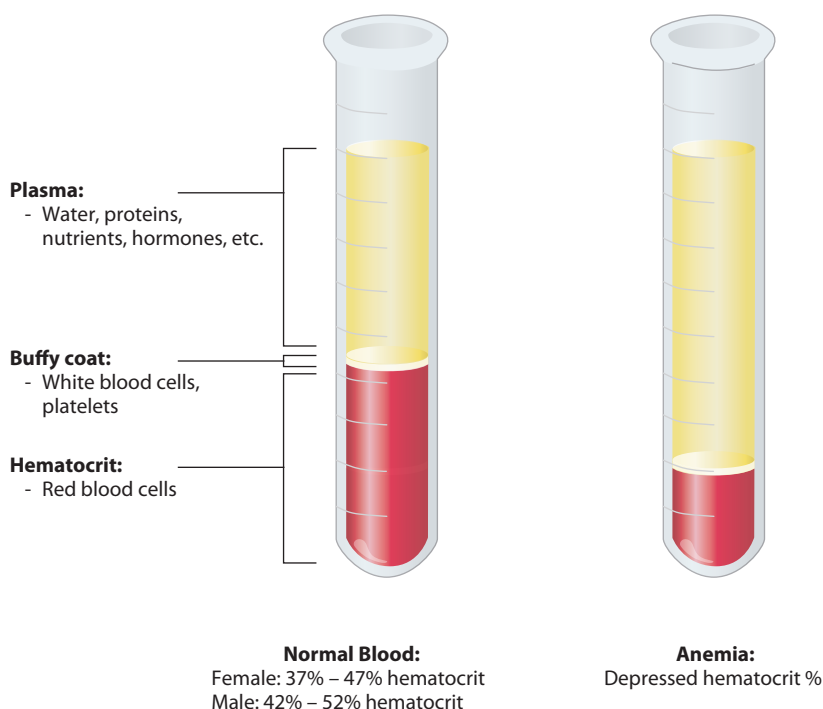


Figure 13.10 Measurement of hematocrit.
Source: Slideshare.net.

are thought to parallel the decrease in the amount of iron found in stores. Plasma ferritin concentrations less than about 15 $\mu\text{g/L}$ are associated with iron deficiency and with bone marrow iron deficits. However, if inflammation or infection is present, the plasma ferritin concentration rises, an occurrence unrelated to iron stores. Thus, plasma ferritin may appear within normal range or high while the body's iron status is quite low; measurement of the levels of C-reactive protein or alpha-1 acid glycoprotein in the blood can be used to detect the presence of inflammation or infection. Once iron stores are depleted, plasma ferritin concentrations no longer reflect the tissue iron pool.

As iron deficiency progresses into the second stage, iron stores typically remain low, and transport iron decreases. Thus, plasma ferritin concentrations continue to be diminished, and circulating iron begins to decrease. Because iron circulates in the blood bound to transferrin, with iron deficiency, transferrin saturation decreases; levels less than 16% are diagnostic of deficiency (remember normal saturation is about 30–40%). Transferrin saturation can be calculated by multiplying the serum iron concentration by 100 and then dividing by the total iron-binding capacity (TIBC). TIBC represents the amount of iron that plasma transferrin can bind and normally ranges from ~250 to 400 $\mu\text{g/dL}$. Levels greater than 400 $\mu\text{g/dL}$ suggest iron deficiency. Serum iron concentrations, which represent the amount of iron bound to transferrin and other blood proteins, are also affected with iron deficiency, decreasing to $<50 \mu\text{g/dL}$ (normal range $\sim 50\text{--}180 \mu\text{g/dL}$).

As circulating iron diminishes, functional or cellular iron also becomes limited. With diminished iron, free protoporphyrin concentrations in erythrocytes rise. Protoporphyrin is a precursor of heme (for hemoglobin) and accumulates within red blood cells when iron is not available. Erythrocyte protoporphyrin levels greater than 70 $\mu\text{g/dL}$ red blood cells are associated with iron deficiency. In iron deficiency, the number of transferrin receptors on the cell surface, especially of immature red cells, also increases. The increased receptor number represents an up-regulation to enable cells to better compete for transferrin-bound iron. With iron deficiency, concentrations of serum transferrin receptors (sTfR), truncated forms of the membrane receptor protein, increase to greater than 8.0 mg/L and are thought to be directly proportional to the functional tissue (i.e., cellular) iron deficit after depletion of iron stores.

In the final stages of iron deficiency, anemia occurs. With anemia, blood hemoglobin concentrations drop below the lower limit of normal, which is typically 12 g/dL and 13 g/dL for females and males, respectively. Hematocrit concentrations with anemia also decrease below the lower limit of normal, less than about 37% and 40% for women and men, respectively. Hematocrit values are normally about three times higher than hemoglobin values. Characterization of red blood cells with respect to size (mean corpuscular volume, or MCV) and amount of hemoglobin they contain (mean corpuscular hemoglobin, or MCH, and mean corpuscular hemoglobin concentration, or MCHC)

typically shows that they are smaller and lower in hemoglobin than normal in the final stages of iron-deficiency anemia. Descriptions of these assessments follow.

- MCV (fL) represents the size of the red blood cell. It is calculated by dividing hematocrit by red blood cells and then multiplying by 10.
- MCH (pg/rbc) represents the average hemoglobin content of each individual red blood cell. It is calculated by dividing hemoglobin by number of red blood cells and then multiplying by 10.
- MCHC represents the amount of hemoglobin in grams per deciliter (%) of red blood cells. It is calculated by dividing hemoglobin by hematocrit and then multiplying by 100.

Thus, red blood cells are pale (hypochromic) and small (microcytic) with iron-deficiency anemia. Figure 13.9 illustrates the changes that occur in the various measurements.

Other assessments that can be useful in assessing iron status, especially with inflammatory conditions, include the ratio of serum soluble transferrin receptors to the log of ferritin, with derived values greater than 2 suggesting anemia of chronic disease with iron deficiency. A ratio less than 1 is indicative of anemia of chronic disease without iron deficiency. The presence of inflammation is most often determined by elevations in C-reactive protein (CRP) concentrations (> about 0.5 or 1 mg/dL). A functional iron deficit is also suggested by low reticulocyte hemoglobin concentrations (less than about 28 pg).

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13.2 ZINC

The human body contains about 1.5–3.0 g of zinc. Zinc is found in all organs, tissues (especially muscle and bone), and body fluids. Zinc, a metal, can exist in several different valence states, but it is almost universally found as the divalent ion (Zn^{2+}) in the human body.

Sources

Zinc is found in foods complexed with nucleic acids and with amino acids that are part of peptides and proteins. The zinc content of foods varies widely (Table 13.4). Very good sources of zinc are red meats (especially organ meats) and seafood (especially oysters and mollusks). Other relatively good animal sources of zinc include poultry, pork, and dairy products. Animal products are thought to provide 40–70% of zinc consumed by most people in the United States. Whole grains and legumes provide moderate amounts of zinc. Cereals, some of which may be fortified, are thought to provide about 30% of the zinc in the U.S. diet. Fruits contain little zinc. Zinc from plant sources is also absorbed to a lesser extent than zinc from animal sources (e.g., meat). The Daily Value for zinc used on food and supplement labels is 11 mg.

Processing of certain foods may affect zinc availability. Heat treatment can cause zinc in food to form complexes that resist hydrolysis, thereby making zinc less available for absorption. Maillard reaction products—that is, amino acid–carbohydrate complexes resulting from browning, for example—are particularly notable for inhibiting zinc's availability for absorption.

Zinc supplements are available in several forms including oral tablets and lozenges, throat or nasal sprays, and nasal gels. Zinc is typically found in supplements as zinc oxide, zinc sulfate, zinc acetate, zinc chloride, and zinc gluconate. These various forms provide differing amounts of zinc. Zinc gluconate, for example, is approximately 14.3% zinc, whereas zinc sulfate is 23% zinc, zinc acetate is 30% zinc, and zinc chloride is 48% zinc. Zinc chloride and zinc sulfate are very soluble, as is zinc acetate. In contrast, zinc carbonate and zinc oxide are fairly insoluble.

Table 13.4 Zinc Content of Select Foods*

Select Foods/Food Group	Zinc (mg)
Seafood	
Oysters, farmed (3 oz)	38.4
Crabmeat (3 oz)	3.2
Shrimp (3 oz)	1.4
Cod, Atlantic (3 oz)	0.5
Salmon, farmed (3 oz)	0.4
Tuna, light, canned in water (3 oz)	0.7
Meat and poultry	
Liver, beef (3 oz)	4.5
Chicken, dark meat (3 oz)	2.4
Chicken, white meat (3 oz)	1.0
Beef, top sirloin (3 oz)	4.6
Beef, ground (3 oz)	5.3
Veal, ground (3 oz)	3.3
Pork, ground (3 oz)	2.7
Eggs and dairy products	
Egg, poached (1 large)	0.6
Milk, 1% fat (1 cup)	1.0
Cheese, variety (1 oz)	0.5–1.2
Legumes , variety (½ cup)	0.8–2.7
Nuts , variety (1 oz)	0.9–1.6
Grains and cereals	
Rice and pasta (1 cup)	0.7–1.2
Quinoa (½ cup)	1.0
Bread, whole wheat (1 slice)	0.5
Bread, white, enriched (1 slice)	0.3
Vegetables (1 cup)	0.1–0.7
Fruits	< 0.1

*Data represent cooked foods, except for fruits, nuts, and milk.

Source: U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov>

Multivitamin/mineral supplements contain up to about 15 mg of zinc, while single-nutrient supplements provide about 10–100 mg of zinc. Zinc supplements for oral consumption should be consumed on an empty stomach, without simultaneously ingesting other mineral supplements such as iron or calcium. Abdominal pain (e.g., gastric irritation), dyspepsia, nausea and vomiting, and diarrhea are commonly reported side effects with the use of zinc supplements.

Topical zinc products, which usually provide zinc as zinc oxide or zinc chloride, may be used in wound management and include paste bandages, occlusive adhesive dressings, alginates, stockings, and zinc-saline dressings. The percutaneous absorption of zinc depends on the skin's integrity and requires (in the case of zinc oxide or chloride) the acidic moisture on the skin's surface to release the zinc. If skin is not intact, as may occur with burns or other

injury, much more zinc is absorbed. In the presence of an intact skin barrier, zinc binds to the sulfhydryl groups in epidermal keratin upon release from the zinc oxide or chloride salt in the acidic environment. A small amount of the zinc will then penetrate beyond the superficial keratinocytes and enter circulation in a process that takes about 60 minutes from the time of topical application. The remaining zinc not entering the circulation binds to metallothionein (which is induced with topical zinc application) in epidermal keratinocytes and will eventually be sloughed off with skin cell turnover.

In addition to dietary sources, endogenous zinc (up to about 4.5 mg) from pancreatic, intestinal, and biliary secretions is released into the GI tract with food ingestion and augments the zinc present in food sources and dietary supplements. The secreted zinc can be reabsorbed and represents an important means for body zinc homeostasis.

Digestion, Absorption, Transport, and Storage

Figure 13.11 provides an overview of zinc digestion, absorption, and transport as well as some of zinc's fates in the enterocyte.

Digestion

Zinc needs to be hydrolyzed from amino acids and nucleic acids before it can be absorbed. Zinc is believed to be liberated from these food constituents during the digestive process, most likely by the acidic environment of the stomach and upper duodenum and by proteases and nucleases in the stomach and small intestine.

Absorption

Zinc absorption occurs primarily in the proximal small intestine, that is, the duodenum and upper jejunum. Two mechanisms (carrier-mediated transport and diffusion) are responsible for intestinal zinc absorption (Figure 13.11). The primary means of absorption with usual intakes, which is up to about 7–9 mg of zinc, is saturable and carrier mediated [1]. The protein carrier Zrt- and Irt-like protein 4 (ZIP4) is the major transporter of zinc across the enterocyte brush border membrane and is expressed throughout the GI tract. Zinc intake influences ZIP4. With high zinc intakes, ZIP4 is degraded more rapidly to down-regulate absorption, whereas zinc restriction enhances ZIP4 mRNA stability, rapidly induces ZIP4 synthesis, and shifts ZIP4 proteins to the brush border membrane. The enhanced ZIP4 synthesis is mediated by up-regulation of the transcription factor Kruppel-like factor 4 (KLF4), which binds to the promoter region on the ZIP4 gene.

In addition to carrier-mediated transport, paracellular (meaning between cells) diffusion of zinc through the

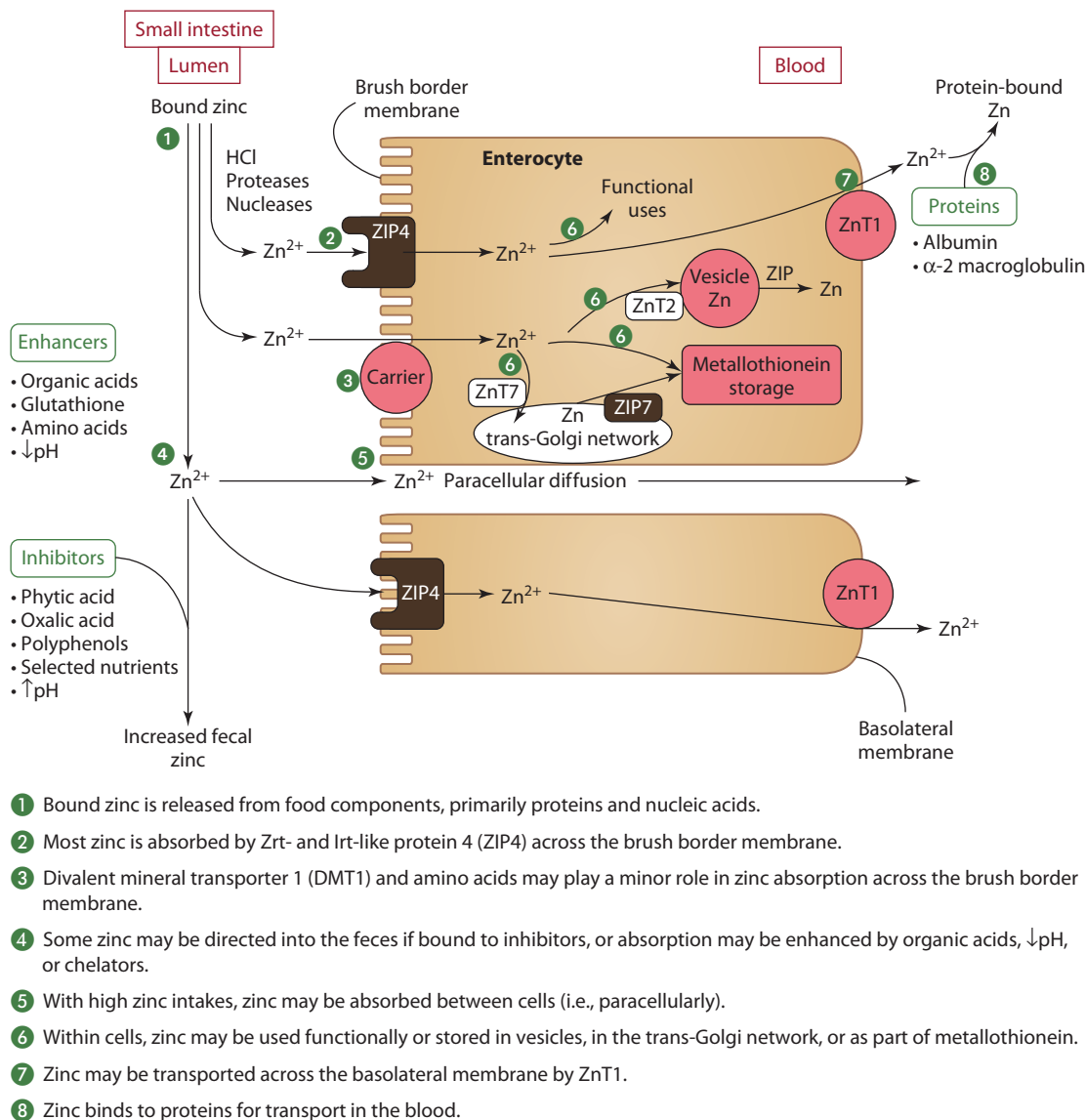


Figure 13.11 Digestion, absorption, enterocyte use, and transport of zinc.

tight junctions of the enterocytes enables absorption. This process is thought to contribute to the absorption of zinc when zinc intakes (typically 20 mg or more) exceed the capacity of the ZIP4 carriers.

A mutation in ZIP4 causes the disorder acrodermatitis enteropathica. The condition is characterized by poor zinc absorption and is clinically manifested by skin lesions (which often become infected), especially on the face, knees, and buttocks; impaired growth; and low plasma zinc concentrations, representing signs and symptoms of zinc deficiency. If untreated, the condition can be fatal. The provision of high doses of zinc (30–150 mg/day) that can be absorbed by paracellular diffusion typically helps compensate for the impaired ZIP4 transporters.

Although other carrier proteins, such as DMT1, present in the GI tract bind zinc, they are not thought to contribute except in a minor capacity to zinc absorption. Similarly,

some zinc may bind to amino acids such as histidine and perhaps enter the enterocyte using amino acid transporters; however, this contribution to zinc absorption is minor. ZIP11 is also found on the brush border membrane, and its synthesis is up-regulated with zinc restriction; however, at this time, its role in zinc absorption is thought to be small, if any [1].

Overall, about 20–50% of zinc is absorbed from the typical U.S. diet. However, fractional zinc absorption varies from approximately 10 to 80%; at higher intakes absorption diminishes, whereas at lower intakes absorption increases. For example, 100% of zinc may be absorbed at an intake less than 1 mg, whereas about 40% may be absorbed with a zinc intake of 12 mg [1]. This up- and down-regulation of absorption as well as the ability to increase and decrease zinc excretion are important for maintaining zinc homeostasis in the body.

Factors Influencing Zinc Absorption

As is the case with iron, chelators bind to zinc. Whether these substances are enhancers or inhibitors of zinc absorption depends on the digestibility and absorbability of the zinc–chelate complexes that form.

Enhancers of Zinc Absorption Chelators including organic acids—like citric acid and picolinic acid—and prostaglandins may bind and promote zinc absorption. Pancreatic secretions are thought to contain an unidentified constituent that enhances zinc absorption. In addition, glutathione—a tripeptide composed of cysteine, glutamate, and glycine—and products of protein digestion, such as tripeptides and amino acids, are purported to serve as chelators. Zinc typically binds to sulfur (e.g., cysteine alone, or as part of glutathione) and nitrogen (e.g., histidine) that reside within the structure. Amino acids that chelate zinc help to maintain its solubility in the GI tract; whether zinc bound to amino acids can be absorbed using amino acid transporters is unclear.

Absorption of zinc is also enhanced by an acidic environment. Conversely, the use of antacid medications that treat heartburn, GERD, and ulcers raise gastric and proximal intestinal pH and decrease zinc absorption. These medications include:

- H_2 receptor blockers, such as Axid (nizatidine), Tagamet (cimetidine), and Pepcid famotidine)
- Proton pump inhibitors, such as Prevacid (lansoprazole), Prilosec (omeprazole), and Nexium (esomeprazole).

Inhibitors of Zinc Absorption In addition to a more alkaline environment, which diminishes zinc absorption, many compounds in food complex with zinc to inhibit its absorption. Some examples of inhibitors include:

- *Phytic acid* found in plant foods, particularly legumes, lentils, nuts, seeds, and whole-grain cereals. It binds to zinc (as well as other minerals) via oxygen within the compound's phosphate groups. The zinc–phytic acid complex is large, insoluble, and poorly absorbed. Reductions in zinc bioavailability are greatest when the phytate-to-zinc molar ratio is greater than 15 to 1; the aforementioned plant foods typically contain such phytate-to-zinc ratios [2]. Calculation of the ratio is done as follows: “phytic acid content of the food” / “molecular weight of phytic acid, which is 660” ÷ “zinc content of the food” / “atomic weight of zinc, which is 64.5” [2]. The fermentation of foods, such as bread, reduces its phytic acid content and improves zinc absorption. Figure 13.12 depicts the binding of zinc by phytic acid. Individuals in the United States consuming primarily plant-based high-phytate diets may absorb less than adequate amounts of dietary zinc.

- *Oxalic acid (oxalate)*, found in a variety of foods, most notably spinach, chard, berries, chocolate, and tea. The binding of zinc by oxalic acid is shown in Figure 13.12.
- *Polyphenols* (such as tannins and gallic acid), found in tea and coffee, and some *fibers*, found in whole grains, fruits, and vegetables. The impact of these substances on an individual's zinc status varies with the amount consumed and other sources of zinc in the diet. Most individuals in the United States consume well under the recommended amount of dietary fiber.
- *Other minerals such as iron and calcium.* The negative influence of iron and calcium is incompletely understood. Iron in amounts of 20–25 mg or more, or in a ratio with zinc of 2 (or higher) to 1, impairs zinc absorption when ingested in solution but not as part of a meal [3]. Thus, in practice, to maximize zinc absorption, a zinc supplement should not be consumed at the same time as a nonheme iron supplement on an empty stomach. The effects of calcium on zinc absorption and balance are equivocal. Some studies have shown that ingestion of calcium (500 mg–2 g) as calcium carbonate, hydroxyapatite, or calcium citrate malate has no effect on zinc absorption, whereas other studies providing similar amounts of calcium as milk, calcium phosphate, and calcium carbonate found reductions in net zinc absorption and zinc balance. Results appear to vary with the forms and amounts of the nutrients provided, the study populations, and the simultaneous presence/absence of other inhibitors such as phytate in the diet. To minimize the likelihood of interactions, it is prudent to not take mineral supplements at the same time, to avoid the ingestion of calcium supplements at meals providing significant amounts of zinc, and to ensure adequate dietary intake of zinc from bioavailable foods.

Intestinal Cell Zinc Use

Zinc entering the enterocyte has several possible fates. The zinc may be:

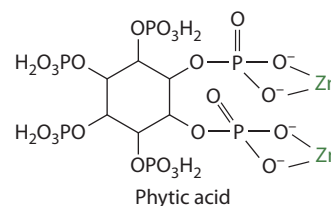
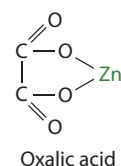


Figure 13.12 The binding of zinc by oxalic acid and phytic acid.

- Used functionally within the enterocyte (see the section “Functions and Mechanisms of Action”)
- Stored or sequestered in the enterocyte
- Transported through the cytosol and across the basolateral membrane into the blood for delivery to other tissues.

If not used functionally within cells such as enterocytes, zinc is largely sequestered or bound to proteins, so little zinc is found free. Zinc transporters function to remove zinc from the environment; that is, they lower intracellular (cytosolic) zinc concentrations, mediating both zinc efflux from cells and movement of zinc into intracellular compartments. Intracellularly, zinc is sequestered in vesicles, secretory granules, endosomes, or the trans-Golgi network (complex). The intracellular trafficking protein ZnT7 in the enterocyte is thought to be involved in zinc movement into the trans-Golgi network, while ZIP7 may transport the mineral out of the trans-Golgi network; however, the exact roles of these two transporters have not been clearly established. ZnT2 appears to sequester zinc in endosome-like vesicles within enterocytes. Overall, the intracellular trafficking of zinc is not well delineated.

The protein metallothionein (sometimes called thionein if free of metals) serves as zinc’s main short-term storage protein and as an intracellular chelator. Metallothionein contains an unusually high content (30%) of cysteine, which functions in metal binding. Metallothionein is thought to both transport zinc to zinc-requiring enzymes and store zinc transiently. The metallothionein-bound zinc can be released for cellular use, but if not used, it is lost into the feces with the sloughing of enterocytes that is part of normal intestinal cell turnover. Metallothionein is covered in further detail in the “Storage” section.

Zinc needed for extraintestinal use is carried across the basolateral membrane of the enterocyte for release into the blood. Zinc transporter 1 (ZnT1), which does not require sodium or ATP, preferentially transports zinc out of duodenal and jejunal cells, likely in exchange for H^+ or K^+ ; ZnT1 is also found on several other cell membranes. The synthesis of ZnT1 is increased with high dietary zinc intake but does not appear to be affected with decreased zinc intake. In addition to ZnT1, a sodium-zinc exchanger has been identified that appears to direct sodium-dependent active extrusion of zinc from some cells. DMT1, found on the basolateral membrane of enterocytes, may also play a minor role in intestinal cell zinc efflux into the blood.

Transport

Zinc passing into portal blood from the intestinal cell is mainly transported loosely bound to albumin. Most zinc is then taken up by the liver, where the mineral is initially concentrated. Zinc leaving the liver binds mainly (about 70–75%) to albumin, with the remaining zinc bound more tightly to α -2-macroglobulin. Two amino acids—histidine

and cysteine—also loosely bind and transport (in a ternary complex as histidine–zinc–cysteine) less than 1% of zinc in the blood. Plasma zinc concentrations range from about 70 to 120 $\mu g/dL$ (10 to 18 $\mu mol/L$), with plasma containing about 3 mg of zinc. Plasma zinc concentrations decrease after eating, as well as under conditions of infection and trauma.

Multiple transporters, including at least 14 ZIPs and 10 ZnTs, facilitate cellular zinc uptake and release, yet many of the mechanisms remain unclear. ZIP carriers 1, 2, 4, 5, 6, 7, 8, and 14 appear to be involved in cellular zinc uptake from extracellular locations and the release of zinc from intracellular stores, both to effect increased cytosolic zinc concentrations. ZIP14, for example, transports zinc into hepatocytes, and its activity appears to be increased as part of the acute-phase reactant response (as occurs with infections and trauma). The transporter ZIP5 is expressed in the intestinal cell as well as the pancreas, liver, and kidneys. Intestinal ZIP5 is found on the basolateral membrane, where it facilitates serosal to mucosal zinc transport. In other words, ZIP5 moves zinc from the blood into the intestinal cell. The zinc transporter ZnT6, found on the enterocyte’s brush border membrane, is thought to mediate the exocytosis of zinc from the intestinal cell back into the lumen for ultimate excretion in the feces. Not all ZIP carriers, however, solely transport zinc; many, such as ZIP14, which transports both Fe^{2+} and Zn^{2+} , carry other minerals as well.

Storage

Zinc is found in all body tissues and organs, most notably the liver, kidneys, muscle, skin, and bones. Within cells, about 30–40% of zinc is bound to proteins in the nucleus, about 50% is in the cytosol, and the remaining zinc is found in cell membranes. The zinc content of most soft tissues including muscle, brain, heart, and lungs is relatively stable. This soft-tissue zinc does not respond to or equilibrate with other zinc pools to release zinc if dietary zinc intake is low. Similarly, although zinc is found in bones as part of apatite, bones release the mineral very slowly and cannot be depended on to supply zinc during dietary deprivation. Instead, when dietary zinc intake is insufficient, catabolism of selected “less essential” zinc-containing metalloproteins (enzymes) and liver metallothionein occurs to enable the release and redistribution of zinc to meet particularly crucial needs for the mineral.

Zinc is stored attached to metallothionein, which contains a high proportion of cysteine residues that bind metals, including not only zinc (seven atoms/molecule), but also copper, cadmium, and mercury. Metallothionein is found in most body tissues, including the liver, pancreas, kidneys, intestine, keratinocytes, and red blood cells. Various forms of the protein exist and are designated by number as metallothionein (MT)-1 through MT-4. MT-1 and MT-2 appear to be the most common tissue

forms. Although metallothionein is thought to provide a short-term storage pool of zinc, other roles have also been attributed to the protein. Metallothionein may regulate zinc distribution by serving as a transporter or chaperone, thus transferring zinc to enzymes, gene-regulatory molecules, or other acceptor proteins. Metallothionein also exhibits antioxidant-type functions. For example, the protein is known to scavenge free hydroxyl radicals. In times of cell injury/stress, metallothionein synthesis increases and helps control free radical concentrations. This response is part of the body's acute-phase response, and the enhanced thionein gene expression results in part from the increased release of glucagon and the cytokine interleukin 1 (which is synthesized and secreted by monocytes and activated macrophages). The induction in thionein gene transcription during infection promotes zinc storage and prevents bacterial use of the mineral. Should zinc be needed within cells, it may be released from metallothionein by the action of lysosomal proteases. At an acidic pH, these proteases degrade metallothionein to release the zinc for use by cells.

Zinc regulates the gene expression of thionein. More specifically, metal regulatory or response elements (MREs), consisting of particular nucleotide sequences, are found in the promoter region of the thionein gene. A metal transcription factor dependent on zinc interacts with the MRE to induce thionein synthesis. See the "Gene Expression" section for a more complete description of how zinc affects gene transcription.

Functions and Mechanisms of Action

Zinc plays a vital role facilitating hundreds of biochemical reactions. Because of its expansive enzymatic functions, zinc impacts most metabolic pathways including carbohydrate, protein, nucleic acid, and lipid metabolism. As a structural component of thousands of transcription factors, zinc has profound effects on gene expression and in turn impacts numerous physiologic processes in the body.

Zinc-Dependent Enzymes

As a component of metalloenzymes, zinc provides two primary functions: (1) structural integrity to the enzyme by binding directly to amino acid residues and thereby stabilizing the enzyme's tertiary structure and/or (2) participation in the reaction at the catalytic site. Zinc appears to be part of more enzyme systems than all the rest of the trace minerals combined; over 300 enzymes from every enzyme class (oxidoreductases, hydrolases, lyases, isomerases, transferases, and ligases) require zinc. A few of these zinc-dependent enzymes are listed in Table 13.5 and are described hereafter.

Carbonic Anhydrase: Acid–Base Balance Carbonic anhydrase, found primarily in erythrocytes and in renal tubule cells, is essential for acid–base balance/buffering

Table 13.5 Select Functions of Zinc

Metalloenzyme component
Carbonic anhydrase
Alkaline phosphatase
Alcohol dehydrogenase
Carboxypeptidases
Aminopeptidases
Delta aminolevulinic acid dehydratase
Superoxide dismutase
Phospholipase C
Polyglutamate hydrolase
Matrix metalloproteinases
Polymerases
Kinases
Nucleases
Transcriptases
Gene expression: zinc fingers
Membrane/cytoskeletal stabilization
Immune function
Sexual maturation
Fertility and reproduction

and respiration. The enzyme has a high affinity for zinc: About eight to nine times more zinc is found associated with this enzyme in red blood cells than is found in the plasma. The enzyme catalyzes the following reaction, thereby allowing the rapid disposal of carbon dioxide: $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-$. The H^+ dissociated from carbonic acid reduces oxyhemoglobin as oxygen is released to the tissues; the bicarbonate passes into the plasma to participate in buffering reactions. Concentrations of the enzyme are not significantly affected by zinc deprivation, but activity in red blood cells diminishes with low zinc (3.8 mg/day for several weeks) diets [4]. (See also "Acid–Base Balance" in Chapter 12.)

Alkaline Phosphatase: Phosphate Release Alkaline phosphatase contains four zinc atoms per enzyme molecule. Two of the four atoms are required for enzyme activity. The other two are needed for structural purposes in maintaining proper protein conformation. The enzyme is found mainly in bones and in the liver, with small amounts in the plasma. Alkaline phosphatase lacks substrate specificity, hydrolyzing monoesters of phosphates from various compounds. Enzyme activity decreases with zinc deficiency.

Alcohol Dehydrogenase: Nonspecific Aldehyde Synthesis Alcohol dehydrogenase contains four zinc atoms per enzyme molecule, with two of the four required for catalytic activity and two required for structural purposes (protein conformation). This enzyme, which generally lacks specificity, is important in the NADH-dependent conversion of alcohols to aldehydes. For

example, in vitamin A metabolism, the enzyme converts retinol to retinal, which is needed for the visual cycle and especially night vision. In addition, the enzyme converts ethanol to acetyl aldehyde, a reaction important in alcohol metabolism.

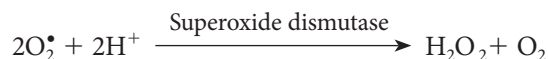
Carboxypeptidases A and B and Aminopeptidases: Protein Digestion Carboxypeptidases A and B, exopeptidases secreted by the pancreas into the duodenum, are necessary for protein digestion. Zinc is bound tightly to carboxypeptidases and is essential for enzymatic activity; consequently, enzyme activity decreases with zinc deficiency. Figure 13.13 shows the zinc-containing portion of the carboxypeptidase A enzyme.

Aminopeptidases consist of a group of enzymes also involved in protein digestion. Aminopeptidases typically contain one or two zinc atoms, needed for catalytic activity. The enzymes cleave amino acids from the amino (N)-terminal end of proteins or polypeptides that are being digested in the intestinal tract.

Delta (Δ)-Aminolevulinic Acid Dehydratase: Heme Synthesis Δ -aminolevulinic acid dehydratase, needed for heme synthesis, is also zinc dependent. This enzyme is made up of eight subunits, each of which binds one zinc atom. Zinc is essential for the maintenance of free thiols ($-\text{SH}$) in the enzyme because zinc prevents the oxidation of thiol groups and consequently disulfide bond formation within the enzyme. The enzyme catalyzes the condensation of two Δ -aminolevulinic acids to form porphobilinogen (see Figure 13.7). Lead, if present in the body in high concentrations as occurs with lead poisoning, replaces zinc in the dehydratase and diminishes heme synthesis.

Superoxide Dismutase: Antioxidant Superoxide dismutase (SOD1 or CuZn-SOD) found in the cell cytosol requires two atoms each of zinc and copper for function; zinc

appears to have a structural role in the enzyme. An extracellular form of the enzyme (SOD3) that is also zinc and copper dependent has been characterized and appears to be more sensitive to zinc than is the cytosolic form of the enzyme. The extracellular form is found in the plasma, lymph, synovial fluid, and lungs; it exists in equilibrium between cell surfaces and the plasma. Both the cytosolic and extracellular forms of superoxide dismutase serve important antioxidant defense roles in the body by catalyzing the removal of superoxide radicals, $\text{O}_2^\bullet$:



Further information on this enzyme is found in the section on copper, in the “Functions and Mechanisms of Action” subsection.

Phospholipase C: Phospholipid Metabolism Phospholipase C requires three zinc atoms for catalytic activity. This membrane-bound enzyme selectively hydrolyzes the glycerophosphate bond of phosphatidylinositol 4,5-bisphosphate (PIP_2), producing diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3). Both products, DAG and IP_3 , are important second messengers that control many cellular processes and are substrates for synthesis of other signaling molecules.

Polyglutamate Hydrolase: Folate Digestion Polyglutamate hydrolase, also called γ -glutamylhydrolase or pteroyl-glutamate hydrolase, is a zinc-dependent enzyme necessary for folate digestion in the GI tract. Folate, a B vitamin, is found in foods bound to several (i.e., poly) glutamic acid residues. For folate to be absorbed, all but one of the glutamic acids must be removed. The enzyme polyglutamate hydrolase catalyzes the removal of glutamic acids from folate. Poor zinc status can diminish folate absorption.

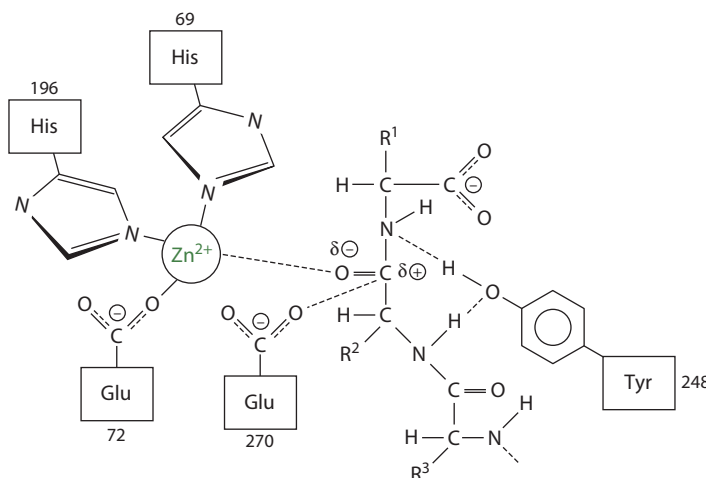


Figure 13.13 Partial structure of carboxypeptidase A.

mTOR regulates insulin-signaling and protein-synthesis pathways that occur when insulin binds to cell receptors.

Some of zinc's other diverse roles include effects on basal metabolic rate. A decrease in thyroid hormones and basal metabolic rate has been observed with consumption of a zinc-restricted diet. Zinc is also important for taste. It is a component of gustin, a protein involved in taste acuity, and carbonic anhydrase, which is found in salivary glands and taste buds.

Cell survival and immune function (both cell-mediated and humoral) are also influenced by zinc. Consequently, much attention has focused on zinc's role in cancer development and treatment. The literature in this area is extensive, consistently showing an association between zinc deficiency and incidence of cancer. The prevailing thought is that immune dysfunction allows tumor cells to grow without initiating the normal immune response. A specific example of zinc's role is illustrated through its actions on thymulin, a zinc-dependent hormone peptide that regulates the differentiation of maturing T cells in the thymus gland and the function of maturing T cells in the blood (including cytokine release). With zinc deficiency, thymulin activity diminishes, thus increasing the risk of cancer development. The use of targeted zinc supplementation as a cancer treatment shows promise, but experts caution that excessive zinc can have immunosuppressive effects [5].

Selected Pharmacological Uses

Colds The relationship between immunity and zinc has led to many studies examining the use of zinc to treat colds. Zinc supplementation (orally as a lozenge or as a nasal spray or gel) appears in some, but not all, studies to reduce the duration and severity of cold symptoms. The benefits, if found, have been more likely if supplementation started within the first 24 hours of the onset of symptoms. Typical zinc dosages used to treat colds approach 75–80 mg/day, with supplementation recommended for up to 1 week. Caution, however, should be exercised if taking these products, given the possibility of side effects (see the “Toxicity” section) and unanswered questions regarding appropriate dosing and treatment duration.

Eye Health Zinc's presence in the eye and its cofactor roles in antioxidant enzymes have prompted its inclusion in studies examining nutritional supplementation and eye health, primarily age-related macular degeneration (see Chapter 9 under “Vitamin C” for a description of the condition). The benefits of multivitamin supplementation—including zinc (initially 80 mg and later 25 mg), vitamin C (500 mg), vitamin E (273 mg/400 IU), beta-carotene (15 mg), and copper (2 mg)—on age-related macular degeneration was examined as part of a large, randomized, placebo-controlled 5-year clinical trial

known as the Age-Related Eye Disease Study (AREDS) and a subsequent trial, AREDS2. In the AREDS trial the inclusion of zinc along with vitamins C and E and beta-carotene produced the greatest benefits [6]. The AREDS2 trial involved individuals with intermediate or advanced age-related macular degeneration and provided nutrient supplements containing reduced zinc (25 mg), no beta-carotene, and added lutein (110 mg), zeaxanthin (2 mg), eicosapentaenoic acid (660 mg), and docosahexaenoic acid (330 mg). AREDS2 reported no further reductions (vs. the first AREDS) in the risk of progression to advanced age-related macular degeneration with the changes in nutrient supplementation [7].

Interactions with Other Nutrients

Substances that interact with zinc in the GI tract to inhibit its absorption have been addressed in the section on absorption. Other types of interactions between zinc and selected nutrients are presented in this section. Zinc and vitamin A interact in a couple of ways. From the discussion on zinc functions, you may remember that zinc is required for alcohol dehydrogenase structure and activity. Retinol, the alcohol form of vitamin A, serves as a substrate for this enzyme, which converts retinol to retinal (retinaldehyde), the aldehyde form of vitamin A. Conversion to retinal is necessary for its function in the body. In addition, zinc is necessary for the hepatic synthesis of retinol-binding protein, which transports vitamin A in the blood. Zinc deficiency is associated with both decreased mobilization of retinol from the liver (even with adequate liver vitamin A stores) as well as decreased plasma retinol-binding protein concentrations.

The detrimental effect of excessive zinc intake on copper absorption is thought to be attributable to zinc's stimulation of the synthesis of metallothionein, which has a higher affinity for copper than for zinc. With increased intestinal concentrations of metallothionein induced by high zinc levels, ingested copper readily binds to the metallothionein within the enterocyte and becomes “trapped,” preventing its passage into the plasma. The increased risk of copper deficiency precipitated by zinc supplementation led to the Tolerable Upper Intake Level for elemental zinc of 40 mg daily [8].

Diminished calcium absorption has been observed with the ingestion of zinc supplements when calcium intake is low (<300 mg/day of calcium). Conversely, calcium intake >600 mg/day may enhance zinc absorption [9].

Cadmium, if present in high concentrations in the body, can be toxic and particularly damaging to the kidneys. The toxicity of cadmium is related primarily to its ability to displace zinc from critical enzymes and other proteins. Cadmium is similar to zinc and can bind with high affinity to metallothionein and zinc finger proteins.

In zinc deficiency, an increase in cadmium absorption and accumulation can occur. On the other hand, adequate zinc intake is associated with lower cadmium exposure by preventing cadmium absorption [10].

Excretion

Zinc is lost from the body primarily via the GI tract, kidneys, and skin. Most zinc is excreted through the GI tract in the feces; the amount lost increases or decreases depending on body zinc concentrations, although absorption is also regulated to control body zinc homeostasis. Zinc in the feces comes from unabsorbed dietary zinc, sloughed intestinal cells, and unabsorbed endogenous zinc from digestive tract secretions.

Regulated intestinal excretion of zinc is accomplished by ZIP5, which facilitates the movement of zinc from the blood across the basolateral membrane and into the enterocyte. The action of ZnT6 on the enterocyte's brush border membrane then mediates the exocytosis of zinc from the intestinal cell into the lumen for excretion in the feces. Fecal zinc losses range from 3 mg/day among adults ingesting very low dietary zinc up to about 4.6 mg with dietary zinc intakes of 15 mg; higher dietary zinc intakes promote greater fecal losses of zinc [11].

Small amounts of zinc are also lost via the kidneys and skin (dermal) as well as in semen and menses. Most zinc filtered by the kidneys is reabsorbed by the tubules. ZnT1 is thought to control renal zinc resorption. About 0.3–0.7 mg of zinc/day is typically excreted in the urine. The zinc appearing in the urine is believed to be derived from the small percentage of plasma zinc that is complexed with histidine and cysteine. It is not affected by dietary zinc except with severe deficiency. Zinc losses of ~0.4–0.7 mg/day occur with exfoliation of skin and with sweating. Other minor routes of zinc loss include (for men) semen (1 mg/ejaculate) and (for women) menses (0.1–0.5 mg total). Hair contains ~0.1–0.2 mg zinc/g of hair [8].

Recommended Dietary Allowance

Zinc recommendations are based on the intake needed to maintain balance as well as on estimates of zinc absorption and body losses. Total daily zinc losses for adult men and women were calculated at 3.84 mg and 3.3 mg, respectively [8]. Zinc losses for men consisted of 0.63 mg urinary zinc, 0.54 mg integumental and sweat zinc, 0.1 mg semen zinc, and 2.57 mg endogenous intestinal zinc; for women, urinary zinc losses were 0.44 mg, integumental and sweat zinc losses were 0.46 mg, menses zinc losses were 0.1 mg, and endogenous intestinal zinc losses were 2.3 mg [8]. To account for absorption, the daily requirements for zinc for adult men and women were set at 9.4 mg and 6.8 mg, respectively, and the RDAs were set at 11 mg and 8 mg, respectively. The RDA for zinc during pregnancy is 11 mg/day to cover the calculated need for growth of the fetus and placenta [8]. The zinc recommendation for lactating women is 12 mg/day [8]. The inside front cover of the book provides additional RDAs for zinc for other age groups.

Deficiency

Signs and symptoms of zinc deficiency observed in children are growth retardation, skeletal abnormalities, poor wound healing, diarrhea, skin rash/lesions/dermatitis (especially around body orifices), and delayed sexual maturation. Some signs and symptoms of deficiency in adults include anorexia, diarrhea, lethargy, depression, skin rash/lesions/dermatitis, hypogeusia (blunting of sense of taste), alopecia (some hair loss; remaining hair make take on a reddish hue), vision problems, and impaired immune function, protein synthesis, and wound healing (Figure 13.15). Treatment of deficiency in adults typically requires oral zinc supplementation; doses of 10–20 mg/day are recommended. Although higher doses (often up to 50 mg given two to three times per



Figure 13.15 Dermatitis associated with zinc deficiency.

day) may be prescribed, use of such doses is more likely to impair copper status.

Some population groups—especially older adults, vegetarians, and those with alcoholism and with limited income—have been found to consume less than adequate amounts of zinc. Alcohol ingestion additionally reduces intestinal zinc absorption and increases urinary zinc excretion. Thiazide diuretics increase urinary excretion of zinc; consequently, the use of such medications for prolonged periods and in high doses may contribute to deficiency and require increased intakes to maintain zinc status. Additional conditions associated with an increased need for intake include trauma, sickle-cell anemia, and disorders causing malabsorption such as Crohn's disease, short bowel syndrome, celiac disease, and liver failure, as well as surgical bariatric procedures, especially Roux-en-Y gastric bypass and duodenal switch, used to treat obesity. Diarrhea and intestinal fistulas also substantially increase fecal zinc losses; supplementation with up to 20 mg of zinc/day may be needed under such conditions.

Toxicity

Excessive intakes of zinc cause toxicity. An acute zinc toxicity (such as from 570 mg of elemental zinc) produces some of the following symptoms: metallic taste, headache, nausea and vomiting, epigastric pain, abdominal cramps, and bloody diarrhea. In addition, reduced immune function and alterations in copper and iron status may occur. Chronic ingestion of zinc in amounts of about 40 mg (lower for some people) results in a copper deficiency (see the “Interactions with Other Nutrients” section) as well as neurologic problems such as numbness, weakness, ataxia, and spastic gait [8]. Excessive zinc absorbed from the overuse of zinc-containing denture creams has also been associated with toxicity. Additionally, zinc used intranasally (spray or gel) has been reported to cause anosmia (permanent loss of smell) in some individuals. The Tolerable Upper Intake Level for zinc has been set at 40 mg daily based on its interaction with copper [8].

Assessment of Nutriture

Evaluating zinc nutriture is difficult, owing to homeostatic control of body zinc. A variety of indices have been used to assess zinc status, including measurements of zinc in red blood cells, leukocytes, neutrophils, and plasma or serum. The most common basis for assessment is serum or plasma zinc, with fasting concentrations less than about 70 $\mu\text{g}/\text{dL}$ (10 $\mu\text{mol}/\text{L}$) suggesting deficiency. A cutoff of 50 $\mu\text{g}/\text{dL}$, however, may better predict clinical signs of zinc deficiency [12]. Low fasting plasma zinc concentrations indicate that little zinc is present in the exchangeable zinc pool and may reflect a loss of tissue zinc, especially from the liver. Plasma zinc concentrations must be interpreted

with caution because concentrations are influenced by many factors unrelated to zinc depletion, including meals, time of day (diurnal variation), stress, infection, and medications such as steroid therapy. In fact, postprandial (after eating) plasma zinc concentrations have been found to be more sensitive to low dietary zinc intake than fasting plasma zinc concentrations.

Metallothionein has also been used to assess zinc status. Concentrations of metallothionein respond to changes in dietary zinc. For example, liver and red blood cell metallothionein concentrations diminish as dietary zinc intake decreases and are thought to reflect zinc status or stores. Serum zinc and serum metallothionein concentrations can be used to indicate poor zinc status if both are low. Elevations in serum metallothionein coupled with low serum zinc, however, usually suggest an acute-phase response, and in such conditions these indices are not reliable.

Urinary zinc excretion remains fairly constant over a range of intakes and is thought to be a useful marker of status in those with moderate to severe zinc deficiency [8]. Low hair zinc may be associated with chronic intake of dietary zinc in suboptimal amounts; however, the concentration of zinc in hair depends not only on delivery of zinc to the root but also on the rate of hair growth, which is affected by other conditions, including protein status.

Measurement of the activity of zinc-dependent enzymes has also been employed as an index of zinc status. Studies using enzymes as indicators typically have measured carbonic anhydrase or alkaline phosphatase, which “hold” zinc less securely than other zinc metalloenzymes. Ideally, measurements of activity should be taken before and after zinc supplementation.

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13.3 COPPER

The copper content of the human body ranges from about 50 to 150 mg. Copper is found in all body tissues and most secretions. In the body, the mineral is found in two valence states, the cuprous state (Cu^{1+}) and cupric state (Cu^{2+}).

Sources

The copper content of food varies widely, reflecting the origin of the food and the conditions under which the food was produced, handled, and prepared for use. The richest sources of copper are meats (especially organ meats like liver) and shellfish (especially oysters and lobster), as shown in Table 13.6. Plant food sources rich in copper include nuts (especially cashews), seeds, legumes, and dried fruits. Potatoes, whole grains, and cocoa (chocolate) are also good sources. In contrast, milk and dairy products are poor sources of the mineral. In the United States, the median copper intake from foods by adults ranges from about 1,000 to 1,600 $\mu\text{g}/\text{day}$ [1]. The Daily Value for copper used on food and supplement labels is 0.9 mg.

The main form of copper found in mineral-fortified food products and supplements is copper sulfate; however, cupric oxide is also found in some supplements. The use of cupric oxide as a dietary source is discouraged because the copper has been shown to be unavailable for absorption from the GI tract of animals; in fact, it is no longer used as a copper supplement in animal nutrition [2]. In addition to copper sulfate, other bioavailable forms of copper include cupric chloride, cupric acetate, copper carbonate, copper gluconate, and copper–amino acid chelates. Multivitamin/mineral supplements typically provide about 0.5–2 mg of copper. Single-nutrient supplements contain about 2–3 mg of copper.

In addition to dietary sources, copper from endogenous sources is released with the secretion of digestive tract juices. For example, the copper contents of saliva and gastric juice are $\sim 400 \mu\text{g}$ and 1 mg per day, respectively; pancreatic juice contains up to 2 mg/day; and bile released into the duodenum contributes another 2.5 mg/day of

Table 13.6 Copper Content of Select Foods*

Select Foods/Food Group	Copper (mg)
Seafood	
Oysters, Eastern (3 oz)	4.9
Lobster (3 oz)	1.3
Crabmeat (3 oz)	0.7
Shrimp (3 oz)	0.4
Salmon, farmed (3 oz)	0.05
Eggs and dairy products	
Egg, poached (1 large)	0.04
Milk, 1% fat (1 cup)	0.02
Cheese, variety (1 oz)	0.01–0.12
Grains and cereals	
Rice, and pasta (1 cup)	0.11–0.20
Bread, white, enriched (1 slice)	0.04
Bread, whole wheat (1 slice)	0.17
Meat and poultry	
Liver, beef (3 oz)	12.1
Chicken, dark meat (3 oz)	0.07
Chicken, white meat (3 oz)	0.04
Beef, ground (3 oz)	0.07
Pork, ground (3 oz)	0.04
Legumes, variety (½ cup)	0.16–0.30
Nuts, variety (1 oz)	0.11–0.62
Fruits	0.02–0.11
Vegetables, cooked (1 cup)	0.02–0.06
Potato, baked with skin (1 medium)	0.33
Spinach (½ cup)	0.16
Other, cocoa powder (1 Tbsp)	0.20

*Data represent cooked foods, except for fruits, nuts, and milk.

Source: U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov>

copper [3]. The reabsorption of secreted copper is important for body copper homeostasis.

Digestion, Absorption, Transport, and Storage

Figure 13.16 provides an overview of copper digestion, absorption, and transport as well as some of copper's fates in the enterocyte.

Digestion

Most copper in foods is found as Cu^{2+} and is bound to organic components, especially amino acids that make up food proteins. Thus, digestion is needed to free the bound copper before absorption can occur. Hydrochloric acid and pepsin facilitate the release and reduction of bound copper in the stomach. Additional proteolytic

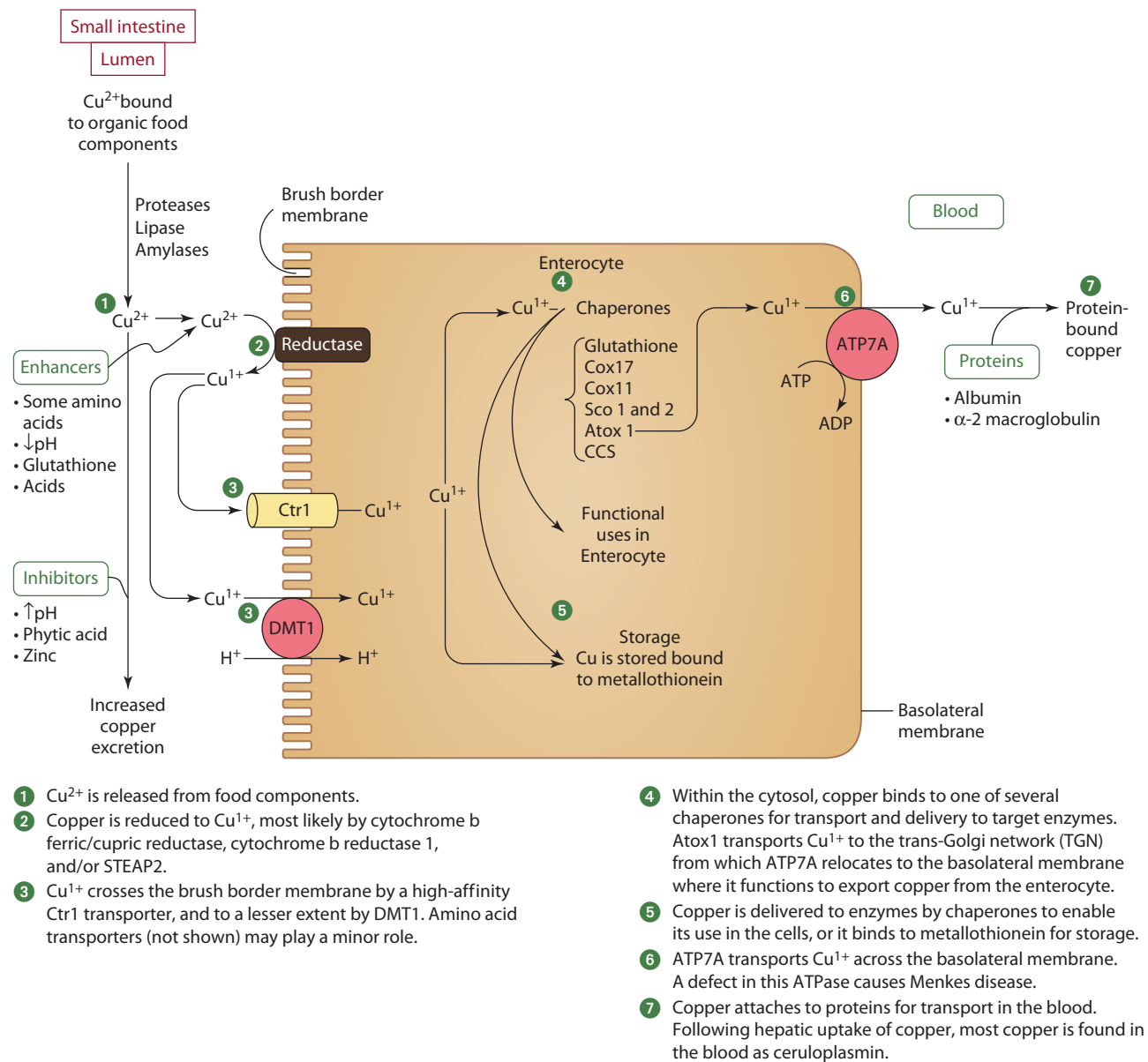


Figure 13.16 Overview of copper digestion, absorption, enterocyte use, and transport.

enzymes in the small intestine hydrolyze proteins further to release copper.

Absorption

Copper is absorbed primarily in its reduced cuprous (Cu^{1+}) state from the proximal small intestine, especially the duodenum. While a small amount may be absorbed from the stomach, gastric copper absorption is thought to contribute relatively little to the overall absorption of the mineral.

The reduction of copper from Cu^{2+} to Cu^{1+} may occur in the acidic environment of the stomach, but more likely results from the action of one or more reductases, including cytochrome *b* ferric/cupric reductase, six-transmembrane epithelial antigen of the prostate

2 (STEAP2), and cytochrome *b* reductase 1 found on intestinal cell membranes. Vitamin C may also facilitate the reduction.

Copper absorption across the enterocyte's brush border membrane is accomplished by one or more carrier proteins. One such carrier is copper transporter 1 Ctr 1 (also designated hCtr1), which enables copper in its Cu^{1+} state to be absorbed through a gated channel. Ctr1 is found not only on the enterocyte's brush border membrane, but it is also associated with vesicles in the cytosol and is found on most extraintestinal cell membranes to facilitate copper uptake. Synthesis of Ctr1 appears to be regulated by transcription factor Sp1 and is responsive to the body's copper status. However, the mechanism by which body copper influences Sp1 and Ctr1 synthesis is not clear.

In addition to Ctr1, divalent metal transporter 1 (DMT1) also serves as a copper transporter, specifically cotransporting (symporting) Cu^{1+} and H^+ across the enterocyte's brush border membrane. Other copper transporters, yet to be identified, may also facilitate intestinal copper absorption.

The GI tract absorbs about 50–80% of ingested copper. Fractional absorption of copper increases as copper intake decreases, and vice versa. Absorption, for example, may average about 20–25% when copper intake is high, such as >5 mg/day, but increases to over 50% when intake is <1 mg/day [4]. Copper absorption was calculated at 75% with an intake of 350 μg of copper, an amount that is about one-half of the adult requirement for the mineral [1].

Factors Influencing Copper Absorption

Copper transport across the enterocyte's brush border membrane may be influenced by a variety of dietary components, with some having a positive effect and others exerting a negative influence on absorption.

Enhancers of Copper Absorption Examples of chelators that facilitate copper absorption include amino acids, especially histidine and cysteine. Whether copper bound to these amino acids can be absorbed through amino acid carrier systems is not clear. Copper also forms complexes with sulfhydryl groups in cysteine residues that are found within the tripeptide glutathione. Glutathione is found both within the lumen of the GI tract and intracellularly.

The presence of organic acids in foods also improves copper absorption. Citric, gluconic, lactic, acetic, and malic acids act as chelators to improve solubilization and thus absorption of copper. Citric acid, for example, forms a stable complex with copper and improves its absorption. A more acidic environment, as found in the proximal intestine versus the distal intestine, also favors copper absorption.

Inhibitors of Copper Absorption Just as an acidic environment facilitates copper absorption, the opposite is true of an alkaline environment. Excessive use of medications to treat heartburn, GERD, and ulcers raise pH and can thus diminish copper absorption. The main classes of medication are:

- H_2 receptor blockers, such as Axid (nizatidine), Tagamet (cimetidine), and Pepcid (famotidine)
- Proton pump inhibitors, such as Prevacid (lansoprazole), Prilosec (omeprazole), and Nexium (esomeprazole).

Copper atoms in an alkaline medium often bind to hydroxides (OH), forming insoluble compounds that are not readily absorbable. In addition to these pH effects,

substances found in foods may bind to copper to diminish its absorption. Phytic acid (see Figure 13.12), found mainly in plant foods (cereals, legumes, etc.), is a known inhibitor of the absorption of copper.

Other minerals, including zinc, iron, and calcium, also impede copper absorption. In particular, the use of zinc supplements, typically in amounts of about 40 mg or more (but sometimes less), impairs copper absorption and diminishes copper status. The detrimental effect of excessive zinc intake on copper absorption is thought to result from zinc's stimulation of metallothionein synthesis in intestinal cells. Metallothionein normally binds zinc and serves as an intracellular storage form of zinc. However, metallothionein more avidly binds copper than zinc, and thus reduces the transfer of copper from the lumen of the GI tract across the basolateral membrane into the blood. Copper deficiency induced by high zinc intake can be difficult to correct. For example, when zinc (110–165 mg) supplements were taken for 10 months, discontinuation of the zinc and 2 months of oral copper supplementation failed to correct the copper deficiency. Intravenous administration of cupric chloride for 5 days (total dose of 10 mg) was needed to bypass the intestinal cells and correct the deficiency, suggesting that the correction of a zinc-induced copper deficiency is a slow process [5]. The effects of supplemental iron and/or iron fortification on copper absorption remain unclear, with conflicting reports depending on the forms and amounts of the nutrients provided and the populations being studied.

Intestinal Cell Copper Use

Once within the enterocyte, copper binds to other compounds, which serves to minimize the damaging effects of free copper ions that can occur through nonenzymatic reactions (see the “Other Roles” section). Intracellular copper can bind to amino acids (especially histidine and cysteine), glutathione (a tripeptide composed of glycine, cysteine, and glutamate, which transports Cu^{1+}), and/or protein chaperones.

Copper entering the enterocyte has several possible fates. The copper may be:

- Stored or sequestered in the enterocyte
- Used functionally within the enterocyte
- Transported through the cytosol and across the basolateral membrane into the blood for delivery to other tissues.

Copper can be stored temporarily within enterocytes (and other cells) in cytosolic vesicles; the protein Ctr2 serves to transport copper out of the cell cytosol and into cytosolic vesicles for temporary storage. Short-term storage of copper also occurs attached to glutathione or as part of the protein metallothionein (discussed further in the “Storage” section). If not released from metallothionein,

the bound copper will be lost into the feces with intestinal cell turnover, approximately every 3 days. Copper's uses in the intestinal cell are similar to its uses in the body and are discussed in the "Functions and Mechanisms of Action" section.

Copper transport across the intestinal cell's basolateral membrane occurs primarily via active transport as Cu^{1+} by the ATPase ATP7A. ATP7A is expressed in most body cells with the exception of hepatocytes, which express a similar transporter, ATP7B. Mutations in the *ATP7A* gene result in Menkes disease, an X-linked disorder characterized by defective copper efflux from cells. Menkes disease, with an estimated prevalence of 1 in 40,000–350,000, results in increased intestinal cell copper concentrations and impaired delivery of copper to peripheral tissues. Symptoms become apparent at about 2–3 months of age. The condition is characterized by laxity of joints, "steely or kinky" hair, impaired growth, vascular problems, and neurological problems including progressive neurodegeneration, severe intellectual disability, and seizures. While some symptoms may be partially alleviated by intravenous administration of copper, the condition is usually fatal within the first few years of life.

Transport

From the intestinal cells, copper is transported in portal blood to the liver bound to the proteins albumin (specifically the N-terminal amino group) and α -2-macroglobulin, which binds two copper atoms and may have a higher affinity for the mineral than albumin.

The uptake of copper into the liver (and other tissues) occurs by multiple carrier proteins, including Ctr1, Ctr2, DMT1, and likely unidentified carriers [6]. After transport into the cell, the copper binds to glutathione, metallothionein, or directly to chaperones. Copper chaperones are soluble intracellular proteins that bind intracellular copper and deliver it to various, but specific, locations following cellular copper uptake. The chaperone atox1 transports Cu^{1+} to the trans-Golgi network, where ATP7B directs the copper for insertion into ceruloplasmin as well as other cuproenzymes. In the liver, most copper is used for the synthesis of ceruloplasmin, which contains six copper atoms, giving the protein a blue color. Although copper does not appear to influence ceruloplasmin synthesis, ceruloplasmin activity is diminished without sufficient copper, and ceruloplasmin's half-life is shortened.

Ceruloplasmin is released from the liver and constitutes about 60–70% of circulating copper in the blood; the remaining copper circulates loosely bound to albumin and α -2-macroglobulin for delivery to tissues. Plasma ceruloplasmin concentrations typically range from about 20 to 50 mg/dL; serum copper concentrations normally range from about 70 to 150 $\mu\text{g/dL}$ (10 to 24 $\mu\text{mol/L}$).

Storage

The liver is the main organ that stores copper and that controls copper homeostasis in the body. However, compared to other trace minerals, relatively little copper (up to about 150 mg) is found in the body. On a percentage basis, the skeleton, followed by muscles and then the liver, contains the most copper; on a concentration basis, the organs with the most copper per gram are the kidneys, liver, brain, and skeleton.

Within cells, the protein metallothionein serves as a copper storage pool. Metallothionein binds 12 copper atoms (as well as zinc atoms) per molecule; it is also thought to regulate copper uptake and cellular zinc distribution and to scavenge free hydroxyl radicals, thereby acting in an antioxidant capacity. Copper positively influences metallothionein synthesis in the liver, kidney, and brain, but not intestine. The mechanism by which copper influences metallothionein synthesis is not well defined.

Functions and Mechanisms of Action

The essentiality of copper is due, in part, to its participation as an enzyme cofactor, either at the enzyme's active site (perhaps as an intermediate in electron transfer) or at the enzyme's allosteric regulatory site. An important aspect of copper's regulatory function depends on chaperone proteins, which direct intracellular trafficking of copper to sites where it is needed. Some of the identified chaperones for copper include:

- Cyclooxygenase (cox) 17, cox 19, cox 23, and cox 11
- Sco1 and Sco2
- CCS (copper chaperone for superoxide dismutase)
- atox1 (also called hAtx or Hah1).

Six chaperone proteins are involved in the transport of Cu^{1+} to cytochrome *c* oxidase; these proteins include cox 17, cox 19, and cox 23, as well as the mitochondrial membrane proteins cox 11, Sco1, and Sco2. CCS, which is found in the mitochondria and cytosol, delivers Cu^{1+} to superoxide dismutase. Atox1 ferries Cu^{1+} to ATPases ATP7A and ATP7B, which are necessary for cellular export of copper. More specifically, atox1 transports Cu^{1+} to the trans-Golgi network, where ATP7B then directs it for insertion into cuproenzymes such as ceruloplasmin. This section addresses a few of the body's cuproenzymes (copper-requiring metalloenzymes; Table 13.7).

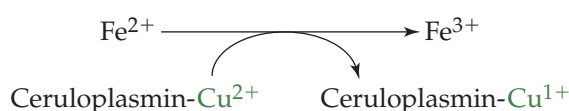
Ceruloplasmin: Iron Utilization

Ceruloplasmin, also known as ferroxidase, is critical for iron utilization in the body. This oxidase is found in the blood and bound to receptors on the plasma membranes of cells, where it oxidizes minerals, most notably ferrous (Fe^{2+}) iron but also manganese (Mn^{2+}). Its role in

Table 13.7 Select Functions of Copper

Ceruloplasmin and hephaestin: iron oxidation
Superoxide dismutase: antioxidant
Cytochrome c oxidase: ATP production
Amine oxidases: oxidation of biogenic mono- and diamines
Lysyl oxidase: collagen and elastin cross-linking
Dopamine monooxygenase/hydroxylase: norepinephrine (catecholamine) synthesis
Tyrosinase: melanin pigment production
Peptidylglycine α -amidating monooxygenase: activation of selected hormones/peptides
Factors V and VIII: blood clotting
Immune function

the oxidation of iron (Fe^{2+} to Fe^{3+}) is critical for cellular iron release and binding to transferrin for transport to the body's tissues.



An absence of functioning ceruloplasmin due to genetic mutations impairs iron utilization and is characterized by increased iron deposition in tissues, especially the liver, pancreas, and brain, and by low serum iron concentrations. Copper metabolism is not affected.

Another proposed function of ceruloplasmin is as an antioxidant or modulator of the inflammatory process. As modulators of the inflammatory process, acute-phase reactant proteins like ceruloplasmin increase in the blood in response to severe infections and other inflammatory events, such as injury. This rise in blood levels is important because during infections, for example, phagocytosis of invading organisms by white blood cells generates superoxide radicals, among other damaging compounds. These compounds, while normally produced by cells, are generated in larger amounts with infections and inflammation and must be eliminated by ceruloplasmin, superoxide dismutase, or other enzymes to prevent excessive damage to body cells.

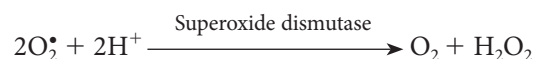
Hephaestin: Iron Utilization

Hephaestin, like ceruloplasmin, oxidizes iron. However, this copper-containing ferroxidase protein is present on the basolateral membrane of enterocytes where it oxidizes iron to its ferric state to enable transport attached to transferrin (see Figure 13.3). The protein may also be present in other tissues.

Superoxide Dismutase: Antioxidant

Superoxide dismutase (SOD) is a copper- and zinc-dependent enzyme (although another form in the mitochondria is manganese dependent). Copper and zinc are linked

to the enzyme by an imidazole group and histidine and aspartate residues. Copper (Cu^{2+}) is found at the enzyme's active site, where the superoxide substrate binds to the enzyme. Removal of copper, but not zinc, results in reduced cytosolic superoxide dismutase activity. Specifically, superoxide dismutase catalyzes the removal (dismutation) of the superoxide radicals ($\text{O}_2^{\bullet -}$). During the reaction, copper is reduced along with the oxygen radical to initially generate molecular oxygen (O_2) and then, by reoxidation, hydrogen peroxide (H_2O_2).



Superoxide radicals along with other free radicals can cause peroxidative damage to the phospholipid component of cell membranes, especially disrupting unsaturated double bonds in fatty acids, as well as damaging other cellular components. Superoxide dismutase therefore provides an important protective function. The enzyme is found in the cytosol of most cells (SOD1). Additionally, an extracellular form (SOD3) is found in the blood and other body fluids (e.g., lymph, synovial fluid, and lungs); it is also secreted and bound to heparan sulfate on the surface of cells and is found in relatively large concentrations in the arterial wall. Increased peroxidation of cell membranes is observed with copper deficiency.

Cytochrome c Oxidase: ATP Production

Cytochrome c oxidase contains three copper atoms per molecule. One subunit of the enzyme contains two copper atoms and functions to receive electrons from cytochrome c and then transfer the electrons to the second subunit. The second subunit contains another copper atom and is involved in reducing molecular oxygen and ultimately in ATP production. Cytochrome c oxidase (which also requires iron) functions in the terminal oxidative step in mitochondrial electron transport. The final step transfers an electron so that molecular oxygen (O_2) is reduced to form water molecules, thus facilitating ATP production. Severe copper deficiency impairs the activity of this enzyme.

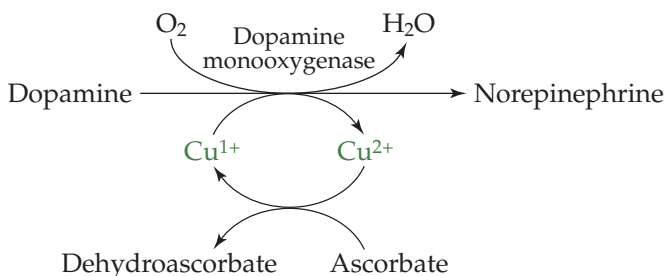
Lysyl Oxidase: Collagen Synthesis

Lysyl oxidase is secreted by connective tissue cells in bone, blood vessels, and other tissues. It promotes cross-links between connective tissue proteins, including collagen and elastin. Specifically, lysyl oxidase catalyzes the removal (oxidative deamination) of the epsilon (ϵ)-amino group of lysyl and hydroxylysyl residues of a collagen and elastin polypeptide and the oxidation of the terminal carbon atom of an aldehyde to form cross-links. The cross-linking is needed to stabilize the extracellular matrix. Lysyl oxidase activity decreases with inadequate copper intake, negatively affecting the strength of connective tissues. While lysyl oxidase is an amine oxidase, it is listed here, separate

from the other section addressing amine oxidases because of its different physiological role in the body.

Dopamine β -Monooxygenase/Hydroxylase, Tyrosinase, and Peptidylglycine α -Amidating Monooxygenase: Catecholamine, Pigment, and Neurotransmitter/Neuropeptide Activation

Catecholamine and Pigment Synthesis In tyrosine metabolism (see Figure 6.10), the production of the catecholamine norepinephrine and of melanin pigments requires copper-dependent enzymes. Norepinephrine synthesis, which occurs mainly in the adrenal medulla and neurons, begins with tyrosine, which is converted in an iron-dependent reaction to 3,4-dihydroxyphenylalanine (also called L-dopa). The L-dopa is then decarboxylated to form dopamine. To synthesize norepinephrine from dopamine, the enzyme dopamine β -monooxygenase/hydroxylase, which contains up to eight copper atoms per molecule, is required for the hydroxylation reaction as shown. Vitamin C serves as a cosubstrate and reduces the copper. Norepinephrine functions in the body as both a hormone and neurotransmitter, affecting a wide range of physiological processes.



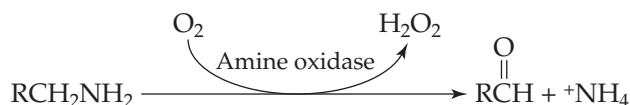
While in some cells L-dopa is used to form dopamine, in melanocytes, which are found in the epidermal layer of skin and in the eyes and hair, L-dopa can be oxidized by the copper-dependent enzyme tyrosinase (also called catechol oxidase) to produce dopaquinones. The dopaquinones then polymerize to form pigments called melanin. Melanin provides color to the iris of the eye, skin, and hair. A mutation in the tyrosinase enzyme causes the condition called albinism, which is characterized by lighter than normal skin color, white hair, and light-colored eyes.

Neurotransmitter/Neuropeptide Activation The amidation of some peptide hormones, such as bombesin, calcitonin, gastrin, and cholecystokinin, is necessary for hormone function. The amidation requires the copper-dependent enzyme peptidylglycine α -amidating monooxygenase, which is found mostly in the brain. This enzyme cleaves a carboxy terminal glycine residue off peptides that have a C-terminal glycine. The amino group of glycine is retained by the peptide as a terminal amide. The oxidized residue is released as glyoxylate. Peptidylglycine α -amidating

monooxygenase also requires vitamin C to reduce Cu^{2+} back to Cu^{1+} and is shown in Figure 9.5. Because of the divergent roles that these hormones play in the body once activated by amidation, copper indirectly has wide-ranging effects on multiple body processes.

Amine (Monoamine and Diamine) Oxidases: Biogenic Amine Degradation

Amine oxidases, including mono- and diamine, are also copper dependent and found in the blood and body tissues. The enzymes catalyze the oxidation of biogenic amines, such as tyramine, histamine, and dopamine, as well as serotonin (5-hydroxytryptamine), norepinephrine, and polyamines, to form aldehydes and ammonium ions (NH_4 is generated from the cleaved amine group). In the reaction, oxygen (O_2) is reduced to form hydrogen peroxide (H_2O_2). Because of the wide range of biogenic amines oxidized by copper-dependent amine oxidases, suboptimal copper status may result in a broad range of neurological and physiological manifestations.



Other Roles

Copper also plays a variety of other roles in the body, some of which are not well characterized. The blood clotting proteins (factors) V and VIII both contain copper atoms. Copper is also involved in angiogenesis, immune system function, nerve myelination, and endorphin action. Copper is thought to influence gene expression by binding to specific transcription factors, which in turn bind to promoter sequences on DNA. Once the copper-bound transcription factors interact with DNA, transcription may be enhanced or suppressed. These interactions, however, are not well characterized.

Pro-Oxidant Role

As a pro-oxidant, free copper ions behave similarly to iron. Copper reacts with superoxide radicals and catalyzes the formation of hydroxyl radicals through the Fenton reaction: $Cu^{1+} + H_2O_2 \rightarrow Cu^{2+} + OH^- + \cdot OH$. Associated with the generation of reactive oxygen species is increased oxidative damage to DNA (base oxidation and strand breaks), proteins, and lipids (peroxidation), especially membrane lipids.

Interactions with Other Nutrients

Copper is known to interact with a number of dietary constituents. Those that affect copper absorption have been described previously. Additional interactions with iron and molybdenum that are unrelated to intestinal copper absorption are discussed here.

The importance of copper in normal iron metabolism is evidenced by the microcytic anemia that results from prolonged copper deficiency. Microcytic anemia is caused by impaired mobilization and use of iron, stemming from the reduced ferroxidase activity of hephaestin and ceruloplasmin, which oxidize iron to its trivalent (Fe^{3+}) state. With copper deficiency, the activity of ceruloplasmin and the expression of hephaestin are reduced, and iron remains mostly trapped within cells. Thus, copper deficiency results in a secondary iron-deficiency anemia, and treatment with copper (and not iron) is required to correct the problem.

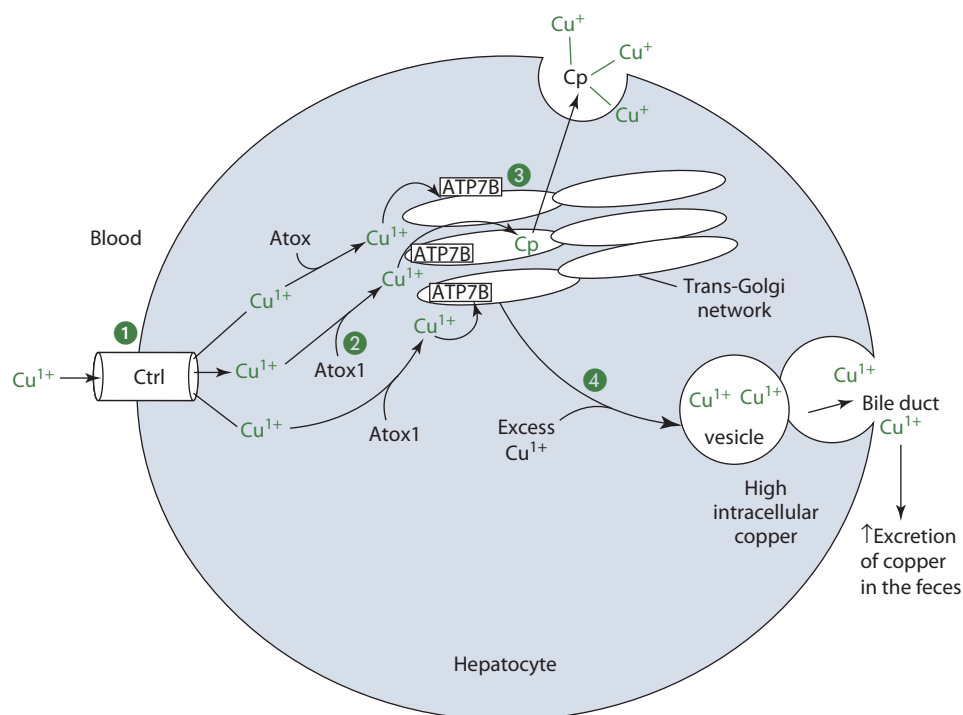
Another nutrient that interacts with copper is molybdenum. The mineral, when given as tetrathiomolybdate, interferes with copper utilization and consequently has been shown to be beneficial in the management of Wilson's disease (see "Toxicity") [7].

Excretion

Copper is excreted primarily (~95%) through the bile into the feces. In fact, biliary copper excretion is regulated by the liver to maintain copper balance (homeostasis). Thus, with high dietary copper intake, biliary copper excretion via the

feces increases, and with low dietary copper intake, fecal copper excretion decreases. Fecal copper losses range from about 0.5 to 2.5 mg/day [3].

The ATPase ATP7B, which is usually positioned in the trans-Golgi network, in hepatocytes plays a major role in copper excretion. Concentrations of ATP7B are controlled, at least in part, by "copper metabolism (Murr1) domain containing 1," abbreviated COMMD1, and X-linked inhibitor of apoptosis (XIAP). When the copper content of hepatic cells is low or normal, atox1 takes copper to the trans-Golgi network where it is directed into the secretory pathway for incorporation into ceruloplasmin and other apocuproenzymes. In the presence of high copper, the copper gets "stored" likely in cytosolic vesicles. Additionally, ATP7B translocates from the trans-Golgi network and facilitates the fusion and exocytosis of the copper-containing vesicles and the exocytosis of lysosomal copper across the hepatic canalicular membrane for excretion into the bile (Figure 13.17). This bile containing the excess copper is secreted into the duodenum; however, the copper in the bile is bound to bile components and thus cannot be reabsorbed from the small intestine. Wilson's disease, an inherited disorder of copper metabolism, is characterized by defective biliary copper excretion and thus copper toxicity. The disorder results from a mutation(s) in



- 1 Ctrl transports copper into the hepatocytes from the blood.
- 2 Atox1 functions as a chaperone to take Cu^{1+} to the trans-Golgi network.
- 3 Within the trans-Golgi network (TGN), copper is incorporated into ceruloplasmin (Cp) and other cuproenzymes. ATP7B transports the proteins into the secretory pathway.
- 4 In the presence of excess copper, ATP7B moves from the TGN to copper-containing cytosolic vesicles (and lysosomes—not shown) to direct the secretion of the copper from these sites into the bile duct.

Figure 13.17 The role of ATP7B in copper use and excretion in the liver.

ATP7B and is discussed further in the “Toxicity” section. Other conditions such as cholestasis, however, can impair bile flow, diminish copper excretion, and increase the likelihood of copper toxicity.

Other minor routes of copper excretion/losses include the urine, sweat and skin cell desquamation, menses (for women), semen (for men), hair, and nails. Copper loss via the urine is small ($<60 \mu\text{g/day}$). Urinary copper excretion does not typically change with changes in copper intake except under extreme conditions. Similarly, only small amounts ($<50 \mu\text{g/day}$) of copper are lost in sweat and with desquamation of skin cells. Women experience trace losses of copper in normal menstrual flow; however, a woman's copper status, unlike her iron status, is not compromised by menstruation. Together, losses from menses or semen, along with losses from hair and nails, are not thought to exceed surface losses [1].

Recommended Dietary Allowance

The results of depletion and repletion studies, along with other studies permitting factorial analysis of obligatory losses over a range of intakes, have enabled estimates of copper requirements. Based on a copper requirement for adults of $700 \mu\text{g}$, a 30% coefficient of variation of the requirement, and rounding to the nearest $100 \mu\text{g}$, an RDA for copper was set at $900 \mu\text{g/day}$ [1]. Recommendations during pregnancy and lactation are $1,000 \mu\text{g/day}$ and $1,300 \mu\text{g/day}$, respectively [1]. RDAs for copper for other age groups are found on the inside front cover of the book.

Deficiency

Various clinical manifestations are associated with copper deficiency. Most commonly recognized signs and symptoms include:

- Microcytic anemia (small red blood cells)
- Macrocytic anemia (abnormally large red blood cells)
- Normocytic anemia (fewer, normal-sized red blood cells)
- Leukopenia (specifically neutropenia, a lower-than-normal number of neutrophils).

With each type of anemia, the red blood cells are low in hemoglobin, causing muscle weakness and fatigue. The majority of patients with copper deficiency present with anemia and leukopenia; furthermore, these blood manifestations of copper deficiency do not improve with iron supplementation [8].

Other manifestations of copper deficiency may include hypopigmentation or depigmentation of skin and hair, impaired immune function, blood vessel/connective tissue and bone abnormalities, altered cholesterol metabolism, hypotonia, and cardiovascular and pulmonary dysfunction (Figure 13.18).

The likelihood of copper deficiency increases in persons consuming excessive amounts of zinc (especially greater than 40 mg/day). In addition, deficiency is more likely with prolonged use of medications (such as proton pump inhibitors) that increase the intestinal pH and thus diminish copper absorption. Additionally, persons with conditions that promote increased loss of copper from the body, such as nephrosis, or that decrease the copper absorption, such as GI malabsorptive disorders (e.g., celiac disease, Crohn's disease, and ulcerative colitis), are at greater risk of deficiency. Bariatric surgical procedures, such as Roux-en-Y, also increase the likelihood of deficiency secondary to decreased absorption.

Copper deficiency usually responds to oral supplementation with copper. Ingestion of doses providing about $1.5\text{--}3 \text{ mg}$ of copper, taken once or twice daily for about 1–3 months, is usually sufficient to correct deficiency. However,



Figure 13.18 Skin depigmentation associated with copper deficiency.

higher doses or intravenous copper administration is sometimes needed, especially if the copper deficiency is zinc induced.

Toxicity

Copper toxicity is fairly rare in the United States, although acute poisonings have occurred because of water contamination and accidental ingestion. A Tolerable Upper Intake Level for copper was set at 10 mg/day by the Food and Nutrition Board [1], although intakes below this level, such as 5 mg, may cause GI discomfort. Ingestion of a large dose of copper (such as 64 mg elemental copper provided by 250 mg of copper sulfate) may cause acute toxicity, characterized typically by epigastric pain, nausea and vomiting, diarrhea, weakness, lethargy, and anorexia. Other symptoms of toxicity with prolonged intake of high amounts of copper include hematuria (blood in urine), liver damage resulting in jaundice, and kidney damage resulting in oliguria (little urine production) or anuria (no urine production) [9]. Chronic copper ingestion of 30 mg daily for 2 years followed by 60 mg daily for a year resulted in liver failure in a young man who self-prescribed copper supplements [10].

Wilson's disease, a genetic disorder resulting from a mutation(s) in the gene coding for ATP7B, is characterized by copper toxicity and is lethal if not treated. The estimated prevalence worldwide is between 1 in 30,000 and 1 in 100,000 individuals. The most common mutation in Caucasians is C3207A, which disrupts the binding of ATP to the transporter. The absence or dysfunction of ATP7B in turn disrupts copper excretion into the bile and into the secretory pathway for incorporation into cuproenzymes such as ceruloplasmin. Thus, in Wilson's disease, copper accumulates in the liver but also leaks out (unbound, not as part of ceruloplasmin) into the blood and is deposited in joints and other organs, especially the brain, kidneys, heart, and eyes (cornea).

Symptoms of Wilson's disease are usually not apparent until at least 7 years of age. Deposition of copper in the liver leads to cirrhosis, inflammation and liver failure, and in the kidneys causes renal failure. The copper deposition in the corneas results in Kayser-Fleischer (greenish or brownish gold) rings visible in the eyes. Osteoarthritis occurs with copper deposits in joints. Neurologic dysfunction (such as seizures and movement disorders) and/or psychiatric problems (including personality changes) may also occur secondary to copper deposition in the brain. At present, Wilson's disease is treated primarily with systemic chelation medications, such as D-penicillamine or triethylenetetramine therapy, to bind body copper and increase its urinary excretion. Avoidance of high-copper foods is recommended. Additionally, zinc supplements (such as 50 mg zinc as zinc acetate or zinc sulfate given three times a day) along with molybdenum supplements (such as thiomolybdate given in six doses of 20 mg each)

may also be recommended to decrease intestinal copper absorption and to enhance urinary copper excretion, respectively [11].

Assessment of Nutriture

Copper status is best assessed using multiple indicators. Serum, plasma, or red blood cell copper is frequently used, but these indicators are likely inadequate to assess short-term changes in copper status. The lower end of the normal range for serum copper concentrations is about 70 $\mu\text{g/dL}$ (10 $\mu\text{mol/L}$). The change in plasma or serum copper concentration that occurs when subjects consume inadequate copper varies considerably between individuals and is further affected by several factors unrelated to diet. An extremely low copper intake (~ 0.38 mg/day), however, appears to be sufficient to significantly decrease not only plasma copper but also ceruloplasmin concentration and activity as well as urinary copper excretion [12]. Changes in serum ceruloplasmin concentration and activity are also used to assess copper status. In fact, decreased concentrations of serum ceruloplasmin (< 20 mg/dL) are often an early manifestation of copper deficiency.

Response of serum ceruloplasmin to copper supplements may also be used to assess copper status; however, because ceruloplasmin is an acute-phase reactant protein, it may be elevated secondary to inflammation and not representative of copper status. Typically, provision of supplemental copper first normalizes serum copper and the neutrophil count, then serum ceruloplasmin [13]. Ceruloplasmin concentration increases following supplementation only in copper-deficient subjects. Another useful indicator of copper status is measurement of the activity of copper-dependent enzymes such as superoxide dismutase in the red blood cell. Superoxide dismutase activity is sensitive to longer-term copper deficiency [14]. Platelet copper concentrations, along with platelet or leukocyte cytochrome *c* oxidase and skin lysyl oxidase activity, are responsive to changes in copper status.

Copper concentration in hair does not correlate with either serum or organ copper, even though copper concentration in hair is reduced with a prolonged period of copper deficiency. Hair copper concentration is not thought to be useful as an indicator of copper status. Similarly, urinary copper excretion, which is normally very low, is responsive to change, but only when intake is so low that other indicators have already declined [12,14].

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13.4 SELENIUM

Selenium is a nonmetal, but with metalloid properties. It exists in several oxidation states, including Se^{2-} , Se^{4+} , and Se^{6+} . The chemistry of selenium is similar to that of sulfur; consequently, selenium can often substitute for sulfur. The total body selenium content ranges from 5 mg to over 20 mg [1].

Sources

Perhaps more than any other essential element, selenium varies greatly in its soil concentration throughout the regions of the world. Consequently, the selenium content of plant foods grown in these soils is highly variable and directly dependent on soil concentration. Similarly, the selenium content of meat, milk, eggs, and other animal products depends on the amount of selenium in animal feed. Plant materials fed to livestock are usually grown in the same geographic region as the plant foods consumed directly by humans. Selenium deficiency therefore occurs in large geographic regions of the world, as indicated in the “Box” feature.

The richest sources of selenium are generally organ meats and seafoods, followed in descending order by muscle meats, cereals and grains, eggs, and milk/dairy products (Table 13.8). Fruits and most vegetables are typically poor sources of the element; however, some plants, such as wheat, broccoli, onions, asparagus, cabbage, and garlic, hyperaccumulate selenium from the soil, and thus

Table 13.8 Selenium Content of Select Foods*

Select Foods/Food Group	Selenium (μg)
Meat and poultry (3 oz)	~15–40
Beef, top sirloin (3 oz)	28
Beef, ground (3 oz)	18
Chicken, white meat (3 oz)	21
Turkey, white meat (3 oz)	26
Pork, ground (3 oz)	40
Seafood	
Swordfish (3 oz)	58
Grouper, halibut, pollock, snapper (3 oz)	40–47
Salmon, Atlantic, farmed (3 oz)	35
Cod, Atlantic (3 oz)	32
Tuna, yellowfin (3 oz)	92
Tuna, light, canned in water (3 oz)	68
Shrimp (3 oz)	40
Clams, mixed species (3 oz)	54
Oysters, Eastern (3 oz)	34
Nuts and Seeds	
Almonds (1 oz)	1
Cashews (1 oz)	6
Brazil (1 oz)	543
Sunflower seeds (1 oz)	23
Eggs and dairy products	
Egg, poached (1 large)	15
Milk, 1% fat (1 cup)	8
Yogurt, Greek, lowfat (5.3 oz)	14
Cottage cheese, 2% fat ($\frac{1}{2}$ cup)	13
Cheese, variety (1 oz)	4–8
Grains	
Bread, white, enriched (1 slice)	6
Bread, whole wheat (1 slice)	13
Cereals, fortified (1 cup)	15
Pasta, enriched (1 cup)	33
Rice, white, enriched (1 cup)	12
Couscous ($\frac{1}{2}$ cup)	22
Quinoa ($\frac{1}{2}$ cup)	5
Other	
Peanut butter (2 Tbsp)	2
Spinach ($\frac{1}{2}$ cup)	5
Broccoli (1 cup)	3
Legumes, variety ($\frac{1}{2}$ cup)	1–5

*Data represent cooked foods, except for fruits, nuts, and milk.

Source: U.S. Department of Agriculture, Agricultural Research Service. FoodData Central, 2019. <https://fdc.nal.usda.gov>

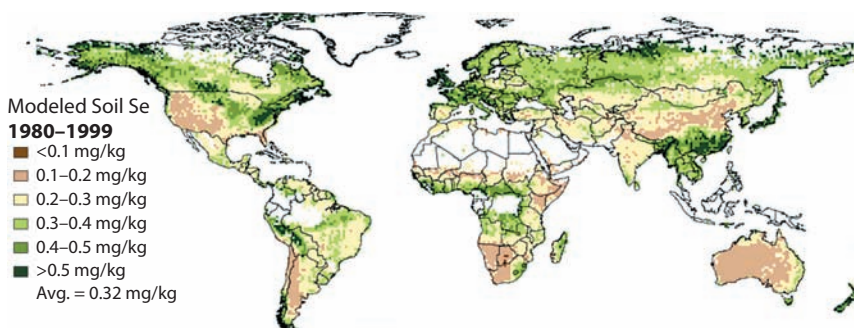
may provide significant amounts. The selenium content of cereals varies from less than 10 μg to over 80 $\mu\text{g}/100\text{ g}$ based on the selenium content of the soil in which the cereals were grown. Additionally, although fish is generally an

THE SHIFTING SANDS OF SELENIUM

The amount of selenium in food is highly variable, a direct result of the selenium concentration of the soil in which crops are grown. Soil selenium is thus the main determinant of selenium status in crops, livestock, and humans. Large regions of the earth are low in selenium, whereas other regions have high selenium. But why is selenium distributed so unevenly over the earth's surface? What geological and environmental factors contribute to selenium distribution?

Rocks that make up the earth's crust are generally very low in selenium. In a listing of 88 naturally occurring elements in the earth's crust, selenium is placed 70th, confirming its classification as a trace mineral [1]. It follows that the mineral content of soil reflects the type of rocks that exist in various regions of the world. Slightly higher levels of soil selenium would be expected in regions where the underlying bedrock consists of selenium-rich sedimentary rocks [1]. But these geological differences alone cannot explain the vastly uneven distribution of selenium on the earth's surface (see Figure).

One explanation may be found above the earth's surface and in the ocean. A significant amount of selenium resides in the atmosphere and the ocean—and it is constantly moving. Marine selenium is the largest natural source of selenium entering the atmosphere. In the ocean, selenium is methylated by marine organisms and released as volatile gaseous molecules, primarily dimethyl selenide, dimethyl selenyl sulfide, and dimethyl diselenide. These



Modeled soil selenium concentration for the years 1980–1999. Source: Jones GD, Droza B, Greve P, et al. Selenium deficiency risk predicted to increase under future climate change. *PNAS*. 2017; 114:2848–53.

molecules are oxidized in the atmosphere to inorganic species, including Se(0) , SeO_2 , and H_2SeO_4 [2]. Following global moisture recycling patterns, atmospheric selenium is then deposited on the earth's surface through rainfall. Not surprisingly, many of the selenium-deficient regions of the world receive relatively low annual rainfall. In one pivotal study, the regional soil selenium concentrations in China were associated with monsoon-derived precipitation, thus highlighting the role of climate–soil interactions to selenium distribution [3].

Another factor contributing to global selenium distribution is due to anthropogenic (human-generated) activities. One such activity is the use of fertilizers that adds selenium to agricultural soil. This may be done intentionally in selenium-deficient regions, but the unintentional addition of selenium to selenium-rich soil could increase the risk of toxicity. Metal-processing

industries and burning of fossil fuels are also common anthropogenic sources of selenium in the environment [1].

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2. Suess E, Aemisegger F, Sonke JE, et al. Marine versus continental sources of iodine and selenium in rainfall at two European high-altitude locations. *Environ Sci Technol*. 2019; 53:1905–17.
3. Blazina T, Sun Y, Voegelin A, et al. Terrestrial selenium distribution in China is potentially linked to monsoonal climate. *Nat Commun*. 2014; 5:4717.

excellent source of selenium, the mercury content, if high, in some fish limits selenium's bioavailability secondary to the formation of unabsorbable mercury–selenium complexes. Another very rich source of selenium is the Brazil nut, each nut containing as much as 90 μg of selenium. Experts caution, however, that regular consumption of even an ounce (about six to eight nuts) increases the likelihood of toxicity (see the section on "Toxicity"). Selenium intakes in the United States are typically adequate.

Selenium occurs naturally in foods and in the body in both organic and inorganic forms. The organic forms, selenomethionine and selenocysteine, represent selenium analogues of the sulfur-containing amino acids methionine

and cysteine, respectively (Figure 13.19). The element substitution is made possible due to the chemical similarity between selenium and sulfur. In plants, these selenium analogues become incorporated into plant proteins. Consumption of plant foods provides mostly selenomethionine, with lesser amounts of selenocysteine and selenium methyl selenocysteine, and some inorganic forms of selenium such as selenite (H_2SeO_3) and selenate (H_2SeO_4). Animal products tend to contain selenium primarily as selenocysteine. Another inorganic form of selenium is selenide ($(\text{H}_2\text{Se}$ or $\text{HSe}^-)$). Although not an important dietary source of selenium, selenide serves as a critical metabolite in the body, as discussed in the section "Metabolism."

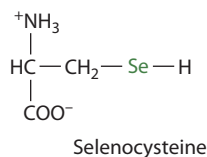
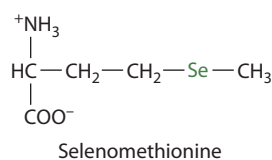


Figure 13.19 Selenomethionine and selenocysteine.

Supplements provide selenium as selenomethionine, selenium methyl selenocysteine, sodium selenate, sodium selenite, or selenium-enriched yeast (which usually contains a mixture of different forms of selenium). Multivitamin/mineral supplements typically provide 50–200 µg of selenium. Selenium supplements sold as a single nutrient contain similar amounts and forms as is used in the multivitamin/mineral preparations. The various forms of selenium, however, do not appear to equally increase the body's selenoproteins; inorganic selenite availability is about two-thirds that of selenomethionine [2]. The Daily Value for selenium used on food and supplement labels is 55 µg.

Digestion, Absorption, Transport, and Storage

Digestion and Absorption

Selenium, in organic and inorganic forms, is efficiently absorbed throughout the small intestine, including the duodenum, jejunum, and ileum, and does not require digestion prior to its absorption into enterocytes. Selenoamino acid absorption, which occurs through amino acid transport systems, is estimated to be over 80%, with selenomethionine typically better absorbed (at about 97%) than selenocysteine. Selenate is absorbed by active, sodium-dependent transport, primarily in the distal small intestine. Selenite absorption occurs by passive diffusion, primarily in the jejunum. Usually greater than 90% of dietary selenate and greater than 50% of dietary selenite are absorbed into enterocytes. Selenium absorption does not appear to be regulated and thus does not play a role in body selenium homeostasis. Within the intestinal cell, some selenite may be reduced using glutathione or other thiols to form selenide.

Factors Influencing Selenium Absorption Factors enhancing selenium absorption include vitamins C, A, and E, as well as the presence of reduced glutathione in the intestinal lumen. Heavy metals such as mercury and phytic acid are thought to inhibit selenium absorption through chelation and precipitation.

Transport

Selenoamino acids are likely transported across the basolateral membrane using amino acid transporters; however, the mechanism(s) by which inorganic selenium crosses the enterocyte's basolateral membrane to enter portal blood is largely unknown. Within portal blood, selenoamino acids travel free (unattached) to the liver and other tissues. Selenite entering portal blood is thought to be taken up by red blood cells and reduced to selenide, which is then released back into portal blood for transport to the liver bound to albumin. Some selenite, and likely other inorganic forms of selenium, upon leaving the intestinal cells, may also attach to the sulfhydryl groups of apoproteins found on the surface of lipoproteins for travel to the liver. The liver takes up about 50% of absorbed selenium on first pass [3].

Once in the liver, organic and inorganic forms of selenium are metabolized. Selenium is transported in the blood mainly as part of selenoprotein P, which contains 50–80% of selenium in the plasma. Other transporters including albumin also deliver selenium to tissues for use. Selenoprotein P has a half-life of about 3 hours. Receptors for selenoprotein P are found on the cell membranes of some body tissues. An apoprotein E receptor (ApoER2) governs selenoprotein P uptake into the brain and testes. In the kidneys, a megalin receptor mediates selenoprotein P uptake, most likely by receptor-mediated endocytosis. Selenoprotein P is described further in the “Functions and Mechanisms of Action” section. Plasma selenium concentrations typically range from about 60 to 150 µg/L (note µg/L = ng/mL).

Storage

Tissue selenium concentrations vary to some extent, with amounts typically higher in the kidneys, liver, and spleen and lower in the pancreas, heart, brain, and lungs. Skeletal muscle, bones, and red blood cells also contain fairly large quantities of the mineral. The form(s) in which selenium is found in these tissues is not clear.

Metabolism

Within tissues such as the liver and to some extent in other tissues such as the kidneys, selenoamino acids and inorganic forms of selenium undergo metabolism (Figure 13.20). Selenate ingested from the diet is reduced to selenite. Selenite, either obtained directly from the diet or produced from selenate, is further reduced to selenide. The reduction of selenite (but not selenate) is facilitated by thioredoxin reductase and glutathione; how selenate is reduced to selenite remains unclear.

Selenomethionine, which is derived from the diet, may be either “stored” in tissues as selenomethionine in an amino acid pool, used for protein synthesis (just as the amino acid methionine is used), catabolized in multiple reactions

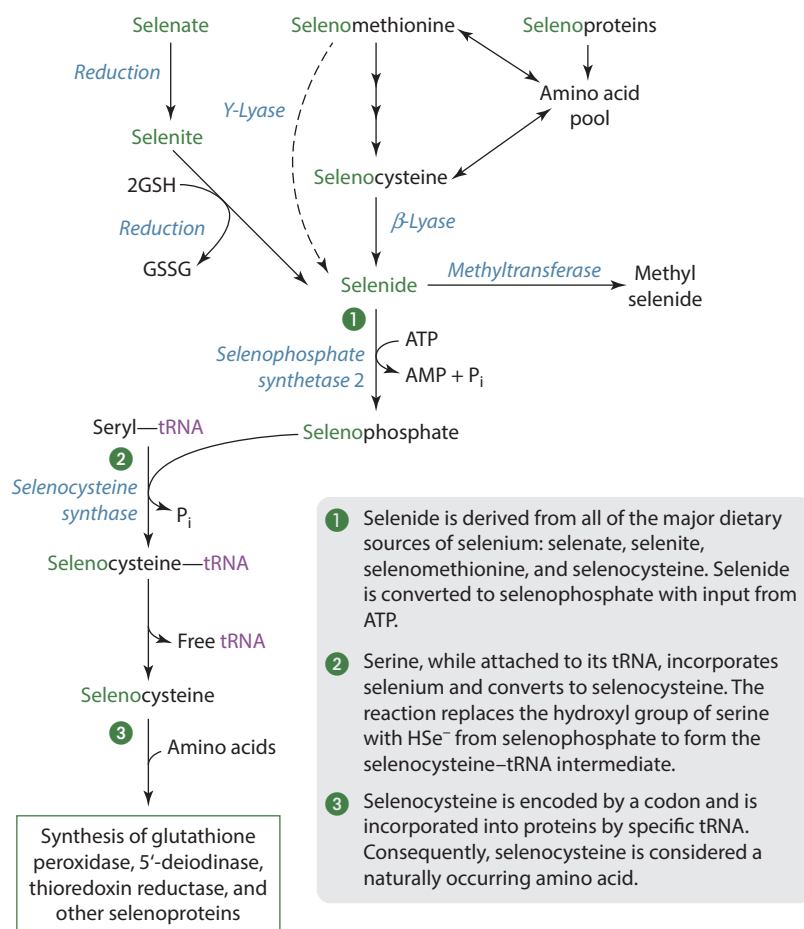


Figure 13.20 Selenum metabolism in the liver.

(referred to as trans-selenation) to yield selenocysteine, or directly lysed at the γ -position to generate selenide. This latter reaction is thought to be induced with excessive selenium intakes. Selenocysteine, which is derived either from selenomethionine metabolism or from the diet, is degraded primarily by selenocysteine β -lyase, producing selenide.

As depicted in Figure 13.20, selenide is an important intermediate in the synthesis of the body's selenium-dependent enzymes (selenoenzymes). Selenide is derived from selenite, selenate, selenomethionine, and selenocysteine. During normal metabolism, selenide can be methylated and excreted in the urine as a pathway for maintaining selenium homeostasis. However, its conversion to selenophosphate is critical for the synthesis of selenium-dependent enzymes.

The form of selenium required for selenoprotein synthesis is selenocysteine. Interestingly, selenocysteine obtained directly from the diet or selenomethionine degradation cannot be utilized. Instead, the selenocysteine must be synthesized in the body from the amino acid serine, while the serine is attached to transfer (t) RNA; the selenium donor is selenophosphate (Figure 13.20).

Synthesis of selenophosphate is catalyzed by selenophosphate synthetase 2—itsself a selenocysteine-containing enzyme—with input from ATP. Next, while attached

to tRNA, serine is converted into a selenocysteine-tRNA intermediate (abbreviated Sec-tRNA^{[Ser]^{Sec} or simply tRNA^{SEC}). Should selenophosphate not be produced in sufficient quantities, the subsequent generation of all selenoproteins becomes diminished. A total abolishment of selenoprotein synthesis is lethal. Furthermore, diminished activity of even some selenoproteins also results in massive cellular damage due to the decrease of the antioxidant/cell redox reactions normally provided by the selenoproteins.}

The incorporation of selenocysteine that is made while attached to its tRNA involves a novel process whereby the UGA stop codon is reprogrammed to be read as a sense codon. This reprogramming requires:

- 1 tRNA^{SEC}
- 2 A selenocysteine insertion sequence (SECIS) in the 3' untranslated section of the mRNA of selenoproteins
- 3 SEC insertion sequence binding protein 2 (SBP2)
- 4 Elongation factor selenocysteine (EFsec).

The selenocysteine insertion sequence is found in the 3' untranslated part of the mRNA of selenoproteins. The attachment of the SEC insertion sequence binding

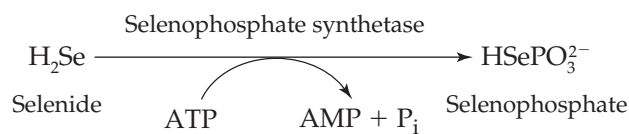
protein 2 to the insertion sequence on the mRNA enables interaction with a specific elongation factor and with the tRNA^{SEC}. In the presence of these elements, selenocysteine is incorporated at the UGA codon, and UGA is not read as a stop codon. Because selenocysteine is encoded by a codon and is incorporated into proteins by specific tRNA, it is widely considered as the 21st naturally occurring amino acid in the genetic code. The amino acid's three-letter abbreviation is SEC and one-letter abbreviation is U.

Functions and Mechanisms of Action

The better-characterized roles of selenium are related to its functions as an integral part of specific proteins/enzymes in the body. Over 25 genes encoding these selenoproteins have been identified in humans. These proteins primarily function in antioxidant capacities, and thus regulate cell redox status. The next sections describe some of the selenium-dependent enzymes and their metabolic roles.

Selenophosphate Synthetase 2: Selenoprotein Synthesis

Selenophosphate is a key compound needed in the body to synthesize the other selenocysteine-containing proteins/enzymes such as glutathione peroxidase, deiodinase, thioredoxin reductase, and selenoprotein P, among others. At least two forms of selenophosphate synthetase have been identified. One form (designated SPS1) does not contain selenocysteine and is thought to recycle selenium from selenocysteine. The selenophosphate synthetase 2 isoform contains selenocysteine and catalyzes the synthesis of selenophosphate from selenide, as shown here:



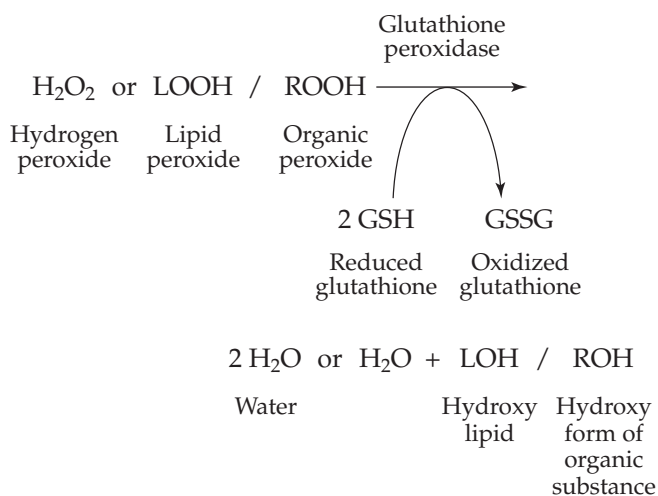
Mutations in selenophosphate synthetase 2 or defective selenophosphate synthetase 2 activity significantly reduces selenoprotein synthesis. This in turn leads to increased reactive oxygen species generation and cellular apoptosis.

Glutathione Peroxidase (GPx): Antioxidant

One of the most clearly established functions of selenium is as an integral part of the enzyme glutathione peroxidase. Several glutathione peroxidase enzymes (designated GPx followed by a number) have been characterized, and each catalyzes the same basic reaction but in different tissues. Seven GPx isozymes have been identified and most are selenium dependent, containing selenocysteine. Within cells, glutathione peroxidase is found mainly (~70%) in the cytosol and to a lesser extent (~30%) in the mitochondrial matrix; however, GPx4 is found predominantly associated with cell membranes. GPx1 is found in most body tissues

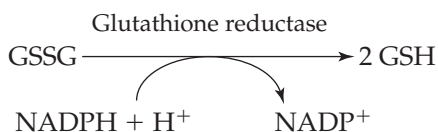
but most notably in the liver, kidneys, and red blood cells. GPx1 concentrations are reduced with selenium deficiency to a greater extent than other GPx isozymes. GPx2 is found mainly in the GI tract and liver. GPx6 is found in olfactory epithelium. GPx3 originates in the kidney but is released into the plasma (extracellular); GPx3 accounts for 10–30% of selenium in the plasma and has a half-life of about 12 hours. Serum selenium concentrations of about 70 or 80 ng/mL and a selenium intake of about 37 μ g as selenomethionine or 66 μ g as selenite/day are needed to optimize GPx3 activity [4].

Glutathione peroxidase catalyzes the removal of hydrogen peroxides (H_2O_2) and organic (including lipid) hydroperoxides (designated ROOH). GPx4 functions in a similar capacity, removing phospholipid hydroperoxides (designated LOOH) associated with membranes. Organic peroxides are derived from nucleic acids and other molecules, including unsaturated fatty acids; however, a peroxide derived from fatty acids may be designated as a lipid peroxide. Hydrogen peroxides are generated in many cells throughout the body as part of normal metabolism and may be generated in large amounts by activated white blood cells as they phagocytize foreign substances. If not removed, these peroxides typically damage cellular membranes and other cell components including proteins and DNA. Glutathione, a tripeptide of glycine, cysteine, and glutamate found in most body cells, is needed in its reduced form (GSH) for the glutathione peroxidase-catalyzed reaction. Reduced glutathione, which is thought to be the most abundant antioxidant found in cells, furnishes the reducing equivalents, as shown in the following reactions.



The oxidized glutathione (GSSG) that is formed as a result of glutathione peroxidase activity must be regenerated back to its reduced form (GSH). This regeneration is imperative for cells to maintain appropriate redox states. Glutathione reductase, a flavoenzyme, catalyzes this reduction in a reaction dependent on $\text{NADPH} + \text{H}^+$, which is derived from the pentose phosphate pathway (hexose

monophosphate shunt). The regeneration of reduced glutathione is shown here:



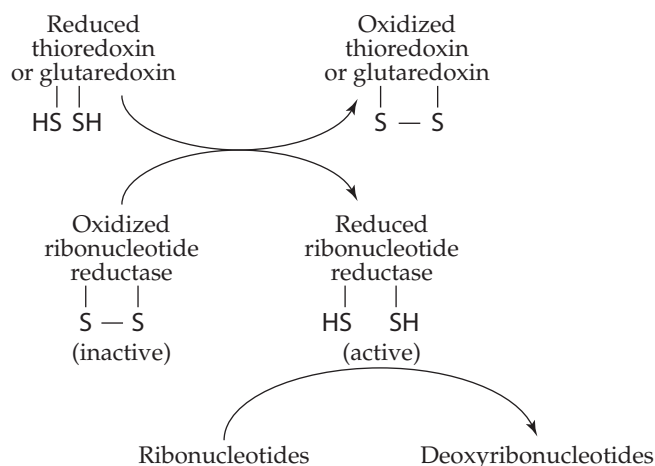
Selenoprotein P: Antioxidant

Selenoprotein P, a glycoprotein, is synthesized mostly in the liver but also in the brain; it is found throughout the body, including in association with capillary endothelial cells. The protein transports selenium from the liver to some tissues for use, but also exhibits antioxidant functions such as peroxynitrite (ONOO) radical disposal. Peroxynitrite is synthesized by activated white blood cells (e.g., at infection sites) from superoxide radicals ($\text{O}_2^\bullet$) and nitrogen monoxide (NO). If not inactivated, peroxynitrite causes DNA single-strand breaks and lipid peroxidation, among other damage. Selenoprotein P may also catalyze the reduction of membrane phospholipid hydroperoxides to alcohols, similar to the function of GPx4.

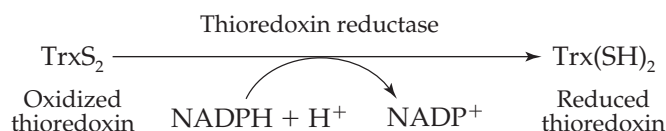
Selenoprotein P, unlike most selenoenzymes (which contain one to four selenium atoms as selenocysteine), contains up to 10 selenocysteine residues. However, under conditions in which selenium is limited, selenoprotein P may be synthesized with fewer selenocysteine residues. In other words, instead of having 10 selenocysteines, selenoprotein P may only have two or three or so selenocysteines if sufficient selenium is not available in the cells. Moreover, when selenium is limited, selenoprotein P appears to preferentially receive selenium over other selenoenzymes such as glutathione peroxidases. Selenoprotein P is optimized at a serum selenium concentration of about 120 ng/mL; a selenium intake (as selenomethionine) of about 37 $\mu\text{g}/\text{day}$ was not sufficient to optimize selenoprotein P activity [4].

Thioredoxin Reductase: Antioxidant

Thioredoxin reductase is a flavoenzyme (containing FAD) that, like glutathione peroxidase, selenoprotein P, and selenophosphate synthetase 2, contains selenocysteine at its active site. The enzyme is found in three forms designated by numbers: form 1 is typically located in the cytosol, form 2 is present in the mitochondria, and form 3 is found primarily in the testes. Within cells, thioredoxin reductase helps maintain the redox state (i.e., prevents oxidative stress) by reducing oxidized thioredoxin (TrxS-SxTr) and oxidized glutaredoxin, as well as a multitude of other oxidized molecules and protein substrates. Some of these protein substrates include transcription factors, receptors, and enzymes. An example of an enzyme substrate is ribonucleotide reductase, needed in the conversion of ribonucleotides to deoxyribonucleotides in DNA synthesis.



Specifically, thioredoxin reductase transfers reducing equivalents from NADPH through its bound FAD to reduce disulfide bonds (S-S) within the oxidized substrate, as shown next.



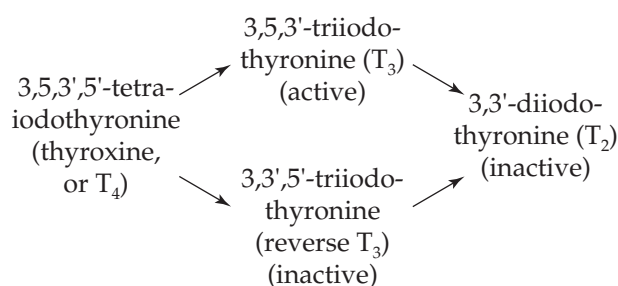
Some of the transcription factors, which contain cysteine in their DNA binding region and which rely on the thioredoxin-thioredoxin reductase system (composed of thioredoxin, thioredoxin reductase, and NADPH), include activator protein (AP) 1, p53, and nuclear factor κB (NF κB). Additionally, thioredoxin and glutaredoxin are thought to modulate intracellular signaling cascades that in turn affect other cellular processes. Thioredoxin, for example, inhibits apoptosis through interactions with apoptosis signal-regulating kinase (ASK) 1 and thus affects cell division, proliferation, and longevity.

Iodothyronine 5'-Deiodinases (IDI or DI): Thyroid Hormone Synthesis

Selenium is also necessary for iodine metabolism, including the generation of thyroid hormones, as a constituent of a group of enzymes called deiodinases. The deiodinases are selenocysteine-containing enzymes with the selenocysteine present at the active site. Three types of deiodinases have been characterized. Type 1 is found mainly in the thyroid gland, pituitary gland, liver, and kidneys. Type 2 is found in a larger group of tissues including the thyroid gland, brain/central nervous system, heart, pituitary gland, skeletal muscle, and brown adipose tissue. Type 3 is found mainly in the brain and a few other tissues such as skin. Deiodinase activity is optimized at serum selenium concentrations of about 60 ng/mL [5].

5'-deiodinases catalyze the deiodination (removal of iodine) from the 5 or 5' positions of thyroid hormones and some of their metabolites. Deiodinases 1 and 2 remove one

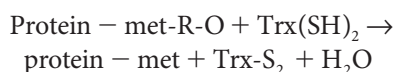
of four iodines in the thyroid hormone thyroxine (T_4) to produce 3,5,3'-triiodothyronine (T_3). T_3 is the body's primary regulator of metabolism as well as of normal growth and development; a selenium deficiency decreases T_3 concentrations and increases T_4 concentrations. Deiodinase 2 provides for the production and use of T_3 within specific tissues. Such function in individualized tissues enables specific adaptations; for example, expression of deiodinase 2 in brown adipose tissue increases in response to cold, resulting in increased heat production. Surplus T_3 is degraded to inactive T_2 (also called 3,3'-diiodothyronine), and T_4 is degraded to inactive reverse (r) T_3 by deiodinases 1 and 3.



For further information regarding thyroid hormone metabolism, see the “Iodine” section.

Methionine R-Sulfoxide Reductase (formerly Selenoprotein R): Antioxidant

Methionine sulfoxide reductases catalyze the reduction of R- and S-forms of methionine sulfoxide; however, it is the R-form of the enzyme that contains selenocysteine. The enzyme, found in both the cytosol and nucleus of cells, regulates cellular redox levels. Specifically, the enzyme reduces methionine R-sulfoxides (oxidized methionines) using reduced thioredoxin as well as GSH-dependent glutaredoxin. Methionine (met) sulfoxides (met-R-O) are generated in proteins when free radicals cause oxidation of methionine residues. The reaction catalyzed by methionine R-sulfoxide reductase is as follows:



The presence of the sulfoxide within the protein damages the protein so that it is unable to perform its normal function; however, methionine repair and thus return of normal protein function occurs with the action of methionine sulfoxide reductase.

Other Selenoproteins

Several other selenoproteins containing at least one selenocysteine have been identified, but little is known about their functions. Table 13.9 provides an overview of some of the characteristics and proposed functions of these selenoproteins. Reference [6] provides an excellent review of selenoproteins.

Table 13.9 Proposed Functions/Characteristics of Select Selenoproteins

Well-established/better-characterized selenoproteins

Selenophosphate synthetase 2: selenoprotein synthesis
 Glutathione peroxidase: antioxidant
 Selenoprotein P: antioxidant
 Thioredoxin reductase: cellular redox state maintenance
 Iodothyronine 5'-deiodinases: thyroid hormone synthesis and metabolism
 Methionine R-sulfoxide reductases: oxidative damage repair

Lesser-characterized selenoproteins and their possible roles

W: in skeletal and heart muscles, binds glutathion—antioxidant role and muscle growth
 N: in muscle cells, endoplasmic reticulum—calcium regulation and antioxidant role
 H: antioxidant role and transcription factor to up-regulate selenoprotein expression
 M: in neuronal cells and in endoplasmic reticulum—antioxidant, regulator of calcium release and protein folding roles
 K and 15: role in process and/or folding of proteins in the endoplasmic reticulum
 S: in endoplasmic reticulum—removal of misfolded proteins and antioxidant function
 T: in endoplasmic reticulum—calcium mobilization and neuroendocrine function
 O, I, and V: unclear roles

Other Roles: Disease Prevention

Low serum selenium concentrations or impaired selenium status have been inversely associated with increased risk of heart disease and some cancers. Yet, selenium supplementation studies for the prevention of these diseases report inconsistent findings and, if benefits are reported, they are mostly confined to those with low baseline serum selenium concentrations. A U-shaped curve between serum selenium concentrations and disease risk has been demonstrated such that plasma selenium concentrations less than about 120 (or 130) $\mu\text{g/L}$ as well as above about 160 (or 150) $\mu\text{g/L}$ are associated with increased disease risk, whereas concentrations in between these cutoff values are associated with reduced disease risk [5,7]. Higher serum selenium concentrations in some studies have been associated with an increased risk for diabetes, hypertension, and some cancers as well as overt selenium toxicity. Reductions in the risk of cardiovascular diseases also have been reported with serum selenium concentrations ranging from 55 to 145 $\mu\text{g/L}$ [8]. At present, however, scientific studies do not support the use of selenium supplements for the prevention of cancer or heart disease. Increased dietary selenium intakes are recommended for those with poor selenium status or low serum selenium concentrations.

Interactions with Other Nutrients

Selenium may help prevent some toxic effects associated with some metals such as arsenic and mercury. Selenium, when consumed in adequate amounts, may reduce

arsenic-associated skin lesions and arsenic-associated oxidative tissue damage. The interaction of selenium and mercury is a central feature in mercury toxicity. Mercury can bind to the selenium site of thioredoxin reductase and glutathione peroxidase, thus disrupting the cell's redox environment and antioxidant capabilities. Dietary amounts of both selenium and mercury determine the extent of mercury's damaging effects. Adequate selenium intake can be protective when mercury intake is relatively low; however, excessive mercury intake can overwhelm normal selenium metabolism, creating a selenium deficiency state. Selenium supplementation is necessary to mitigate the toxic effects of mercury [9].

Excretion

Urinary excretion of selenium enables body selenium homeostasis with urinary excretion directly proportional to selenium intake when intake is adequate. The major urinary metabolites are methylated forms of selenium, with the most prevalent form being the selenosugar methylseleno-N-acetylgalactosamine ($\text{CH}_3\text{Se-GalN}$). Other urinary metabolites, especially with higher intakes of the mineral, include methylselenol (CH_3SeH), dimethylselenide [$(\text{CH}_3)_2\text{Se}$], and trimethylselenonium [$(\text{CH}_3)_3\text{Se}^+$]. The selenosugar is also excreted in feces.

Selenium losses through the lungs and skin also contribute to selenium excretion to a small extent. Pulmonary elimination (i.e., exhalation in the breath) of selenium increases when selenium is consumed in large amounts. The main form of selenium that is exhaled is dimethylselenide, which is quite volatile and has a garlicky odor.

Recommended Dietary Allowance

The Food and Nutrition Board set a Recommended Dietary Allowance for selenium for adults of 55 $\mu\text{g/day}$ [10]. Based mostly on balance studies as well as on repletion studies of men with selenium deficiency in regions of China, the adult requirement for selenium was determined to be 45 μg . The requirement was based on calculation of the amount of selenium necessary to plateau concentrations of selected selenoproteins, primarily GPx, in the plasma. To set the RDA, a 20% coefficient of variation was added, and the final number was rounded to the nearest five. RDAs for selenium for pregnancy and lactation were set at 60 μg and 70 μg , respectively [10]. The inside front cover of the book provides RDAs for selenium for other age groups. Studies, however, suggest that the current RDA for selenium for adults may be suboptimal, with some recommending as much as about 200 μg of selenium/day [4,11].

Deficiency

Widespread selenium deficiency occurs in certain regions of the world, including parts of China, central Africa, and Europe. The deficiency is linked to Keshan disease and Kashin-Beck disease. Keshan disease is characterized by cardiomyopathy involving cardiogenic shock, congestive heart failure, or both, along with multifocal necrosis of heart tissue, which becomes replaced with fibrous tissue. Infection by coxsackie virus appears to be a cofactor in the development of Keshan disease. In the absence of sufficient selenium, mutations occur in benign strains of the virus. These mutations cause the virus to become virulent; the presence of the virus is thought to account for some of the symptoms of Keshan disease. Kashin-Beck disease is characterized by osteoarthropathy involving chronic degeneration and necrosis of the joints and of epiphyseal-plate cartilages of the legs (primarily knees and ankles) and arms (mostly fingers, hands, and elbows). Several factors, including selenium deficiency, are thought to contribute to the development of Kashin-Beck disease.

In the United States, selenium deficiency has been observed in people receiving total parenteral nutrition. Some deficiency symptoms included poor growth, muscle pain and weakness, loss of pigmentation of hair and skin, and whitening of nail beds (Figure 13.21). The observed poor growth may be associated with selenium's role in thyroid hormone metabolism. Individuals with HIV and cirrhosis are at increased risk of deficiency secondary to malabsorption and increased diarrhea-induced losses of selenium. Selenium deficiency is typically treated with supplements providing 100–200 μg of selenium/day. Absorption of selenomethionine is typically greater than that of selenite. However, those with impaired liver function (and thus possibly an impaired ability to metabolize selenomethionine) may respond better to supplementation with selenite.



Figure 13.21 Leukonychia (whitening of the nail bed) associated with selenium deficiency.

DR P. MARAZZI/Science Source

Impaired or low selenium status is also found in those with critical illness and with some inflammatory conditions. Selenium deficiency is sometimes associated with reductions in immune system function and the body's anti-inflammatory response. Selenium is thought to perhaps modulate both processes through some of its redox functions. The thioredoxin-thioreductase system's control of gene expression via transcription factors enables the blocking of proinflammatory cytokine production. Additionally, the mineral provides transient pro-oxidant actions by inducing apoptosis and/or cytotoxic effects against proinflammatory or hyperactivated cells (such as polymorphonuclear neutrophils), among other actions [12,13]. Selenium supplementation in individuals with a variety of inflammatory conditions has not consistently yielded benefits. Supplementation with high doses of selenium in those with critical illness has in some, but not all, studies been associated with reductions in the inflammation and lower mortality, but current evidence for supplementation is considered disputable [12,14].

Toxicity

Selenium toxicity, called selenosis, has been observed both in miners and in people who consumed excess selenium from supplements. Signs and symptoms of toxicity include nausea and vomiting, fatigue, diarrhea, hair brittleness and loss, brittle and thickened nails, muscle cramps, paresthesia, interference in sulfur metabolism (primarily oxidation of sulfhydryl groups), and inhibition of protein synthesis [15]. Acute poisoning from ingestion of gram amounts of selenium is lethal, with damage occurring to most organ systems [15]. A Tolerable Upper Intake Level of 400 $\mu\text{g}/\text{day}$ has been set by the Food and Nutrition Board [10].

Assessment of Nutriture

Serum or plasma selenium concentrations are widely used for assessment, with concentrations less than about 70 ng/mL suggestive of inadequate recent intake [10]. Urinary selenium excretion is also used as an indicator, with concentrations less than 15 $\mu\text{g}/\text{L}$ indicative of inadequate recent intakes and those greater than about 50 $\mu\text{g}/\text{L}$ suggestive of excessive selenium ingestion. Whole-blood selenium concentrations represent longer-term intake (about 120 days, the lifespan of the red blood cell). Toenails can be used as an indicator of even longer-term (months to a year) intakes and are considered better than hair concentrations due to more consistent growth rate and less contamination from shampoo and other personal care products.

The activities and concentrations of some selenoproteins have also been used to assess selenium status. Selenoprotein P and glutathione peroxidase in tissues (GPx1) and in the plasma (GPx3) are commonly used. Selenoprotein P and glutathione peroxidase concentrations decrease in the plasma as selenium deficiency worsens, thus serving as an index of selenium status in populations with low intake [15,16]. However, selenoprotein P is thought to be a better indicator of selenium status than GPx; GPx achieves maximal activity at a selenium intake considerably lower than selenoprotein P [4].

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13.5 CHROMIUM

Chromium, a metal with a ubiquitous presence in air, water, and soil, exists in several oxidation states from Cr^{2-} to Cr^{6+} . Trivalent chromium, Cr^{3+} , is the most stable oxidation state and is often found attached to ligands containing nitrogen, oxygen, or sulfur to form hexacoordinate or octahedral complexes. It is this trivalent form of chromium that is most important in humans.

Sources

In foods, chromium exists in the trivalent form. Few foods, however, have been analyzed to accurately quantify chromium content; this task is further complicated by the variability of chromium within foods. Good dietary sources of chromium are generally thought to include meats and grains (especially whole grains) along with some vegetables and fruits. Some examples of the approximate chromium content of some foods include:

- Beef and turkey breast (3 oz), 2 μg ;
- Whole-wheat bread (1 slice), 1 μg ;
- Orange juice (1 cup), 2 μg ;
- Grape juice (1 cup), 7 μg ;
- Banana and apple (1 medium), 1 μg ;
- Potatoes, mashed (1 cup), 3 μg ;
- Broccoli (1/2 cup), 11 μg ; and
- Green beans (1/2 cup), 1 μg .

Relatively large amounts of chromium may be present in some red wines. Additionally, chromium is found in selected spices such as cinnamon, cloves, bay leaves, and turmeric; in tea and beer; and in some yeast preparations. Most adults in the United States are estimated to ingest 23–54 μg of chromium daily [1].

Chromium is available in multivitamin/mineral supplements and in single-nutrient supplements as inorganic salts (such as chromium chloride) and as an organic compound complexed with acetate, nicotinate, citrate, picolinate, or amino acids. The bioavailability of the inorganic salt, chromium chloride, is lower than the organic complexes. The multivitamin supplements usually contain about 25–120 μg , whereas the individual-nutrient supplements provide about 50–200 μg of chromium. Although all forms appear to be absorbed and used, different forms of the supplement appear to affect tissue concentrations differently. For example, chromium picolinate has been touted as superior to other forms of chromium; however, urinary chromium excretion from chromium picolinate is also higher than other supplemental forms, suggesting poor tissue uptake

[2]. The Daily Value for chromium used on food and supplement labels is 35 μg .

Digestion, Absorption, Transport, and Storage

Digestion and Absorption

Chromium, Cr^{3+} , may be released from food components in acidic solutions, as found in the stomach. Chromium is absorbed throughout the small intestine, especially in the jejunum. Although the mode of absorption in humans is not known, chromium is thought to be absorbed by passive diffusion, by a carrier-mediated transporter, and perhaps by endocytosis. Absorption estimates for chromium range from 0.4 to 2.5%. The percent of chromium absorbed is influenced by the absolute intake, with fractional absorption increasing as chromium intake decreases [1].

Factors Influencing Chromium Absorption Like that of other trace minerals, the absorption of chromium may be influenced by dietary factors. Within the stomach, amino acids or other molecules may chelate chromium. Amino acids such as phenylalanine, methionine, and histidine as well as picolinic acid (picolinate) act as chelators to improve chromium absorption [3]. These chelations typically help chromium remain soluble and prevent olation (see next paragraph) once it reaches the alkaline pH of the small intestine. Lipophilic compounds such as picolinate may enhance Cr^{3+} absorption through the cell's lipid membranes. Vitamin C and niacin may also enhance chromium absorption.

Chromium in a neutral or alkaline environment may react with hydroxyl ions (OH^-), which readily polymerize to form high-molecular-weight compounds in a process called olation. This reaction readily occurs with prolonged use of medications to treat heartburn, GERD, and ulcers. These medications (including proton pump inhibitors and H_2 receptor blockers) increase gastric pH, resulting in chromium precipitation and thus reduced absorption. Phytic acid, found mostly in grains, legumes, nuts, and seeds, also binds to and diminishes chromium absorption.

Transport

In the blood, Cr^{3+} , like Fe^{3+} , binds to transferrin. If transferrin sites are unavailable due to occupation by iron, for example, albumin is thought to transport chromium. Globulins and possibly lipoproteins may also transport the mineral if present in very high concentrations. Some chromium may also circulate unbound in the blood. Plasma chromium concentrations are typically low, ranging from 1 to 5 $\mu\text{g/L}$. The uptake of transferrin-bound chromium into cells is thought to occur like that of iron (see Figure 13.6).

Storage

The body contains ~4–6 mg of chromium. Tissues especially high in chromium include the liver, spleen, and bone and to a lesser extent the kidneys, heart, and pancreas. Tissue chromium concentrations appear to decline with age.

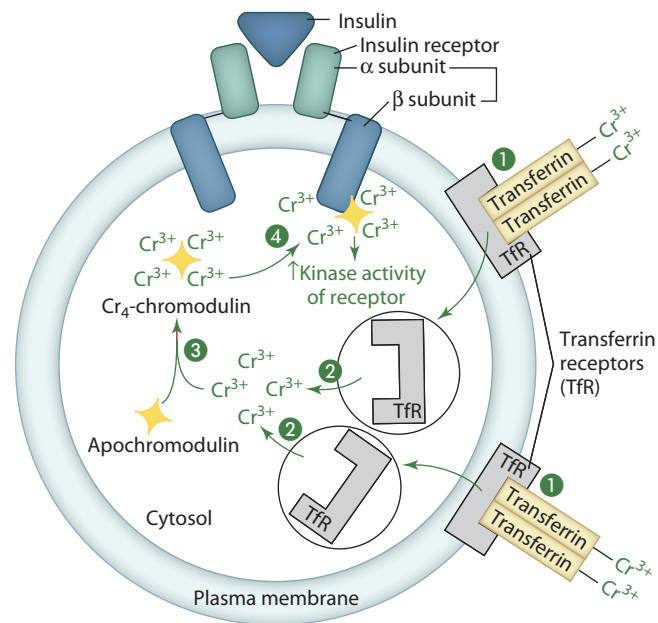
Functions and Mechanisms of Action

Chromium is thought to potentiate the action of insulin; however, the mechanism by which potentiation occurs is still unclear. For decades, this biological action of chromium was believed to be attributable to its complexing with nicotinic acid and amino acids to form the organic compound **glucose tolerance factor** (GTF). GTF was first identified in brewer's yeast, but this factor has never been purified, nor has its exact structure been characterized.

While it is still thought to potentiate the action of insulin, more recent studies have suggested that the biologically active form of chromium is a low-molecular-weight chromium-binding substance called chromodulin. Chromodulin is thought to be produced in response to insulin secretion, which stimulates chromium uptake by cells. Once within the cell, chromium atoms (four) bind to apochromodulin, an oligopeptide that is thought to be composed of glycine, cysteine, aspartate, and one or more glutamates. Once the four chromium atoms bind to the apochromodulin, the complex is called holochromodulin (Cr^4 -chromodulin) or chromodulin. This proposed process is shown in Figure 13.22.

Insulin signaling within cells is regulated, in part, by the phosphorylation of tyrosine residues on selected proteins. More specifically, insulin initially binds to the alpha subunit of an insulin receptor; this binding leads to autophosphorylation of specific tyrosine residues on the beta subunit of the insulin receptor and stimulates tyrosine kinase activity of the receptor and of other cytosolic protein substrates (such as insulin receptor substrate 1) involved in signal transduction. For example, the activation of phosphatidylinositol-3-kinase results from the binding of insulin receptor substrates to the enzyme's regulatory subunit; this in turn enables a variety of insulin-dependent cellular activities such as GLUT4 translocation for cellular glucose uptake and protein synthesis, among others.

Chromodulin is thought to bind to the cytosolic beta subunit of the insulin receptor, where it stimulates or amplifies the kinase activity of the beta subunit of the insulin receptor. Chromodulin may also stimulate the tyrosine kinase activity of other enzymes involved in insulin signaling to effect GLUT4 translocation and improved cellular glucose uptake [4,5]. It has been suggested that chromodulin may act as a second messenger, but only in rodents fed pharmacological doses of chromium [6].



- 1 Transferrin delivers Cr^{3+} to transferrin receptors (TfR) on cell membranes.
- 2 Cr^{3+} is released inside the cell.
- 3 Four Cr^{3+} atoms complex with chromodulin to form holo chromodulin or Cr^4 -chromodulin.
- 4 Cr^4 -chromodulin functions to increase the kinase activity of the beta subunit of the insulin receptor and other cytosolic tyrosine kinases.

Figure 13.22 Proposed role of chromium (Cr^{3+}) as part of chromodulin in potentiating insulin's reactions.

Skeletal muscle cells treated with selected forms of chromium have also been shown to exhibit increased insulin receptor gene expression, improved protein anabolism, decreased protein degradation, and improved insulin sensitivity, although the results of studies on the effects of chromium supplementation on insulin sensitivity have been conflicting [4,7,8].

Another possibly biologically active form of chromium in the body is chromate. Chromate or chromic acid (CrO_3), which contains the hexavalent form of chromium, can be produced within the body from oxidation of Cr^{3+} by oxidants such as hydrogen peroxide and free radicals. Chromate, similarly to vanadate, may inhibit the activity of phosphotyrosine phosphatase to prolong or enhance insulin signaling; however, the methodological approaches used in chromate studies as well as in some of the studies examining chromodulin's mechanism(s) of action have been questioned [4].

In addition to its proposed association with insulin, Cr^{3+} may also affect gene expression and/or maintain the structural integrity of nuclear strands [9]. The expression of a handful of genes in adipose tissue of diabetic

mice has shown to be altered in response to niacin-bound Cr^{3+} [4,8].

Chromium and Health

Diabetes A plethora of studies and meta-analyses of studies continue to document mixed findings regarding the effectiveness of chromium supplements on improvements in measures of glucose control (primarily glycosylated hemoglobin and fasting glucose concentrations) in individuals with type 2 diabetes. Similar mixed results have been demonstrated in studies examining the effectiveness of chromium supplementation on blood lipid concentrations in those with hyperlipidemias. Consequently, unless a person is chromium deficient (which is difficult to assess; see the section “Assessment of Nutriture”), supplementation is not currently recommended.

Weight Loss/Body Composition Changes Chromium as a supplement has also been purported to effect changes in body composition, weight, and strength performance. However, most well-controlled studies providing chromium supplementation have shown no significant effects on strength gains, muscle accretion, or fat loss and have shown conflicting results on weight loss; in fact, the Federal Trade Commission ordered the discontinuation of such claims [10].

Excretion

Most chromium (~95%) is excreted from the body in the urine. In absolute terms, urinary chromium excretion ranges from ~0.2 to 0.4 $\mu\text{g}/\text{day}$ [2]. In addition to urinary losses, small amounts of chromium are lost with desquamation of skin cells. Fecal chromium represents mostly unabsorbed dietary chromium, not endogenous chromium excreted via the bile into the feces.

Adequate Intake

The Adequate Intakes (AIs) for chromium for adult men and women through age 50 years are 35 μg and 25 μg , respectively; these values drop to 30 μg and 20 μg for men and women, respectively, over 50 years of age [1]. During pregnancy and lactation, intakes of 30 μg and 45 μg of chromium, respectively, are recommended [1]. AIs for chromium for other age groups are provided on the inside front cover of the book.

Deficiency

Chromium deficiency was originally described in adults receiving total parenteral nutrition (TPN) intravenously. Signs and symptoms of deficiency included weight

loss, peripheral neuropathy, elevated plasma glucose concentrations or impaired glucose use (also called insulin resistance, which may be characterized by hyperinsulinemia), and high plasma free fatty acid concentrations. While the reversal of these symptoms with chromium supplementation was viewed as evidence of chromium's essentiality, it is now apparent that the parenteral nutrition solution given to correct the deficiency provided pharmacological amounts of chromium, 2–6 $\mu\text{g}/\text{day}$, more than would typically be absorbed from the diet [5,11].

Toxicity

Oral supplementation of up to about 1,000 μg of chromium as Cr^{3+} appears to be safe [1]. However, the use of chromium (Cr^{3+}) picolinate has been associated with chromosomal and organ damage. Chromium-induced DNA damage includes adducts, single- and double-strand DNA breaks, and inter- and intrastrand cross-links, among other types of destruction. Organ damage, specifically renal failure and hepatic dysfunction, has been reported in those ingesting chromium picolinate supplements providing 600–2,400 μg of chromium [1]. Renal problems were also documented in some individuals with higher than normal concentrations of chromium in their blood and urine due to long-term receipt of parenteral nutrition contaminated with high amounts of chromium [11]. Infants and children receiving TPN intravenously are susceptible to chromium toxicity. In fact, it is recommended that chromium be eliminated from TPN solutions for infants and children [12]. No Tolerable Upper Intake Level for chromium has been established by the Food and Nutrition Board to date [1].

Assessment of Nutriture

No specific tests are currently available to determine chromium status. Fasting plasma chromium is not in equilibrium with tissue chromium. Responses of plasma chromium to an oral glucose load are inconsistent. Urinary chromium appears to reflect only recent intake, not status.

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13.6 IODINE

Iodine, a nonmetal, is typically found and functions in its ionic form, iodide (I^-). Hence, the term *iodide* is used throughout this section. The human body contains about 15–20 mg of iodide, most (70–80%) of which is found in the thyroid gland. The mineral, however, is also a component of topical agents used as antiseptics. Iodine tincture and iodine compounds, such as povidone iodine, have broad-spectrum anti-infective actions against yeast, fungi, bacteria, viruses, spores, and protozoa.

Sources

The iodide concentration in foods is extremely variable because, as is so often the case, it reflects the regionally variable soil concentrations of the element and the amount and nature of fertilizer used in plant cultivation. Thus, the iodide content of grains, vegetables, and fruits varies with the iodide content of the soil, and the iodide content of meat depends on the iodide of the plants that animals eat.

The amount of iodide in drinking water is an indication of the iodide content of the rocks and soils of a region and closely parallels the incidence of iodine deficiency among the inhabitants of that region. For example, the iodide content of water from goitrous areas in India, Nepal, and Sri Lanka ranged from 0.1 to 1.2 mg/L, compared to 9.0 mg/L found in nongoitrous Delhi [1]. In the United States, before salt was fortified with the mineral beginning in the 1920s, people living in the Great Lakes and Rocky Mountain areas had iodine-poor diets. Iodized salt was introduced in 1924 in the state of Michigan [2]. Yet, in 2007, 29–50% of the world's population was thought to be iodine deficient, especially those living in mountainous regions

(including the Himalayas, European Alps, and the Andes) and lowland noncoastal regions (such as central Africa and Eastern Europe) [3].

Iodide is found in seafoods, although large differences in iodide content exist between marine (ocean) fish and freshwater fish. Edible marine fish contain about 30–300 μg of iodide/100 g, in contrast to only 2–4 μg of iodide/100 g in freshwater fish. In addition to seafoods, dairy products represent a major source of iodide in the American diet. Grains are generally low in iodide but are consumed in large quantities and therefore contribute significant amounts of iodide in the American diet [4]. Some bread products contain added iodates, IO_3^- , to improve gluten cross-linking. Table 13.10 provides the approximate iodide content of selected foods.

Individuals needing to restrict dietary iodide intakes, as may be needed in the treatment of thyroid cancer, are typically instructed to limit dietary intake to less than about 50 μg of iodide/day. Most commonly restricted foods include all seafoods, iodized salt, eggs and dairy products, and soybean/soybean products such as tofu, soy milk, and soy sauce.

Table 13.10 Iodine Content of Select Foods*

Select Foods/Food Group	Iodine (μg)
Seafood	
Shrimp (3 oz)	85
Crab (3 oz)	88–283
Fish, variety (3 oz)	20–358
Seaweed, nori, dried (1 oz)	417
Seaweed, wakame, dried (1 oz)	4,770
Seaweed, kombu, dried (1 oz)	>100,000
Meat and poultry	
Beef (3 oz)	9–14
Pork (3 oz)	3–6
Chicken (3 oz)	5–7
Eggs and dairy products	
Milk, 1% fat (1 cup)	73
Yogurt, Greek (5.3 oz)	59
Cheese, variety (1 oz)	4–20
Egg, poached (1 large)	27
Grains	
Bread, white or whole wheat (1 slice)	1–8
Pasta, variety (1 cup)	5–47
Other	
Legumes, variety ($\frac{1}{2}$ cup)	1–4
Nuts, variety (1 oz)	4–6

*Data represent cooked foods, except for nuts and dairy products.

Source: Public Health England. Composition of foods integrated dataset (CoFID), 2019. <https://www.gov.uk/government/publications/composition-of-foods-integrated-dataset-cofid>

Iodized salt ($\frac{1}{4}$ teaspoon) supplies about 70 μg of iodide. The mineral is usually added to salt as potassium iodate or iodide. About 90% of households in North and South America use iodized salt; in contrast, less than half of households in Europe do. In the United States, processed foods are typically manufactured with noniodized salt. Restricting salt intake, as may be necessary for people being treated for hypertension, can negatively affect iodine status [5].

Iodine in multivitamin/mineral supplements is provided usually as potassium or sodium iodide and in quantities similar to the Daily Value, which for iodine is 150 μg . However, the use of iodine derived from kelp (seaweed), which is present in some supplements, can result in significantly more or less supplemental iodide than the amount stated on the label [6]. The use of the medication amiodarone for some types of arrhythmias also provides pharmacological amounts of iodide. Iodine is further provided in large amounts as a contrast agent for some radiological procedures such as computerized tomography (CT) scans. The use of the iodinated contrast agents during a single X-ray procedure can result in well over 10,000 μg of infused iodine, an amount several thousand times higher than recommended intakes and Tolerable Upper Intake Levels [6].

Digestion, Absorption, Transport, and Storage

Dietary iodine (I) is either bound to amino acids (sometimes termed organified) or found free, primarily as iodate (IO_3^-) or iodide (I^-). During digestion, organic bound iodine is released and converted to iodide. Iodate, for example from breads or iodized salt, is usually reduced to iodide by glutathione within the GI tract. Small quantities of iodinated amino acids and other organic forms of iodide that escape digestion may be absorbed, but not as efficiently as the iodide ion. The thyroid hormones thyroxine (T_4) and triiodothyronine (T_3) are also absorbed unchanged, with a bioavailability of about 70%, which allows T_4 medication to be administered orally.

Iodide is absorbed rapidly, mostly from the stomach and to a lesser extent from the duodenum. Overall, absorption of iodide is greater than 90%. Following absorption, free iodide appears in the blood, from which it is capable of permeating all tissues. The mineral selectively concentrates in the thyroid gland, with lesser amounts found in the ovaries, placenta, skin, and salivary, gastric, and mammary glands. Absorption of iodine applied topically is thought to be negligible, except in cases where the skin is not intact, as may occur in individuals with burns, and in cases of prolonged, repeated exposure [6].

The thyroid gland traps iodide most aggressively, by way of an active transport system against an iodide gradient that

is often 20–50 times the plasma concentration. Specifically, iodide is taken up into the thyroid gland by a Na^+/I^- symporter located in the basolateral membrane of the thyroid gland; the transporter carries two sodiums and one iodide, with the sodium gradient generated by the Na^+/K^+ ATPase serving as the driving force. The thyroid gland contains 70–80% of the total body iodide and takes up about 120 μg of iodide/day. Because the thyroid gland and its synthesis of the thyroid hormones are the focal points of iodide metabolism, information on the transport of iodide into nonthyroidal tissue is sparse. Iodide uptake by other tissues, such as salivary glands, likely occurs by an active transport mechanism.

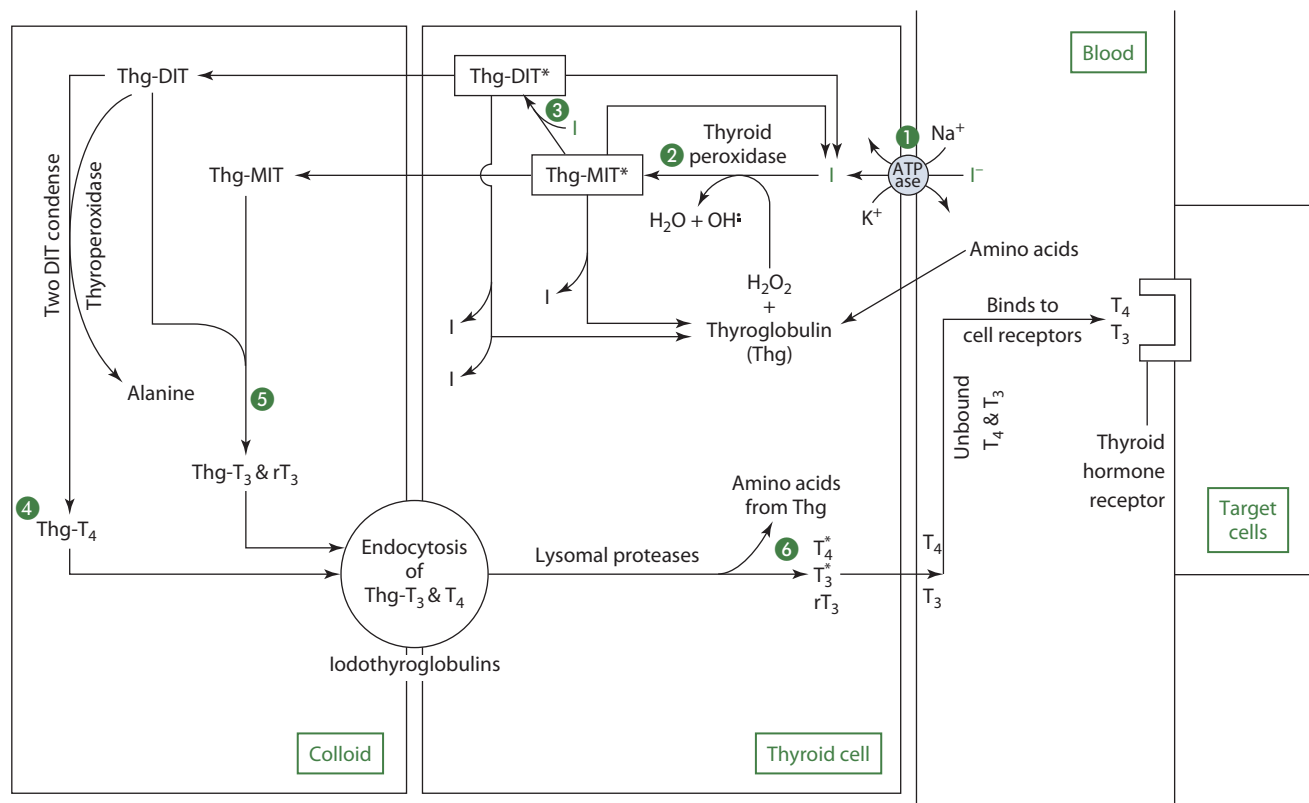
Functions and Mechanisms of Action

The main function of iodide is in the synthesis of the thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3). As long as the daily iodide intake is above about 50 μg , uptake of iodide by the thyroid gland is usually sufficient for hormone production [7]. Thyroid-stimulating hormone (also called thyrotropin), released from the pituitary gland, helps to regulate thyroid hormone production and secretion. Four atoms of iodide are needed for each T_4 , and three atoms of iodide for each T_3 . Amino acids are also needed for the synthesis of the thyroid hormones.

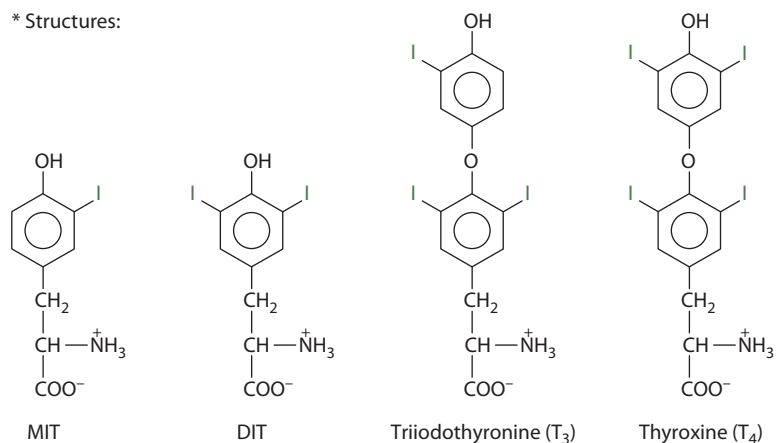
Thyroid Hormone Synthesis

The thyroid gland is made of multiple acini, also called follicles. The follicles are spherical in shape and are surrounded by a single layer of thyroid cells. The follicles are filled with colloid, a proteinaceous material. The events in thyroid hormone synthesis are shown in Figure 13.23 and described here:

- The thyroid cells actively (through an Na^+/K^+ -ATPase pump) take up iodide from the blood.
- Once within the cell, iodide (I^-) is oxidized to iodine (I), which is then bound to the number 3 position of tyrosyl residues of the glycoprotein thyroglobulin (a process called organification of the iodine). This binding of iodine to the tyrosyl residues is catalyzed by the heme iron-dependent enzyme thyroperoxidase and generates thyroglobulin-3-monoiodotyrosine (Thg-MIT). Hydrogen peroxide acts as the electron acceptor.
- Next, MIT is iodinated in the number 5 position by thyroperoxidase to form thyroglobulin-3,5-diiodotyrosine (Thg-DIT).
- In the colloid, two DITs condense or couple to form Thg-3,5,3',5'-tetraiodothyronine (Thg- T_4), with the elimination of an alanine side chain. Thyroperoxidase also catalyzes this coupling reaction. DIT also condenses or couples with MIT to form 3,5,3'-triiodothyronine (T_3) and reverse T_3 (rT_3).



* Structures:



- I^- is actively transported into the thyroid cell.
- I is bound to a tyrosine residue on thyroglobulin to form thyroglobulin-3-monoiodotyrosine (Thg-MIT).
- Thg-MIT is iodinated to form Thg-DIT, thyroglobulin-3, 5-diiodotyrosine, which (4) condenses with another Thg-DIT in the colloid to form Thg-T₄.
- Thg-DIT also can condense with Thg-MIT to form Thg-T₃ and reverse (r)T₃.
- T₄ and T₃, active thyroid hormones, are released into the blood following endocytosis of Thg-T₃ and Thg-T₄ back into the thyroid cell and hydrolysis of the Thg by proteases.

Figure 13.23 Overview of iodine intrathyroidal metabolism and hormonogenesis.

DIT and MIT not used for thyroid hormone synthesis in the thyroid cells are deiodinated, and the iodine is made available for recycling.

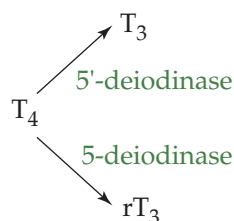
To release the thyroid hormones into the blood, iodothyroglobulin must be resorbed in colloid droplets by endocytosis back into the thyroid cell. Within the thyroid cell, the iodothyroglobulin (Thg-T₄) and (Thg-T₃)

is hydrolyzed by lysosomal proteases, and T₄ and T₃ are released into the blood.

Transport of Thyroid Hormones in the Blood

Three transport proteins bind and transport T₄ and T₃ in the blood. The protein thyroxine-binding globulin has the smallest capacity but the greatest affinity for T₄ and

T_3 . Albumin and transthyretin (also called prealbumin) also transport the thyroid hormones. A very small fraction ($<0.1\%$) of T_4 and T_3 in the blood is not bound to transport proteins; it is this free form that binds to cell receptors and affects physiological processes. The plasma concentration of T_4 is nearly 50 times that of T_3 , but T_3 is many times (~ 20 – 100) more potent on an equal molar basis. Several tissues—the liver, kidneys, brain, pituitary, and brown adipose, to name a few—deiodinate T_4 to generate T_3 , although most T_3 in the blood has been synthesized in the liver from T_4 . The conversion of T_4 to T_3 is catalyzed by the selenium-dependent enzyme 5'-deiodinase; this conversion is impaired with selenium deficiency.



For a more in-depth description of thyroid hormone synthesis, see the reviews by Visser [8] and Vanderpas [9].

Thyroid Hormone Functions

The multiple effects of the thyroid hormones result from the hormones' occupancy of nuclear receptors, with subsequent effects on gene expression. The receptors appear to be the same in all tissues, binding T_3 more avidly than T_4 and requiring fivefold to sevenfold higher concentrations of T_4 to achieve comparable physiological effects. Thyroid hormones bind to DNA as monomers, homodimers, and heterodimers, such as with retinoid X receptors (discussed under "Vitamin A" in Chapter 10). Additionally, the hormone receptor complex may interact with the DNA through zinc fingers. Although the mechanisms of action of the thyroid hormones are unclear, biological effects occur in response to increased mRNA and protein synthesis triggered by the thyroid hormone receptor complex interactions with DNA.

The effects of thyroid hormones on metabolism are many and varied. Thyroid hormones stimulate oxygen consumption, the basal rate of metabolism, and body heat production and are necessary for normal nervous system development and linear growth. Directly or indirectly, most organ systems are under the influence of these hormones (Table 13.11).

Prophylactic Use of Iodide

Iodide supplementation, as potassium iodide, is recommended in large (pharmacologic) amounts by the Centers for Disease Control and Prevention upon exposure or risk of exposure to radioactive iodine, as may occur during nuclear accidents [10]. Ingestion of doses of up to

Table 13.11 Select Physiological Effects of Thyroid Hormones

Adipose tissue: enhances lipolysis
Muscle: enhances contraction
Bone: promotes anabolism (growth and development)
Cardiovascular system: increases heart rate
Gastrointestinal tract: stimulates nutrient digestion and absorption
Metabolism: stimulates metabolic rate and cellular oxygen consumption in metabolically active tissues

130 mg of iodide, depending on age, is suggested as quickly as possible and enables "saturation" of the thyroid gland with mineral. The uptake of this supplemental iodide by the thyroid gland (which occurs to a greater extent in those that are deficient) reduces the likelihood that the gland takes up the released radioactive iodine and reduces the person's subsequent risk of thyroid cancer [10].

Interactions with Other Nutrients

The metabolism of the thyroid hormones is largely dependent upon a group of three selenium-dependent iodothyronine 5'-deiodinases. Impaired selenium status results in altered thyroid hormone metabolism and function. The roles of these deiodinases are described further under the section of this chapter addressing selenium, specifically "Iodothyronine 5'-Deiodinases (IDI or DI)."

Like selenium deficiency, iron and vitamin A deficiencies may magnify the effects of inadequate iodine. Heme iron is a component of the enzyme thyroperoxidase, which attaches iodine to tyrosine residues on thyroglobulin, and which then conjugates the thyroglobulins for the production of the thyroid hormones. Iron may also be involved in the binding of T_3 to nuclear receptors. Thus, iron deficiency impairs thyroid hormone synthesis and functions. Similarly, vitamin A deficiency reduces iodine uptake by the thyroid gland and decreases the synthesis of thyroglobulin and the coupling of iodotyrosine residues to form T_4 [11].

Another well-established interaction is that between iodide and goitrogens. Substances that interfere with iodide metabolism to inhibit thyroid hormoneogenesis are called goitrogens because their effect is to secondarily augment thyroid-stimulating hormone release and consequently thyroid gland enlargement. Goitrogens may affect iodide uptake by the gland, organification of the iodide, or hormone release from the thyroid cells. Some natural foods are goitrogenic, as discovered many years ago when it was observed that rabbits fed a fresh cabbage diet developed goiters that could be reversed by iodine supplementation. It was later shown that cruciferous vegetables, including cabbage, kale, cauliflower, broccoli, rutabaga, turnips, Brussels sprouts, and mustard greens, contain glucosinolates, which compete

with iodide for uptake into the thyroid gland. Perhaps the only food to be identified directly with goiter etiology is cassava, which is consumed in large quantities in many developing countries. Cassava contains a cyanogenic glucoside, linamarin, which later became known as goitrin (Figure 13.24). The linamarin, once hydrolyzed within the GI tract, releases cyanide, which is then metabolized to thiocyanate (SCN^-). Thiocyanate competes with iodide for uptake into the thyroid gland. Cyanogenic glucosides are also found in lima beans, flaxseed, linseed, sorghum, and sweet potatoes.

Other goitrogenic compounds that compete with iodide's active transport into the thyroid cells include halide ions such as bromide (Br^-) and astatide (At^-). Perchlorate (ClO_4^-), along with perrhenate (ReO_4^-) and pertechnetate (TcO_4^-), interferes with organification as well as iodide uptake; perchlorate is a known contaminant in drinking water. Lithium (Li^+), used to treat some psychiatric disorders, inhibits thyroid hormone release from the gland. Some other classes of goitrogens that interfere with iodide metabolism include polycyclic hydrocarbons and phenol compounds derived from coal.

Excretion

The kidneys have no mechanism to conserve iodide, and they therefore provide the major route (~80–90%) for iodide excretion. Usual urinary iodine excretion exceeds 100 $\mu\text{g}/\text{L}$ if intake is adequate [12]. Fecal excretion of iodide is relatively low. Some iodide is also lost in sweat, a loss that can be of consequence in hot, tropical regions where iodide intake may be only marginally adequate.

Recommended Dietary Allowance

Because of its important link to thyroid function, iodide nutriture has been investigated thoroughly for over half a century. Dating as far back as the 1930s, requirements have been published based on results of balance studies and on calculations of average daily urinary losses. Adult daily requirements established by those early studies ranged from 100 to 200 μg . The amount of iodide to prevent goiter is estimated at 50–75 $\mu\text{g}/\text{day}$ or ~1 $\mu\text{g}/\text{kg}$ of body weight, and has not changed significantly over the years. The RDA for iodine is 150 $\mu\text{g}/\text{day}$ for adults [12]. Although the recommendations apply equally to both sexes, iodide needs are higher during pregnancy and lactation: 220 μg and 290 μg per day, respectively [12]. The inside front cover of this book provides additional RDAs for iodine for other age groups.

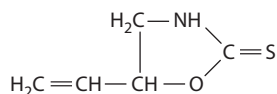


Figure 13.24 Goitrin.

Deficiency

Thyroid Hormone Release as Related to Iodide Deficiency

The release of thyroid hormones by the thyroid gland is highly regulated. Thyrotropin-releasing hormone secreted by the hypothalamus acts on the pituitary gland to stimulate thyroid-stimulating hormone (TSH). TSH, in response to thyrotropin-releasing hormone, is secreted from the anterior pituitary and increases the uptake of iodide from the blood into the thyroid gland to enhance T_4 production. TSH output is regulated by T_4 through negative feedback to the pituitary. A decline in the blood level of T_4 triggers the release of TSH, whereas elevated T_4 inhibits the release of TSH and thyrotropin-releasing hormone.

Iodine Deficiency and Iodine Deficiency Disorders

Iodine deficiency, known as goiter, prevails in many areas of the world and is associated most often with dietary insufficiency of iodine, typically less than about 10–20 μg of iodine/day. High goitrogen intake may also be a contributing factor. About 200–300 million people worldwide are iodine deficient [13]. The condition is characterized by enlargement (hyperplasia) of the thyroid gland (Figure 13.25). This enlargement is caused by overstimulation by TSH. Iodide deficiency depletes the thyroid gland's iodide stores and therefore reduces the output of T_4 and T_3 . The reduced blood T_4 concentrations trigger continuous release of TSH, resulting in hyperplasia of the thyroid gland. The growth of the gland is self-restricting, however, because in its enlarged state it traps and processes available iodide more efficiently. The gland can return to normal size over time (months to years) as dietary iodide is increased to adequate amounts.

Because iodide deficiency also affects growth, development, and other health indices, the term *iodide deficiency disorders (IDDs)* has been coined. Iodine deficiency in a fetus results from iodide deficiency of the mother; two types of cretinism can result. Neurological cretinism in the infant is characterized by mental deficiency, hearing loss or deaf mutism, and motor disorders such as spasticity and muscular rigidity [7]. Hypothyroid cretinism results in thyroid failure. Early treatment of cretinism with iodine can often correct the condition.

Defects in the Na^+/I^- symporter have been characterized and result in the absence of thyroid hormone production. Hypothyroidism, goiter, and intellectual disability, among other signs and symptoms of iodide deficiency, develop if the condition goes untreated.

The addition of iodide to table salt and the administration of iodized oil, potassium iodide or iodine, and iron salts have done much to alleviate the problem of endemic goiter in some goitrous regions of the world. Yet iodide deficiency continues to be a major health problem in many underdeveloped countries and, in many countries, may be coupled with selenium and iron deficiencies. In the United



Dr. Ivan Polunin/Avalon/Alamy Stock Photo

Figure 13.25 Large nodular goiter due to iodine deficiency (North Malaya).

States, the restriction of salt intake, as may be necessary for people being treated for hypertension, or use of salt that is not iodized may increase a person's risk for developing iodine deficiency; this risk is further increased if the diet does not include dairy products and seafood [5]. Additionally, in the United States, pregnant women may not consume sufficient iodide to meet the increased needs of the mineral during pregnancy. See Patrick [14] for a review of the history of iodine deficiency.

Toxicity

A Tolerable Upper Intake Level for iodine has been set at 1,100 μg (1.1 mg)/day [12]. Poor monitoring and oversupplementation of iodine in several countries with supplementation programs have resulted in excessive intakes. In addition, excessive intakes may result from the overconsumption of foods, such as kelp, that are naturally very high in iodine. Some signs of acute iodide toxicity include burning of the mouth, throat, and stomach; nausea and vomiting; diarrhea; and fever. High iodine intake or infusion of large amounts of iodine secondary to radiological

procedures results in excess iodine stores in the body and potentially impaired function of the thyroid gland, including both hyper- and hypothyroidism as well as inflammation of the thyroid (thyroiditis). See reference [6] for a review of iodine toxicity.

Assessment of Nutriture

Assessment of iodide status is generally directed at populations living in areas suspected of being iodide deficient, although individuals may be assessed if thyroid problems are suspected. Several methods are used for iodine assessment. Urinary iodine excretion represents an indicator of recent iodine intake; in fact, daily urinary iodine can be used to calculate iodine intake using the following formula:

$$\text{Daily iodine intake} = \text{urinary iodine} \times 0.0235 \times \text{body weight},$$

with urinary iodine measured in $\mu\text{g/L}$ and weight measured in kg [12]. Usual urinary iodine excretion exceeds 100 $\mu\text{g/L}$ if intake is adequate. Urinary iodide excretion of 100 $\mu\text{g/L}$ corresponds to an iodide intake of about 150 μg [7]. Median urinary iodine concentrations of $<100 \mu\text{g/L}$ suggest inadequate iodine intake and iodine insufficiency (or mild iodine deficiency) in a population. Urinary iodine concentrations less than 50 $\mu\text{g/L}$ are usually associated with insufficient thyroid hormone secretion and are indicative of moderate iodine deficiency, while concentrations less than 20 $\mu\text{g/L}$ are considered severe [7]. As dietary iodine intake increases, urinary iodine concentrations also rise. Urinary iodine concentrations of 300 $\mu\text{g/L}$ or higher have been associated with increased thyroid volume, which in turn indicates thyroid dysfunction [6].

Thyroid size, measured by ultrasonography or by palpation, is also used to assess goiter, and indirectly iodine status. Enlargement of the gland is associated with suboptimal iodide status; however, the size of the gland may take months to years to return to normal in response to treatment with iodine supplementation [7]. Thus, this indicator is typically used along with urinary iodine excretion. In addition to urinary iodide excretion and measurement of the thyroid gland, serum TSH concentrations are an especially sensitive indicator of iodine status in newborn infants from at-risk populations. Serum TSH concentrations greater than about 5 $\mu\text{U/L}$ in a population suggest deficiency. Finally, serum concentrations of thyroglobulin, which rise with iodide deficiency as the size of the thyroid gland enlarges, may be used to assess iodine status. Serum thyroglobulin concentrations greater than 10 $\mu\text{g/L}$ suggest inadequate iodine intake.

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13.7 MANGANESE

Although widely distributed in nature, manganese exists primarily as Mn^{2+} or Mn^{3+} in only trace amounts (about 10–20 mg) in the body.

Sources

The best food sources of manganese are plant foods and shellfish. Whole-grain cereals, nuts, and leafy vegetables are considered manganese-rich foods:

- Wheat bran-based breakfast cereals (1 cup), about 1.7 mg;
- Wheat germ ($\frac{1}{4}$ cup), 2.0 mg;
- Oatmeal (1 cup), 1.3 mg;
- Almonds, pecans, cashews, and hazelnuts (1 oz), about 0.5–1.8 mg;
- Spinach, collard greens, and turnip greens ($\frac{1}{2}$ cup), about 0.8–1.7 mg;
- Pineapple (1 cup), 2.3 mg;
- Pineapple juice ($\frac{1}{2}$ cup), 1.3 mg;
- Blueberries (1 cup), 0.9 mg;
- Tea, brewed (1 cup), 0.4–1.6 mg; and
- Legumes (1 cup, cooked), 0.7–1 mg.

The manganese content of grain products varies widely, due partly to plant species differences and partly to the efficiency with which the milling process separates the manganese-rich and manganese-poor parts of the grain. White flour, for example, has a much lower manganese concentration than the wheat grain from which it was produced. A slice of whole-wheat bread provides 0.30 mg of manganese, whereas a slice of white bread contains only 0.15 mg. The usual intake of manganese among Americans ranges from about 2 to 9 mg/day. The Daily Value for manganese used on food and supplement labels is 2.3 mg. Supplements provide manganese as manganese gluconate, manganese sulfate, manganese ascorbate, and amino acid chelates of manganese. Multivitamin/mineral supplements typically contain about 2–5 mg of manganese.

Digestion, Absorption, Transport, and Storage

Whether manganese is bound to food components that need to undergo digestion to release manganese prior to its absorption is not clear. Manganese absorption, likely as Mn^{2+} , is typically less than about 5%, with absorption percentage decreasing as intake increases. Females may absorb greater amounts than males for unclear reasons. Manganese absorption appears to be quickly saturable and is thought to involve a low-capacity, high-affinity, active carrier protein such as divalent metal transporter 1 (DMT1) and/or Zrt- and Irt-like protein 14 (ZIP14). Diffusion may contribute to absorption at higher manganese intakes. Absorption likely occurs throughout the length of the small intestine. Both regulation of intestinal absorption and control of excretion enable manganese homeostasis in the body.

Relative to many of the other trace minerals, little information is available on factors influencing manganese absorption. Low-molecular-weight ligands, such as histidine and citrate, enhance manganese absorption. In contrast, but as with other divalent cations, fiber, phytic acid, and oxalic acid may precipitate manganese in the GI tract, making it unavailable for absorption. Iron also appears to compete with manganese for absorption, likely using DMT1. Copper also decreases manganese absorption and retention.

Manganese entering into portal circulation from the GI tract may either remain free or become bound as Mn^{2+} to α -2-macroglobulin before traversing the liver, where it is almost totally removed. Upon release from the liver, some manganese in the blood may (1) remain free as Mn^{2+} ; (2) be bound as Mn^{2+} to albumin, α -2-macroglobulin, β -globulin, or γ -globulin; or (3) be oxidized by ceruloplasmin to Mn^{3+} and complexed with transferrin. Whole blood manganese concentrations normally range from about 4 to 15 ng/mL [1]. The mineral has a half-life of about 10–42 days.

Manganese is efficiently cleared from the blood. Manganese transport into cells is facilitated by DMT1 (ubiquitously expressed), ZIP14 (expressed primarily in the intestine and liver), and ZIP8 (expressed in the liver, kidneys, lungs, and testes). In addition, uptake of manganese bound to transferrin occurs through transferrin receptors (see the “Cellular Iron Uptake” section).

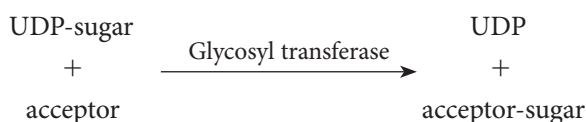
Within cells, manganese is found primarily as Mn^{2+} in the mitochondria; unlike iron and copper, manganese is not readily oxidized within tissues. Manganese is found in most organs and tissues (including hair) and does not tend to concentrate significantly in any particular one, although its concentration is highest in the bones, liver, pancreas, and kidneys. In bones, which may contain 25–40% of total body stores, manganese is found as part of the apatite compounds.

Functions and Mechanisms of Action

Manganese, like some other trace elements, functions both as an enzyme activator and as a constituent of metalloenzymes. In the activation of enzyme-catalyzed reactions, manganese may bind to the substrate, to ATP, or to the enzyme directly, inducing conformational changes. Enzymes from nearly every class—including transferases, kinases, hydrolases, oxido-reductases, ligases, and lyases—can be activated by manganese in this manner and are numerous and diverse in function. However, largely because the activation of many enzymes is not manganese specific, a manganese deficiency does not impair the activity of most of these enzymes. The metal is typically replaced by other divalent cations like magnesium. An exception to this apparent lack of specificity is the manganese-specific activation of the glycosyl transferases. Examples of roles that manganese-dependent enzymes perform in the body are described in the next section.

Bone, Cartilage, and Connective Tissue Synthesis

Two manganese-dependent transferases important for connective tissue synthesis are xylosyl transferase and glycosyl (also called galactosyl or galacto) transferase. Glycosyl transferase is especially important for the synthesis of glycosaminoglycans, such as chondroitin sulfate, which attach to proteins to form proteoglycans. Remember that proteoglycans are important structural components of connective tissues such as cartilage and bone. Specifically, glycosyl transferase catalyzes the transfer of a sugar moiety (galactose) from uridine diphosphate (UDP) to an acceptor molecule, as shown by the general reaction:



Manganese deficiency is associated with impaired glycosyl transferase activity.

Manganese also activates the hydrolase prolidase, a dipeptidase with specificity for dipeptides. Prolidase is found in dermal fibroblasts and is important for collagen formation.

Urea Synthesis

Arginase, a hydrolase that requires four manganese atoms per molecule, is a cytosolic enzyme responsible for urea formation. The enzyme is found in high concentrations in the liver, the site of the urea cycle. The enzyme cleaves arginine to generate urea and ornithine. Low manganese diets in animals have been shown to decrease arginase activity.

Carbohydrate/Nutrient Metabolism

Pyruvate carboxylase, a ligase/synthetase that contains four manganese atoms, converts pyruvate to the TCA cycle intermediate oxaloacetate. Because magnesium can replace manganese in pyruvate carboxylase, minimal changes in pyruvate carboxylase activity occur with manganese deficiency.

Phosphoenolpyruvate carboxykinase (PEPCK), a lyase activated by manganese, converts oxaloacetate to phosphoenolpyruvate and carbon dioxide. This reaction is important in gluconeogenesis. The activity of PEPCK decreases in animals with manganese deficiency.

The enzyme isocitrate dehydrogenase requires either manganese or magnesium. This enzyme catalyzes the conversion of isocitrate to alpha-ketoglutarate in the TCA cycle; $NADP^+$ is needed for the reaction.

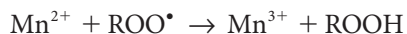
Amino Acid Metabolism

Glutamine synthetase, activated by manganese or magnesium, is important in the synthesis of glutamine from glutamate.

Antioxidant Roles

Superoxide dismutase, a manganese-dependent (Mn^{3+} -SOD) oxido-reductase metalloenzyme (not manganese activated), functions in a manner similar to copper- and zinc-dependent superoxide dismutase to prevent lipid peroxidation by superoxide radicals. The Mn-SOD is found in the mitochondria, whereas copper-zinc-SOD is found extracellularly and in the cell cytosol. Thus, Mn-SOD likely eliminates superoxides before they damage mitochondrial function. The activity of the electron transport/respiratory chain generates large amounts of superoxide radicals, necessitating substantial Mn-SOD activity. Cellular ultrastructural abnormalities associated with manganese deficiency are likely caused by uncontrolled lipid peroxidation in the membranes because of reduced Mn-SOD activity or simply by reduced availability of manganese to directly

scavenge free radicals. Manganese, one of a few minerals able to scavenge free radicals, quenches peroxyl radicals, as shown in this equation:



Low-manganese diets in animals have been shown to decrease Mn-SOD activity. Moreover, Mn-SOD knockout mice die shortly after birth, an indication of Mn-SOD's essential protective role in the body.

Other Roles

Manganese may also act as a modulator of second-messenger pathways in tissues. For example, manganese increases cAMP accumulation through binding to ATP and ADP. Manganese can activate guanylate cyclase, and manganese may affect cytosolic calcium levels and thus regulate calcium-dependent processes.

Interactions with Other Nutrients

Only a few interactions between manganese and other trace elements are thought to be of significance nutritionally. One such relationship—that between manganese and iron—is detailed in the section on absorption. However, the interaction is reciprocal; that is, iron in excess inhibits manganese absorption, and manganese, when ingested in amounts about four to eight times the recommended intake, decreases iron absorption up to about 40%. Interactions may also occur between manganese and calcium and between manganese and zinc; however, because of the paucity of information and the divergent results from various studies, the nature of such interactions remains undefined.

Excretion

Manganese is excreted primarily (~90%) via the bile in the feces. Excess absorbed manganese from the diet is quickly released by the liver into the bile to maintain homeostasis. Very little manganese is excreted in the urine, less than 1 µg/L. Small losses also occur through sweat and skin desquamation [2].

Adequate Intake

The recommendation for manganese intake is based on median intake because data are insufficient to calculate requirements for the mineral. Recommended Adequate Intakes of manganese are 2.3 mg for adult men and 1.8 mg for adult women daily [3]. With pregnancy and lactation, recommendations increase to 2.0 mg and 2.6 mg per day, respectively [3]. The inside front cover of the book gives additional recommendations for manganese for other age groups.

Deficiency

Manganese deficiency is associated with diverse physiological malfunctions. In humans, manganese deficiency generally does not develop unless the mineral is deliberately eliminated from the diet. Studies in which men received either 0.11 mg or 0.35 mg manganese/day for 39 days resulted in negative manganese balance. While both levels of dietary manganese are deficient, the diet was also devoid of vitamin K, making it difficult to separate the effects of the manganese and vitamin K deficiencies [2].

Symptoms and signs of deficiency include nausea and vomiting; dermatitis; decreased serum manganese; decreased fecal manganese excretion; increased serum calcium, phosphorus, and alkaline phosphatase (thought to be associated with skeletal bone changes); decreased growth of hair and nails; changes in hair and beard color; poor bone formation and skeletal defects; decreased clotting proteins; and altered carbohydrate and lipid metabolism [2,3]. Other problems associated with deficiency include ataxia, loss of equilibrium, cell ultrastructure abnormalities, compromised reproductive function, abnormal glucose tolerance, and impaired lipid metabolism [2,3].

Toxicity

The Tolerable Upper Intake Level for manganese is 11 mg/day [3]. Exposure to high levels of oral, parenteral, and air manganese may result in toxicity. Additionally, individuals with liver failure are at greater risk for toxicity because manganese homeostasis is maintained largely by the liver with excretion of excess manganese occurring via the bile. Manganese toxicity secondary to liver failure is characterized by manganese accumulation within the liver and other organs such as the brain; accumulation in the brain results in neurologic abnormalities [4]. Motor and cognitive dysfunction also occurs with manganese toxicity. Neonates receiving parenteral nutrition are thought to be at higher risk for manganese toxicity because of diminished biliary manganese excretion [5]. Symptoms of manganese toxicity with chronic exposure to airborne manganese include cough, bronchitis, pneumonitis, and reduced lung function, as well as symptoms resembling Parkinson's disease including tremors, diminished memory capacity, and loss of coordination/difficulty walking [6]. Other signs and symptoms of toxicity include insomnia, headache, increased forgetfulness, anxiety, mood changes, compulsive behaviors, reduced response speed/reaction time, rapid hand movements, and gait disturbance [4,6].

Assessment of Nutriture

Assessment of manganese status is typically based on concentrations of manganese in the plasma/serum and whole blood. Serum concentrations have been found to

be somewhat sensitive to large variations in intake but do not necessarily correlate with intake or status [7]. Enzyme activity, primarily Mn-SOD and arginase, has also been used but has not been shown to be a good indicator of manganese status [7].

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13.8 MOLYBDENUM

Molybdenum, a metal, is found in the body primarily in two valence states, Mo^{4+} and Mo^{6+} . The need for molybdenum was established in humans through the observation that a genetic defect in enzymes that required molybdenum as a cofactor resulted in severe pathology.

Sources

Molybdenum is widespread among foods but, as with many other minerals, the molybdenum content of a plant food varies depending on the concentration of molybdenum in the soil. Better dietary sources of molybdenum are legumes, which can provide up to 184 $\mu\text{g}/100\text{ g}$; meat, fish, and poultry, which contain up to $\sim 129\text{ }\mu\text{g}/100\text{ g}$; and grains and grain products, which provide up to $\sim 117\text{ }\mu\text{g}/100\text{ g}$ [1,2]. Nuts and vegetables usually contain less than 50 $\mu\text{g}/100\text{ g}$, but fruits and dairy products are especially low in molybdenum, providing less than 12 $\mu\text{g}/100\text{ g}$ [1,2].

The molybdenum content of multivitamin/mineral supplements ranges from about 25 to 45 μg and is provided as ammonium or sodium molybdate. Individual-nutrient supplements provide varying amounts, up to about 1,000 μg (1 mg) of molybdenum. The form of molybdenum in single-nutrient supplements is usually as sodium or ammonium molybdate or as an amino acid chelate. The Daily Value for molybdenum is 45 μg .

Digestion, Absorption, Transport, and Storage

Molybdenum—found in foods primarily as molybdate (MoO_4^{2-})—does not appear to require digestion prior to its absorption into the small intestine, which occurs mostly in the proximal region. The mechanism by which molybdenum is absorbed is thought to be passive, although carrier-mediated uptake may also occur. Molybdenum absorption increases with increasing dietary intake over a range of 22–1,490 $\mu\text{g}/\text{day}$ [3]. Molybdenum absorption from foods ranges from ~ 50 to 85% and, from the supplement ammonium molybdate, absorption is over 90% [3]. Sulfates (SO_4^{2-}), if present in large quantities, may compete with molybdate for absorption. Transport of molybdenum in the blood is thought to occur as molybdate, which binds to albumin and α -2-macroglobulin.

The molybdenum content of human tissues is quite low, averaging 0.1–1.0 $\mu\text{g}/\text{g}$ of wet weight; the human body contains about 2 mg of the mineral. Molybdate uptake by tissues is thought to occur by one or more carriers, including one that perhaps also transports sulfate. The liver, kidneys, and bones contain the most molybdenum in terms of both absolute amount and tissue concentration. Other tissues, such as the small intestine, lungs, spleen, brain, thyroid and adrenal glands, and muscle, also contain the element. In tissues, molybdenum is found as molybdate, free molybdopterin, and molybdopterin that is bound to enzymes.

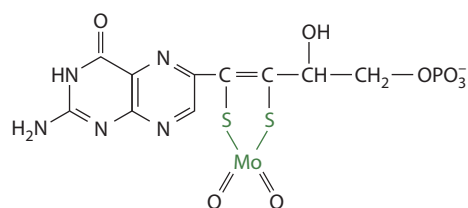
Functions and Mechanisms of Action

The biochemical role of molybdenum centers around the redox function of the element and its necessity as a cofactor in the form of molybdopterin for four metalloenzymes (sulfite oxidase, aldehyde oxidase, xanthine oxidoreductase, and amidoxime reductase), all of which catalyze oxidation-reduction reactions. Each of the reactions involves a transfer of two electrons, which results in a change in the molybdenum oxidation state (from 4+ to 6+ and vice versa).

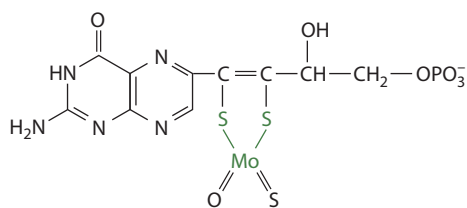
Molybdenum is attached to enzymes as a part of molybdopterin, an alkylphosphate-substituted pterin to which molybdenum is coordinated through two sulfur atoms. Molybdopterin anchors the molybdenum to the apoenzyme at its catalytic site. The molybdenum is further bonded either to two oxygen molecules (called dioxomolybdopterin) or to one oxygen and one sulfur (called oxosulfidomolybdopterin), as shown in Figure 13.26. The inability to synthesize molybdopterin because of a genetic defect is usually lethal, as discussed in the section “Deficiency.”

Sulfite Oxidase: Sulfur Metabolism

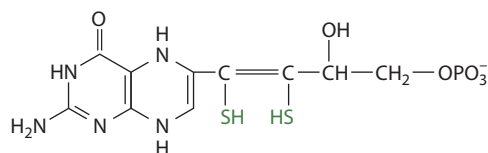
Sulfite oxidase, a mitochondrial intermembrane enzyme found in many body tissues—especially the liver, heart, and kidneys—has iron-sulfur clusters, two molybdopterin



Molybdopterin—the dioxo form



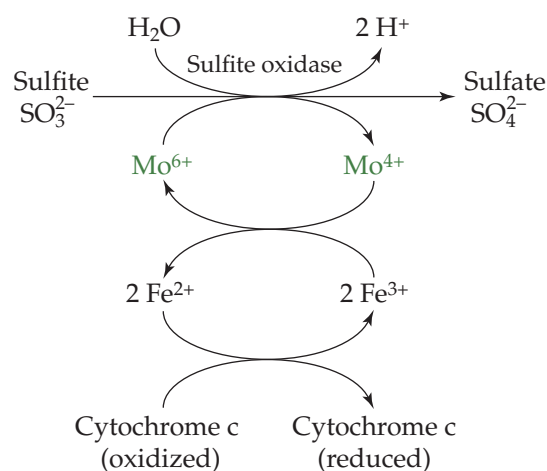
Molybdopterin—the oxosulfido form



Pterin—without the molybdenum attached

Figure 13.26 Molybdopterin structures.

(dioxo cofactor form), and two cytochrome residues. The enzyme catalyzes the terminal step in the metabolism of the sulfur-containing amino acids, methionine and cysteine, in which sulfite (SO_3^{2-}) is converted to sulfate (SO_4^{2-}), as shown here:



Cytochrome *c* is the physiological electron acceptor for the reaction.

In addition to originating from the catabolism of methionine and cysteine, sulfites are found in the diet, where they are added to some foods as an antimicrobial agent. Sulfate generated from this reaction in the body is typically excreted in the urine or used for the synthesis of sulfoproteins, sulfolipids, and mucopolysaccharides (a component of mucus).

Aldehyde Oxidase: Various Roles

Aldehyde oxidase is a molybdoenzyme (oxosulfido form) that is similar to xanthine oxidoreductase (see the next section) in size, cofactor composition, and substrate specificity. It presumably functions in the cytosol of liver cells as a true oxidase, using exclusively molecular oxygen as its physiological electron acceptor. The enzyme's primary substrates are thought to include a variety of aldehydes, including, for example, the retinal form of vitamin A and the pyridoxal form of vitamin B₆, as well as acetaldehyde and other drugs. Other enzymes, such as an NADH-dependent aldehyde dehydrogenase also found in the liver, are thought to catalyze reactions similar to those catalyzed by this aldehyde oxidase.

Xanthine Oxidoreductase: Hydroxylation of Purines, Pteridines, and Pyrimidines, among Other Compounds

Xanthine oxidoreductase is an iron-dependent (containing two iron–two sulfur centers) enzyme that exists as a homodimer of two identical subunits and that also requires FAD and molybdopterin in the oxosulfido cofactor form. The molybdenum is necessary for the oxidative capacity of the enzyme; inactivation of the enzyme results if the mineral is not present. The enzyme catalyzes the conversion of hypoxanthine to xanthine and the conversion of xanthine to uric acid, as part of purine degradation as shown in Figure 13.27. Both reactions are oxidative hydroxylations with two electrons passed from the substrate to the molybdenum in the enzyme. Electrons are then transferred to FAD via the iron–sulfur centers, and then are typically passed onto NAD^+ , forming NADH. However, when conformational changes occur in the enzyme, the enzyme becomes an oxidase (xanthine oxidase) and molecular oxygen becomes the electron acceptor instead of NAD^+ and hydrogen peroxide (H_2O_2), or a superoxide radical ($\text{O}_2^{\cdot-}$) is formed.

Xanthine oxidoreductase is found in a variety of tissues, including the liver, lungs, kidneys, and intestine, and is also capable of hydroxylating other purines as well as pteridines, pyrimidines, and other heterocyclic nitrogen-containing compounds. Conversion to xanthine oxidase occurs following the oxidation of sulfhydryl groups or by proteolysis. Healthy tissues may contain about 10% of their total xanthine enzymes in the oxidase form [4].

Reductions in xanthine oxidoreductase activity cause no apparent clinical effects. The genetic disorder xanthinuria, in which large amounts of xanthine are excreted in the urine, provides evidence of the body's ability to tolerate low xanthine oxidoreductase activity. The condition is essentially free of clinical manifestations, except for the possible development of kidney calculi (stones) caused by the high urinary xanthine concentration.

The effects of xanthine oxidase activity, however, can be damaging with administration of oxygen for ischemia (a

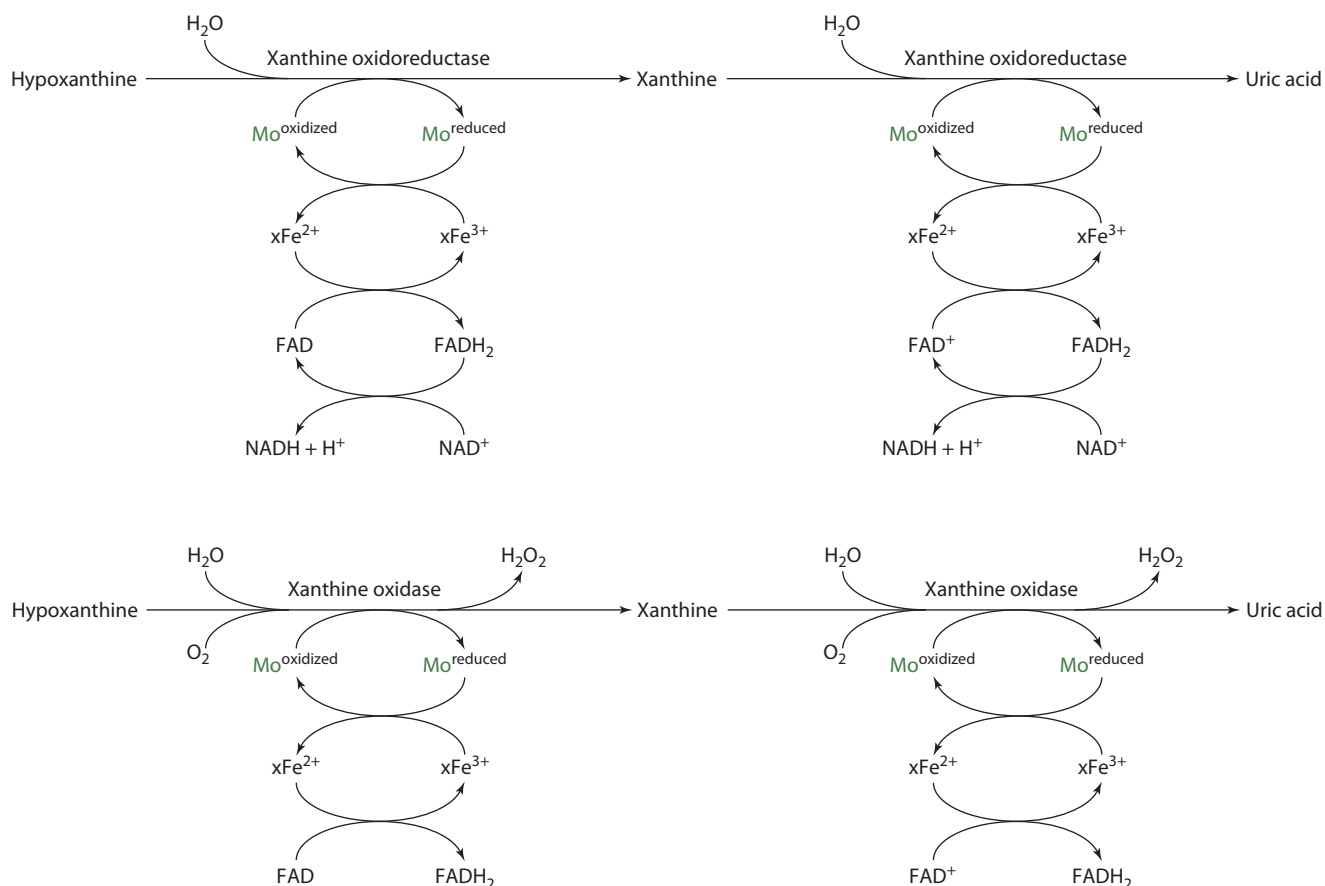


Figure 13.27 Molybdenum's role in hypoxanthine and xanthine degradation.

local or temporary deficient blood supply and thus relative oxygen deprivation). Xanthine oxidase activity generates large amounts of hydrogen peroxide, which further induces damage (called reperfusion injury) in the ischemic tissues.

Amidoxime Reductase: Drug Metabolism

Amidoxime reductase is found attached to the outer mitochondrial membrane, where it complexes with cytochrome *b5* and molybdopterin [5]. The enzyme complex reduces N-hydroxylated amidines and hydroxyamines. N-hydroxylated drugs, for example, are synthesized by drug companies to improve the solubility and absorption of drugs into intestinal cells. Within the body, however, the drug must be reduced to exert its desired effects. Amidoxime reductase enables such a reaction (Figure 13.28).

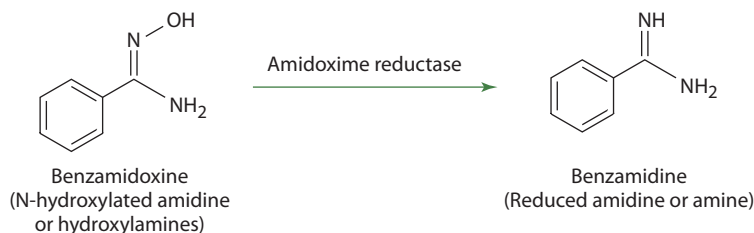


Figure 13.28 The reduction of N-hydroxylated compounds such as benzamidoxime by the molybdenum-dependent enzyme amidoxime reductase.

Interactions with Other Nutrients

The most notable interaction in humans involving molybdenum is with copper. Ingestion of molybdenum as tetrathiomolybdate inhibits copper utilization, and use of the mineral has been shown to be of benefit in the treatment of Wilson's disease [6,7]. Molybdenum's ability to induce copper deficiency also inhibits angiogenesis through down-regulating the expression of vascular growth factors involved in angiogenesis signal pathways [6-10]. Because angiogenesis provides blood to tumors and enables their growth, substances like molybdenum that can prevent copper-induced angiogenesis have exhibited promising results such as inhibition of tumor growth in some animal studies [9,10].

Excretion

Most molybdenum is excreted as molybdate in the urine. Urinary excretion of molybdenum increases as dietary molybdenum intake increases. In other words, little molybdenum is retained in the body when dietary intake is high, and the kidneys are responsible for molybdenum homeostasis. Small amounts of molybdenum are excreted from the body in the feces by way of the bile. Small amounts of the mineral can also be lost in sweat (20 $\mu\text{g}/\text{day}$) and in hair (0.01 $\mu\text{g}/\text{g}$ of hair).

Recommended Dietary Allowance

Based on balance studies as well as depletion and repletion studies providing varying amounts of dietary molybdenum, an Estimated Average Requirement for molybdenum for adults was set at 34 $\mu\text{g}/\text{day}$ [11]. The RDA for molybdenum for adults (men and women) is 45 $\mu\text{g}/\text{day}$ (130% of the requirement), with 50 $\mu\text{g}/\text{day}$ suggested during pregnancy and lactation [11]. The inside front cover of the book provides additional RDAs for molybdenum for other age groups.

Deficiency

Molybdenum deficiency is rarely encountered, although the condition has been documented in a patient maintained for 18 months on total parenteral (intravenous) nutrition. Molybdenum deficiency was associated with high blood concentrations of methionine, hypoxanthine, and xanthine, as well as low blood concentrations of uric acid. Additionally, urinary concentrations of sulfate were low, and those of sulfite were high. Treatment with 300 μg of ammonium molybdate (providing 163 μg of molybdenum) resulted in clinical improvement and normalized sulfur amino acid metabolism and uric acid production.

Genetic defects in enzymes involved in molybdopterin synthesis disrupt both cofactor production and molybdenum use in the molybdenum-dependent enzymes. Defects in a few of the enzymes involved in the four-step set of reactions required for molybdopterin synthesis have been characterized along with a review of cases [12]. The condition, which is evident at birth, results in severe and progressive neurological deterioration. It is further characterized by microcephaly, progressive encephalopathy, intractable seizures, facial dysmorphic features, and poor feeding [12]. Isolated sulfite oxidase deficiency presents similarly to the cofactor deficiency. The genetic disorder is characterized by poor feeding, hypoactivity, dyspnea, dislocation of the ocular lenses, attenuated brain growth, seizures, severe neurological damage, and death in childhood. Elevated levels of urinary sulfite and thiosulfate, along with biochemical manifestations reflecting aberrant sulfur amino acid metabolism and sulfite oxidation, are also present.

Toxicity

Molybdenum appears to be relatively nontoxic with intakes up to about 1,500 $\mu\text{g}/\text{day}$ [11,13]. However, symptoms such as gout (inflammation of the joints caused by the accumulation of uric acid) have appeared in some people living in regions that contain high soil molybdenum levels and in those with occupational exposure to molybdenum [14]. A Tolerable Upper Intake Level for molybdenum has been set at 2 mg/day [11].

Assessment of Nutriture

Molybdenum appears to distribute itself fairly equally between the plasma and red blood cells; however, the use of these as indicators of molybdenum status has not been validated. Similarly, while urinary molybdenum concentrations increase with increased molybdenum intake, urinary molybdenum is not necessarily reflective of molybdenum status.

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Perspective

NUTRIENT–DRUG INTERACTIONS

Nutrient–drug interactions represent nutrient-induced changes in the kinetics of a drug or drug-induced changes in nutrient metabolism or nutritional status. Such interactions can be extremely detrimental. Some nutrient interactions can lead to failure of the drug to perform its desired actions or to drug toxicity. Alternately, some drug interactions can promote nutrient deficiencies or toxicities. In addition to these direct interactions, drugs may also influence nutrition status indirectly by, for instance, diminishing appetite, altering taste, or promoting nausea, vomiting, or diarrhea. This Perspective reviews some examples of foods and nutrients that affect the absorption, distribution, metabolism, actions (functions), or excretion of drugs, as well as some examples of drugs that affect the absorption, metabolism, or excretion of nutrients. Table 1 provides an overview of some of the interactions presented in this Perspective.

EFFECTS OF FOODS AND NUTRIENTS ON DRUG ABSORPTION

Foods or nutrients in foods can alter drug absorption by serving as a physical

barrier or through effects on transit time (i.e., motility of the GI tract), secretions, drug dissolution, chelation, or carrier uptake, among other effects. Many drugs should be taken without food or beverages to prevent interference with drug absorption. For example, the absorption of Fosamax (alendronate), used to treat osteoporosis, is greatly diminished with concurrent ingestion of food or beverages (other than water). Similarly, the antibiotics erythromycin and penicillin (ampicillin), along with the antihypertensive drugs Capoten (captopril) and Univasc or Uniretic (moexipril), should be ingested only with water; food should not be consumed for at least 1 hour. On the other hand, the absorption of many other drugs is enhanced with coingestion of food or even specific dietary nutrients such as those provided by a high-fat meal.

Foods or antacids that contain relatively large amounts of magnesium, calcium, zinc, iron, and aluminum need to be avoided or should be ingested separately (by several hours) from antibiotics such as Achromycin and Sumycin (tetracycline antibiotics), Cipro

(ciprofloxacin), Maxaquin (lomefloxacin), and Levaquin (levofloxacin), and from other groups of antibiotics and antifungals such as Nizoral (ketoconazole). The divalent and trivalent minerals in the antacids or from the foods chelate (bind to) and decrease the absorption of the drugs. An appropriate GI tract pH is important for dissolving or absorbing some drugs. Thus, ingesting foods or antacids that can promote GI secretions or alter pH may be detrimental.

EFFECTS OF FOODS ON DRUG METABOLISM

Many drugs that undergo substantial first-pass metabolism in the GI tract are affected by coingestion of grapefruit juice. Some of these medications include the immunosuppressants Neoral and Sandimmune (cyclosporine); some HMG-CoA reductase inhibitors (used to treat high blood cholesterol) such as Zocor (simvastatin), Mevacor (lovastatin), and Lipitor (atorvastatin); Pletal (cilostazol), which is used to treat intermittent claudication and peripheral vascular disease; VePesid (etoposide), which is used to treat some cancers; and

Table 1 An Overview of Some Select Drug–Nutrient/Food Interactions

Drug(s)	Nutrient(s)/Food(s)
Antibiotics: tetracycline, Achromycin, Sumycin, Cipro, Maxaquin, and Levaquin; Antifungal: Nizoral	Calcium, magnesium, zinc, iron, and aluminum
Immunosuppressant: Neoral; some HMG-CoA reductase inhibitors: Zocor, Mevacor, and Lipitor; Anti-intermittent claudication: Pletal; Antimigraine: Relpax	Grapefruit juice
Anti-Parkinson's: Dopar, Larodopa, Sinemet, and Parcopa	Protein and vitamin B₆
Monoamine oxidase inhibitors: Parnate and Nardil; Antituberculosis: Isoniazid	Amine-containing foods: aged cheeses; smoked, salted, and pickled fish; sausage, salami, pepperoni, corned beef, and bologna; meat extracts; wines; and chocolate, among others
Anticoagulants: Coumadin	Vitamin K
Bronchodilators: Theo-24, Theolair, Uniphyll, and Elixophyllin	Caffeine and vitamin B₆
Antimanic: Eskalith, Lithobid, and Lithotabs	Sodium
Bile-acid sequestrants: Questran	Fat, fat-soluble vitamins A, D, E, and K; folate; iron; magnesium; calcium; and zinc
Antituberculosis: Isoniazid	Vitamin B₆
Anticonvulsants: Phenobarbital, Dilantin, and Phenytek	Vitamin D and folate
H₂ receptors blockers: Tagament and Pepcid; Proton pump inhibitors: Prilosec and Prevacid	Iron, zinc, calcium, magnesium, and vitamin B₁₂
Loop diuretics: Lasix and Bumex	Potassium, chloride, magnesium, thiamin, and sodium

Relpax (eletriptan), which is used to treat migraines. The exact compound or compounds in grapefruit juice that cause the interaction are not clear. Grapefruit juice is rich in many phytochemicals, especially flavonoids such as the flavanone naringenin and its glycoside naringin and the flavanol kaempferol. Ingesting grapefruit juice is thought to decrease (down-regulate) the isozyme of cytochrome P450 known as CYP 3A4, which is found in the intestine. This enzyme normally begins intestinal cell metabolism of many drugs. Consequently, the down-regulation of this enzyme by ingestion of grapefruit juice causes the drugs to be absorbed without any metabolism and causes blood concentrations of the drugs to be much higher than desired. The high blood concentrations of the drug, in turn, can result in undesirable side effects, including toxicity. Similarly, inhibition of intestinal non-CYP3A enzymes as well as enterocyte transport proteins has been demonstrated *in vitro* and may alter drug metabolism.

In addition to alterations in drug distribution caused by grapefruit juice, a high protein intake (two to three times recommendations) alters the distribution of the anti-Parkinson drugs Dopar and Larodopa (levodopa) and Sinemet and Parcopa (levodopa and carbidopa). The effects are thought to result from competition for carriers at the blood–brain barrier between the drug and large neutral amino acids (such as phenylalanine, tyrosine, and tryptophan). These large neutral amino acids appear in the blood following consumption of large amounts of protein. Vitamin B₆ can also increase the metabolism of levodopa by enhancing its conversion to dopamine before the drug crosses the blood–brain barrier. Vitamin B₆ is found in liver and other protein-rich foods, such as meats and legumes, as well as seeds and whole grains. Thus, ingesting levodopa with large amounts of protein or vitamin B₆, or regularly consuming a diet high in protein or vitamin B₆ while taking levodopa, is contraindicated.

EFFECTS OF FOODS AND NUTRIENTS ON THE ACTIONS OF DRUGS

Some foods or nutrients can enhance or oppose the actions of drugs. Foods that contain amines, especially tyramine, dopamine, or histamine, are known to interact

with a group of drugs known as monoamine oxidase inhibitors (MAOIs), which are used mostly to treat some forms of depression. MAOIs such as Parnate (tranylcypromine sulfate) and Nardil (phenelzine sulfate) prevent the enzyme monoamine oxidase from catabolizing amines in the diet as well as amines made endogenously. Amines consist of vasoactive or pressor amines (e.g., tyramine, serotonin, and histamine) and neurotransmitters or psychoactive amines (e.g., dopamine and norepinephrine). The antituberculosis drug INH (isoniazid) exhibits MAOI-like activity. The problem arises when people on MAOIs or INH eat foods high in amines, especially tyramine or histamine, which may be found in fairly large quantities in some foods. Consuming these foods ordinarily presents no problem because the amines can quickly be inactivated by monoamine oxidase. However, in people taking MAOIs or isoniazid, these reactions do not occur. Consequently, high dietary amine intake coupled with high endogenous norepinephrine may result in excessive vasoconstriction, manifested as severe headache, acute hypertension or a hypertensive crisis, and cardiac dysrhythmia. People taking MAOIs are counseled against ingesting foods high in amines, such as aged cheeses (cheddar, Camembert, Stilton, and Boursault), yeast extracts (e.g., Marmite), and brewer's yeast. Smoked, salted, or pickled fish such as herring or cod, as well as sausage, salami, pepperoni, corned beef, and bologna, are also high in tyramine. Foods moderately high to high in tyramine include meat extracts; tenderizers; red wines, including Chianti, vermouth, sherry, and burgundy; and cheeses such as blue, natural brick, Brie, Gruyère, mozzarella, Parmesan, Romano, and Roquefort. Broad beans (fava and Chinese pea pods), chocolate, large amounts of caffeine, liver (chicken or beef), and selected fruits may also contain large amounts of tyramine. Histamine is not typically found in large quantities in foods, with the exception of improperly stored or spoiled fish. Dopamine is found in fava and broad beans and snow peas.

A nutrient known to antagonize the action of the anticoagulant Coumadin (warfarin) is vitamin K. Coumadin works by inhibiting reactions in the vitamin K cycle that generate the active form of vitamin K needed for blood clotting. By inhibiting

production of active vitamin K, the drug prolongs the clotting time of blood. Large amounts of vitamin K oppose the actions of the drug, promoting blood clotting and leading to drug resistance. Ingesting very large quantities of foods rich in vitamin K, including green vegetables, some legumes (soybeans, garbanzo beans), and liver, should be avoided.

Caffeine—a component of coffee, tea, many soft drinks, and chocolate—counters the actions of tranquilizers and may exacerbate some adverse effects of the bronchodilators Theo-24, Theolair, Uniphyll, and Elixophyllin (theophylline). Specifically, large amounts of caffeine coupled with use of theophylline promote increased nervousness, insomnia, and tremors. (These drugs also interfere with vitamin B₆ metabolism.)

EFFECTS OF FOODS AND NUTRIENTS ON DRUG EXCRETION

Sodium and the mineral lithium in the antimanic drugs Eskalith, Lithobid, and Lithotabs (lithium carbonate) are known to interact in the kidneys. Specifically, the sodium and lithium compete with each other for reabsorption into the tubules of the kidneys. Thus, a high intake of sodium promotes lithium excretion and thereby diminishes the effects of the drug, whereas a low intake of sodium promotes reabsorption of lithium and thereby enhances the likelihood of drug toxicity.

EFFECTS OF DRUGS ON NUTRIENT ABSORPTION

Drugs may alter the absorption of nutrients through several mechanisms. For example, drugs may alter the transit time of nutrients through the GI tract, speeding up or slowing down the passage of its contents. Typically, when contents move quickly through the GI tract, fewer nutrients are absorbed; when contents move slowly, more nutrients are absorbed. Changes in the pH of the GI tract may also alter nutrient absorption. For example, H₂ receptor blockers such as Tagamet (cimetidine) and Axid (nizatidine) and proton pump inhibitors such as Prilosec (omeprazole) and Prevacid (lansoprazole), which decrease hydrochloric acid secretion into the stomach and thus increase gastric pH, diminish the absorption of several nutrients, especially iron. H₂ receptor blockers and proton pump inhibitors are used to treat ulcers and GERD.

The ability of some drugs to chelate or adsorb nutrients or GI secretions such as bile also diminishes nutrient absorption. For example, bile acid sequestrants such as Questran (cholestyramine) adsorb bile and thus decrease the absorption of fat and the fat-soluble vitamins A, D, E, and K and the carotenoids. In addition, the drug chelates other nutrients (including folate) and some divalent minerals (including iron, magnesium, calcium, and zinc). Bile acid sequestrant drugs are used to treat high blood cholesterol concentrations, a risk factor for heart disease.

EFFECTS OF DRUGS ON NUTRIENT METABOLISM

In addition to altering nutrient absorption, drugs may alter the metabolism of nutrients in body tissues. Isoniazid, used in the treatment of tuberculosis, for example, diminishes the conversion of pyridoxine (vitamin B₆) to its functional coenzyme form in the liver and thus can cause a vitamin B₆ deficiency. Another group of drugs known to alter vitamin metabolism includes

the anticonvulsants phenobarbital and phenytoin (Dilantin and Phenytek). These drugs alter the metabolism of vitamin D, leading to (in severe cases) the deficiency conditions rickets and osteomalacia if vitamin supplements (as 25-OH cholecalciferol) are not taken. Specifically, the anticonvulsants are thought to diminish the hepatic conversion of vitamin D as cholecalciferol to 25-OH cholecalciferol. Interestingly, these anticonvulsants also affect the vitamin folate by diminishing its absorption in the intestine.

EFFECTS OF DRUGS ON NUTRIENT EXCRETION

Drugs may increase or decrease the excretion of nutrients from the body. Loop diuretics used to treat high blood pressure such as Lasix (furosemide) and Bumex (bumetanide) promote the urinary excretion of sodium and water (important in lowering blood pressure); however, the drugs may also increase the urinary losses of potassium, chloride, thiamin, zinc, and magnesium. Dietary replacement of the minerals, especially potassium, is

important to prevent low blood potassium concentrations.

SUMMARY

Nutrient–drug interactions can severely affect both nutritional status and the effectiveness of pharmacological treatment. Although accredited health care facilities are mandated to educate patients about interactions between foods and drugs, many individuals remain unaware of such interactions and their consequences.

Suggested Readings

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NONESSENTIAL TRACE AND ULTRATRACE MINERALS

LEARNING OBJECTIVES

- 14.1** Identify particularly good food and beverage sources of fluoride, boron, silicon, and vanadium.
- 14.2** Describe known roles of fluoride, boron, silicon, and vanadium in humans.
- 14.3** Describe toxicities associated with fluoride, boron, silicon, and vanadium.

AS CHAPTER 13 EXPLAINED, TRACE ELEMENTS are those minerals that are needed by the body in amounts less than 100 mg/day, and ultratrace elements are those elements with estimated, established, or suspected requirements of less than 1 mg/day. The identification of ultratrace elements that are essential for humans represents ongoing areas of investigation. Several ultratrace elements, copper, chromium, iodine, molybdenum, and selenium, which have established essentiality and thus an Adequate Intake or Recommended Dietary Allowance set by the Food and Nutrition Board, were discussed in the preceding chapter. The elements that are addressed in this chapter—fluoride, boron, silicon, and vanadium—are not, at present, considered essential, although for each, some evidence suggests a possible need for humans. Figure 14.1 shows the location of these elements in the periodic table. Table 14.1 provides an overview of selected functions, food sources, and deficiency symptoms for these nutrients. A brief discussion is also provided for the element cobalt, which is needed but only as part of vitamin B₁₂ (cobalamin).

14.1 FLUORIDE

Whereas fluorine (F⁻) is a gaseous chemical element, fluoride (F⁻), as a negative charged anion, is typically found bound to a metal, nonmetal, or organic compound in soil and rocks, from which it leaches into ground water. The term *fluoride* is used throughout this section (analogous to the use of the terms *iodide* and *chloride* previously). Fluoride, although present in the body in tiny amounts, is not considered an essential nutrient since the element has not been shown to be essential for life and no biochemical role has been identified for the element. Fluoride is included in this chapter because it has been shown to exert a beneficial effect, as discussed in the “Functions and Deficiency” section.

Sources

The fluoride content of most foods is low, usually less than about 0.05 mg/100 g. Tea and seafoods tend to contain the most fluoride. Tea is rich in fluoride because tea leaves accumulate the element in fairly high amounts, ranging widely from

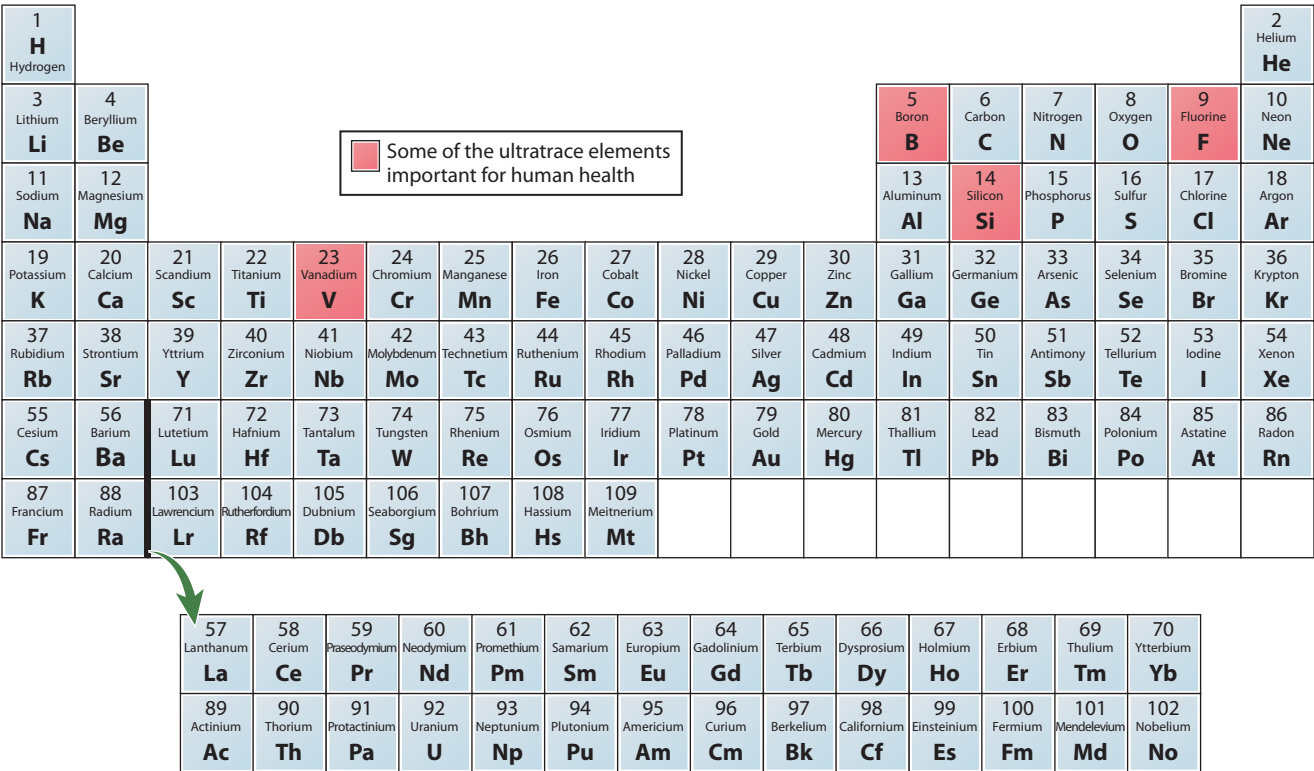


Figure 14.1 The periodic table highlighting important nonessential trace and ultratrace elements.

3.2 to 400 mg/kg [1]. Brewed tea typically contains from 1 to 6 mg of fluoride/L, with decaffeinated forms higher in fluoride than caffeinated. Relatively high amounts of fluoride are also found in some fish ranging from about 0.01 to 0.17 mg/3.5 oz, including some canned fish (such as sardines with bones) providing from about 0.2–0.4 mg/3.5 oz. Other seafoods like clams, lobster, crab, and shrimp also represent sources of fluoride. Crab and shrimp (canned), for example, both provide about 0.2 mg fluoride/3.5 oz portion [2]. See Table 14.2 for the fluoride content of some other food groups.

Community drinking water, the primary dietary source of the element, has been fluoridated since about 1945 in the United States due to the discovery of the inverse relationship between fluoride intake and the incidence of dental caries. Current recommendations for fluoridation are 0.7 parts fluoride per million parts water (ppm), or mg/L.

Because of the use of fluoridated water in some food processing, several beverages, including some ready-to-use infant formulas, bottled water, and juices (to name a few), contain fluoride; however, overall, the fluoride content of beverages varies greatly.

Fluoride added to drinking water is typically in the form of sodium fluorosilicate. Sodium fluoride, sodium monofluorophosphate, and stannous fluoride are used in toothpaste. The fluoride content of toothpaste generally ranges from 1,000 to 1,500 ppm, although prescription-strength versions providing 2,000 to 5,000 ppm of fluoride (as sodium fluoride) are also available [1]. Some mouth-rinses also provide fluoride, usually as sodium fluoride, in amounts ranging from 100 to 500 mg/L [1].

Supplemental fluoride, usually as sodium fluoride, in the form of tablets, liquid drops and lozenges are available by prescription, and targeted for individuals (mostly

Table 14.1 Nonessential Trace and Ultratrace Elements: Select Functions, Deficiency Symptoms, and Food Sources

Mineral	Possible Physiological Roles	Select Deficiency Symptoms in Animals	Food Sources
Fluoride	Reductions in dental caries	Growth and infertility	Tea, fish/seafood, and drinking water (variable)
Boron	Bone development, immune system function, and brain function	Impaired bone health, cognitive/brain function, and immune response	Fruits, vegetables, legumes, and nuts
Silicon	Connective tissue and bone formation	Decreased collagen; long bone and skull abnormalities	Beer, whole grains, selected fruits, lentils, and root vegetables
Vanadium	Mimics insulin action (pharmacologic effect)	Impaired fertility; reduced survival, growth, and development	Fish, shellfish, grains, black pepper, parsley, mushrooms, and dill seed

Table 14.2 Fluoride Content of Select Food Groups

Food Group	Fluoride Content Range (mg/100 g)
Dairy products	0.002–0.082
Meat and poultry	0.004–0.092
Grain products	0.008–0.201
Potatoes	0.008–0.084
Green leafy vegetables	0.008–0.070
Legumes	0.015–0.057
Root vegetables	0.009–0.048
Other vegetables	0.006–0.017
Fruits	0.002–0.013
Fats and oils	0.002–0.044

Source: Taves, DR. Dietary intake of fluoride ashed (total fluoride) v. unashed (inorganic fluoride) analysis of individual foods. *Brit J Nutr.* 1983; 49:295–301.

young children and adolescents) who live in areas with drinking water that has a low fluoride content and who are at high risk for the development of dental caries. Dosages provided by the supplements range from about 0.25 to 1 mg fluoride [1].

Adults living in areas of the United States where water is fluoridated obtain the majority (almost 75%) of dietary fluoride from drinking water [3]. A cup (8 oz) of fluoridated water provides about 0.24 mg of fluoride. Usual fluoride intake by adults in America ingesting fluoridated water ranges from about 1.4 to 3.4 mg fluoride/day [4].

Absorption, Transport, Tissue Uptake, Storage, and Excretion

Fluoride in some foods may be bound to proteins and must be hydrolyzed by pepsin or other proteases before absorption can occur. Additionally, calcium and magnesium when present in high amounts may form insoluble complexes with fluoride (when provided as sodium fluoride) and decrease its absorption. Fluoride absorption occurs in both the stomach and small intestine by passive diffusion. Over 90% of the element is absorbed, typically quite rapidly (within 90 minutes of ingestion) when it is consumed as fluoridated water or toothpaste. When consumed with food, fluoride absorption diminishes to about 50–80%.

Fluoride is transported in the blood free (unbound) as ionic fluoride and distributed rapidly to body tissues. Most fluoride is found in bones and teeth. The quantity of fluoride taken up by tissues increases as the amount of absorbed fluoride increases; however, the percentage retained is typically low due to accelerated urinary excretion. Skeletal growth rate influences fluoride balance, exemplified by the fact that young, growing children incorporate more fluoride into the skeleton and excrete less in the urine than adults.

Ionic fluoride is rapidly excreted in the urine, with only minor losses occurring in sweat. Urinary fluoride excretion normally ranges from about 0.2 to 1.1 mg of fluoride/L urine.

Functions and Deficiency

Fluoride, both topically and systemically delivered, exerts several functions that help to reduce the development of dental caries (cavities). Fluoride that is ingested as fluoridated water and from foods (and swallowed toothpaste and/or oral supplements, if used) is provided to tissues systemically (i.e., from the blood) but is also provided to teeth topically. The topical provision occurs because systemically delivered fluoride enters saliva, where it is present in concentrations of about 0.01–0.05 ppm and “bathes or washes” the teeth throughout the day. Fluoride is also “provided to” teeth (and the oral cavity) topically from the use of toothpaste, mouthrinses, gels, or dental applications.

Dental caries result from the actions of cariogenic bacteria, which are present in the oral cavity, including in plaque on the tooth surface. These bacteria ferment sugars present on teeth and in the mouth and, in the process, generate acids. These acids are destructive to the tooth’s enamel surface and inner layers (including dentine and soft pulp) and lead to the development of dental caries. The provision of fluoride can reduce the incidence of dental caries in both children and adults.

Systemically delivered fluoride is incorporated into the structure of developing teeth in infants and children, making the teeth stronger and more resistant to the actions of cariogenic bacteria. Fluoride replaces some of the hydroxide ions in hydroxyapatite $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ in the enamel of teeth and in bone to form fluoroapatite. Ions in hydroxyapatite can be replaced during initial crystal formation or by displacement from previously deposited mineral according to the following equation: $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + x\text{F}^- \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 - x\text{F}_x^-$. In bone and dental enamel, the ratio of substitution of F^- for OH^- is from about 1:20 to 1:40. Fluoride can also be incorporated into spaces around the hydroxyapatite to increase mineral density and thus strength and to increase resistance of teeth to the actions of bacteria. In general, fluoride promotes the following actions.

- The presence of fluoroapatite (vs. hydroxyapatite) within the crystalline structure of teeth makes it less acid soluble and increases the tooth’s resistance to acid demineralization and the development of dental caries.
- The presence of topical fluoride accelerates the growth/rem mineralization of a new surface on partially demineralized subsurface crystals in enamel; these actions make the teeth more resistant to acid demineralization and the formation of dental caries.

- The presence of fluoride interferes with enzymatic activity required for sugar fermentation by cariogenic bacteria in the oral cavity, actions that reduce acid production and the formation of dental caries.

Manifestations of fluoride deficiency in humans include only the findings from decades of studies reporting a higher prevalence of dental caries in individuals living in areas without water fluoridation and substantial reductions in the prevalence of dental caries (up to 60% depending on the group studied, age, and level of fluoridation) in individuals living in areas with adequate water fluoridation. The U.S. Public Health Service recommends fluoridation of water in the range of 0.7 to 1.2 mg fluoride/L (ppm) to achieve reductions in dental caries.

Recommended Intake, Toxicity, and Assessment of Nutriture

With the goal of minimizing risk for dental caries in the population without causing side effects, Adequate Intakes (AIs) of 4 and 3 mg of fluoride/day for adult males and females, respectively, were established [4]. The inside front cover of the book provides additional AIs for fluoride for other age groups.

The Tolerable Upper Intake Levels for fluoride range from 1.3 mg/day for children age 1–3 years to 10 mg/day for children age 8 years and older and adults [4]. Fluoride-containing toothpaste, if swallowed, can provide substantial amounts of the element and is often the cause of toxicity. Even the ingestion of small amounts of fluoride-containing toothpaste can result in the ingestion of fluoride in amounts exceeding recommended intakes, especially in young children. Consequently, many manufacturers recommend that toothpaste be kept out of reach of children, that only a “pea-sized” amount be used for toothbrushing, and that toothbrushing is supervised.

Acute toxicity (from accidental ingestion of fluoride supplements or excessive amounts of toothpaste) manifests as nausea, vomiting, and diarrhea, with higher intakes associated with acidosis and cardiac arrhythmias. A fluoride intake of as little as 5 mg/kg body weight has been associated with adverse effects, and doses of 15 mg/kg body weight can be fatal [5].

Fluoride toxicity associated with chronic ingestion of excessive fluoride is referred to as fluorosis. Fluorosis affecting the enamel of teeth (termed dental fluorosis) occurs when fluoride ingestion is excessive during tooth development. While the mechanism(s) are not clear, dental fluorosis results in mottling of the tooth surface, appearing usually as white spots or striations. In more severe cases, subsurface porosity, pitting, weakened teeth, and permanent brown spots can occur.

Excessive fluoride ingestion for prolonged time periods also damages skeleton tissues, causing bone deformities,

restricted joint movement, abnormal/excessive bone formation and mineralization, and increased bone fracture risk. Some of these effects result from fluoride accumulation in joints. The functions of several nonskeletal tissues, including skeletal muscle, brain, nerve, and kidneys, are also damaged with excessive fluoride [5].

Plasma and urinary fluoride concentrations can be monitored to determine toxicity and fluoride exposure, respectively, but not the body’s fluoride status. Normal plasma ionic fluoride concentrations range from about 0.01 to 0.2 $\mu\text{g/mL}$. Serum fluoride concentrations of 190 ng/mL have been associated with toxic effects on bone (abnormal bone formation and mineralization) [6]. Urinary fluoride excretion of 15 mg/L is associated with a fluoride exposure of about 20–30 mg ; however, this level of exposure is likely to occur over one or more decades [6].

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14.2 BORON

Boron is found in nature not in elemental form, but as a compound with other elements, most often oxygen. It is present in water and soil, including the earth’s crust. Boron, as boric acid and sodium borate ($\text{Na}_2\text{B}_4\text{O}_7 \cdot \text{H}_2\text{O}$; called borax), was used to preserve foods such as fish, meat, cream, butter, and margarine for over 50 years—that is, until the 1920s, when it was decided that its use was dangerous. Today, boron is commonly found in products used as an antiseptic, bactericide, and cleaning agent, among others. Boron is considered a beneficial bioactive element for humans. It is deemed “probably essential” by the World Health Organization. Essentiality requires the identification of the element’s biochemical function.

Sources

Foods of plant origin including fruits, vegetables, nuts, and legumes represent good sources of boron (Table 14.3). Coffee (providing nearly 0.1 mg/cup), wine, cider, and

Table 14.3 Boron Content of Select Food Groups¹

Food Group (included foods)	Boron Content Range (mg/100 g)
Nuts (almonds, cashews, hazelnuts, walnuts)	1.1–2.8
Fruits (apples, apricots, bananas, grapes, tomatoes)	2.1–2.7
Other fruits (peaches, pears, oranges)	0.2–0.5
Dried fruit (raisins)	4.5
Vegetables (broccoli, carrots, celery)	1.4–2.5
Other vegetables (onions and potatoes)	0.2
Legumes	1.6
Lentils	0.7
Dairy (cheese and milk)	0.2
Eggs	0.4

¹Source: Khaliq H, Juming Z, Ke-Mei P. The physiological role of boron on health. *Biol Trace Elem Res*. 2018; 186:31–51.

beer also contribute to dietary intake. Animal foods such as meat, fish, and dairy products are relatively poor sources of the element, usually providing less than about 0.6 mg of boron/100 g [1].

Boron appears in foods as sodium borate or as organic borate esters. Dietary boron intake ranges from about 0.8–1.5 mg/day, although intakes may be as high as about 5 mg/day [2,3]. Boron is also found as a contaminant/ingredient in some antibiotics, gastric antacids, lipsticks, lotions, creams, and soaps; it is not, however, typically absorbed through the skin.

Some multivitamin/mineral supplements as well as individual single-ingredient supplements provide elemental boron in amounts ranging from about 0.15 to 6 mg. The amount of elemental boron is shown on the supplement facts label; however, no %Daily Value is listed because of the absence of an AI or Recommended Dietary Allowance. The form of the boron within supplements varies. Some examples include sodium borate, sodium tetraborate, calcium fructoborate (a sugar-borate-ester), boron citrate, boron amino acid complexes (with aspartate and glycine), and boron ascorbate [4]. Information on bioavailability of these various forms is not available.

Absorption, Transport, Tissue Uptake, Storage, and Excretion

Boron, as boric acid (also called orthoboric acid $[B(OH)_3]$), is rapidly and almost completely (greater than 85%) absorbed from the small intestine by passive diffusion. Boron appears in the blood primarily as boric acid with levels rising within about 6 hours after ingestion (sodium tetraborate). Plasma boron concentrations usually range from about 20–95 ng/mL.

Tissues taking up higher amounts of boric acid [and, depending on the pH, possibly the borate monovalent anion, $B(OH)_4^-$] include bones, nails, and hair; however,

the element does not tend to accumulate in tissues [5]. Tissue uptake of boric acid occurs by diffusion. The body is thought to contain about 3–20 mg of boron. Tissue concentrations of boron are under homeostatic control, with the kidneys serving to eliminate excess boron and maintain plasma concentrations. The element is excreted primarily (greater than 80%) in the urine as boric acid, with lesser amounts lost in the feces and only tiny amounts lost in sweat and in the breath. The half-life of boron elimination is about 21 hours [1].

Functions and Deficiency

Boron is thought to exert its influence on select body functions through the formation of diester complexes, with several biomolecules containing cis-hydroxyl groups including S-adenosylmethionine (SAM), adenosine diphosphate (ADP), nicotinamide adenine dinucleotide (NAD^+), and NAD-generated cyclic ADP-ribose. Through its interactions with these biomolecules, boron exerts multiple effects. Some examples include:

- Maintaining the strength and integrity of cell membranes
- Maintaining bone composition and structure
- Modulating the effects of oxidative stress in response to injury and infection
- Maintaining selective cognitive and immune system functions
- Assisting in steroid hormone metabolism.

Boron forms complexes with cell membrane components such as glycoproteins, glycolipids, and phosphoinositides (a subfraction of phospholipids). Such interactions are thought to strengthen the integrity of cell membranes and may influence cell signaling, among other roles. The binding of boron to NAD^+ and cyclic ADP ribose affects calcium release and intracellular signaling pathways. In bone, boron has been shown to enhance the activity of osteoblasts by increasing calcium flux as well as enhancing the formation of bone's extracellular matrix. (Note: Boron's role in promoting collagen and proteoglycan synthesis extends its actions into helping with wound healing.) Bone composition, structure (especially trabecular bone), and strength (especially cortical bone) are positively influenced by boron. Defects in bone growth and development occur with boron deficiency in animals.

Beneficial effects of boron on immune and brain functions have been observed. Boron promotes anti-inflammatory actions in response to injury or infection by reducing production of reactive oxygen species and enhancing immune cell production and activity. Boron deprivation in animals is associated with diminished production of lymphocytes and several cytokines. Brain (central nervous

system) function is also influenced by boron. Boron deprivation results in changes in electroencephalograms and selected cognitive processes affecting attention, mental alertness, and short-term memory, among other skills in adults. The element also appears to assist in steroid hormone (especially estrogen and testosterone) metabolism, increasing hormone half-life and availability.

Recommended Intake, Toxicity, and Assessment of Nutriture

Recommendations for boron intake have not been established. An Acceptable Daily Intake of 0.16 mg/kg body weight (about 11.2 mg/day for a person weighing 70 kg) was established by the European Food Safety Authority [4,6]. Daily intakes up to 0.4 mg/kg body weight (about 28 mg for a person weighing 70 kg) are thought by the World Health Organization to be safe [6]. Intakes of at least 0.4 mg/kg body weight are suggested based on the findings of improved bone and brain function and response to oxidative stress following consumption of a diet providing 0.2–0.4 mg boron/kg body weight [6].

Acute boron toxicity (such as from accidental consumption of boron-containing cleaning products) results in nausea, vomiting, diarrhea, dermatitis, and lethargy and may also cause (with intakes around 20 g or higher) organ damage and death [2,4,5]. Chronic boron toxicity (about 25–76 mg/kg body weight) is associated with vomiting, diarrhea, and abdominal pain as well as headache, restlessness, dermatitis, and poor appetite, and in infants is associated with anemia, dermatitis, alopecia, and seizures [2,4,5]. A Tolerable Upper Intake Level of 20 mg of boron/day has been established for adults [2].

Urinary boron excretion is thought to be a good indicator of recent boron intake within an intake range of 0.35–10 mg of boron/day [2]. Because plasma concentrations rise in response to increased dietary boron, they may be representative of status during periods of low intake.

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14.3 SILICON

Silicon, second only to oxygen in earthwide abundance, is found in nature usually as silica, also known as silicon dioxide (SiO_2) because of its attraction for oxygen. Sand, granite, and quartz are among the most well known of the silicates found in nature. Whereas early investigations concentrated on silicon's toxic effects, such as silicon-related urolithiasis (stones in the urinary tract) and particularly silicosis (a respiratory condition caused by the inhalation of dust), since about the mid-1970s research has focused on the possible functional roles of silicon. Although the element has not yet been found to be essential (as it is not required to sustain life and no specific biochemical function for silicon has been identified), silicon does appear to be needed/beneficial for bone formation (as discussed under “Functions and Deficiency”).

Sources

Plant foods are typically richer in silicon than those of animal origin. Whole cereal grains (especially oat bran) contain higher amounts of silicon (about 2–10 mg/100 g serving) than most other food groups. Selected fruits (such as bananas, pineapple, mango, and dried fruits) also contribute higher amounts of the element to the diet along with some vegetables (such as green beans, spinach, and root vegetables). Lentils, soybeans, and nuts, as well as sugar cane, provide dietary silicon. The element is also found in seafood (especially mussels), water (hard), and a variety of beverages (tea, coffee, juices, wine, beer, etc.) [1]. Beer (8.25 mg silicon/12 oz) is relatively high in silicon because of its presence in the hops and barley and its solubilization in the beer-making process. Dietary silicon intake by adults in the United States is thought to range from about 24 to 33 mg/day [2,3].

Silicon is present in some medications (including antacids, antidiarrheal agents, and analgesics where it may be added as an inert ingredient), usually as magnesium silicate or magnesium trisilicate. Silicon absorption from magnesium trisilicate, however, is poor. Silicon, as silicate, is also used as an additive in various foods, for example, to prevent caking or as a thickening, stabilizing, and/or clarifying agent. As an additive, it may be present as, for example, calcium silicate, sodium aluminosilicate, or magnesium trisilicate. Implants, cosmetics (creams, lipsticks, etc.), powder (talcum), and toothpaste also may contain silicon as silica, silicates, silicone, and/or magnesium hydrogen silicate. These forms of silicon are not thought to be absorbable via the skin, with the exception of silicones (found in some creams and in implants).

Absorption, Transport, Storage, and Excretion

Silicon in water and most beverages is found as orthosilicic acid, also called monosilicic acid, $\text{Si}(\text{OH})_4$. This form of silicon is readily absorbed (about 50%) from the proximal small intestine either paracellularly or via transcellular pathways [2]. Silicon in whole grains and grain products is also well absorbed, while that found in some fruits is not. The bioavailability of the element for intestinal absorption varies depending on the form of silicon and its solubility as well as factors such as pH and the cation/mineral content of the meal. Polymerized forms of orthosilicic acid are not well absorbed, usually less than 1% [4]. Phytolith silica, found in some plant foods, requires digestion, and its absorption ranges from less than 2% up to about 41% [4]. The extent of silicon absorption from foods with silicate-containing additives is not clear; however, studies suggest at least some of the silicon may be digested to form the absorbable orthosilicic acid.

Once absorbed, orthosilicic acid is found mostly free (i.e., not bound to proteins) in the blood. The element is taken up by the red blood cells, liver, lungs, skin, and bones, with slower entry occurring into the heart, muscles, spleen, and testes. Generally, silicon concentrates in connective tissues, including bone, skin, blood vessels, cartilage, and tendons (with some in hair and nails). Total body silicon is estimated at about 1–2 g [3]. Most silicon is excreted fairly rapidly from the body via the urine as orthosilicic acid and as magnesium orthosilicate. Urinary silicon excretion is significantly correlated with dietary silicon intake [4].

Functions and Deficiency

Silicon is involved in bone and connective tissue formation. Within the extracellular matrix of bone and connective tissues, the element interacts with various glycoaminoglycans (that make up proteoglycans) and facilitates their cross-linking with collagen. These actions are thought to contribute to bone formation and to enhance calcium deposition into bone (and thus improve mineralization). While further studies are needed to identify its mechanism of action, mostly animal studies suggest silicon improves bone strength (including bone mineral density) and reduces bone fragility. Silicon may also play roles in collagen synthesis. Silicon intake in human studies has been inversely associated with osteoporosis [5,6]. Potential benefits in terms of the element's ability to reduce intestinal absorption and enhance excretion of aluminum have linked silicon indirectly with neurological conditions [7].

Silicon deficiency in animals results in smaller, less flexible long bones and in skull deformation characterized by reductions in collagen in the connective tissue matrix.

Silicon deficiency also weakens blood vessels and negatively impacts skin integrity and wound healing.

Recommended Intake, Toxicity, and Assessment of Nutriture

The requirement for silicon is largely unknown, although estimates range from about 10 to 25 mg/day [8,9]. No Tolerable Upper Intake Level has been established for silicon, although a safe upper level of 700 mg/day has been suggested [3].

Toxicity from ingested silicon has been associated with the formation of silicon-containing kidney stones; however, it is chronic (years) use of large amounts of silicon-containing antacids (e.g., magnesium trisilicate, which provides up to about 6.5 mg of elemental silicon per tablet) that appears to contribute to the rare development of kidney stones [2,3].

Silicosis occurs from the inhalation of particulate crystalline silica and silicates, including quartz, and man-made silicates, such as asbestos. Silicosis is characterized by a progressive fibrosis or scarring of the lungs leading to severe respiratory problems and increased risk of lung cancer. Based on inhalation as the means of exposure (not dietary intake), silica is classified as a “known human carcinogen.” The effects of exposure to silica nanoparticles (in humans) are an active area of investigation as the use of nanoparticles grows. *In vitro* studies have linked silica nanoparticles with cellular apoptosis through multiple pathways [10].

As in the case of most of the ultratrace elements, levels of silicon in biological fluids of healthy adults have been reported but may not accurately represent nutriture. Serum silicon concentrations usually range from about 11 to 31 $\mu\text{g}/\text{dL}$ [11].

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14.4 VANADIUM

Vanadium was discovered in the early 1800s, named for a Swedish goddess of beauty and fertility, Vanadis. The element occurs in several oxidation states from V^{2+} to V^{5+} . In solution, vanadium produces a range of colors from yellowish orange in its pentavalent state to blue in its divalent state. In the human body, vanadium is found primarily in its pentavalent V^{5+} state as vanadate (HVO_4^{2-}), also called monovanadate (VO_3^- , VO_4^{3-} , HVO_4^{2-} , or $H_2VO_4^-$) or in its less toxic, more stable tetravalent V^{4+} state as vanadyl (VO^{2+}). While the element has not been found to be essential in humans, research continues to unravel vanadium's biological actions.

Sources

The vanadium content of foods, which contain the element primarily as tetravalent vanadyl and pentavalent vanadate, is very low and consequently so is the average dietary intake. Vanadium intake in the U.S. diet is thought to range from about 10 to 60 $\mu\text{g}/\text{day}$. The richest sources of the element are fish and shellfish, such as oysters, which contain up to about 12 $\mu\text{g}/100\text{ g}$ and cereals and grain products with up to 15 $\mu\text{g}/100\text{ g}$ [1,2]. Sweeteners provide up to 4.7 $\mu\text{g}/100\text{ g}$ [1,2]. Other vanadium-rich foods include mushrooms, black pepper, parsley, dill seed, and canned apple juice. Most fats and oils contain particularly low levels of vanadium, less than 0.3 $\mu\text{g}/100\text{ g}$ [1]. Beer and wine also provide some vanadium. Supplements are available providing vanadium as, for example, vanadyl sulfate, sodium metavanadate, ammonium metavanadate, and sodium orthovanadate.

Absorption, Transport, Storage, and Excretion

Absorption of vanadium varies with its oxidation states. Vanadate may be reduced to the tetravalent vanadyl in the acidic environment of the stomach before being absorbed by diffusion in the proximal small intestine. Alternately, vanadate may be absorbed directly via the same anion transport carrier system as used by phosphate and then

reduced intracellularly by glutathione. Compared to vanadyl, vanadate is three to five times more efficiently absorbed. Overall, vanadium absorption is generally less than 5%, and, when ingested in pharmacological doses, less than 1% is absorbed.

In blood cells, the plasma, and other body fluids, vanadium may be present as pentavalent vanadate but is usually converted to vanadyl using glutathione, NADH, and ascorbic acid as reducing agents. In the plasma, vanadyl and vanadate bind preferentially to transferrin and albumin. Lesser amounts may be bound to lower-molecular-weight compounds such as citrate, lactate, phosphate, and oxalate, as well as to glycine, histidine, and immunoglobulin G.

Vanadium enters cells by multiple mechanisms. Vanadium may be taken up by diffusion via anion transport systems/channels used by phosphate and sulfate. Uptake also occurs via the monocarboxylate transporter (MCT)1 and organic anion transporter (OCT). Cellular uptake of vanadium bound to transferrin occurs via receptor-mediated endocytosis, with intracellular release of vanadium requiring transport by divalent metal transporter (DMT)1. Similar to its metabolism elsewhere, intracellular vanadate is reduced primarily by glutathione to tetravalent vanadyl, which is then almost exclusively bound to intracellular ligands, such as phosphates and iron-containing proteins.

The total body pool of vanadium is about 100–200 μg , with most tissues containing less than 10 ng of vanadium/g wet weight. The element is found mainly in the liver, spleen, and kidneys, with lesser amounts in the lungs, heart, and brain. Longer-term storage occurs in muscles, adipose tissue, and bone. In bone, which contains about 50% of the body's vanadium, the element can replace phosphate in hydroxyapatite [3,4].

Renal excretion is the major route for the elimination of absorbed vanadium, with urinary vanadium excretion occurring in amounts generally less than 0.8 $\mu\text{g}/\text{day}$ [5]. In addition, small amounts of vanadium are excreted in the bile.

Functions and Deficiency

No specific biochemical function has been identified for vanadium. Vanadium's effects *in vivo*, however, are predictable from a consideration of its aqueous chemistry. The element as vanadate competes with phosphate at the active sites of phosphate transport proteins, phosphohydrolases, and phosphotransferases. As vanadyl, the element competes with other transition metals for binding sites on metalloproteins (including enzymes) and small ligands such as adenosine tri- and diphosphate (ATP and ADP) and nicotinamide adenine dinucleotide (NAD). For example, when vanadate binds to the ATP hydrolysis site on Na^+/K^+ -ATPase, an enzyme involved in the transport of ions against a concentration gradient, enzyme activity is inhibited. In muscle, vanadate forms ternary complexes

with myosin and ADP and thus inhibits interactions with actin to affect muscle function. Vanadium's interaction with protein kinases and phosphatases disrupt signal pathways, cellular metabolism, and numerous physiological processes.

Vanadium participates in redox reactions, resulting in the generation of reactive oxygen and nitrogen species. The resulting reactive species initiate damage to DNA, chromosomes, and other cellular components.

In pharmacological doses, vanadium mimics the actions of insulin. However, it is important to note that pharmacological activity is generally manifested at a concentration threshold that is considerably greater than that required to fulfill the need for essentiality. In its actions, vanadate is not thought to interact with the insulin receptor, but instead interacts with cytosolic and plasma membrane protein kinases to affect insulin's intracellular signaling pathways. This activation of cytosolic protein kinases enhances glucose and lipid metabolism, while activation of the plasma membrane tyrosine kinases trigger phosphatidylinositol-3-kinase (PI3K). Vanadate activates Akt signaling through inhibition of tyrosine phosphatases, thereby prolonging the activity of the phosphorylated enzymes and enhancing the insulin-signaling pathway. (Note: Akt is a protein involved in insulin signaling; some of the other signaling and transcription factors affected by vanadium include mitogen-activated protein kinases [MAPKs] and extracellular nuclear factors—kappa B [NF- κ B]). Among other effects, activation of Akt by vanadium promotes translocation of the glucose transporter GLUT4 to cell membranes to facilitate glucose uptake into cells. Vanadium also stimulates hepatic glycogen and lipid synthesis and inhibits gluconeogenesis and lipolysis.

Conflicting reports can be found in the scientific literature regarding vanadium's effectiveness in reducing/normalizing fasting blood glucose concentrations in adults with type 2 diabetes. The differences (no effect vs. reduction) may be attributed to multiple factors such as forms of vanadium provided as well as varying dosages and study time periods, among others. However, a phase II clinical trial providing vanadium as bis(maltolato)oxovanadium IV (BMOV) to adults with type 2 diabetes was discontinued with the occurrence of adverse effects on kidney function [6]. Moreover, while studies have been conducted providing vanadyl sulfate to adults with type 1 diabetes and shown reductions in insulin needs and fasting blood glucose, concerns raised over tissue accumulation and toxicity from long-term vanadium use (vs. use of insulin) are noteworthy [4,6,7].

In addition to ongoing investigations on the use of vanadium in pharmacological doses to treat diabetes, multiple other uses for the mineral have/are being studied. Some of these include antineoplastic, antiparasitic, and anticholesterol activities as well as cardio- and neuroprotective

roles, to name a few [4]. Deficiency of vanadium in some animals impairs fertility/reproduction as well as survival, growth, and development.

Recommended Intake, Toxicity, and Assessment of Nutriture

Daily intakes of up to 100 μ g of vanadium are considered safe. A Tolerable Upper Intake Level of 1.8 mg of vanadium/day has been established [8]. Vanadium intakes of about 7–10 mg result in mostly gastrointestinal manifestations—nausea, vomiting, diarrhea, and abdominal cramps. Green tongue syndrome from deposition of green-colored vanadium on the tongue may also occur. Depending on multiple factors (dose, duration, form, etc.), vanadium toxicity (related in part to its pro-oxidant activities) can lead to cell and tissue/organ damage [4].

Methods to assess vanadium status have not been established. Blood vanadium concentrations typically range from about 0.4 to 2.8 ng/mL but may be greater than 500 ng/mL in those ingesting vanadium supplements [1]. Urinary excretion of vanadium averages about 8 μ g/day [5].

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14.5 COBALT

Little evidence exists that cobalt plays a role in human nutrition other than its being a part of vitamin B₁₂ (cobalamin). Although ionic cobalt can substitute for other metals in metalloenzyme activity *in vitro*, no evidence exists that

it acts in that capacity *in vivo*. In this respect, the metal is unique among the elements, in that the requirement in humans is not for an ionic form of the metal but for a pre-formed metallovitamin that cannot be synthesized from dietary metal. Therefore, it is the vitamin B₁₂ content of foods and the diet, rather than the cobalt present, that is important in human nutrition.

SUMMARY

Fluoride, boron, silicon, and vanadium represent elements that are not yet considered essential for humans.

- Foods and beverages relatively high in these elements include tea (fluoride), beer (silicon), fish/seafood (fluoride and vanadium), and foods of plant origin (boron, silicon, and vanadium).
- Fluoride and boron are fairly well absorbed, and silicon is moderately well absorbed; this is in contrast with the absorption of vanadium, which is typically quite low.
- The elements are found in multiple body tissues, especially teeth (fluoride), bones (fluoride, boron, silicon), and connective tissues (silicon).
- Possible roles for boron and silicon include bone formation, composition, and/or strength and, in the case of fluoride, a more clearly established role in the prevention of dental caries. Other suspected roles of boron extend to cell membrane integrity, selected aspects of brain and immune system function, steroid hormone metabolism, and modulation of oxidative stress.
- Vanadium, in contrast with these other elements, in physiological amounts appears to interfere with cellular metabolism through interactions with phosphate and other metal ions. Possible uses of vanadium are primarily focused on provision of the element in pharmacological doses for the management of diabetes and perhaps other conditions. However, the risks associated with its toxicity may be greater than any benefits gained from its use.
- Adequate Intake recommendations by the Food and Nutrition Board are available only for fluoride and by the World Health Organization and European Food Safety Authority for boron.
- Tolerable Upper Intake Levels have been established for fluoride and, based on animal data, for boron and vanadium.

Perspective

NO, SILVER IS *NOT* ANOTHER ESSENTIAL ULTRATRACE MINERAL: TIPS TO IDENTIFYING BOGUS CLAIMS AND SELECTING DIETARY SUPPLEMENTS

Although found in the environment (and thus natural) and often worn as jewelry, the mineral silver is nonessential for human life. Silver has no known biochemical role or physiological function in the body. Yet, a quick search of “silver supplements” on the Internet will yield close to 20 million related websites. Many of these sites proclaim the benefits from ingesting supplements providing silver—colloidal silver (a liquid suspension of tiny silver particles), ionic silver, native silver, or silver protein, among other forms of the element—as a cure for viral and bacterial infections, arthritis, gastrointestinal problems (such as gastroesophageal reflux), and various skin ailments including dry skin, rashes, and dermatitis. What the manufacturers of the oral silver supplements *do not* tell you is often more important—that is, the side effects or health hazards of silver supplements.

Television appearances (on *The Today Show*, *Oprah*, news broadcasts, etc.) of the “man who turned blue” from the regular consumption of a liquid colloidal silver supplement provided visual proof of one of the supplement’s detrimental effects—argyria. Argyria is a permanent (i.e., irreversible) bluish or grayish discoloration of skin, nails, gums, and conjunctiva (the membrane that covers the white part of the eye) that results from the ingestion of silver. The condition is not reversed upon discontinuation of the product or by medical interventions. Moreover, the exact amount of silver that induces argyria is not clear. However, based on a review of cases of argyria associated with silver consumption, the Environmental Protection Agency established an oral reference dose of 5 μg of silver/kg body weight/day [1]. This corresponds to a daily intake of 341 μg of silver for a person weighing 150 lb (68.2 kg). Other health hazards associated with consumption of silver—all unlikely to

be disclosed by the manufacturer of the product—include brain, nerve, liver, and kidney damage; gastrointestinal problems; and headaches and seizures. Silver is a heavy metal; thus, with oral ingestion, the mineral accumulates in organs (causing destruction) and gets deposited in arteries (which can lead to atherosclerosis/heart disease).

Silver does, however, have a few approved medical uses. The mineral is used in *topical* ointments and in bandages/gauze dressings for the treatment of some burns, wounds, and infections of the skin; none of these silver-containing medical products, however, is ingested orally.

Silver supplements are just one example of the thousands of dietary supplements marketed to Americans to improve well-being and appearance and to prevent or cure diseases or ailments. The advertisements for supplements appear in stores, newspapers, and magazines and on television; they also “pop up” on computers and other electronic devices while a user browses the Internet. Added to the harmful effects that many of these supplements have on the body is their economic impact: These products are not cheap. Health fraud is estimated to cost consumers over \$25 billion each year.

The Dietary Supplement Health and Education Act (DSHEA) of 1994 considers dietary supplements, including minerals, vitamins, herbs or other botanicals, amino acids, dietary substances (such as enzymes), metabolites, constituents, and extracts, to be products ingested orally that contain dietary ingredient(s) intended to supplement the diet (not medications) [2]. As a consequence, the manufacturers of dietary supplements do not have to secure approval to sell products, nor do they have to demonstrate to any regulatory agency that the supplements are safe

(unless they contain a dietary ingredient that was not sold prior to 1994). Moreover, manufacturers of supplements can list claims about their product on the label; specifically allowed are claims of general well-being, of structure/function (i.e., how the nutrient affects human body structure or function), and of benefit related to a classical nutrient deficiency disease [3]. Along with such claims, the supplement manufacturer must provide a disclaimer on the label stating that “this statement has not been evaluated by the Food and Drug Administration” and that “this product is not intended to diagnose, treat, cure, or prevent any disease.” In addition, the manufacturer must have substantiation that the claim is truthful and not misleading and must notify the U.S. Food and Drug Administration (FDA) that its product bears such a claim within 30 days of marketing the product [3].

Unfortunately, many consumers assume all dietary supplements are safe, and many fraudulent supplements remain on the market for a long time, causing injury or adverse reactions among users before the FDA accumulates enough evidence that a particular supplement is unsafe and removes it from the market. Consequently, the buyer must beware. Consumers must learn to identify bogus dietary supplement (as well as weight-loss diet) claims to avoid being scammed and potentially harmed. The Food and Nutrition Science Alliance (a coalition of four professional organizations: the Academy of Nutrition and Dietetics [formerly the American Dietetic Association], the American Society for Clinical Nutrition, the American Society for Nutritional Sciences, and the Institute of Food Technologists) developed “Ten Red Flags of Junk Science” to help consumers identify nutrition misinformation [4]. They include:

1. Recommendations that promise a quick fix
2. Dire warnings of danger from a single product or regimen
3. Claims that sound too good to be true
4. Simplistic conclusions drawn from a single study
5. Recommendations based on a single study
6. Dramatic statements that are refuted by reputable scientific organizations
7. Lists of “good” and “bad” foods
8. Recommendations made to help sell a product
9. Recommendations based on studies published without peer review
10. Recommendations from studies that ignore individual or group differences.

Many of these “red flags” are apparent in advertisements for dietary supplements, such as colloidal silver, which are frequently found on the Internet. (Remember: There are no rules to posting information on the Internet; anyone can create a website and post content.) Manufacturers’ websites often *guarantee a quick fix* to problems, and it is not uncommon to see *promises of dramatic or even miraculous results, with cures to one or more diseases* (often those for which medical science has no cure). These claims, of course, are *too good to be true* and incorrectly suggest “one product can do it all.” In addition, the products are often marketed as being “natural” (implying natural is “better,” when in reality many “natural” substances are dangerous and many artificial or synthetic ones are not) and as containing “specialized formulas,” making them superior to other products. Colloidal silver, for example, may be marketed as being better absorbed than other forms of silver because it contains nanoparticles (meaning it has a particle size between 1 and 100 nm) and because it is mined from secret (nondisclosed) natural sources. Finally, *proof* of the dietary supplement’s effectiveness is usually provided by the manufacturer in the *form of testimonials or anecdotes from satisfied consumers*. Lacking are scientific data regarding the supplement’s benefits provided by multiple well-designed, double-blind, placebo-controlled studies.

Each year hundreds of manufacturers of dietary supplements receive letters from the FDA warning against the promotion of products with unsubstantiated (false or misleading) claims. Products that present a *direct health threat* to consumers are usually targeted first by the FDA and Federal Trade Commission (which regulates the advertising of products), followed by those that *present indirect health hazards*. To facilitate the identification of harmful products, adverse events associated with the use of dietary supplements should be reported to the FDA’s MedWatch program [5].

As a means of gaining some reassurance that a product is of sufficient quality and potency, consumers can look for a U.S.P. (U.S. Pharmacopoeia) symbol or designation on the product’s label. This designation indicates that the manufacturer followed established U.S.P. standards for quality, purity, strength, packaging, labeling, and storage. The NSF Public Health and Safety Organization [6] and Consumer Lab [7] also provide information on certified dietary supplements and product reviews. Other considerations when choosing a vitamin and/or mineral supplement are as follows:

- In what form is the nutrient provided in the supplement, and how does this compare with the form of the nutrient provided in other supplements? For example, for the B-vitamin folate, does the supplement provide folic acid, methylfolate, or formyl folate? Are any claims being made about the form of the vitamin/mineral?
- How much of the nutrient is provided in the supplement, and how does this compare with recommendations? Is the amount provided above the Tolerable Upper Intake Level or can you tell? Using the example of folate, a supplement may provide 400 µg DFE folic acid (equal to the Daily Value), but is this 400 µg dose equivalent to another supplement providing 5 mg methylfolate? Are there dangers associated with taking this form of the vitamin in this quantity? Research the information from reputable sources; do not depend on the manufacturer’s

label claims or in many cases the salesperson.

- Is the product and its ingredients manufactured in the United States, and is this information provided on the product’s label? If the product or its ingredients are not made in the United States, what are the manufacturing practices of the country of origin?
- Has the supplement been recalled? Information on recalls can be obtained from the FDA website at <https://www.fda.gov/food/recalls-outbreaks-emergencies/recalls-foods-dietary-supplements>.

The ultimate decision on whether to purchase and consume a supplement should be made with caution and considerable research. Remember the “Ten Red Flags of Junk Science,” some of the other tips suggested in this Perspective, to be skeptical, and to rely on reputable information sources in your evaluation of dietary supplements before spending your money and potentially jeopardizing your health.

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5. FDA’s MedWatch program. <http://www.fda.gov/medwatch>
6. Public Health and Safety Program. Certified Products and Systems. <http://info.nsf.org/Certified/Dietary/>
7. Consumer Lab. www.consumerlab.com

Additional Reading

1. O'Dwyer DD, Vegiraju S. Navigating the maze of dietary supplements – Regulation and safety. *Topics in Clinical Nutrition*. 2020; 35:248–263.

Recommended Websites

National Council Against Health Fraud, www.ncahf.org
National Institutes of Health, Office of Dietary Supplements, <https://ods.od.nih.gov>

Quackwatch: Your Guide to Quackery, Health Fraud, and Intelligent Decisions, www.quackwatch.org
U.S. Food and Drug Administration, www.fda.gov

GLOSSARY

Achlorhydria Lack of hydrochloric acid in gastric juice.

Activation energy Energy introduced into the reactant molecules to activate them to the transition state so that an exothermic reaction can take place.

Acute Having a rapid or sudden onset.

Adequate Intake (AI) Recommended daily dietary nutrient intake based on the nutrient intake levels of healthy people; an AI is thought to exceed the daily requirement for a given nutrient.

Alkalosis Condition in which the pH of the blood is higher than approximately 7.45, the upper end of the normal range.

Alkoxyl radical ($\text{RO}^\bullet$ or $\text{LO}^\bullet$) Monovalent radical consisting of an alkyl group united with oxygen. Alkyl groups are derived from alkanes (a class of hydrocarbons in which the molecule contains only carbon and hydrogen atoms that are joined by single covalent bonds) by the removal of one hydrogen atom and have the general formula $\text{C}_n\text{H}_{2n+1}^\bullet$.

Allele Copy of the particular gene inherited from a parent.

Amenorrhea Absence of at least three consecutive menstrual cycles.

Amphibolic pathway Pathway that is involved in both the catabolism and the biosynthesis (anabolism) of carbohydrates, fatty acids, and/or amino acids.

Amphipathic Molecule that has a polar (hydrophilic) region at one location and a nonpolar (hydrophobic) region at another.

Amphoteric Capable of reacting as either an acid or a base.

Anaplerotic reaction Reaction that involves replenishing or restoring a substrate (e.g., the conversion of pyruvate to oxaloacetic acid).

Android obesity Excess body fat that accumulates centrally around the trunk. Also called *apple-shaped obesity*.

Anomeric carbon Carbon that comprises the carbonyl function that is capable of forming a ring structure with the OH group on the highest-numbered chiral carbon of a monosaccharide.

Anorexigenic Capable of producing anorexia or diminishing appetite.

Anticodons Three-base sequences of nucleotides within transfer RNA (tRNA) molecules.

Antral Pertaining to the antrum, the lower or distal portion of the stomach.

Apical At or near the apex; pertaining to the intestinal lumen side of an enterocyte.

Apolipoprotein Protein component of a lipoprotein particle; also called *apoprotein*.

Apoptosis Organized series of events that, once triggered, leads to cell death.

Arcuate nucleus Subcortical region of the brain that secretes appetite-enhancing neuropeptide Y and appetite-suppressing melanocortins.

Aromatic compound Organic compound that contains a benzene ring.

Ataxia Impaired muscle coordination, especially when trying to perform voluntary muscular movements.

Atheroma Mass of plaque consisting of degenerated, thickened arterial intima, occurring in atherosclerosis.

Autolysis Digestion of intracellular components (including organelles) by lysosomes.

Autophagy Breakdown or digestion of the body's proteins, such as those found in the blood or within cells.

Autosomal or autosome Pertains to non-sex chromosomes. There are 44 (i.e., 22 pairs) of autosomal chromosomes.

Autosomal dominant Inheritance pattern of a gene on a chromosome other than the sex chromosomes in which you need only one copy of the gene to express the trait.

Beriberi Condition resulting from a thiamin deficiency.

Bile Body fluid made in the liver and stored in the gallbladder that participates in emulsifying fat and forming micelles for fat absorption.

Bitot's spots Small, white, foamy-looking accumulations of sloughed cells and secretions in the eye that are associated with a vitamin A deficiency.

Buffer Compound that ameliorates a change in pH.

Calpain Calcium-dependent protease involved in protein turnover in the body.

Carboxylation Addition of a carboxyl group to a molecule.

Caspases Family of cysteine proteases involved in the degradative events during apoptosis (cell death).

Catabolism Process by which organic molecules are broken down.

Cathepsins Group of enzymes involved in breaking down or digesting the body's proteins.

Cells Basic units for all organisms that arise from preexisting cells.

Cerebrosides Sphingolipids containing a single galactose or glucose unit at the terminal hydroxyl.

Ceremide Simplest sphingolipid containing no attached group at the terminal hydroxyl.

Chaperones Soluble intracellular proteins that bind to and deliver minerals or vitamins to specific intracellular locations.

Chelators Small organic compounds that form a complex with another compound, such as a mineral.

Chemiosmotic theory Theory that most ATP synthesis occurs in a process whereby protons move down an electrochemical gradient, and the energy generated is used to phosphorylate ADP to make ATP.

Chiral carbon Carbon atom with four different atoms or groups covalently attached to it.

Chronic Long and drawn out in duration or recurring over a long period of time.

Chylomicron Type of lipoprotein that transports lipids and lipid-soluble vitamins from the intestine into the lymph and then the blood for use by body cells.

Chylomicron remnant Portion of a chylomicron that is left after blood lipoprotein lipase removes part of its triglycerides.

Chyme Partially digested food.

Codon Three-base sequence in a DNA or mRNA molecule that specifies the location of a single, particular amino acid in a polypeptide chain.

- Cohort** Group of individuals that share common characteristics.
- Colloids** Substances comprised of very small particles that are suspended uniformly in a medium.
- Complementary base pairing** Pairing of nucleotide bases in two strands of nucleic acids; A pairs with T or U, while G pairs with C.
- Complete protein** Protein that contains all the essential (indispensable) amino acids in the approximate amounts needed by humans.
- Cori cycle** Metabolic pathway in which lactate (produced by anaerobic glycolysis in muscle and red blood cells) is released into the bloodstream and transported to the liver where the lactate is converted to glucose by gluconeogenesis. The glucose is released into the bloodstream and taken up by muscle and red blood cells where it can enter glycolysis, thus completing the cycle.
- Cross-over study** Longitudinal study in which subjects receive a sequence of different treatments. Each subject receives all treatments (including placebos), usually in a random order.
- Cytochromes** Heme-containing proteins that serve as electron carriers (e.g., in oxidative phosphorylation or the cytochrome P450 system).
- Cytokines** Generic term for nonantibody protein messengers released from a macrophage or lymphocyte that is part of an intracellular immune response.
- Cytoskeleton** Microtubules and microfilaments in the cell that provide internal reinforcement and communication.
- Cytosol (cytoplasm)** Continuous aqueous solution of the cell and the organelles contained in it.
- Deamination** Removal of an amino (NH_2) group from an amino acid.
- Dehydrogenases** Enzymes that catalyze reactions in which hydrogens and electrons are removed from a reactant.
- Desaturation** Process of converting a saturated compound to an unsaturated one.
- Dietary fiber** Nondigestible (by human digestive enzymes) carbohydrates and lignin that are intact and intrinsic in plants.
- Dipeptidylaminopeptidase** Protein-digesting enzyme that breaks apart dipeptides.
- Diphosphatidylglycerol** Phosphatidylglycerol esterified through the C-1 hydroxyl group of the glycerol moiety to the head phosphoryl group of another phosphatidic acid molecule; also called *cardiolipin*.
- Direct calorimetry** Method of measuring the dissipation of heat from the body.
- Disaccharides** Sugars formed by combining two monosaccharides through a glycosidic bond between the hydroxyl group of one monosaccharide and the hydroxyl group of another.
- Double-blind study** Experiment in which neither the person administering the treatment nor the subject knows which treatment (placebo or experimental) the subject is receiving.
- Dowager's hump** Deformity of the spine characterized by a humpback or being bent forward; also called *kyphosis*.
- Eicosanoids** Biologically active substances derived from linoleic and α -linolenic (n-6 and n-3) essential fatty acids.
- Electron transport chain** Sequential transfer of electrons from reduced coenzymes to oxygen that is coupled with ATP formation and occurs within the mitochondria.
- Elongation** (1) Extension of the polypeptide chain of the protein product during protein synthesis. (2) Addition of carbons (in two-carbon increments) to a fatty acid chain.
- Endocrine** All of the body's hormone-secreting glands.
- Endocytosis** Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.
- Endopeptidase** Enzyme that hydrolyzes amino acids linked to other amino acids in the interior of a peptide or protein.
- Endoplasmic reticulum (ER)** Network of membranous channels pervading the cytosol and providing continuity between the nuclear envelope, the Golgi apparatus, and the plasma membrane.
- Endothermic reaction** Reaction in which the products have more free energy than the reactants; it therefore requires energy.
- Enkephalins** Peptides that bind to opioid receptors found in the brain and gastrointestinal tract.
- Enterocyte** Intestinal cell.
- Enterohepatic circulation** Movement of a substance, such as bile, from the liver to the intestine and then back to the liver.
- Enzymes** Protein catalysts that increase the rate of a chemical reaction in the body.
- Epidemiology (Epidemiological studies)** Science concerned with studying those factors that influence the frequency and distribution of disease in a defined human population.
- Estimated Average Requirement (EAR)** Amount of a nutrient thought to meet the requirements of 50% of healthy individuals in a specified age and gender group.
- Eukaryotic cells** Cells with a defined nucleus surrounded by a nuclear membrane.
- Exercise** Planned, structured physical activity to enhance physical fitness.
- Exocytosis** Process by which compounds may be released from cells.
- Exons** Segments of a gene that code for a sequence of nucleotides in a specific molecule of mRNA.
- Exopeptidase** Enzyme that hydrolyzes amino acids off the terminal end of a peptide or protein.
- Exothermic reaction** Reaction in which the reactants have more free energy than the products; it therefore gives off energy as heat.
- Exudate** Fluids that have exuded (been forced or pressed) out of a tissue or its capillaries.
- Ferment** To break down substrates anaerobically to yield reduced products and energy.
- Fermentation** Anaerobic breakdown of carbohydrates and protein by bacteria.
- Free energy** Potential energy inherent in the chemical bonds of nutrients.
- Free radical** Atom or molecule that has one or more unpaired electrons.
- Functional fiber** Nondigestible carbohydrates that have been isolated, extracted, or manufactured and have been shown to have beneficial physiological effects in humans.
- Gangliosides** Sphingolipids containing an oligosaccharide at the terminal hydroxyl, with sialic acid attached to the oligosaccharide chain.
- Gap junctions** Channels between cells.
- Gene** Section of chromosomal DNA that codes for a single protein.
- Genome** Sum of all the chromosomal genes of a cell.
- Ghrelin** Hormone secreted by the stomach and duodenum that signals hunger.
- Glucagon** Hormone secreted by the pancreas in response to decreasing blood glucose concentration. Promotes glucose secretion by the liver, thus normalizing blood glucose concentration.
- Gluconeogenesis** Formation of glucose by primarily the liver or kidney from noncarbohydrate precursors.

- Glucose tolerance factor (GTF)** Chromium-containing compound whose structure has yet to be characterized but may potentiate the action of insulin in the body.
- Glycemic index** Relative number assigned to a particular food indicating its effect on blood glucose concentration above baseline (fasting level) compared to a reference food, usually pure glucose.
- Glycemic load** Glycemic load equals the glycemic index times the grams of carbohydrate in a typical portion of a food.
- Glycocalyx** Layer of glycoprotein and polysaccharide that surrounds many cells.
- Glycogenesis** Pathway by which glucose is converted to glycogen.
- Glycogenolysis** Pathway by which glycogen is enzymatically broken down to glucose.
- Glycolysis** Pathway by which glucose is converted to pyruvate.
- Glycoproteins** Proteins covalently bound to a carbohydrate.
- Glycosaminoglycan** Unbranched polysaccharide consisting of alternate units of two different sugars.
- Glycosidases/carbohydrases** Digestive enzymes that hydrolyze polysaccharides to their constituent monosaccharide units.
- Golgi apparatus (network)** Part of the cell responsible for modifying macromolecules synthesized in the endoplasmic reticulum and packaging them to be transported to the cell surface or cytosol.
- Gynoid obesity** Excess body fat that accumulates around the hips and thighs. Also called *pear-shaped obesity*, occurring mostly in women.
- Hartnup disease** Hereditary disorder in which tryptophan absorption and excretion are abnormal.
- Heat stress** Metabolic response to heat exposure, resulting in heat rash, heat cramps, heat exhaustion, and heat stroke.
- Hemochromatosis** Inherited disorder characterized by excessive iron absorption and iron overload in the body.
- Heterodimers** Complexes formed between two or more different receptors or molecules.
- Hexose monophosphate shunt** See *Pentose phosphate pathway*.
- Homeostasis** Tendency toward stability in the internal environment of the body.
- Homodimers** Complexes formed between two of the same receptors or molecules.
- Hormones** Chemical messengers synthesized and secreted by endocrine tissue (glands) and transported in the blood to target tissues or organs.
- Hydride ion** Anion of hydrogen containing one proton and two electrons that participates in the reduction-oxidation reaction, $\text{NAD}^+ \leftrightarrow \text{NADH}$.
- Hydrogen atom** Chemical element of hydrogen containing one proton and one electron (electrically neutral), often abbreviated as $\frac{1}{2}\text{H}_2$.
- Hydrogen ion** Cation of hydrogen containing a single proton and no electrons, referred to simply as a proton and abbreviated H^+ .
- Hydrolases** Enzymes that catalyze cleavage of bonds between carbon atoms and some other kind of atom by the addition of water.
- Hydroperoxyl (perhydroxyl) radical** ($\text{HO}_2^\bullet$ or $\text{H}-\text{O}-\text{O}^\bullet$) Protonated superoxide radical.
- Hydrophilic** Molecule or part of a molecule having a strong affinity for water and other polar substances. See *Amphipathic*.
- Hydrophobic** Molecule or part of a molecule that repels water but has strong affinity for nonpolar substances. See *Amphipathic*.
- Hydroxyapatite** Crystal-lattice-like substance with the formula $\text{Ca}_{10}(\text{PO}_4)_6\text{OH}_2$ that is found in bones and teeth.
- Hydroxyl radical** ($^\bullet\text{OH}$) Oxygen-centered radical that can be generated in the body when it is exposed to γ rays, low-wavelength electromagnetic radiation.
- Hypercalciuria** Excessive urinary calcium excretion.
- Hyperglycemia** Above-normal blood glucose level.
- Hyperkalemia** High concentrations of potassium in the blood.
- Hyperlipidemia** General term for an elevated blood level of any lipid.
- Hyperphosphatemia** High concentrations of phosphorus in the blood.
- Hyperplasia** Abnormal cell proliferation.
- Hyperpnea** Abnormal increase in the rate and depth of breathing.
- Hypertrophy** Enlargement of the size of cells to increase the size of an organ or tissue.
- Hypocalcemia** Low concentrations of calcium in the blood.
- Hypochondriasis** Abnormal anxiety about one's own health.
- Hypoglycemia** Below-normal blood glucose level.
- Hypokalemia** Low concentrations of potassium in the blood.
- Hyponatremia** Low concentrations of sodium in the blood.
- Hypoxia** Inadequate oxygen supply to tissues.
- Immunoproteins** Proteins made by plasma cells that help destroy foreign substances in the body; also called *immunoglobulins* or *antibodies*.
- In vitro** In a test tube or culture (outside the body).
- In vivo** Within the body.
- Incomplete protein** Dietary protein source that is missing or contains insufficient amounts of one or more indispensable amino acids needed for protein synthesis in the body. May also be called *low-quality proteins* and are generally derived from plants.
- Indirect calorimetry** Measurement of the consumption of oxygen and the expiration of carbon dioxide by the body, used to estimate metabolic rate.
- Insulin** Hormone secreted by the pancreas in response to rising blood glucose derived from food. Promotes glucose uptake into muscle and adipose tissue, thus normalizing blood glucose concentration.
- Intermediate filaments** Strong, ropelike cytoskeletal fibers that are made of protein and that function to provide mechanical stability for cells.
- International Unit (IU)** Quantity of a nutrient that produces a particular biologic effect. The amount varies among different nutrients. Sometimes used with vitamins A, D, and E.
- Introns** Noncoding regions of a gene.
- Ion** Electrically charged atom or group of atoms; positively charged ions are called *cations*, and negatively charged ions are called *anions*.
- Ischemia** Deficiency of blood in a tissue.
- Isoforms** Proteins that have different amino acid sequences but perform the same biological function.
- Isomer** One of two or more different chemical compounds that have the same molecular formula.
- Isomerases** Enzymes that catalyze the interconversion of optical or geometric isomers.
- Isoprenoid** Structure of the side chains of five-carbon units, as found in vitamins E and K.
- Isozymes** Enzymes that have different amino acid sequences but catalyze the same reaction.
- Keratinocytes** Cells that produce the protein keratin.
- Ketoacidosis** Blood acidosis due to excessive ketone bodies.
- Ketogenesis** Process of producing ketone bodies.

Ketone bodies Compounds (acetoacetate, β -hydroxybutyrate, and acetone) formed during the oxidation of fatty acids in the absence of adequate four-carbon intermediates.

Ketosis Condition resulting in elevated ketone body concentration in the blood.

Krebs cycle See *Tricarboxylic acid cycle*.

Kyphosis Deformity of the spine characterized by a humpback or being bent forward; also called *dowager's hump*.

Lanugo Fine, soft, lightly pigmented hair that is usually found on a fetus toward the end of pregnancy but may appear on malnourished individuals.

Leptin Polypeptide hormone secreted by adipose tissue that reduces hunger through hypothalamic mechanisms.

Leptin resistance Concept that, despite increased circulating leptin levels and adequate leptin receptors, the feeling of hunger that persists that food intake does not diminish.

Leukotrienes Biologically active compounds derived from linoleic or α -linolenic acids (n-6 and n-3 essential fatty acids).

Ligands Small molecules or minerals that bind to a larger molecule.

Ligases Enzymes that catalyze the formation of bonds between carbon and other atoms.

Limiting amino acid Amino acid within a protein with the lowest amino acid or chemical score; present in a protein in the lowest amount, compared with a reference amount.

Lingual Pertaining to the tongue.

Lipophilic State of being attracted to lipids and thus repelled by water.

Lipoproteins Complexes of lipids and proteins that play a role in the transport and distribution of lipids.

Lyases Enzymes that catalyze cleavage of carbon-carbon, carbon-sulfur, and certain carbon-nitrogen bonds without hydrolysis or oxidation-reduction.

Lysosomes Cell organelles that contain digestive enzymes.

Macronutrient Dietary nutrients that supply energy, including fats, carbohydrates, and proteins.

Marasmus Malnutrition caused by prolonged intake of a diet deficient in energy (kcal).

Metabolic syndrome Clustering of risk factors for cardiovascular disease and type 2 diabetes, including elevated blood pressure and obesity.

Micelle Stable aggregate of lipid molecules that exist in equilibrium within an aqueous solution. Micelles form during lipid digestion and contain bile salts, free fatty acids, 2-monoacylglycerols, lysophospholipids, cholesterol, phytosterols, and other foodborne lipids.

Microfilament Solid cytoskeletal structure made of a double-helix polymer of the protein actin that plays a role in cell motility.

Microflora Bacteria adapted to living in a specific environment, such as the intestines.

MicroRNAs Small noncoding RNAs that silence gene expression by binding to mRNA to inhibit its translation and/or promote its degradation.

Microtubules Hollow, cylindrical cytoskeletal structures composed of the protein tubulin that act to support the cell structure.

Microvilli Extensions of intestinal epithelial cells designed to present a large surface area for absorbing dietary nutrients.

Mitochondria Cellular organelles that are the site of energy production by oxidative phosphorylation and the site of the tricarboxylic acid (Krebs) cycle; they are surrounded by an outer membrane that is very permeable and an inner membrane that is only selectively permeable.

Mole (abbreviated mol) Quantity of a substance that is equal to its molecular weight or atomic weight expressed in grams. Thus,

1 mol of a substance such as glucose is equal to the molecular weight of glucose (e.g., 180.16 g) or, for an element, 1 mol of hydrogen, for example, is equal to its atomic mass (weight), 1.08 g.

Monosaccharides Simplest form of carbohydrates, which cannot be reduced in size to smaller carbohydrate units.

Motility Movement.

Mucins Glycoproteins found in some body secretions, such as saliva.

Mutation Change in the structure of a gene; the change can affect a single nucleotide in a gene or affect multiple nucleotides such as may occur with the deletion, insertion, or rearrangement of sections of genes or chromosomes.

Natriuresis Excretion of large amounts of sodium in the urine.

Nervous system System of nervous tissue made up of neurons and glial cells.

Nitrogen dioxide ($\cdot\text{NO}_2$ or $\cdot\text{ONO}$) Nitrogen- and oxygen-containing radical, formed from a reaction between nitric oxide and molecular oxygen, in which one of the two oxygen atoms possesses an unpaired electron.

Nitrosation Substitution of a hydrogen atom in an organic compound with a nitroso group ($-\text{N}=\text{O}$).

Nitrosothiol (RSNO) Compound, which is typically organic, that contains a nitroso group ($-\text{N}=\text{O}$) attached to a sulfur atom of a thiol.

Nuclear envelope Set of two membranes that contain nuclear pores and surround the cell nucleus.

Nucleoli Regions of the nucleus containing condensed chromatin and sites for synthesizing ribosomal RNA.

Nucleotides Phosphate ester of the 5'-phosphate of a purine or pyrimidine in N-glycosidic linkage with ribose or deoxyribose, occurring in nucleic acids.

Nystagmus Constant, involuntary movement of the eyeball.

Obesogens Endocrine-disrupting chemicals in the environment, including the diet, that alter lipid metabolism by binding to hormone receptors.

Observational study Epidemiological study in which the assignments of subjects into control or treated groups is outside of the investigator's control. Inferences about the possible effects of the treatment are drawn from the differences between the two groups.

Oligosaccharides Short chains of monosaccharide units joined by covalent bonds.

Oncogenes Genes capable of causing a normal cell to convert to a cancerous cell.

Oncosis Pre-lethal pathway accompanied by cellular swelling, organelle swelling, and increased membrane permeability that lead to cell death.

Ophthalmoplegia Paralysis of the ocular muscles.

Orexigenic Pertaining to increasing or stimulating the appetite.

Osmolality Measure of solute particle numbers expressed as osmoles of solute per kg of solvent (osm/kg).

Osmolarity Measure of the solute particle numbers expressed as osmoles of solute particles in 1 L of solution (osm/L). In dilute aqueous solutions as found in the human body, only a small numerical difference exists between osmolarity and osmolality.

Osmoles (osm) Number of moles of each particle in solution.

Osmosis Net movement of the solvent (such as water) from a solution of lesser concentration to one of greater when the two solutions are separated by a membrane that selectively prevents passage of solute molecules but is permeable to the solvent.

Osmotic pressure Property of a solution that is proportional to the nondiffusible solute concentration.

- Osteoblasts** Bone-forming cells.
- Osteoclasts** Cells that break down or resorb bone.
- Osteomalacia** Disorder characterized by bone-mineralization defects that may occur in adults because of inadequate vitamin D intake.
- Oxidation** Enzymatic reaction in which oxygen is added to, or hydrogen and its electrons are removed from, the reactant.
- Oxidative phosphorylation** Pathway in the mitochondria that makes ATP from ADP and P_i .
- Oxidoreductases** Enzymes that catalyze all reactions in which one compound is oxidized and another is reduced.
- Parenchymal cells** Functional cells of an organ such as the liver.
- Paresthesia** Sensation of burning, tingling, or pricking (like pins and needles) associated with peripheral nerve damage.
- Pellagra** Condition that results from niacin deficiency.
- Pentose phosphate pathway** Pathway that metabolizes glucose-6-phosphate to pentose phosphate, producing NADPH.
- Peroxisomes** Cell organelles containing enzymes that perform oxidative catabolic reactions.
- Peroxyl radical** (O_2^{2-}) Radical that contains a peroxyl ($—O—O—$) group.
- Peroxynitrate** (O_2NOO^*) Nitrogen- and oxygen-containing radical that is generated from a reaction between nitrogen dioxide and a superoxide radical and that typically decomposes to form singlet oxygen and nitrogen dioxide.
- Peroxynitrite** ($ONOO^-$) Nitrogen- and oxygen-containing radical, formed by a reaction between nitric oxide and superoxide radicals, that can decompose to generate hydroxyl and nitrogen dioxide radicals or react with carbon dioxide to produce carbonate and nitrogen dioxide radicals.
- Petechiae** Skin discolorations caused by ruptured small blood vessels.
- Phagocytosis** Endocytotic process in which material is engulfed into a cell.
- Phospholipids** Lipids that belong to a class of lipids containing phosphate and one or more fatty acid residues.
- Phosphorolysis** Cleavage of a chemical bond with the addition of phosphoric acid, analogous to hydrolysis (an example is the sequential release of individual glucose units from glycogen).
- Phosphorylation** Metabolic process of adding a phosphate group to an organic molecule.
- Phytochemical** Biologically active, non-nutritive substance that is found in plants.
- Phytol tail** Structure of the side chains of vitamins E and K.
- Pinocytosis** Uptake of a substance into a cell through the formation of vesicles derived from the plasma membrane.
- Plasma** Liquid portion of blood that has been separated from the particulate portion (through the removal of cells and platelets).
- Plasma membrane** Phospholipid bilayer encapsulating a cell.
- Polar molecule** Molecules having an overall electronegative charge due to the arrangement of the bonded atoms. Polar molecules have a slightly negative charge; nonpolar molecules exhibit no electronegativity; some molecules have both a polar region and nonpolar region.
- Polymer** Substance with a high molecular weight, made up of a chain of repeating units.
- Polysaccharides** Long chains of monosaccharide units that may number from several into the hundreds or thousands.
- Porphyria** Nitrogen- and iron-containing nonprotein portion of hemoglobin.
- Postprandial** Occurring after a meal.
- Prebiotics** Nondigestible food ingredients that serve as substrates to promote the colonic growth and/or activity of selected health-promoting species of bacteria.
- Preprandial** Occurring before a meal.
- Probiotics** Products that contain specific strains of microorganisms in sufficient numbers to alter the microflora of the gastrointestinal tract, ideally to exert beneficial health effects.
- Prokaryotic cells** Primitive cells that do not contain a defined nucleus.
- Propagation** Ongoing generation of free radicals following the initiation stage of free radical formation.
- Prophylactic** Substance or regimen that helps to prevent disease or illness.
- Prospective study** Epidemiological study in which subjects are selected on the basis of factors that are to be examined in the future for possible effects on some outcome.
- Prostaglandins** Biologically active compounds derived from linoleic or α -linolenic acids (n-6 or n-3 essential fatty acids).
- Proteases** Enzymes that digest (break down) proteins.
- Protein kinases** Family of enzymes that transfers a phosphate group to another protein from ATP.
- Proteoglycans** Large molecules made up of proteins and glycosaminoglycans.
- Proteolytic** Pertaining to the breakdown of protein.
- Quenching** Process by which electronically excited molecules, such as singlet molecular oxygen, are inactivated.
- Receptors** Macromolecules (usually proteins) that bind a signal molecule with a high degree of specificity that triggers intracellular events.
- Recommended Dietary Allowance (RDA)** Average daily dietary intake level of a nutrient that is thought to be sufficient to meet the nutrient requirements of about 97% of healthy individuals.
- Reflex** Involuntary response to a stimulus.
- Reperfusion** Resupply of an organ or tissue with oxygen, nutrients, or both.
- Replication** Synthesis of a daughter duplex DNA molecule identical to the parental duplex DNA.
- Resin** Compound that is usually solid or semisolid and usually exists as a polymer.
- Respiratory quotient (RQ)** Ratio of the volume of CO_2 expired to the volume of O_2 consumed.
- Rhodopsin** Vitamin A-containing protein found in the eye.
- Rickets** Condition in infants and children that results from vitamin D deficiency.
- Ryanodine receptor** Calcium channel in the sarcoplasmic reticulum of muscle that opens to permit the release of calcium.
- Sarcoplasmic reticulum** Smooth endoplasmic reticulum that is found in muscle cells and is the site of the calcium pump.
- Scurvy** Condition resulting from vitamin C deficiency.
- Seborrheic dermatitis** Inflammatory skin condition.
- Sense strand** Strand of DNA that serves as a template for mRNA.
- Serum** Pale yellow, clear fluid portion of blood from which the clotting factors (fibrinogen) have been removed.
- Short-chain fatty acids** Fatty acids typically containing two to four carbons.
- Sideroblastic anemia** Inherited disorder that affects red blood cell production and function.
- Signal-lipidomics** Branch of the emerging field of lipidomics, which studies the pathways and networks of cellular lipids. Studies the lipidomics of various signaling sites of cell membranes, which often involve polyunsaturated fatty acids such as docosa-hexaenoic acid.

Signal transduction Cascade of intracellular reactions resulting from the binding of an extracellular molecule to its cell-surface receptor, resulting in a genetic or metabolic response.

Singlet (molecular) oxygen Electronically excited radical in which one of oxygen's electrons is excited to an orbital above the one it normally occupies.

Sphingolipids Class of lipids that contain the amino alcohol sphingosine as a backbone structure, with a fatty acid attached to the amino group.

Sphingomyelin Sphingolipid containing phosphocholine at the terminal hydroxyl.

Splanchnic Pertaining to the internal organs (viscera) in the abdominal cavity. The splanchnic organs or portal-drained viscera typically include the liver, stomach, intestines, and spleen.

Standard reduction potential Tendency of a molecule to donate or receive electrons.

Steatorrhea The presence of an excessive amount of fat in the feces.

Stellate cells Storage cells of the liver.

Stereoisomers Group of compounds that have the same structure but different configurations.

Sterols Subclass of lipids that contain a cyclopentanoperhydrophenanthrene ring system, a hydroxyl group, and a side chain.

Substrate-level phosphorylation Process of transferring a phosphate group from one organic molecule to another.

Superoxide radical Oxygen-centered free radical, $O_2^{\cdot-}$.

Teratogenic Capable of causing birth defects in a fetus.

Tetany Condition resulting from inadequate blood calcium concentrations, characterized by prolonged muscle contraction.

Thalassemia Hereditary form of anemia associated with defective synthesis of hemoglobin.

Thermogenesis Production of heat within the body.

Thermoregulation Process whereby a regulatory mechanism keeps heat production and loss about equal.

Thromboxanes Biologically active compounds derived from linoleic or α -linolenic acids (n-6 or n-3 essential fatty acids).

Tolerable Upper Intake Level Highest daily-intake level that is likely to cause no risk of adverse health effects to most individuals in the general population.

Total fiber Sum of dietary fiber plus functional fiber that is in a food.

Transcaltachia Rapid intestinal calcium absorption stimulated by the active form of vitamin D.

Transcription Process by which the genetic information (base sequence) in a single strand of DNA is used to specify a complementary sequence of bases in an mRNA chain.

Transcription factors Auxiliary proteins that bind to specific sites in the DNA and alter the transcription of nearby genes.

Transducin G-protein found in the eye that responds to changes in opsin and is involved in the visual cycle.

Transferases Enzymes that catalyze reactions not involving oxidation and reduction in which a functional group is transferred from one substrate to another.

Transition state Energy level at which reactant molecules have been activated and can undergo an exothermic reaction.

Translation Process by which genetic information in an mRNA molecule specifies the sequence of amino acids in the protein product.

Translocation Movement of a compound or agent across a cell membrane, such as the intestinal cell, and into the blood.

Transport proteins Proteins that transport (carry) nutrients in blood or into and out of cells or cell organelles.

Tricarboxylic acid cycle Aerobic metabolic cycle in the mitochondria that produces ATP; also called the *citric acid cycle* or *Krebs cycle*.

Tubular maximum (such as for phosphorus—TmP) Amount (mmol) of phosphorus reabsorbed per unit time.

Tumor necrosis factor Cytokine released by immune cells and mast cells that causes destruction of tumors and migration of neutrophils toward the site of bacterial infections.

Ubiquinol Alcohol form of ubiquinone, a fat-soluble molecule that functions in electron transport and ultimately ATP generation; also called *coenzyme Q₁₀* or *CoQ₁₀*.

Ubiquitin Protein that attaches to other proteins within cells or tissues to promote the degradation of the protein.

VO₂ max Maximal uptake of oxygen, as measured during a test with increasing work intensity.

Xenobiotics Foreign chemicals such as drugs, carcinogens, pesticides, food additives, pollutants, or other noxious compounds.

Xerophthalmia Dryness of the conjunctiva and keratinization of the epithelium of the eye following inflammation of the conjunctiva associated with vitamin A deficiency.

Zwitterion Dipolar ion that has both negatively and positively charged regions, such as an amino acid. The ion has no net charge in solution.

Zymogen Inactive form of an enzyme, also referred to as a *proenzyme*.

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