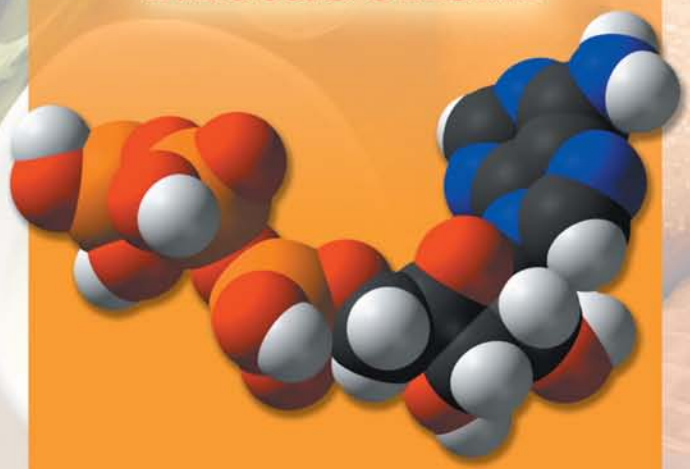
ADVANCED NUTRITION

Macronutrients, Micronutrients, and Metabolism



Carolyn D. Berdanier
Janos Zempleni

CRC Press
Taylor & Francis Group

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Preface

As in the original editions of this text, Advanced Nutrition has been written for the advanced student who has a background in biochemistry and physiology but may or may not have a background in nutrition and dietetics. The book is divided along traditional lines, addressing each of the major nutrient classes. In addition, there are chapters on food intake, cell cycle, nutritional biochemistry and nutrition genomics, and epigenetics. Woven throughout the text are topics of clinical interest such as obesity, diabetes, lipemia, renal disease, etc., as these conditions are influenced by nutrition. Some medical terms, food science terms, metabolic terms, physiologic terms, and drug terms are included. Because nutrition and foods are integrated sciences, not all of these terms will be useful to all readers. However, there should be a large number useful to many readers. The third edition brings together both the macronutrients and the micronutrients texts. It has been updated and revised for ease of understanding. A Web address has been included to give the reader access to the extensive Tables of Food Composition maintained by the USDA. In addition, a Web address for Dietary Reference Intakes has been given to again provide the most current recommendations for nutrient intakes. These recommendations are in a state of flux. As the information base expands with respect to nutrient use and need, the DRIs are changed to reflect this newer knowledge.

It is hoped that you, the reader, will find this book an essential addition to your library.

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The author would like to express her appreciation to all the readers of the earlier editions who were so kind as to write with their comments. Appreciation is also extended to our spouses, Reese Berdanier and Sabine Zempleni, who put up with our many hours at the computer preparing this edition. Their patience and support are much appreciated. Lastly, this edition would not have been possible without the encouragement and support of Randy Brehm, our gracious editor at Taylor & Francis Group.

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Carolyn D. Berdanier is a professor emerita of nutrition at the University of Georgia in Athens. She earned her B.S. degree from Pennsylvania State University, and M.S. and Ph.D. degrees from Rutgers University. After a postdoctoral fellowship year with Dr. Paul Griminger at Rutgers, she served as a research nutritionist at the USDA Human Nutrition Institute in Beltsville, Maryland. At the same time she also served as an assistant professor of nutrition at the University of Maryland. Following these appointments she moved to the University of Nebraska College of Medicine and then in 1977 joined the faculty of the University of Georgia where she served as department head, Foods and Nutrition, for 11 years. She stepped down from this position to resume full time research and teaching with a special interest in diabetes. Her research has been funded by a variety of funding agencies.

Dr. Berdanier has authored over 150 research articles, contributed 40 chapters to multiauthored books, prepared 45 invited reviews for scientific journals, and has edited/coauthored or soleauthored 15 books. She has served on the editorial boards of the FASEB Journal, the Journal of Nutrition, Biochemistry Archives, Nutrition Research, and the International Journal of Diabetes Research. She serves as an ad hoc reviewer for articles in her specialty for a wide variety of scientific journals.

Janos Zempleni is an associate professor of molecular nutrition in the department of Nutrition and Health Sciences at the University of Nebraska, Lincoln. He earned his B.S. and Ph.D. degrees in nutritional sciences from the University of Giessen, Germany. Dr. Zempleni was a postdoctoral fellow at Innsbruck University, Austria (Department of Pediatrics), Emory University School of Medicine (biochemistry), and the University of Arkansas in medical sciences (departments of pediatrics and biochemistry and molecular biology). Dr. Zempleni has spent his entire academic career investigating vitamin metabolism in mammals. His current research focus is on novel modification of histones that he discovered, biotinylation of lysine residues, and its role in gene regulation, stress resistance and lifespan, and genomic stability. This research has been published in more than 100 papers, book chapters, and meeting proceedings. His work is currently funded by the NIH, NSF, and USDA. Dr. Zempleni was the recipient of the 2006 Mead Johnson Award from the American Society for Nutrition (ASN), and he is the 2007–2008 chair of the Nutrient-Gene Interactions Research Interest Group of ASN. He also coedited the most recent edition of the Handbook of Vitamins. Dr. Zempleni is an active member of the American Physiological Society, the American Association for the Advancement of Science, the American Nutrition Society, and the Society for Experimental Biology and Medicine. He is a member of Sigma XI and Gamma Sigma Delta.

1 Energy

After water, the most important requirement to sustain life is energy. Life in the human can continue for ~60 days without food energy whereas without water for 3–4 days, the human dies. Energy is expressed in heat units known as calories or joules. Energy used/consumed or expended by mammals *should* be expressed as kilojoules or kilocalories; however, these units are sometimes misused.

DEFINITION

Classical nutritionists use the term *Calorie* or *kilocalorie (kcal)* to represent the amount of heat required to raise the temperature of 1 kilogram of water 1°C.[1] The international unit of energy is the joule. One Calorie or kcalorie is equal to 4.184 kilojoules or 4.2 kjoules (kJ). There are cogent reasons to express energy in terms of kjoules. Nutritionists have realized that the energy provided by food is used for more than heat production. It is also used for mechanical work (muscle movement) and for electrical signaling (vision; neuronal messages) and is stored as chemical energy. The joule is 107 ergs, where 1 erg is the amount of energy expended in accelerating a mass of 1 g by 1 cm/s. The international joule is defined as the energy liberated by one international ampere flowing through a resistance of one international ohm in one second. Even though the use of joules or kilojoules is being urged by international scientists as a means to ease the confusion in discussions about energy, the student will still find the term Calorie or kcal in many texts and references. In some texts, the term *calorie*, spelled with a lower case "c," is used. This heat is actually 1/1000th of the heat unit spelled with an upper case "C." Physicists use the term *calorie* to represent the amount of heat required to raise the temperature of one gram of water 1°C. Note that this definition uses 1 g, not 1 kg, as stated previously. Even though it is not correct, the term *calorie* is used in some nutrition literature when in fact Calorie or kilocalorie (kcal) is intended.

When a small sample (usually 1 g) of food is burned (oxidized) in the presence of oxygen, heat is produced. This complete oxidation is measured in a bomb calorimeter (Figure 1.1) and represents the gross energy (GE) value of that particular food. The bomb calorimeter is a highly insulated, box-like container. All the heat produced during the oxidation of a dried sample of food is absorbed by a weighed amount of water surrounding the combustion chamber. A thermometer registers the change in the chamber temperature. The instrument's name is derived from the design of the combustion chamber, which is, indeed, a small bomb. Figure 1.1 shows a cross section of this instrument.

Some corrections have been applied to these energy values. For example, the energy value of a protein food is higher than the actual biologic value because the end products of oxidation in the body must be excreted as urea, a process that costs energy. The combustion or oxidation of protein yields 5.6 Calories (23.5 kJ)/g; the energy yield from the oxidation of protein after correction for urea formation and digestive loss by the body is about 4 Calories (~16.8 kJ)/g. Corrections for digestive losses are also applied to the values obtained for the combustion of lipids and carbohydrates. The value of 4.1 Calories (17.22 kJ)/g is rounded off to 4 (16.8 kJ) for carbohydrates, and the value of 9.4 (39.5 kJ) for lipids is rounded off to 9 (37.8 kJ). Listed in Table 1.1 are some common foods and their energy value. The energy value of a wide variety of foods can be accessed using the web address: www.nal.usda.gov/fnic/foodcomp/Data/foods,82 nutrients. Individuals wishing to plan their energy intake through the control of portion size can do so using these data.

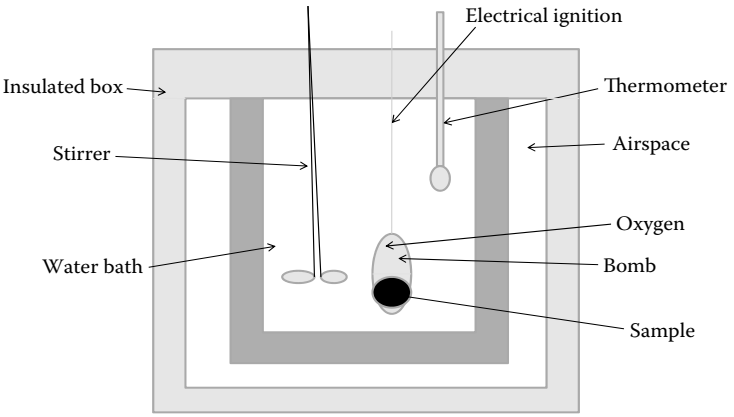


FIGURE 1.1 The bomb calorimeter.

TABLE 1.1
Energy content of selected human foods

Item	Amount	Food kcal[a]	Energy kJ
Milk (3.3% fat)	8 oz	150	628
Skim milk	8 oz	85	356
Butter	1 oz	204	854
Egg	50 g	80	335
Bacon	2 slices	85	356
Hamburger, lean	3 oz	185	774
Apple	1 medium	80	335
Avocado	216 g	370	1548
Banana	119 g	100	418
Grapefruit	241 g	50	209
Orange	131 g	65	272
Bread, white	25 g (1 slice)	70	293
Brownie with nuts	20 g	95	397
Peanut butter	16 g	95	397
Green beans	1 cup, 125 g	30	126
Corn	1/2 cup, 83 g	65	272
Tomato, raw	135 g	25	105

[a]1 kcal = 4.184 kJ.

Source: www.nal.usda.gov/fnic/foodcomp/Data/food.82nutrients

The objectives of digestion, absorption, and metabolism in the animal system are to convert the chemicals in foods to the chemicals in the body. The conversion of food chemicals to body chemicals is stepwise and not 100% efficient. Energy exchange and conversion are an integral part of the process that converts the food chemicals to the body chemicals. Some of the chemical energy from food is trapped in high-energy storage compounds, but, at each step of the conversion process, some of the energy escapes as heat. This heat, generated by the body in the course of oxidizing various food components or converting them to body components, serves to maintain body temperature. However, because this heat is also lost from the body through radiation as well as through the various excretory processes, these losses must be replaced. Hence, the daily need for food and its associated energy content. Figure 1.2 illustrates the continuity of energy losses and gains by the living system. Energy is gained from oxidation of the fats, carbohydrates, and proteins consumed as food. This energy is transmitted via ATP (and other high-energy transfer compounds) to a variety of essential macro- and micromolecules in the body, which, in turn, are needed to sustain its optimal function. As these functions also consume and release energy, more must be provided by the diet. Over and over this cycling occurs; this is the nature of the dynamic state of the living body. No reaction or process is ever completed and stops. The only time this happens is at death.

ENERGY NEED

The Food and Nutrition Board of the National Academy of Sciences has made recommendations for a range of energy intakes but because there is a degree of uncertainty about a firm intake recommendation, the board felt that a range of acceptable macronutrient intakes would suffice. For children age 1–3 a range of fat, carbohydrate, and protein intake as a percent of the total intake of energy is 30–40, 45–65 and 5–20, respectively. For children age 4–18, the recommendations are 25–35, 45–65, and 10–30, respectively. Lastly for adults, the ranges are 20–35, 45–65, and 10–35, respectively. In contrast to the recommended intakes for the other essential nutrients, there can be no "safety factor" in the energy intake recommendation. In fact, the energy need is so variable that an intake that would result in obesity in one person might be totally inadequate for another. Genetics and lifestyle choices have profound effects on energy balance and energy need. Periodically, recommendations are updated by the National Research Council's Food and Nutrition Board

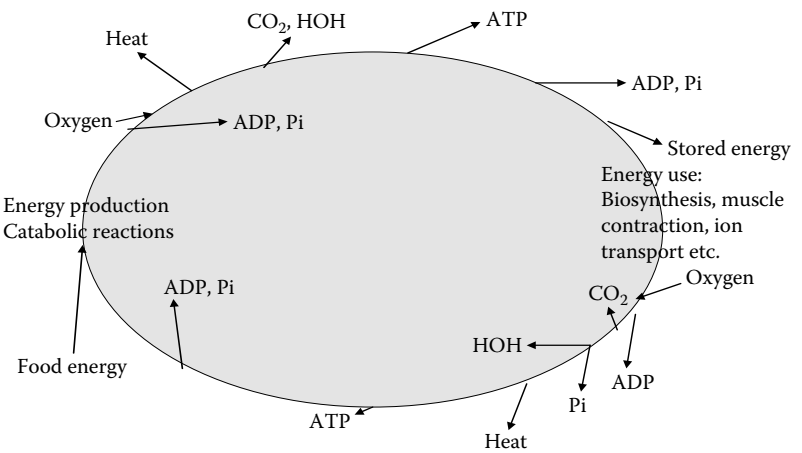


FIGURE 1.2 Overview of energy gains and losses showing the high-energy compound ATP as the medium of exchange. Other high-energy compounds (e.g., GTP, UTP, and creatine phosphate) are also involved in the cycle of energy intake and expenditure.

Gross energy of food – (Energy of feces and urine) = apparent digestible energy
Metabolizable energy = Heat increment + basal energy

Digestible energy – Heat increment = net energy = basal energy + utilizable energy + energy balance
Heat increment = Diet-included thermogenesis + Heat production

FIGURE 1.3 Energy equations representing various losses of energy from the body.

and published by the National Academy Press (Washington, D.C.). Even though there is no current fixed recommendation for energy intake (although past boards provided one) the current and future research might provide sufficient information to allow the board to devise a new one. For the latest information the reader should go to the National Academy of Sciences Web site (www.nap.edu). The intake recommendations should be different according to gender and age but they also should differ with respect to body size, genetic background, and daily activity. In addition, the types of food consumed (i.e., high-fiber foods or essential fatty acid deficient foods) and prescribed medications such as orlistat or steroids, or disease states (i.e., irritable bowel syndrome, food allergies, hypertension, diabetes—and the drugs used to control these problems), etc., may affect energetic efficiency that, in turn, could influence the use of the energy consumed. Thus, a prescription or recommendation for energy intake is, at present, not provided.

Perhaps the best way of understanding the dimensions of the energy requirement is to examine what happens to the energy provided by food when it is consumed.

The energy provided by food (gross energy, GE) can be determined using a bomb calorimeter as already mentioned. Not all of this energy is available to the body. Some is lost in the preparation of the food, and some is lost through digestion. The energy value of feces and urine is subtracted from the gross energy of the food, and this is defined as the apparent digestible energy (DE) available to the body[2] (see Figure 1.3). During digestion, energy is lost to the system through the breakdown of food to its appropriate absorbable components. Where energy is needed for absorption (active transport), this is also deducted from the gross energy of the food. It is difficult to accurately measure these losses, therefore, animal nutritionists typically correct the gross energy of food by deducting the energy of the feces and urine. Aliquots of the consumed food as well as the urine and feces can be collected and assessed for the energy content using the bomb calorimeter. Generally, the urinary and fecal losses amount to about 10% of the gross energy of the diet if that diet is a mixture of fats, proteins, and carbohydrates. An additional deduction of the energy value of the urine accounts for energy lost as a result of metabolism of the food mixture. Again, this is an estimate, not an absolute value. When the deductions for these losses are made, the energy available to the body is the digestible energy (DE). Thus, DE is the gross energy minus the energy lost in feces and urine. Most tables of food composition of human foods make the correction for these losses. The energy of the urine and feces is deducted from the gross energy because these excreta represent or approximate the food energy lost via the digestive tract and the urinary tract. The energy of the feces consists of exfoliated intestinal cells, the residual of food not absorbed, and the residual enzymes and lubricants used in the course of digestion and absorption. The energy of the urine represents the energy cost of excreting nitrogen as well as energy value of some small metabolites that are the end products of degradative one-way reactions.

Metabolizable energy (ME) consists of the energy needed to keep the body warm (the heat increment) and to run the body processes. The heat increment (HI) is composed of the heat released

through basal work done by the body—for example, heart muscle contraction—and the heat released as food is oxidized and converted to usable body components. The latter is sometimes referred to as the specific dynamic action of food, or diet-induced thermogenesis (DIT). In animal nutrition, the heat increment is defined as the increase in heat production following the consumption of food by an animal in a thermoneutral environment. Heat production is related to the surface area of the body producing the heat and is generally expressed in terms of body size or weight to the 0.75 power ($W^{0.75}$).[3] Utilizable energy (UE) is that which is used for body work. Net energy (NE) likewise has two components. One is basal energy (BE) or the absolute minimum amount of energy that is needed to keep the body alive, whereas the other is the energy that is either available for storage or is withdrawn from storage (energy balance, EB). If weight is neither gained nor lost, the body is in energy balance. If weight is lost, the body is in negative energy balance, and if weight is gained, the body is in positive energy balance. This concept of balance is one that is used by classical nutritionists considering a variety of essential nutrients. Methods to measure protein balance, calcium balance, and so forth have been devised. They are practical for many situations because the methodology does not usually require that the animal under study be killed. For the energy balance equation, there are some terms the reader should know because these terms are used outside of the calculations used for energy balance. They are as follows:

Digestive energy. The energy lost through digestion can be measured by collecting the feces and urine and determining its energy content. Some of the fecal energy is due to the desquamated intestinal cells and intestinal flora (and their end products) and some from the nondigested portion of food. However, for the purposes of determining the energy balance of the individual, all of these components of fecal energy can be grouped together because they represent energy lost from the body.

Basal energy. By definition, basal energy reflects the energy used to sustain life. It is the sum of the energy lost as heat or used by anabolic and catabolic processes that are involved in body maintenance. It can be measured directly as energy lost as heat using a whole-body calorimeter[4–8] or indirectly by measuring oxygen consumption and carbon dioxide release.[9–12] More recent iterations of the respiratory gas technique includes the use of doubly labeled water.[13–17] Basal energy excludes the energy used for growth, production/reproduction, lactation, movement, or work. The clinical definition for basal energy is the amount of energy used by the body at rest (not asleep), in the postabsorptive state (not starving), at sexual repose, and in a comfortable (thermoneutral) environment where neither shivering nor sweating occurs. When measured indirectly via the measurement of oxygen consumption, this is referred to as the basal metabolic rate of oxygen use or BMR.

The measurement of energy need based on energy lost as heat or on the consumption of oxygen needed to oxidize fats, carbohydrates, and proteins to provide energy has been well studied. The energy need is additive.[18] That is, energy is needed to maintain the body (basal energy) plus energy is needed to sustain the body's various activities, i.e., voluntary movement, work, growth, reproduction, and lactation. Many methods have been developed for estimating the basal energy requirement. Some of these are listed in Table 1.2. The methods vary from very simplistic ones based on height and weight to those based on actual measures of heat production, or estimates of heat production based on the heat needed to evaporate water lost by the body. Each method has its advantages and disadvantages. In those methods where actual measurements are made, the basal energy need must be measured in the postabsorptive state and the energy needed for growth and activity added to it. The postabsorptive state occurs after consumed food is digested and absorbed, but before the state of starvation ensues. Starvation involves a series of hormonal responses that can affect the fuel used by the different tissues.

In the human, the postabsorptive state is about 12 to 14 h after the last meal. In the mouse, it is 10 h; the rat, 17 h; guinea pig, 22 h; rabbit, 60 h; pig, 96 h; and the ruminant, 5–6 d. The time needed

TABLE 1.2
Methods and equations used for calculating basal energy need

	Method	Equation or units for expression
1.	Heat production, direct calorimetry	kcal (kJ)/m2 (surface area)
2.	Oxygen consumption; indirect	O_2 cons./W $^{0.75}$
3.	Heat production; indirect	Insensible water loss (IWL) = insensible
		Weight loss (IW) + (CO_2 exhaled – O_2 inhaled)
		Heat production = IWL × 0.58 × (100/25)
4.	Energy used; indirect	Basal energy = $\frac{\text{creatinine N (mg/d)}}{0.00482\,(\text{W})}$
5.	Estimate (energy need not measured; Harris Benedict equation)	BMR = 66.4730 + 13.751W + 5.0033L – 6.750A (men)
		BMR = 655.0955 + 9.563W + 1.8496L – 4.6756A (women)
6.	Estimate (energy need not measured)	BMR = 71.2W0.75 (men)
		BMR = 65.8W0.75 (women)

Note: W = weight in kg; L = height in cm; A = age in years.

to achieve the postabsorptive state depends on the length of the digestive tract and whether the animal is a ruminant (cow, sheep, goat) or has a sizable "fermentation vat" within the gastrointestinal tract. In some species, for instance, the rat and mouse, the cecum serves this function, whereas in others (i.e., the horse) an enlarged intestinal tract has this function. At any rate, the postabsorptive state is characterized by the respiratory quotient (RQ). The RQ is the ratio of CO_2 released to O_2 consumed. In the post absorptive state it is around of 0.85, which indicates that a mixture of fat and carbohydrate is being oxidized. If only fat were being oxidized, the RQ would be about 0.7. If only carbohydrate were being oxidized, the RQ would be 1.0. If energy need was being assessed by measuring heat production, the postabsorptive state would be that heat released at the point (in a continuous measurement) where the slope of the line changes, as shown in Figure 1.4.

As mentioned earlier, energy released as heat can be determined directly by using a calorimeter. This instrument is similar in concept to the bomb calorimeter used to measure the gross energy of food. It is nothing more than a very large insulated box with sensors that can detect very small differences in temperature. It can also be designed to include measures of spontaneous activity and in the case of small animals, can measure food and water intake. The size of the subject determines the

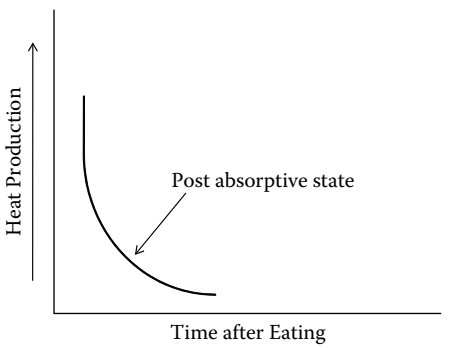


FIGURE 1.4 Changes in heat production mark the postabsorptive state.

size of the box. A small animal such as a mouse would need a very small box, whereas a cow or a horse would need a very large one. Using a calorimeter is extremely tedious and the instrumentation is very expensive. Much of the collected data can be computerized.[19,20]

When human heat production is assessed, the subjects live in the box for various periods of time from a few hours to several days depending on the objectives of the experiment. The heat emitted from the body is detected by sensors in the walls of the chamber and measured. There are corrections for clothing that might affect the measurement of heat released by the body. The food energy available to the subject is carefully measured before consumption, and the weight and height of the subject is known. The body weight/height measurements are used to calculate body surface area. To determine insensible body weight loss over the observation period, the subject is carefully weighed before and after the observation period. Any weight lost is considered to be the insensible weight loss. The feces and urine are collected, and their energy content determined. Usually a 24-h period of observation is used for the acquisition of basic measurements, then the subject may be asked to consume different foods or different amounts of foods or engage in different activities to determine the effects these changes have on heat production. Smaller time intervals can be used to assess heat production as a result of specific treatments such as the responses to hormone treatments or specific dietary ingredients or as a result of physical activity. The basal energy is presumed to be that heat produced by the subject upon waking, whereas the heat production of a sedentary life is that measured during the interval after waking. Heat production during sleep is also measured. Usually sleep induces a 10% decrease in heat production.

The calorimeter chamber is kept at a temperature that elicits neither sweating nor shivering. This temperature range is called the zone of thermic neutrality. Both sweating and shivering are energy-using processes. Sweating allows the individual to lose heat energy through water evaporation and loss. Shivering generates heat through surface muscle contraction and relaxation. Both processes are important to body heat regulation at environmental temperatures above and below the zone of thermic neutrality. If one were to measure energy loss above, at, and below this zone, increased energy loss would be observed at each extreme, as shown in Figure 1.5.

The temperatures that mark the limits of the zone of thermic neutrality can vary between species as well as between individuals within a species. The zone of thermic neutrality is influenced by body covering. The winter coat of many animals is longer and thicker than the summer coat. The

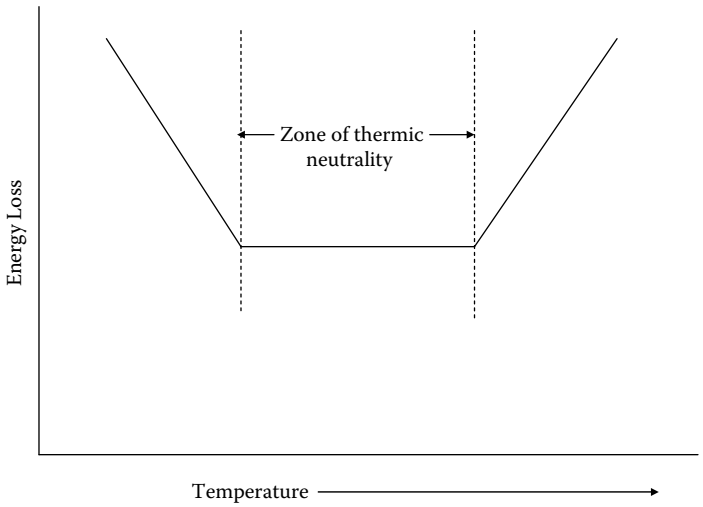


FIGURE 1.5 Zone of thermic neutrality. Energy is lost when the environmental temperature is below or above this zone.

winter hair or fur coat traps a layer of air around the body, which insulates it against the lowered environmental temperature. Humans do the same thing with their clothing choices. Heavier, more-insulating clothing is selected for winter wear keeping the human more comfortable when outside in cold weather. These strategies affect the temperature at which shivering as a heat-generating mechanism begins. In addition, many animals adopt social behaviors such as huddling that work to decrease the need to generate heat via shivering. Some animals go into hibernation (a deep, long-lasting sleep) that reduces their energy need and heat output. In the summer, the reverse occurs to allow heat dissipation without the need for sweating to facilitate body heat loss. Some species do not sweat but use panting to release excess energy. Animals lose their heavy hair coat while humans wear minimal clothing. Air conditioning serves to keep the environmental temperature in the thermal neutral zone. For humans, these environmental controls (heating/air conditioning) serve to decrease energy wasting, and this, in turn, affects energy balance, adding to the amount of energy available for storage as fat and for use to maintain the body's metabolism.

Directly measuring the energy lost as heat from the body is not always convenient or possible. Few clinical settings can afford the time and expense to assess energy needs in this way. There is an alternative. Metabolic processes not only produce heat but also consume oxygen. The consumption of oxygen and heat production are direct correlates of each other. Oxygen consumption can thus be measured as an indirect method of assessing energy use and need (see Table 1.2). The oxidation of energy-providing nutrients (fats, carbohydrates, and proteins) requires oxygen, which is used to make water and carbon dioxide. For every molecule of oxygen used to make water, there is an associated release of energy that is either trapped in the high-energy bond of ATP or released as heat.[22] The process whereby water and ATP are synthesized simultaneously is called oxidative phosphorylation (abbreviated, OXPHOS). Actually, there are two processes—one, respiration, joins hydrogen and oxygen ions to make water, and the other, ATP synthesis, uses some of the energy so released to synthesize ATP. These coupled processes occur in the mitochondria of the cell (see Chapter 3, "Nutritional Biochemistry").

Because each ATP has an energy value of about 30.5 kJ or 7.3 kcal/mol, the amount of energy each sequence will produce or use can also be estimated. Going one step further, the oxygen consumed can be measured and the amount of heat energy released estimated. This, then, is the basis for the indirect measure of energy expenditure through the measurement of oxygen consumed. The basal oxygen consumption can be measured, as can the increase in oxygen consumption with activity. The difference between growing and nongrowing animals can be obtained so that the energy cost of growth can be estimated.[21] Similarly, if a person or animal is either gaining or losing weight, the energy cost of this weight gain (or loss) can be estimated.[10] There are other uses for measuring oxygen consumption or respiratory rate. For example, the 24-h carbohydrate oxidation rate can be used to predict *ad libitum* food intake.[23]

The details of each of the major metabolic pathways are given in Chapter 3. Each pathway is characterized by the number of ATPs it uses or produces.[22,24,25] Not all of the ATPs are produced by the mitochondrial oxidative phosphorylation process. Some are produced as a product of substrate oxidation. Shown in Table 1.3 are the number of ATPs used or produced by these pathways. This number assumes that the reducing equivalents produced enter the OXPHOS system, which produces three ATPs if site 1 is used and two ATPs if site 2 is used.

As can be noted in Table 1.3, if the metabolic mixture consumed and oxidized is known, the energy change can be calculated. Rarely is this possible. Usually, the oxygen consumed and the carbon dioxide produced can be measured, and it can be determined whether the subject is gaining or losing weight and whether the subject is in nitrogen balance. Nitrogen balance means that the nitrogen from the protein in the food is replacing that of the proteins being degraded in the body. (The nitrogen balance concept is discussed in greater detail in Chapter 6.) Energy balance has a similar meaning. The energy used by the body is replaced by the energy provided by the diet. A subject in energy and nitrogen balance is neither losing nor gaining weight.

TABLE 1.3
ATP produced or used by the major metabolic pathways

Pathway	ATPs produced
Glycolysis	
Gluco or hexokinase	−1
Phosphofructokinase	−1
Glyceraldehyde 3 phosphate dehydrogenase-2 $NADH^+H^+$ produced	+6
Phosphoglycerate kinase	+2
Pyruvate kinase	+2
Krebs cycle	
Pyruvate dehydrogenase ($2NADH^+H^+$)	6
Isocitrate dehydrogenase ($2NADH^+H^+$)	6
a Ketoglutarate ($2NADH^+H^+$)	6
Succinate thiokinase	2
Succinate dehydrogenase (2FADH2)	4
Malate dehydrogenase ($2NADH^+H^+$)	6
Urea synthesis	
Carbamyl synthetase	−4
Protein synthesis	
Amino acid tRNA binding	2/amino acid
Amino acid tRNA bound to ribosome	−1/amino acid
Amino acid chain formation	0
RNA degradation	−2
Fatty acid synthesis	
Formation of methyl malonyl CoA	−1
Fatty acid oxidation	
Activation of fatty acid (thiokinase)	−1
Acyl-CoA dehydrogenase (FADH)	2/acetyl unit
L(+), b hydroxyacyl CoA dehydrogenase (NAD^+H^+)	3
Krebs (citric acid or TCA) cycle	24/acetyl unit

When glucose is the sole substrate being oxidized, then the RQ is 1 because the oxidation of glucose uses and releases equal amounts of oxygen and carbon dioxide.

This complete oxidation to CO_2 and HOH will produce 36 molecules of ATP. When fatty acids are being oxidized, the RQ is close to 0.7. This is because fatty acids require far more oxygen for their oxidation than they produce as carbon dioxide.

$$\text{Palmitic acid, } C_{16}H_{22}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ HOH$$

$$RQ = 16\ CO_2/23\ O_2 = 0.7$$

When the fatty acids are mobilized from the stored triacylglycerols and the RQ is computed on this basis, a slightly higher RQ results. For example, using a theoretical triacylglycerol consisting of two molecules of stearic acid and one of palmitic acid attached to a glycerol backbone, the equation is as follows:

$$C_{55}H_{106}O_6 + 76.5\ O_2 \rightarrow 55\ CO_2 + 53\ HOH$$

$$RQ = 55\ CO_2/76.5\ O_2 = 0.719$$

A molecule of glucose will, when oxidized to pyruvate, yield a net of 8 molecules of ATP (2 molecules of ATP are needed for glycolysis). When glucose is oxidized to CO_2 and HOH, 36 molecules of ATP are formed. A fatty acid such as palmitate will yield a net of 129 molecules of ATP (2 molecules of ATP are needed for activation). If each ATP has an energy value of 7.3 kcal (30.5 kJ)/high-energy bond, then, in theory, glucose oxidation to pyruvate should yield 7.3 kcal (30.5 kJ/mol) × 8 ATP or 58.4 kcal (244.3 kJ) per molecule. Complete glucose oxidation should yield 262.8 kcal (1099.6 kJ) and palmitate should yield 7.3 kcal × 129 ATP or 941.7 kcal (3940 kJ) trapped in the high-energy bond of ATP. However, these figures do not correct for the energy trapped in the ATP molecule through substrate phosphorylation, nor do they correct for energy lost as heat. The assumption is also made that the reducing equivalents produced by these catabolic reaction sequences will be sent to the OXPHOS system for ATP synthesis. This does not always occur. When palmitate is oxidized in a bomb calorimeter, 2609 kcal (10,958 kJ) of heat is released per molecule. Compare this to the 941.7 kcal (3940 kJ) calculated above for the in vivo oxidation of palmitate. Obviously, in the living system, not all of the energy of nutrient oxidation is captured in the high energy bond of ATP, GTP or UTP; most of it is released as heat. The difference between the in vitro and in vivo oxidation of palmitate has to do with the energetic efficiency of the living system. Some energy is lost when the carbon is joined to the oxygen to make carbon dioxide; some is lost when water is made and, of course, a lot is used to make the high energy bond of ATP and its related compounds, GTP and UTP. If a ratio were to be made of the amount of energy captured in the ATP to that totally available (941.7:2609), the finding would be that about 36% of the total inherent energy of palmitate can be captured in the chemical bonds of ATP versus ~64% that is released as heat. Note that palmitate oxidation produces more than three times the amount of ATP than does glucose.

The ratio of glucose to fatty acids being oxidized will be reflected in the measured RQ. Most humans consuming a mixed diet will have an average RQ of about 0.87. It will vary throughout the typical feeding-fasting, day-night cycle followed by most people who work during the day and sleep at night. Just before breakfast, the RQ will be at its lowest, whereas after the evening meal it will likely be at its highest. This, of course, is dependent on the composition of the meals consumed as well as on their spacing. As mentioned in the beginning of this section, the basal heat production or oxygen consumption is usually measured in the postabsorptive state. This usually means a measurement made before breakfast and it also usually means that the metabolic fuel mix is that of glucose (from glycogen) plus fatty acids (from the fat depots). Other species may have different patterns. For example, the rat, a nocturnal animal will have been very active during the night and will rest during the day. The day–night cycle in RQ will therefore be reversed.

ENERGY RETAINED

After the heat production associated with exercising muscles and the inefficiency of energy capture as described above are accounted for, two other components must be included. These are the energy that is either stored in the fat and glycogen depots and the energy needed to sustain growth or production.[25a] This is quite straightforward. If weight is neither gained nor lost, then the energy balance (energy retained) is zero. If weight is gained as fat, the energy value of that fat must be accounted for. Body composition studies can provide the estimate for fat gained or lost. If the fat is deposited as preformed fat, that is, the fat in the diet is hydrolyzed at the gut, transported to the depot, and reesterified for storage, the energy cost of this storage is minimal. This is a very efficient way to accumulate body fat stores. On the other hand, if glucose is used to make the stored

triacylglycerols, the process is quite inefficient. To make one molecule of tripalmitin (palmitate is a 16 carbon fatty acid), 12 molecules of glucose and 45 molecules of ATP would be needed to make 3 molecules of palmitoyl CoA. Another half molecule of glucose and four ATPs would be needed to make the glycerol phosphate. Altogether, 12.5 moles of glucose plus 49 moles of ATP would be necessary to make 1 mol of tripalmitin. To generate the ATP for this synthesis, 1.4 mole of glucose would have to be completely oxidized because one mole would give only 36 ATPs and 49 are needed. Tripalmitin synthesis from glucose is energetically more expensive because only four of the six glucose carbons are used to make the 16-carbon fatty acid, palmitate. It will take four glucoses to make one palmitate and 12 glucoses to make three palmitates.

$$12 \text{ glucose} + 45 \text{ ATP} + O_2 \rightarrow 3 \text{ palmityl CoA} + 24 \text{ } CO_2$$

$$0.5 \text{ glucose} + \text{ATP} \rightarrow \alpha \text{ glycerophosphate} + \text{ADP}$$

$$3 \text{ palmityl CoA} + \alpha \text{ glycerophosphate} \rightarrow \text{tripalmitin}$$

1 mol of glucose completely oxidized yields 36 ATP and 49 are needed. Thus, 49/36 = 1.4 moles of glucose are needed to provide the ATP or $1.4 \times 294.8 = 412.72$, and $12 + 0.5 + 1.4 = 13.9$ mole of glucose are needed to produce 1 mol of tripalmitin; each glucose has a value of 294.8 kcal or 1238 kJ.

Table 1.4 illustrates these calculations.

However, if this cost was computed using the energy value of tripalmitin (7597 kcal or 31,907 kJ) corrected for the energy value of all the glucose needed to make the palmityl CoA and all the ATP used, the cost would be $(7597 - 4068 - 412.72) \div 7597$ or 41% of the total energy value of the tripalmitin. To store preformed tripalmitin would cost 10 ATP, each with a value of 7.3 kcal or 30.5 kJ. The total cost would be 73 kcal or 305 kJ. If the total energy value of tripalmitin was 7597 (31,907 kJ), then the efficiency of storing preformed fat would be $(7597 - 73) \div 7597$ or ~98%.

Using protein for energy is even more costly and less efficient because the protein that is degraded for energy must first be broken down into its constituent amino acids, and these, in turn, must be deaminated and the amino group used to make urea. It takes 5 ATPs to make one molecule of urea. Approximately 20% of the gross energy of a typical protein is lost because of the need to synthesize urea. So, energetically, this is a very expensive process. Nonetheless, many humans overconsume food protein and, of course, when this occurs, the surplus protein is degraded. The carbon skeletons of the deaminated amino acids can either be oxidized for energy or used to synthesize body fat or used to synthesize glucose (gluconeogenesis). The latter process would only use a very small number of these carbon skeletons.

TABLE 1.4

Efficiency of storing energy from dietary fat versus that using glucose for triglyceride synthesis (tripalmitin)

Cost
3 pal + 3ATP + 3CoASH → 3 Pal CoA + 3AMP (2 ATP/palmitate → pal CoA)
4ATP + 0.5 glucose → αGP + 4ADP
3 Pal CoA + αGP → tripalmitin
10ATP/36ATP per mole glu = 0.28 moles glucose
0.5 + 0.28 = 0.78 moles glucose + 3 moles palmitate + 10 moles ATP → 1 mole tripalmitin

Protein synthesis is likewise very expensive energetically. Each amino acid that is incorporated into a polypeptide chain requires an ATP for its activation. In a protein containing many hundred amino acids, the requirement for ATP is enormous. ATP is also required for the synthesis of the mRNA coded for the protein being synthesized. Because protein synthesis is so energy dependent, the energy requirement to support growth (protein synthesis) can be quite large.

Using dietary protein for body protein synthesis can be either very efficient or very inefficient, depending on the amino acid content of the diet and the body proteins being synthesized. This aspect of metabolism will be described in Chapter 6. However, the energetics of protein deposition using dietary protein is quite straightforward.

Disregarding the energy cost of amino acid oxidation, urea formation, and the growth process, if the energetic efficiency of protein turnover is examined, an efficiency of about 82% is found. This figure is arrived at using the assumption that the dietary protein is used *completely and exclusively* to replace degraded body protein. For ease of description, let us take a hypothetical amount (100 g) of protein that is to be degraded and replaced. This 100 g would have a gross energy value of 570 kcal or ~2384 kJ. For every amino acid incorporated into this protein, 5 ATPs would be used plus 1.2 ATPs for rearrangements of the structure. This would mean an energy cost of about 694 kcal or ~2904 kJ. Dividing the energy value of the protein produced by the cost of its production gives an efficiency of about 82%. Practically speaking, for the average human adult in the United States, the energy efficiency of protein turnover is of little importance. The typical U.S. diet is not limiting with respect to protein or energy.

ENERGETIC EFFICIENCY

The food that provides the energy does so by providing fuel for oxidation to provide heat, ATP, CO_2, and water. The heat produced through this oxidation is needed to keep the body warm, but it is also a measure of the energy need. That is, the heat produced (and that can be measured) is the energy that is lost from the body and must be replaced. Of the inherent energy of palmitate, 36% is trapped by the body when this fuel is used, and 64% is released as heat (see discussion above). This 40/60 (rounded figures) distribution of energy available to the body versus that released as heat is an average distribution. More or less can occur in each category. The pattern of distribution is influenced by the composition of the diet,[25–28] age or life stage,[29–33] and the genetics of the consumer.[34–44] An example of the effect of diet composition on energetic efficiency occurs when an essential fatty acid-deficient diet is fed. This deficiency state is characterized by a decrease in the efficiency of use of food for body weight gain (see Chapter 8). On the other hand, growing individuals are more efficient than nongrowing individuals. Genetically obese individuals are likely more efficient at storing fat than are genetically thin people.

Feed efficiency is a term used to designate the efficiency with which an animal (or human) uses the food it consumes to build new tissue as it grows or rebuild body tissue that is lost through the normal conditions of life. Those animals that can gain more body weight on less food are more efficient than those animals that require more food for the same gain. Meat-animal production research has been devoted to increasing energetic efficiency using dietary and nondietary techniques. Selective breeding, for example, of beef animals has resulted in bovines that grow rapidly and consume less food per pound of weight gained than animals used for this purpose a century ago. Whereas it used to take three years to produce a bovine of a size suitable for use, it now takes 2 years or less to produce a meat animal ready for market. Furthermore, the meat produced today is of higher quality in terms of tenderness, flavor, and nutrient content than that produced 100 years ago. Other meat animals (sheep, pigs, chickens, goats) likewise have been selectively bred to produce a rapidly growing, energetically efficient animal. With the growing emphasis today on the production of meat with a lower fat content, animal scientists are continuing to use genetics and dietary maneuvers to produce an animal that meets the consumers' demands.

Just as the diet and genetics of the meat animal determine its energetic efficiency, so, too, can these same effects be expected in humans. Genetic and dietary factors interact and, in so doing, determine the rate and extent of longitudinal growth as well as the development of muscle mass and fat mass. At present, the segregation of humans by genotype although theoretically possible, is not practical on a population basis.[41–43] However, genes that appear to be important in the development of obesity (excess fat mass) are being identified (see www.ncbi.nih.gov/OMIM). How diet, particularly the sources of energy, can affect the phenotypic expression of these obesity-related genes is being explored.[45]

UTILIZABLE ENERGY

The energy expended to do the body's work is referred to as utilizable energy. This is the energy used by muscles as they contract in the course of voluntary activity or work. This energy is sometimes referred to as the activity increment. That is, it is the energy cost associated with body movement, which is added to the basal energy need when the energy requirement is calculated.[46] For sedentary persons, only a small increment is needed but for very active persons, a large increment will be needed to sustain activity. As much as a 200% increment above basal energy need may be required to sustain a high level of activity and maintain body weight. Shown in Table 1.5 are some representative activities and their associated energy cost given as heat units per kilogram body weight per hour.

In the course of muscle contraction, oxygen is used for the oxidation of metabolic fuels by the working muscle. Heat is released, and water and carbon dioxide are produced. The more active a person is, the more oxygen is needed for the oxidation of metabolic fuel by the muscle. Trained athletes are more efficient in their use of metabolic fuels than are nontrained, sedentary individuals. Training involves an increase in use of specific muscle groups as well as an increase in lung and heart action. Muscle contraction, which is usually supported by the oxidation of glucose (from blood glucose or glycogen breakdown) in the sedentary person, can use fatty acids as fuel after training. During exercise, the respiratory quotient (RQ) in the trained individual decreases. This means that fat is being oxidized because the oxidation of fatty acids results in a lower RQ than the oxidation of glucose. Fatty acid oxidation by working muscle will spare glucose and will decrease the rate of lactate production. The muscle does not store large amounts of lipid for use during work, but it can use fatty acids liberated from fat depots through the action of the catabolic hormones. Part of training is to increase muscle fatty acid oxidation while also increasing the glycogen–glucose reserve in the muscle. The so-called glycogen-loading technique used in training endurance athletes, dictates the exhaustion of the muscle glycogen store followed by rest and glycogen repletion just prior to the competitive event. During the rest/repletion period a high-carbohydrate diet is consumed. This exhaustion/repletion routine results in an increased supply of glucose from glycogen within the muscle. Because the first phase of glycolysis is anaerobic, an enlarged supply of glucose from glycogen can be oxidized in the absence of oxygen, and thus exhaustion, which is characterized by an oxygen debt, is delayed. Training or adaptation to exercise or work is thus characterized by decrease in RQ due to both an increase in the oxidation of fatty acids and an increase in the anaerobic use of glucose from glycogen. Table 1.6 summarizes the effects of exercise on metabolism, whereas Table 1.7 gives some typical results of a comparison of trained and untrained subjects with respect to their oxygen and glucose use during a bout of exercise. Note that the untrained and trained subjects consumed the same amount of oxygen, but the trained subjects had a lower RQ. This means that the trained subjects were oxidizing some fatty acids. That fatty acids were used is seen in the last column, showing that the trained subjects consumed less glucose per minute than did the untrained subjects.

Training also has effects on a number of hormones. One study by Jurimae et al.[47] reported that plasma ghrelin levels were significantly increased following exercise by trained male athletes. Ghrelin is a small peptide involved in growth hormone secretion and in energy homeostasis. It is a

TABLE 1.5
Energy cost of activities, exclusive of basal metabolism and the influence of food

Activity	kcal/kg/h	(kJ)	Activity	kcal/kg/h	(kJ)
Bedmaking	3.0	12.6	Playing cards	0.5	2.1
Bicycling (century run)	7.6	31.9	Playing ping pong	4.4	18.4
Bicycling (moderate speed)	2.5	10.5	Piano playing (Mendelssohn's *Song Without Words*)	0.8	3.3
Boxing	11.4	47.9	Piano playing (Beethoven's *Appassionata*)	1.4	5.9
Carpentry (heavy)	2.3	9.7	Piano playing (Liszt's *Tarantella*)	2.0	8.4
Cello playing	1.3	5.5	Reading aloud	0.4	1.7
Cleaning windows	2.6	10.9	Rowing	9.8	41.0
Crocheting	0.4	1.7	Rowing in race	16.0	66.9
Dancing, moderately active	3.8	16	Running	7.0	29.3
Dancing, rhumba	5.0	21	Sawing wood	5.7	23.8
Dancing, waltz	3.0	12.6	Sewing, hand	0.4	1.7
Dishwashing	1.0	4.2	Sewing, foot-driven machine	0.6	2.5
Dressing and undressing	0.7	2.9	Sewing, electric machine	0.4	1.7
Driving car	0.9	3.8	Singing in loud voice	0.8	3.3
Eating	0.4	1.7	Sitting quietly	0.4	1.7
Exercise, Very light	0.9	3.8	Skating	3.5	14.6
Light	1.4	5.6	Skiing (moderate speed)	10.3	43.1
Moderate	3.1	13	Standing at attention	0.6	2.5
Severe	5.4	22.7	Standing relaxed	0.5	2.1
Very severe	7.6	31.9	Sweeping with broom, bare floor	1.4	5.9
Fencing	7.3	30.7	Sweeping with carpet sweeper	1.6	6.7
Football	6.8	28.6	Sweeping with vacuum sweeper	2.7	11.3
Gardening, weeding	3.9	16.4	Swimming (2 mi/h)	7.9	33.1
Golf	1.5	6.3	Tailoring	0.9	3.8
Horseback riding, walk	1.4	5.9	Tennis	5.0	20.9
Horseback riding, trot	4.3	18.0	Typing, rapidly	1.0	4.2
Horseback riding, gallop	6.7	28.0	Typing, electric typewriter	0.5	2.1
Ironing (5 lb iron)	1.0	4.2	Violin playing	0.6	2.5
Knitting sweater	0.7	2.9	Walking (3 mi/h)	2.0	8.4
Laboratory work	2.1	8.8	Walking rapidly (4 mi/h)	3.4	14.2
Laundry, light	1.3	5.4	Walking at high speed (5.3 mi/h)	8.3	34.7
Lying still, awake	0.1	0.4	Washing floors	1.2	5.0
Office work, standing	0.6	2.5	Writing	0.4	1.7
Organ playing (1/3 handwork)	1.5	6.3			
Painting furniture	1.5	6.3			
Paring potatoes	0.6	2.5			

TABLE 1.6
Summary of the effects of training on metabolism and body composition

Decreased RQ during exercise
Decreased fat mass
Increased muscle mass[a]
Increased fatty acid mobilization during exercise
Increased glucose synthesis by the liver
Increased aerobic capacity of muscle
Improved glucose tolerance
Decreased need for insulin to facilitate glucose use
Decreased heat production via activation of uncoupling proteins in muscle[a]

[a]May be localized; depends on the types of muscles used for the activity.

TABLE 1.7
Effect of training on oxygen uptake and fuel use

Subject	O_2 L	RQ CO_2/O_2	Glucose mmol/min
Trained	3.0	0.90	10.6
Untrained	3.0	0.95	13.3

gut hormone produced by the stomach and signals satiety information to the hypothalamus and also signals the release of growth hormone. Growth hormone stimulates protein synthesis and lipolysis. Plasma ghrelin levels are often reduced in obese individuals and substantially elevated in starving or malnourished people. It is likely that the increased muscle mass of the trained athlete can be explained in part by the exercise effect on not only ghrelin but also on the increased release of leptin, growth hormone, testosterone, and insulin-like growth factor (IGF-1). All of these hormones play a role in energy homeostasis as well as on the partitioning of energy and nutrients toward body protein and fat.

THERMOGENESIS

Another way energy is lost from the body as heat is the heat lost as one or more of the uncoupling proteins are activated. In early years, it was thought that this heat was associated with the degradation of the food components in preparation for its use by the body for its maintenance. Hence, it was called *diet-induced thermogenesis* (DIT). Now, however, there seems to be another explanation having to do with the production and release of proteins called uncoupling proteins.[48–58] The first of these, UCP_1, was identified in the brown fat depots. These brown fat depots are located at the base of the neck, along the backbone and, in males, across the shoulders. The brown fat cell differs from the white fat cell in that it contains many more mitochondria in the cytosol than does the white cell. Both cell types have stored lipid droplets that, if sufficiently large, can push all of the other organelles off to the side of the cell. In contrast to the white cell depots, the brown fat depots are highly innervated and vascularized. This increase in vascularization gives the depot its brownish color and name.

As described above, much of the structure of the white cell is similar to that of the brown fat cell.[48] Whereas white depots are distributed throughout the body, brown depots are found in just a few locations. Brown fat cells, if allowed to accumulate lipid, may change in appearance and look like white fat as the lipid droplets enlarge. Histochemical studies have failed to reveal any qualitative differences between the fat depots of the two cell types. More brown fat can be observed in newborns than in adults, and it is thought that thermoregulation in the immature animal is dependent on the activity of the brown fat depot with respect to its ability to generate heat. Both cell types can store lipid, and this lipid can be readily mobilized. However, when it mobilizes its stored lipid, the brown fat oxidizes its lipid in situ rather than releasing it for utilization by other tissues. When stimulated, the brown fat can produce more heat during metabolism than can the white fat cell. The heat produced through the action of the UCP is especially important to newborns because they lack the insulation provided by the subcutaneous fat store. This fat layer helps conserve body heat. Additional heat production is provided by the newborn's metabolic processes, which are relatively immature with respect to energy conservation. Heat loss from these processes can be quite large. As the newborn matures biochemically as well as physiologically, the importance of these uncoupling proteins in energy balance subsides. The baby begins to accumulate a subcutaneous fat layer and no longer has to endure the penalty of an inadequate energy store. Glycogen accumulation and utilization patterns mature as well, so that the infant and young child can be fed less frequently.

The brown fat depot is not the only tissue that contributes to thermoregulation.[52,53,55] Heat production due to uncoupling protein also occurs in muscle and white adipose tissue. Uncoupling proteins 2 and 3 (UCP_2 and UCP_3) have been identified. Their distribution is quite different from that of UCP_1. Whereas UCP_1 is located in the brown adipocytes, UCP_3 is found in skeletal muscle; UCP_2 is found both in brown fat pads and white fat pads as well as muscle.[52–56] The expression of these uncoupling proteins is downregulated by endurance exercise training.[55] UCP_2 not only has a role in thermogenesis but also, in an inverse relationship, regulates hydrogen peroxide generation, mitochondrial reactive species, and therefore the redox state and oxidative stress of the cell.[54] As UCP_2 rises, peroxides levels fall. If muscle UCP_2 rises in skeletal muscle, utilization of lipid by that muscle falls.[53] Dietary fat type can affect brown fat thermogenesis.[58] Diets rich in polyunsaturated fats are less thermogenic than diets rich in saturated fats.

ABNORMAL ENERGY STATES

Starvation and Undernutrition

The basic metabolic response to starvation of an otherwise healthy individual is conservation. As the gut receives less food, its emptying time is reduced. First, the stomach, then the duodenum, the jejunum, the ileum, and the large intestine lose their contents and shrink in size. Simple mono- and disaccharides are the first to disappear, followed by the products of the progressively more complex nutrients: polysaccharides, proteins, and lipids. As the sugars disappear, there is less stimulus for insulin release and basal insulin levels are approached. As glucose is less available from the gut, the body begins to mobilize its glycogen stores and when they are close to being depleted, the body will begin to mobilize its stores of triacylglycerol and, to a lesser extent, its body protein. It can use certain of the amino acids and glycerol from the triacylglycerols to synthesize glucose via gluconeogenesis and utilize the carbon skeletons of deaminated amino acids plus the fatty acids liberated from the triacylglycerols for fuel. In 1915, Benedict,[59] in an extensive monograph on the metabolic responses to starvation, reported that the body carbohydrate (glycogen and glucose) provided only a small fraction of the total fuel needed for maintenance. Cahill[60] later estimated that a 70-kg man had approximately 75 g of glycogen stored in the muscle and liver, which provides approximately 300 kcal (1255 kJ) during the initial 24 h of starvation. The glycogen and the glucose synthesized via gluconeogenesis thus provide only a small percentage of the 2000–2500 kcal (8368–8577 kJ) needed per day for maintenance. Benedict[59] estimated that mobilizable body protein could provide

about 15% of the body's fuel needs and that the fat stores of the adipose tissue provided the rest. Although Benedict did not have today's sophisticated technology at his disposal, his estimates were remarkably close to those of Cahill. Cahill[60] estimated that a "normal" 70-kg man required about 2000 kcal (8368 kJ) per day to maintain his body and that he had sufficient fuel stores to sustain life for about 80 d. As shown in Figure 1.6, adapted from Cahill's paper, most of the energy comes from the lipid stored in the adipose tissue. While Cahill and others evaluated the energy losses from the whole animal, interorgan fuel fluxes have been studied as well. Figure 1.6 illustrates these fluxes during the first 24 h of starvation.

Lipids, primarily triacylglycerols, are hydrolyzed to fatty acids and glycerol through the action of hormone-sensitive lipase. This enzyme, located on the interior aspect of the fat cell membrane, is activated by the catabolic hormones, epinephrine, and glucocorticoid, and its activity is increased when glucagon is high and insulin is low. The glycerol is converted to glucose via gluconeogenesis in the liver and kidney. Most of the glucose formed in the kidney is used in situ whereas hepatic glucose production can supply glucose to the rest of the body. The fatty acids are oxidized to ketones and then to carbon dioxide and water. Whereas the liver and some other tissues can oxidize the fatty acids completely to carbon dioxide and water, the muscle is unable to do so and thus ketones, the end products of muscle fatty acid oxidation, rise. These ketones can be used by the brain as a fuel when the supply of glucose becomes more limited. For example, after about 40 days of starvation, the brain will obtain nearly 65% of its energy from the ketones. These fuel fluxes are diagrammed in Figure 1.7. Under normal (i.e., fed) conditions, the central nervous system (CNS) uses 115 g of glucose/day whereas erythrocytes, bone marrow, renal medulla, and peripheral nerves use about 36 g of glucose/day. Most of this glucose can be synthesized through gluconeogenesis from glycerol, lactate, and selected amino acids during the early phase of starvation. However, as the starvation continues, the body attempts to protect its protein component and the amino acid substrates for gluconeogenesis

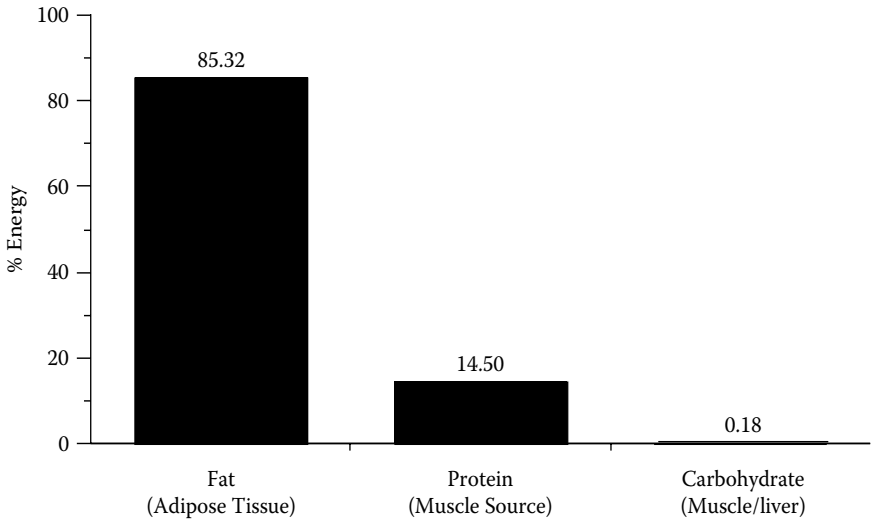


FIGURE 1.6 Sources of metabolic fuel for starving human body. The lipid utilized is primarily the fatty acids released from triacylglycerol by the adipose tissue depots; protein is mainly that raided from the muscle; carbohydrate utilized is that released by the liver and muscle glycogen and that synthesized by the kidney and liver via gluconeogenesis from selected amino acids and from the glycerol moiety of triacylglycerol released by the adipose tissue. (Adapted from Cahill, G.F., Herrera, M.G., Morgan, A.P., Soeldner, J.S., Levy, P.L., Reichard, G.A., and Kipnis, D.M. [1966]. *J. Clin. Invest.* 45: 1751–1769; Saudek, C.D., and Felig, P. [1976]. *Am J. Med.* 60: 117–126.)

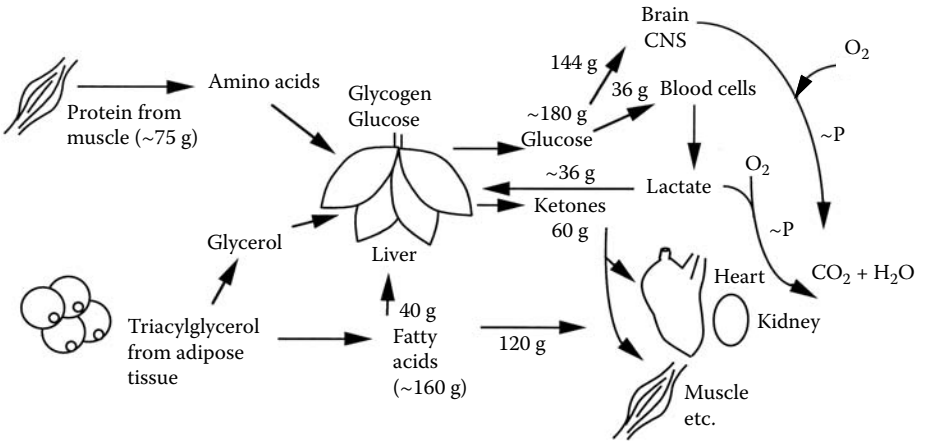


FIGURE 1.7 Intertissue fuels fluxes after 24 hours of starvation. Note the central position of the liver. This organ uses glycerol, lactate, fatty acids, and amino acids to produce ketones and glucose, which serve as fuel sources for blood cells, the brain and central nervous system, heart, kidney, and muscle. These fuels are oxidized to carbon dioxide and water, requiring only the presence of molecular oxygen and energy from the high-energy phosphate bonds as provided by the adenine and guanine nucleotides.

become less available. This, coupled with the rising ketone level (ketones can cross the blood–brain barrier), serves to induce the utilization of the ketones by the brain as a metabolic fuel.

Proteolysis, initially increased, after 48 h of starvation, is suppressed by rising levels of growth hormone as the body attempts to conserve its body proteins. The initial proteolysis, however, serves to provide the needed amino acids for the synthesis of enzymes needed for survival (enzymes needed for energy mobilization and conservation). Once these mechanisms are established, body protein is conserved. The temporal relationship of fuels and the hormones that control their availability is diagrammed in Figure 1.8.

Protein-Energy Malnutrition

Starvation is the extreme state of malnutrition that occurs when the individual is provided little or no food to nourish the body. Between starvation and the state of adequate nourishment to meet nutrient needs, there are graded levels of inadequate nutrient intake. Although infants and children of third-world nations come to mind when malnutrition is pictured, people of all ages in all countries are vulnerable. Where the intake of macronutrients is inadequate, the syndrome is called protein-calorie malnutrition (PCM) or, more correctly, protein-energy malnutrition.

Chronic malnutrition is characterized not only by energy deficit (energy need exceeding energy intake) but also by a deficit in the protein intake and the intake of micronutrients. The needs for these nutrients and energy are determined by the age and health status of the individual. Rapid growth, infection, injury, and chronic debilitating disease can drive up the need for food and the nutrients it contains. Infants and young children have been described as having PCM. Just as infection or trauma potentiates the needs for protein, energy, and micronutrients in third-world children, these conditions also increase the need for nutrients in developed nations. Injury and sepsis both increase energy needs. Both adults and children may have increased nutrient needs under these circumstances and if these needs are not met, varying degrees of malnutrition or PCM will be observed. Malnutrition has been documented in hospitalized patients in the United States, and this malnutrition may have a negative effect on the time course of recovery as well as on mortality.

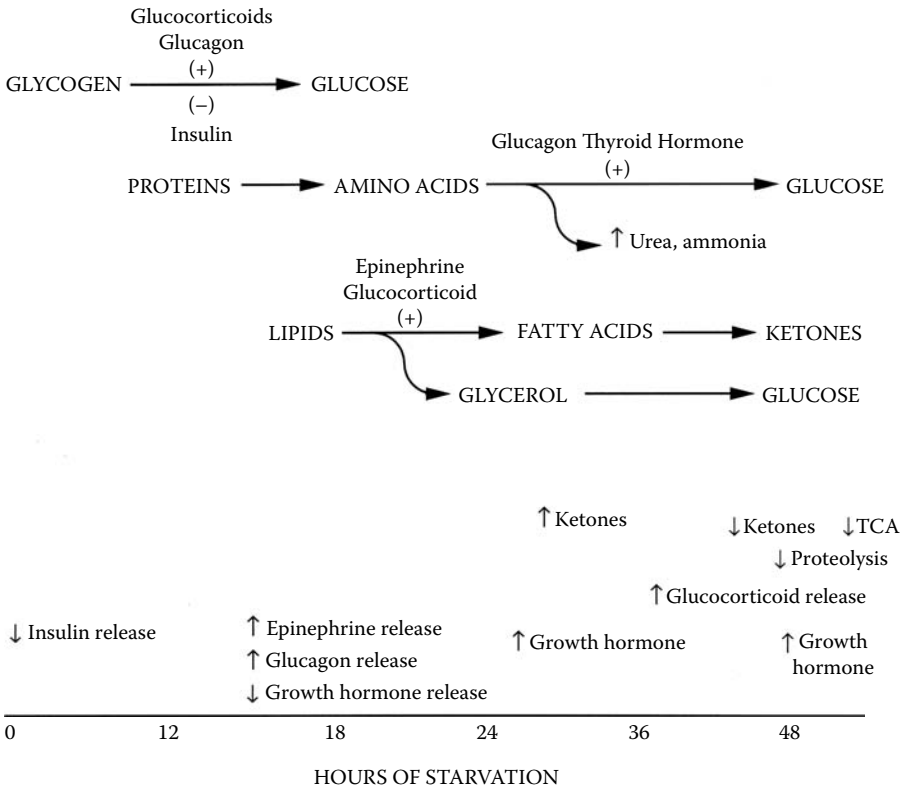


FIGURE 1.8 Temporal relations of fuels and hormones during starvation. During the first 24 h, there is a decrease in blood glucose levels and an increase in proteolysis. After 18 h, measurable increases in gluconeogenesis due to glucose production from glycerol can be observed. There is a rise in lipolysis, initially stimulated by epinephrine but then maintained by rising glucocorticoid, glucagon, and growth hormone levels. As these hormones rise, they serve to decrease peripheral tissue sensitivity to insulin, and decreased glucose tolerance can be observed. With rising growth hormone levels, proteolysis is decreased and the body attempts to conserve body protein. By 48 h, ketosis, having been high, now begins to decline as the body adapts to using ketones and fatty acids as metabolic fuels.

Trauma and Energy Needs

Many years ago a disproportionate catabolic response to trauma was reported. Since that time, physicians, nutritionists, and physiologists alike have accepted this as an obligatory and necessary response to illness or injury. With the advent of parenteral and enteral feeding techniques, it has been possible to show that this loss, formerly thought to be obligatory, is not. Indeed, losses due to surgery, infection, or burn have been shown to be minimized and even reversed (in some cases) with the provision of adequate nutritional support. This implies then, that the catabolic response to trauma or illness is more a response to inadequate nutrition in the face of a greatly increased set of nutrient requirements than to the trauma or illness itself.

The endocrine and metabolic responses to injury are closely related (Figure 1.9). Increased production of some hormones and the inhibited release of others are integral parts of the body's basic defense mechanism. The primary function of this mechanism is to provide a continuous fuel supply to the CNS and the required substrates for the repair of body tissue. While the body may respond to trauma with an increased release of the hormones that regulate metabolism through cAMP or through the phosphatidylinositol cycle, this response, in comparison with the overall

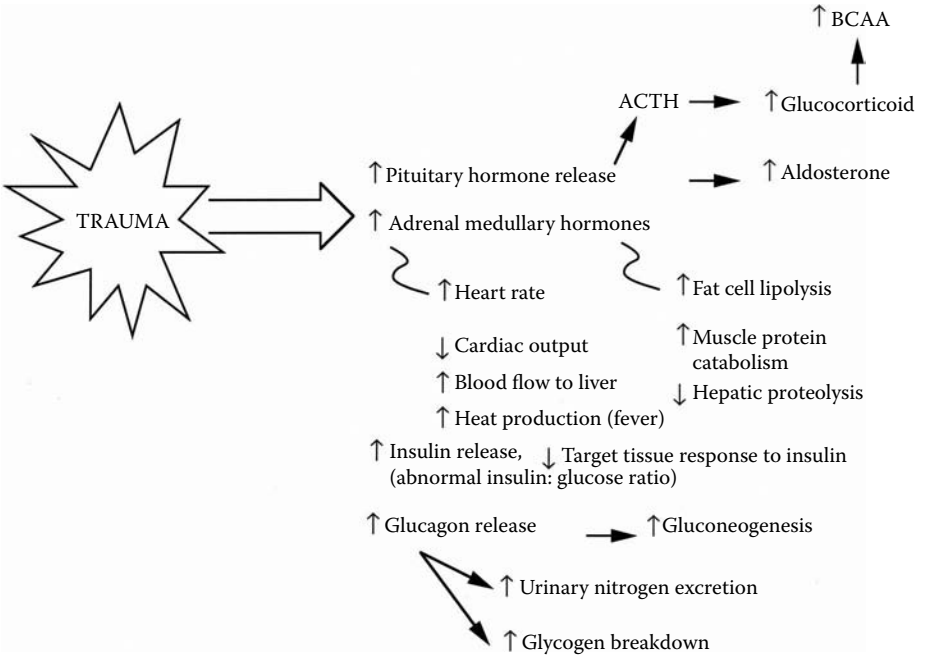


FIGURE 1.9 Trauma elicits both hormonal and metabolic responses that result in increased energy need. ACTH, adrenocortico stimulating hormone; BCAA, branched chain amino acids.

metabolic response to injury or illness, is relatively short-lived. It appears, therefore, that the hormonal responses to injury serve as initiators or inducers of metabolic events that must occur if recovery is to proceed. As relief from the trauma occurs, these hormonal responses recede and other metabolic control mechanisms assume command. These, in turn, give way to the normal metabolic control mechanisms as recovery proceeds. Aging patients and children likely will have a more exaggerated metabolic response to trauma than young, otherwise healthy adults. Although the sequence of hormonal and metabolic response may be similar, the time frame and intensity of response will differ. Sometimes these differences can be life threatening if close monitoring is not practiced. The elderly may already have peripheral tissue insulin resistance, which is made worse by the trauma. The child may be more likely to quickly exhaust energy stores and have little reserve. In each instance, the difference between death and survival may rest with the provision of adequate nutrition support in terms of amounts of energy, glucose, and amino acids plus appropriate amounts of insulin to facilitate the return to normal fuel homeostasis.

The temporal changes in the choice of metabolic fuels are characterized by subtle transitions from one fuel source to another. These are orchestrated by the hormonal response to the trauma or infection, which in turn may be influenced by the preinjury nutritional status of the patient and the nutrients provided during the recovery period.

Basal energy requirements can increase by as much as 200% in traumatized patients. Part of this increase can be attributed to the thermogenic effects of the catecholamines released in response to the injury. In addition, increases in energy requirement may be due to the increased energy needed to support protein synthesis. Protein synthesis is highly dependent on energy intake. New proteins are needed for tissue repair and for the inflammatory and immunologic responses of the body to infection or injury or both. If the diet is inadequate, the synthesis of these proteins occurs at the expense of muscle protein. Amino acids not used for protein synthesis are deaminated and the resulting ammonia converted to urea. Urea production likewise is energetically expensive. Thus,

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large increases in both protein synthesis and ureagenesis represent an increase in the basal energy requirement and an increase in the protein requirement in the traumatized or severely ill individual. Unless this additional energy and protein are provided, weight loss will occur.

Obesity

Whereas with starvation, inadequate energy intake and trauma represent negative energy balance (more energy is being used than is being consumed), obesity represents that condition where more energy is consumed than is expended. This is positive energy balance. This excess energy is stored as fat in the body. How is body fat determined? Is an increase in body weight due to only an increase in fat store or could this increase be due to a change in lean body mass? There are a variety of methods that will indicate the composition of the body. Some of these are direct and useful in small rodents, whereas others are useful for ongoing studies of body composition change.

Body Composition

Of the four major body constituents—fat, protein, water, and ash (minerals)—water is by far the most abundant.[62–71] Normal bodies usually consist of 16–20% protein, 3–5% ash (mineral matter), 10–12% fat, and 60–70% water. Age, diet, genetic background, physical activity, hormonal status, and gender can affect not only the proximate composition of the whole body—that is, the magnitude of each of these components—but also their distribution.

Regardless of the methods used to estimate lean body mass, total body water, and percent body lipid, it is generally agreed that the composition of the human body, as well as the bodies of other species, can vary. Age, sex, degree of physical activity, and diet have all been shown to affect body composition. For example, a 175-g fetus contains 154 g of water (88%); a 3.5-kg baby contains 2.4 kg of water (69%); and a 70-kg man contains 42 kg of water (60%). As the human progresses from conception to birth to maturity, the percentage of water decreases and, as mentioned previously, the percentage of fat increases. Actively growing tissue has a high water content and very little fat.

Differences in water content on the basis of sex have also been observed in adults. Females tend to have less water and more fat than do males. These differences can be attributed in part to the effects of the female sex hormones on fat synthesis and deposition and, in part, to the tendency of young adult males to engage in more-strenuous physical activities that increase their muscle mass and hence their bodies' water content.

Diet, particularly energy-rich diets that increase fat deposition, affects body composition. For example, Brozek and Keys[63] found that in overfed men who gained weight, 14% of the weight gain was due to an increase in extracellular fluid, 24% was an increase in cellular components, and 62% was an increase in fat. When humans are fed energy-poor diets, changes in body composition can also be observed. Berlin et al.[64] followed one such subject for 60 days and, as might be anticipated, showed a loss in body weight, body fat, and body water. These losses are shown in Figure 1.10. Weight loss and regain (weight cycling) alter body composition, particularly the distribution of the body fat. There is a tendency to accumulate subcutaneous fat at the expense of visceral fat by obese people who lose weight and then regain it, yet their total body fat following regain is not different from that prior to weight loss.

Although each cell type has a unique composition and organization, altogether they contribute to the composition of the whole body. Frequently, nutritionists wish to know whether dietary and nondietary factors affect body composition. Researchers might want to know, for example, whether changes in dietary protein can affect the protein content of the whole body or whether a certain exercise routine affects the body fat content, or how age affects bone mineral content. In small animals, body composition can be determined directly. The fat content is the difference in body weight before and after extraction with a fat solvent. Typically a 2:1 mixture of

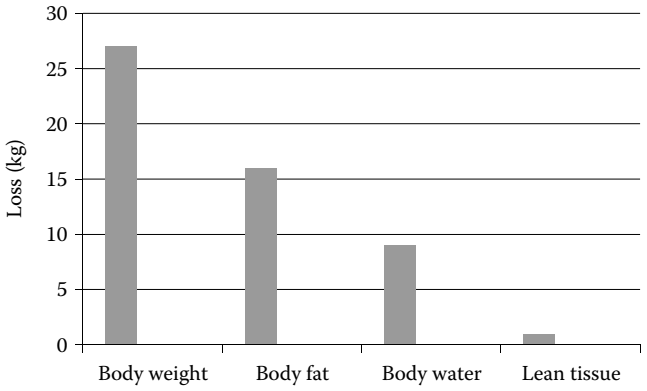


FIGURE 1.10 Changes in body weight, total body water, and body density, and calculated fat and lean tissue solids in a subject consuming 800 calories per day. (Adapted from Berlin, N.I., Watkin, D.M., and Gevirtz, N.R. [1962]. *Metabolism*. 11: 302–14.)

chloroform:methanol is used. Similarly, percentage of body water can be determined as the difference in body weight before and after drying.

The percent of the body that is mineral (ash) is determined as the difference in body weight before and after all the organic matter is oxidized using a muffle furnace. This furnace can achieve heat up to 3000° and will oxidize everything except the mineral matter. Finally, the protein content of the body is determined by measuring the nitrogen content and multiplying by 6.25 to convert to protein and then calculating percentage protein.

Body composition is not measured directly using the whole body but rather using a sample taken from a homogenate of the whole body. In this manner, all components (water, ash, fat, protein) in the same body can be determined using a small sample size.

A limited amount of data are available (Table 1.9) on the body composition of the adult human as determined through direct measurements; however, most of the data in the literature on humans are based on indirect or derived values for each of the major body components. Sequential estimates of body fat, bone density, body water, or protein content are possible using sophisticated instrumentation together with established methods for estimating the magnitude of the fat store and the lean body mass.

The data in Table 1.8 were collected in the early 1950s from persons who had donated their bodies for scientific research.[72,73] Ages and causes of death varied widely. Four of the ten patients had died from heart disease, two were accident victims. The others died from a variety of degenerative diseases. The values were obtained from many very small samples of tissues taken as representative of each tissue type.

It is not always practical, however, to determine the body composition directly. The animal under study may be too large for convenient handling, or one may wish to make sequential measurements in the same body, i.e., to study changes in body composition in response to dietary or nondietary (e.g., exercise) treatments, or one may wish to work with humans. In the latter instance, direct measurements would be out of the question. Indirect methods must be used.

Lean body mass (LBM) can be calculated if one assumes that the fat-free body has a constant water content (72%) and that the neutral fat is stored dry. Thus, the formula

$$\text{LBM (kg)} = \text{Total body water (TBW, kg)}/0.72$$

TABLE 1.8
Proximate composition of adult humans, as percentage

Water	50–70
Fat	4–27
Protein	14–23
Ash	4.9–6

TABLE 1.9
General formulas for calculating body fatness from skinfold measurements

Males

% Body Fat = $29.288 \times 10^{-2}(x) - 5 \times 10^{-4}(x)^2 + 15.845 \times 10^{-2}$ (Age)

Females

% Body Fat = $29.699 \times 10^{-2}(x) - 43 \times 10^{-5}(x)^2 + 29.63 \times 10^{-3}$ (Age) + 1.4072

Note: X= sum of abdomen, suprailiac, triceps and thigh skinfolds, and age is in years.

Source: Data from Jackson, A.S. and Pollack, M. L. 1985. *Phys. Sports Med.* 13: 76–90.

can be used. The figure 72% is an average figure derived from careful direct measurements of the water content of lean tissue. Total body water (TBW) and body fat must also be determined. TBW can be measured by infusing heavy water (deuterium), allowing this to equilibrate within the body and then withdrawing a sample of blood. This is called the "water dilution method." Knowing the concentration of the deuterated water, the volume that was infused, and the concentration in the blood sample withdrawn, one can calculate the volume of dilution of the infused labeled water. This will provide a value for TBW. The calculation of TBW is as follows: $C_1V_1 = C_2V_2$ where V_2 is the volume in which the solute is distributed or the TBW. Dividing TBW by 0.72 (as per the preceding equation) gives an estimate of the lean body mass. LBM is also equal to the total body mass minus its fat content. These methods may not be applicable to all situations and in fact, may be impractical for studies using large number of subjects or subjects unwilling to provide a small sample of lean tissue for the determination of its water content.

Lean body mass can also be predicted from skeletal measurements and from bone weight. Sophisticated techniques using ultrasound, neutron activation analysis, infrared interactance, dual energy x-ray absorptiometry, computer-assisted tomography, magnetic resonance imaging, or bioelectrical impedance are available, and, as the instrumentation improves, these methods may become practical in the clinical setting.[65–71] Presently, considerable effort is being expended to validate these methods because they are noninvasive and allow for the sequential determination of changes in one or more of the major body components as a result of a change in diet, activity, age, or endocrine status. In addition, these noninvasive methods could be useful in assessing population groups in relation to fat mass, bone density, or adipose tissue distribution. For example, Sohlström et al.[69] used magnetic resonance imaging (MRI). They compared these estimates with those obtained from underwater weighing and from body water dilution studies. The three methods gave similar results with MRI and body weight dilution providing 1.4 ± 2.9% less and 4.7 ± 4.0% more, respectively, than underwater weighing for total body fat. However, because MRI can provide information about where this fat is found, this method is superior to the others. In MRI, images are created by a combination of electromagnetic radiation and a magnetic field. Individual body segments such

as shoulders, chest, hips, thighs, etc., can be examined as discrete entities. Thus, the amount and distribution of fat can be detailed.

Dual energy x-ray absorptiometry (DEXA) is another of the newer methods being developed. It has been used to estimate bone density and soft-tissue composition.[65,71] The advantage of this method is that it eliminates the need to use the assumption that the body exists as a two-pool system. The one pool is the fat and the other is the fat-free mass (or lean body mass). Bone density as a fraction of the fat-free mass can be distinguished and quantitated using DEXA. The result is that bone mass and density can be quantitated, as can the fat mass, and the remaining tissue is more legitimately the lean body mass. One can then distinguish and quantitate the muscle mass using its creatine content.

If the major component of the lean is assumed to be the muscle, muscle mass can be determined using the dilution of radioactive creatine or creatine labeled with a heavy nonradioactive isotope.[68] This has been used successfully in rats and may be applicable to humans because creatine is almost exclusively located in the muscle. Labeled creatine must be infused and, after a set interval, a muscle biopsy obtained. The total muscle mass can be calculated using the equation as follows:

$$\frac{\text{Total } {}^{14}\text{C creatine infused}}{[{}^{14}\text{C creatine}] \text{ in sample}} = \frac{\text{Muscle sample size}}{\text{total muscle mass}}$$

On the assumption that lean body mass has a constant potassium content and that neutral fat does not bind the electrolyte, lean body mass can be estimated by measuring the body content of the heavy potassium isotope ^{40}K or by measuring the dilution of ^{42}K (the radioactive isotope) in the body cells.[70] The former requires a whole-body scintillation counter, whereas the latter can be determined in a small tissue (muscle biopsy) sample. The formula for calculating lean body mass is as follows:

$$\text{LBM} = \frac{\text{Total K content}}{\text{Concentration of K/kg tissue}}$$

Either of these methods may underestimate the lean body mass because of the lack of correction for the small amounts of potassium in the extracellular fluids.

Lean body mass and percent fat can be estimated using measures of body density or specific gravity of the individual. The fat-free body will have a specific gravity of 1.1000. This will decrease as the body increases its fat content because fat has a lower (~0.92) specific gravity than the fat-free body mass. Thus, the fatter the subject, the lower the specific gravity or density. The body density can be determined in the adult using Archimedes' principle. The subject is weighed in air and again when immersed in water. The difference in the two body weights is the weight of the body that is water. Because water has a density of 1 (1 mL of water weighs 1 g), the volume of the water displaced represents both the volume of the body immersed and its density. The immersion weight/unit volume of water displaced then is diluted by the air weight/unit volume of water displaced, which in turn is the specific gravity of the subject. Corrections for the residual air in the lungs and intestines must be made. There are a number of reports on body density and body fat using this technique. Age affects body density and percent body fat. In one study of women of different ages, Young et al.[74] has reported that young (16–40 years of age) women had body densities of 1.0342 g/mL to 1.0343 g/mL and percent body fats of 28.69 to 28.75. Older women (50–70 years of age) had body densities that ranged from 1.0095 g/mL to 1.0050 g/mL and percent body fats of 41.88 to 44.56.

Body fat can be estimated using the Siri et al.[75] equation:

$$\% \text{ body fat} = 2.118 - 1.354 - 0.78 \times \%\text{TBW/ body weight density}$$

where 2.118, 1.354, and 0.78 are constants and the density (g/cc), body weight (kg), and TBW (kg) are determined.

Other prediction equations for percent fat from specific gravity are available.[76] The Pace–Rathbun[77] method calculates % fat = 100 − TBW/0.732. The Pace–Rathbun method is based on TBW only and compensates for the structural lipids (those in cell membranes, etc., as contrasted to the depot lipids) by adding 3%. This gives a lean body mass that is somewhat different from that of Siri et al.[75]

Underwater weighing as described above is based on the difference in density of the different body components.[74] Estimating fat stores in this way is cumbersome or not feasible in many clinical settings or under conditions of field surveys. Researchers using this method have made some correlations between this estimate and estimates of body fatness using the measurements of skinfold thicknesses at key locations, that is, places where subcutaneous fat can be assessed using calipers to estimate the skinfold thickness. The fold below the upper arm (triceps fold) and the fold at the iliac crest are frequently used locations. Other locations include the abdominal fold and the thigh fold. Equations (Table 1.9) have been derived to calculate body fatness using these measurements.

For population surveys where close estimates of body fat, protein, lean body mass, etc., are not critical, simpler estimates of body fatness are frequently used. Using the patient's body weight and height one can compare these values with those considered desirable for men and women. The first such tables were developed by the Metropolitan Life Insurance Company, which made the assumption that young (age 20–30 years) people applying for life insurance (and found insurable) were healthy. They then took the body weights of these people and arranged them according to height for both males and females. They called these weights "desirable" weights because they were associated with the lowest mortality due to disease. Because the weight range for each height was so large, they later subdivided each weight-for-height range into thirds and presented these thirds as being representative of small, medium, and large frame sizes. This table has been found useful by many in estimating desirable body weight, but the user must remember that this table was not based on actual measurements of skeletal size. The table is based only on heights and weights of individuals in the third decade of life who wanted (and could afford) life insurance. In this respect, there is a bias in the table. Minority groups were largely underrepresented in the database used for these tables.

A broader database using subjects of all ages, economic status, both sexes, and from minority and majority cultural/ethnic groups has been obtained by the National Health and Nutrition Examination Surveys (NHANES). These surveys are conducted at intervals by the Centers for Disease Control of the U.S. Department of Health and Human Services. These surveys have collected data not only from young adult men and women but also from children and aging adults. These weights and heights have been used to create tables giving weight ranges for males and females. In addition, NHANES made more detailed measurements of skinfold thickness, skeletal size and density, and a variety of biochemical and physiological features using a representative subset of the population assessed. The NHANES tables therefore have a broader database than the Metropolitan Life Insurance tables. Despite the difference in databases used to construct the tables, both are useful in evaluating humans in terms of desirable body weight.

Perhaps more popular now is the use of body mass index (BMI). This is useful in that it is an index of the body weight (kg) divided by the height (meters) squared (wt/ht^2). BMI correlates with body fatness and with the risk of obesity-related disease or diseases for which obesity is a compounding factor.[76] Overweight is defined as BMI between 25 and 30 and obesity is a BMI over 30. The BMI varies with age. A desirable BMI for people age 19–24 is between 19 and 24, whereas that for people age 55–64 is between 23 and 28. Although simple in concept, this term does not assess body composition per se. It provides a basis only for assessing the health risks associated or presumed to be associated with excess body fatness. BMI applies only to normal individuals, not the super athlete or the body builder, who may be quite heavy yet have little body fat.

Although total body fatness is an important risk factor for several degenerative diseases, the distribution of the stored fat may impact upon these disease states as well. Males and females differ in the pattern of body fat stores. Males tend to deposit fat in the abdominal area, whereas females tend to deposit fat in the gluteal area. Measuring the waist and hip circumference allows computation of the waist-to-hip ratio (WHR). As this ratio increases, so does the risk for cardiovascular disease, diabetes mellitus, and hypertension. In men, if the WHR is greater than 0.90 and in women greater than 0.80, the risk for cardiovascular disease increases significantly.

The assessment of excess fat stores has been addressed in a variety of ways. In small rodents direct measurement of body fat is possible; however, with larger animals it is not. Obesity, or excess fat stores, develops for a variety of reasons. Although excess body fat stores, or obesity, is considered a risk factor in a number of diseases, we have no permanent cure for the disorder. Listed in Table 1.10 are some of the reasons that excess body fatness develops. Obesity is probably the result of an interaction between genetics and environment. An individual may have inherited one or more mutated genes that influence the efficiency with which that individual uses the intake energy and converts it to stored energy. When provided with an ample food supply, these individuals may become fat. However, if the food supply is limited, the excess fat store might not develop. In addition, there may be cultural overrides that influence the phenotypic expression of an individual genotype. Individuals may consciously limit food intake and increase physical activity so as not to phenotypically express their genotype. Obesity, therefore, may be considered a multifactorial phenotype.

Genetics of Obesity

Research on the genetic basis for excess body fatness is very active.[34–43,78,79] Many candidate genes have been identified as being associated with excessive body fat (www.ncbi.nlm.nih.gov OMIM). Several investigators have shown that the familial trait for body fatness has a much stronger influence on body composition than environmental influences such as culture, socioeconomic status, or food-intake patterns. Stunkard and associates [39,40] as well as Bouchard and Despres [35,36] have published extensively on the responses of twins to dietary manipulation and exercise. They found that identical (monozygotic) twins are more nearly alike in these responses than are siblings or nonidentical (dizygotic) twins. These and other workers[34] have also examined the home environment with respect to body fatness. This work attempts to answer the question of whether people become overly fat because of environmental influences such as the daily coaching by the parents to eat or not eat. Most revealing in this respect are the studies of monozygotic and dizygotic twins reared by their biological parents or by adoptive parents. In one study, adopted children and their biological and adoptive parents were compared with respect to body weight and body fatness, whereas in other studies twins reared together or apart were compared. Allison and colleagues[34] have calculated that the heritability of the body mass index is between 0.50 and 0.70, depending on the definition of obesity and body mass index used. Overfeeding studies of twins have been conducted by Bouchard and associates that showed that identical twins will gain a similar amount of weight, whereas siblings or unrelated subjects will be highly variable. After the overfeeding period, there were also genetic differences in weight-loss patterns. This is also true for those attempting to lose weight via energy restriction. Some people can lose weight far more easily than others. These differences are probably of genetic origin. All these studies showed that the genetic influence on body fatness far outweighed the environmental influence.

A number of genetic diseases are characterized by obesity. The Prader–Willi, the Bardet–Biedl, the Laurence–Moon, the Cohen, the Boyeson, and the Wilson–Turner syndromes are all characterized by obesity as well as by other abnormalities. All of these syndromes are rare and none seem to correlate with any of the mutations listed in Table 1.10. The Prader–Willi syndrome occurs as a result of a partial deletion of the long arm of chromosome 15. It occurs in 1/5000 to 1/10,000 live births. Individuals with this syndrome are developmentally delayed, have poor muscle tone and are growth retarded. Once the child with Prader–Willi syndrome reaches the age of 2 or 3,

TABLE 1.10
Suggested causes of obesity

Mutations in genes for	
	Leptin, leptin receptor
	Cholesystokinin
	Adipsin, adiponectin
	Ghrelin
	Tumor necrosis factor (TNFa)
	Neuropeptide Y (NPY), NPY receptor
	Corticotropin-releasing hormone (CRH), CRH receptor
	Adipocyte-specific transcription factor C/EBPa
	Agouti protein
	Carboxypeptidase E
	Phosphodiesterase
	β-adrenergic receptor
	Growth hormone receptor
	Glucocorticoid receptor
	Insulin receptor
	UCPs
	Melanocortin 3 receptor
Hormonal imbalance	
	Excess glucorticoid
	Excess insulin
	Hypothyroidism
	Polycystic ovary disease
Other causes	
	Injury to the brain stem or hypothalamus
	Social/cultural feeding behaviors
	Inactivity linked to overeating

other behavioral features develop. The child has a persistent food-seeking behavior that can be self-destructive. Patients with Bardet–Biedl syndrome, although obese, have a different set of characteristics. Most are mentally retarded with polydactyly (extra fingers/toes) and hypogonadism. The prevalence of this condition is about 1/17,500 live births. These patients do not have the aggressive, persistent food-seeking characteristic of the patient with Prader–Willi syndrome. Patients with the Laurence–Moon syndrome are similar to those with the Bardet–Biedl syndrome except that they are frequently diabetic and paraplegic. They do not have the polydactyly feature. The prevalence of this disorder as well as the others listed above is of the order of 1/20,000. As might be anticipated, all of these disorders are characterized by a shortened life span.

A number of spontaneous genetic errors in the animal kingdom have obesity as one of their characteristics.[41,78,79] Genetically obese rats, mice, dogs, and desert animals have been described. In the rodent species, the mode of inheritance and, in some instances, the chromosomal location of genes for obesity have been found. Obesity can be inherited via an autosomal recessive or dominant or sex-linked mode. In each of these mutations, an error occurs that affects energy balance. Errors in the perception of hunger and/or satiety by the brain can explain the excess food intake

(hyperphagia) that characterizes several of these mutants. Inappropriate hunger signals, satiety signals, and their receptors have all been implicated. The central integrator of this seemingly unrelated set of characteristics may reside in that group of compounds called the cytokines. This group of small peptides has intercellular, interorgan signaling functions that in turn define them as hormones. These compounds have receptors in specific cells or target cells, and current evidence suggests that mutations in either the genes for the target cell receptors or for the cytokine itself or for its production and release could explain a number of clinical conditions. The cytokines, their receptors, and their signaling mechanisms are listed in Table 1.11. The interleukins (IL-1 through 8) are mainly concerned with the immune system, although one or two may also play a role in adipocyte apoptosis (programmed cell death). The cytokines of interest to researchers in energy balance are TNF (α and β), leptin. TNF is found in many cell types but of interest is the TNF α. This cytokine induces adipocyte apoptosis. Thus, this cytokine has a two-pronged attack on energy balance. First, through suppressing hunger it reduces energy intake and second, because it stimulates adipocyte apoptosis, it has the effect of reducing fat stores.

Another cytokine that has a similar mode of action is leptin. Leptin is produced by the adipocyte and transported to the hypothalamus, where it serves to signal satiety. Mutations in the gene for leptin have been found in genetically obese rodents as well as in humans. Leptin gene mutation is uncommon. However, mutations in the leptin receptor in the brain have also been found in several obese rat and mouse strains, and they are presumed to be more common than mutations in the leptin gene. Mutations in either gene result in obesity due to a lack of this satiety-inducing cytokine.

Leptin also can stimulate adipocyte apoptosis so, like TNF α, it affects both food intake and energy store through an effect on the number of fat cells available for fat storage.[19,78] Leptin upregulates the muscle UCP_3 and thus increases heat production via an effect on OXPHOS. In addition, leptin regulates fatty acid homeostasis. It suppresses fatty acid deposition in nonadipocytes. Counteracting TNFα and leptin is neuropeptide Y (NPY), which stimulates appetite. Although present in most neural tissues, the NPY that is closely associated with feeding is synthesized by cells of the arcuate nucleus. It is released primarily in the paraventricular nucleus, whereupon it stimulates feeding. NPY has the opposite effect of corticotropin-releasing hormone (CRH) within the hypothalamus with respect to signaling feeding. Leptin regulates both of these compounds. It downregulates NPY and upregulates CRH.

Greater than normal food intake may also characterize the genetically obese human. Yet, there are many overfat people who are not hyperphagic. There are those who cannot dissipate their surplus intake energy as heat, i.e., thermogenesis and who do not tolerate cold well. The common thread to these two conditions is the apparent inability of tissues to uncouple oxidative phosphorylation so as to release heat to keep the body warm. Thus, ATP is continuously synthesized, in turn transferring its energy to the synthesis of fat. Obesity has also been attributed to a failure of these cells to respond to the stimulatory effects of norepinephrine and is associated with an anomalous central regulation of the sympathetic input to this tissue.

It has been suggested that genetically obese individuals become obese because they are unable to increase their heat production when overfed or when suddenly thrust into a cold environment.[49–57] It has been hypothesized further that genetically obese animals develop subcutaneous fat pads as insulation against heat loss or gain, thereby circumventing their relative inability to thermoregulate. To increase their insulation layer, they must overeat to provide the requisite substrates for lipogenesis. Such an hypothesis has some elements of validity. The ob/ob mouse, for example, is unusually sensitive to cold and is incapable of increasing its heat production when suddenly exposed to cold or injected with norepinephrine. If gradually exposed to cold, these mice can slowly adapt to the gradual change in temperature. These features of the ob/ob mouse precede the development of both hyperphagia and obesity and have been attributed to a mutation in the gene for leptin.[41] These mice do not produce normal leptin, and this deficiency means that there is no leptin to travel to the brain to suppress feeding. Hence, these mice are hyperphagic. These mice also do not turn on thermogenesis very well when exposed to cold. This is because leptin has another role—that of

TABLE 1.11
Cytokines, their receptors, and their signaling mechanism

Cytokine	Receptor distribution	Signaling mechanism
IL-2	T cells, B cells, monocytes, myeloid precursors, NK cells	High-affinity binding induces phosphorylation of tyrosine kinase
IL-1	T cells, B cells, fibroblasts, endothelial cells, keratinocytes	(a) A GTP-binding motif suggests G protein activity (b) May involve cAMP and PLC activation
IL-5	B cells, eosinophils, basophils	Cellular tyrosine kinase induces phosphorylation of several proteins, and this praline-rich receptor protein may be one of them
IL-4	T cells, B cells, monocytes, macrophages, granulocytes, megakaryocytes, erythroid products, NK cells, fibroblasts, endothelial cells	Cellular tyrosine kinase induces phosphorylation of this serine and praline-rich receptor protein
IL-6	T cells, B cells, monocytes, fibroblasts, megakaryocytes, hepatocytes, keratinocytes, mesangial cells	Tyrosine kinase-mediated signaling system
IL-8	Neutrophils, basophils, monocytes, T cells (3 Types of receptors)	Tyrosine kinase-mediated signaling system
IL-3	Myeloid precursors, basophils, mast cells, macrophages, megakaryocytes	Involves tyrosine kinase, protein kinase C
IL-7	Two types: high- and low-affinity, B cell progenitors, thymocytes	Tyrosine kinase
IL-3	Basophils, myeloid precursors, mast cells, macrophages, megakeryocytes	Tyrosine kinase
SCF	Pluripotent stem cells, lymphoid precursors, myeloid precursors, megakaryocyte precursors, erythroid precursors	SCF-binding initials dimerization of receptors. Also involves the tyrosine kinase, phosphorylation of PLC γ, c-raf, and PI-3 kinase. Ca^{++} is also involved as a second messenger
Ghrelin	Synthesized in stomach Receptors are in hypothalamus	Transfers information from stomach to hypothalamus, and influences growth hormone release in response to changes in energy homeostasis
Cholecystokinin	Receptors are in brain, gall bladder, and endocrine pancreas	Stimulates bile release and pancreatic exocrine enzyme release; signals satiety
Adiponectin	Released by adipose tissue; receptors in insulin target tissues	Increases insulin sensitivity
LIF	Hematopoietic stem cells, megakaryocyte progenitors, osteoblasts, neurons, myoblasts, adipocytes, embryonic stem cells, hepatocytes, kidney epithelial cells, placental cells	Involves the tyrosine kinase system
G-CSF	Granulocytes, pluripotent stem cells, myeloid progenitors, T cells, fibroblasts, endothelial cells	Involves G proteins and the tyrosine kinase
EPO	Erythroid precursors, megakaryocytes	Unknown

TABLE 1.11 (continued)
Cytokines, their receptors, and their signaling mechanism

GM-CSF	Myeloid precursors, monocytes, macrophages, eosinophils, neutrophils, megakaryocytes, endothelial cells, osteoblasts, Langerhans cells	Involves the tyrosine kinase system
M-CSF	Monocytes, macrophages	Involves the tyrosine kinase system
TGF α, β	Several related cytokines and receptors. Found in most cells	Involves the tyrosine kinase system
NPY	Found in most neural cells	Involves the tyrosine kinase system
TGF α, β	Found in many cell types	Unknown
Leptin	Found in brain cells as well as other cell types	Unknown

stimulating the production of UCPs.[19] Because the mobilization of body fuel (induction of lipolysis and glycogenolysis) is not abnormal in the obese ob/ob mouse, it would appear that only the release of heat when these fuels are oxidized is defective.[51–53] In other words, more energy is trapped in the chemical bonds of high-energy compounds than is released as heat. This, of course, is related to the increased energetic efficiency of these animals as well, because they also gain more fat per unit food consumed (and release less heat) than lean animals.

Impaired thermoregulation has also been reported in genetically obese Zucker rats.[78] These rats produce ample amounts of leptin but they have a mutation in the gene that encodes the leptin receptor.[41] Thus, although they produce the cytokine it is without effect because its receptor is aberrant. It cannot bind the leptin and signal the cell accordingly. Zucker rats, like ob/ob mice, are hyperphagic and obese.[79] Similarly, they do not respond well to sudden cold exposure. Again, the missing link is not leptin but its leptin receptor that in turn affects satiety signaling and UCP production. Zucker obese rats and ob/ob mice are not the only animals affected. Lower body temperatures in neonates and an impaired ability to increase thermogenesis in response to overeating, cold exposure, or to norepinephrine, have been reported in a variety of genetically obese animals models. Perhaps obese humans may also have this defect. A few humans have been described with leptin defects, but whether leptin or its receptor can explain all the cases of obesity is doubtful. When stimulated by cold or infusions of norepinephrine, normal-weight subjects increased their heat production and obese subjects did not.

Normal-weight humans appear to regulate their body fat mass by increasing their heat production when overfed or exposed to cold. Several investigators developed an animal model that showed that when overfed, thermogenesis was stimulated. They showed that when offered a variety of energy-rich snack foods, normal animals increased their heat production to maintain a normal weight.[58] However, this mechanism is far from perfect in that, over time, these overfed animals did become fatter than their control-fed littermates. Nonetheless, the observation that heat production will increase in response to fluctuations in energy intake is a very interesting facet of energy balance. The mechanism whereby heat production is increased above basal involves the synthesis and release of UCPs. These proteins are not always present and active. In the starved animal, for example, they cannot be found.[56,57] This means that extra heat production induced by UCPs can be switched on and off. The synthesis of UCPs is rapid. Exposure to cold or hyperthyroidism or birth or starvation-refeeding triggers a rapid and marked increase of messenger RNA for these proteins as well as a marked increase in the level of protein. In most animals, the increase in the level of UCPs parallels an increase in nonshivering thermogenesis. In lean animals, surplus food intake seems to signal UCP synthesis. The regulation of this synthesis occurs at the level of messenger RNA transcription.[50] The genes that encode these proteins have been isolated and sequenced. The

protein completely traverses the inner mitochondrial membrane and has a C-terminal region that projects from the outer surface of the membrane. It works by dissipating the proton gradient that is developed when pairs of reducing equivalents are passed down the respiratory chain to make water. The energy that is developed by the respiratory chain is not captured in the high-energy bond of ATP but is, instead, released as heat. The control of its uncoupling function is through the external C terminal region that contains the nucleotide (ADP)-binding site. If UCP binds the nucleotide, it is not available to the F_0F_1 ATP synthase for ATP synthesis. Fatty acids interact with the nucleotide binding site so that ADP binding is increased. These fatty acids are released from the stored triacylglycerols via a hormone-stimulated cyclic AMP mechanism. This explains how norepinephrine can stimulate brown fat thermogenesis (BAT). Norepinephrine works by increasing the cAMP levels in the cytosol. This, in turn, stimulates the activity of the cytosolic lipoprotein lipase, which serves to release the fatty acids from the stored lipid. Norepinephrine is particularly effective with respect to intracellular fatty acid release in the brown fat. Other lipolytic hormones that work through increasing adenylate cyclase activity and cAMP levels also work to increase heat production. Diets rich in polyunsaturated fatty acids potentiate these hormone effects on this process.

Although insulin is not thought of as a lipolytic hormone, it, too, has a role in regulating the synthesis and activity of the UCPs and heat production. Both the synthesis of the uncoupling protein and the ability of various tissues to assist in the regulation of body temperature (via the UCPs that help to dissipate the energy provided by excess food intake) is impaired in the insulin-deficient animal. Once insulin is restored, the impairment is corrected. Of interest is the observation that many genetically obese rats and mice are hyperinsulinemic. This hyperinsulinemia is probably due to insulin resistance of the target tissues.

As mentioned in the sections on starvation and anorexia, hormonal balance is important to the regulation of energy balance and normal body weight. Hypercortisolism and hyperinsulinism are associated with obesity. Cortisol and its related compounds, corticosterone and cortisone, play an important role in the regulation of lipogenesis as well as in the signaling of apoptosis. Although these hormones are usually catabolic hormones, there are circumstances when they stimulate anabolic processes, such as fat synthesis.

Finally, there are social and cultural influences that can ensure or potentiate genetic tendencies to develop obesity. Anthropologists and medical historians have identified examples of cultural groups that consider excess body fat a mark of beauty as well as an indication of economic status within their society. Examples of this are the various statuary of different ages all the way from the upper Paleolithic period through the Renaissance to the 18th and 19th centuries. Women have been represented with large bellies and breasts that, to the eye of the contemporary observer, are overfat. Men, too, are of ample proportions. In fact, there is an old Pennsylvanish (Pennsylvania Dutch) expression that indicates the desire of men to have overweight women as wives: "A fat wife and a full barn never did a man harm." Even today there are cultures, notably in Africa, that have customs that include preparing girls for marriage by fattening them. Malcom, for example, described this practice for elite Efik girls in traditional Nigeria, as well as the custom in Kenya of demanding high bride prices for fat brides. In these cultures, female fatness may not only be a testament to the families' wealth, it may also be a symbol of maternity and nurturance. This is important to the woman if the only way she gains status is through motherhood. A fat woman is thus assumed to be very maternal and nurturing.

With respect to societal determined fatness and the values of fatness, it can be assumed that the fatter a person is in a society that values fatness, the more likely that person will be to marry and produce children carrying genetic tendencies to be fat and who will be similarly taught to eat enough to be fat also. Lean people will be less likely to contribute to the gene pool because they will be considered less-desirable mates. Although people in the United States, as well as other developed nations, may not have these values, there is no doubt that eating behaviors can be taught. If young children are constantly reminded and coached to overeat, there may be a continuing stimulus to overconsume food. Added to this may be cultural and social dictates with respect to physical

activity. A decrease in energy expenditure ensures a positive energy balance that may well result in excess body fatness.

Frequently, those who are overfat are told that they could become lean if they would reduce their food intake. However, simply restricting one's food intake does not cure the problem; it merely treats the result of the positive energy balance—excess body fat. Once this fat is lost, the formerly obese person frequently abandons the restricted diet, returns to previous eating habits and, as a result, returns to the prior weight. In some, there may also be an increase in body fatness, not just a return to the prior body weight.

Set Point Theory in Body Weight Regulation

The idea that each body has its own unique size and weight has been discussed, denied, and supported by a wide variety of researchers. The hypothesis that adult body weight is closely regulated at its own unique level was developed from observations of both humans and animals.[80–83] The mechanisms that serve to regulate this steady-state body weight are not fully known.

Healthy adult humans change very little over the years in their body weight. They may be overweight and/or overfat but, for most humans, that weight is maintained for years until some event occurs that results in a body weight change. In women, pregnancy or menopause, two normal physiological events, may perturb the system sufficiently to establish a new steady-state body weight (or new set point) which, again, will be defended tenaciously. A change in the endocrine system, an insult to the body, or a conscious decision to eat more (or less) over a prolonged period (months to years) are other examples of events that might perturb the system sufficiently to result in a new set point—a new body weight that is maintained from that time on.

Similarly, animals appear to regulate their body weight within fairly tight limits. Much of the research on the set-point hypothesis has used rats and mice. Studies using rats that were either over- or underfed revealed that they had a body weight that was related to their food intake. That is, if they were forced to consume more energy than they would voluntarily consume, they would become overfat. If they were underfed, they would be leaner than normal. After these feeding treatments were discontinued, the rats that were overfed significantly reduced their food intake and used their fat stores to provide their energy needs whereas those rats that were underfed dramatically increased their voluntary food intake until they gained the weight they would have gained had they not been food restricted. When both these groups of rats attained the weight of their untreated controls, they resumed normal feeding behavior.

Although the body weight returned to normal in these over- or underfed rats after the treatment was terminated, the composition of the body was not the same as their untreated counterparts. The percent of the body that was fat was affected. Those rats that were underfed recovered by significantly increasing the synthesis and deposition of body fat. This recovery was faster than the recovery of body protein. In the overfed rats, the body protein normalized within days of cessation of the overfeeding, yet the body fat content remained elevated weeks after overfeeding ended.

Other studies have used parabiotic rats or mice to study the consequences of overfeeding or underfeeding on body weight and composition. Parabiosis is a technique where two weanling animals are joined together surgically at the skin so that they have a common circulation. Hervey, Harris, and others have used this technique to answer the question of whether there are blood-borne factors that are involved in the regulation of feeding and body weight. Genetically obese animals have been joined to genetically lean ones, as have normal weight partners in which one was either over- or underfed, or was lesioned in either the feeding center of the hypothalamus or the satiety center. In each of these instances, the feeding behavior of both as well as their body weight and composition were monitored. In each instance where the one partner overate and became obese, the other underate or starved and subsequently lost its body fat as well as its lean body tissue. These results were interpreted as indications that there are blood-borne factors generated by the fat store that signal the feeding behavior. Indeed, some of these blood-borne factors have been identified

(leptin, NPY, TNFα, etc.). In the preceding section, a number of rodents (as well as other species) were described that carry mutations that result in obesity. Some are characterized by hyperphagia (abnormally increased food intake) and some are diabetic as well as obese. Still others are hypertensive and obese. Some develop renal disease, and others do not. The range of phenotypes is large, suggesting that more than one mutation can exist having obesity as part of its phenotype. The results of studies using hyperphagic genetically obese animals suggested that these obese animals neither sent nor received appropriate satiety signals or were not able to respond to them by decreasing their food intake. Likely the nonresponsivity (that is, an error in the signaling system) is the explanation, as parabiotic pairs using genetically lean and obese rats behaved like the force-fed and voluntary feeding pairs. Although the genetically obese partner was hyperphagic and obese, the lean partner ceased eating and eventually starved to death.

It is apparent that although there may be controls that influence feeding and body weight, these controls may not fully regulate body fatness. That is, body weight may be set but body fat may change depending on food intake and physical activity. This may explain why aging humans may gain body fat while decreasing food intake to maintain their body weight. As humans age, they decrease their physical activity and their body composition changes; they lose muscle mass and gain body fat. Their body shape changes as well. They may observe an increase in the size of their fat mass in the abdomen and on the thighs and buttocks. Again, epidemiologists have noted the differences in health risks associated with the location of the excess fat stores; the so-called apple and pear shapes. Those persons whose fat stores are distributed equally between storage sites on shoulders, arms, abdomen, hips and thighs are said to be "apples." Their risk of developing obesity-associated disorders is greater than that in people who have accumulated fat stores at sites below the waist, the "pears." The depots differ in the degree of fatty acid turnover. That is, they differ in how readily they can release their stored fat for use by other tissues. In humans, studies of cells isolated from the femoral, gluteal, and omental (thigh, buttocks, abdomen) depots revealed significant differences in free fatty acid release. On the basis of the rate of free fatty acid release and the size of the depots, the half-life of the fat depot in the femoral area was calculated to be 305 days. For the gluteal depot it was 326 days and for the omental depot it was 134 days. Estimates of the half-life of the fat in the other depot sites have not been made. An estimate of half-life is the estimate of time needed to exhaust one-half of the fat store. As the human uses the stored lipid, more lipid is synthesized to replace that which was used. Hence, the term fatty acid turnover means that fatty acids are both used and replaced. If they are used at a greater rate than they are replaced, a net fat loss will occur. As can be seen, however, different depots will shrink at different rates depending on their location. In addition to the aforementioned differences in depot fat use, there are also genetic and sex differences in the extent and location of the fat depots. Women, for example, have larger subcutaneous fat stores than men. Men have larger omental fat depots than women of the same age and weight.

Morbidity of Severely Obese People

Health care professionals have observed countless instances of the codevelopment of excess body fat with diabetes mellitus, hypertension, and cardiovascular disease.[33,84] Some have termed this codevelopment as metabolic syndrome. Epidemiologists have reported that obesity and overweight are risk factors in the development of these diseases. However, there are some inconsistencies with respect to the relationship of obesity to cardiovascular disease and total mortality. Studies by the CDC and those by Sjostrom[84] suggest that weight loss by the obese does not positively affect life span. Long-term studies of mortality by formerly obese people conducted by the CDC suggest the reverse. Their preliminary report indicates an increase in mortality in people who have consciously reduced their body fat and remained lean. This report has raised serious questions about the efficacy of weight loss with respect to life span extension.

Treatment of Obesity

In almost no other area of medicine have there been so many failures as have occurred in the treatment of obesity. Fully 90% of all those who lose weight regain it within 5 years. Data from the Chicago Gas and Electric Study suggest that one cycle of loss and regain is a risk factor for death from coronary heart disease independent of body fatness. The gain–loss group, when compared with subjects who neither gained nor lost weight, had 1.8 times the risk of death from heart disease. This suggests that weight cycling is not a healthy behavior. If weight is to be lost, it must stay lost if health benefits are to be gained. Often this does not occur. Weight cycling consists of intermittent periods of food restriction followed by periods of "normal" eating patterns. These patterns may include periods of gorging or binge eating. One of the major effects of calorie restriction on metabolism is a reduction in resting metabolic rate (RMR). This lowers the overall energy requirement and increases energy efficiency, thus allowing a greater percentage of dietary energy to be partitioned into fat synthesis upon refeeding.

The effects of weight cycling on energy efficiency may be due to the composition of the weight loss during calorie restriction. One of the consequences of rapid weight loss, especially when induced by very low-calorie, low-carbohydrate diets, is the loss of body protein or lean body mass. This is especially true when the individuals are physically inactive. Maintenance of lean body mass is an energy-expensive process. Lean body mass is the most metabolically active tissue in the body with respect to energy demands, accounting for the majority of calories to support the basal energy requirement (i.e., 60–70% of daily basal energy requirements for adults). Therefore, the less body protein, the lower the energy requirement. If weight loss consists of significant amounts of body protein, then the formerly overfat person will have a lower basal energetic requirement and an increased energy efficiency in terms of the weight regain as fat.

One of the responses of cycled humans is the tendency to overeat during the initial few days of the refeeding period. This suggests that the regulation of food intake is affected by the weight loss. The regulation of food intake is discussed in Chapter 2. Signals sent to the brain by the starved body seem to set the stage for hyperphagia (increased food intake above normal) once food is no longer restricted. These signals must be fairly enduring because this hyperphagia is of about the same duration as the duration of the restriction period. The origin of these signals is not known, but no doubt they exist because food intake is an event regulated by the CNS. Studies of starved and refed rats showed that these rats had a preference for dietary fat if given a choice of several energy sources. As a result of this selection, cycled rats regained more body fat than if the food offered was rich in carbohydrate and/or protein. Again, this suggests the involvement of signals from the brain directing the individual to select energetically rich food. This signal, coupled with the increased efficiency of the body in retaining the ingested energy, helps to explain why the fat regain occurs in people who have restricted their energy intake to lose weight. Food restriction puts into place a metabolic machinery geared to save as much energy as possible and to stimulate the brain to signal the body to consume energy-rich foods. Thus, even though the patient tries to control eating and food intake, the body seeks to return to its prior overfat state. Constant vigilance is required of the patient to override these biological signals that direct the body to be fat. However, even when the patient carefully monitors food-energy intake and consciously decides to regulate it, weight regain may occur due to the body's increased energetic efficiency and its tendency to synthesize and store fat in preference to protein. Here is where a good exercise program might be useful. Exercise, on a regular basis, stimulates muscle protein development and increases energy expenditure. Exercise can be a useful adjunct to energy-intake restriction because it redirects energy loss from the lean body mass. In the sedentary individual, weight loss occurs at the expense of both fat and protein components of the body. In the exercising food-restricted individual, the weight loss is primarily fat loss. Further, mild to moderate exercise seems to suppress food intake. Thus, food restriction together with exercise is additive in a beneficial way with respect to the loss and regain of body fat.

TABLE 1.12
Drugs that may have antiobesity properties

	Example	
Antinutrition drugs		**Effect**
1. Gastric emptying inhibitors	(—) threochlorocitric acid	Delays gastric emptying, induces satiety
2. Glucosidase inhibitors	Acarbose, miglitol	Inhibits carbohydrate digestion
3. Inhibitors of lipid uptake	Cholestyramine	Binds bile acids, disrupts micelle formation
4. Pseudonutrients	Olestra	Fat substitute with less energy
	Artificial sweeteners	Sugar substitute, no energy
	Bulking agents, fibers	Induce satiety at lower energy intake
5. Lipase inhibitor	Xenecal	Inhibits hydrolysis of triacylglycides, promotes lipid malabsorption
Drugs that affect nutrient partitioning		
1. Growth hormone		Stimulates protein synthesis
2. Testosterone		Stimulates protein synthesis in males only
3. Anabolic steroids		Stimulates gain of lean body mass and suppresses fat deposition
4. α2-adrenergic antagonists		Enhances lipolysis
5. Thermogenic drugs:		Reduce metabolic efficiency; increase heat production
Dinitrophenol		Metabolic poison; not recommended
β2- and β3-adrenergic agonists	BRL-26830A, Terbutaline	Stimulates protein synthesis and lipolysis; has serious side effects
Appetite suppressors		
1. β-phenethylamine derivatives	Fastin, Dexatrim	Interferes with hunger signaling via norepinephrine
2. Serotonergic agents	Fenfluramine, fluoxetine	Increases serotonin release and signals satiety
3. Amine reuptake inhibitor	Sibutramine	Blocks reuptake of norepinephrine and 5-HT and suppresses appetite

Drugs for the Treatment of Obesity

Pharmacologic approaches to the treatment of obesity have captured the interest of numerous pharmaceutical companies worldwide.[85,86] Table 1.12 lists some of these drugs. Drugs that target those mechanisms involved in hunger and satiety as well as drugs that target feed efficiency have been developed. The latter category includes compounds that interfere with normal digestion and absorption. Unfortunately, none of these compounds has a significant antiobesity effect. Glucosidase inhibitors as well as lipase inhibitors have been developed. The glucosidase inhibitors acarbose and miglitol, for example, inhibit the action of amylase and thus reduces its activity. This means that the long-chain (straight and branched) starches found in many plant products are not digested as quickly as usual. In theory, this retardation of digestion should result in a retardation of absorption of the end products of starch hydrolysis. Unfortunately, this may not result in a reduction in intake energy. If starch digestion is not completed by the enzymes of the small intestine, flora of the large intestine take over and produce fatty acids that are then absorbed and used for energy.

Lipase inhibitors (Xenecal) or fat absorption inhibitors (cholestyramine, neomycin, perfluorooctyl bromide) also serve to reduce energy intake. Cholestyramine binds bile acids and disrupts micelle formation. This results in an inhibition of fat absorption and increased fecal fat loss. An

over-the-counter drug that has recently been released, Alli, is a weight loss drug sold in a lower dose than its parent drug, Orlestat. It was originally approved in 1999 as Xenical. The drug interferes with fat absorption and can help with weight loss if used as part of an overall weight loss program that includes exercise and the use of a low-fat diet. Because it inhibits fat absorption, very uncomfortable side effects are experienced if the food plan is not low fat. Gastrointestinal discomfort (bloating, gas, loose to runny stools) occurs, and some individuals may have more problems with this discomfort than others.

Neomycin, an antibiotic, reduces fat absorption as well. However, it has the side effect of reducing the intestinal mucosa. This can result in diarrhea. Perfluorooctyl bromide, a product used as a contrast medium for gastrointestinal x-ray studies, also blocks fat absorption as well as amino acid and glucose absorption. Although useful in studying the functional state of the gastrointestinal tract, because it interferes with the absorption of all the macronutrients, its usefulness as an antiobesity drug is very limited.

Food companies have expanded into the "antiobesity business" by developing noncaloric sweeteners, reduced-caloric sweeteners, fat substitutes, and bulking agents. All of these products are designed to reduce the energy value of food products. Aspartame, cyclamate, saccharin, and acesulfame K are sweeteners without energy value. Theoretically, aspartame could have an energy value as it is a dipeptide of phenylalanine and aspartic acid, but because it is 180 times sweeter than sucrose and is unstable in water and to heat, it is unlikely that significant amounts of this product will be consumed. So, for practical purposes, this sweetener would not contribute energy in any significant amount to the daily intake. They are used in soft drinks as well as a number of snack foods. Canned fruit and frozen desserts are also prepared with these noncaloric sweeteners. Some of the sugar alcohols—for instance, mannitol—are also in use in prepared foods to reduce their energy content. Likewise, fat substitutes, such as the sucrose polyester olestra, are used to reduce the energy value of some foods. Bulking agents such as guar, pectin, and fiber are used as energy diluents in processed foods. All of these pseudonutrients can effectively reduce energy intake when incorporated into a well-balanced diet that is energy restricted. This will help the overweight individual lose excess fat.

Listed in Table 1.12 are some hormones that affect protein synthesis as well as fat storage. None of these should be used therapeutically to stimulate weight loss. However, they are included in the table because fat weight loss may be a secondary effect when these hormones are used for other reasons. For example, growth-hormone supplements may be given to the growth-deficient child who may be very short and fat. The supplemented child will then grow and as this growth occurs, the fat depots shrink. In males, the percent body fat is inversely related to circulating testosterone levels. In females, increased upper abdominal and visceral fatness is associated with rising levels of testosterone. Glucocorticoid excess (Cushing's disease) usually results in an accumulation of abdominal fat, whereas glucocorticoid deficiency (Addison's disease) is characterized by a depletion of fat stores. Thyroid hormone excess likewise is characterized by a reduction in fat stores, whereas thryoid hormone deficiency has the reverse feature. Hypothyroid individuals have enlarged fat depots. Among the pharmaceutical products we have drugs that are thermogenic. In other words, these are drugs that stimulate energy wastage as heat. None of the known thermogenic drugs are without risk. Frequently, β-adrenergic compounds have serious side effects such as tremors. One of these, Terbutaline, is useful in asthma treatment. When used for this purpose, weight loss has been reported.

One group of drugs acts through the CNS in the regulation of hunger and satiety. The first of these is the β-phenethylamine derivatives, which work through affecting either the release or reuptake of norepinephrine. These drugs suppress appetite. Some, however, have undesirable side effects on the cardiopulmonary system and may induce insomnia. In addition, with long-term use they lose effectiveness. The second group of drugs is the serotonergic drugs. These suppress appetite by increasing serotonin levels in that part of the brain (the hypothalamus) that controls food intake. Dry mouth and insomnia are side effects and, in some instances, pulmonary hypertension has been reported as a result of the use of fenfluramine. Fluoxetine, another of the serotonergic drugs, blocks

serotonin reuptake. It, too, is an appetite suppressant. This drug is mainly used as an antidepressant, not as an antiobesity drug. Subutramine, an amine uptake inhibitor, blocks the reuptake of norepinephrine, thereby suppressing food intake. All of the drugs described above have some measure of risk associated with their use. Some are not available at all because they are too risky to use. Others are available by prescription only and should be used only under the supervision of a physician. Even when used appropriately, they may not result in significant and lasting weight loss.

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2 Regulation of Food Intake

In the preceding chapter, the health concerns surrounding obesity were discussed, along with the observation that it is sometimes characterized by hyperphagia (overeating). In this chapter, food intake and its regulation will be discussed not only from the physiological point of view but also from the sociocultural angle. It is important for those who study the processes by which man consumes and utilizes food to realize how complex the subject is. An individual does not ingest thiamine, vitamin A, protein, and selenium—those nutrients necessary for his well-being; he ingests food, be it sirloin steak, fresh juicy peaches, or chocolate-covered ants. People do not choose to eat a green salad just because it is nutritionally sound to do so, but because of complex conscious and subconscious motivations peculiar to each individual. This chapter is designed to explore the psychological and physiological roots of the regulation of food intake. This has relevance to our understanding of not only the development of obesity but also our understanding of the reverse problem, anorexia nervosa. Understanding the motivation for eating or not eating underpins our understanding of the whole gamet of nutrition-related problems.

PSYCHOLOGICAL ASPECTS OF FOOD INTAKE

Man consumes food, not nutrients. Although specific nutrients are necessary for the growth and maintenance of the human organism, it would be shortsighted to attempt to study man's basic nutritional needs without an appreciation of those factors that influence his intake of a sufficient variety of foods to obtain nutrients. Most animals eat primarily to satisfy this nutritional need; humans' motivation for eating (or not eating) frequently satisfies nonnutritional needs. Although their desire to eat (or not eat) may have physiological origins, their selection of the foods they consume is based on a combination of forces arising from their culture, their families, their educational level, their economic circumstances, and their individual needs and idiosyncrasies.[1,2]

Culture is the integrated pattern of human behavior that is transmitted to succeeding generations. It dictates the role people play in society, as well as their responsibilities to themselves, their peer groups, and their families. Food habits are largely determined by one's culture. Many habits have existed for centuries and have been maintained as an integral part of a cultural heritage. The Judaic dietary law, based on passages in Leviticus and Deuteronomy in the Old Testament, have very specific regulations about meat consumption. The prohibitions include the flesh of birds and animals of prey, reptiles, creeping insects, animal blood, any animal that does not chew its cud and have a cloven hoof, and any species from the water than does not have fins and scales. This eliminates eagles, ostriches, snakes, lizards, grasshoppers, camels, pigs, rabbits, sharks, oysters, clams, shrimp, and mussels. A large portion of the world's population has its dietary habits controlled by the teachings found in Buddhism, Hinduism, and Jainism (a sect of dissenters from Hinduism). These philosophies support the belief that all life is sacred. This precludes killing animals for food. Such a prohibition does vary from religion to religion, and even within a religion varies from cultural group to group. For example, the Hindu reveres the cow and will not kill it for food. However, when a cow or oxen dies, members of the lowest class in Hindu society, the Dalits (it is no longer legal in India to call them "Untouchables"), may take the animal, skin it for leather, and eat the meat. Because this population ordinarily is the poorest in India, this distribution of energy and protein serves to ensure their survival.

Not only do cultural/religious practices prohibit the eating of certain foods, cultural practices also influence the foods that are eaten. The Jamaican enjoys plantain, ackee (a local fruit, poisonous when unripe, that looks and tastes like scrambled eggs), eggplant, papaya, mangoes, fish, lobster, naseberries, and otaheite apples. The Otomi Indians of the Mezquital Valley in Mexico make their meals from tortillas and from local plants such as malva, hediondilla, nopal, maguey, garambullo, yucca, purslane, pigweed, sorrel, wild mustard flowers, lengua de vaca, sow-thistle, and cactus fruit. They drink an intoxicating beverage, pulque, made from the century plant. A North American raised in a different culture would look askance at this diet and view it as unpalatable and perhaps nutritionally unacceptable. However, nutritional analyses of the Otomi Indians' diet showed it to be better balanced than that of an urban group from the United States. Persons from large sections of east and south Asia and tropical Africa refuse to drink milk; other African groups, on the contrary, prize milk as a precious food and serve it only to adult men. The Masai, an African tribal group, not only drink the milk from their cattle but draw blood from the jugular vein and drink that also. Entomophagy, the eating of insects, is accepted in many cultures. The Australian bushmen consume sugar ants and witchetty grubs. Local inhabitants from Central Africa eat fresh and fried termites, and some Japanese groups eat dytiscid beetles (fried and made into a sauce with sugar), grasshoppers, maggots, pupae of the wasp, and the larvae of silkworms.

Within a given culture, the family has a significant influence on food acceptance.[2] This happens not because there is an active effort by elders to teach the children but because the children see the same daily ritual of food preparation. Unconsciously they assimilate it. In primitive societies, meals are important daily social events; females prepare the food, and it is distributed on the basis of sex and age. In these societies as well as in our own, important family social events (christenings, weddings, funerals, etc.) are celebrated with food. These family practices become part of the cultural heritage and influence food choice.

Similarly, in progressive industrial societies, food customs are assimilated by children through the practices of their parents. As technological advances increase the complexity of a society, food as an element in its culture becomes less important. Sociologists who study contemporary society have observed that mealtimes have become less relevant as a time for family social interaction. In the United States, many meals are consumed outside the family environment; convenience foods, sandwiches, fast foods, soft drinks, and many such items are picked up by individuals on a regular basis throughout the day.

Forces outside the family influence food choices. Advertising, peer pressure, lifestyle, and age may well be more important determinants of food choices than family food practices. There has also been a change in the role of food in festive occasions. Whereas 100 years ago a large meal might have been served to celebrate an important life event, such as a wedding or a christening, today these events might be celebrated with a cocktail party that includes light finger foods and beverages, both alcoholic and nonalcoholic. As in less industrialized societies, children observe these practices and copy them.

The impact that the family has on food choices is often a reflection of the educational level of the one who selects and prepares the food. If this person is limited in education, the diet frequently shows a poor supply of nutrients.

In more recent times in the United States, a definite correlation has been shown between the education of a homemaker and the nutritional intake of her family. The data for this correlation came from surveys conducted to examine the extent of malnutrition in the United States. Initially, the Ten State Survey mandated by the federal government conducted from 1968 to 1970 was used for this correlation. Then, on a regular basis, the Centers for Disease Control has conducted the Health and Nutrition Examination Survey (HANES). For the most recent information on these surveys visit the NHANES Web site: http://www.cdc.gov/nchs/r&d/nchs_datalinkage/nhefs_data_htm. At this site there are various subcategories to access. Food intake (food recalls), clinical tests, physical examinations, anthropometric examinations, medical histories, and educational and financial status have

been evaluated. The surveys showed that the fewer the years of education the homemaker had, the greater the number of nutritional deficiencies in the diet of the family.

Economic circumstances also exert considerable influence on food choices. The Australian aborigine, living in an arid barren land, is very poor. He hunts for the food he consumes. To increase his food supply, his culture has evolved to include a host of insects as part of the daily diet. At the other extreme, in a more affluent society considerable amounts of money are spent on foods of no outstanding nutritional value. Caviar is a prime example. In 2007, Beluga caviar cost over $1300 per pound. Caviar is considered a prestigious food by many Americans and Europeans, and they delight in both serving it to guests and eating it themselves. It has no greater nutritional value than cheese, eggs, or hamburger but does impart a certain social status to the consumer.

Additionally, there are a host of other interrelated factors that influence food choices: geography, climate, methods of distribution, and storage facilities. Although the New Zealanders and the Danes live many miles apart, both their diets include an abundance of dairy products. This is a function of, in part, the similarities in their geography and climate. Citrus fruits are easily grown in Egypt. However, the food distribution methods are antiquated. As a result, many people in the country do not enjoy these fruits because they spoil in the process of being shipped. In contrast, in the United States, because of rapid transportation systems, both native grown foods and imported foods are frequently enjoyed and foods produced in one segment of the country are available throughout the nation and in all seasons. It is not unusual to see fresh strawberries in the market year round. These berries may be local in the appropriate season or may be from South America or from some distant point in the country when the local crop is no longer available.

Finally, among the factors that influence food choices are an individual's physiological and psychological idiosyncrasies. An example of the former is the situation in which individuals lack the intestinal enzyme, lactase, which is necessary to digest the milk sugar, lactose. When they drink milk, they experience abdominal bloating, cramps, and diarrhea. Needless to say, they elect to exclude fresh milk from their diet. Countless food allergies have been observed: allergies to wheat, corn, peanuts, chocolate, eggs, strawberries, tomatoes, soy products, to name a few. If an allergy can be identified, frequently a very difficult task, the item is eliminated from one's diet. Sometimes a food item is eliminated instinctively without medical documentation simply because an individual senses a relationship between his food intake and his sense of well-being.

Throughout history, there have been individuals who possess bizarre appetites.[3] During the 19th century, one Jeremiah Johnson, a mountain man, wandered the unexplored West, living off the land and the livers of Crow Indians. According to folklore, during the course of his travels, he devoured the livers of 247 Indians. In Hungary in the early 1600s the Countess Elisabeth de Bathory had a penchant for the blood of young, buxomy virgins, for through their blood she hoped to regain her youth. In her efforts to do so, she is reported to have killed 650 girls, drinking their blood and using it as a fluid in which to bathe.

All in all, humans' reasons for selecting the foods they eat is a very complex issue with apparently little consideration given to their nutritional requirements. The food one person might consider an elegant meal, another would not even touch. Human behavior added to the study of nutrition makes a difficult subject even more complex.

PHYSIOLOGICAL ASPECTS OF FOOD INTAKE

Sensory Perception of Food

In addition to the social, cultural, and economic influences on food intake, the selection of foods involves a complex interaction among the special senses: reactions of the eye, ear, nose, and mouth, and the sensations of pain and touch are all involved. The sensation of hunger, coupled with the appearance, texture, smell, and taste of food, which, in many ways, are inextricably bound to one's cultural heritage, determine whether the hand will reach out and grasp the food, transport it to

the mouth, and consume it. The appearance of the food, its color, its consistency, and its temperature are perceived by the sensory system, which includes the eye, the sense of touch, the sense of temperature, and the sense of smell. These impressions are reconciled with a person's acceptance or rejection of the food based on his social/cultural background. If the mashed potatoes are green or lumpy or burned, an individual will likely reject them. The food product simply does not conform to the expectation of the consumer for the characteristics of that food.

Temperature, taste, texture, and smell are perceived via sensory receptor systems located in the nose and mouth. A receptor is a defined organization of molecules, usually a protein, within a membrane or cellular organelle that recognizes and binds compounds or elements needed by the cell. Receptors serve to translate or transmit or initiate the sending of a message to other parts of the cell or to other parts of the body. For example, the sensory receptors in the oral cavity perceive food attributes such as texture and taste. The taste/texture translates the stimulus into an electrochemical message that is relayed to the brain. Currently, far more is known about the anatomy of the area in which these events take place than about the physiology of the events themselves.

Appearance

Part of the social/cultural force that influences one's acceptance of food is the defined expectation of an acceptable food. In part, this expectation is based on the appearance of that food. Does it have the desired size and shape, and most important, is it the expected color?[4] Work done on the relationship between food acceptance and color has shown a striking dependence of one on the other. One study showed that when jellies were colored in an atypical manner, the fruit flavors were incorrectly identified. In another study, the flavoring of colorless syrups was incorrectly identified by most of a group of 200 pharmacy students; they were even less able to identify the correct flavor if the solutions were given unusual colors. In a third study, a trained panel of wine tasters showed a dependence on color in their evaluation of wine. Food coloring was added to dry white table wine to simulate the appearance of sauterne, sherry, rosé, claret, and Burgundy. The panel judged the rosé-colored wine to be the sweetest and the claret-colored wine to be the least sweet. Interestingly, subjects who seldom drank wine and participated in the same experiment did not relate color to sweetness.

So important are these visual aspects of food that the USDA food quality grading standards are based on the appearance of food. For example, color is an important characteristic for the grading standards of beef and of fruits. Other visual characteristics, such as the presence or absence of blemishes and bruises, are also important.

Food scientists have spent considerable time trying to relate visual characteristics to measurable physical parameters that determine the acceptance or rejection of a given food. Appearance may provide a clue about the juiciness of an apple or the tenderness of a steak; these properties, of course, are also determined in the mouth and perceived there as differences in texture.

Texture

The texture of food plays an important role in food acceptance because the sense of touch is highly developed in the mouth. Texture, traditionally defined in terms of how a food "feels" in the mouth, is perceived by four different sets of receptors: the pain, tactile, pressor, and sound receptors. The pain receptors may be activated if foods are extremely hot or cold or rich in such seasonings as cayenne pepper. The active ingredients in cayenne pepper are capsaicins that act through chemically burning the surfaces of the mouth and/or tongue.

The tactile receptor receives messages about the geometrical characteristics of the food. The size, shape, and frequency of food particles will be ascertained and, if these characteristics are expected, the food will be accepted. If, however, the ice cream is gritty, the tactile receptors will perceive this, and the food may be rejected. These tactile receptors are located in the skin of the tongue, oral cavity, and throat. Not only do they perceive characteristics such as "grittiness" or

"lumpiness," they also detect differences in moisture and fat content. These latter characteristics may describe the richness, moistness, or slipperiness of a given food.

Texture is also perceived by pressor receptors located in the muscles, tendons, and joints of the mouth, jaws, and throat. These receptors are elements of the kinesthetic sense. The characteristic resistance to chewing, as in a tough piece of meat, is an example of the perception of texture by the kinesthetic sense. "Hard or tough to chew" means extreme physical resistance to the actions of the teeth and jaws. Strenuous exertion by the voluntary muscles is required; this, in turn, is perceived as changes in the position, movement, and tension of the teeth and jaws. The kinesthetic sense is difficult to study because it is not easily located and identifiable. However, through the use of such drugs as cocaine, which blocks the muscle receptors, it has been learned that the oral kinesthetic sense originates as much from the joints as from the muscles. Four sets of receptors are involved: two in the muscles, one in the tendon, and one in the fascia associated with a muscle. There are free nerve endings (also called *pressor receptors*) in the muscles that are activated by the chewing of such hard items as nuts, crackers, or bones, and that stimulate a sense of motion.

Some textural characteristics are sensed by sound receptors. The sounds a food makes when chewed contributes to the acceptability of the item. The crunch of crisp celery or the snap of a fresh potato chip contribute to the enjoyment of that food. The stimulation of tactile and kinesthetic receptors and of the auditory receptors play important roles in the evaluation of textural characteristics of the food. Individuals will vary in their preferences for smooth, chewy, crisp, hot, cold, or crunchy textures; all of these attributes, however, are based not on taste, smell, or appearance but on mouth "feel" and "food sounds." In addition, cultural influences will contribute to the textural expectations of individuals. For example, a soft, smooth, bland-textured food may be associated with the food needs of infants or invalids and may not be accepted by the young male with a strong "macho" self-image. Juicy, chewy textures requiring exertion of the jaw muscles may be very acceptable to the young adult but are much less acceptable to the school-age child.

Smell (Olfaction) and Taste (Gustation)

Olfaction and gustation are intimately related. Persons who have lost their sense of smell, as frequently happens with a cold, complain that food is not as tasty as when they are well. This is because part of their appreciation of food has decreased through a temporary impairment of their ability to smell.

Among the special senses, the sense of smell is the most sensitive. The average person can detect one part in a trillion parts of air for some high-potency odorants. For example, ethyl mercaptain (ethanethiol) can be detected at 4.0×10^{-11} mg/mL air. However, the perception of a particular aroma quickly diminishes if the aroma persists. This process is called *olfactory adaptation* and begins the first second after an aroma is perceived.

The perception of smell is a subjective phenomenon. Depending upon a person's expectations, an item can have an intrinsically pleasant or unpleasant aroma. One example of such subjectivity is the scent of a gardenia: many people enjoy it but some find the aroma too strong or overpowering.

Because the perception of smell is so highly subjective, it is difficult to study it either qualitatively or quantitatively. Some physiologists contend that whereas taste perception involves the differentiation of four primary tastes, smell involves many primary odors. However, little progress has been made toward identifying and classifying these odors.

The perception of taste is, as is the perception of smell, highly subjective.[4,5] Despite this, physiologists have established that man perceives four primary tastes: sour, salty, sweet, and bitter. Chemicals that can elicit any one of these tastes are called tastants. The tastants must be in solution in order to be perceived. Both water-soluble and lipid-soluble compounds can serve as tastants.

Different substances evoke each of the primary tastes. The chemicals that elicit a sour taste are acidic compounds; the hydrogen ion, rather than the associated anion, actually stimulates the receptor. Generally, the sourness is proportional to the concentration of the hydrogen ion. A more acidic

compound will trigger a stronger response than a neutral compound. An anion of an inorganic salt produces a salty taste. The halides, chloride, fluoride, bromide, and iodide are usually associated with a salty taste.

A variety of chemicals, mostly organic, trigger the sensation of a sweet taste; sugars, glycols, alcohols, aldehydes, ketones, amides, esters, amino acids, sulfonic acids, halogenated acids, and the inorganic salts of lead and berryllium. The sweetest compound known is the *n*-propyl derivative of 4-alkoxy-*e*-aminonitrobenzene. Such organic compounds as the glycosides amygdalin (found in almond kernels) and naringin (found in citrus fruit), and the alkaloids caffeine, quinine, strychnine, and nicotine taste bitter.

The average person is more receptive to a bitter taste than to a sour, salty, or sweet taste. For example, a 0.000008 M quinine solution tastes bitter, but a much higher concentration (0.0009 M) of hydrochloric acid is required to taste sour, and an even higher concentration (0.01 M) of sodium chloride to taste salty, or of sucrose to taste sweet.

Additionally, the pleasantness of a given taste relates to the concentration of the tastant. For example, as the concentration of sucrose in a solution is increased, its taste changes from unpleasant to pleasant; the pleasant sensation of sweet arises only at higher concentrations. In contrast, a bitter taste can be pleasant at low concentrations but become unpleasant at high ones. In small quantities, the white membrane of orange or grapefruit sections enhances the flavors of these fruits; however, they are seldom eaten alone because of their bitter taste.

Within a given taste modality, some chemicals can be tasted at a lower concentration than others. This can be expressed quantitatively by measuring the detection threshold and, from this, calculating the relative taste indices. Table 2.1 gives the relative taste indices of several substances with the intensities of each of the four primary sensations referred to a reference compound: the acidic substances to hydrochloric acid; the sweet substances to sucrose; the bitter ones to quinine; and the salty ones to sodium chloride. Each of these reference compounds is assigned an index value of 1.

Each of these primary tastes is perceived through the organ of taste, the taste cell. The taste cell is about 1/30 mm in diameter and 1/16 mm in length. It contains two kinds of cells: the receptor cell and the supporting cell. The gustatory receptor cells, also called the taste buds, are barrel-shaped, modified epithelial cells. From one end of each taste cell protrudes several microvilli or taste hairs.

TABLE 2.1
Relative taste indices of different substances

Sour substances	Index	Bitter substances	Index	Sweet substances	Index	Salty substances	Index
Hydrochloric acid	1	Quinine	1	Sucrose	1	NaCl	1
Formic acid	1.1	Strychnine	3.1	4-propoxy-3-amino nitrobenzene	5000	NaF	2
Chloracetic acid	0.9	Nicotine	1.3	Saccharin	675	$CaCl_2$	1
Lactic acid	0.85	Phenylthiourea	0.9	Chloroform	40	NaBr	0.5
Tartaric acid	0.7	Caffeine	0.4	Fructose	1.7	NaI	0.35
Malic acid	0.6	Pilocarpine	0.16	Alanine	1.3	LiCl	0.4
Potassium H tartrate	0.58	Atropine	0.13	Glucose	0.8	NH_4Cl	2.5
Acetic acid	0.55	Cocaine	0.02	Maltose	0.45	KC1	0.6
Citric acid	0.46	Morphine	0.02	Galactose	0.32		
Carbonic acid	0.06			Lactose	0.3		

Source: Adapted from Guyton, A.C. (1971). *Textbook of Medical Physiology*. 7th ed., W. B. Saunders, Philadelphia, p. 639.

These microvilli extend through a taste pore within the tongue's surface to contact the fluids of the mouth. The other end of each taste cell is innervated with gustatory nerve fibers. One cell may be innervated with several fibers or several fibers may innervate one cell; there is no one-on-one line of communication between the individual taste cell and the central nervous system (CNS).

The traditional view of taste held that its perception was mediated only by taste buds found in the papillae on the surface of the tongue, and that there were specific areas where each modality, and only that modality, was perceived. This is not wholly true. Taste buds are actually found several places within the oral cavity—on the surface of the tongue, palate, pharynx, and larynx, and sometimes on the cheeks.

The tongue, although it perceives all four of the taste modalities, is the most sensitive to salty and sweet. The palate, on the other hand, is more sensitive to the sour and bitter tastes than to the salty and sweet tastes. The pharynx also detects all four tastes but not to the same extent as the tongue and palate. Scattered over the entire oral cavity of an adult are approximately 10,000 taste buds. The taste cells within the taste buds are short-lived cells with a rapid turnover rate. In the human the taste cell has an approximate lifetime of 250 h. The ability to quickly regenerate is in marked contrast to most other elements of the nervous system. As one grows older, the rate at which taste cells are regenerated is decreased and, as a consequence, there is a concomitant decrease in quantity. With a decrease in number of taste cells, taste acuity declines.

The anatomical features involved with gustation have been well studied, as have the tastants that elicit the sensation. Organic chemists have long known that small structural changes can alter tastes. For instance, the sugars listed in Table 2.1 are structurally similar. The taste response they elicit, however, varies from very sweet (β-D-fructose) to bitter (β-D-mannose). The most remarkable feature of these compounds is that mutarotation around the anomeric carbon of α-D-mannose to make β-D-mannose changes the taste perceived from sweet to bitter.

Saccharin is sweet, but its *N*-alkylated derivatives are tasteless. The alkali metal salts of cyclamate (cyclohexyl amine sulfate) are sweet, and the amine salt is nearly tasteless. It has been found that the dipeptide L-aspartyl-L-phenylalanine methyl ester (aspartame) and certain related compounds are nearly 200 times sweeter than sucrose. Because this compound is composed of natural amino acids and is quite low in calories, it offers intriguing possibilities as a synthetic sweetener. If any other amino acids are substituted for the L-aspartate (even the closely related compound L-glutamate), the resulting product is tasteless. However, sweetness is maintained if phenylalanine is replaced by methionine or tyrosine. The dipeptide free acid of the methyl ester is not sweet, nor is the ethyl ester as sweet as the methyl ester.

As with smell, the taste process includes both a preneural and neural phase. The preneural event involves an interaction between the tastant and the receptor cell. It is generally believed that this is a steric interaction between these two sites, possibly involving conformational changes.[4] Then, in some incompletely understood manner, this triggers the neural phase, which is the depolarization of specific taste nerves and the passage of impulses to the brain.

For the auditory and visual senses, medical specialties have evolved to diagnose and treat, where possible, deviations from normal. Similar specialties have not been developed to solve problems of taste and smell, nor have great strides been made in the diagnosis and treatment of disorders of these senses. However, abnormalities in taste perception have provided a means for verifying and enlarging the understanding of the mechanism of the taste sensation.

Anatomic abnormalities of both the palate and the tongue have been associated with a decrease in taste sensitivity. Patients with abnormal palate structures have significantly elevated thresholds for sour and bitter tastes but not for sweet and salty ones. An increase in threshold means that a greater concentration of the tastant is required for detection and recognition of that tastant by a subject. However, not all patients with anatomic abnormalities of the hard or soft palate exhibit taste disturbances. For example, those with gross clefts of the back part of the hard palate detect and recognize all four modalities of taste.

People who wear dentures report an increase in the detection and recognition thresholds for sour and bitter tastes, but no change in their response to sweet and salty tastes. Dentures fit close to the palate and cover part of the mouth area containing taste receptors. As a result, there may be an artificial masking of taste receptor sites in these parts of the mouth and taste perception may be affected.

Abnormalities on the surface of the tongue, such as lichen planus and tumors, cause a decrease in taste acuity. Diseases that affect the nerve supply to the tongue (such as postdiptheritic neuritis, sarcoidosis, and Bell's palsy), severe trauma, and irradiation of the oral cavity as part of treatment for a malignancy will result in a decrease in taste sensitivity. In all of these abnormal states, there is a reduction or omission in the number of taste receptor cells, which has been given as the reason for the decrease in taste acuity. Interestingly, however, congenital underdevelopment or absence of the tongue is not accompanied by a decrease in taste acuity.

Speculation about the specific functions of the taste bud in taste perception have resulted from studies of patients with Type I familial dysautonomia (Riley–Day Syndrome). In this syndrome, the tongue's surface is smooth; the sulcus terminalis, taste buds, and fungiform and circumvallate papillae are missing, and the number of unmyelinated free nerve endings is greatly diminished. Patients demonstrate significantly raised detection and recognition thresholds; some cannot consistently distinguish between water and saturated solutions of sodium chloride, sugar, urea, and 0.03 M-hydrochloric acid. However, when treated with methacholine (an α-adrenergic drug), these patients have normal taste perception while the drug remains in their system. This has led to the suggestion that the taste buds function as a chemical sieve. It is proposed that taste buds have pores of a small, controlled size through which chemical stimuli may reach the nerves. Numerous factors, not yet identified, may control this pore size. In patients with familial dysautonomia, treatment with methacholine causes an increase in membrane permeability, including that of the lingual surface, which, in turn, allows a tastant to reach the unmyelinated, free nerve endings in the tongue. Thus, the taste threshold is lowered, and the patient is more responsive to the tastant.

The divalent cations—particularly copper, zinc, and nickel—have been reported to affect taste sensitivity. When given to patients with hypoguesia, some improvement occurs. Observations of serum and tissue levels of copper in patients with rheumatoid arthritis and Wilson's disease have led to conjectures about copper's role in the regulation of taste acuity. Patients with either of these diseases are often treated with D-penicillamine. With this therapy, patients having Wilson's disease experience no change in their taste acuity; however, patients with rheumatoid arthritis frequently report a decrease. D-penicillamine therapy is associated with a decrease in serum and tissue copper levels. For arthritic patients, this does indeed happen. Not so in patients with Wilson's disease, which is characterized by abnormally high levels of serum copper. Penicillamine simply reduces this high level to a normal level. The taste acuity of arthritic patients, if given oral copper sulfate, returns to normal. Thus, copper appears to be directly involved with taste acuity and its depletion leads to hypoguesia.

Recent reports indicate that taste dysfunction may be associated with impaired zinc absorption and decreased levels of zinc in the saliva. Oral therapy of zinc or nickel returns the taste acuity to normal.

Steroid hormones have also been implicated in the taste mechanism through studies of diseases of the endocrine system. Patients with Addison's disease, decreased adrenal cortical function, or panhypopituitarism have lowered detection thresholds. Patients with Addison's disease are sometimes able to detect concentrations of tastants as low as 0.01% of that perceived by normal subjects. In both cases, the heightened taste sensitivity returns to normal when the missing steroids are given. The mechanism by which the steroids influence taste perception is not known.

A comprehensive, unified theory of taste perception has not yet been realized. But the taste of food, as well as its appearance, texture, and smell, is intimately involved in humans' desire to eat.

Neuronal Signals for Hunger and Satiety

Internal cues regulate food intake through a number of signals and responses that ultimately result in the initiation or cessation of feeding. These cues are in addition to those described above, which involve the cerebrum. Both short-term and long-term controls are exerted that, over time, serve to regulate the food intake of normal individuals so that they neither gain nor lose weight. Food-intake control rests, in part, with the integration of a variety of hormonal and nonhormonal signals that are generated both peripherally and centrally. The hypothalamus is thought to be the main integrator of these signals. Other discrete areas are also involved. The hypothalamus is located beneath the thalamus, a part of the forebrain, close to the pituitary. The hypothalamus is involved in both the initiation and cessation of food and water intake. It serves as an endocrine organ that produces the hormones that, in turn, modulate the release of hormones from the posterior pituitary. It also releases other hormones, called *releasing factors* or *tropins*, which control the activity of the anterior pituitary. The area of the brain that includes the thalamus, hypothalamus, and pituitary has been called the center of existence, because it controls much of what is known as instinctive behavior. In addition to its regulatory effect on appetite, satiety, and thirst, the hypothalamus serves, through its effect on the pituitary, as the main subcortical control center for the regulation of the parasympathetic and sympathetic systems; for the regulation of heart rate; and for the regulation of vasodilation and vasoconstriction, two important processes for the maintenance of body temperature. If the body temperature rises, vasodilation (increased blood flow through skin capillaries), along with increased respiration and increased sweat loss, occurs, thus increasing body heat loss. Conversely, if body temperature is below normal, vasoconstriction (decreased blood flow) occurs and body heat is conserved. Vasoconstriction, vasodilation, and heart rate are also important to the regulation of blood pressure. Indirectly, the hypothalamus regulates the activity of the gastrointestinal system, the emotions, and spontaneous behavior.

The role of the hypothalamus in the control of eating behavior has been well studied. As early as 1840, extreme obesity in man was reported to occur in patients with hypothalamic tumors. Recognition of the involvement of the ventromedial hypothalamus in the regulation of food intake did not come until it was shown that if the ventromedial hypothalamic area was destroyed or lesioned, the animals overate, with a resulting increase in body fat. If the lateral hypothalamus was lesioned, animals became both adipsic (had no thirst response) and aphagic (did not eat). This relationship of feeding behavior to drinking behavior can be understood when the consequences of dehydration due to absence of fluid intake are realized. In adipsic animals, saliva production is significantly reduced; thus, laterally lesioned animals have difficulty, initially, in swallowing dry food. As the lesioned animal recovers or adapts to the lesion, he drinks when he eats dry food but does not eat when water deprived. In addition, the lesioned rat does not drink in response to serum hyperosmolarity (increased levels of solutes in the blood), hyperthermia (increased body temperature) or hypovolemia (decreased blood volume). Animals with lesions in the lateral hypothalamus do not respond to reductions in blood sugar levels (via insulin injections) and will die in severe hypoglycemia rather than eat readily available food. This observation suggested that both the lateral and ventromedial nuclei in the hypothalamus interact via chemical signals to control eating and drinking. Since eating and the cessation of eating are under hypothalamic control, it is reasonable to assume that this behavior is initiated or stopped by a series of signals emitted from or received by this tissue. The nature of this signal system is rather complex. As research continued in this area, it was learned that other areas of the brain are involved. The paraventricular nucleus located slightly in front of the dorsomedial nucleus appeared to be involved in the regulation of glucose intake as it relates to the maintenance of glucose homeostasis. The dorsomedial nucleus, found on either side of the third ventricle, seems to be involved in the control of body size but not body fat and, because of this involvement, is likely to play a role in food intake. In addition, the area postrema of the brain stem and the caudal medial nucleus have been implicated in food-intake regulation. All of these studies implicating various portions of the brain in the regulation of food intake were conducted using

brain lesioning experimental protocols. These protocols, however crude, provided the basis for the concept that feeding behavior was coordinated by the CNS in response to factors in the blood and that likely this was a neuroendocrine system.[6,7]

Studies of energy balance in relation to brown fat thermogenesis (BAT) supported this concept because BAT could be stimulated by infusions of norepinephrine. Stimulation of BAT was energy wasting and led to body weight loss if that stimulation persisted over a period of time. Again, this was a gross measurement, not one likely to occur on a regular basis in normal individuals. Nonetheless, it gave a hint of the involvement of the CNS in the regulation of energy balance. Because energy intake is an integral part of the energy balance, it suggested that neuroendocrine factors were indeed involved in the regulation of feeding behavior.

That energy balance was related to food intake was not a novel concept, yet it was one that investigators in a wide variety of fields had to study together in order to make sense of it all. There had to be an integration of the peripheral and central signaling systems that could then explain why animals (and man) increased or decreased their food consumption.[8,9] Models such as that shown in Figure 2.1 were devised to allow for the further investigation of these systems that then contributed to our understanding of how food intake is controlled. The nature and identity of the signals were not known but using a variety of techniques they were presumed to occur.

Several different approaches were used to elucidate these signals. Among these were the use of the parabiotic technique.[10–14] This involved the surgical pairing (skins of each animal were sutured together on one side so that the animals shared their interstitial fluids but nothing else) of two animals that differed in either food intake or genetic background. In the first paradigm, two young animals were surgically connected. One was force fed an overabundance of food.[11,12] The second member of the pair was allowed to eat ad libitum. The first member became fat. This animal stored the excess energy intake as fat. The second member of the pair ate very little and became very thin. The thin member of the pair had decreased adipocyte lipogenic activity.[11] In another paradigm, the members of the pairs were siblings that differed in their genetic background: one carried the homozygous recessive fatty gene whereas the other did not.[10,13,14] Both were allowed ad libitum access to food. The fatty member of the pair was hyperphagic. Hyperphagia is one of the traits of the genetic obesity. The other member ate very little. In both paradigms there clearly was a signal circulating

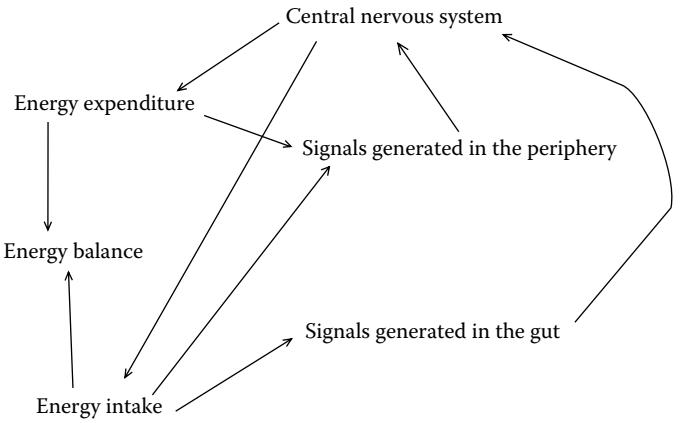


FIGURE 2.1 A general scheme for integrating the various signals for food intake with the regulation of energy balance. In this scheme energy balance is central to determining energy intake. Food, when consumed, stimulates the production and release of signals that travel to the brain and, in turn, elicit signals that regulate energy expenditure and also serve to reduce subsequent food intake.

from the obese animal to the lean partner influencing its appetite since its food intake was significantly less than control parabiosed animals without the obesity gene.

That there was a blood-borne factor was now clearly evident but the identity of this factor and its mechanism of action was not known until some investigators interested in genetic obesity began looking at possible genes that if mutated could phenotype as obesity.[15–17] Truett et al mapped the rat fatty (fa) gene to chromosome 5 and showed that it was homologous to the db gene in the mouse.[15,16] Later Zhang et al. showed that the ob gene in mice mapped to chromosome 6.[17] Chromosome 6 had been identified years ago as the location of the ob gene and chromosome 4 for the db gene by scientists at the Jackson Laboratory in Bar Harbor, Maine. The phenotype of all three mutations included hyperphagia as well as obesity and Type 2 diabetes. Although the mutations were mapped to different locations on the chromosomes, the common phenotype of hyperphagia suggested a possible linkage. That linkage turned out to be leptin and its receptor. The mutation in the ob/ob mouse was a mutation in the gene that encoded leptin, whereas the mutation in the fa/fa rat and the db/db mouse was in the gene for the leptin receptor.[18–21] Leptin deficiency in humans is rare yet it has been reported.[22] Leptin receptor defects in humans are more likely.

Leptin is a cytokine produced and released by the adipose tissue. A cytokine is a soluble polypeptide or glycoprotein that is similar to a hormone but is not necessarily produced by a recognized endocrine organ. In the case of leptin one could argue that it is a hormone. It is released by the adipose tissue and travels to the brain and to tell the brain that satiety has been attained. In this respect it is a hormone, as by definition, a hormone is a substance released by one tissue having an effect on a tissue some distance from its point of origin. One could also argue that in this instance the adipose tissue is an endocrine organ and the brain the target tissue. The brain has multiple receptors for leptin. The hypothalamus, in particular, has an abundance of the long form of the receptor on its surface with an intracellular domain of 303 amino acid residues.[21] This intracellular domain probably has signaling properties, and when leptin is bound to the extracellular domains, cell signaling occurs that results in the perception of satiety by the individual.[23–25]

As research in leptin developed it was discovered that it had other functions in addition to signaling satiety. It also signals apoptosis of adipocytes,[26] and serves as the link between adipose tissue mass and the central neural network.[27] With age, leptin responsiveness falls, whereas serum leptin levels rise[28,29]; this may explain the change in energy balance that occurs as people (and animals) age.

As with any hormone, there are numerous additional linkages for leptin as it function within the metabolic and endocrine networks.[30] Leptin receptors are found in many tissue types. In particular, they can be found in the paraventricular nucleus (PVN) that contains neurons that produce thyrotropin-releasing hormone (TRH) secretion by the anterior pituitary. TRH simulates the release of thyroid-stimulating hormone (TSH) that stimulates the synthesis and release of the thyroid hormones. These hormones in turn stimulate metabolic rate, lipolysis, oxidative phosphorylation, and energetic efficiency. Leptin has been shown to elicit these same changes in metabolism.[25,30–33]

Leptin is a potent stimulator of growth hormone release. This hormone has effects on metabolic efficiency and in particular on protein homeostasis.[34] Glucocorticoid mediated lipolysis is responsive to leptin[35] and, conversely, leptin gene expression is activated by glucocorticoids.[36] Central leptin administration increases glucorticoid release.[37] Leptin has been shown to modulate glucose homeostasis as well by affecting insulin action.[38] Thus, it should come as no surprise that leptin directly alters lipid partitioning, and the homeostasis of all of the macronutrients.[33,39]

Leptin, as mentioned, is a major player in the regulation of food intake, yet food or food components can have effects on leptin production and release. For example, zinc deficiency regulates leptin production and release.[40,41] No doubt other nutrients will be shown to have this effect as well when a deficiency of their intake is characterized by a decrease in food intake.

Although leptin has been identified as a major satiety hormone, other neuropeptides have been identified as well. Among these are neuropeptide Y, which has been shown to have the opposite effect; it stimulates feeding.[28,42,43] Ghrelin is another. Ghrelin is a satiety factor that is responsive to protein intake as well as physical activity.[44] In humans with metabolic syndrome, a

carbohydrate-restricted diet that induces weight (fat) loss resulted in increases in ghrelin levels, as well as increases in another of the satiety-signaling compounds, cholecystokinin.[45] Cholecystokinin is released by cells in the intestine.

Lastly, leptin has a role in oxidative stress.[46–48] Leptin stimulates angiogenesis and exerts an atherogenic effect through the generation of oxidative stress in endothelial cells. Leptin appears to regulate peroxisome proliferator activated receptor γ, tumor necrosis factor α, and uncouling protein 2 in adipose tissue. These are proinflammatory responses and are typical of the pathophysiology of degenerative disease. This action of leptin might explain the association of diabetes, atherosclerosis, and obesity that commonly occurs if, in fact, the obesity is due to an abnormality in the leptin system. If there is too much circulating leptin produced because of receptor insensitivity due to either age or perhaps some polymorphism in the leptin receptor, this leptin could then be available to stimulate chronic disease development.

Although the literature is rapidly expanding with respect to the neurological signals that regulate satiety and hunger, there still has to be an overall understanding of feeding and satiety that includes not only the signals for hunger and those for satiety, but also signals for the continuation of feeding once feeding has commenced. One theory suggests that eating is a response to variations in the level of circulating glucose.[49] This theory, called the *glucostatic theory*, proposes that cellular energy requirements determine feeding behavior. In particular, it suggests that brain cells, which use glucose almost exclusively as their metabolic fuel, are exquisitely sensitive to fluctuations in blood glucose levels, and, as such, will activate the "feeding center" to initiate feeding when blood glucose levels dip below normal, or activate the "satiety center" to stop feeding when blood glucose levels are high. Support for this theory comes from the observation that feeding is initiated in animals injected with insulin and from the observation that gold thioglucose (which destroys the satiety center of the hypothalamus) is ineffective in diabetic animals. In the latter case, eating continues because gold thioglucose does not get into the hypothalamic cells of diabetic animals to destroy the satiety center. This occurs because the penetration of gold thioglucose into the hypothalamic cells is insulin dependent. This is a very simplistic approach to food-intake regulation, but it has provided a framework for the building of a more cohesive explanation of the systems that operate to ensure energy balance. More recently, Pannacciulli et al. showed that carbohydrate oxidation and balance predicts subsequent ad libitum food intake and can influence short-term weight change.[50] They concluded that carbohydrate balance is a contributing metabolic factor affecting food intake.

Another school of thought is that eating behavior is controlled by the fat-cell size and number.[51] This is called the *lipostatic theory*. It speculates that there is a set point for each animal for the number of fat cells and their fat content, and that when this set point is reached, the animal ceases to eat. In the absence of eating, these fat stores are mobilized and used until a lower set point is reached and feeding again commences. This theory is supported by observations that starved animals ate large amounts of food when realimented after starvation until they regained their prestarvation weight; then, they resumed their prestarvation eating behavior. In other words, they overate to fill their fat depots, then ate only enough to maintain these depots. Modulation of food intake by signals arising in adipose tissue has been an important component of our understanding of energy balance as discussed above.

It is possible that the theories described above (as well as some others) may be integrated into one to more readily allow for the understanding of the signals needed to initiate, maintain, and stop feeding. In addition to the cytokines already described and released by the adipocytes, the brain, and the gastrointestinal tract, there are their cognate receptors that also play a role in the initiation, maintenance, and cessation of feeding. Most of the hormonal signals have short-term effects on feeding, but do not affect overall long-term food consumption. These signals may truly be hunger or satiety signals, but one must not confuse a satiety signal with a food-intake-inhibition signal or confuse a hunger signal with a food-intake-initiation signal. The initiation may occur but not be sustained sufficiently to result in significant food consumption. Similarly, inhibition may occur but may not significantly alter overall food intake. Therefore, not only the stop and start signals need

to be examined, but so, too, does the overall control of food intake that has a long-term effect on energy balance. Sustaining feeding or food abstinence may involve not only the factors discussed above but may also include those listed in listed in Table 2.2. Initiation may be hormonally induced but feeding may be sustained because the food is found to be pleasing as per the discussion on the hedonistic qualities of food—its taste, smell, texture, etc. Similarly, cessation of feeding even though hormones have signaled initiation may occur if the food is not palatable or acceptable.

Hormones that can enhance food intake at one level can suppress it at another. Insulin is a prime example. Thyroxine is another. Normal individuals given a low dose of insulin will experience hunger. However, large doses of insulin can provoke a serious hypoglycemia that will have the opposite effect. Campfield and Smith have studied the signals for feeding that occur in the rat. They have shown that feeding is initiated when the brain perceives a small fall in blood glucose. Transient declines within the normal range of blood glucose levels were found to precede meal initiation. This feeding response could be attenuated if blood glucose levels were elevated via an intravenous infusion of glucose. Preceding the transient fall in blood glucose was a transient insulin spike that probably was responsible for the transient fall in glucose. Individuals soon after a high-glucose meal will feel satiated; their blood and brain glucose levels have risen, as has blood insulin, and their appetite is suppressed. Other hormones are involved as well. In hypothyroidism, hunger signals are

TABLE 2.2
Factors that affect food intake

Enhancers	Suppressors	
Insulin	Leptin	Corticotropin-releasing hormone
Testosterone	Estrogen	Phenylethylamines[a]
Glucocorticoids	Mazindol	Neurotensin
Thyroxine	Substance P	Bombesin
Low serotonin levels	Glucagon	Cyclo-his-pro
Dynorphin	Satietin[b]	High-protein diets
Bendorphin	Serotonin	High blood glucose
Neuropeptide Y	GHRH	Enterostatin
Galanin	Fluoxetine	Calcitonin
Opioid peptides	CCK	Amino acid imbalance
TRH	Anorectin	TNFα
Histidine (precursor of histamine)		Pain
Desacetyl-melanocyte-stimulating hormone		
Tryptophane (precursor of serotonin)		
Antidepressants[c]		
Somatostatin		
TSH		

[a] These are drugs and, except for phenylpropanolamine, are controlled substances. Many have serious side effects. They are structurally related to the catecholamines. Most are active as short-term appetite suppressants and act through their effects on the central nervous system, particularly through the β-adrenergic and/or dopaminergic receptors. This group includes amphetamine, methamphetamine, phenmetrazine, phentermine, diethylpropion, fenfluramine, phenylpropanolamine. Phenyl-propanolamine-induced anorexia is not reversed by the dopamine antagonist haloperidol.

[b] This is a blood-borne factor.

[c] All of these drugs are controlled substances, and their use as antidepressants must be carefully monitored. This group includes amitriptyline, buspirone, chlordiazepoxide, chlorpromazine, cisplatin, clozapine, ergotamine, fluphenazine, impramine, iprindole, and others that block 5-HT receptors.

poorly perceived. The patient, although not anorexic, does not have a strong drive to eat. In contrast, hyperthyroidism is characterized by strong, almost unremitting hunger.

Within this framework are a number of afferent and efferent systems that influence food intake by providing information to the brain and relaying instructions via neuronal signals from the brain to the rest of the body.[52-54] Food intake can be increased or decreased with reciprocal effects on the CNS when these peptides are administered. Galanin, neuropeptide Y, opioid peptides, growth-hormone-releasing hormone, and desacetyl-melanocyte-stimulating hormone increase food intake, whereas insulin excess, glucagon, leptin, cholecystokinen, anorectin, corticotropin-releasing hormone, neurotensin, bombesin, cyclo-his-pro, and thyrotropin-releasing hormone reduce food intake. Several of these hormones or peptides have specific actions with respect to the intake of specific food components. For example, increases in neuropeptide Y result in increased carbohydrate intake, whereas increases in the level of galanin and opioid peptides increase fat intake. Fat intake is suppressed when the blood level of enterostatin rises. Rising blood levels of glucagon suppress protein intake. All of the above are short-term signals that appear to regulate food selection as well as the amount of food consumed. Although most of these studies have been done in carefully prepared experimental animals (usually rats), there is sufficient indirect evidence to suggest that short-term food intake is similarly regulated in man. In humans, serotoninergic agents are being developed for use as treatments for obesity and eating disorders. These agents are successful because they either block the binding of serotonin (5-hydroxytryptamine, 5-HT) to its receptor, or upregulate the receptors' binding affinity. The 5-HT receptors are widespread throughout the cerebral cortex, limbic system, striatum, brain stem, choroid plexus, and almost every other region of the CNS. Because serotonin suppresses feeding if the receptor is blocked, feeding is enhanced. Thus, drugs that block these receptors are useful in treating anorexia (decreased desire to eat), especially the anorexia that accompanies anxiety, depression, obsessive–compulsive disorders, panic disorders, migraine, and chemotherapy emesis. In contrast, drugs that potentiate the binding of 5HT to its receptor will result in a suppression of appetite and may be useful in treating the hyperphagia of Prader–Willi syndrome and that associated with genetic obesity. As the database on leptin and its receptor expands, doubtless there will be drugs that either interfere with this system or potentiate it. The latter might be useful in the treatment of obesity, whereas the former might be helpful in treating anorexia.

Drugs, particularly those used in cancer chemotherapy, frequently have appetite suppression as a side effect. In part, this reduction in food intake may be due to disease- or drug-induced changes in taste and aroma perception and, in part, due to the effects of the disease or drugs on the CNS, particularly the adrenergic and serotonergic receptors. Several of the drugs listed in Table 2.2 are appetite suppressants and are chemically related to the catecholamines. As indicated, some of these drugs can be addictive and are therefore controlled substances. It appears that none of the drugs listed in Table 2.2 are free of side effects.

Some steroids affect food intake. Adrenalectomized animals or humans with Addison's disease, both having glucocorticoid-deficient states, do not perceive normal hunger signals. If without food for extended periods of time, these individuals are difficult to realiment. However, once eating commences, a normal feeding pattern will be maintained. In excess, glucocorticoid stimulates feeding, and patients with Cushing's disease (excess glucocorticoid production) or patients who are receiving long-term glucocorticoid treatment will report increased hunger and food intake. Patients with Cushing's disease are often characterized by large fat depots across the shoulders and in the abdomen. In addition, obese patients are frequently characterized by excess blood levels of both glucocorticoids and insulin. As noted above, these hormones stimulate appetite and feeding.

Within the normal range, doses of testosterone and estrogen, although also steroids, have opposite effects with respect to food intake. In experimental animals, day-to-day variations in food intake by females will follow the same pattern as their day-to-day variation in estrogen level. When estrogen is high, food intake is suppressed and vice versa. Women who are anestrus due to ovariectomy or who are postmenopausal frequently lose their day-to-day estrogen-mediated food-intake

pattern.[55] With this loss is a more even (and somewhat increased) food intake and subsequent body fat gain. This has been found as well in castrated female rats, dogs, and cats.

The gain in body weight as fat may be explained by the loss in food-intake control exerted by the estrogens rather than by an estrogen-inhibiting effect on lipogenesis. Testosterone increases food intake marginally, but also stimulates protein synthesis and spontaneous physical activity. As a result, body fat does not increase. As testosterone levels decline in males with age, protein synthesis declines and the body tends to sustain its fat synthetic activity. This results in a change in body composition with an increase in body fat stores. The age-related decline in testosterone production may not be accompanied by a decline in food intake.

Although food intake can vary from day to day in response to minor day-to-day variations in food supply, activity, and hormonal status, body weight is relatively constant.[56] The mechanisms that control body weight are very complex and the fine details of this regulation are far from clear. However, suffice it to say, major long-term deviations in either food intake or physiological state can affect body weight or energy balance. If food intake (energy intake) is curtailed for days to months, body weight will fall; similarly, if food intake is dramatically increased, body weight will increase. This relationship assumes no change in body energy demand. As described in the section on trauma, the energy requirement can be increased up to tenfold by major illness despite the fact that the patient may be recumbent and perhaps sedated. Similarly, individuals who have markedly changed their activity levels will see this affect their energy balance.

If strenuous exercise is added without an increase in food intake, this will increase energy expenditure and negative energy balance, or weight loss will occur. In most individuals, therefore, long-term changes in energy balance, either through changes in intake or expenditure, will result in a body weight change.

Anorexia Nervosa and Bulimia

That food intake can be consciously controlled is evident in the condition known as anorexia nervosa. This condition is frequently observed in adolescent females and is related to their inaccurate perception of their body fatness. They become obsessed with the desire to be thin and either refuse to eat and adequately nourish their bodies or they eat, then force themselves to regurgitate the food. Self-induced vomiting is called bulimia. Additional behavior related to an obsession with body image includes the excessive use of laxatives and diuretics and the extensive participation in exercise designed to increase energy expenditure. Although the patients may be eating some food, they are not consuming enough food to meet their macro- and micronutrient requirements. Because of this, they are in negative energy and protein balance. Their serum leptin levels are low[57-59] as are serum serotonin levels.[60] These patients are characterized by little body fat. Because ovulation requires a minimal amount of fat in the body, ovulation ceases.[55] Amenorrhea, hypothermia, and hypotension also develop and, if unrecognized and untreated, anorexics may starve to death. Some people, for whatever reason, self-recognize and resume eating.

In many respects, anorexic people have physiological/biochemical features that are similar to those patients described in the section on starvation. Their catabolic hormone levels are high, and their body energy stores are being raided as a result. Insulin resistance due to the catabolic hormones is observed. Liver and muscle glycogen levels are low. Fat stores are minimal. As the weight loss proceeds further, these individuals have reduced bone mass, decreased metabolic rate, decreased heart rate, hypoglycemia, hypothyroidism, electrolyte imbalance, elevated free fatty acid and cholesterol levels, peripheral edema, and finally, cardiac and renal failure. When their fat stores fall below 2% of total body weight, they will die. This 2% represents the lipids essential to the structure and function of membranes as well as those complex lipids that compose the CNS. With this scenario in mind, the clinician faces the challenge of reversing the condition. Just as it is difficult to reverse starvation-induced changes in the metabolism of unintentionally starving humans (see sections on starvation, protein-calorie malnutrition, and trauma in Chapter 1), reversing the weight loss

of anorexic patients presents some special challenges. The energy requirements for weight regain in anorexic patients are highly variable and depend largely on the physiological status of the patient at the time of treatment initiation and on the preanorexia body weight. Those patients who had been obese prior to their self-induced anorexia regained their lost weight faster than patients who had been of normal body weight.

Successful treatment of anorexia nervosa is associated with increases in bone mineral density as well as improvement in other measures of body composition.[61] Pharmaceutical agents to stimulate appetite and reverse depression (if present) can be used. If the person is clinically depressed, treatment of the depression will frequently have a positive effect on food intake. This is not always true, however. In contrast, treatment of the anorexia with appetite-stimulating drugs, nutritional support, and counseling can reverse the condition of weight loss and, secondarily, positively affect the depression. Again, this is not always true. The outcome of the treatment depends on the time at which it is instituted. If anorexia nervosa is recognized early in the sequence of hormonal and metabolic change, then the chances of success are much greater than if treatment is initiated after irreversible tissue changes have occurred. Controversy exists as to the success of treatment as well as the accuracy of diagnosis, but it is generally agreed that aggressive treatment can achieve reversal in 50% of the cases. Mortality is estimated in 6% of cases. This leaves an estimate of approximately 44% who recover spontaneously without medical intervention. Treatment success also depends on the degree of self-prescribed food-intake restriction. Total food abstinence is far more threatening than mild abstinence. Included in the mortality figure of 6% are those who commit suicide. This implies a relationship between the development of depression and anorexia—two self-destructive behaviors that represent abnormalities in the CNS.

Restoring the weight loss of the anorexic patient follows a slightly different pattern from the weight regain by traumatized individuals and formerly obese individuals. In the latter groups, the fat regain precedes the protein regain. In fact, in the genetically obese individual, fat regain takes precedence over protein regain. In the recovering anorexic who was not genetically obese prior to anorexia, protein regain keeps pace with fat regain. As both synthetic processes utilize micronutrients, these must be provided at levels similar to those prescribed for growing children. Recovering anorexics are "growing" new tissue to replace that which was raided during the energy-deficit period. They must consume sufficient nutrients to support this regrowth.

Bulimic and nonbulimic anorexics differ in their weight recovery. Those who were bulimic recover their lost weight more rapidly than those who were anorexic only. This is probably due to the difference in rate of weight loss. Those anorexics who were also bulimic were more severely starved and lost weight faster than nonbulimic anorexics. Because of this, bulimics are more likely to be diagnosed and treated sooner than nonbulimic anorexics. In anorexics, as with prolonged starvation, gut absorptive capacity is compromised due to a loss of cells lining the gastrointestinal tract. In the early phase of treatment, malabsorption is likely to occur. For this reason, the diet offered during recovery must be gradually increased with respect to its energy content. A gradual 300 kcal (1255 kJ) three times weekly increase from an initial 1200 kcal (5,040 kJ) diet which includes about 3 g sodium is recommended. The recovery diet should also be a lactose-free diet and should be offered in six or more small meals over 24 h. A low-fat diet is sometimes recommended, but this depends on the genetic background and pre-anorexia health status of the patient. Those with diabetic tendencies might not fare as well if faced with a low-fat, high-carbohydrate diet.

The medical history of the patient and family will provide clues as to the most appropriate diet design. The recovering anorexic requires more food than the recovering bulimic anorexic. The recovering anorexic has lost more absorptive cells than the bulimic anorexic. Of interest is the report that even after weight regain, the recovered anorexic has a higher than normal energy requirement and, if this is not met, will begin to lose weight once again. This suggests that not all anorexia nervosa is self-inflicted. It may begin with a conscious effort to consume less food but then may continue because of a change in the signals for food-intake initiation and cessation and a change in the efficiency with which the body uses the food consumed.

Abnormal Appetite: Pica

Humans, as well as some lower animals, will sometimes or habitually consume items of no nutritional value. In some cases, the item in question will have a deleterious effect on the person's health. This habit is called *pica*, after the Latin word for magpie, a bird that will consume all manner of food and nonfood items. Pica has been observed for centuries and was described by Aetius of Amida in 1542. Many different items are consumed; however, the most common are clay (*geophagia*), laundry starch (*amylophagia*), or ice (*pagophagia*). A number of studies on the prevalence of pica have shown that up to 70% of some population groups may have this habit. Pregnant women as well as children are the most frequently affected, and black women are three to four times more affected than white women of the same socioeconomic group. The most common cravings were for laundry starch (as much as 8 oz/d) and clay. When both men and women were studied, few men exhibited the practice, and it has been suggested that men use liquor or tobacco to meet their nonfood oral needs.

The question of why pica exists has not been satisfactorily answered. From the various epidemiological studies, age, sex, social status, and race appear to be important factors in the development of the habit. Several studies have noted that pica was associated with anemia. Pica has been reported to be accompanied by frequent nosebleeds and other spontaneous losses of blood accompanied or preceded an increased craving for certain food and nonfood items. Clay, rice, French fries, ice, green vegetables, bread, hot tea, and grapefruit were mentioned as being consumed in large quantities by these patients. The patients were treated for their anemias with iron supplements and were tested for their iron-binding capacity. Some of the patients had low uptakes of iron, whereas others were normal.[62,63] Those with poor iron-binding capacities were usually the clay eaters; those with normal iron-binding capacities were ice cream eaters. Clay, even the small amount residing in the gastrointestinal tract of patients having no access to clay while hospitalized, could have adsorbed the oral iron supplements. Thus, it seems unlikely that an innate, lowered iron-binding capacity was responsible for either the anemia or the pica. However, pica does appear to follow the development of anemia rather than precede it.

In addition to anemia, other conditions have been observed in pica patients. Muscular weakness and low serum potassium levels have been reported in geophagic patients. Both these conditions could be attributed to the binding of potassium in the intestine by the clay. This may also be true in patients consuming large quantities of laundry starch.

A more serious aspect of pica is the consumption of paint chips (*plumbism*) by young children. If the paint contains lead oxide as the pigment, lead intoxication can develop. This is characterized by anemia, low serum iron and copper values, growth depression, ataxia, kidney damage, coma, convulsions, and death. The ataxia, stupor, coma, and convulsions reflect the effect of lead on the CNS. This can be understood as the effect of lead on hemoglobin synthesis. Both copper and iron utilization are impaired, and the anemia typical of lead intoxication is microcytic and hypochromic in character. In addition, lead may replace either copper, iron, or calcium in a number of tissues and, because it is metabolically inert, inhibit the functionality of that tissue. In the case of hemoglobin synthesis, it becomes obvious that the oxygen-carrying capacity of the red blood cells is decreased. Those tissues with a high oxygen requirement, that is, the neural tissue, will be the most affected. Thus, one can understand the neuromuscular response to chronic lead ingestion. If neuronal tissue suffers from prolonged oxygen deprivation, it will die, and this damage is irreversible. Subjects with lead poisoning can be treated with compounds such as EDTA that will bind the circulating lead and allow the body to excrete the EDTA–lead complex. It is not possible, however, to rid the body of all of its accumulated lead nor to protect the patients from future ill effects of their lead-induced pathology. Lead will remain in its storage sites, such as bone, and, when mobilized, will have untoward effects.

In the United States today, the majority of lead-intoxication cases are young children, ages 1 to 6, with pica. Adults who work in lead-related industries or consume lead-contaminated illicit

beverages are also affected. Increasing the levels of lead exposure generally increases the blood and tissue lead levels, yet, individual variations due to age, sex, and nutritional status occur. The factors that determine the fractions of the body where lead is deposited have not been determined. It is known that well-nourished individuals are more resistant to the deleterious effects of lead than are poorly nourished individuals.

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SUPPLEMENTAL READING

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3 Nutritional Biochemistry

In order for the body to access nutrients, food must first be broken down into usable components. Food is first digested and absorbed via a variety of mechanisms as will be described in the chapters specific for each nutrient. The use of the food components in the macronutrient class involves both the breakdown of the macromolecules (catabolism) and the resynthesis of needed molecules (anabolism). Together, these actions form the pathways of intermediary metabolism. Nutrient intake can affect these pathways in a variety of ways: A large intake of a given macronutrient—glucose, for example—can affect the initial reaction sequences of the glycolytic, glycogenic, and hexose monophosphate shunt pathways through substrate induction. In contrast, should the diet be deficient in glucose, the glucose-synthesizing pathway, gluconeogenesis, and the glycogenolytic pathway would be stimulated. In addition to dietary effects on intermediary metabolism, there are hormonal effects that in turn can be affected by dietary status or by environmental conditions. In a number of instances there are genetic effects on these pathways as well (see Chapter 5). Before these influences can be explained, however, the pathways themselves must be described.

GLYCOLYSIS

The glycolytic pathway for the anaerobic catabolism of glucose can be found in all cells in the body. This cytosolic pathway begins with glucose, a 6-carbon unit, and through a series of reactions, produces two molecules of pyruvate. The pathway is shown in Figure 3.1. The pathway is stimulated by insulin and is less active when glucocorticoid is present.[1] The pathway also produces ATP by substrate phosphorylation as well as through its contribution (via the α-glycerophosphate shuttle) of reducing equivalents to the respiratory chain. Because two of the steps require ATP (the initial kinase step and the phospho-fructokinase step), the net ATP production of the glycolytic sequence is eight ATPs. The control of glycolysis is vested in several key steps. The first step is the phosphorylation of glucose through the formation of glucose-6-phosphate. In the liver and pancreatic β cell, this step is catalyzed by the enzyme glucokinase.[2–4] A molecule of ATP is used and magnesium is required. Glucose-6-phosphate is a key metabolite. It can proceed down the glycolytic pathway, or move through the hexose monophosphate shunt, or be used to make glycogen. How much glucose-6-phosphate is oxidized directly to pyruvate depends on the type of cell, the nutritional state of the animal, its genetics, and its hormonal state. Some cell types—the brain cell for example—do not make glycogen. Some people do not have shunt activity in the red cell because the code for glucose-6-phosphate dehydrogenase has mutated such that the enzyme is not functional. Insulin-deficient animals likewise might have little shunt activity due to the lack of insulin's effect on the synthesis of its enzymes. All these factors determine how much glucose-6-phosphate goes in which direction.

Two enzymes are used for the activation of glucose: glucokinase and hexokinase. Both these enzymes are present in the liver.[2–4] Although hexokinase activity is product inhibited, that is, inhibited by glucose-6-phosphate, glucokinase is not. The hexokinase in the nonhepatic tissues must be product-inhibited to prevent the hexokinase from tying up all the inorganic phosphate in the cells as glucose-6-phosphate. The Km for glucokinase is greater than that for hexokinase, so the former is the main enzyme for the conversion of glucose to glucose-6-phosphate in the liver. It is also the main enzyme for the phosphorylation of glucose in the pancreatic islet cells. In the islet cell, glucokinase serves as the glucose sensor that signals the need to release insulin.[3] The other enzyme will phosphorylate not only glucose but other 6-carbon sugars as well. The amount of fructose

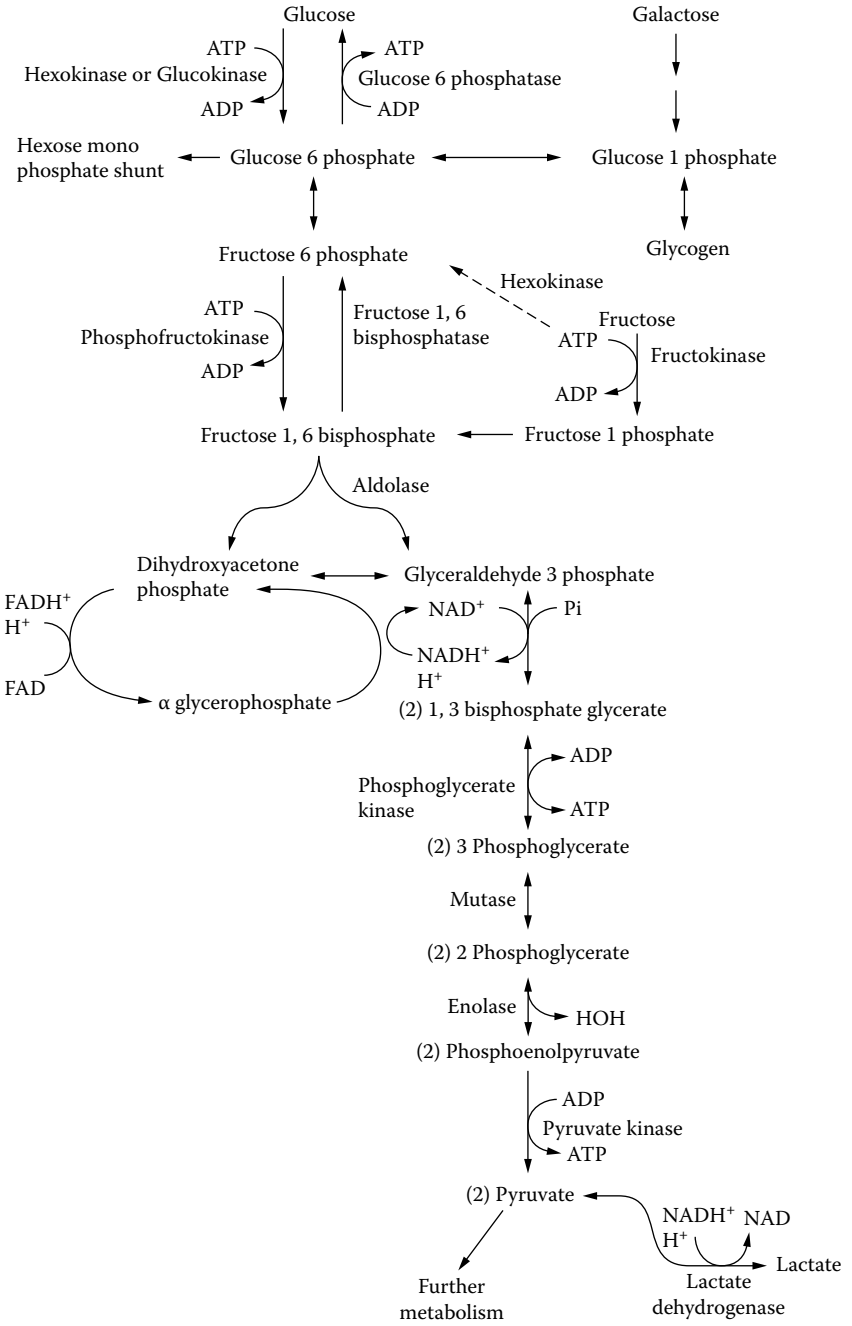


FIGURE 3.1 The glycolytic pathway.

phosphorylated by hexokinase to fructose-6-phosphate is small in comparison with the phosphorylation of fructose at the carbon 1 position catalyzed by fructokinase.

Glucose-6-phosphate is isomerized to fructose-6-phosphate and then is phosphorylated once again to form fructose-1,6-bisphosphate. Another molecule of ATP is used and again magnesium is an important cofactor. Both kinase reactions are rate controlling reactions in that their activity determines the rate at which subsequent reactions proceed. The phosphofructokinase reaction is

unique to the glycolytic sequence, whereas the glucokinase or hexokinase step is not. Thus, one could argue that the formation of fructose-1,6-bisphosphate is the first committed step in glycolysis. Glycolysis is inhibited when phosphofructokinase is inhibited.[6–17] This occurs when levels of fatty acids in the cytosol rise,[13] as in the instance of high rates of lipolysis and fatty acid oxidation or with the feeding of high-fat diets.[7] Phosphofructokinase activity is increased when levels of fructose-6-phosphate rise[5–8,11] or when cAMP levels rise. Exercise stimulates the flux of fructose-6-phosphate through the phosphofructokinase reaction.[17] Stimulation occurs also when fructose-2,6-bisphosphate levels rise.[14–16] Phosphofructokinase is activated by divalent ions.[18] In any event, glycolysis then proceeds with the splitting of fructose-1,6-bisphosphate to dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate.

At this point, another rate-controlling step occurs. This step is one that shuttles reducing equivalents into the mitochondria for use by the respiratory chain. This is the α-glycerophosphate shuttle. This shuttle carries reducing equivalents from the cytosol to the mitochondria. DHAP picks up reducing equivalents when it is converted to α-glycerol phosphate. These reducing equivalents are produced when glyceraldehyde-3-phosphate is oxidized in the process of being phosphorylated to 1,3-diphosphate glyceraldehyde. The α-glycerophosphate enters the inner mitochondrial membrane, whereupon it is converted back to DHAP and releases its reducing equivalents to FAD, which in turn transfers the reducing equivalents to the mitochondrial respiratory chain. The reason that this shuttle is rate limiting is due to the need to regenerate NAD^+ in the cytosol. Without NAD^+, the glycolytic pathway ceases. NAD^+ itself cannot pass through the mitochondrial membrane so substrate shuttles are necessary.

Another means of producing NAD^+ is by converting pyruvate to lactate. This is a nonmitochondrial reaction catalyzed by lactate dehydrogenase. It occurs when an oxygen debt is developed, as happens in exercising muscle. In these muscles, more oxygen is consumed than can be provided. Glycolysis is occurring at a rate faster than can be accommodated by the respiratory chain that joins the reducing equivalents transferred to it by the shuttles to molecular oxygen, making water. If more reducing equivalents are generated than can be used to make water, the excess is added to pyruvate to make lactate. Thus, rising lactate levels are indicative of oxygen debt.

Lactate levels are elevated in individuals with mitochondrial dysfunction due to mitochondrial DNA mutation.[19] In this instance, the reason is due to inefficient mitochondrial OXPHOS that cannot handle all the reducing equivalents sent to it for the synthesis of water.[20]

There are other shuttles that also serve to transfer reducing equivalents into the mitochondria. These are the malate–aspartate shuttle and the malate–citrate shuttle. Neither of these are rate limiting with respect to glycolysis. The malate–aspartate shuttle has rate-controlling properties with respect to gluconeogenesis, whereas the malate–citrate shuttle is important to lipogenesis.[21]

Once 1,3-bisphosphate glycerate is formed, it is converted to 3-phosphoglycerate with the formation of 1 ATP. The 3-phosphoglycerate then goes to 2-phosphoglycerate and then to phosphoenolpyruvate. These are all bidirectional reactions that are also used in gluconeogenesis. The phosphoenolpyruvate (PEP) is dephosphorylated to pyruvate with the formation of another ATP. Because of the great energy loss to ATP formation at this step, this reaction is not reversible. Gluconeogenesis uses another enzyme, phosphoenolpyruvate carboxykinase (PEPCK), to reverse this step. Glycolysis uses pyruvate kinase to catalyze the reaction. At any rate, pyruvate can now be activated to acetyl CoA, which can enter the mitochondrial citric acid cycle.

The glycolytic pathway is dependent on both ATP for the initial steps of the pathway, the formation of glucose-6-phosphate and fructose-1,6-bisphosphate, and on the ratio of ATP to ADP and inorganic phosphate, Pi.[4] In working muscle, the continuance of work and the continuance of glycolysis depend on the cycling of the adenine nucleotides and the export of lactate. ATP must be provided at the beginning of the pathway, and ADP and Pi must be provided in the latter steps. If the tissue runs out of ATP, ADP, or Pi, or accumulates lactate and H^+, glycolysis will come to a halt and work cannot continue. This is what happens to the working skeletal muscle. Exhaustion sets in when glycolytic rate is downregulated by an accumulation of lactate. An interesting clinical

condition arises in some humans and in pigs that have a genetic error in the regulation of their lactate export and adenine nucleotide cycling. This error is unnoticed unless the individual is anesthetized with an anesthesia such as halothane. When anesthetized, body temperature rises precipitously, and muscle rigor and acidosis quickly follow. This condition is known as malignant hyperthermia and occurs in one child in 15,000 and one adult in 50,000–100,000. It is also a problem in swine, resulting in poor-quality meat. Unless it is quickly recognized and measures are taken to reduce the body temperature and combat the acidosis, death will result. The biochemical explanation has to do with ATP, ADP, and Pi cycling and the export of lactate from the muscle cell. Apparently, the anesthesia affects several exchange mechanisms in the membranes within and around the muscle cell. Affected is the coupling of respiration to ATP synthesis by the mitochondria, and the energy normally trapped in the high-energy bond of the ATP molecule is released as heat. Probably the ADP generated in the early ATP-dependent steps of glycolysis is not sent into the mitochondria for rephosphorylation into ATP, and instead is recycled through substrate phosphorylation. Because ADP is exchanged (via several different exchange mechanisms) for ATP, the cell must make some adjustments in its metabolism. Muscle tends to use the glycolytic pathway with the excess product, pyruvate, sent back to the liver as lactate. Although muscle does not lack mitochondria, its mitochondrial activity cannot keep pace with its glycolytic flux when the muscle is working hard and long, or in this instance, when the person (or pig) with the genetic disease of malignant hyperthermia is anesthetized with halothane, which acts as a mitochondrial uncoupler. Hence, the pyruvate/lactate spillover. The lactate must exit the cell via a lactate/H^+ symport. This requires a normal or accelerated blood flow. If the lactate and H^+ are not carried away by the blood, they accumulate. H^+ inhibits the activity of phosphofructokinase and lactate acts as a feedback inhibitor of glycolysis. When an anesthesia such as halothane is used on susceptible individuals, there is a further disruption in the control of glycolysis. There is an accelerated futile cycling between fructose-6-phosphate and fructose-1,6-phosphatase, as well as a dramatic decrease in glycolytic flux. The decrease in flux is secondary to the lactate and H^+ accumulation. Under halothane anesthesia, blood flow is reduced. This, coupled with the genetic error(s) in the membranes, results in the phenotypic expression of the malignant hyperthermic genotype.

HEXOSE MONOPHOSPHATE SHUNT

The hexose monophosphate shunt provides an alternate pathway for the use of glucose-6-phosphate. It is an important pathway because it generates reducing equivalents carried by $NADP^+$ and because it generates a phosphorylated ribose for use in nucleotide synthesis. It is estimated that approximately 10% of the glucose-6-phosphate generated from glucose is metabolized by the shunt.

As can be seen in Figure 3.2, the shunt contains two NADP-linked dehydrogenases, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The rate-limiting steps in the reaction sequence are catalyzed by these two enzymes.[21] In the instance where there is an active lipogenic state, these reactions provide about 50% of the reducing equivalents needed by the lipogenic process (see section on lipogenesis). In fact, if one wanted a quick assessment of the lipogenic response to a variety of treatments, one could measure the activity of these enzymes using an aliquot of a tissue homogenate plus enough substrate and magnesium to optimize the conditions of the assay. There is an excellent correlation between this dehydrogenase activity and lipogenesis.[22–24]

Lipogenesis is not the only process that requires reducing equivalents carried by NADP. The microsomal P450 enzymes need them, as does the maintenance of glutathione in the reduced state. The glutathione system in the red cell maintains the redox state and integrity of the cell membrane. If sufficient reducing equivalents are not produced by the shunt dehydrogenase reactions to reduce glutathione, the red cell membrane integrity is lost and hemolytic anemia results.[25] Glutathione reduction is important to the red blood cells' function of carrying oxygen and exchanging it for

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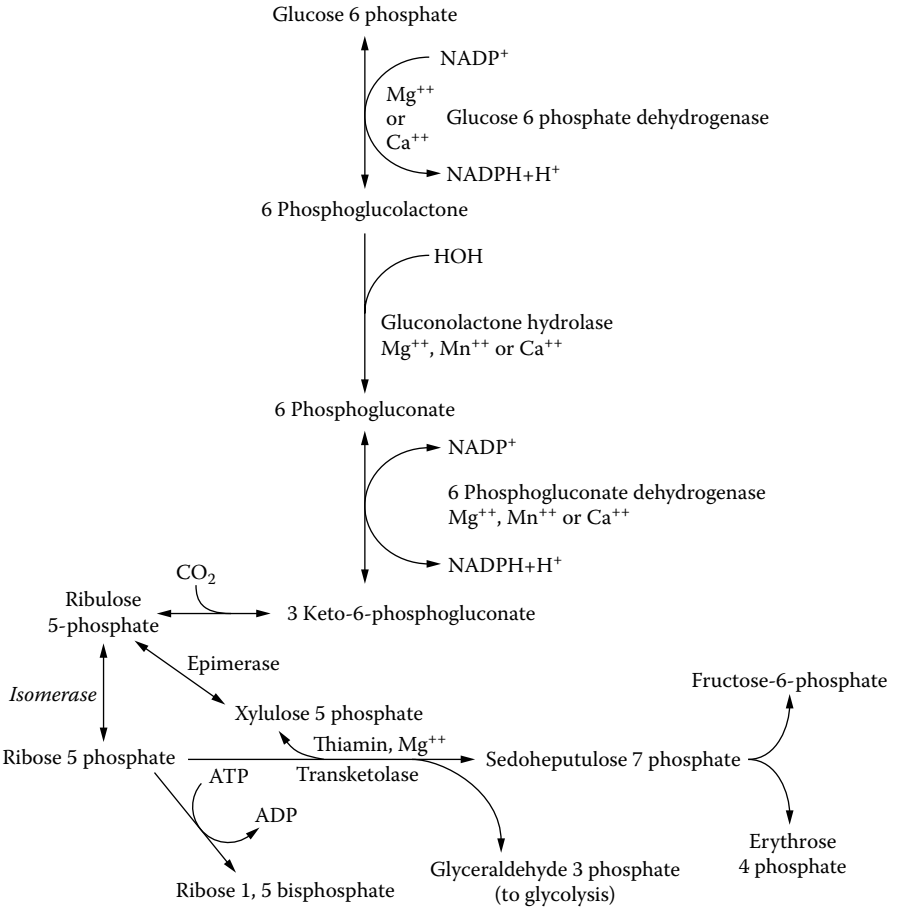


FIGURE 3.2 Reaction sequence of the hexose monophosphate shunt commonly referred to as the "shunt."

carbon dioxide. There are a number of genetic mutations in the code for red cell glucose-6-phosphate dehydrogenase.[26,27] The code is carried as a recessive trait on the X chromosome and thus only males are affected. These mutations are usually silent. That is, the male having a defective red cell glucose-6-phosphate dehydrogenase does not know he has the problem unless his cells are tested or unless he is given a drug such as quinine or one of the sulfur antibiotics that increase the oxidation of $NADPH^+H^+$. When this happens, $NADPH^+H^+$ is depleted and is not available to reduce oxidized glutathione. In turn, the red cell ruptures. In almost all cases, the affected male has sufficient enzyme activity to meet the normal demands for $NADPH^+H^+$. It is only when stressed by these drugs that a problem develops.

In any event, as shown in Figure 3.2, glucose-6-phosphate proceeds to 6-phosphoglucolactone, a very unstable metabolite which is in turn reduced to 6-phosphogluconate. The 6-phosphogluconate is decarboxylated and dehydrogenated to form ribulose-5-phosphate with an unstable intermediate (keto-6-phosphogluconate) forming between the 6-phosphogluconate and ribulose-5-phosphate. Ribulose-5-phosphate can be isomerized to ribose 5-phosphate or epimerized to xylulose 5-phosphate. Xylulose and ribose 5-phosphate can reversibly form sedoheptulose 7-phosphate with release of glyceraldehyde 3-phosphate. This, of course, will be recognized as a component of the glycolytic sequence.

INTERCONVERSION OF DIETARY SUGARS

Fructose

In the course of digestion, the simple sugars glucose, fructose, and galactose are released from sucrose, maltose, and lactose. These sugars are usable by most cells after they have been converted to glucose. There are specific cells that have specific needs for these monosaccharides. The testes need fructose for sperm production and the mammary cells need galactose for lactose production. In each instance, there are reactions that are of interest to the nutrition scientist.[28] Shown in Figure 3.3 is the metabolism of fructose. Although two enzymes are available for the phosphorylation of fructose, one of these, fructokinase, is present only in the liver. Just as hexokinase has a lower Km for glucose than glucokinase for the substrate glucose, so, too, does hexokinase with respect to fructose and fructokinase. The Km for fructokinase is so high that, in fact, most of the dietary fructose that enters the portal blood is metabolized in the liver. This is in contrast to glucose, which is metabolized by all the cells in the body. As a result, sucrose-rich, low-fat diets fed to rats or mice will result in a fatty liver. These diets contain ~65% total weight as sugar and only 5% as fat. The fatty liver occurs because the dietary overload of fructose from sucrose exceeds the capacity of the liver to completely oxidize it to CO_2 and HOH, so it uses the sugar metabolites as substrate for fatty acid and triacylglyceride synthesis. Until the hepatic lipid export system increases sufficiently to transport this lipid to the storage depots, the lipid accumulates, hence, the fatty liver. Normal rodents adjust to this fructose intake so that after a few weeks the liver returns to its normal (~4%) fat content. Those rodents with diabetic tendencies do not adapt and the fatty liver persists throughout their lives. Humans rarely consume this much sugar with so little dietary fat. There are circumstances, however, that are analogous to this rodent paradigm. The human who is provided nutrition support via parenteral feeding is one such instance. The parenteral feeding solution is rich

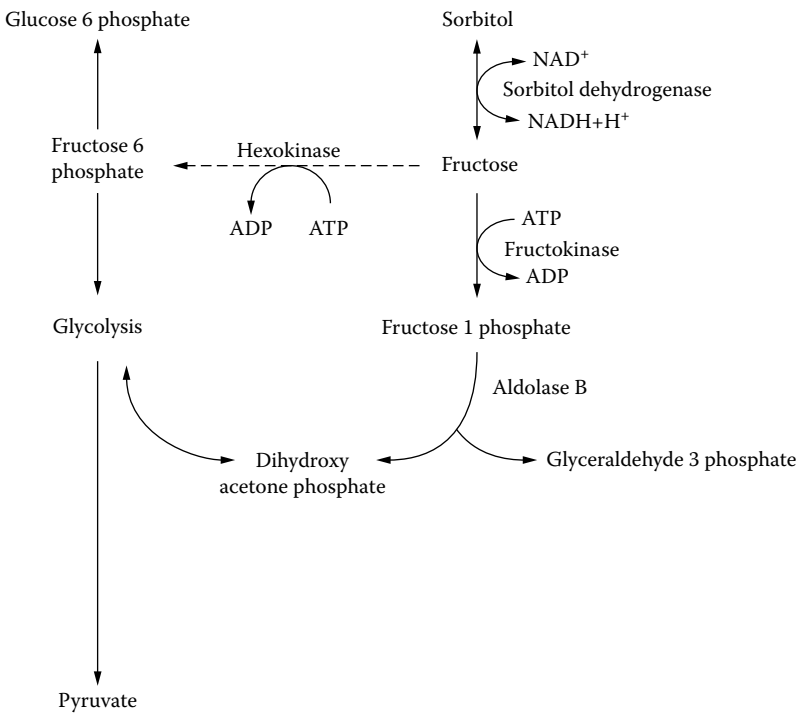


FIGURE 3.3 Metabolism of fructose.

in sugar (glucose) and contains only enough fatty acid to meet the essential fatty acid requirement (see Chapter 8). There is concern that patients so treated will have metabolic consequences similar to that described for the rat fed a 65% sugar, 5% fat diet. However, with continual monitoring of the patient on parenteral nutrition support, such untoward metabolic response can be avoided.

Concern for the normal human also has developed because of the observed increase in consumption of beverages and foods containing high-fructose syrups. Nutrition scientists began to question whether this dietary change might have untoward effects.[28] However, just as normal rats can adapt to such dietary change, so too can the normal human. Of greater concern is the increase in energy intake occasioned by this high consumption of sweet beverages. Instead of consuming water or other sugar-free energy-free beverages, consumers are increasing their energy intakes by consuming large amounts of sweetened beverages. This can lead to energy imbalance with a consequent gain in body fat.

The pathway for fructose metabolism shows three enzymes, any one of which, if mutated, results in a condition known as fructosemia. The first is relatively harmless—that is, a mutation in the gene for fructokinase. As discussed above, hexokinase will phosphorylate fructose and, if the dietary burden is light, the disease of fructosemia will be harmless. Its characteristics of elevated blood and urine levels of fructose are of little concern.

A second mutation has been identified in the gene for aldolase B. This is the enzyme that catalyzes the splitting of fructose-1-phosphate to glyceraldehyde phosphate and dihydroxyacetone phosphate. Recall that aldolase A catalyzes the splitting of fructose-1,6-bisphosphate. Aldolase A is found in all tissues, whereas aldolase B is located only in the liver. The mutation is such that the enzyme has a reduced affinity for its substrate, fructose-1-phosphate. The results of this reduced affinity include hypoglycemia, due to an inhibition of glycogenolysis by fructose-1-phosphate. This hypoglycemia is not responsive to glucagon stimulation. Prior to the identification of the aldolase B gene mutation, this form of fructosemia was misdiagnosed as one of the glycogen-storage diseases because of the presence of hypoglycemia in the face of ample liver glycogen stores. In addition to the disturbance in glycogenolysis, patients with this disorder vomit after a fructose load, have elevated levels of urine and blood fructose, grow poorly with evidence of jaundice, hyperbilirubinemia (high levels of bilirubin in the blood), albuminuria (albumin in the urine), amino aciduria (amino acids in the urine), and some patients may have damaged renal proximal convoluted tubules.

The third mutation involves only the liver enzyme fructose-1,6-bisphosphatase. The muscle enzyme is normal in activity. This enzyme is a key enzyme in the hepatic gluconeogenic pathway, so, as one might expect, hypoglycemia is one of the characteristics of a mutation in this enzyme. Other characteristics include an enlarged liver, poor muscle tone, and increased blood lactate levels. All of these mutations are uncommon and all are autosomal recessive traits. Heterozygotes are not detectable.

Galactose

Another monosaccharide of importance, especially to infants and children, is galactose, a component of the milk sugar, lactose. In the intestine, lactose is hydrolyzed to its component monosaccharides, glucose and galactose. Galactose is converted to glucose and eventually enters the glycolytic sequence as glucose-6-phosphate. This pathway is shown in Figure 3.4. Galactose is phosphorylated at carbon 1 in the first step of its conversion to glucose. It can be isomerized to glucose-1-phosphate or converted to UDP-galactose by exchanging its phosphate group for a UDP group. This UDP-galactose can be joined with glucose to form lactose in the adult mammary tissue under the influence of the hormone prolactin. However, usually the UDP-galactose is converted to UDP-glucose and then used to form glycogen. When glycogen is degraded, glucose-1-phosphate is released, which, in turn, is isomerized to glucose-6-phosphate and enters the glycolytic sequence.

Three autosomal recessive mutations in the genes for enzymes involved in galactose conversion to glucose have been reported. Galactosemia results in each instance. One of these mutations involves the gene for galactose-1-phosphate uridyl transferase. Three variants have been described.

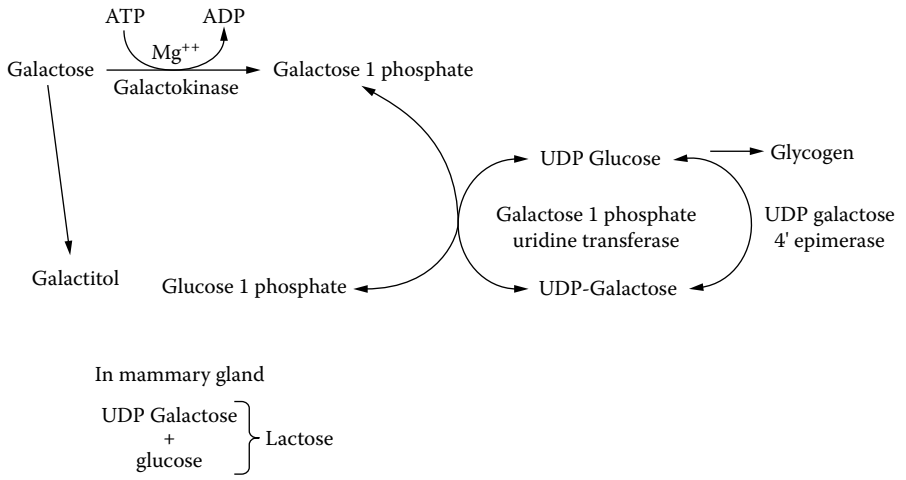


FIGURE 3.4 Conversion of galactose to glucose.

One of these variants is fairly innocuous in that the mutation occurs only in the enzymes found in the red cell. This variant is called the *Duarte variant*, and affected individuals have 50% less red cell galactose-1-phosphate uridyl transferase activity than normal individuals. These people have no other discernible characteristics.

The second variant is far more severe in its effects on the patient. The enzyme in the liver is abnormal and does not function to convert galactose-1-phosphate to UDP galactose. As a result, galactose-1-phosphate accumulates, and some is converted to the sugar alcohol galactitol via NADH aldose reductase action. In this disease, cataracts in the eye form, accompanied by mental retardation and increased tissue levels of galactose-1-phosphate, galactitol, and galactonic acid. These last metabolites are excreted in the urine. Also characteristic of this mutation in galactose-1-phosphate uridyl transferase, are decreased blood glucose levels, decreased glycogenesis, decreased mutase activity and decreased pyrophosphorylase activity. Because UDP-galactose is necessary for the formation of the galactoyl lipids, chondroitin sulfate formation is decreased. A mutation in the gene for galactokinase also results in accumulations of galactose, and galactitol and cataracts. Two variants of this gene have been reported. The galactitol accumulation was found to be the causative agent in the formation of cataracts. Except for cataract formation due to galactitol accumulation, no other symptoms have been described for this form of galactosemia.

Finally, two mutations in the gene for UDP galactoepimerase have been reported. The defect is present only in the red blood cell and the patient is symptom-free.

Mannose

Although the main dietary monosaccharides are glucose, fructose, and galactose, mannose is also present in small amounts. Mannose found in food is converted to fructose via phosphorylation and isomerization.

$$\text{Mannose} \rightarrow \text{Mannose-6-phosphate} \rightarrow \text{Fructose-6-phosphate} \rightarrow \text{Glycolysis}$$

To date, no genetic errors that would have effects on health have been described with respect to this sugar. Occasionally, the diet provides other sugars as well. These are the pentoses, xylose, and xylulose, found in plums, cherries, and grapes. Mannose and the sugar alcohols are used to produce reduced-sugar or reduced-energy food products. Many of these sugar alcohols are

nondigestible/nonabsorbable carbohydrates but, because the gut flora can utilize them and provide short-chain fatty acids for absorption, they have an energy value ranging from 1.8 to 4 kcal/g (8 to 17 kJ/g) depending on the constituents of the polyol. Maltitol (4-*O*-α-d-glucopyranopyl-d-glucitol), for example, contains one glucose and one glucose alcohol. It has an energy value of about 2 kcal/g (8.4 kJ/g). Lactitol, on the other hand, has a higher energy value of approximately 3.6 kcal/g (15 kJ/g). Xylose is converted to the sugar alcohol xylitol through the action of the NADP-linked enzyme xylose/xylulose dehydrogenase, and the xylitol is excreted in the urine. However, a mutation in the gene for this enzyme has been detected and characterized by the presence of xylose or xylulose in the urine instead of xylitol. This has no clinical significance, except if the urine is screened for reducing sugars without discriminating for glucose just after such a person has consumed the fruits containing these sugars. If the person is not studied more carefully, there is a slight chance of the misdiagnosis of diabetes, based on the presence of these reducing sugars in the urine.

GLYCOGENESIS AND GLYCOGENOLYSIS

Glucose is an essential fuel for working muscle as well as for the brain. To ensure a steady supply of this fuel, some glucose is stored in the liver and muscle in the form of glycogen.[29] This is a ready supply of glucose accessed when these tissues are stimulated to release its glucose from its glycogen by the catabolic hormones glucagon, epinephrine, the glucocorticoids, and thyroxine, or by the absence of food in the digestive tract.

Because the glycogen molecule has molecules of water as part of its structure, it is a very large molecule and cumbersome to store in large amounts. In fact, detailed studies of the nature of the body's fuel stores have shown that the average 70-kg man has less than an 18-h fuel supply stored as glycogen while that same individual might have up to a 2-month supply of fuel stored as fat.[30] Nonetheless, the synthesis of glycogen and its release of glucose in time of need are important aspects of carbohydrate nutrition. Shown in Figure 3.5 is an overview of glycogenesis.

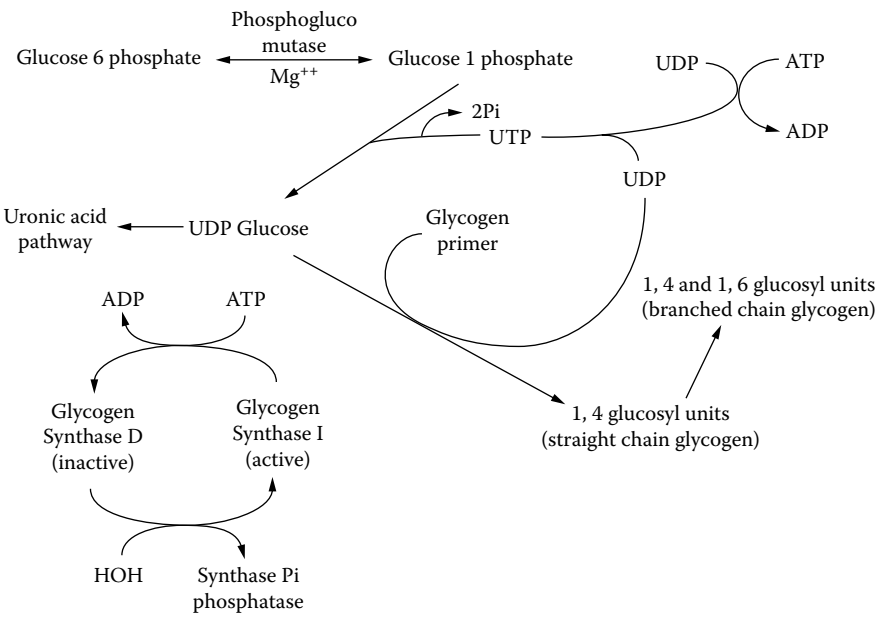


FIGURE 3.5 Glycogen synthesis (glycogenesis).

Figure 3.6 gives details of the glycogenolytic cascade. Muscle and liver glycogen stores have very different functions. Muscle glycogen is used to synthesize ATP for muscle contraction, whereas hepatic glycogen is the glucose reserve for the entire body, particularly the central nervous system (CNS). The amount of glycogen in the muscle is dependent on the physical activity of the individual. After bouts of strenuous exercise, the glycogen store will be depleted, only to be rebuilt during the resting period following exercise. One aspect of physical training is concerned with the expansion of the muscle glycogen store. Through repeated depletion–repletion routines, athletes hope to expand the size of this store so as to increase their endurance. Athletes follow an exercise/recovery routine as well as a dietary carbohydrate routine (a normal carbohydrate diet followed by a carbohydrate-rich diet just prior to competition) that they hope will improve their performance through increasing their muscle glycogen store.

The different types of muscle store and use glycogen for glucose and ATP generation differently. Overall, about 8% of muscle glycogen is converted to glucose and some of this is released into the bloodstream. The remainder is oxidized via the glycolytic pathway. Myocytes lack glucose-6-phosphatase, so glucose release from gluconeogenesis is not possible. Exercise or "work" by "red" muscle fibers that are richly endowed with mitochondria can oxidize glucose fully to provide ATP and result in water and carbon dioxide. These fiber types are found in the heart muscle and

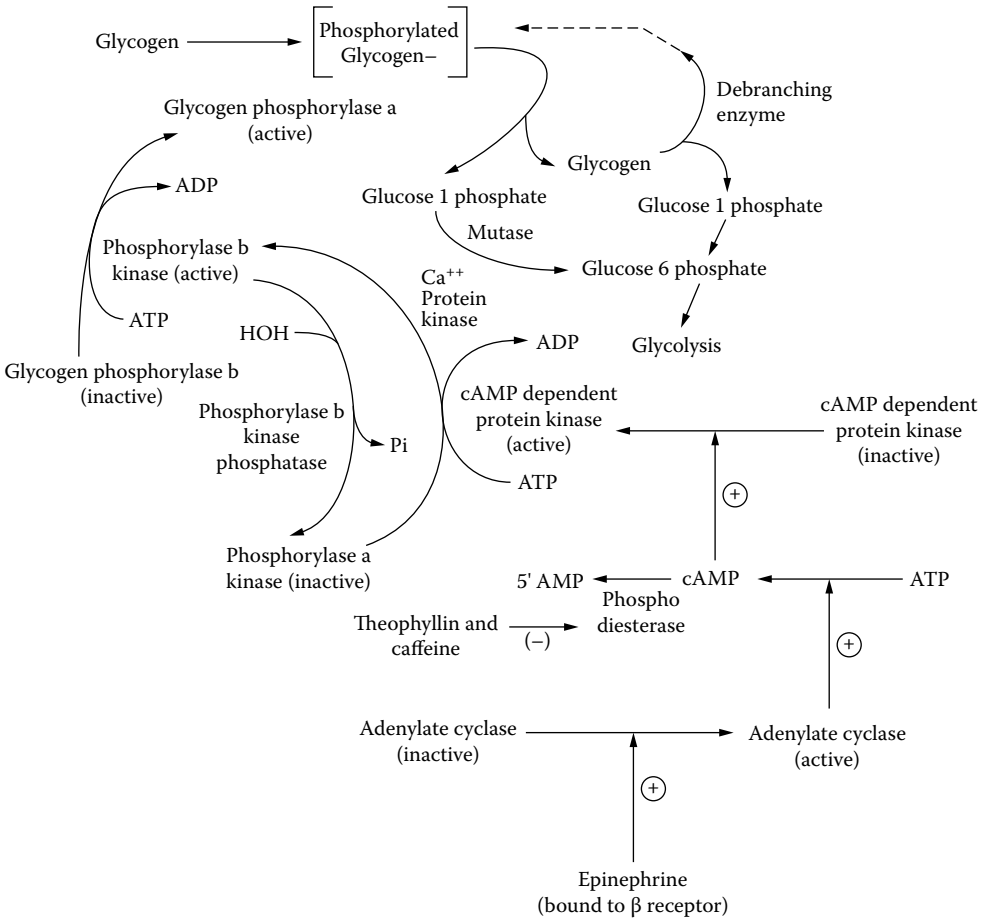


FIGURE 3.6 Stepwise release of glucose molecules from the glycogen molecule.

the long muscles of legs and arms. White muscle, in contrast, has a tremendous capacity for glycogenesis, glycogenolysis, and glycolysis, but has far fewer mitochondria than found in red muscle. Hence, work by white muscle results in an accumulation of lactate released to the bloodstream, as the glucose being used cannot be completely oxidized to $C0_2$ and water. Although red muscle can continue to work indefinitely, given its generous blood supply and mitochondrial complement, white muscle cannot. It can provide the sudden burst of activity needed for sprinting, for example, but its endurance is limited. Most muscle groups in the human body are mixtures of red and white fibers and, therefore, physical training will capitalize on the fiber type best used for the type of competition (i.e., sprinting vs. marathon running) anticipated.

Hepatic glycogen stores are dependent on nutritional status. They are virtually absent in the 24-hour-starved animal,[30,31] and are replenished within hours of ad libitum feeding. Clusters of glycogen molecules with an average molecular weight of 2×10^7 form quickly when an abundance of glucose is provided to the liver. As noted, the amount of glycogen in the liver is diet dependent. In fact, there is a 24-hour rhythmic change in hepatic glycogen that corresponds to the feeding pattern of the animal.[31] In nocturnal animals such as the rat, the peak hepatic glycogen store will be found in the early morning hours, whereas the nadir will be found in the evening hours just before nocturnal feeding begins. In humans accustomed to eating during the day, the reverse pattern will be observed.

Glycogen synthesis begins with glucose-1-phosphate formation from glucose-6-phosphate through the action of phosphoglucomutase.[32] If there are elevations in blood glucose, there will be elevations in hepatic glucose-6-phosphate, and this in turn will stimulate glycogen synthesis. Thus, it could be argued that the level of glucose-6-phosphate is rate controlling with respect to glycogenesis.[32] Glycogen synthesis can also occur using 3-carbon precursors, which traverse the gluconeogenic pathway before being used for glycogen synthesis.[33] These 3 carbon intermediates are generated in the extrahepatic tissues as well as the liver itself. The proportion of glycogen synthesis from glucose-6-phosphate compared to that from the 3 carbon intermediates depend on the amount of glucose consumed as well as the physiological state of the animal.[34] Low- glucose diets seem to enhance the glycogen synthesis from the trios phosphates, whereas high-glucose loads favor the direct synthesis of glycogen from glucose-6-phosphate.

In the direct path for glycogenesis, glucose-1-phosphate is converted to uridine diphosphate glucose (UDP-glucose), which can then be added to the glycogen already in storage (the glycogen primer). UDP-glucose can be added through a 1,6 linkage or a 1,4 linkage. Two high-energy bonds are used to incorporate each molecule of glucose into the glycogen. The straight-chain glucose polymer is composed of glucoses joined through the 1,4 linkage and is less compact than the branched-chain glycogen, which has both 1,4 and 1,6 linkages, as shown in Figure 3.7. The addition of glucose to the primer glycogen with a 1,4 linkage is catalyzed by the glycogen synthase enzyme, whereas the 1,6 addition is catalyzed by the so-called glycogen-branching enzyme (Amylo $1\rightarrow4$, $1\rightarrow6$ transglucosidase). Once the liver and muscle cells achieve their full storage capacity, these enzymes are product inhibited and glycogenesis is "turned off." Glycogen synthase is inactivated by a cAMP-dependent kinase and activated by a synthase phosphatase enzyme that is stimulated by changes in the ratio of ATP to ADP.[34] Glycogen synthesis is stimulated by the hormone insulin and suppressed by the catabolic hormones. The process does not fully cease, but operates at a very low level. Glycogen does not accumulate appreciably in cells other than liver and muscle, although all cells contain a small amount of glycogen. Note in Figure 3.5 that a glycogen primer is required for glycogen synthesis to proceed. This primer is carefully guarded so that some is always available when glycogen is synthesized. This means that glycogenolysis never fully depletes the cell of its glycogen content.

Glycogenolysis is a carefully controlled series of reactions referred to as the *glycogen cascade* (Figure 3.6). It is called a "cascade" because of the stepwise changes in activation states of the enzymes involved.[35] To release glucose for oxidation by the glycogenolytic pathway, the glycogen must be phosphorylated. This is accomplished by the enzyme glycogen phosphorylase,

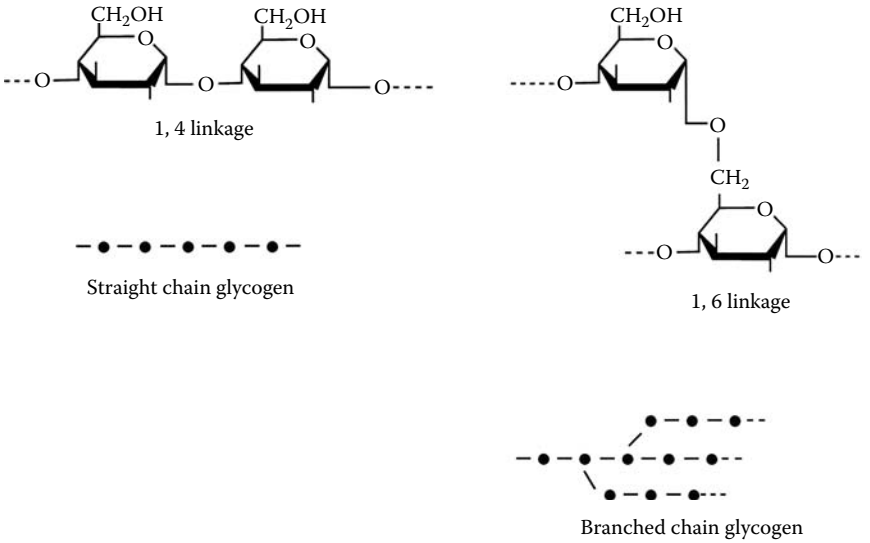


FIGURE 3.7 Glycogen structures.

which exists in the cell in an inactive form (glycogen phosphorylase b) and is activated to its active form (glycogen phosphorylase a) by the enzyme phosphorylase b kinase. In turn, this kinase also exists in an inactive form, which is activated by the calcium-dependent enzyme protein kinase, and active cAMP-dependent protein kinase. These activations each require a molecule of ATP. Finally, the cAMP-dependent protein kinase must have cAMP for its activation. This cAMP is generated from ATP by the enzyme adenylate cyclase which, in itself, is inactive unless stimulated by a hormone such as epinephrine, thyroxine, or glucagon. As can be seen, this cascade of activation is energy dependent, with three molecules of ATP required to get the process started. Once started, the glycolytic pathway will replenish the ATP needed initially as well as provide a further supply of ATP to provide the necessary energy. As mentioned, the liver and muscle differ in the use of glycogen. This also affects how ATP is generated within the glycogen-containing cell and how much is generated by cells that do not store glycogen.

In part, our understanding of how glycogen is used or how glycogenolysis occurs has come about because of some spontaneous mutations in the genes coding for the enzymes involved. Although these mutations in several instances are devastating to the affected person, they have shed light on the control of the overall balance of glycogenesis and glycogenolysis.

One very rare autosomal recessive disease is due to a mutation in the gene for the branching enzyme. This results in an accumulation of straight-chain glycogen which, because it is less compact than the normally branched-chain glycogen, results in an enlarged liver. This disorder is called *amylopectinosis* and is usually grouped with the other disorders characterized by enlarged glycogen stores. However, this disorder affects synthesis rather than degradation (which is normal) and there is not an excess of glycogen. Infants with this disorder die from cardiac or liver failure, rarely surviving their first year.

Five mutations in the genes that code for the enzymes of glycogenolysis have been described. These include a mutation in the lysomal α-1,4-glucosidase (also called acid maltase), which results in a generalized excess of glycogen not only in the liver and muscle but also in the viscera and central nervous system. This disorder is called *Pompe's disease*, and is characterized by an enlarged liver and heart, and extreme muscular weakness. Death due to heart failure is common.

A mutation in the gene for the debranching enzyme (amylo-1,6-glucosidase) results in the accumulation of highly branched short-chain glycogen in the muscles and liver. The usual glycogen has

a branch point at every fourth glucosyl residue in the interior of the molecule and farther apart on the outer regions. The glycogen stored in people lacking the debranching enzyme have their branch points very close together. This occurs when glucose is mobilized from glycogen and only the outer limbs of the molecule are used. Without a functioning debranching enzyme, the remaining inner core is untouched. These cores accumulate and are responsible for the enlarged liver and heart. Because the glycogen gives up only a small amount of its glucose, the muscle that needs it for ATP synthesis is very weak. This condition is called *Forbes disease.*

McArdle's disease affects only the muscle, and the enzyme lacking is the muscle phosphorylase. Patients with this disorder are intolerant to exercise and accumulate glycogen in the muscle but not the liver. This disorder was discovered in military recruits during World War II, when, in a few isolated instances, young men were found who could not endure the rigorous physical training of boot camp. Unfortunately, the first few were thought to be malingerers and were forced to exercise to death by their drill instructors. When their deaths were investigated, the reason for their exercise intolerance was understood. The mutation in the muscle phosphorylase gene might not have been discovered otherwise.

In the liver, a mutation in phosphorylase is far more serious. This disorder, known as *Her's disease*, results in growth retardation, an enlarged liver due to glycogen accumulation, and elevated serum lipids. In both McArdle's disease and Her's disease, glycogen is not phosphorylated because of a mutation in the enzyme that catalyzes the initial step in glycogenolysis. This is also the case for another glycogen-storage disease, but, instead of a mutation in the gene for phosphorylase, the mutation is in the gene for the phosphorylase kinase, the enzyme that is responsible for the activation of glycogen phosphorylase b to glycogen phosphorylase a. Without the addition of energy through the hydrolysis of ATP, the glycogen phosphorylase cannot transfer this phosphate to the glycogen molecule and produce a molecule of glucose-1-phosphate. People with this disorder have the same symptoms as those with Her's disease and, in addition, have increased rates of gluconeogenesis and hypoglycemia when without food for long periods of time. They also have decreased phosphorylase activity in hepatocytes and leukocytes.

All of these disorders in glycogen synthesis and degradation are rare and all appear as autosomal recessive traits. Their long-term outlook is not very good. Some nutritional manipulations have been tried to reduce the hypoglycemic episodes that characterize some of these disorders. These include a continual nasogastric drip of a starch suspension. Whether this therapy is effective over the long term has not been established. Because the hypoglycemia is due to an inability to normally mobilize the glycogen-glucose, care must be given to avoid prolonged periods without food. The glucose that is needed by all the body's cells must be provided either by the diet or through an active gluconeogenic process.

GLUCONEOGENESIS

The provision and maintenance of normal blood glucose levels in the absence of food involves not only glycogenolysis but the synthesis of glucose from noncarbohydrate precursors. This process, called gluconeogenesis, is shown in Figure 3.8. Gluconeogenesis occurs primarily in the liver and kidney. Most tissues lack the full complement of enzymes needed to run this pathway. In particular, the enzyme phosphoenolpyruvate carboxykinase is not found or is in short supply. Shown in Figure 3.8 are the enzymes that are unique to gluconeogenesis. The other reactions shown use the same enzymes as glycolysis (see Figure 3.1) and do not have control properties with respect to gluconeogenesis. The rate-limiting enzymes of interest are glucose-6-phosphatase, fructose-1,6-bisphosphatase, and phosphoenolpyruvate carboxykinase (PEPCK). Pyruvate kinase and pyruvate carboxylase are also of interest because their control is a coordinated one with respect to the regulation of PEPCK.

The capacity to synthesize glucose in times of need is absolutely essential to survival. Blood glucose must be maintained so that those tissues, i.e., brain, that have an absolute requirement

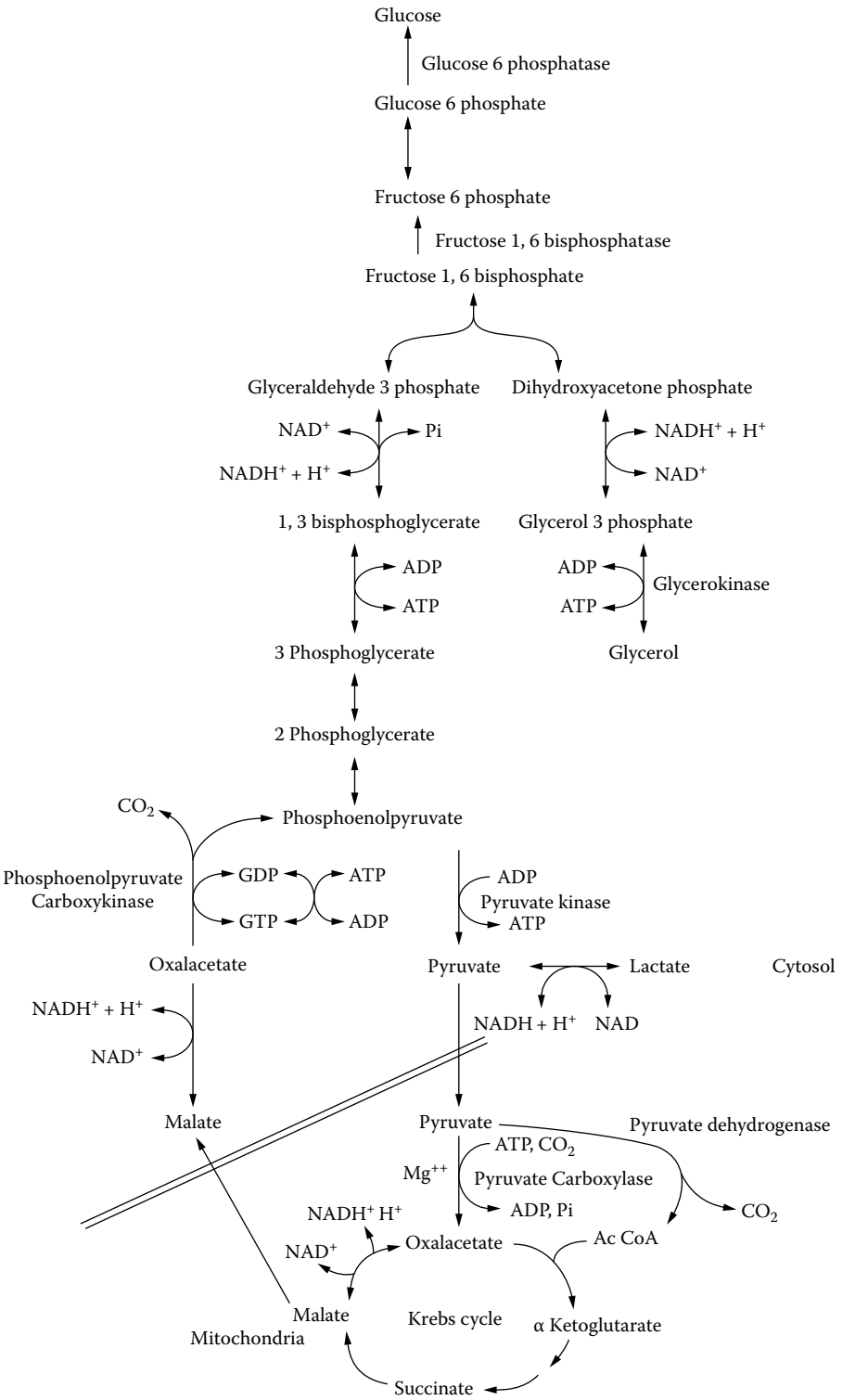


FIGURE 3.8 Pathway for gluconeogenesis.

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for this fuel can continue to function. Although gluconeogenesis is essential, it must be carefully controlled so that excess glucose is not produced. The process is energetically very expensive. If it were not closely regulated, undue protein catabolism with its associated ammonia load could be devastating because it uses as substrates the carbon chains of deaminated amino acids. Fortunately, a number of fail-safe controls that do not allow this to occur are in place.

Gluconeogenesis can use the carbon skeletons from a number of metabolites of glucose (lactate, pyruvate, and some of the triose phosphates) and also those from deaminated amino acids. Not all of the amino acids enter the gluconeogenic sequence at the same place. Shown in Table 3.1 are the amino acids that serve as substrates for glucose synthesis. Some enter as pyruvate, whereas others enter the Krebs cycle at various points.

CORI AND ALANINE CYCLES

The pathway shown in Figure 3.8 provides glucose to all cells in the body. Under normal dietary conditions, the glucose synthesized in the kidney is used by it as fuel to run its metabolism. Only under conditions of prolonged starvation (more than 48 h) will the kidney contribute significant amounts of glucose to the circulation. Thus, circulating glucose produced by gluconeogenesis comes from the liver. There are two important metabolite or substrate cycles that are crucial to the effective regulation of blood glucose levels. One is the Cori cycle, shown in Figure 3.9, and the other is the alanine cycle, shown in Figure 3.10. The Cori cycle involves the use of glucose by the red blood cell. Glucose is oxidized via glycolysis to two molecules of lactate. The lactate is delivered to the liver, which converts it back to glucose via gluconeogenesis. The red cell produces two ATPs through the glycolytic process, whereas the liver uses six ATPs to resynthesize the glucose.

The alanine cycle differs from the Cori cycle in that the exchange is between muscle and the liver.[36] Because the muscle cell has mitochondria, it can use the reducing equivalents generated by the glycolytic sequence to generate 4–6 moles of ATP. Rather than lactate, the muscle cell transaminates the surplus glycolytic product, pyruvate, to alanine and sends this alanine to the liver. Once in the hepatocyte, this alanine is deaminated (the amino group used for urea synthesis), and the resultant pyruvate used to resynthesize glucose via gluconeogenesis. These cycles are important in glucose homeostasis because they provide the means for supplying glucose to tissues that need it and which cannot complete its oxidation.

To participate in these cycles, the peripheral cells must release lactate, pyruvate, or alanine as their metabolic end product of glycolysis.[37] Further, the use of the $NADH^+H^+$ generated by the glycolytic sequence differentiates whether the Cori cycle or the alanine cycle is used. If the former, the $NADH^+H^+$ is used to reduce pyruvate to lactate. In the latter, the $NADH^+H^+$ is used as part of a

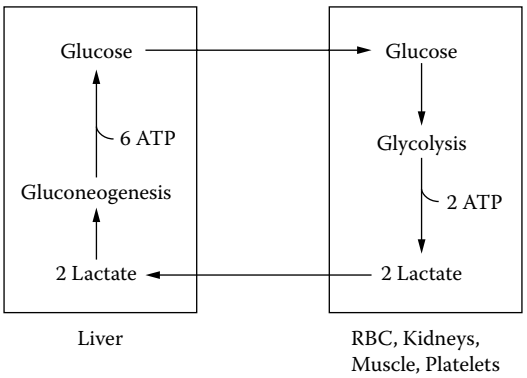


FIGURE 3.9 The Cori cycle.

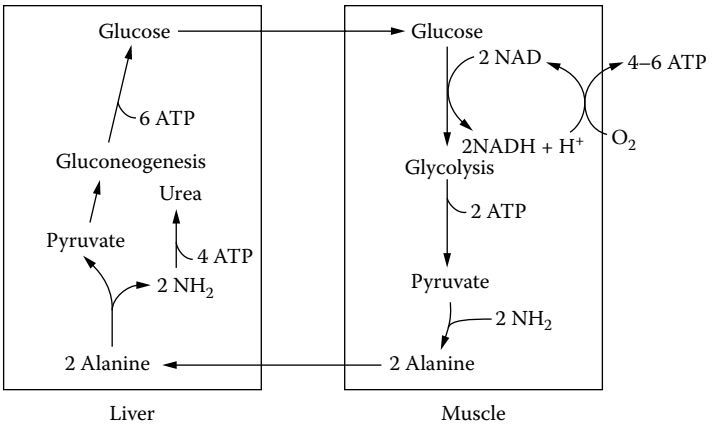


FIGURE 3.10 The alanine cycle. Both the Cori cycle and the alanine cycle serve to ensure a supply of glucose to cells that require it.

shuttle system for the entry of reducing equivalents into the mitochondrial respiratory chain. This $NADH^+H^+$ is not available for pyruvate reduction. Pyruvate is thus available for transamination and is converted to alanine, which, in turn, is used by the liver for glucose synthesis. Although the alanine cycle is an important mechanism in the regulation of blood glucose level, other amino acids can also serve as glucose precursors as shown in Table 3.1. People who consume high-protein, high-fat diets frequently use their excess protein intake to provide the carbon skeletons for glucose synthesis. In addition, when people have been without food for several days, the body proteins are catabolized for energy and to provide glucose precursors. As can be seen in the gluconeogenic pathway shown in Figure 3.8, as well as in the Cori and alanine cycles (Figures 3.9 and 3.10), gluconeogenesis uses more ATP than glycolysis produces.

TABLE 3.1
Amino acids that contribute a carbon chain for the synthesis of glucose

Amino acid	Enters as
Alanine	Pyruvate
Tryptophane → alanine	Pyruvate
Hydroxyproline	Pyruvate
Serine	Pyruvate
Cysteine	Pyruvate
Threonine	Pyruvate
Glycine	Pyruvate
Tyrosine	Fumarate
Isoleucine	Succinyl CoA
Methionine	Succinyl CoA
Valine	Succinyl CoA
Histidine → glutamate	α-ketoglutarate
Proline → glutamate	α-ketoglutarate
Glutamine → glutamate	α-ketoglutarate
Arginine → glutamate	α-ketoglutarate

The difference in ATPs used and produced implies that the mitochondria are important to the regulation of the pathway. Indeed, one shuttle, the malate–aspartate shuttle shown in Figure 3.11, has rate-controlling properties with respect to gluconeogenesis.[38–40] The malate–aspartate shuttle works to transport reducing equivalents into the mitochondria. Malate is transported into the mitochondria, whereupon it gives up two reducing equivalents and is transformed into oxalacetate. Oxalacetate cannot traverse the mitochondrial membrane, so it is converted to α-ketoglutarate in a coupled reaction that also converts glutamate to aspartate. Aspartate travels out of the mitochondria (along with ATP) in exchange for glutamate. Once out in the cytosol, the reactions are reversed. Aspartate is reconverted to glutamate, and α-ketoglutarate reconverted to oxalacetate. The oxalacetate, in turn, can be reduced to malate or decarboxylated to form phosphoenolpyruvate. Measurement of the activity of this shuttle has revealed that the more active the shuttle, the more active is gluconeogenesis. This is because the shuttle provides a steady supply of oxalacetate via α-ketoglutarate in the cytosol. This oxalacetate cannot get there any other way. As mentioned, it is generated by the Krebs cycle in the mitochondria, but cannot leave this compartment.

Oxalacetate is essential to gluconeogenesis because it is the substrate for PEPCK, which catalyzes its conversion to phosphoenolpyruvate (PEP).[41–44] This is an energy-dependent conversion that overcomes the irreversible final glycolytic reaction catalyzed by pyruvate kinase. The activity of PEPCK is closely coupled with that of pyruvate carboxylase.[45–47] Whereas the pyruvate kinase reaction produces 1 ATP, the formation of PEP uses two ATPs: one in the mitochondria for the pyruvate carboxylase reaction and one in the cytosol for the PEPCK reaction. PEPCK requires GTP provided via the nucleoside diphosphate kinase reaction, which uses ATP. ATP transfers one high-energy bond to GDP to form ADP and GTP.

The enzyme PEPCK has been studied extensively as scientists have tried to understand the gluconeogenic process.[48,49] In starvation or uncontrolled diabetes, PEPCK activity is elevated, as is gluconeogenesis. Starvation elicits a number of catabolic hormones that serve to mobilize tissue energy stores as well as precursors for glucose synthesis. Uncontrolled diabetes elicits similar hormonal

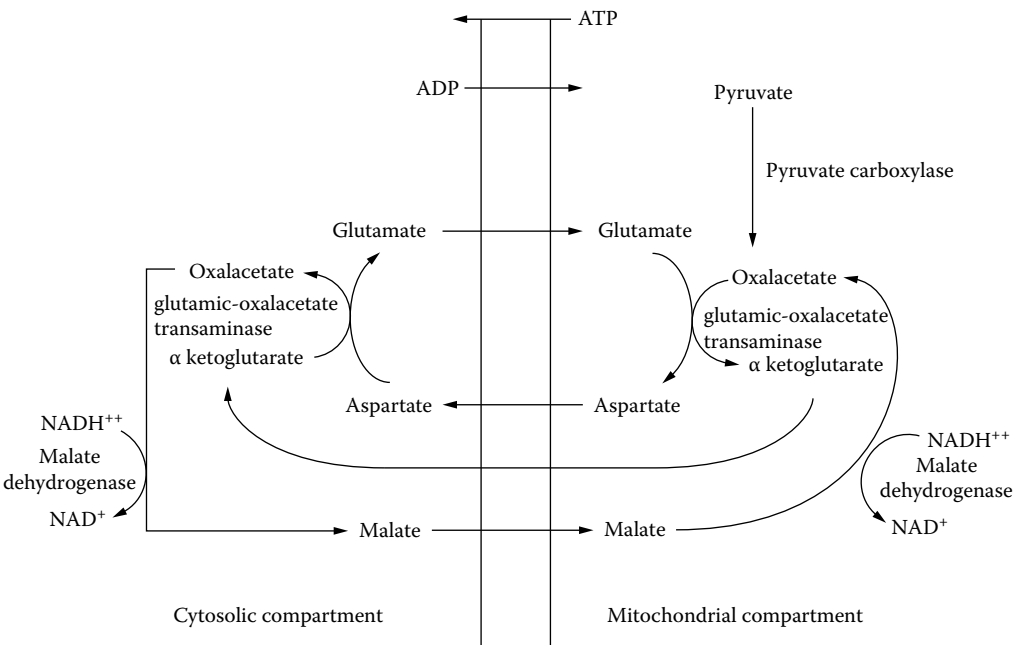


FIGURE 3.11 The malate aspartate shuttle.

responses. In both instances, the synthesis of the PEPCK enzyme protein is increased. Unlike other rate-limiting enzymes, PEPCK is not regulated allosterically or by phosphorylation–dephosphorylation mechanisms. Instead, it is regulated by changes in gene transcription of its single copy gene from a single promoter site. This regulation is unique because all of the known factors (hormones, vitamins, metabolites) act in the same place. They either turn on the synthesis of the messenger RNA for PEPCK or they turn it off.[49] What is also unique is the fact that only liver and kidney cells translate this message into active enzyme protein, which catalyzes PEP formation. Other cells and tissues have the code for PEPCK in their nuclear DNA but do not synthesize the enzyme. Instead, these cell types synthesize the enzyme that catalyzes glycerol synthesis. In effect then, only the kidney and liver have an active gluconeogenic process.

The next few steps in gluconeogenesis are identical to those of glycolysis but are in the reverse direction. When the step for the dephosphorylation of fructose-1,6-bisphosphate occurs, there is another energy barrier and instead of a bidirectional reaction catalyzed by a single enzyme, there are separate forward and reverse reactions. In the synthesis of glucose, this reaction is catalyzed by fructose-1,6-bisphosphatase and yields fructose-6-phosphate. No ATP is involved but a molecule of water and an inorganic phosphate are produced. Rising levels of fructose-2,6-bisphosphatase allosterically inhibit gluconeogenesis while stimulating glycolysis.[50,51] AMP likewise inhibits gluconeogenesis at this step.[52]

Finally, the removal of the phosphate from glucose-6-phosphate via the enzyme complex glucose-6-phosphatase completes the pathway to yield free glucose. Again, this is an irreversible reaction that does not involve ATP. The glucose-6-phosphate moves to the endoplasmic reticulum, where the phosphatase is located.[53] Should there be a mutation in the gene for the translocation of glucose-6-phosphate to the endoplasmic reticulum or in its phosphatase, glucose cannot be released and hypoglycemia will result. This reaction is also used when glycogenolysis provides the phosphorylated glucose. In either instance, free glucose is not released to the circulation. The resultant disease is called *Von Gierke's disease*. In addition to hypoglycemia, this rare, autosomally recessive disease is characterized by an enlarged liver due to excess glycogen stores, elevated blood lipids, decreased sensitivity to insulin, brain damage, and a shortened life span. About 25% of all patients with some form of glycogen-storage disease have Von Gierke's disease. Aside from providing many small meals throughout the day and night in an attempt to prevent the hypoglycemia, dietary maneuvers can achieve little else.

Gluconeogenic activity can be influenced by dietary composition. As already mentioned, the consumption of high-fat and high-protein diets result in elevated gluconeogenic rates. Rats have been fed a 64% sucrose or cornstarch with 6% corn oil or coconut oil.[55–57] Gluconeogenesis was increased in rats fed the sucrose diet and further increased when the fat source was hydrogenated coconut oil.

OXIDATIVE PHOSPHORYLATION

Oxidative phosphorylation (OXPHOS) occurs in the mitochondria.[56] It is the coupled process used for the production of water using the reducing equivalents released by the various metabolic steps in intermediary metabolism and the trapping of some of the energy released when water is made in the high energy bond of ATP. Not all of the energy is trapped; actually only a small percentage is. The rest is released as heat and it is this heat that keeps the body warm. OXPHOS has been extensively studied over the last 50 to 60 years. Much attention has been paid recently to the health problems associated with mitochondrial malfunction.[19]

MITOCHONDRIAL STRUCTURE AND FUNCTION

The number, size, and shape of mitochondria within the cell are influenced by many factors: cell type, age, genotype, hormones, and nutritional state. Estimates vary from 200 to 20,000 depending

on the cell type and the species. However, within a given cell type and within the same animal, the number is relatively constant. Liver cells contain between 800 and 1200 mitochondria, whereas bone cells contain far fewer mitochondria and ova contain many. The ova can have as many as 20,000 mitochondria per cell.

Mitochondria are scattered throughout the cytosol and are in proximity to other organelles that have a high ATP requirement. Thus, electronmicrographs of liver cell cross sections often show mitochondria near the nucleus and ribosomes where protein synthesis is taking place. High rates of protein synthesis demand large amounts of ATP, because every amino acid incorporated into the protein macromolecule must first be activated by ATP. Thus, if the protein being synthesized contains 300 amino acid residues, 300 molecules of ATP will be required in addition to that used for RNA transcription and translation. Similarly, one will find large numbers of mitochondria in muscle located close to the contracting muscle fibrils. The ATP molecules produced by the mitochondria need diffuse only a very short distance to where they are used. ATP can also be provided to the contracting muscle by a shuttle known as the *creatine phosphate shuttle*. Creatine is shuttled into the mitochondria and converted to creatine phosphate by the mitochondrial creatine kinase. The newly synthesized creatine phosphate is then shuttled back to the cytosol whereupon it is then hydrolyzed to provide energy, ATP (using cytosolic ADP) and creatine. Creatine phosphate then serves as a cytosolic source of high-energy phosphate during fast-twitch muscle contraction.

Mitochondria are frequently located near fat droplets that serve as sources of metabolic fuel. Stored triacylglycerides are hydrolyzed in the cytosol to glycerol and fatty acids. The fatty acids are then transferred, via the acylcarnitine transferase mechanism, into the mitochondria and oxidized. Mitochondria, in addition to serving as the central integrators of metabolism through the production of ATP, are the sites for the citric acid cycle and fatty acid oxidation, and initiate urea cycle as well as gluconeogenesis. These latter pathways are completed in the cytosol.

Mitochondria are of many shapes. In the brown fat cell, they are spherical, in hepatocytes they are oval, in the kidney they are cylindrical, and in fibroblasts they are threadlike. The organelle consists of two membranes separated by a space called the *intermembrane space*. The membranes of the mitochondria differ not only in appearance but in function as well. The outer membrane is smooth and somewhat elastic, whereas the inner membrane is, as mentioned, convoluted. These convolutions serve to increase the surface area of the inner membrane, which is covered with regularly spaced spheres on stalks called *inner membrane spheres*. These spheres are not present on the outer membrane. The two membranes have been separated and their functional components studied. Within the inner membrane is the matrix, which can change in volume and organization as it changes its respiratory activity. The matrix contains, in addition to its carriers and enzymes, DNA and ribosomes with which it synthesizes 13 subunits of the respiratory chain and F_1F_0 ATPase.

RESPIRATION AND THE CITRIC ACID CYCLE

Respiration is the process by which aerobic cells obtain energy from the oxidation of fuel. When coupled with ATP synthesis, the process is called *oxidative phosphorylation* or OXPHOS.[57] Carbon dioxide, water, heat, and ATP are the products of this process.

Glycolysis, as well as fatty acid and amino acid oxidation, results in the production of an activated 2-carbon residue, acetyl CoA. This common metabolic intermediate is joined to oxalacetate to form citrate and is then processed by the citric acid cycle. This cyclic sequence of reactions is found in every cell type that possesses mitochondria. The cycle is catalyzed by a series of enzymes and yields reducing equivalents (H^+) and carbon dioxide. It is illustrated in Figure 3.12. Reducing equivalents are produced when α-ketoglutarate is produced from isocitrate, when α-ketoglutarate is decarboxylated to produce succinyl CoA, when succinate is converted to fumarate, and when malate is converted to oxalacetate. These reducing equivalents, one pair for each step, are carried to the respiratory chain by way of either NAD or FAD. Reducing equivalents from succinate are carried by FAD to site II of the respiratory chain, whereas those produced by the oxidation of the other

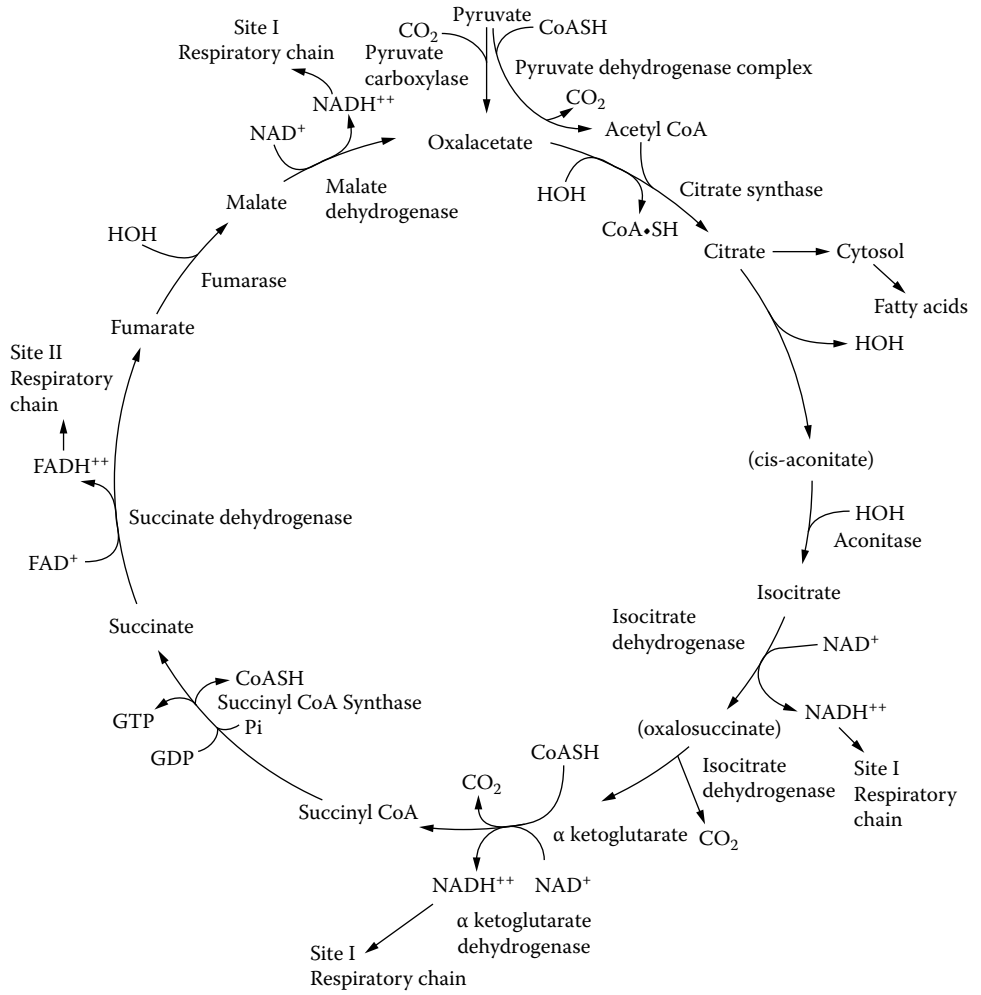


FIGURE 3.12 Krebs citric acid cycle in the mitochondria. This cycle is also called the tricarboxylate cycle (TCA). Abbreviations used: HOH, water; FAD, flavin adenine nucleotide; $FADH^{++}$, flavin adenine nucleotide, reduced; NAD, niacin adenine dinucleotide; $NADH^{++}$, niacin adenine dinucleotide reduced; GTP, guanosine triphosphate; GDP, guanosine diphosphate; CoA, coenzyme A; CO_2, carbon dioxide.

substrates are carried by NAD to site I. Once oxalacetate is formed, it can then pick up another acetate group from acetyl CoA and begin the cycle once again by forming citrate. Thus, for every turn of the cycle, two carbon dioxide molecules and eight pairs of reducing equivalents are produced. As long as there is sufficient oxalacetate to pick up the incoming acetate, the cycle will continue to turn and reducing equivalents will continue to be produced and these, in turn, will be joined with molecular oxygen to produce water, the end product (with carbon dioxide) of the catabolic process.

The preceding simplistic description of the citric acid cycle implies that it is free of controls, and, given adequate supplies of substrates, enzymes, and molecular oxygen, proceeds unhindered. This is not true. There are numerous controls in place that regulate the cycle. The citric acid cycle produces the reducing equivalents needed by the respiratory chain, and the respiratory chain must transfer these hydrogen ions and their associated electrons to the oxygen ion to produce water. In doing so, it generates the electrochemical gradient (the proton gradient) necessary for the formation

of the high-energy bonds of ATP. Obviously, then, these processes, citric acid cycle, respiratory chain, and ATP synthesis, are regulated coordinately.

RESPIRATORY CHAIN

The pairs of electrons produced at the four steps described above in the citric acid cycle, as well as electrons transferred into the mitochondria via other processes, are passed down the respiratory chain to the ultimate acceptor, molecular oxygen. The respiratory chain is shown in Figure 3.13. The enzymes of the respiratory chain are particularly complex. They are embedded in the mitochondrial inner membrane and are difficult to extract and study. They catalyze the series of oxidation-reduction reactions that together are known as the respiratory chain.

Each reaction is characterized by a redox potential that can be calculated using the Nernst equation:

$$E_h = E_o^1 + 2.303\ RT/nP \log n\ [(\text{electron acceptor})/(\text{electron donor})]$$

where E_h = observed potential, E_o^1 = standard redox potential (pH = 7.0, T = 25°, 1.0 M concentration), R = gas constant (8.31 J deg^{-1} mol^{-1}), T = Temperature (°K), n = number of electrons being transferred, P = Faraday (23,062 cal, V^{-1} = 96,406 J V^{-1}).

The more positive the potential, the greater the affinity of the negatively charged acceptor for the positively charged electrons. It is this potential that drives the respiratory chain reactions forward toward the formation of water. Each succeeding acceptor donor pair has a higher affinity for the electrons than the preceding pair, until the point in the chain where the product, water, is formed. By comparison with the preceding pairs, water itself has little tendency to give up its electrons, and, unless this water is immediately removed, the chain reaction will stop. Of course, water does not accumulate in the mitochondria; it leaves as quickly as it is formed and thus does not feed back to inhibit the chain. If it did accumulate, it would dilute the concentration of the chain components. When water accumulates, the mitochondrion swells; if in excess, the mitochondrion would burst and die. This does not happen. Obviously, then, water must exit the organelle as soon as it is formed so as to maintain optimal osmotic conditions.

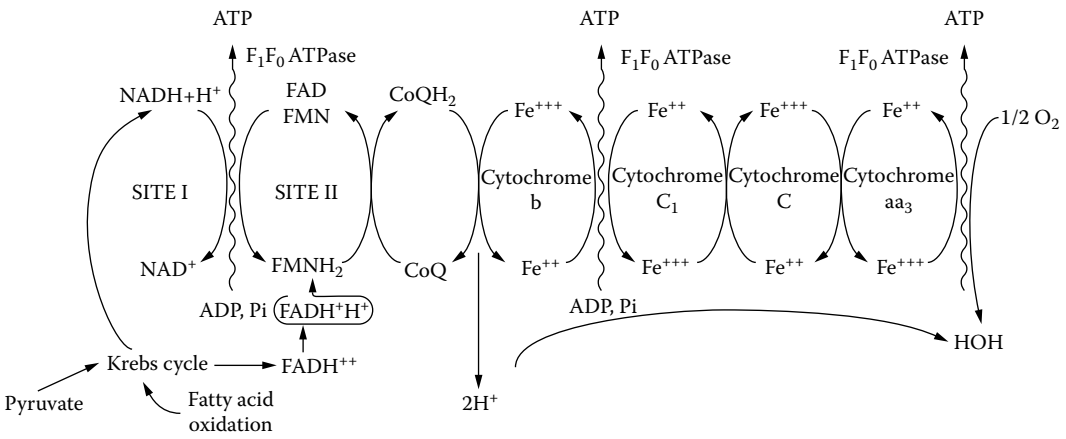


FIGURE 3.13 The respiratory chain showing the points where sufficient energy has been generated to support the synthesis of 1 molecule of ATP from ADP and Pi. Each of the segments generates a proton gradient. This energy is captured by the F_0 portion of the ATPase and transmitted to the F_1 portion of the ATPase. If uncouplers are present, the proton gradient is dissipated and all of the energy is released as heat.

Although the respiratory chain usually proceeds in the forward direction (toward the formation of water) due to the exergonic nature of the reaction cascade, it should be realized that, except for the final reaction, all of these steps are fully reversible. In order to be reversed, sufficient energy must be provided to drive the reaction in this direction. For example, the reducing equivalents derived from succinate are usually carried by FAD (as FAD^+H^+). These can be transferred to NAD (as $NADH^+H^+$) with the concomitant hydrolysis of ATP. Electron transport across the other two phosphorylation sites can also be reversed, again, only if sufficient energy is provided.

Overall regulation of respiration or the activity of the intact respiratory chain is vested in the availability of the phosphate acceptor, ADP. A rapid influx of ADP into the mitochondrial compartment is what is needed to ensure a rapid respiratory rate. For example, the working muscle uses the energy provided by the hydrolysis of ATP and creatine phosphate. Creatine phosphate is split to creatine and a phosphate group and ATP is hydrolyzed to ADP and Pi. The ADP travels into the mitochondria and stimulates respiration and is used to resynthesize ATP.

ATP SYNTHESIS

As electrons pass down the respiratory chain, ATP is synthesized. The sequential reactions of the respiratory chain generate an electrochemical gradient of H^+ ions across the inner mitochondrial membrane. This gradient serves as the means for coupling the energy flow from electron transport and water formation to the formation of ATP. This involves the mitochondrial membrane. The membrane, and the enzymes and transporters that are embedded in it, couple the energy gradient of the electron flow of the respiratory chain to the synthesis of ATP.[56,57] To do this, the membrane must be intact and in the form of a continuous closed vesicle. If the membranes are disrupted, coupling will not occur. Respiration may occur, but ATP synthesis will not. The enzymes of the respiratory chain are arranged so as to transport hydrogen ions from the matrix across the inner membrane so that the gradients will develop in proximity to the F_1F_0 ATPase (ATP synthase, Figure 3.14) complex and provide a proton gradient sufficient to drive ATP synthesis by causing a dehydration of ADP and Pi. This energy capture in the ATP molecule is only a fraction of the energy generated by the respiratory chain. The rest of the energy is released as heat.

The components of oxidative phosphorylation are divided into five complexes. Complexes I through IV plus ubiquinone (Q) and cytochrome C comprise the respiratory chain. Complex V is the F_1F_0 ATPase. The electron carriers are the quinoid structures (FMN, FAD, Q) and the transition metabolic complexes. At three stages in the chain, oxidative energy is conserved (Figure 3.13) via coupled vectorial proton translocations and the creation of a membrane proton gradient ($\Delta\mu H^+$). This protonic energy is what drives the synthesis of ATP. Should this energy be dissipated, as happens when agents such as dinitrophenol or one of the uncoupling proteins are present, ATP synthesis does not occur, or occurs to a more limited extent. Uncoupling takes place because protons are transported through the membrane from the intramembrane space to the matrix. This "short circuits" the normal energy flow through complex V. In such a circumstance, electrons continue down the respiratory chain but the protonic energy thus generated is not captured in the high-energy phosphate bond. Instead, this energy is released as heat. There are variations or degrees of protonic energy capture in vivo that are related to the presence or graded absence of naturally occurring agents that serve to either inhibit the action of complex V or uncouple the respiratory chain energy-generation process from the energy-capture process of ATP synthesis.

UNCOUPLING PROTEINS

Uncoupling proteins (UCPs) are inner mitochondrial membrane transporters that dissipate the proton gradient, thus releasing heat.[58] The uncoupling proteins are similar in size and homologous in both amino acid sequence and gene sequence for the majority of their structures. Three distinct UCPs have been identified so far. UCP1 is uniquely expressed in brown adipose tissue. It uncouples

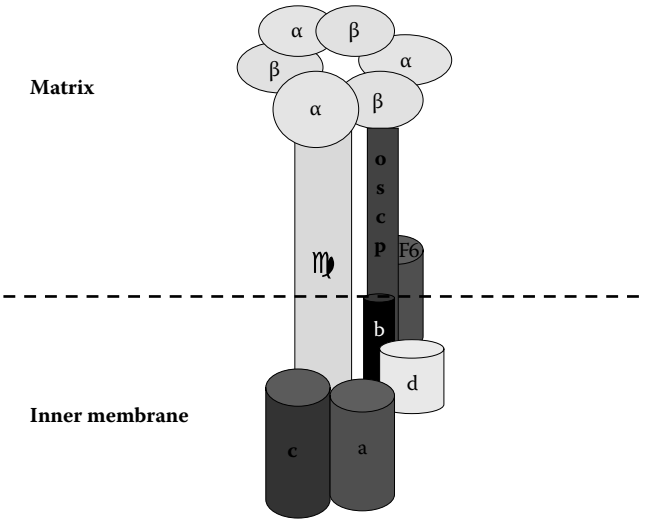


FIGURE 3.14 The F_1F_0 ATPase. Note that the lower portion is embedded in the inner mitochondrial membrane, whereas the upper part protrudes out into the matrix. The two parts can be separated. The upper part is the F_1 part.

OXPHOS in this tissue and this action can be stimulated by norepinephrine.[59,60] Cold exposure and starvation, two events characterized by an initial rise then fall in norepinephrine, stimulate UCP1 release and heat production by the brown fat. After the initial use in heat production, there is a fall as the animal attempts to conserve energy. UCP homologs have also been found in skeletal muscle and white adipose tissue. However, the control of their release and their function in the heat economy of the whole animal is somewhat different. UCP2 is found in heart, liver, muscle, and white adipose tissue. It is subject to dietary manipulation and, as well, probably functions in body weight regulation. UCP3 is found in skeletal muscle and is downregulated during energy restriction or starvation. Actually, this down-regulation probably explains the increased metabolic efficiency that occurs in the food-restricted individual. Following refeeding, some of this increased efficiency is immediately lost, whereas the rest disappears over time. During the starvation period, the mRNA for these UCPs are up regulated, but the message is not translated.[61] Translation does not occur immediately when food is restored but takes up to 10 days to appear, thus allowing for maximum food efficiency during the immediate recovery period. This up regulation of the UCP mRNA may also play a role in the rapid weight regain following food restriction.

Long-chain free fatty acids can act as partial uncoupling agents.[62] A large influx of fatty acids increases the volume of the mitochondrion, and this in turn serves to spacially separate the respiratory chain and the ATPase. Other naturally occurring compounds can also reduce coupling efficiency. These agents increase body heat production. This is an important defense reaction against invading pathogens. Increased body heat (fever) serves to reduce the viability of these pathogens. Some of these compounds are catabolic hormones, and their effect on coupling is dose dependent. These include the thyroid hormones, glucocorticoids, sex hormones, catecholamines, insulin, glucagon, parathyroid hormone, and growth hormone. Some of these hormones have direct effects on mitochondrial respiration and coupling through affecting the synthesis and activation of the various protein constituents of the five complexes. These effects are listed in Table 3.2. Other hormones have their effects on the exchange of divalent ions, notably calcium, and/or on the phospholipid fatty acid composition of the inner mitochondrial membrane. Some hormones, thyroid hormones,

for example, influence enzyme synthesis/activation, calcium flux, *and* membrane lipid composition. Clearly, this is a very carefully regulated system with checks and balances to ensure the continuity of life. Without this careful control, survival during times of energy stress could not be assured.

METABOLIC CONTROL: OXIDATIVE PHOSPHORYLATION

Metabolic regulation is defined as the sum of all of those processes and reactions designed to provide a continuous supply of substrates for the maintenance of cell life. It includes both catabolic and anabolic processes as well as both energy-producing and energy-using pathways. In other words, it is the sum of all these reactions and their controls that defines the steady state. Living cells must interdigitate these pathways so that they are able to survive times of energy deficit and surfeit. They must be able to replace their functional parts as they are used up or worn out or damaged.

The regulation of metabolism can be viewed either as the controls exerted on a single reaction in the cell or as the control of an animal's response to a change in its environment. Neither view is wholly correct because regulation can and does occur at many levels in the body. The simplest level of control is that which is exerted over a single enzymatic reaction. This reaction is controlled by the amount of available substrate, the amount and activity of the enzyme that catalyzes the reaction, the presence of appropriate amounts of required cofactors or coenzymes, and the accumulated product.

One can calculate the strength of the control exerted by the enzyme that catalyzes a reaction or by factors that affect that enzyme using measurements of substrate, products, cofactor amounts, coenzyme amounts, and enzyme amounts using the equation for the calculation of the flux control coefficient. This is an expression of the rate at which a substrate passes to a product through one or more reactions in a series.

Control strength can vary among enzymes in a given reaction sequence, and this can be estimated using metabolic control analysis. The elasticity and responsivity of a reaction or series of reactions to perturbations in reactants or environment can also be calculated. It is expressed as $\varepsilon^{y}_{k} = x\partial v/v\partial x$, where v is the rate of the reaction and x is a variable that modified v. Thus, the flux through a single reaction or a series of reactions can be estimated. Elasticity, response, and control coefficients determine how a steady-state response will be affected by changes in one or more constituents or conditions needed by this reaction or reaction sequence. This provides a quantitative approach to assessing metabolic control.

TABLE 3.2
Effects of hormones on the coupling of respiration to ATP synthesis

Hormone	Effect
Thyroxine	Increase
Triodothyronine	Increase/decrease; dose dependent
Epinephrine	Decrease
Norepinephrine	Decrease
Insulin	Increase
Glucocorticoid	Increase/decrease; dose dependent
Glucagon	Increase
Parathyroid hormone	Increase; dose dependent
Growth hormone	Increase

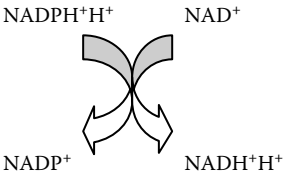


FIGURE 3.15 Transfer of reducing equivalents from $NADPH^+H^+$ to NAD^+ via transhydrogenation.

OXIDATIVE PHOSPHORYLATION

Changes in the dietary or hormonal status of the individual result in large changes in the cytosolic pathways for glucose and fatty acid use as well as changes in protein turnover. All of these are orchestrated by changes in oxidative phosphorylation, which is governed by these changes in cytosolic activity. Starvation and diabetes, for example, result in decreased cytosolic glycolytic activity and lipogenesis while increasing fatty acid oxidation in the mitochondrion. These coordinated decreases and increases have in common a change in compartment redox state (ratio of oxidized to reduced metabolites) and phosphorylation (ratio of ATP to ADP) state. Reducing equivalents generated in the cytosol and carried by NAD+ or NADP+ must be transferred to the mitochondria for use by the respiratory chain. Through transhydrogenation (Figure 3.15), reducing equivalents generated in the cytosol through NADP-linked enzymatic reactions are transferred to NAD. In turn, because NAD cannot traverse the mitochondrial membrane, these reducing equivalents must be carried on suitable metabolites into the mitochondrial compartment. Several shuttle systems exist, with the malate–aspartate shuttle (Figure 3.11) thought to be the most important. The shuttle requires a stoichiometric influx of malate and glutamate and efflux of aspartate and α-ketoglutarate from the mitochondria. Alterations in the rate of efflux of α-ketoglutarate can significantly alter the shuttle activity in terms of the rate of cytosolic NADH utilization. α-Ketoglutarate efflux is dependent on mitochondrial ATP/ADP ratios and on the concentration of cytosolic malate. Shuttle activity is controlled by cytosolic ADP levels, malate levels, and availability of NADH.

Malate is exchanged for citrate (Figure 3.16) or for phosphate. The malate–citrate exchange or the malate-citrate shuttle involves the transport of malate into the mitochondria, where it is converted to citrate via oxaloacetate. The citrate is then transported out of the mitochondria and the reactions reversed. This exchange is thought to be particularly active in lipogenic states, as it provides citrate to the cytosol for citrate cleavage. Because the cleavage of citrate provides the starting acetate for fatty acid synthesis, this exchange plays a key role in lipogenesis. As shown in Figure 3.16, other exchanges also take place to varying degrees. One can recognize the components of the malate shuttle among these exchange systems as well as identify the system for the exchange of adenine nucleotides. When the activity of the malate–aspartate shuttle is increased, the redox state of the cytosol is decreased, as is lipogenesis, whereas gluconeogenesis is increased. All of these exchanges of metabolites are related to each other via a coordinated control exerted by the phosphorylation state, the latter, in turn, being controlled by the ADP/ATP exchange.

Of importance to the control of glycolysis is the α-glycerophosphate shuttle (Figure 3.17). This shuttle is located at the outer side of the mitochondrial inner membrane. Transport of α-glycerophosphate and dihydroxyacetone phosphate across the mitochondrial inner membrane is not required. The activity of the shuttle is related to the availability of cytosolic α-glycerophosphate and the activity of the mitochondrial FAD-linked α-glycerophosphate dehydrogenase. It is particularly responsive to thyroid hormone, which is thought to act by increasing the synthesis and activity of the mitochondrial FAD-linked α-glycerophosphate dehydrogenase.

Adenine nucleotide translocation, or the exchange of adenine nucleotides across the mitochondrial membrane (Figure 3.16), also plays a role in the regulation of oxidative phosphorylation.

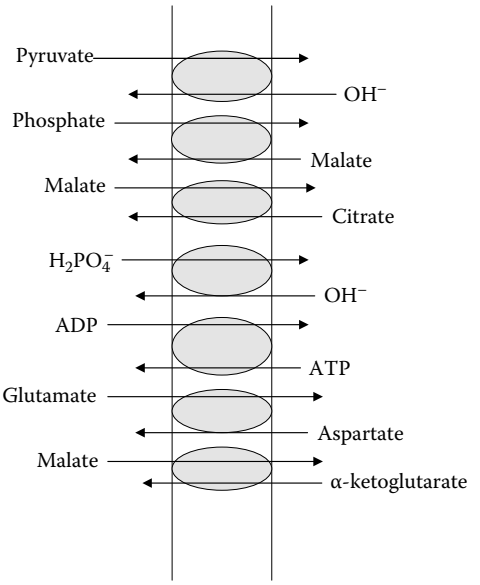


FIGURE 3.16 Metabolite exchanges across the mitochondrial membranes. The first one (top) is the monocarboxxylate transporter; the second is the dicarboxxylate transporter; the third is the tricarboxylate transporter; the fourth is the phosphate exchanger; the fifth is the adenine nucleotide translocase; the next is the aspartate glutamate transporter and the last is the malate-α-ketoglutarate transporter. The last two can be recognized as being involved in the malate shuttle. The cytosol is on the left and the mitochondria compartment is on the right.

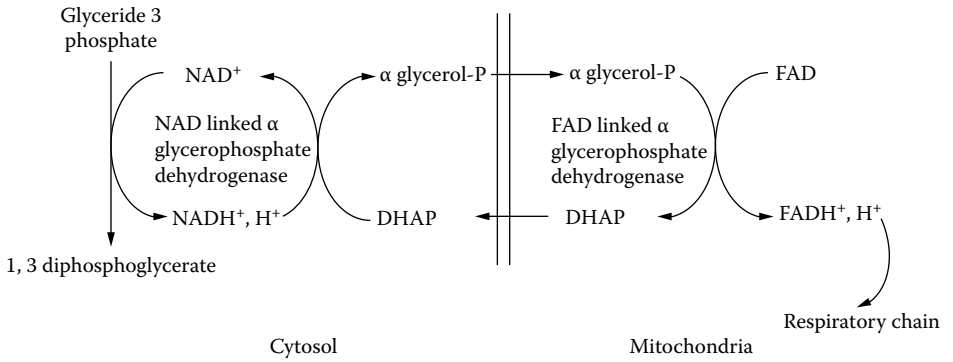


FIGURE 3.17 The α glycerophosphate shuttle.

Although any one of the adenine nucleotides could theoretically exchange for any other, this does not happen. AMP movement is very slow (if it occurs at all). Under certain conditions, ADP influx into the mitochondria is many times faster than ATP efflux. This occurs when the need for ATP within the mitochondria exceeds that of the cytosol. An example of this occurs in starvation, where ATP is needed to initiate urea synthesis and fatty acid oxidation, and to support gluconeogenesis. Under normal dietary conditions, however, the exchange of ADP for ATP is very nearly equal. For every 100 molecules of ADP that enter the mitochondrial compartment, about 87 molecules of ATP exit.

The number of molecules of ATP produced by the coupling of the respiratory chain to ATP synthesis depends on where the reducing equivalents enter the chain. If they enter carried by NAD,

they are said to enter at site I. If they enter carried by FAD, they are said to enter at site II. Site I substrates are those that are oxidized via NAD-linked dehydrogenases. Pyruvate, for example, is a site I substrate, whereas succinate is a site II substrate. Reducing equivalents entering at site I will generate the energy for the synthesis of three molecules of ATP. Those entering at site II will generate enough energy to result in two molecules of ATP. Knowing how a substrate provides its reducing equivalents to the respiratory chain allows estimation of how many ATPs will be generated by a particular reaction sequence.

FATTY ACID SYNTHESIS

In humans consuming a typical American diet containing 35 to 45% of its energy as fat, very little fatty acid synthesis occurs. However, in certain circumstances, for example, humans consuming a very low-fat diet that is high in refined sugar, synthesis can and does occur. The magnitude of this synthesis is dependent on a variety of factors, of which diet is one. In laboratory rats accustomed to a very low-fat diet composed mainly of cereal grains, lipid synthesis occurs at a significant rate. Furthermore, in people who are obese, it is suspected that significant fatty synthesis also occurs despite their high-fat intake. In humans, primates, and birds, the liver is the primary site for fat synthesis. In rats and mice, lipogenesis occurs in the adipose tissue as well as in the liver. In pigs, lipogenesis occurs primarily in the fat depots. In those humans having a very high synthetic rate, a fatty liver may be observed. This is a very rare situation and diet as a cause of a fatty liver in humans is almost nonexistent with few exceptions. Humans sustained by parenteral nutrition (nutrients provided through a catheter placed in the subclavian vein) or those who derive a significant (more than 60%) amount of their energy from carbohydrate, may have high lipogenic rates and a fatty liver if the export of these newly synthesized lipids via the very-low-density lipoprotein (VLDL) is impeded.

If VLDL synthesis keeps pace with the high lipogenic rate, a fatty liver does not develop. In rats fed high-sugar (65% by weight) diets, lipogenesis is quite high. However, normal rats will adapt to this diet so that the initial fatty liver will disappear as the hepatic lipid output increases to match the increased synthesis.

Fatty acid synthesis (Figure 3.18) begins with acetyl CoA, which arises from the oxidation of glucose or the carbon skeletons of deaminated amino acids. Acetyl CoA is converted to malonyl CoA with the addition of 1 carbon (from bicarbonate) in the presence of the enzyme acetyl CoA carboxylase.[63] The reaction uses the energy from 1 molecule of ATP and biotin as a coenzyme. This reaction is the first committed step in the reaction sequence that results in the synthesis of a fatty acid. In many respects, it resembles the carboxylation of pyruvate, the first committed step in gluconeogenesis. In both reactions, activated carbon dioxide, attached to the biotin–enzyme complex, is transferred to the methyl end of the substrate. Although most fatty acids synthesized in mammalian cells have an even number of carbons, this first committed step yields a 3-carbon product. This results in an asymmetric molecule that becomes vulnerable to attack (addition) at the center of the molecule with the subsequent loss of the terminal carbon. The vulnerability is conferred by the fact that the carboxyl group at one end and the −CoA group at the other end are both powerful attractants of electrons from the middle carbon group. This leaves the carbon in a very reactive state, and a second acetyl group carried by a carrier protein with the help of phosphopantethine, which has a sulfur group connection, can be joined to it through the action of the enzyme malonyl transferase. Subsequently, the "extra" carbon is released via the enzyme β-ketoacyl enzyme synthase leaving a 4-carbon chain still connected to an SH group at the carboxyl end. This SH group is the docking end for all the enzymes that the fatty acid synthase complex comprises. These enzymes catalyze the addition of two carbon acetyl groups in sequence to the methyl end of the carbon chain until the final product, palmityl CoA, and then palmitic acid, is produced. Members of this fatty acid synthase complex include the aforementioned malonyl transferase and β-ketoacyl synthase, β-ketoacyl reductase, which catalyzes the addition of reducing equivalents carried by FMN, and an acyl transferase. Upon completion of these six steps, the process is repeated until the chain length is 16

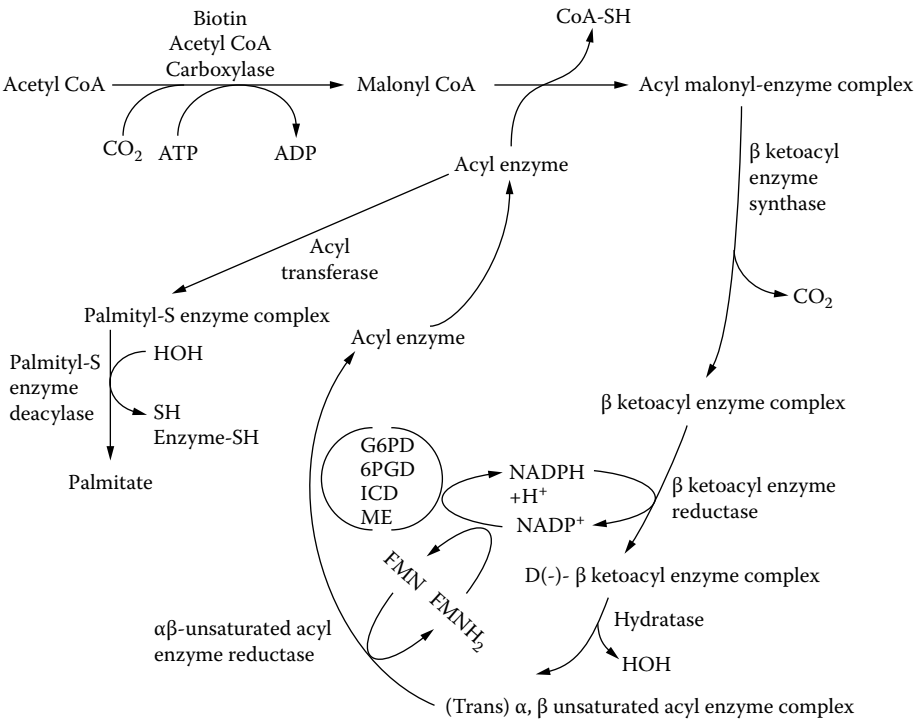


FIGURE 3.18 Fatty acid synthesis from acetyl coA.

carbons long. At this point, the SH-acyl carrier protein is removed through the action of the enzyme palmityl-*S*-enzyme deacylase, and the palmitic acid is available for esterification to glycerol to form a mono-, di-, or triacylglyceride.

Fatty acid synthesis does not occur as an uncontrolled sequence of reactions. It has a number of direct and indirect controllers. As mentioned above, the synthesis of malonyl CoA is the first committed step in the pathway. It also is the first rate-limiting reaction. Acetyl CoA carboxylase as a rate-limiting enzyme has been studied in detail. It is synthesized as an inactive protomer which, when citrate levels increase, aggregate to form the active enzyme. Other tricarboxylates can stimulate aggregation but citrate is the preferred anion. Citrate, a citric acid cycle intermediate in the mitochondria, is exchanged for malate or pyruvate. The malate–citrate shuttle is very active in cells also having a very active lipogenic process. This shuttle also has rate-limiting properties. Pyruvate, from the oxidation of glucose via the glycolytic sequence, is also actively exchanged for citrate in the lipogenic cell. Citrate is cleaved through the action of the ATP-citrate lyase (citrate cleavage enzyme) producing oxalacetate and acetyl CoA. This acetyl CoA is the beginning substrate for fatty acid synthesis. Feedback inhibition of acetyl CoA carboxylase is exerted when palmitoyl CoA accumulates.

Acetyl CoA carboxylase is sensitive to the phosphorylation state of the cytosol. High levels of ATP are needed for the carboxylation of acetyl CoA to form malonyl CoA. Thus, high phosphorylation states (high concentrations of ATP relative to ADP and inorganic phosphate) are a characteristic of high rates of lipogenesis. Acetyl CoA carboxylase is also controlled by a cAMP-mediated phosphorylation–dephosphorylation mechanism in which the phosphorylated enzyme is less active than the dephosphorylated enzyme. Insulin promotes dephosphorylation whereas glucagon promotes phosphorylation. Thus, when insulin levels are high, one would anticipate high rates of lipogenesis and the reverse when insulin levels are low and glucagon levels are high. One might also anticipate an increase in lipogenesis in hyperinsulinemic individuals, and, indeed, obese people as

well as genetically obese experimental animals are characterized by both. As a corollary, one might also anticipate that the consumption of a high-glucose, low-fat diet that stimulates insulin release and also provides ample glucose for conversion to fatty acids would be characterized by high rates of lipogenesis, particularly in the liver. In rodents, this anticipation is justified. However, in humans, there is some discussion as to whether the human diet is sufficiently high in simple sugar and sufficiently low in fat to have the same sort of lipogenic response. Species differences in habitual diet composition as well as species differences in biological time frame complicate the discussion. A 24-hour day in a rat is roughly equivalent to a 30-day time span in the human, with respect to total life span. The peaks and nadirs in the lipogenic rates in the rat will thus be further apart within a 24-hour period than will lipogenic rates in the human. Thus, one can elicit an increase in acetyl CoA carboxylase activity and in lipogenesis in the rodent with a short interval (1 to 2 days) of high-glucose diet feeding where that same interval for the human will show little, if any, change.

Genetic factors also influence acetyl CoA carboxylase activity.[64] In the human, the gene for acetyl CoA carboxylase is located on chromosome 17. Should this gene mutate, a nonfunctional enzyme will be produced and de novo fatty acid synthesis will not occur. Such an inborn error has been reported in humans. However, since humans consuming a high-fat diet, such as that consumed in the United States, synthesize very little lipid de novo, the absence of a functioning acetyl CoA carboxylase is not as devastating as the absence of an important rate-limiting step in some other pathway. In contrast, such a deficiency in rats, animals that usually consume a low-fat, high-carbohydrate diet, would seriously impair survival unless the rat was fed a human-like diet.

Another control of fatty acid synthesis is exerted by the complex of enzymes known as the fatty acid synthase.[65] All of the dietary and hormonal factors described earlier for activating or inhibiting acetyl CoA carboxylase have the same effects on the fatty acid synthase complex. Further, phosphorylated sugars have allosteric effects on this enzyme complex. An allosteric effect is one that promotes (or inhibits) the activity of an enzyme or enzyme complex by binding to a site other than the active catalytic site that promotes (or inhibits) the activity of the active catalytic site. An allosteric effector may not be involved in the reaction itself, but, through its binding, causes a change in the conformation of the enzyme. In the case of inhibition, such a conformational change may "hide" the active catalytic portion of the molecule. In promotion, the reverse occurs; the "business end" of the molecule is readily accessible to substrate, cofactors, and coenzymes.

Dietary ingredients affect the activity of fatty acid synthase through effects on the supplies of acetyl CoA, but they have other effects as well. These effects occur at the level of the genes for fatty acid synthase. Diets high in simple sugars increase the synthesis of fatty acid synthase. Diets rich in polyunsaturated fatty acids decrease this synthesis. Clarke and Jump[65] reviewed the literature on this aspect of fatty acid synthesis and concluded that polyunsaturated fatty acids suppress the rapid transcription of genes coding for fatty acid synthesis. They speculate that in liver cells there is a nuclear fatty acid-binding protein that selectively binds 18:2 (w6 or N6) polyenoic fatty acids and their metabolites. Upon binding, the putative trans acting protein would bind to a *cis*-acting element linked to the fatty acid synthase (or S14) gene which, in turn, would result in a suppression of gene transcription. This, in turn, would result in less enzyme activity.

Numerous publications report in detail how the various enzymes in metabolism work in vitro. However, one must appreciate the fact that most in vitro studies of enzyme activity are conducted with idealized amounts of substrate and cofactors, and with little subsequent product removal. Usually the enzyme is studied at saturation, a condition that seldom occurs in the living organism. In vivo, enzymes are rarely saturated; they usually are working so rapidly that 10–20% saturation is more likely. This allows for greater control of the flux of metabolites through the system by factors other than the amount and activity of the enzyme per se. Thus, as measured in vitro, in cells having 50% less of a given enzyme than normal cells, the flux of substrate through the pathway in vivo would not necessarily be slower. In fact, the inheritance of a number of the genetic diseases characterized by an absence of activity of a certain enzyme can be tracked within a given family by the activity of that enzyme in unaffected homozygotes and heterozygotes. Heterozygotes will have

reduced enzyme activity yet be normal with respect to their metabolism. This may also be true for the fatty acid synthase complex in animals fed polyunsaturated fatty acids. That is, they may have less enzyme but the flux of acetyl CoA through the fatty acid synthesis pathway as reflected in the total amount of accumulated lipid in the tissue may differ from animals fed a low-polyunsaturated-fatty-acid diet. There are many reasons that this occurs. Suffice it to say that the amount of rate-limiting enzyme does not always predict the activity of the pathway of which it is a part or the amount of product the pathway produces that accumulates.

Despite the gaps in our knowledge about the details of acetyl CoA carboxylase and fatty acid synthase, the reaction sequence shown in Figure 3.18 is well established, as are the effects of certain dietary ingredients and hormones on fatty acid synthesis in living creatures. We know that the fatty acid chain is continually bound to the fatty acid synthase complex and is sequentially transferred between the 4′-phosphopantetheine (pantothenic acid) group of the acyl carrier protein and the sulfhydryl group of a cystein residue on ketoacyl-ACP synthase during the condensation step.

There are other aspects of fatty acid synthesis that should be mentioned. Although the hexose monophosphate shunt NADP-linked enzymes are not rate controlling, it is recognized that the reactions catalyzed by these enzymes provide about 50% of the reducing equivalents needed in the reaction sequence catalyzed by the fatty acid synthase complex. The remaining reducing equivalents are provided by the NADP-linked isocitrate dehydrogenase (~10%) and the malic enzyme (~40%) Fatty acid synthesis is one of the few synthetic pathways that incorporate reducing equivalents into the product. Most pathways for the synthesis of large molecules release reducing equivalents as part of their condensation reactions. Because fatty acid synthesis incorporates these reducing equivalents, one can measure the rate of incorporation of ^{3}H (from radio-labeled water) into palmitate. The pathway for the synthesis of fatty acids, shown in Figure 3.18, has been well studied. It produces the 16-carbon saturated fatty acid palmitate. This fatty acid is then the substrate for a variety of other reactions to produce the full array of endogenously produced fatty acids found in the body. Palmitate can be elongated, desaturated, oxidized, and esterified. Descriptions of all of these processes follow.

FATTY ACID ELONGATION

Elongation occurs in either the endoplasmic reticulum or the mitochondria. The reaction differs, depending on where it occurs and in what species. Mammalian de novo fatty acid synthesis from excess dietary carbohydrate results mainly in palmitic, stearic, and oleic acid. Some longer-chain fatty acids are produced by specific tissues. Oleic acid is usually produced by the desaturation of palmitic acid, followed by elongation rather than the desaturation of stearic, although both reactions do occur. Chain elongation following desaturation of stearic acid to oleic acid will produce eicosenoic (20:1ω9), erucic (22:1ω9), and nervonic (24:1ω9) acids. These are of the ω9 series of fatty acids. Fatty acid elongation and desaturation of the ω9 series in the endoplasmic reticulum essentially stops with the formation of 22:3 ω9. In the ω7 series, elongation stops at 18:3ω7. The ω6 series ends with the formation of arachidonate, whereas the ω3 series continues on up to the formation of 22:6ω3. In the endoplasmic reticulum, the reaction sequence is similar to that just described for the cytosolic fatty acid synthase complex. The source of the 2-carbon unit is malonyl CoA and NADPH provides the reducing power. The intermediates are CoA esters not the acyl carrier protein 4′-phosphopantetheine. The reaction sequence (Figure 3.19) in the brain can proceed to produce fatty acids containing up to 24 carbons. In the mitochondria, elongation uses acetyl CoA rather than malonyl CoA as the source of the 2-carbon unit. It uses $NADH^{+}H^{+}$ as the source of reducing equivalents and uses, as substrate, carbon chains of less than 16 carbons. Mitochondrial elongation is the reversal of fatty acid oxidation, which also occurs in this organelle.

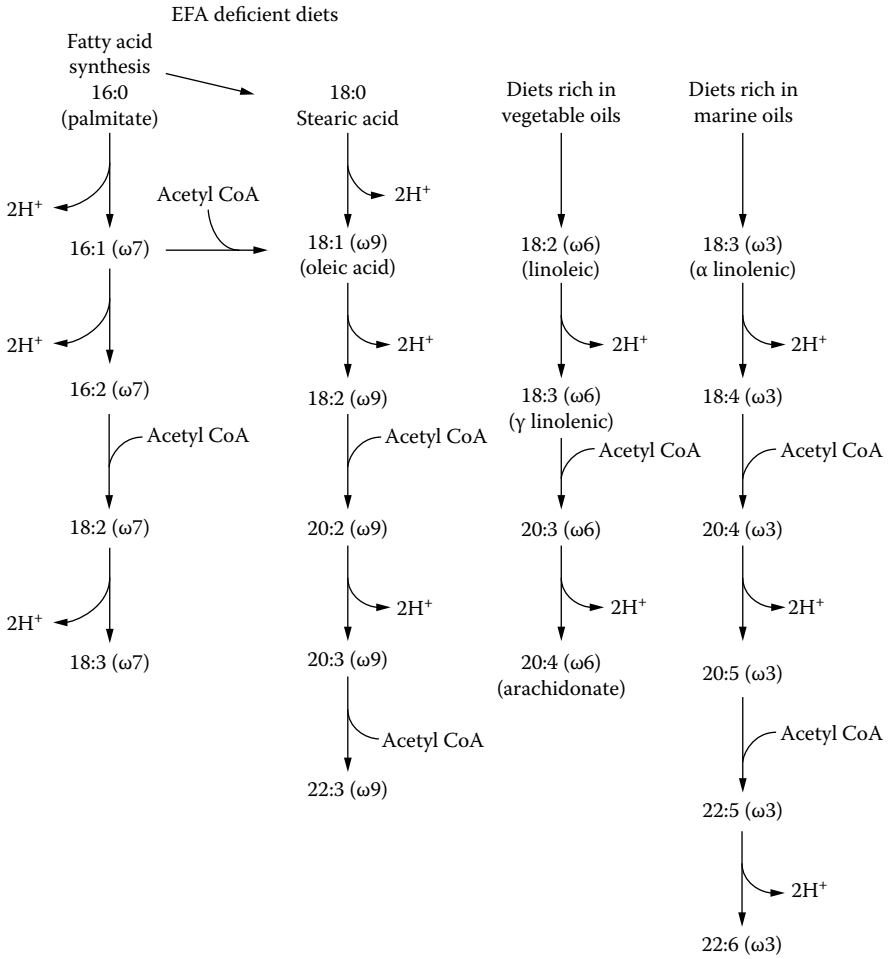


FIGURE 3.19 Pathways for synthesis of long-chain polyunsaturated fatty acids (PUFA) through elongation and desaturation. Not all of these reactions occur in all species. Cold-water ocean plants and simple cell organisms for example, can synthesize (through desaturation and elongation) long-chain polyunsaturated fatty acids. These become part of the food chain and end up in the lipid components of marine fish and mammals consumed by humans. The ω symbol is the same as the n symbol. Thus, 18:2ω6, linoleic acid, could also be written 18:2n6.

FATTY ACID DESATURATION

Desaturation occurs in the endoplasmic reticulum and microsomes. The enzymes that catalyze this desaturation are the Δ4, or Δ5, or Δ6 desaturases. Again, desaturation is species specific. Mammals, for example, lack the ability to desaturate fatty acids in the n6 or n3 position. Only plants can do this, and even among plants there are species differences. Cold-water plants can desaturate at the n3 position, where land plants of warmer regions cannot. The cold-water plants are consumed by cold-water creatures in a food chain that includes fish as well as sea mammals. These, in turn, enter the human food supply and become sources of the n3 or omega 3 fatty acids in the marine oils. In animals, desaturation of de novo synthesized fatty acids usually stops with the production of a monounsaturated fatty acid with the double bond in the 9–10 position counting from the carboxyl

end of the molecule. Hence, palmitic acid (16:0) becomes palmitoleic acid (16:1), and stearic acid (18:0) becomes oleic acid (18:1).

In the absence of dietary linoleic and linolenic acids, the essential fatty acids (EFA), most mammals will desaturate eicosenoic acid to produce eicosatrienoic acid. Increases in this fatty acid with unsaturations at ω7 and ω9 positions characterize the tissue lipids of EFA-deficient animals. They are sometimes called *mixed-function oxidases*, because two substrates (fatty acid and NADPH) are oxidized simultaneously. These desaturases prefer substrates with a double bond in the ω6 position but will also act on ω3 fatty acid bonds and on saturated fatty acids. Desaturation of de novo synthesized stearic acid to form oleic acid results in the formation of a double bond at the ω9 position. This is the first committed step of this desaturation–elongation reaction sequence. Oleic acid can also be formed by the desaturation and elongation of palmitic acid.

Fatty acid desaturation can be followed by elongation and repeated such that a variety of mono- and polyunsaturated fatty acids can be formed. These fatty acids contribute fluidity to membranes because of their lower melting point. An increase in the activity of the desaturation pathway is a characteristic response of rats fed a diet high in saturated fatty acids. The body can convert the dietary saturated fatty acids to unsaturated fatty acids thus maintaining an optimal P:S ratio in the tissues. Dietary fat composition can regulate desaturase activity, and this regulation correlates with the level of plasma triacylglycerides. These dietary effects are independent of weight status. That is, dietary fat effects on desaturase activity are independent of the effects of weight loss on this enzyme.[66]

There are two unsaturated fatty acids the body cannot make. These are the so-called essential fatty acids, linoleic (18:2, ω6) and linolenic (18:2ω3) acids. In addition, felines cannot make arachidonic acid. The consequences of inadequate essential fatty intake are both subtle and various (see Chapter 8). In the absence of linoleic acid in the diet, microsomal desaturation and elongation activity increases and Δ5,8,11-eicosatrienoic acid (20:3ω9) accumulates. These fatty acids are synthesized in the plant kingdom and, when consumed by animals, find their way into the food chain. Linoleic acid is used to make arachidonic acid (20:4ω6), whereas linolenic acid is used to make eicosapentanoic acid (20:4ω3) through elongation and desaturation. Figure 3.19 shows the overall pathway for the synthesis of these unsaturated fatty acids including the conversion of linoleic to arachidonic acid.

The activity of the desaturases can be increased through feeding saturated fat or high-sugar diets. Both dietary maneuvers increase the need to synthesize unsaturated fatty acids. Desaturase activity is stimulated by insulin, triiodothyronine, and glucocorticoid. Desaturase activity is decreased when high polyunsaturated fats are fed.

FATTY ACID ESTERIFICATION

Most fats in food, as well as those stored in the adipose tissue depots and those present in small amounts in other tissues, exist as triacylglycerides. Triacylglycerides are hydrolyzed to their component fatty acids and glycerol and reesterified at each of several points in their metabolism. The process of fat absorption by the small intestine involves hydrolysis of the dietary glycerides and reesterification prior to entry into the bloodstream as either chylomicrons or VLDL. This hydrolysis and reesterification also occurs when lipids arrive at the hepatocyte, the myocyte, or the adipocyte. The triacylglyceride is hydrolyzed by interstitial lipoprotein lipase and the fatty acids are transported into the target cell and reesterified to glycerol-3-phosphate. In the fat cell, this glycerol-3-phosphate usually is a product of glycolysis rather than the glycerol liberated when the triacylglycerol is hydrolyzed. The liberated glycerol usually passes back to the liver, which has a very active glycerokinase to phosphorylate it. In the liver, the phosphorylated glycerol is either used as a substrate for glucose synthesis or recycled into hepatic phospholipids or triacylglycerides or oxidized to CO_2 and water.

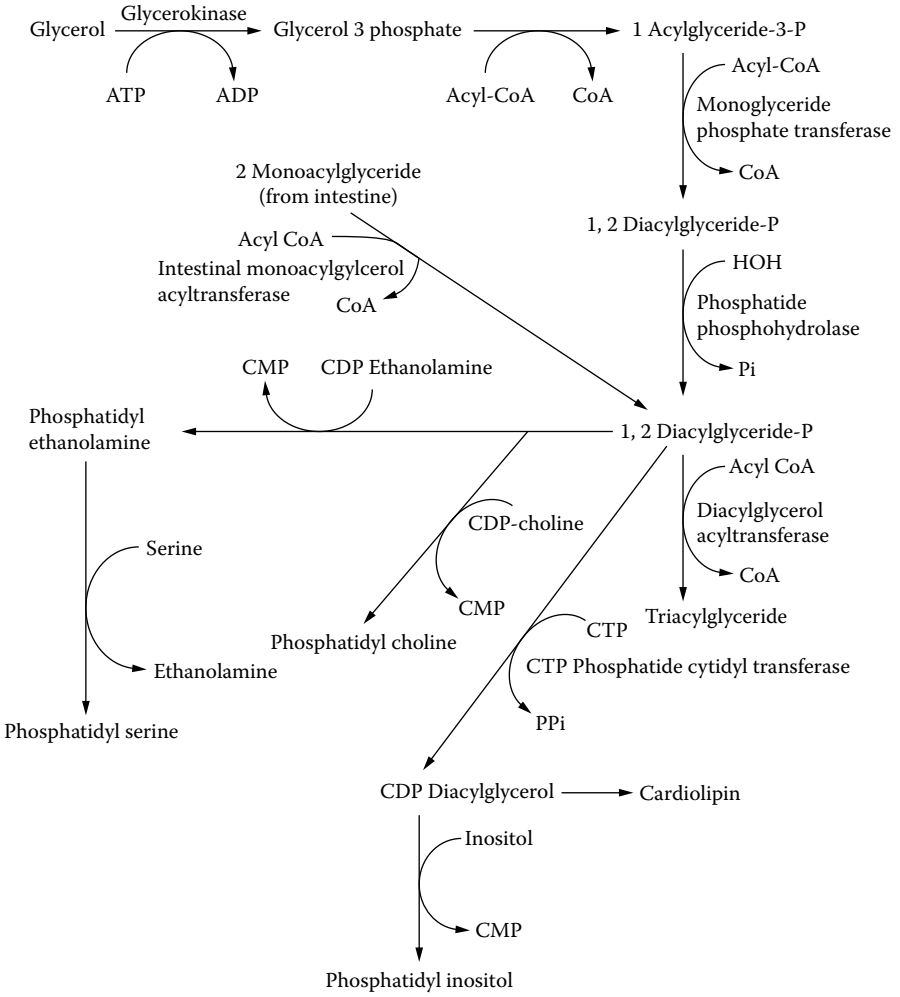


FIGURE 3.20 Pathways for the synthesis of triacylglycerides and phospholipids.

The formation of triacylglycerides, regardless of the source for the glycerol-3-phosphate, follows the same pattern in all tissues. With some modification, it is the pathway used for the synthesis of phospholipids. These pathways are shown in Figure 3.20.

Triacylglycerides are formed in a stepwise fashion. First, a fatty acid (usually a saturated fatty acid) is attached at carbon 1 of the glycerophosphate. The phosphate group at carbon 3 is electronegative and, because it pulls electrons toward it, leaves carbon 1 more reactive than carbon 2. The fatty acid (as an acyl-CoA) is transferred to carbon 1 through the action of a transferase. The attachment uses the carboxy end of the fatty acid chain and makes an ester linkage releasing the CoA. Now the molecule has electronegative forces at each end: the phosphate group on carbon 3 and the oxygen plus carbon chain at carbon 1. Now carbon 2 is vulnerable and reactive and another carbon chain can be attached. In this instance, the fatty acid is usually an unsaturated fatty acid. At this point, the 1,2-diacylglyceride-phosphate loses its phosphate group so that carbon 3 is now reactive. The 1,2-diacylglyceride can either be esterified with another fatty acid to make triacylglyceride or can be used to make the membrane lipids phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, cardiolipin, and phosphatidylserine.

FATTY ACID OXIDATION

The hydrolysis of stored lipid is catalyzed in a three-step process by one of the lipases specific to mono, di, or triacylglycerol. Intracellular hormone-sensitive lipase hydrolyzes fatty acids one at a time from all three carbon positions of the glycerol moiety and these fatty acids are then available for oxidation. Interstitial hormone-insensitive lipase has a similar mode of action. The lipases that act on the phospholipids to release arachidonic acid for eicosanoid synthesis or to release inositol-1,3,4-phosphate and diacylglyceride or other components of the phospholipids also provide fatty acids for oxidation—but that is not their primary role.

The lipases in the adipose tissue are the key to the regulated release of fatty acids from the stored triacylglycerides. These fatty acids can be oxidized in situ by the adipocyte but usually they are released for transport to other tissues as energy sources. The fatty acids are carried by albumin or by lipoproteins to where they are needed. At the target cell, the fatty acids are liberated from the triacylglycerides carried by the lipoproteins through the action of lipoprotein lipase or liberated from albumin by an (as-yet) undefined mechanism. The liberated fatty acids diffuse through the plasma membrane, bind to the cytosolic fatty acid-binding protein and migrate through the cytoplasm to the outer mitochondrial membrane or to the peroxisomes, microsomes, or the endoplasmic reticulum. At each of these destinations, they are activated by conversion to their CoA thioesters. This activation requires ATP and the enzyme acyl-CoA synthase or thiokinase. There are several thiokinases that differ with respect to their specificity for the different fatty acids. The activation step is dependent on the release of energy from two high-energy phosphate bonds. ATP is hydrolyzed to AMP and 2 molecules of inorganic phosphate. Figure 3.21 shows the initial steps in the oxidation of fatty acids.

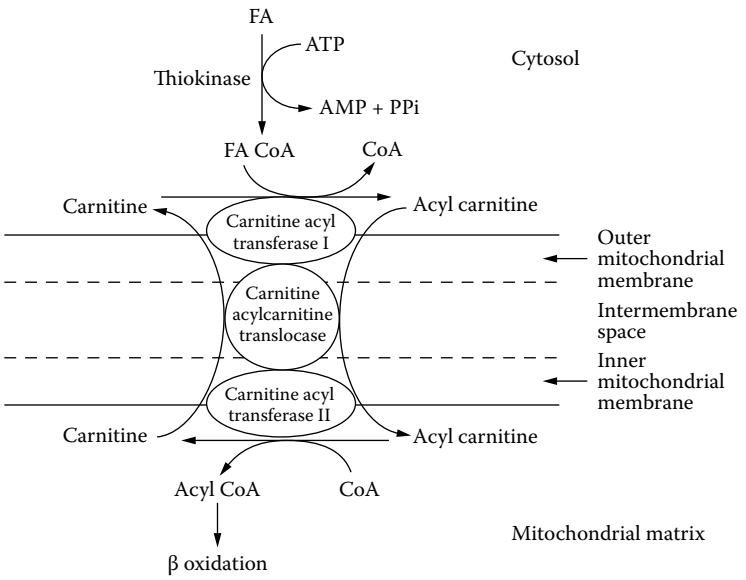


FIGURE 3.21 Mechanism for the entry of fatty acids into the mitochondrial compartment fatty acid is activated and is bound to carnitine with the release of CoA. The acyl carnitine is then translocated through the mitochondrial membranes into the mitochondrial matrix via the carnitine acylcarnitine translocase. As 1 molecule of acylcarnitine is passed into the matrix, 1 molecule of carnitine is translocated back to the cytosol and the acylcarnitine is converted back to acyl-CoA. The acyl-CoA can then enter the β-oxidation pathway shown in Figure 3.22.

An important member of the system for the transfer of fatty acids into the mitochondrial or peroxisomal or endoplasmic reticulum for oxidation is the long-chain carnitine acyltransferase.[67,68] This is a family of enzymes that catalyze the exchange of carnitine for coenzyme A in the fatty acyl-CoA. Conversion of the fatty acyl-CoA to fatty acylcarnitine renders the fatty acid more permeable to the various membranes. The activity of these enzymes are regulated. Malonyl CoA inhibits their activity. However, regulation occurs at the level of mRNA transcription by a variety of transcription factors.

Without carnitine, the oxidation of fatty acids, especially the long-chain fatty acids, cannot proceed. Acyl-CoA cannot traverse the membrane into the mitochondria and thus requires a translocase for its entry. The translocase requires carnitine. Carnitine is synthesized from methionine and lysine as shown in Figure 3.22. Although most of the fatty acids that enter the β oxidation pathway (Figure 3.23) are completely oxidized via the Krebs cycle and respiratory chain to CO_2 and HOH, some of the acetyl CoA is instead converted to the ketones, acetoacetate, and β hydroxybutyrate. The condensation of 2 molecules of acetyl CoA to acetoacetyl CoA occurs in the mitochondria via the enzyme β-ketothiolase. Acetoacetyl CoA then condenses with another acetyl CoA to form HMG CoA. At last, the HMG CoA is cleaved into acetoacetic acid and acetyl CoA. The acetoacetic acid is reduced to β hydroxybutyrate, and this reduction is dependent on the ratio of NAD^+ to $NADH^{++}$. The enzyme for this reduction, β hydroxybutyrate dehydrogenase is tightly bound to the

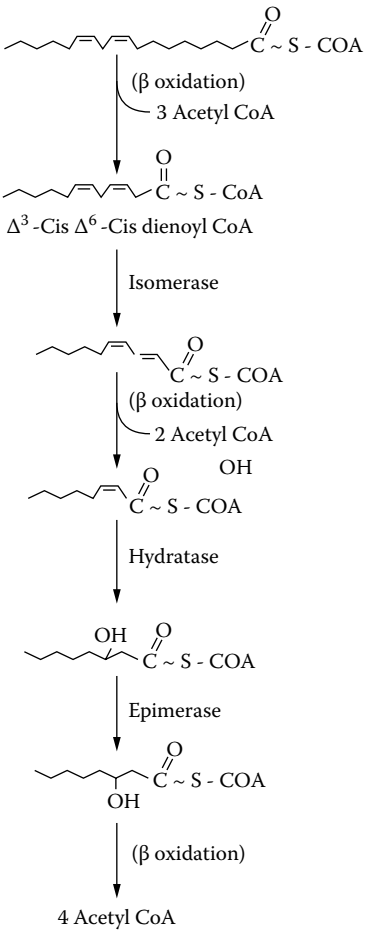


FIGURE 3.22 Pathway for β oxidation of fatty acids in the mitochondria.

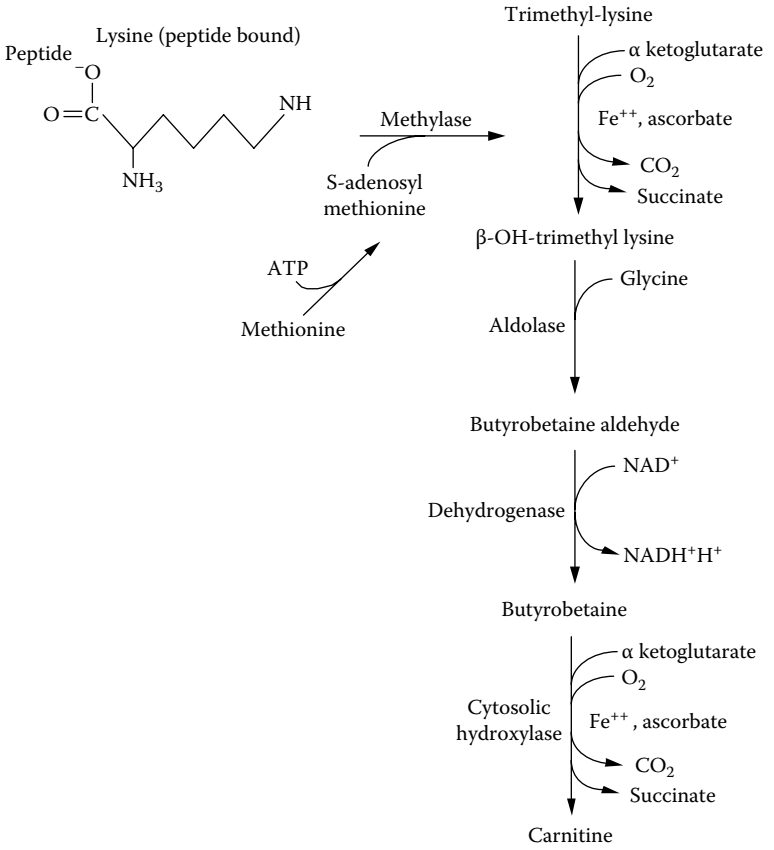


FIGURE 3.23 Synthesis of carnitine from lysine and methionine.

inner aspect of the mitochondrial membrane. Because of its high activity, the product (β hydroxybutyrate) and substrate (acetoacetate) are in equilibrium. Measurements of these two compounds can thus be used to determine the redox state (ratio of oxidized to reduced NAD) of the mitochondrial compartment.

HMG CoA is also synthesized in the cytosol, however, because this compartment lacks the HMG CoA lyase, the ketones are formed only in the mitochondria. In the cytosol, HMG CoA is the beginning substrate for cholesterol synthesis. The ketones can ultimately be used as fuel but may appear in the blood, liver, and other tissues at a level of less than 0.2 mM. In starving individuals, or in people consuming a high-fat diet, blood and tissue ketone levels may rise above normal (3–5 mM). However, unless these levels greatly exceed the body's capacity to use them as fuel (as is the case in uncontrolled diabetes mellitus with levels up to 20 mM), a rise in ketone levels is not a cause for concern. Ketones are choice metabolic fuels for muscle and brain. Although both tissues may prefer to use glucose, the ketones can be used when glucose is in short supply. Ketones are used to spare glucose wherever possible under these conditions.

The oxidation of unsaturated fatty acids follows the same pathway as the saturated fatty acids until the double-bonded carbons are reached. At this point, a few side steps must be taken that involve a few additional enzymes. An example of this pathway is shown in Figure 3.24 using linoleate as the fatty acid being oxidized. Linoleate has two double bonds in the *cis* configuration; β oxidation removes three acetyl units, leaving a CoA attached to the terminal carbon just before the first *cis* double bond. At this point an isomerase enzyme, D3 *cis* D6 trans enoyl CoA isomerase, acts

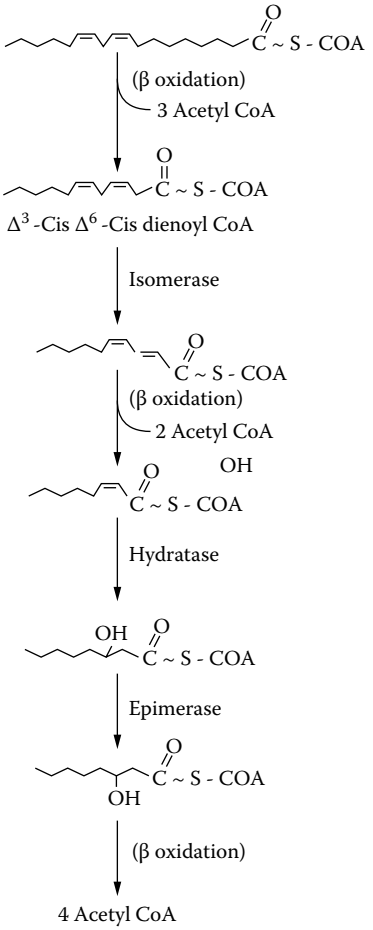


FIGURE 3.24 Modification of β oxidation for unsaturated fatty acid.

to convert the first *cis* bond to a trans bond. Now this part of the molecule can once again enter the β-oxidation sequence, and two more acetyl CoA units are released. The second double bond is then opened and a hydroxyl group is inserted. In turn, this hydroxyl group is rotated to the L position, and the remaining product can then reenter the β-oxidation pathway. Other unsaturated fatty acids can be similarly oxidized. Each time the double bond is approached the isomerization and hydroxyl group addition takes place until all of the fatty acid is oxidized.

Although β oxidation is the main pathway for the oxidation of fatty acids, some fatty acids undergo α oxidation so as to provide the substrates for the synthesis of sphingolipids. These reactions occur in the endoplasmic reticulum and mitochondria and involve the mixed function oxidases because they require molecular oxygen, reduced NAD, and specific cytochromes. The fatty acid oxidation that occurs in organelles other than the mitochondria (i.e., peroxisomes) are energy-wasteful reactions because these other organelles do not have the Krebs cycle, nor do they have the respiratory chain that takes the reducing equivalents released by the oxidative steps and combines them with oxygen. This latter action makes water-releasing energy that is then trapped in the high-energy bonds of the ATP. Peroxisomal oxidation in the kidney and liver is an important aspect of drug metabolism.[68] The peroxisomes are a class of subcellular organelles that are important in the

protection against oxygen toxicity. They have a high level of catalase activity, which suggests their importance in the antioxidant system.

The peroxisomal fatty acid oxidation pathway differs in three important ways from the mitochondrial pathway. First, the initial dehydrogenation is accomplished by a cyanide-insensitive oxidase that produces H_2O_2. This H_2O_2 is rapidly extinguished by catalase. Second, the enzymes of the pathway prefer long-chain fatty acids and are slightly different in structure from those (with the same function) of the mitochondrial pathway. Third, β oxidation in the peroxisomes stops at eight carbons rather than proceeding all the way to acetyl CoA. It may be that peroxisomal oxidation helps the body get rid of fatty acids that are in excess of 20 carbons in length. The peroxisomes also serve in the conversion of cholesterol to bile acids and in the formation of ether lipids (plasmalogens). Of interest are the reports of a rare fatal genetic disorder that is characterized by the absence of the peroxisomes. This is called the *Zellweger syndrome*. Victims of this disorder do not make bile acids or plasmalogens, nor are they able to shorten the very long-chain fatty acids. These biochemical deficiencies are seemingly unrelated to the structural abnormalities observed in liver, kidney, muscle, and brain. Efforts to circumvent the genetic mutation have been unsuccessful. It is possible to detect its presence by the absence of peroxisomal enzymes in amniotic fluid.

CHOLESTEROL SYNTHESIS

As mentioned, the synthesis of cholesterol begins with acetyl CoA. This synthesis occurs in all cells to varying degrees. The liver, adrenals, gonads and intestine have the greatest cholesterol-synthesizing capacity, for obvious reasons. The integration of the cholesterol synthetic pathway and overall fatty acid metabolism is shown in Figure 3.25, whereas the pathway for cholesterol synthesis is shown in Figure 3.26.

When the diet contains a lot of cholesterol, de novo synthesis is almost totally suppressed. Thus, there is an inverse relationship between intake and synthesis. The cholesterol present in the diet is carried by the chylomicrons, not by the VLDL. The high-density lipoproteins (HDL) and the enzyme lecithin: cholesterol acyl transferase (LCAT) are important in the regulation of cholesterol

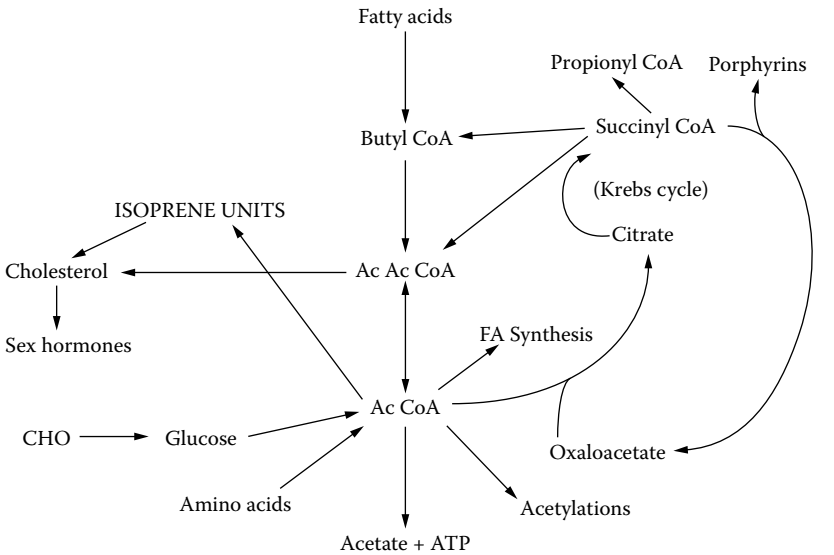


FIGURE 3. 25 Integration of cholesterol and fatty acid metabolism.

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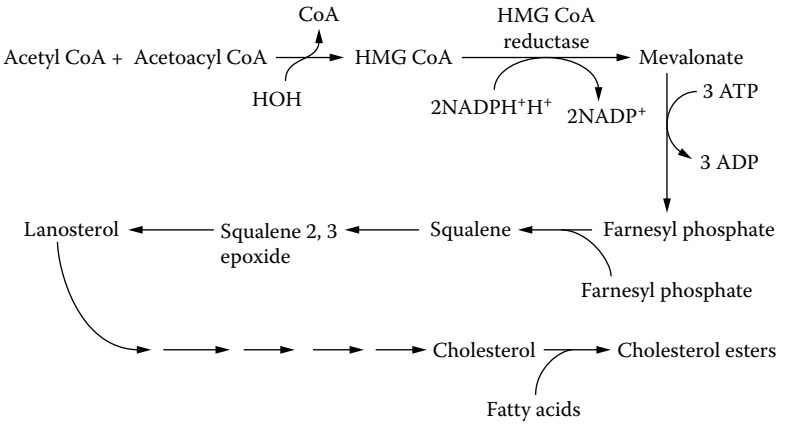


FIGURE 3.26 Cholesterol biosynthesis.

turnover. LCAT catalyzes the transfer of fatty acid in the carbon 2 position of phosphatidylcholine to the 3-hydroxyl group of cholesterol. LCAT is a plasma enzyme and its substrate is the cholesterol carried by HDL. The cholesterol ester generated by the LCAT reaction is carried in the core of the HDL particle, where it is transported to the liver and prepared for excretion as free cholesterol or bile acid in the bile. HDL is thus the vehicle for transporting cholesterol generated in the periphery to the liver for excretion.

Synthesis is controlled primarily at the level of the HMG CoA reductase step.[69] There is feedback inhibition of the enzyme by mevalonate and cholesterol. Mevalonate inactivates the existing enzyme as well as inhibits the activation of the preexisting enzyme. Cholesterol-lowering drugs (the statins) target this step and are quite effective in lowering serum cholesterol levels in hypercholesterolemic patients. Cholesterol originating either from the diet or from de novo synthesis serves as the substrate for the synthesis of a variety of biologically important compounds as shown in Figure 3.27

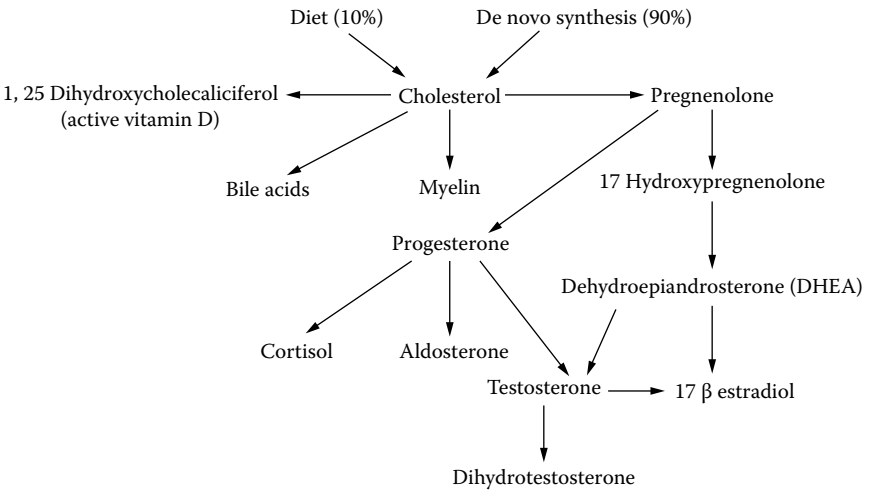


FIGURE 3.27 Cholesterol is the starting substrate for the synthesis of a variety of important compounds: Vitamin D, Bile acids, the sex hormones and the adrenal corticoids.

Cholesterol is not very soluble in water but is carried in the blood by the LDL and VLDL. About 70% of the cholesterol carried by these proteins is esterified to a fatty acid, and the remaining 30% is unesterified. Cholesterol, either from dietary sources or synthesized de novo, is the precursor for the synthesis of bile acids, adrenal steroids, active vitamin D, and sex hormones. Cholesterol is abundant in bile, where the normal concentration is about 380–400 mg/dL. In bile, 96% of the cholesterol exists in the unesterified form. The bile is the chief route for cholesterol excretion. Any cholesterol that is not reabsorbed by the jejunum and ileum is excreted in the feces. Humans consuming a low-cholesterol (~200 mg/dL) diet have approximately 1300 mg/dL returned to the liver. Some of this is the reabsorbed cholesterol via the enterohepatic circulation. Some is carried by the HDL from the periphery as lipids are mobilized and used. The bile contains no lipid or cholesterol-carrying protein, but does contain some phospholipid, whose detergent properties keep the cholesterol in solution. If there is a reduction in the amount of phospholipid in the bile, the cholesterol will fall out of solution and form cholesterol aggregates or gall stones. Bile acids also assist in keeping the cholesterol in solution and again, if there is a reduction in bile acid production, cholesterol will precipitate out and collect in the gall bladder.

The total cholesterol pool in the body is carefully regulated. It comes from two sources: the diet and de novo synthesis. It is excreted as free cholesterol and as bile acids in the bile. When the amount of cholesterol in the diet is reduced, there is a compensatory increase in de novo synthesis by both the liver and the intestine. Once synthesized, it is carried in the blood by lipoprotein B. The liver and intestine are the only tissues that can synthesize apolipoprotein B, hence the cholesterol synthesized in these tissues will be transported primarily by this protein and will be found in the LDL or VLDL lipoprotein class. The LDL is formed when the apolipoprotein C components and triacylglycerides are removed from the VLDL.

AMINO ACID CATABOLISM

Those amino acids in the body's amino acid pool that are neither used for peptide or protein synthesis nor to synthesize metabolically important intermediates are deaminated, and the carbon skeletons are either oxidized or used for the synthesis of glucose or fatty acids.[70] Very little fatty acid synthesis occurs in humans consuming 20–30% of their energy as fat. There are three general reactions for the removal of NH_3 from the amino acids.

1. They can be transaminated with the amino group transferred to another carbon chain via amino transaminases.
2. They can be oxidatively deaminated to yield NH_3.
3. They can be deaminated through the activity of an amino acid oxidase.

The amino acids can be loosely grouped in terms of their catabolism. Valine, leucine, and isoleucine, the branched-chain amino acids (BCAA), are similar in that each has a methyl group on its carbon chain. They can be transaminated with a ketoglutarate to form a keto acids. These acids are considered homologs of pyruvate and are oxidized by a series of enzymes that are similar to those that catalyze the oxidation of a ketoglutarate and pyruvate. The degradative steps are shown in Figure 3.28. Valine ultimately is converted to succinyl CoA, whereas isoleucine ends up as either acetyl CoA or propionyl CoA and leucine catabolism results in HMG CoA (ß-hydroxy ß-methylglutaryl coenzyme CoA). HMG CoA is split to acetyl CoA and acetoacetate. The HMG CoA produced in the catabolism of leucine is not used for cholesterol synthesis because it is produced in the mitochondria and does not travel to the cytosol, where cholesterol is synthesized. Instead, HMG CoA is further metabolized to acetoacetate and acetyl CoA.

Serine, threonine, and glycine are hydroxyamino acids. All three are gluconeogenic precursors. Serine can be deaminated to pyruvate, which then can be transaminated to alanine. Serine can also be demethylated to form glycine, releasing a methyl group useful in one carbon metabolism.

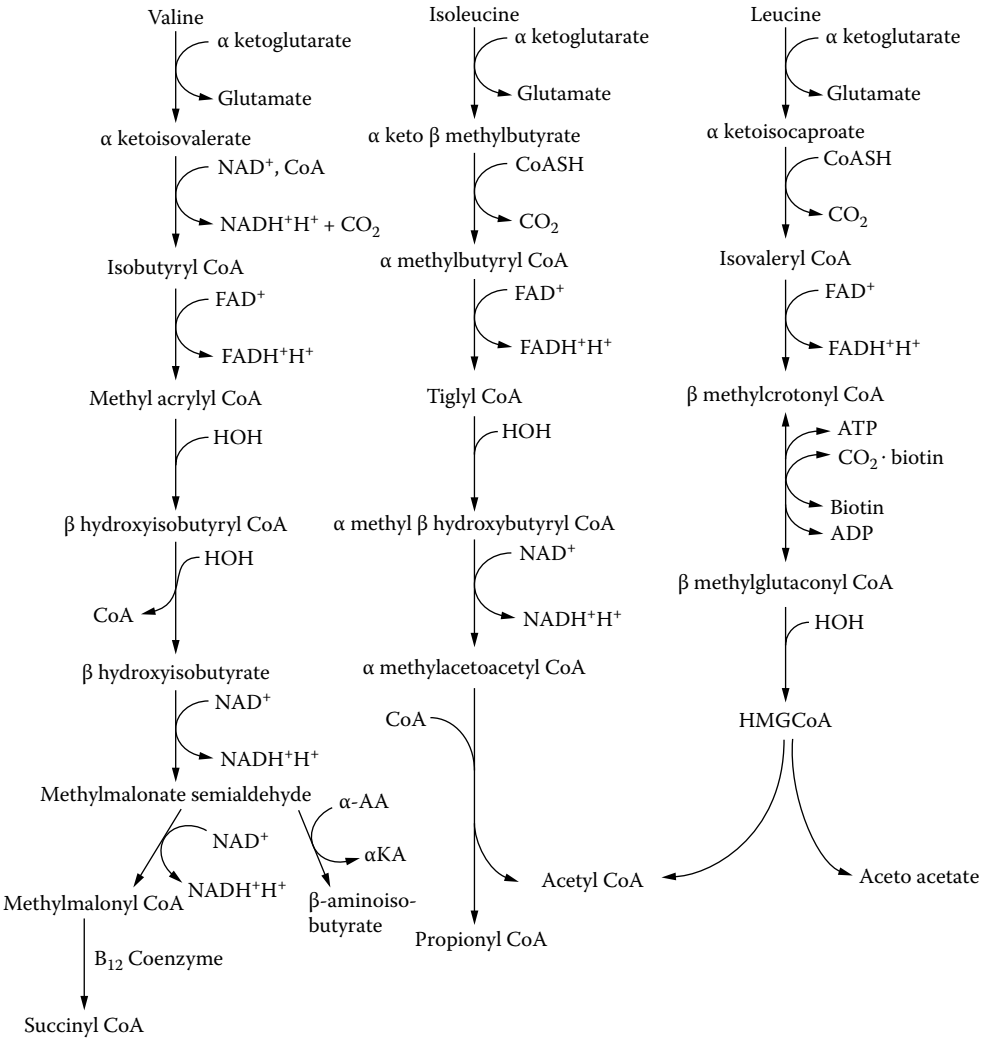


FIGURE 3.28 Catabolism of branched-chain amino acids showing their use in the production of metabolites that are either lipid precursors or metabolites that can be oxidized via the Krebs cycle.

Threonine is degraded to acetyl CoA after deamination, as shown in Figure 3.29. Glutamate may be converted to glutamine via the enzyme glutamine synthetase. This enzyme is a mitochondrial enzyme and serves to fix the ammonia released in this compartment. Rat renal tissue is particularly rich in this enzyme, but human renal tissue is not. In birds and alligators, this enzyme is a cytosolic enzyme rather than a mitochondrial enzyme. Although the brain has some urea cycle activity, it uses glutamine formation primarily to reduce its ammonia level. To do this, it must also synthesize glutamate from α ketoglutarate. If it did not also convert pyruvate to oxalacetate, this synthesis of a ketoglutarate would deplete the brain of citric acid cycle intermediates. Fortunately, carboxylation of pyruvate is very active in brain tissue.

Glutamate is converted to and formed from ornithine and arginine. These two amino acids are essential components of the urea cycle (Figure 3.30). The urea cycle consists of the synthesis of carbamyl phosphate, the synthesis of citrulline, then arginosuccinate, arginine, and, finally, urea. Ornithine and citrulline are shuttled back and forth as the cycle turns to get rid of the excess

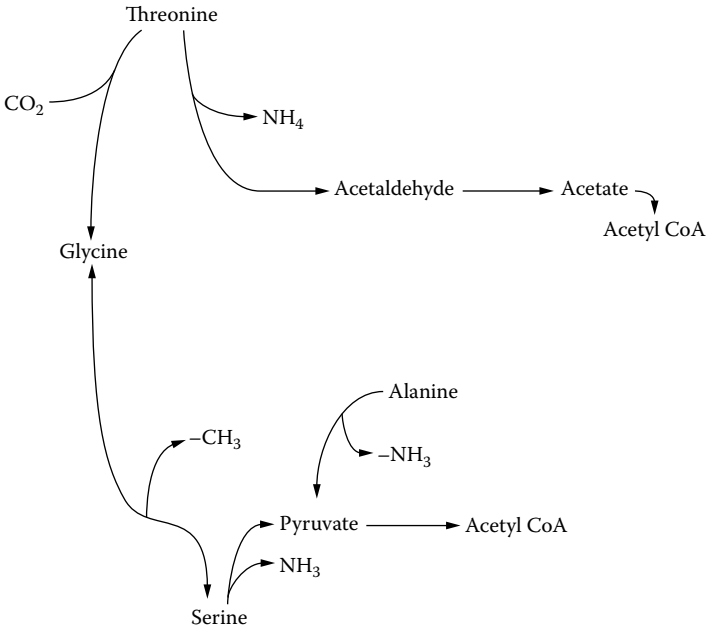


FIGURE 3.29 Catabolism of threonine showing its relationship to that of serine and glycine.

ammonia via urea release from arginine. The cycle functions to reduce the potentially toxic amounts of ammonia that arise when the ammonia group is removed from amino acids. Most of the ammonia released reflects the coupled action of the transaminases and L-glutamate dehydrogenase. The glutamate dehydrogenase is a bidirectional enzyme that plays a pivotal role in nitrogen metabolism. It is present in kidney, liver, and brain. It uses either NAD^+ or $NADP^+$ as a reducing equivalent receiver. It operates close to equilibrium using ATP, GTP, NADH, and ADP depending on the direction of the reaction. In catabolism, it channels NH_3 from glutamate to urea. In anabolism, it channels ammonia to α ketoglutarate to form glutamate. In the brain, glutamate can be decarboxylated to form γ-aminobutyrate (GABA), an important neurotransmitter. The decarboxylation is catalyzed by the enzyme, L-glutamate decarboxylase. Putrescine also can serve as a precursor of GABA either by deamination or via *N*-acetylated intermediates.

The urea cycle is, energetically speaking, a very expensive process. The synthesis of urea requires 3 mol of ATP for every mole of urea formed. The urea cycle is very elastic. That is, its enzymes are highly conserved, readily activated, and readily deactivated. Adaptation to a new level of activity is quickly achieved. Although urea-cycle activity can be high when protein-rich diets are consumed and low when low-protein diets are consumed, the cycle never shuts down completely. The cycle, shown in Figure 3.30, is fine-tuned by the first reaction, the synthesis of carbamoyl phosphate. This reaction, which occurs in the mitochondria, is catalyzed by the enzyme carbamoyl phosphate synthetase. The enzyme is inactive in the absence of its allosteric activator, *N*-acetylglutamate, a compound synthesized from acetyl CoA and glutamate in the liver. As arginine levels increase in the liver, N-acetylglutamate synthetase is activated. This results in an increase in *N*-acetylglutamate. The urea cycle is initiated in the hepatic mitochondria and finished in the cytosol. The urea is then liberated from arginine via arginase and released into the circulation, whereupon it is excreted from the kidneys in the urine. Ornithine, the other product of the arginase reaction, is recycled back to the mitochondria only to be joined once again to carbamyl phosphate to make citrulline. Rising levels of arginine turn on mitochondrial *N*-acetylglutamate synthetase, which

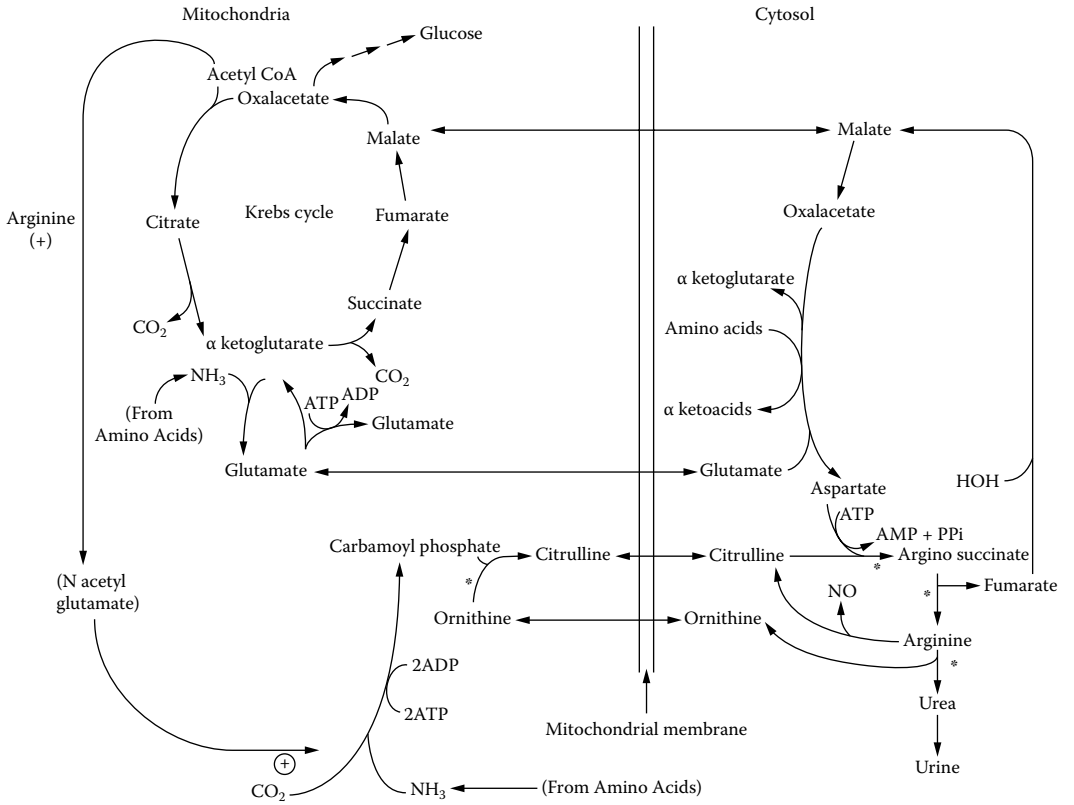


FIGURE 3.30 The urea cycle. Locations of mutations in the urea cycle enzymes are indicated with a star. Persons with these mutations have very short lives, with evidence of mental retardation, seizures, coma, and early death due to the toxic effects of ammonia accumulation. Rate controlling steps are indicated with a circled cross.

provides the *N*-acetylglutamate which, in turn, activates carbamoyl phosphate synthetase and the cycle goes on.

Arginine has many uses in metabolism. Not only is it an essential component of the urea cycle, it is a precursor for nitric oxide (NO), the polyamines, and for proline, glutamate, and creatine. When citrulline is produced from arginine, NO is produced. This occurs not only in the liver but also in other vital organs and the vascular tree. Nitric oxide production by endothelial cells is a vasodilator and thus plays a role in the regulation of smooth muscle tone. Many vasodilators such as the widely prescribed drug for angina pain, nitroglycerine, work by increasing the production of nitric oxide.

Phenylalanine is the precursor of tyrosine. Tyrosine is used for the synthesis of dopamine and the catecholamines (Figure 3.31). Phenylalanine is converted to tyrosine via the hepatic enzyme phenylalanine hydroxylase. A number of mutations in the gene for this enzyme have been found and result in the disease phenylketonuria. This, if not diagnosed within the first few weeks of life, results in mental retardation. Tyrosine, if not used to make one of several hormones, is then deaminated and, through a series of reactions, ends up as fumarylacetate, which is split to provide acetoacetate and fumarate. Tryptophan catabolism shows no similarity to the catabolic pathways of any of the other amino acids. The pathway for tryptophan is shown in Figure 3.32. Tryptophan can be converted to niacin, one of the B vitamins (see Chapter 10). This conversion is not very efficient.

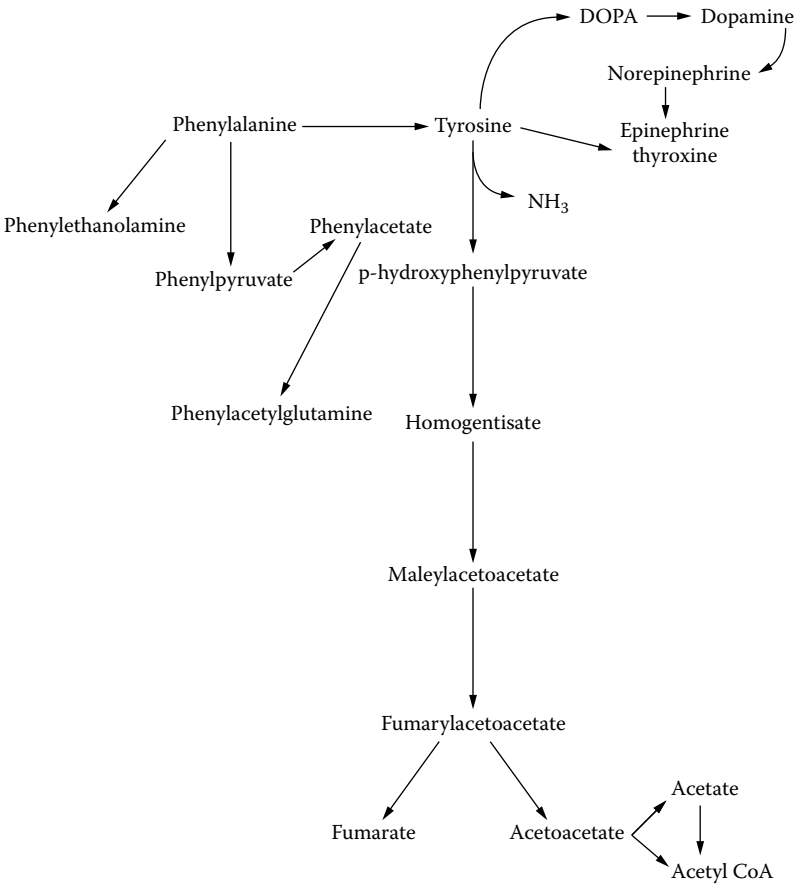


FIGURE 3.31 Phenylalanine and tyrosine catabolism.

There is considerable product feedback inhibition of this reaction sequence by niacin. The metabolism of tryptophan is dependent on adequate intakes of vitamin B_6, pyridoxine. In pyridoxine deficiency, hydroxyanthranilate formation via the enzyme kynureninase is impaired, resulting in a characteristic increase in urinary levels of xanthurenic acid. This is used as a test of vitamin B_6 sufficiency. If subjects are deficient and are given a load dose of tryptophan, these subjects will excrete abnormal amounts of xanthurenate in their urine.

Tryptophan is the precursor of serotonin, an important neurotransmitter that serves a variety of functions in the regulation of smooth muscle tone, especially those smooth muscles of the vascular tree. In so doing, it plays an especially important role in the maintenance of blood pressure and the control of blood supply to the brain and vital organs. Several important drugs that address the problem of clinical depression alter the synthesis of serotonin from tryptophan or alter the action of serotonin once formed. Some of these drugs alter food intake as well. The metabolism of tryptophan is shown in Figure 3.32.

Histidine, an amino acid especially important for muscle protein biosynthesis, is also of great importance in 1-carbon metabolism. The principal pathway of histidine catabolism leads to glutamate formation and is shown in Figure 3.33 (as well as in other figures where glutamate and α ketoglutarate act in transamination). Glutamate is an important component of the urea cycle (Figure 3.30). The decarboxylation of histidine yields histamine. This amine serves to stimulate gastric hydrochloric acid production and to stimulate vasoconstriction. A number of cold remedies and

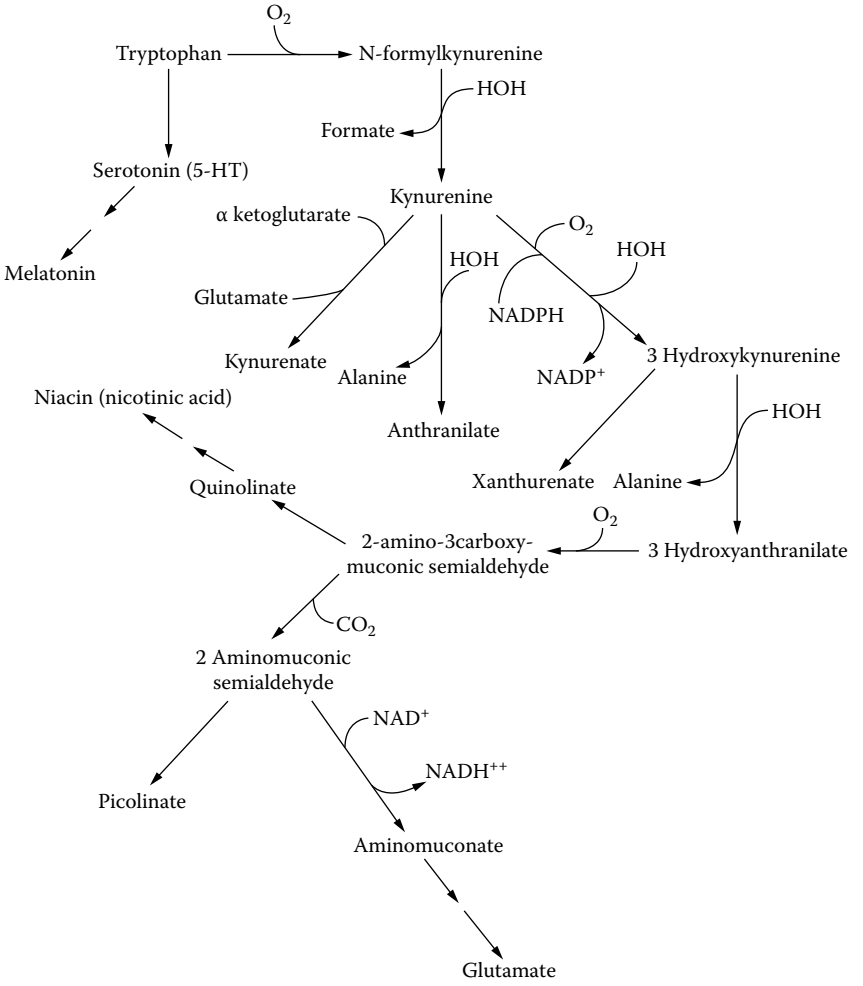


FIGURE 3.32 Catabolism of tryptophan showing conversion to the vitamin niacin. This conversion is not very efficient. Tryptophan catabolism also results in picolinate, which is believed by some to play a role in trace-mineral conservation.

sinus remedies contain substances known as antihistamines that interfere with the vasoconstrictor action of histamine. Histidine in muscle can be methylated and the end product, 3-methyl histidine, can be measured in the urine and used as a measure of muscle protein turnover.

Lysine is one of the two essential amino acids whose amino group does not contribute to the total body amino group pool; the other is threonine. Although lysine can donate its amino group to other carbon chains, the reverse does not occur. Lysine is catabolized to acetoacetyl CoA, which then enters the Krebs cycle as acetyl CoA.

Methionine, cysteine, and cystine are important sulfur group donors. The importance of disulfide bridges in the structure of proteins is described in the sections on protein structure and synthesis (Chapter 6). Methionine is important for carnitine synthesis. The pathway for methionine and cysteine is shown in Figure 3.34. Propionate is also the result of methionine catabolism. Should there be a defect in propionyl CoA carboxylase, propionate will accumulate. This is not the usual situation, for propionate is usually carboxylated to form succinyl CoA. Accumulation of methyl malonate is characteristic of vitamin B_{12} deficiency. Propionate can serve as the substrate for

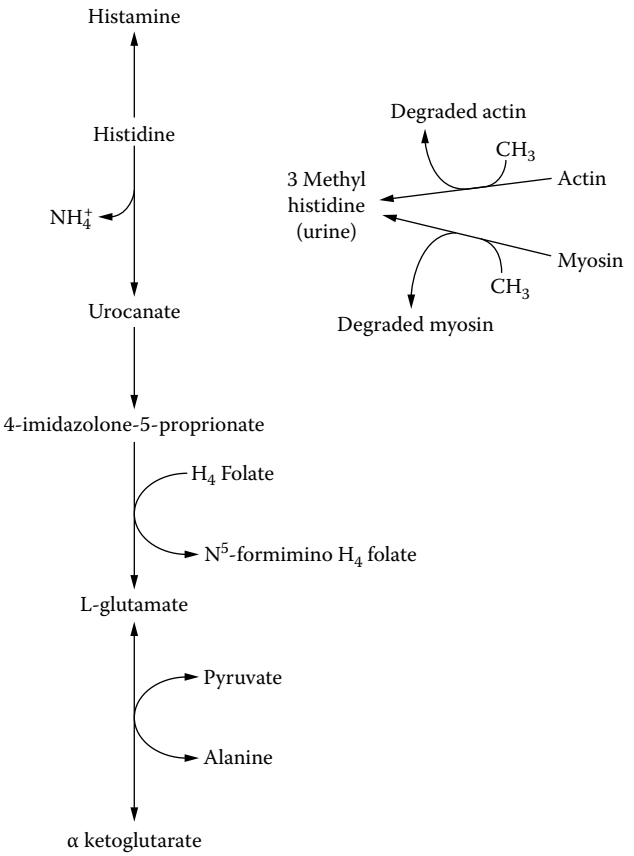


FIGURE 3.33 Catabolism of histidine. Note that 3-methyl histidine is not part of the pathway. This metabolite is formed in the muscle when the contractile proteins actin and myosin are methylated.

long-chain, odd-numbered fatty acids that are incorporated into myelin, the fatty covering of nerves. For some unknown reason, this myelin is abnormal in its function and fails to protect the peripheral nerve endings, which then die. This might explain the peripheral paresia that characterizes B_{12} deficiency. Just as mutations in the genes that encode proteins important to the regulation of energy balance can occur with sometimes-serious, life-threatening effects, so too are there mutations in the genes that encode components of the metabolism of amino acids.

AMINO ACID DERIVATIVES

Creatine Phosphate

The energy needed to drive the energy-dependent reactions of the body is provided by the high-energy bonds of the adenine nucleotides, the guanine nucleotides, and the uridylnucleotides. The concentrations of these nucleotides are carefully regulated and their energy is metered out as needed. Short, quick bursts of energy from these nucleotides are not possible. However, Mother Nature has devised another compound, creatine phosphate, that can do just this. Creatine is formed from glycine, arginine, and *S*-adenyl methionine. The creatine circulates in the blood and can be found in measurable quantities in brain and muscle. The creatine is phosphorylated to form creatine phosphate via the enzyme creatine kinase. Creatine can be converted to creatinine by irreversible

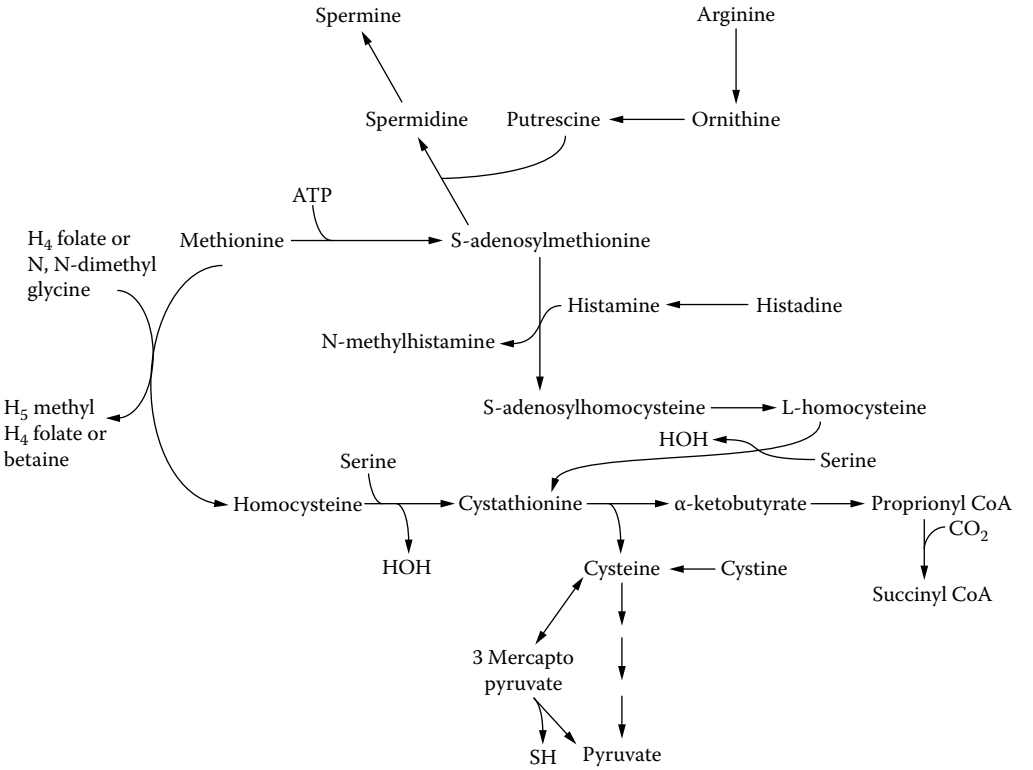


FIGURE 3.34 Conservation of SH groups via methionine–cysteine interconversion. Spermine, putrescine, and spermidine are polyamines that are important in cell and tissue growth.

nonenzymatic dehydration. Upon hydrolysis, creatine phosphate will provide sufficient energy for muscle contraction. This hydrolysis yields creatine and inorganic phosphate. Some of the creatine can be rephosphorylated, whereas the remainder is converted to creatinine that then is excreted in the urine. Creatine phosphate has the same free energy of hydrolysis as ATP. Creatine phosphate is found primarily in the muscle and provides the quick burst of energy needed each time a muscle contracts. Because the muscle activity produces the end product, creatinine, measuring creatinine allows for the estimation of the muscle mass.

Because creatinine excretion is reasonably constant from day to day, researchers use the amount of creatinine in a 24-hour urine collection as an assurance of the completeness of the urine sample. Variations can occur if the generally sedentary subject has an unusually active day. A dramatic increase in muscle use will result in an increase in creatine-phosphate breakdown and an increase in creatinine excretion.

Choline

Choline is a highly methylated compound synthesized from serine. It is an essential component of the neurotransmitter acetylcholine, as well as an essential ingredient of the phospholipid, phosphatidylcholine.

Polyamines

Certain amino acids can be decarboxylated to form the polyamines (see Figure 4.24). Some polyamines are very short-lived compounds that are neurotransmitters. They are quickly broken down so as to limit their effects. The catecholamines fall into this category of polyamines. Other polyamines, putrescine and spermine, bind nucleic acids and other polyanions. They have a role in cell division.

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4 Cell Cycle, Life Cycle

Living creatures consist of one or more cells. The more complex the organism, the more cells and cell types are found in that organism. Higher-order mammals contain a variety of cell types that are organized anatomically into specific tissues, organs, and systems, which have specific functions that are integrated into the whole functioning animal. Nutrition can affect these functions, so it is the purpose of this chapter to describe the functions of cells and their integration into the tissues, organs, and systems that function in the cycle of life.

CELL STRUCTURE AND FUNCTION

The typical eukaryotic cell (Figure 4.1) consists of a nucleus, mitochondria, lysosomes, endoplasmic reticulum, Golgi apparatus, and microsomes surrounded by the cell sap or cytosol. Membranes surround each of these organelles as well as the cell itself. Each segment or organelle has a particular function and is characterized by specific reactions and metabolic pathways. These are listed in Table 4.1. Both the macronutrients and the micronutrients affect these functions. For example, an inadequate intake of folacin (see Chapter 10) will affect the synthesis of the basis that are used for DNA and RNA synthesis. Cell division will be compromised when folacin intake is inadequate. Amino acid deficiency will compromise protein synthesis as will an inadequate intake of energy (see Chapters 1 and 6). As a result growth of the young will be compromised. An inadequate supply of active vitamin D will compromise bone mineralization and subsequent malformations in the skeletal system (see Chapter 9). The kind and amount of carbohydrate in the diet will affect the cytosolic metabolic pathways with subsequent effects on intermediary metabolism (see Chapters 3 and 7). The types and amounts of dietary fat will affect the composition and function of the membranes in and around the cell (see Chapter 8). Altogether, nutrient intake affects the function of the various cells in the body. Some of these effects are long term, whereas others are short term. Some are direct, whereas others are indirect via nutrient effects on cell signaling systems.

Receptors

Among the components of the cell are a group of proteins called *receptors*. There are both plasma and intracellular membrane receptors. Plasma membrane receptors typically bind peptides or proteins. These are called *ligands*. In many instances these compounds are hormones, or growth factors. Upon binding, one of two processes occurs. In one, the binding of the peptide or protein (ligand) to its cognate receptor is followed by an internalization of this ligand–receptor complex. In the other, the receptor is not internalized, but when the ligand is bound to its receptor, it generates a signal that alters cell metabolism and function. Internalization of the ligand allows for its entry into the cell. It can enter either as the intact ligand or as an activated fragment that may target specific intracellular components. If it enters as an intact ligand it can either transfer to another receptor or move to its target site in the cell by itself as it dissociates from its plasma membrane receptor. The receptor in turn is either recycled back to the plasma membrane or it is degraded. Figure 4.2 illustrates this plasma membrane–hormone binding.

There are actually four types of membrane-spanning receptors: (1) simple receptors that have a single membrane-spanning unit; (2) receptors with a single membrane-spanning unit that also involves a tyrosine kinase component on the interior aspect of the plasma membrane; (3) receptors

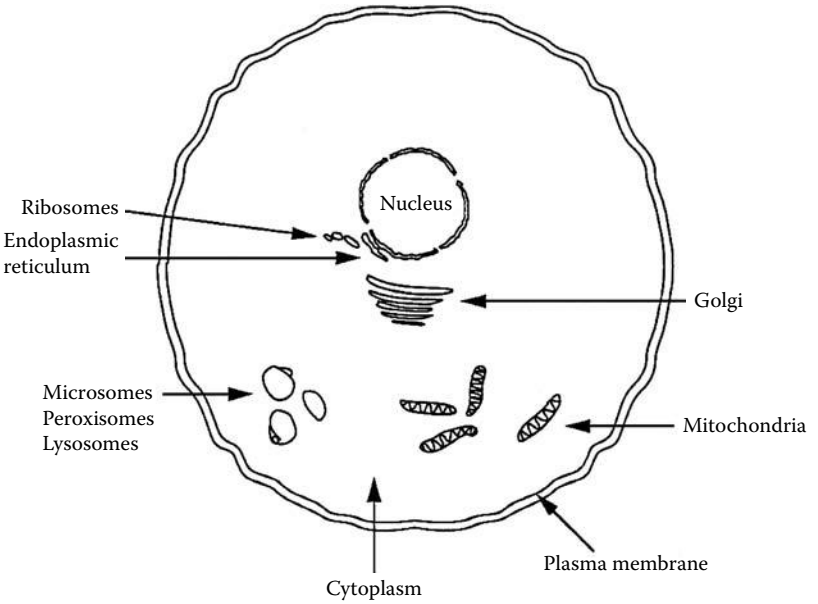


FIGURE 4.1 Typical eukaryotic cell showing representative intracellular structures.

TABLE 4.1
Functions of the organelles/cell fractions of a typical eukaryotic cell

Organelle cell fraction	Function
Plasma membrane	Cell boundary; Holds receptors for a variety of hormones; signal systems begin here; Processes, exports and imports substrates, ions, etc., binds hormones to their respective receptors
Cytosol	Medium for a variety of enzymes, substrates, products, ions, transporters, and signal systems; Glycolysis, glycogenesis, glycogenolysis, lipogenesis, pentose shunt, part of urea synthesis, and part of protein synthesis occurs in this compartment
Nucleus	Contains DNA, RNA, and many proteins that influence gene expression; protein synthesis starts here with DNA transcription
Endoplasmic reticulum	Ca^{++} stored here for use in signal tranduction; glucose transporters accumulate here until needed; has role in many synthetic processes
Golgi apparatus	Sequesters, processes, and releases proteins Export mechanism for release of macromolecules
Mitochondria	Powerhouse of cell; contains DNA that encodes 13 components of oxidative phosphorylation; Krebs cycle, respiratory chain, ATP synthesis, fatty acid oxidation, and the first step of urea synthesis occurs here
Ribosomes	Site for completion of protein synthesis
Lysosomes	Intracellular digestion Protein and macromolecule degradation
Peroxisomes	Suppression of oxygen free radicals; contain antioxidant enzymes
Microsomes	Drug detoxification; detoxification, fatty acid elongation can occur here

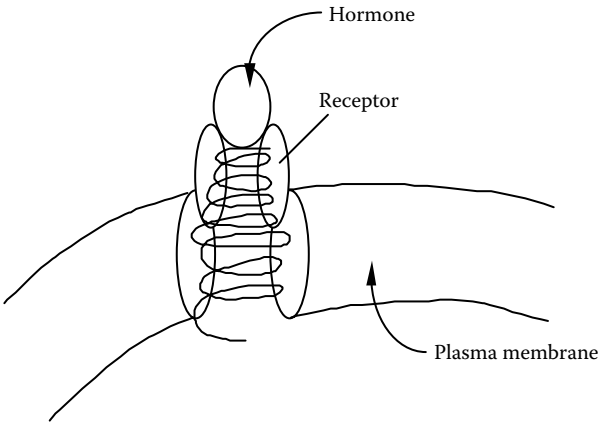


FIGURE 4.2 Schematic representation of a hormone bound to its cognate receptor in the plasma membrane.

with several membrane-spanning helical segments of their protein structures that are coupled to a separate G protein (G proteins are proteins that bind guanosine tri phosphate, GTP or guanosine diphosphate, GDP) on the interior aspect of the plasma membrane; and (4) receptors that not only have membrane-spanning units but also have a membrane-spanning ion channel. Examples of each of these are shown in Table 4.2.

Immunoglobulins typically are moved into the cell via the single membrane-spanning receptor, as are nerve growth factor and several other growth factors. The receptors for these proteins are usually rich in cysteine. The cysteine-rich region projects out from the plasma membrane and is important for the binding of its ligand through disulfide bonds. Insulin and thyroid-stimulating hormone are moved into the cell via a single membrane-spanning unit that has tyrosine kinase activity.

TABLE 4.2
Examples of ligands in four types of plasma membrane receptors

1. In single-membrane spanning unit, no tyrosine kinase	Immunoglobulins
	T cell antibodies
	Insulin-like growth factor (IGF)
	Nerve growth factor (NGF)
	Growth hormone (GH)
2. In single-membrane-spanning unit with tyrosine kinase activity	Thyroid-stimulating hormone (TSH)
	Insulin
	Platelet-derived growth factor (PDGF)
3. In multiple-membrane-spanning unit coupled with G protein	Calcitonin
	Parathormone
	Luteinizing hormone
	Rhodopsin
	Acetylcholine
	Thyrotropin-releasing hormone
4. In multiple-membrane spanning unit coupled with G protein and an ion channel	γ-Aminobutyric acid (GABA)

There is considerable homology between the first type, and this type of receptor in the portion of the receptor that binds the ligand. Where the receptors differ is in the portion that extends into and projects through the interior aspect of the plasma membrane. On the interior aspect of these single-membrane-spanning receptors is a tyrosine kinase domain.

The tyrosine kinase portion of the receptor is involved in the intracellular signaling systems. These can be very complex cascades, as illustrated in Figure 4.3. In this illustration, an ion (Ca^{+}) channel is also depicted, as are the two major intracellular signaling systems, the phosphatidylinositol (PIP) and the adenylate cyclase signaling systems.

Once the ligand has entered the cell, one of two events occurs. If the ligand is a hormone that has its primary effect at the plasma membrane, then its "job" is done. It will then be taken into the cell and degraded via the enzymes of the lysosomes. If the ligand has an intracellular function, it will move to its target within the cell. One of these targets may be DNA. The ligand may be transferred to an intracellular transport protein and/or to another receptor protein that has DNA-binding capacity or may bind directly to the DNA affecting its transcription. These binding proteins make up another group of receptor proteins, the intracellular receptors. These intracellular receptors function in the movement of ligands from the plasma membrane to their respective targets. In this instance, the ligands may be lipid-soluble materials, or minerals or vitamins, or carbohydrates, peptides, or proteins. There is no evidence that these receptors participate in the intracellular signaling cascades except as recipients of their ligands.

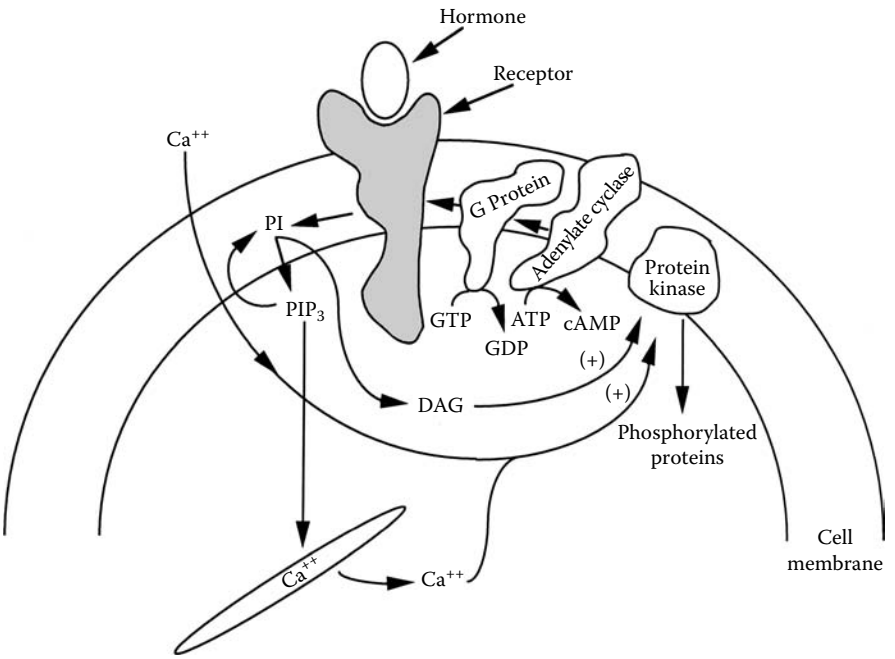


FIGURE 4.3 Hormone-receptor reaction that involves G proteins, adenylate cyclease, proteins, the PIP cycle, and cAMP. When the hormone binds to the receptor, a cascade of events follows. The G protein moves over to the receptor protein and binds GTP. Adenylate cyclase moves over and binds to the G protein and, in the process, energy is released. The cAMP level then rises and through the action of phosphodiesterase is converted to 5′ AMP. ATP is regenerated by the mitochondrial oxidative phosphorylation system or through substrate phosphorylation. The ATP furnishes the phosphate group and the energy for the subsequent phosphorylation of intracellular proteins via the membrane-bound protein, kinase. This enzyme is also stimulated by the calcium ion and diacylglycerol, one of the products of the PIP signaling system.

Intracellular receptors bind such compounds as the retinoids, vitamin D, certain minerals, steroid hormones, thyroid hormone, and some of the small amino acid derivatives that regulate metabolism.[1] As such, they serve to move these materials from their site of entry to their site of action. Sometimes more than one receptor is involved in the action of a specific nutrient. For example, vitamin A is carried into the cell via cellular binding proteins (vitamin A binding proteins). These proteins carry the DNA active form of the vitamin (retinoic acid) to the nucleus or mitochondrion where upon it is then bound to a nuclear or mitochondrial receptor that in turn binds to specific base sequences of the DNA. With some of the nutrient ligands, the intracellular receptor bind other components of the cell (not only DNA) and thereby functions in these other actions of their ligand.

Returning to the retinoic acid receptor, this receptor is a member of a family of receptors known as the steroid super family of DNA-binding receptors. Each of these receptors bind a specific steroid. The family name is something of a misnomer because not all of the ring-structured compounds they bind are steroids or steroid hormones. For example, these receptors bind thyroid hormone (triiodothyronine), retinoic acid, and the peroxisome proliferators (PPARs). In some instances, the receptor protein binds the DNA and more than one of these ring structures at the same time. Figure 4.4 illustrates a generic steroid hormone receptor. In this receptor there are six basic domains that have functional importance with respect to its action in transcription. Of interest is the homology that exists among the various members of this super family of receptors; 60–95% of the amino acid sequence of the zinc finger domain is homologous, 65–75% of the heat shock domain (HSP) is homologous, and 30–60% of the ligand-binding domain is homologous.

The regions where nonhomology exists determine which of the many ligands will be bound. The first hypervariable region provides the identity of the receptor based on its immunoreactivity. This has use in the study of gene expression, especially in the area where investigators are trying to understand how specific nutrients affect the expression of specific genes. Other members of the receptor class of cellular components bind specific ligands and proteins that bind copper or zinc or one or more of the vitamins or one of the many amino acids, fatty acids, carbohydrates, or metabolites. In addition, there are intracellular receptors that bind one or more of these substances but do not bind to DNA. Instead, these binding proteins serve as transporters of their ligands from their point of entry to their point of use. Altogether, the proteins called receptors are important structural components of living cells.

Signal Systems

Once hormones or nutrients are bound to their cognate receptors, changes in cell function occur. How are these changes brought about? As mentioned in the section on plasma membrane receptors, a number of hormones, particularly the protein and peptide hormones, affect cellular metabolism via a signaling system. There are several of these. Some couple to adenylate cyclase, others to guanylate cyclase, and still others involve the phosphatidylinositol pathway. Some use the calcium ion

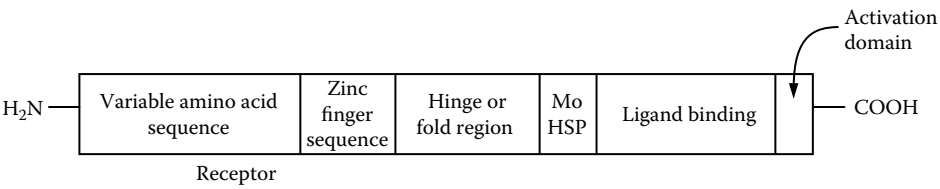


FIGURE 4.4 Generic structure of a receptor having a single polypeptide chain with six. The first is a variable amino acid sequence that confers immunologic identity to the receptor. The second is the zinc finger domain, which is rich in histidine and cysteine. Next is the hinge or fold region, which provides flexibility to the polypeptide chain. This is followed by the ligand-binding domain and the activation domain.

as a second messenger and others use ion channels. Some hormones use elements of all of these in signaling the cell to respond appropriately. Table 4.3 lists some of the hormones and the signaling systems they use. A hormone that uses the G protein as part of its signaling system does so when bound to its cognate receptor. G proteins are proteins that bind the nucleotide guanosine diphosphate or triphosphate (GDP or GTP). When the hormone binds to the receptor, a conformational change occurs that causes the G protein to exchange guanosine triphosphate (GTP) for GDP. This activates the G protein. The activated G protein modulates the catalytic subunit of the membrane-bound adenylate cyclase, which in turn catalyzes the conversion of ATP to cyclic AMP. Cyclic AMP stimulates protein kinase A, which phosphorylates a number of proteins, usually enzymes, thereby amplifying the original signal elicited by the binding of the hormone to the receptor.[2–11]

Some G proteins are coupled to the PIP_3 phospholipase C pathway. Phospholipase C catalyzes the hydrolysis of the membrane phospholipid, phosphatidylinositol releasing two second messengers, diacylglycerol (DAG) and phosphatidylinositol 4,5-phosphate (PIP_2), which is phosphorylated to the 1,4,5-phosphate form (PIP_3). Each of these second messengers has its own targets. PIP_3 migrates to the endoplasmic reticulum and, when bound to its receptor on this organelle, opens up a calcium channel. The calcium ions sequestered in the endoplasmic reticulum are then free to migrate out into the cytoplasm, whereupon they serve as cofactors in several reactions or are bound to calmodulin or stimulate exocytosis. Exocitosis is important to the insulin-producing β cell of the pancreatic islets.[12,13] Insulin is exocytosed, and this exocytosis is calcium stimulated and energy dependent.

DAG, the other second messenger released through the action of phospholipase C, binds to an allosteric binding site on protein kinase C which, in the presence of phosphatidylserine and the calcium ion, becomes activated so that still another group of proteins become phosphorylated.

G proteins also regulate the ion channels in both the plasma membrane and the sarcoplasmic reticulum.[14] There are two types of channels: one that is voltage-gated (depolarization/repolarization) and those that are coupled to specific membrane receptors via G proteins. Altogether, then, hormones that bind to membrane-bound receptor proteins elicit their effects on cell function via an integrated set of intracellular signals. These signal cascades are both simple and complex and involve a number of different proteins, each having a specific role in this signal transduction.

Cell Differentiation

Although all nucleated cells possess the same DNA, some of the specific DNA regions (genes) are not transcribed or translated in all cell types. If translated, some gene products are not very active. Thus, not all cells have the same processes with the same degree of activity. Cellular differentiation has taken place so that muscle cells differ from fat cells, which differ from brain cells, and so forth. In each cell type there are processes and metabolic pathways that may be unique to that cell type. An example is the great lipid storage capacity of the adipocyte, a feature not found in a bone or brain

TABLE 4.3
Some hormones and their signaling systems

Hormone	System
Atrial natriuretic hormone (ANH)	Cyclic GMP
Bradykinin, α2-adrenergic norepinephrine, somatostatin	Cyclic AMP (decreases cAMP)
Adrenalcortical-stimulating hormone (ACTH)	Cyclic AMP and PIP pathway
Acetylcholine	Ion channel
Calcitonin, follicle-stimulating hormone (FSH), glucagon, parathyroid hormone (PTH)	Cyclic AMP (increases cAMP)
Vasopressin, β-adrenergic norepinephrine	Cyclic AMP (increases cAMP)

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or muscle cell, although each of these cells does contain some lipid. Similarly, the capacity to form and retain a mineral apatite (a mixture of different minerals) is characteristic of a bone cell; the synthesis of contractile proteins by muscle cells is also an example of cell uniqueness. Cells differ in their choice of metabolic fuel. Hepatic and muscle cells make, store, and use significant amounts of glycogen. Adipocytes and hepatocytes make, store, and sometimes use triacylglycerols. All of these special features have an impact on the composition of specific organs and tissues in the body that collectively contribute to body composition. Analysis of the composition of specific organs and tissues does not necessarily reflect the whole-body composition. One must consider the function of each organ and tissue in the context of the whole body. Similarly, the determination of the activity of a single process in a single cell type or organ may not necessarily predict the activity (and cumulative result) of that process in the whole body. However, some processes are unique to certain tissues so some exceptions to the above role are possible. For example, one can measure glycogenesis and glycogenolysis in samples of liver and muscle, and be fairly confident that these measures will represent whole-body glycogen synthesis and breakdown. Other cell types may make, store, and use glycogen but not to the extent found in liver or muscle.

With respect to cell function and its contribution to the body's metabolic processes, mention should be made of the differing rates of cell renewal or cell half-life. Cells differ in their life span depending on their location and function.[15] Skin cells (epithelial cells) are short-lived compared with brain cells. The turnover time for an epithelial cell in man is of the order of 7 days. Brain cells are not regenerated and renewal of neural tissue is fragmented. That is, there is turnover of individual cellular components such as the lipid component but the cell once formed is not replaced as an entity, as are the epithelial cells. Epithelial cells, especially those of the skin and the lining of the intestinal tract are shed on a daily basis. Shown in Table 4.4 are some estimates of the life span of several sources of epithelial cells in man and in rats. Whereas the average life span of a human is on the order of 70 years, that of the rat is between 2 and 3 years. Despite this species difference in whole-body life span, the life spans of epithelial cells are surprisingly similar.

Another short-lived cell is the erythrocyte, the red blood cell.[16] It has a half-life of about 60 days. Replacement cells must be made constantly through the process of hematopoiesis. Hematopoisis is responsible for all of the blood cells (white cells, platelets, and red cells) and takes place in the pluripotent stem cells of the bone marrow. Erythropoiesis, red cell proliferation, is dependent on an intact bone marrow environment and on a cascade or network of cytokines, growth factors and nutrients. The main function of the red cell is to carry oxygen to all cells of the body and exchange it for carbon dioxide. A shortfall in the number of red cells or in its oxygen- carrying capacity is called anemia. Table 4.5 lists the nutrients important to normal red cell production.

There are a number of nonnutritional reasons for anemia. These include excessive blood loss, drug-induced red cell destruction, and several genetic diseases, including thalassemia and hemolytic anemia. Some toxic conditions (e.g., lead intoxication) can also cause anemia. The characteristics of nutrition-related anemia are shown in Table 4.6.

TABLE 4.4
Life span (days) of epithelial cells from different organs in man and rat

Cell Type	Human	Rat
Cells lining the gastrointestinal tract	2–8	1.4–1.6
Cells lining the cervix	5.7	5.5
Skin cells	13–100	19.1
Corneal epithelial cells	7	6.9

TABLE 4.5
Nutrients needed for red cell production

Protein
Energy
Vitamins E, A, B_6, B_{12}
Folacin
Iron, copper, zinc
Riboflavin
Niacin

TABLE 4.6
Normal blood values for measurements made to assess the presence of anemia

Measurement	Normal values	Iron deficiency	Chronic disease	B_{12} or folic acid deficiency
Red blood cells	Males: 4.6–6.2			
(million/cu mm);	Females: 4.2–5.4	Low	Low	Low
Hemoglobin (g/ dL)	Males: 14–18	Low	Low	Low
	Females: 12–16			
Hematocrit (vol %)	Males: 40–54%			
	Females: 37–47%	Low	Low	Low
Serum iron	60–280 µg/ dL	Low	Low	Normal
TIBC[a]	250–425 µg/ dL	High	Low	Normal
Ferritin	60	Less than 12	Normal	Normal
Percent saturated	90–100%	Low	Normal to high	Normal
Hypochromia	No	Yes	Slight	None
Microcytes	Few	Many	Slight	Few
Macrocytes	Few	Few	None	Many
RDW (RBC size)	High	High	Normal to low	Very high
Red cell folate	>360 nmol/ L	Normal	315–358	<315
Serum folate	>13.5 mg/ mL	Normal	Normal	Low (<6.7)
Serum B_{12}	200–900 pg/ mL	Normal	Normal	Low
MCV[b]	82–92 cu m	<80	Normal	>80–100

[a]Indirect measure of serum transferrin; iron-binding capacity.
[b]Mean cell volume. When volume increases, the size of the red cell has increased (↑ % of megaloblasts).

It is of interest to note that many of the symptoms of malnutrition include lesions of the skin and anemia. This is because skin cells and red blood cells are among the shortest-lived cells in the body and abnormalities in them are readily apparent.

As noted, cell types differ in their life spans. We do not know all the details of how cells reproduce themselves either in part or whole. Doubtless there are signaling systems in place that control this process. A number of systems have been described that include not only cell multiplication (growth) but also cell death (necrosis or apoptosis). Many of these systems are hormonally controlled. Growth factors including growth hormone, play key roles in new cell production. These factors have been elucidated through the study of conception, embryogenesis, and fetal development. Studies of the growth of animals and humans have also provided information about this process.[17,18]

When growth begins, its velocity is high, then it gradually slows down. When growth ceases (i.e., when the number of new cells appears to equal the number of cells that have died), then a steady state is achieved (maturity). When a steady state has developed, the half-life of the cell can be determined/calculated. By definition, the half-life of the cell (or of any biologically active material) is defined as the amount of time required for half of the number of cells or amount of active material to disappear or be eliminated. Careful measurements of both time and the number of labeled cells or biologic material must be made.[19]

The half-life of a cell can be calculated using measurements of the appearance or disappearance of a labeled material such as thymidine. Thymidine is taken up by the nuclear DNA as the cell prepares to divide or renew itself. It then disappears as the cell dies. The fractional elimination constant (which is the same as the fractional appearance rate) can then be calculated, and the half-life of the item in question derived.[20] Suppose the number of cells initially equals C_o. Over a period of time (t), this number is reduced to half (the half-life).

The equation $\frac{dC}{dt} = k_e C_o$ can be written and solved as follows:

$$C = C_o e^{-kt}$$

$$\ln C = \ln C_o - k_e t$$

Rearranging gives us $\ln (C_o/C) = k_e t$

At one half-life, $C = 0.5C_o$

Therefore, $\ln (2.0) = k_e t_{1/2}$

$$0.693 = k_e t_{1/2}$$

$$t^{1/2} = 0.693/k_e$$

Note that 0.693 is always equal to the natural log of 2. This concept of half-life is part of the overall concept of steady state. Steady state, a feature of living systems, is that state where no net change occurs yet there is a steady flow of materials through the system. There are no net losses or gains, merely maintenance of the status quo. This probably characterizes the steady ebbs and flows of the adult nonpregnant, nongrowing adult. In this individual, there is no body weight gain or loss, no change in skeletal or muscle mass, no measurable changes in body function. Perturbation of steady state occurs from birth to death under well-defined conditions, but in the adult the steady state is the maintenance state.

APOPTOSIS

The half-life of cells varies throughout the body and is dependent on cell type. It depends on a variety of factors including age, nutritional status, health status, genetics, as well as factors not yet identified. Age is important. Growing individuals are in a phase of life in which cell number is increasing exponentially.[18] Once growth and development are complete, this increase in cell number

slows down. In contrast, senescence is characterized by a gradual loss in total cell number as well as losses in discrete cell types. In part, these changes in cell number are analogous to the observations of cell biologists, who have reported on the growth of cells in culture. Cultured cells go through a rapid growth phase doubling in number for 50–100 generations. Eventually, the cells reach a "turning point" and grow poorly or not at all, despite the continual provision of new media. Finally, the culture ceases to thrive and the number of cells begins to decline. At this time, the cells begin to die off slowly through a process called *apoptosis* or programmed cell death.[21-24] Cell cultures that do not become apoptotic are called *immortal* (i.e., they do not have the mechanism for programmed cell death). These cells have been genetically altered so that they can sustain themselves indefinitely. This alteration in the genetic control of the life of a cell is called *transformation*. Transformed cells are powerful research tools and allow for the study of these cells in culture so as to learn about their metabolism. In particular, cell growth has been studied and a number of growth factors have been identified that have relevance to our understanding of how mammalian growth and development occurs. Now, interest is building in learning how cells senesce and die through the process of apoptosis. This is relevant to our understanding of how cell turnover is controlled.

Cell turnover has two parts: cell replacement and cell death. Cell death is either a concerted, all-at-once event or a programmed, gradual process. If the former, cell death is called *necrosis*. This occurs if a tissue sustains an injury, small or large. A small cut in the skin results in necrosis of the injured cells in and around the cut. A ruptured coronary vessel results in the death of the heart muscle supplied by that vessel. If only a small capillary ruptures, the necrosis of the myocardium will be small. If a larger vessel ruptures, damage to the heart can be quite large and indeed may be a mortal event. Necrotic cell death is preceded by cell enlargement and a swelling of all the organelles within the cell. The DNA disintegrates and its nucleotides are degraded.

In contrast, programmed cell death or apoptosis involves a shrinkage of the cell and enzyme-catalyzed DNA fragmentation. This fragmentation can be detected as a laddering when the DNA is extracted from the cell and separated by electrophoresis. Apoptosis is a process that is an integral part of living systems. It is part of the growth process as well as part of the maintenance of cell and tissue function. It is also part of wound healing and recovery from traumatic injury. Adipose tissue remodeling, immune function, epithelial cell turnover, and the periodic shedding of the uterine lining are but a few examples of this process. Apoptosis is viewed as a defense mechanism to remove unwanted and potentially dangerous cells such as virally infected cells or tumor cells. The process involves the mitochondria as the "central executioner."[25,26] The mitochondria produce reactive oxygen (free radicals) as well as other materials that participate in apoptosis. Alterations in mitochondrial function have been observed to occur prior to any other feature of apoptosis. A decrease in mitochondrial membrane potential that, in turn, affects membrane permeability, is an early event. The increase in permeability is followed by a release of cytochrome c that in turn regulates the caspases, which are cysteine proteases.[27] These caspases stimulate the proteolysis of key cell proteins in various parts of the cell. Altogether, the fragmentation of the DNA and the destruction of cell proteins result in the death of the cell via a very orderly process.

Apoptosis is the mechanism used in the thymus to eliminate thymocytes that are self reactive. By doing so, the development of autoimmune disease is suppressed. Indeed, this mechanism is being examined as an explanation of autoimmune, insulin-dependent diabetes mellitus. Apoptosis is being carefully studied to learn about the signals that initiate or suppress it. Figure 4.5 illustrates the process of apoptosis, and Table 4.7 lists some of the signals and regulatory compounds that influence this process.

Many cells can be stimulated to become apoptotic.[28,29] The p53 protein, for example, can induce apoptosis as one of its modes of protecting the body against tumor cells. The p53 protein is a DNA-binding protein. Mutations in tumor cells that inactivate p53 have been found.[30] The result of such mutation is that tumor cells grow and multiply. The gene for p53 likewise can mutate, and this mutation has been associated with tumor development and growth. Apparently, this gene has several "hot spots" (likely places for mutation to occur), which explains its role in carcinogenesis and its loss

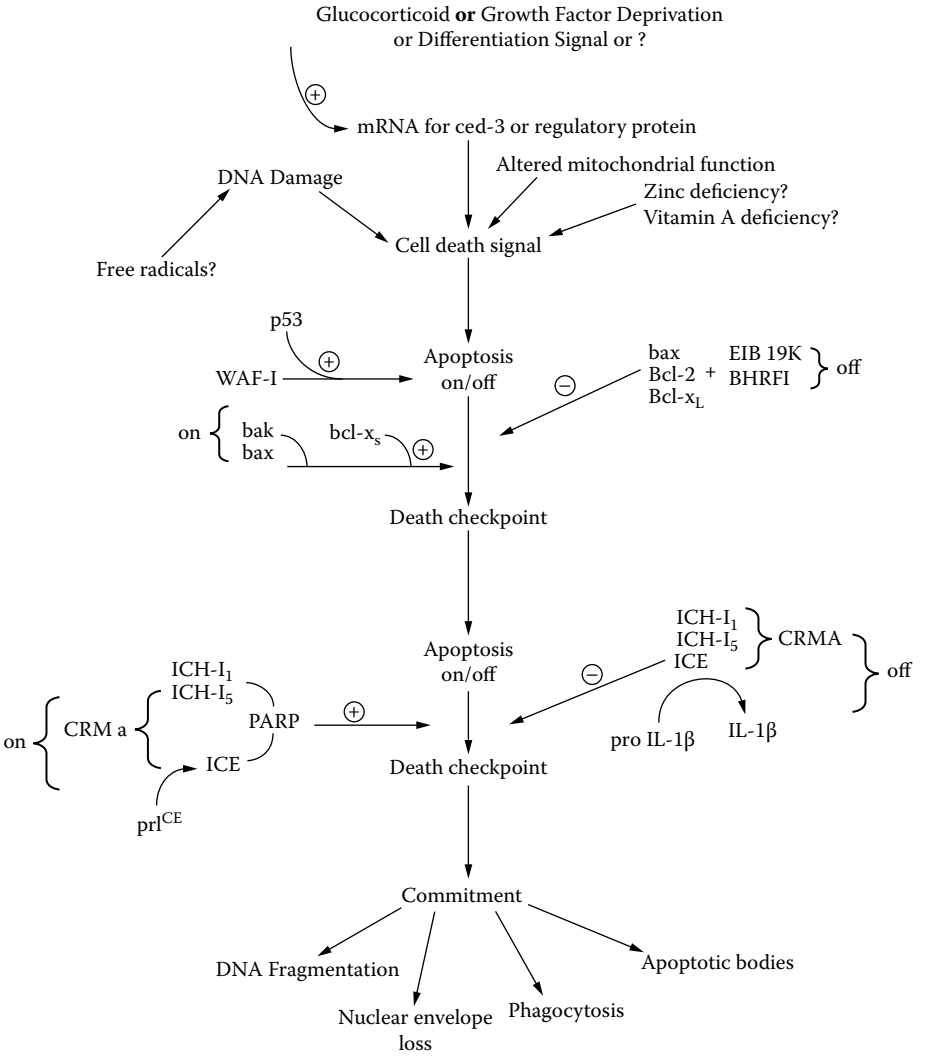


FIGURE 4.5 Flowchart for apoptosis. Note the many question marks, indicating that there are as many unknown factors in this process as there are known.

in normal function. The Bcl-2 protein, a membrane-bound cytoplasmic protein, is another player in this regulation of apoptosis.[28] It is a member of the family of proteins called *protooncogenes*. Its normal function is to protect valuable cells against apoptosis. It is downregulated when cells are stimulated to die. Inappropriately high Bcl-2 protein levels can provoke cell overgrowth. The cell regulates Bcl-2 protein so as to maintain a normal homeostatic state. Bcl-2 and its homologue, Bcl-xL, blocks apoptosis. Both Bcl-2 and Bcl-xL can heterodimerize with Bax or Bcl-xs and, when Bcl-2 or Bcl-xL is overexpressed, an enhancement of oxidative stress mutagenesis can be observed. This occurs because these proteins suppress the apototic process, allowing more exposure of the cell to DNA damage by mutagens.

Nutrients play a role in apoptosis. High levels of zinc (500 μm) have been found to block apoptosis in cultured thymocytes.[31] The zinc blocks the action of glucocorticoids in stimulating apoptosis. Zinc also interferes with tumor necrosis factor (TNF)-induced apoptosis as well as heat-induced

TABLE 4.7
Contributory factors* in the regulation of apoptosis

Factor	Effect	
	Suppress	Stimulate
Age	√	
Apaf-1 protein		√
p53 protein		√
Bcl 2 protein	√	
Bcl-x3 protein		√
Bcl-xL protein	√	
Bax protein		√
Bak protein		√
WAF-1	√	
ced 3 and 4		√
Glucocorticoids		√
ced 9	√	
High zinc	√	
Low zinc		√
Low retinoic acid	√	
High retinoic acid		√
Interleukin 1β		√
Leptin		√
TNFα		√
Insulin	√	
Low manganese		√
IGF_1	√	
Low SOD activity		√
Peroxidized lipid		√

*It may appear that the names of these factors are in "code." Actually, their names were given by the investigators working with them and are somewhat arcane. Some are abbreviations: TNFα is tumor necrosis factor α; IGF_1 is insulin-like growth factor$_1$; SOD is superoxide dismutase.

death. In contrast, low levels of zinc (0.3 to 200 μm) have the reverse effect. That is, at low levels of zinc, apoptosis could be stimulated by TNF, glucocorticoid, and heat exposure. The mechanism whereby zinc has these effects must be multifaceted. Zinc has an important role in transcription control as part of the zinc fingers, but it is not known how this relates to apoptosis.

Manganese, another essential nutrient, also is involved as a cofactor for superoxide dismutase (SOD).[32] In instances where SOD is less active than normal, apoptosis is stimulated coincident with an increase in C2-ceramide, TNFα, and hydrogen peroxide. Peroxides form from unsaturated fatty acids as well as certain of the amino acids. There may be a link, therefore, between the fatty acid intake of the individual and apoptosis of certain cell types. In one study of genetically obese diabetic rats, an increase in fatty acid intake was associated with an increase in β-cell apoptosis.

Retinoic acid (and dietary vitamin A) deficiency stimulates apoptosis.[33,34] The well-known feature of vitamin A deficiency (suppressed immune function) suggests that vitamin A and its metabolite, retinoic acid, serve to influence the sequence of events leading to cell death. This link is made

because it is known that T cell apoptosis is an important defense mechanism, and T cells are part of the immune system. Vitamin A is also an antiproliferative agent, so its effect on abnormal cell growth is two pronged—it stimulates apoptosis and suppresses proliferation.

Genetic regulation of apoptosis involves not only the transcription factors mentioned earlier but also specific genes. Already described are the p53 and Bcl-2 DNA-binding proteins that, like zinc, retinoic acid, and fatty acids, affect gene expression. Add to this list the mitogen-activated protein kinases (p42/44) Erk1 and Erk2. When phosphorylated, these proteins suppress apoptosis in brown adipocytes.[35] Apoptosis is also suppressed by certain of the cytokines (IL6, IL3, interferon-γ) and stimulated by others (leptin).[36,37] Finally, the gene Nedd 2 encodes a protein similar to the nematode cell death gene ced 3 and the mammalian interleukin-1 β-converting enzyme.[38] Overexpression of this gene induces apoptosis.

With the understanding of apoptosis comes the understanding of how and why the body changes in its composition as it ages from conception to death. We have also begun to understand the role of apoptosis (and its control) in the pathophysiology of such degenerative diseases as diabetes.[39,40] All of the factors described here play roles in the gradual changes that occur not only in the pancreas but also in other tissues: muscle, adipose tissue, neural tissue, and so forth. All of these systems are coordinately regulated in the healthy young animal, but as animals (including humans) age, subtle small modulations occur that then cumulatively result in cell, tissue, and lastly, organ dysfunction.

Looking at just the age-related changes in body fat one can observe small but incremental differences in body fat with age. For example, the young animal has little fat, whereas the older one has gained fat sometimes at the expense of body protein. Clearly, the body is continually being remodeled, and quite clearly this remodeling is the result of a combination of many factors including the apoptotic process.

LIFE CYCLE

Just as cells have a finite existence, so, too, do whole bodies. Like the cell with its cycle of formation, maintenance, and death, whole bodies have a life cycle that includes the stage from conception to birth, from birth to growth and development, to adulthood (maintenance) and then to senescence. For the human, the time frame for these stages is long, whereas that for the rat or mouse is relatively short. Yet we have learned much from these shorter-lived species about the processes involved and how nutrition can affect the time frame for these stages of the life cycle. The reader is reminded of the different nutrition needs for these stages. The National Research Council Food and Nutrition Board has set out recommendations for the intakes of each of the essential nutrients that is appropriate for each of the life stages. The recommendations are for daily intakes, yet there can be some variation from day to day as the food choices vary from day to day. In the chapters on the specific nutrients these recommended intakes are stipulated (see Chapters 6 to 12).

Above and beyond these recommendations designed to optimize the health and well-being of the individual, we have a large body of information related to the effects of reduced food intake on development and longevity gleaned from hundreds of studies using rats and mice. When well-balanced diets are provided to rats and mice where only the total energy is reduced, life span is extended.[41–45] Some of these reported effects are listed in Table 4.8. The design of these experiments is critical. If just the amount of food is reduced without accounting for the need for adequate intakes of protein, essential fatty acids, and the micronutrients, nutrient insufficiency will develop. It is critical that the diet be low only in its energy content and nothing else. Life span is then extended. Care must also be exercised when the diets are offered to the experimental animals. If they are offered all at once, then a phenomenon of meal feeding occurs with unexpected results vis a vis fuel flux. If the "meal" is offered during the daylight hours to a nocturnal feeding rat, then there will be a 12-hour time shift in lipogenic capacity and glucose flux. A carefully designed restricted feeding experiment will provide food throughout the 24-hour period so that the animals do not receive (and consume) all their food at once. Another aspect of this feeding paradigm for rats and mice is the

TABLE 4.8
Food restriction effects in rodents

- Increased life span
- Decrease in parathyroid hormone release
- Longer maintenance of serum vitamin D levels
- Lower blood levels of calcium and phosphorus
- Smaller excursions in blood glucose levels throughout the 24-h light cycle
- Greater spontaneous activity
- Reduced weight gain
- Delay in the development of chronic nephropathy
- Smaller fat cells
- Fat cells are more responsive to epinephrine, ACTH, glucagon, and fluoride
- No difference in hepatic protein DNA or RNA content or protein synthetic rates
- Less serum calcitonin
- Decreased serum lipid levels
- Greater bone mass
- Delay in onset of puberty

tight control of their environment. Usually, the environment is carefully controlled with respect to temperature, humidity, light, and pathogen contamination. These rodents are reared in a stress-free environment with respect to cage space and other environmental considerations.

How do these findings relate to humans? Obviously, humans are not kept in a contained environment free of stress. Furthermore, humans eat a variety of foods on an ad libitum basis. Obviously, surfeit feeding with subsequent excess fat gain has been recognized as a detrimental characteristic in humans. However, there are some humans who seem to regulate their food intake so as to avoid eating too much (see Chapter 2). Is there a lifetime benefit to this eating pattern similar to what was reported to occur in rats? We cannot do the critical experiment in humans to answer this question. Yet, it is a tantalizing one.

Growth and Development

From the single fertilized cell to maturity, the human passes through several important stages. From conception to birth is one of these, followed by the growth of infanthood and childhood, followed by adolescence. Each of these stages is characterized by both an increase in cell number and a concerted and coordinated change in cell/tissue/organ characteristics and function. Longitudinal growth of the skeleton is the most obvious feature accompanied by the growth and maturation of the muscles, the internal organs, the reproductive system, and the central nervous system.[17–19] Physical activity, as well as the consumption of a well balanced diet adequate in all the needed nutrients, ensures this coordinated growth pattern. Should one or more of the essential nutrients be inadequate, growth abnormalities will be observed. Familiar images of poorly nourished infants and children throughout the world document these nutritional effects on growth and development. Further, malnourished females have more pregnancy complications and greater numbers of miscarriages, fetal wastage, and fetal malformations than well-nourished females. Their babies likewise have more post natal difficulties than babies of well-nourished mothers. Lactation is compromised as well. Babies of malnourished mothers are more likely to be malnourished as well.

There may be some long-lasting effects of maternal malnutrition on the health and well-being of the children. However, these effects have been hard to document because of the complications

of human life. One carefully conducted study of the fate of Dutch children whose mothers had been severely food-restricted during World War II has been published.[46] Six Dutch cities were cut off from all food supplies for about six months by the Germans. Some women were pregnant at the initiation of food deprivation, whereas others became pregnant during the deprivation period. There were higher rates of spontaneous abortion and miscarriage, higher rates of premature birth, slightly more stillbirths, and far more neonatal deaths and malformations. There were far fewer conceptions during the food-deprivation period. The pregnant women guarded their body stores remarkably even in the face of food deprivation. It is suggested that the people shared what they had and gave preference to the pregnant women with respect to their food supply. Their children were evaluated at entry into the military (20 years later). In contrast to expectation, these progeny were no different from those whose mothers were not food-restricted during this time period. In part, this could be explained by the mothers' nutritional status prior to the food-deprivation period and in part explained by the cultural influences on the food made available to the pregnant women.

In contrast, rodent maternal nutrition has been shown to affect their progeny. Some of the effects are long-lasting, whereas other effects disappear with time. Some of these are listed in Table 4.9.

Whether findings in rodents are applicable to humans is unclear. Rodents differ appreciably from humans with respect to gestation, birth and growth of the progeny. Rodent progeny come in litters; humans usually give birth to single infants. Twins and multiple births occur but not as frequently. Rodent progeny are born in a very undeveloped state. They are blind, have mere stubs for limbs, have no fur and their heads and central nervous systems are in a primitive state. Within a very short period of time fur appears, legs and feet appear, eyes develop and open, and the typical movement and responses of the rodent are apparent. In three weeks they are ready to be weaned from the mother who, under the usual colony conditions, is already pregnant with another litter. These species differences are important if we are to understand whether the maternal diet affects progeny outcome independent of the environment.

Aging

The very nature of life is that aging begins at conception and stops at death. However, for the purposes of this section on the life cycle, the period of interest is the period that occurs after the attainment of maturity when the individual loses the steady state. This period is called *senescence*.

The elderly are the fastest growing segment in our population today.[47,48] Citizens of western, technologically advanced nations are living longer than their ancestors. In the United States, the segment of the population over the age of 65 has grown rapidly. For example, between 1960 and 1990, the total U.S. population increased by 39%. The segment of that population over the age of 65 grew by 89%. The segment of the population over the age of 84 grew by 232%. Figure 4.6 shows the increase in the number of people in the United States over the age of 100. This population segment is rising rapidly.

Due to the increase in the over-65 segment, scientists are beginning to pay much more attention to the nutrition-related health problems facing these people. As well, more nutrition scientists are

TABLE 4.9

Maternal diet effects on progeny (rodents)

- Maternal dietary fat affects fat stores in progeny but this effect disappears with time
- Maternal carbohydrate intake affects glucose metabolism in young pups but not mature ones
- Maternal dietary protein affects fetal development: low protein→poorly developed pups
- Maternal essential fatty acid diet results in fetal resorptions
- Maternal food restriction results in smaller litters

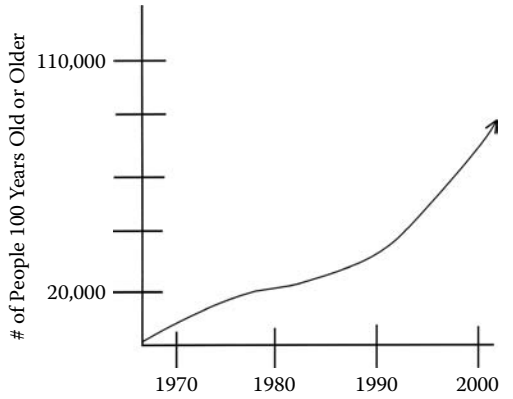


FIGURE 4.6 The number of centenarians in the United States has risen from 4,447 in 1960 to 35,808 in 1990. This group of people is steadily increasing in number.

examining the nutrition needs of this population. Obviously, the health care system will need to be expanded as the elderly segment of the population expands. The costs of elder care and its delivery will need continual reexamination.

As people age, many aspects of their lives change. Upon retirement, their income may be reduced and their health care expenses may increase. Age-related changes in the muscular-skeletal systems of the elderly will probably mean less mobility. Older sensory systems may be less acute. Diminished senses of balance, hearing, smell, sight, and the overall perception of self in society have an impact on health and well-being, as well as on aging individuals' ability to care for themselves. The development of senile dementia of the Alzheimer's type (SDAT) with subsequent losses in both mental and physical function occurs more frequently as the population ages. Shown in Table 4.10 are the predicted age-specific prevalence rates for SDAT. With the significant gain in average life expectancy, a parallel rise in these age-related failures in brain function must be anticipated.

However, as the study by Johnson and colleagues[50] has shown, some deteriorated mental function might not be strictly age-related. It might be due to malnutrition. Older adults may show signs

TABLE 4.10
Predicted age-specific prevalence rates (%) for DSM-III Senile Dementia and SDAT[a]

Age	DSM-III Dementia	SDAT
62.5	0.9	0.2
67.5	1.6	0.4
72.5	2.8	0.9
77.5	4.9	2.1
82.5	8.7	4.7
87.5	15.5	10.8
91.5	24.5	21.0
95.0	36.7	37.4

[a] *SDAT, Senile dementia of the Alzheimer's type. Epidemiology Public Health Issues.*[49]

of inadequate protein, mineral, and vitamin nutriture that impact on mental function. Lack of vitamin B_{12}, folacin, and protein, as well as other problems, have been identified. If these inadequacies can be addressed and nutritional status improved, some of the so-called age-related impaired mental function can be reversed. Some malnutrition can also be attributed to the loss of ability to purchase the needed food—either for economic reasons or because elders may not be able to get to the grocery store to select the foods they need. Their diminished sensory faculties may mean that they can no longer drive and thus must depend on their families and communities for their food supply. They may also be unable to prepare the food or store it appropriately, due to their diminished sensory function. If dentition is poor, the choice of foods might be limited to those items that are easy to swallow with minimal mastication. The absence of dentures or the presence of poorly fitted dentures can be a major impediment to the consumption of the wide variety of foods needed to assure good nutritional status.

How can these problems be identified and corrected? This is not an easy question to answer. The nutrition screening of people over the age of 65 can present some special problems and hurdles. For a variety of reasons, many elders may not be able to read. Thus, acquiring information about their usual food intake has this additional complication. Short-term memory loss may cause some elderly to be unable to recall the food they consumed yesterday or the day before. If elders are blind or have failing vision, they may not be able to complete a diet-recall form or to compile a food-intake diary. A number of innovative techniques must be developed to address this problem. Some researchers have used a picture-sort approach where the subject selects pictures of foods and recalls to the interviewer how frequently that food is consumed. Other researchers have used interview techniques that involve the use of food group patterns as well as individual food-recall methods. Having an observer record the actual food consumed is a possibility, but this method is labor intensive and time consuming unless the elderly are in a group setting such as in an assisted-care facility. Individual observation may not be practical if the elderly person is living independently. In addition, the line between screening and diagnostic testing is blurred. In areas where there is good, widely available geriatric care, the need for such testing is reduced. In other words, where the elderly are well served by the medical community, the need for screening is much less than where they are poorly served. Early indications of health being impacted by malnutrition are usually observed in the former situation and ignored in the latter. Thus, anemia due to mineral or vitamin deficiency might be corrected as elderly people are routinely cared for by their physicians. Osteoporosis, arthritis, and other degenerative diseases that have a nutrition component likewise will be identified and effectively treated.

Good geriatric care also includes the recognition that some of the medications used in managing such chronic diseases as hypertension or diabetes mellitus may interact with certain nutrients,[51] thus placing the patient at risk for malnutrition. Thiazide diuretics, for example, are widely prescribed for the management of hypertension. They are excellent medications for this purpose; however, they increase the loss of potassium, and frequently the elderly need to supplement their diets with potassium-rich foods (citrus fruits, bananas, etc.) or take a potassium supplement. The elderly may develop chemical imbalances in the brain that affect their emotional state. It is not unusual for physicians to prescribe antidepressants. Those drugs that are anticholinergics can have the side effects of dry mouth, altered taste perception, nausea, vomiting, constipation, and reduced appetite. All of these side effects can affect nutritional status because of their influence on food consumption. Similarly, nutritional status can be affected by the use of antacids and laxatives. Some of the elderly believe that a daily large bowel movement is essential. Despite day-to-day variation in the kinds and amounts of foods consumed, there is this belief that a daily movement is a feature of good health. Thus, many elderly are chronic laxative users. This use may disturb gastrointestinal function and promote diarrhea as well as the possibility of an electrolyte imbalance. In turn, this will reduce gut passage time and thus absorptive cell exposure time for nutrients. Again, this can result in impaired nutritional status.

Diseases of the joints and connective tissue are frequently managed with the use of antiinflammatories. Both steroids and nonsteroids are used to reduce the inflammation and associated pain. Aspirin and indomethacin are among the nonsteroidal, over-the-counter medications frequently used. If taken daily in large doses, both can result in iron-deficiency anemia. High aspirin intake can deplete the hepatic iron stores because it interferes with normal blood coagulation. The elderly can have bruises and small "bleeds" that result in blood loss and anemia. A number of other drugs can influence nutritional status. However, many drugs have not been studied at all with respect to their effect on nutritional status especially in the elderly. Nonetheless, interest in drug–nutrient interactions is building as we learn more about the gradual losses in body function that characterize aging.[50]

Aging in itself has effects on the pathways of intermediary metabolism as well as on the endocrine system. These effects are listed in Table 4.11.

Insulin resistance may be a feature of aging. With age the pancreas becomes less responsive to signals for insulin release and the target tissues become less responsive to its action. In part this may be due to age-related, increased plasma membrane phospholipid saturation, but it is also due to age-related increases in fat cell size. As fat cells accumulate stored fat they become less sensitive to the action of insulin in promoting glucose uptake and use. Muscle cells likewise may have age-related changes in membrane fluidity that impair their response to insulin. As well, there is an age-related decrease in muscle use. Working muscles have little need for insulin bound to its receptor to facilitate glucose use. Altogether then, this decrease in muscle activity plus the increase in fat cell size has a negative effect on the insulin–glucose relationship. Glucose levels rise and the β cells of the pancreatic Islets of Langerhans increase their output of insulin to meet the glucose challenge. However, this excess output does little good in reducing blood glucose in the aging, overly fat inactive individual, and it is not uncommon to have Type 2 diabetes develop as a consequence. Age-related impaired glucose tolerance can be mitigated by food restriction and increased activity which reverses the above physiological state. Because insulin is one of the main regulators of intermediary metabolism and because its action is counterbalanced by the glucocorticoids, the catecholamines, the thyroid hormones, glucagon and several other hormones, it should be no surprise that age changes in these hormones occur as well. Again, some of these changes can be attributed to age changes in membrane lipids but some can be attributed to changes in hormone production and in the synthesis and activity of the receptors that mediate their action.

Table 4.12 lists some hormones affected by age. The blood levels of most of these hormones decrease in the aging human and laboratory animal. A few pass through a phase where they are elevated above normal, then fall below normal as aging continues. All of these hormones serve to regulate the metabolism of carbohydrate, lipid, and protein. Age has unique effects on each of these tissues and their respective metabolic processes. The pathways of intermediary metabolism are listed in Table 4.11. Each of these pathways has specific control points, which in turn are affected by age. Although various studies have shown age-related declines in enzyme activities, it should be remembered that these are in vitro measurements in conditions where substrates and coenzymes are in optimal amounts to assure saturation. This rarely occurs in vivo. Hence, a decline in activity may not mean a decline in in vivo function. In a number of instances the age-related decline in activity of a pathway or reaction can be inhibited by chronic food restriction. This inhibition of aging effects on metabolism probably has to do with the decreased fat stores that are the result of food restriction. A decrease in fat store reduces the supply of fatty acid precursors to free radicals, and this may lead to a reduction in error rates in the genetic material as well as a reduction in protein damage with resultant loss of function. In addition, a reduced fat store has effects on peripheral cell responsivity to insulin vis-á-vis glucose use and, of course, in the absence of hyperinsulinemia, there is a decreased release of the antiinsulin hormones. As described above, age-related alterations in hormone balance do occur, and these alterations have an impact on carbohydrate oxidation, glycogen storage, and mobilization, hexose monophosphate shunt, and gluconeogenesis.

As aging proceeds there is a rise in blood lipids coupled with a decrease in adipose tissue lipoprotein lipase. With age, adipocytes have a less competent lipid uptake system due to this decline in

TABLE 4.11
Effects of age on intermediary metabolism and its control

Pathway	Control points	Effects with aging
Glycolysis	Transport of glucose into the cell (mobile glucose transporter)	↓
	Glucokinase	↓
	Phosphofructokinase	↓
	α-Glycerophosphate shuttle	
	Redox state, phosphorylation state	
Pentose phosphate shunt	Glucose-6-phosphate dehydrogenase	↓
	6-Phosphogluconate dehydrogenase	↓
Glycogenesis	Stimulated by insulin and glucose	ND
	High-phosphorylation state (ratio of ATP:ADP)	ND
Glycogenolysis	Low-phosphorylation state	ND
	Stimulated by catecholamines	ND
Lipogenesis	Stimulated by insulin	
	Acetyl-CoA carboxylase	
	High-phosphorylation state	
	Malate citrate shuttle	↓
Gluconeogenesis	Stimulated by epinephrine	
	Malate aspartate shuttle	↓
	Redox state	↑
	Phosphoenopyruvate carboxykinase	↓
	Pyruvate kinase	
Cholesterogenesis	HMG CoA reductase	
Ureogenesis	Carbamyl phosphate synthesis	↑, ↓
	ATP	ND
Citric acid cycle	All three shuttles	ND
	Phosphorylation state	↓
Lipolysis	Lipoprotein lipase	↓
Respiration	ADP influx into the mitochondria	↓
	Ca^{2+} flux	
	Shuttle activities	↓
	Substrate transporters	↓
Oxidative phosphorylation	ADP:ATP exchange	↓
	Ca^{2+} ion	
Protein synthesis	Accuracy of gene transcription	↓
	Availability of amino acids	↓
	ATP	↓

Note: ↑ — increased as the animal ages
↓ — decreased as the animal ages
ND — no data
NC — no change

TABLE 4.12
Hormone changes with age

Hormone	Effects with aging
Thyroxine (T_4)	↓
Triiodothyronine (T_3)	↓
Thyroid-binding globulin	No change or ↑
Thyroid-stimulating hormone (TSH)	↓
Insulin	↑ Followed by ↓
AACTH	↓
Epinephrine	↓
Glucagon	↓
Growth hormone	↓
Estrogen	↓
Testosterone	↓
Cortisol (glucocorticoids)	↓
Pancreatic polypeptide	↓

Note: ↑ increase, ↓ decrease.

lipase activity. In normal aging animals the rates of cholesterol synthesis do not change; however, the uptake of this cholesterol as well as its oxidation and excretion declines. This has the result of an age-related increase in serum cholesterol levels. Genetics plays an important role in these age-related changes in serum lipids (see Chapter 8). Some genotypes are characterized by a sharper decline with age in lipid-uptake processes than other genotypes. For example, those whose lipoprotein receptors are genetically aberrant will, as a result, show a far earlier rise in serum lipids than those whose receptors are fully functional. Some may only have a decline in cholesterol uptake or triacylglyceride uptake, whereas others will have a decline in the uptake of both. Age-related declines in thyroid hormone production, thyroxine conversion to triiodothyronine, glucocorticoid release, and the insulin:glucagon ratio will have effects on fatty acid mobilization and oxidation, and the results of this decline in hormone-stimulated lipolysis are observed as an age-related expansion of the fat stores. Although age has effects on fatty acid synthesis in rats and mice, these effects are minimal in humans consuming the typical Western diet. This is because this diet is relatively rich in fat so the need for its synthesis is almost nonexistent. Humans tend to use the dietary carbohydrate as their primary fuel and the surplus dietary fat is transported to the adipose tissue for storage. Hence, de novo fatty acid synthesis is negligible. In rats and mice this is not the usual situation. The typical rodent diet is low in fat. Most of the energy comes from carbohydrate and deaminated amino acids (those in excess of need for protein synthesis). In the young animal there is considerable protein synthesis, and with age there is a decline in this synthetic activity. With an age-related decline in protein synthesis there is an increased need to rid the body of amino groups, as the surplus amino acids are deaminated for use in gluconeogenesis and lipogenesis. This means that if the protein intake is not reduced to accommodate the decreased need for protein, there will be an increase in the activity of the urea cycle. Studies in aging rats have shown that dietary intake excess of energy and protein are associated with an age-related increase in urinary protein and renal disease that is preceded by, first, an increase, then a fall, in ureogenesis. Food restriction or protein restriction ameliorates these age-related changes in renal function and urea synthesis. In humans,

age has effects on voluntary food intake. Whether these effects are truly age-related or due to other factors (disease, drugs, loneliness, etc.) is difficult to assess.

All of the age-related changes in intermediary metabolism are linked together by age changes in mitochondrial oxidative phosphorylation that in turn are affected by mitochondrial DNA sequence.[52] Age carries with it a progressive change (increase) in mitochondrial membrane saturation, a progressive loss in ATP synthetic efficiency, and an increase in free-radical damage to mitochondrial DNA and its translation products. These progressive changes mean a progressive loss in the tight control of intermediary metabolism exerted by the concentration and flux of the adenine nucleotides. In turn, then, one might expect to find progressive changes as described above in carbohydrate, lipid, and protein metabolism, in addition to, and in response to, the progressive changes with age in the endocrine system and those changes associated with the central nervous system.

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5 Nutrigenomics and Epigenetics

Over the last few decades, there has been a growing interest in unraveling the mysteries surrounding the individuality of nutrient need and tolerance. Scientists have sequenced the human genome. They have steadily been able to map this genome and identify many of the genes of importance to nutrition and metabolism. Nutritionists have been interested not only in how nutrients affect gene expression but also how small differences in the base sequence of a given gene or in the factors that influence gene expression could affect the gene product. Scientists have been especially interested in how specific nutrients functioned as promoters or suppressors of specific genes. This area of research is quite active.

Biochemical individuality has been known to exist for decades, and nutrition scientists have realized that the recommendations of nutrient intakes must allow for this. Hence, the early intake recommendations were made such that nearly all people could avoid the consequences of deficiency if they followed these intake recommendations. Now, however, scientists have come to realize that some intake recommendations are inappropriate for some people. As mentioned in the chapter on energy, a single recommendation of energy intake might be too little for one individual whereas for another it would be too much. This is perhaps the most interesting example of how nutrients and genetic heritage interact to determine the nutrient status of an individual. Other instances can be cited as well. However, before these instances are listed, it is important to consider how genes are expressed and how nutrients affect this expression.

GENE EXPRESSION

The characteristics of an organelle, a cell, an organ or tissue and, indeed, the whole body within a species is vested in the genetic material, DNA. Most of this DNA is found in the nucleus, whereas a small amount is found in the mitochondria.[1,2] It holds the code for the many thousand proteins that give a cell or an animal or a plant its particular characteristics. It dictates the amino acid sequence of each of these proteins. Should there be a change in the sequence of the nucleotides within the DNA, the sequence of amino acids in the resultant protein could be different. In some instances there are no outward effects of these amino acid changes but in other instances changes in function occur. If the protein whose amino acid sequence has been changed is crucial to normal functioning of the organism, then this change is serious, indeed. If, on the other hand, the protein is of minor importance, then a change in its sequence is of no consequence.

The process of protein synthesis using the sequence of bases in DNA provides the basis for understanding genetic differences. It is also the basis for understanding how the unique properties of each cell type are maintained, as the properties that make cells unique are usually conferred by the proteins within them. Some of these proteins are the structural elements of the cell. Others are enzymes that catalyze specific reactions and processes that characterize the cell in question. Still other proteins confer a particular biochemical function on the cell.

DNA is composed of four bases: adenine, guanine, thymine, and cytosine. These bases are condensed to form the DNA chain in a process analogous to the condensation of amino acids that serve as the primary structure of a protein. Species vary in the percent distribution of these bases in their DNA. The chain of nucleotides that makes up the DNA is formed by joining adenine, guanine, thymine, and cytosine through phosphodiester bonds. The phosphodiester linkage is between the 5′ phosphate group of one nucleotide and the 3′ hydroxyl group of the adjacent

nucleotide. This provides a direction (5′ to 3′) to the chain. A typical segment of the chain is illustrated in Figure 5.1. The hydrophobic properties of the bases plus the strong charges of the polar groups within each of the component units are responsible for the helical conformation of the DNA chain. Hydrogen bonding between the bases stabilizes this conformation, as shown in Figure 5.2. The bases themselves interact so that, in the nucleus, the two chains are intertwined. Hence, the term *double helix* applies to nuclear DNA. In the mitochondria, the DNA is also a double strand but is arranged as a double circle with connections between the light and heavy strands. This structure is illustrated in Figure 5.3.

GENE STRUCTURE

The sequence of amino acids in each protein synthesized by the body is determined from a subunit of the DNA molecule known as the gene. It consists of several thousand bases (abbreviated as kb). Each protein has a unique base sequence in its cognate gene. Although only four bases are used for the DNA, the combinations and sequences of the combinations provide a specific code for each and every protein and peptide. It also functions to transmit genetic information from one generation to the next in a given species. Thus, DNA has a broad spectrum of function: It ensures the identity of both specific cell types and specific species. In the nucleus, the DNA is found in the chromosomal chromatin. Chromatin contains very long double strands of DNA and a nearly equal mass of histone and nonhistone proteins. Histones are highly basic proteins varying in molecular weight from

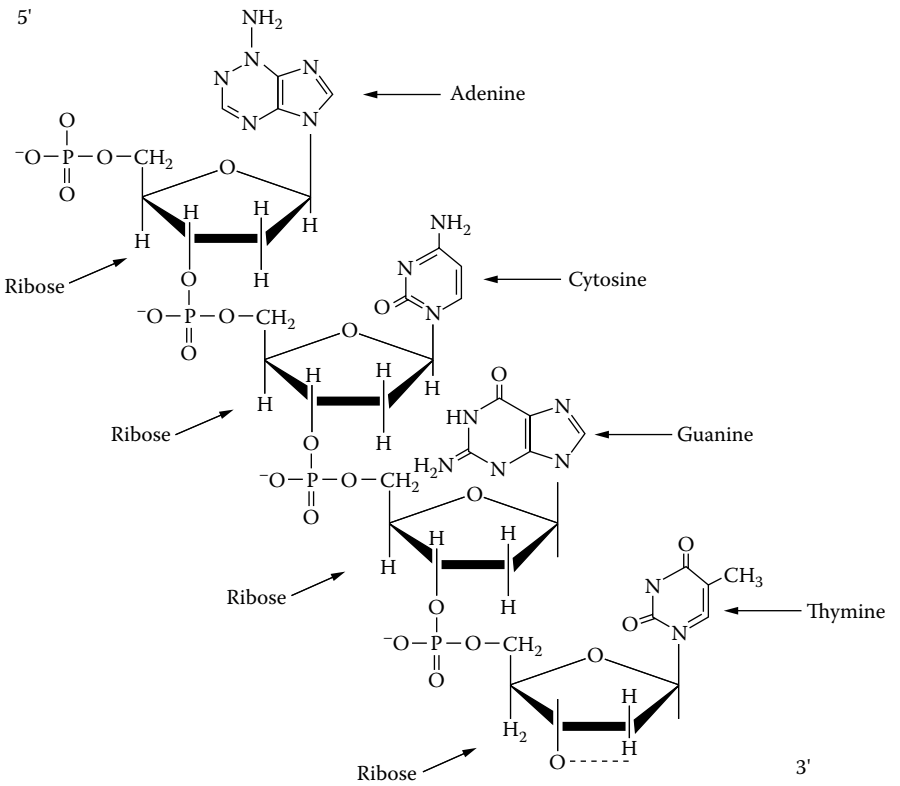


FIGURE 5.1 The bases that make up the DNA polynucleotide chain are joined together by phosphodiester bonds using ribose as the common link between the bases.

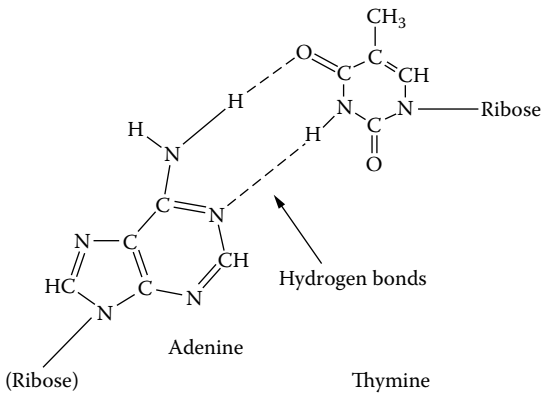


FIGURE 5.2 Hydrogen bonds form between complementary bases: adenine complements thymine; guanine complements cytosine. These bonds stabilize the double-helical array of the two DNA strands.

~11,000 to ~21,000. As a result of their high content of basic amino acids, histones serve to interact with the polyanionic phosphate backbone of the DNA so as to produce uncharged nucleoproteins. The histones also serve to keep the DNA in a very compact form and, as well, to protect this DNA from damage by external agents such as free radicals or viruses or drugs. In mammals, the mitochondrial DNA does not have this protective histone coat.[3] It is "naked" and much more vulnerable to damage. This damage can be quite severe, yet because there are so many copies of the mitochondrial DNA and so many mitochondria in a cell, the effects of this damage might not be apparent.

During cell division, the nuclear DNA, as soon as its replication is completed, becomes highly condensed into distinct chromosomes of characteristic shapes. These chromosomes exist as pairs and are numbered. There are 46 chromosomes in the human. Included in this number are the sex chromosomes, the x and y chromosomes. If the individual has one x and one y he is a male; if two x's are present, she is a female. The chromosomes are the result of a mixing of the nuclear DNA of the egg and sperm. Approximately half of each pair comes from each parent. If identical codes for a given protein are inherited from each parent, the resultant progeny will be a homozygote for that protein. If nonidentical codes are inherited, the progeny will be a heterozygote. Within the heterozygote population, there may be certain codes which are dominant, eye color or hair color, for example. These are dominant traits and are expressed despite the fact that the individual has inherited two different codes for this trait. A mutation in a code that is not expressed is a recessive trait. If, by chance, two identical mutated genes are present that encode a certain protein, the expression of this mutated code will be observed. This is the basis for genetic diseases of the autosomal recessive or dominant type. Autosomal means a mutation in any of the chromosomal DNA except that which is in the X or Y chromosome. A mutation of the DNA in this chromosome is called a *sex-linked mutation*. If it results in a disease, it is called *sex-linked genetic disease*. There is another inheritance pattern based on the mitochondrial genome. Because this genome is primarily of maternal origin, certain of the characteristics of the OXPHOS system will be inherited via maternal inheritance. A number of mitochondrial mutations result in a number of degenerative diseases.

Mutations or Polymorphisms

Changes in the normal sequence of bases in the DNA can occur. If the change results in an aberrant gene product (the protein it encodes) then it is called a *mutation*. If the change has no effect on the gene product then it is called a *polymorphism*. Sometimes a change can occur that results

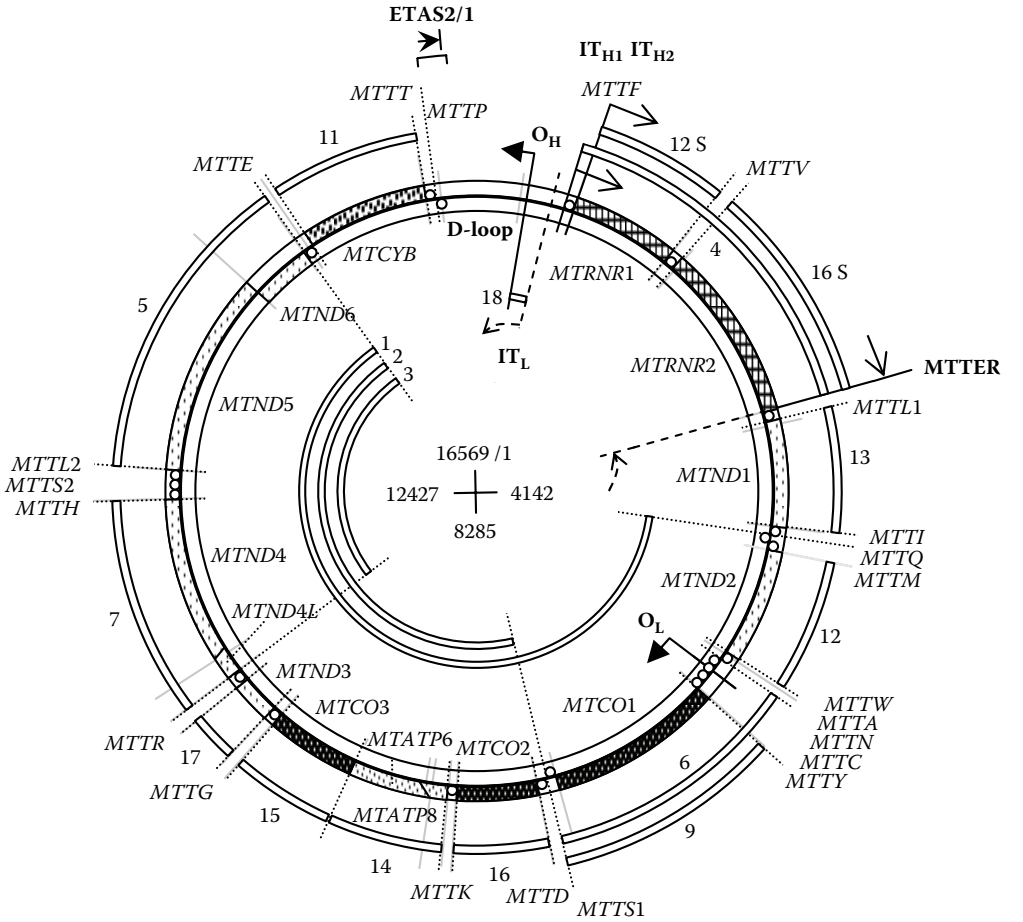


FIGURE 5.3 Genetic and transcriptional map of the human mitochondrial genome.[3] The circle represents the mtDNA duplex. Shaded areas on the outer ring represent those genes encoded on the heavy strand, whereas the light strand is shown as the inner circle. There are 22 tRNA genes shown as open dots. The 7S DNA species is in the control region (the D-loop or the common promoter region for this genome) is shown as a broken line box within the two circles The nucleotides in the human are numbered from 1 to 16,569. Transcripts are numbered according to size, 1 to 18, and include the 12 structural genes that encode subunits of the respiratory chain and the F_1F_0ATPase. These are abbreviated: MTATP6, MTATP8, (subunits of the F_1F_0ATPase) MTND6, MTND5, MTND4, MTND4L, MTND2, MTND1 (subunits of complex 1) MTCO3, MTCO2, MTCO1 (subunits of complex 3). The remaining transcripts are those of the ribosomal proteins (12S and 16S) and the RNA at the R-loop. The site where transcription terminates is indicated by MTERF. This is where the nuclear encoded mitochondrial transcription termination factor binds. (From Taanman J.W., Williams S.L. (2005). In: *Mitochondria in Health and Disease*, C.D. Berdanier (Ed.), Taylor & Francis, Boca Raton, pp. 95–244.

in a change in the amino acid sequence of the gene product but if this change has no effect on the functionality of the product, it, too, is called a polymorphism.

A mutation can be spontaneous or one induced by drugs, a virus, or any one of a number of external variants that target the genetic material of the cell. Mutations can be base substitutions, base rearrangements, base deletions or base additions. In most cases of DNA damage, the damage is random. That is, it does not occur in the same place in every cell. There are some places in the

DNA that are more vulnerable to damage. These are places where there are direct repeats of bases. DNA damage, whether spontaneous or caused by external agents, can be repaired in the nucleus[4] but the mitochondrial DNA has little self-repair.[3] Sometimes repair takes place but is inaccurate. That is, the repair does not fully restore the base sequence to its predamaged state.[4] In this scenario, a base addition or a different base substitution or mismatch repair could occur. This mutation will then become part of the genetic information transmitted to the next generation. Some of the base substitutions have no effect on the gene product. This is because some amino acids have more than one combination of bases (codons) that stipulate a particular amino acid in the gene product (see Table 5.1). Whether the substitution of one or more amino acids for another has an adverse effect on the activity of the protein being synthesized depends wholly on the amino acid in question and on the location of the substitution within the protein structure. For example, a mutation could occur that would result in an amino acid substitution of one neutral amino acid for another (e.g., leucine replacing isoleucine). This substitution might occur in a region outside of the active site(s) of the protein and with little effect on that protein's size and shape. The resultant gene product retains its premutation function, yet has a slightly different amino acid sequence. Such polymorphisms are useful research tools, because they allow population geneticists to track mutation and evolutionary events through related family members. Polymorphisms in the amino acid structures of a given protein occur between species. Sometimes the polymorphism is such that a protein from one species can be given to another with little chance of rejection of that protein by the recipient. Such was the case before the development of human insulin (a biotech reproduction of human insulin). Prior to

TABLE 5.1
Codons for the amino acids

	First base											
Second base	**U**			**C**			**A**			**G**		
U	UUU	Phe		CUU	Leu		AUU	Ile		GUU	Val	
	UUC			CUC			AUC			GUC		
	UUA	Leu		CUA			AUA			GUA		
	UUG			CUG			AUG*	MET		GUG		
C	UCU	Ser		CCU	Pro		ACU	Thr		GCU	Ala	
	UCC			CCC			ACC			GCC		
	UCA			CCA			ACA			GCA		
	UCG			CCG			ACT			GCG		
A	UAU	Tyr		CAU	His		AAU	Asn		GAU	Asp	
	UAC			CAC			AAC			GAC		
	UAA	STOP		CAA	Gln		AAA	Lys		GAA	Glu	
	UAG	STOP		CAG			AAG			GAG		
G	UGU	Cys		CGU	Arg		AGU	Ser		GGU	Gly	
	UGC			CGC			AGC			GGC		
	UGA	STOP		CGA			AGA	Arg		GGA		
	UGG	Trp		CGG			AGG			GGG		

*AUG also serves as a start codon.

this development, insulin was extracted from the pancreas of pigs and cows and prepared for use by insulin-dependent diabetics. Although there were species differences in the amino acid sequence of these insulins, these differences did not prevent their use as a hormone replacement for those who needed it. The species differences were not in the active sites of the hormone nor were they in sites that rapidly induced an immune response.

The synthesis of DNA and RNA depends on the synthesis of the purine and prymidine bases that are building blocks of the nucleic acids. Note that mRNA contains uracil in place of thymidine; the latter is found only in DNA (see below). Purine and prymidine synthesis is nutrient dependent. When insufficient purines and prymidines are provided, cell replacement, growth, and tissue repair is not possible. In this respect one can understand the many symptoms of nutrient deficiency disorders such as pellagra, scurvy, beriberi, and anemia. Short-lived cells that must replicate themselves often will show signs of deficiency far earlier than long-lived cells. Hence, skin lesions and anemia are characteristics of water-soluble vitamin deficiency as well as protein deficiency. Nutrients that can be stored—that is, the fat-soluble vitamins—will have a longer time for symptom development, yet the first signs of deficiency will likewise be seen in the short-lived cells of the epithelia and blood. Many of the micronutrients as well as energy and protein are needed for the synthesis of the purine and prymidine bases that is described as follows:

Synthesis of Purines and Pyrimidines

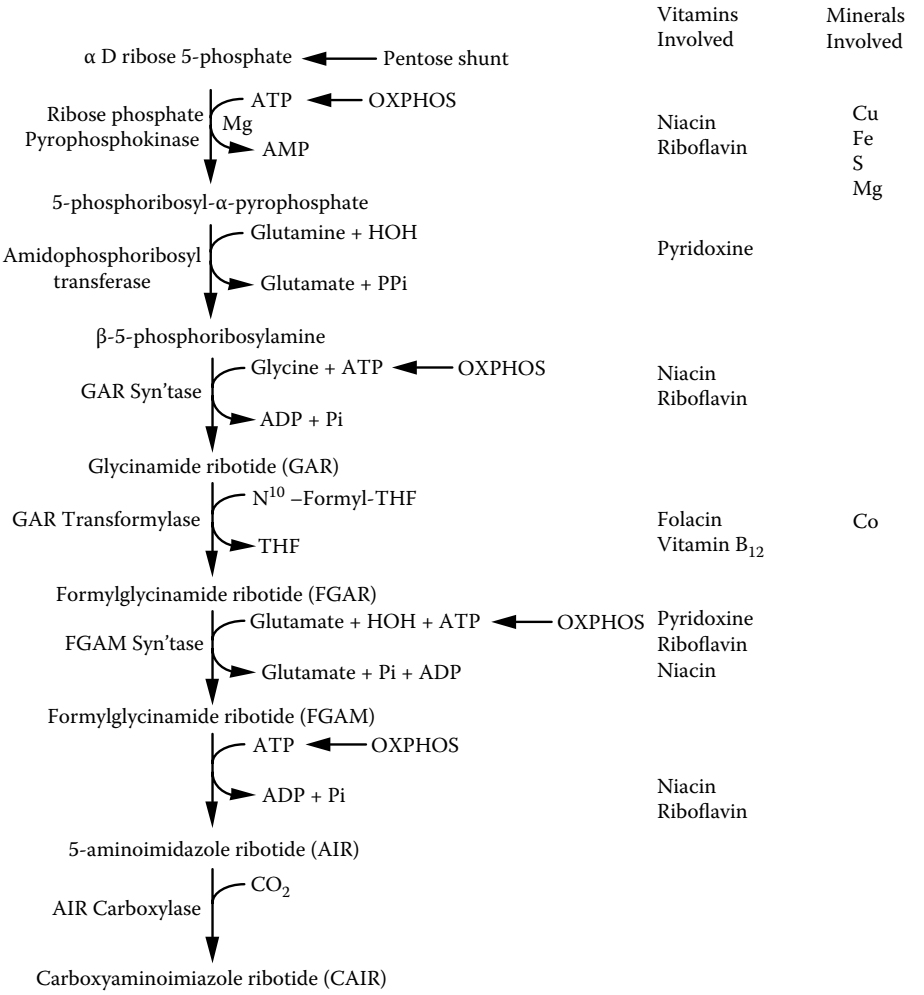
Before pyrimidines and purines can be incorporated into DNA and RNA, they must be synthesized. They are synthesized de novo, and this synthesis requires, both directly and indirectly, a number of vitamins and minerals as well as energy. The purines are adenine and guanine, whereas the pyrimidines are cytosine, uracil, and thymine. Uracil is used for RNA synthesis whereas thymine is used mainly for DNA synthesis. The purines form glycosidic bonds to ribose via the N(9) atoms, whereas the pyrimidines do this using their N(a) atoms. Purine synthesis occurs via the inosine monophosphate pathway (IMP) pathway shown in Figure 5.4. Also shown in Figure 5.4 are the micronutrients needed at each step in the pathway. Where ATP is involved in a reaction step, all of the vitamins that serve as coenzymes in intermediary metabolism are needed. This includes niacin, thiamin, riboflavin, lipoic acid, pantothenic acid, biotin, folacin, vitamin B_{12}, pyridoxine, choline, and inositol. Also needed are the minerals of importance to the redox reactions of oxidative phosphorylation (OXPHOS)—that is, iron, copper and, of course, iodine—as a component of the thyroid hormones (synthesized from the amino acid tyrosine) that regulate OXPHOS, and the selenium-containing enzyme (5′-deiodinase) that converts thyroxine to its active form, triiodothyronine.

Pyrimidine synthesis shown in Figure 5.5. This is a simpler pathway than the purine synthetic pathway. However, one can see where micronutrients are involved here as well. The transamination and one carbon transfer reactions require pyridoxine and folacin, and, of course, all those minerals and vitamins needed as coenzymes for intermediary metabolism are once again called into play so that sufficient energy is available to support the synthetic pathway.

Transcription

Having the codes in the nucleus for the synthesis of protein in the cytoplasm implies a communication between the cytoplasm and the nucleus, and between the nucleus and the cytoplasm. Signals are sent to the nucleus that "inform" this organelle of the need to synthesize certain proteins. Signals include substrates for the needed proteins, nutrients, hormones, and other signaling compounds, some of which have yet to be identified.[5–9] The nucleus directs protein synthesis in the cytoplasm by sending messenger RNA (mRNA) to the cytoplasmic compartment. This process is called *transcription*.

Each gene will have its own unique signals for the initiation of transcription. Some signals are very strong and will stimulate the transcription of a group of related genes. An example of this is



(A)

FIGURE 5.4 Purine synthesis. In this pathway the addition of ribose occurs prior to ring closure and phosphorylation.

the four genes that encode the components of fatty acid synthetase. All are stimulated to transcribe by the same set of signals. Transcription does not occur spontaneously, and it follows that the DNA is quite stable unless signaled to transcribe. Many factors serve to stabilize the structure of the DNA. When DNA is destabilized, transcription is initiated. Unwinding a small portion of the DNA is a necessary step in the initiation of transcription and occurs when the stabilizing factors are perturbed and signals are sent to the nucleus that transcription of a specific gene should begin. Unwinding exposes a small (~17 kb) segment of the DNA (the gene), allowing its base sequence to be available for complementary base-pairing for the synthesis of messenger RNA. The segment that is exposed contains not only the portion of the gene that codes for the corresponding mRNA (*coding region*), but also a sequence called the *promoter region* (Figure 5.6). The promoter region precedes the start site of the coding region, and this is said to be *upstream* of the structural gene. Sequences of bases that bind specific proteins are called *elements.* The proteins that bind to these elements are receptors that in turn bind substances that affect transcription.[6] Some of these substances are nutrients, whereas others may be hormones or other gene activators, enhancers, or silencers. Those

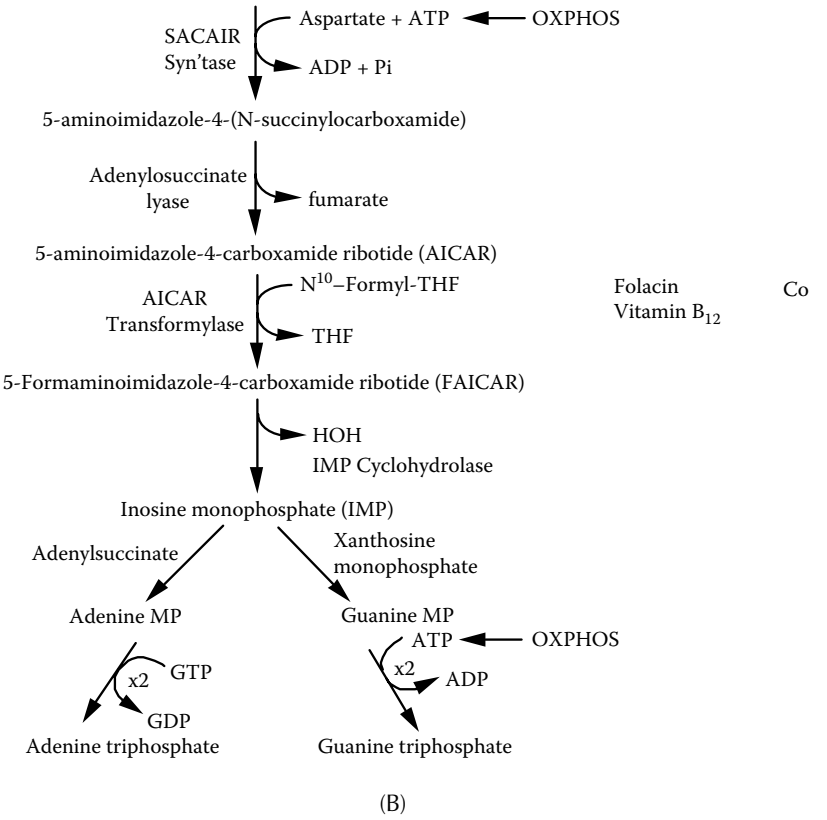


FIGURE 5.4 (continued)

bases following the start site are *downstream*. The nucleotides that code for a specific protein may not be adjacent to each other on the DNA strand but may be located nearby.

Messenger RNA is used to carry genetic information from the DNA of the chromosomes to the surface of the ribosomes (Figure 5.7). It is synthesized through the activity of RNA polymerase II as a single strand in the nucleus.[6–11] Chemically, RNA is similar to DNA. It is an unbranched linear polymer in which the monomeric subunits are the ribonucleoside-5′-monophosphates. The bases include the purines (adenine and guanine) and pyrmidines (uracil and cytosine). Note that thymine is not used in RNA and that uracil is not present in DNA. RNA is single stranded rather than double stranded. It is held together by molecular base pairing and will contract if in a solution of high ionic strength. RNA, particularly the mRNA, is a much smaller molecule than DNA and is far less stable. It has a very short half-life (from minutes to hours) compared to that of nuclear DNA (years). Because it has a short half-life, the purine and prymidine bases must be continually resynthesized.

The synthesis of mRNA from DNA involves three steps: initiation, elongation, and termination. Initiation is the process whereby basal transcription factors recognize and bind to DNA. These factors form a complex with RNA polymerase II. Most of gene expression can be defined as trans-acting factors (proteins) binding to *cis*-acting elements (base sequences) in the promoter regions of genes. Within the promoter, approximately 25 base pairs upstream of the start site, is a consensus sequence called the TATA box, which contain A-T base pairs. One of the basal transcription factors, the TATA-binding protein (TBP), recognizes this sequence of DNA and binds there. This begins the process of transcription initiation. The transacting TBP binds to the *cis*-acting TATA box and a large complex of basal transcription factors, RNA polymerase II, and DNA is formed. Elongation

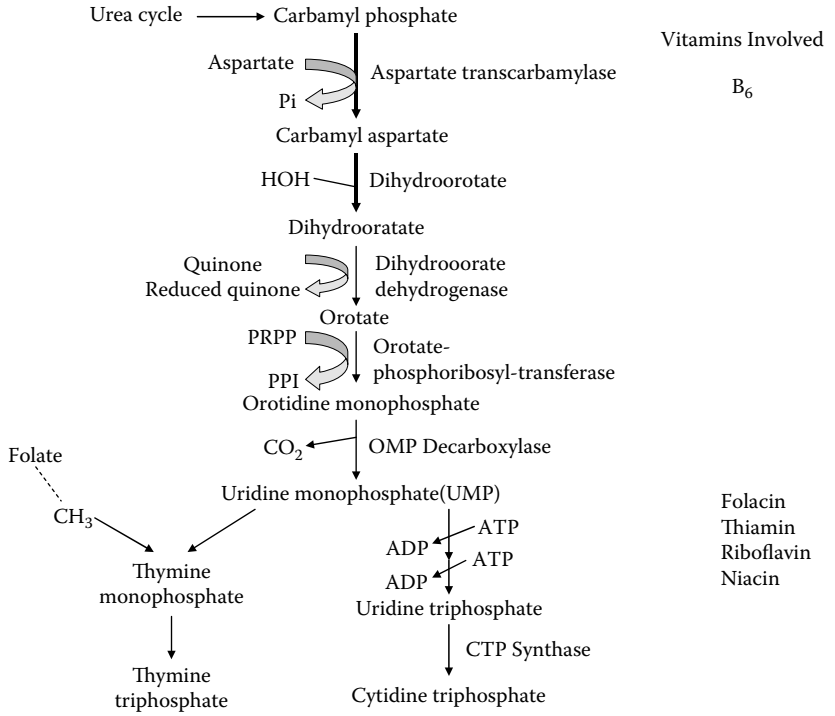


FIGURE 5.5 Pyrimidine synthesis. In this pathway the pyrimidine ring is formed before it is attached to ribose and phosphorylated.

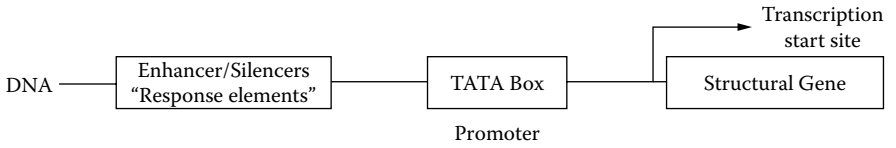


FIGURE 5.6 Detailed structure of the components of a gene that is to be transcribed.

is the actual process of RNA formation through the use of a DNA template in the 5′-to-3′ direction. Shortly after elongation begins, the 5′-end of mRNA is capped by 7-methylguanosine triphosphate. This cap stabilizes the mRNA and is necessary for processing and translation. The third step is the termination of the chain.

Gene expression is a highly controlled process. Its regulation includes transcription control, RNA processing control, RNA transport control, translational control, mRNA stability control, and post-translation control. Each of these control points have nutritionally mediated aspects. In most of the genes studied to date, more nutrients affect transcription than translation or posttranslation processing.

Transcription control is exerted by that portion of the DNA called the promoter region, plus transcription factors that bind either to this region or to an upstream region that in turn affects the activity of either *cis*-acting factors or the polymerase II activity. RNA polymerase II binds to the promoter region just upstream of the start codon for the gene. The promoter region is located in the 5′ flanking region upstream from the structural gene on the same strand of DNA. *Cis*-responsive

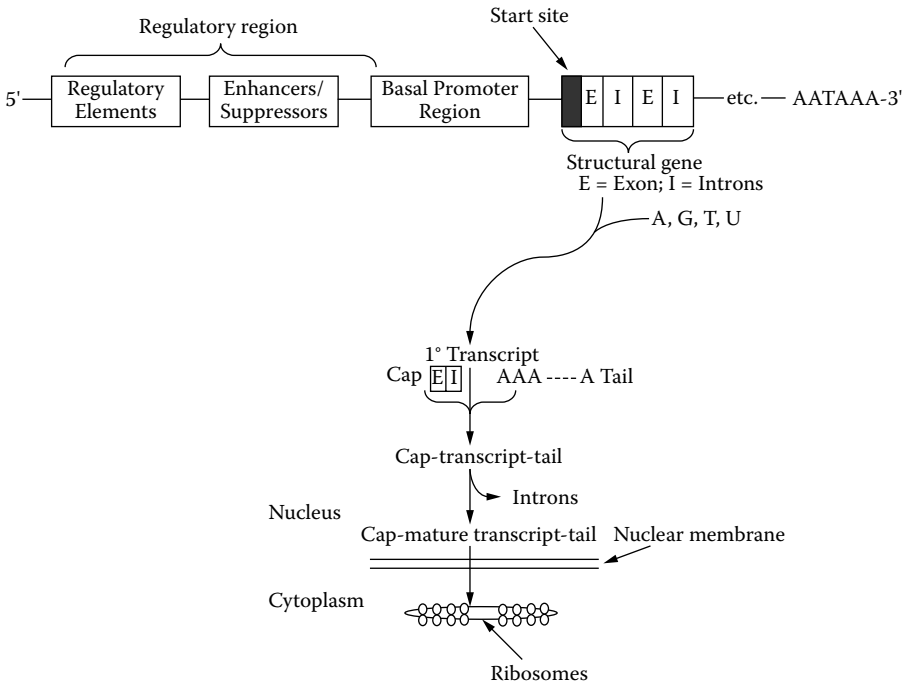


FIGURE 5.7 Detail of the bonding of the zinc ion to histidine and cystine residues of a DNA -binding protein that has zinc fingers.

elements are located about −40 to −200 bp from the start site. Some promoters—for example, TATA, GC, and CCAAT boxes—are common to many genes transcribed by RNA polymerase II. These sequences interact with transcription factors that in turn form preinitiation complexes. The mechanisms of such transcriptional regulation have been reviewed by Semenza[10] and by Johnson et al.[11] *Trans*-acting factors are usually proteins produced by other genes that influence transcription. *Trans*-acting factors can be proteins or peptide hormones or steroid hormone-receptor protein complexes, or vitamin-receptor protein complexes, or minerals, or mineral–protein complexes. The mechanism for binding hormone receptors to specific regions of DNA has been reviewed and described by Freedman and Luisi.[6]

The promoter region contains the start site for RNA synthesis. RNA polymerase II binds to this specific DNA sequence and, under the influence of the various transcription factors, RNA transcription is initiated. The RNA polymerase II opens up a local region of the DNA double helix so that the gene to be transcribed is exposed. One of the two DNA strands acts as the template for complementary base pairing with incoming ribonucleotide triphosphate molecules. The nucleotides are joined until the polymerase encounters a special sequence in the DNA called the *termination sequence*.

At this point, transcription is complete. Following this process, the newly formed RNA is edited and processed. This processing removes nearly 95% of the bases. The resultant shortened RNA then migrates out of the nucleus and becomes associated with ribosomes, whereupon translation takes place.

This outline of transcription has omitted a number of important details with respect to transcription control. For example, the regulation of transcription is exerted by a group of proteins that determine which region of the DNA is to be transcribed. Cells contain a variety of sequence-specific DNA-binding proteins. Nutrients can bind to these proteins and have their effect in this

way. These proteins are of low abundance, and they function by binding to specific regions (elements) on the DNA. The regions are variable in size but are usually between 8 and 15 nucleotides. Depending on the binding protein and the nutrient bound to it, transcription is either enhanced or inhibited, and indeed cell types may differ because of these proteins. Because all cells contain the same DNA, gene expression in discrete cell types is controlled at this point simply by the binding of these very specific DNA-binding proteins. Thus, genes for the synthesis of insulin, for example, could be turned on in the pancreatic β cell, but not in the myocyte, simply because the β cell has the needed specific DNA-binding proteins that the myocyte lacks. At some point in differentiation, the myocyte failed to acquire sufficient amounts of these regulatory factors, and thus can not synthesize and release insulin.

In many instances, specific DNA-binding proteins contain zinc, and the portions on the zinc-contain protein that binds to DNA are referred to as *zinc fingers*.[12,13] Gene expression is regulated by the formation of these zinc fingers, yet they are only a part of this regulation. Most genes are regulated by a combination of regulatory factors. In some, a group of DNA-binding proteins interact to control the activation or inhibition of transcription. Not all of these proteins are of equal power in all instances. There may be a "master" regulatory protein that serves to coordinate the binding of several "lesser" proteins. This is important for the coordinate expression of genes in a single pathway, as happens, for example, in the expression of the genes that encode the multienzyme complex, fatty acid synthetase.

Mutations in genes that encode any one of these transcription factors could result in disease.[14,15] Mutations in genes encoding transcription factors often have pleiotropic effects because these factors regulate a number of different genes. So, too, are the effects of nutrients, which are required components of these transcription factors. An example is the series of genes that encode the enzymes needed for the conversion of a fibroblast to a myocyte.[16] The mammalian skeletal muscle cell is very large and multinucleated. It is formed by the fusion of myoblasts (myocyte precursor cells) and contains characteristic structural proteins as well as a number of other proteins that function in energy metabolism and nerve-muscle signaling. When muscle is being synthesized, all of these proteins must be synthesized at the same time. In proliferating myoblasts, very few of these proteins are present, yet, as these myoblasts fuse, the messenger RNAs for these proteins increase as does the synthesis of the proteins. This indicates that the expression of the genes for muscle protein synthesis is responding to a single regulatory DNA-binding protein. This protein (Myo D1) has been isolated and identified, and occurs only in muscle cells. Should this protein be inserted in some other cell type such as a skin cell or an adipocyte, for example, the same expression will occur. That is, the skin or fat cell will look like a muscle cell. It will take on the characteristics of a myoblast and become a myocyte.

Of interest is the fact that although all of the genes needed for synthesis in the myocyte and its master controller are present, synthesis will not occur or will occur at a very limited rate if one or more of the essential amino acids needed for this synthesis are absent or deficient in the diet. Here is an example of a gene-nutrient interaction that has control properties with respect to muscle protein synthesis, and this interaction ultimately affects the overall process of growth. Turning this situation around, if the master regulator Myo D1 is aberrant, or if one or more of the genes that encode the enzymes needed for protein synthesis in the myocyte have mutated such that the enzyme in question is nonfunctional or only partly functional, muscle development will cease or be retarded. In either instance, abnormal growth will result.

As mentioned, transcription is regulated by both the nearby upstream promoter region and the distant enhancer elements. The upstream enhancer element can include a TATA box and extends for about 100 bp. Enhancer fragments further upstream can bind multiple proteins which, in turn, can influence transcription. These factors are proteins and are labeled JUN, AP2, ATF, CREB, SP1, OTF1, CTF, NF1, SRE, and others.

One well-studied group of DNA-binding proteins is that which binds the steroid hormones.[6,17] These are called the *steroid receptors*, and are members of a group of proteins called the *steroid*

super family of receptor proteins.[17] They bind to specific base sequences called *steroid response elements* (SREs). Included in this super family are receptors for the vitamins A and D, the glucocorticoids, the sex hormones, and the thyroid hormones. This group of receptors is regulated by ligand binding. The ligands are the above-named steroids and hormones. All members of this family of DNA-binding proteins contain two zinc fingers in their DNA-binding domains. Zinc is bound to the histidine- and cysteine-rich regions of the protein that envelops the DNA in a shape that looks like a finger.[12] The zinc finger is shown in Figure 5.8. The zinc ion plays an enormous role in gene expression because of its central use in the zinc finger.

The action of these complexes explain how cells respond to a steroid hormone stimulus. Each receptor protein consists of about 100 amino acids and zinc. As mentioned, they recognize a specific DNA sequence. For some members of this family of proteins, the transcription-enhancing domain is localized at the amino terminus of the polypeptide chain. This is the part of the molecule that binds to the promoter region of the DNA of a specific gene. At the carboxy terminus is the binding site for the hormone. Steroid hormones act in binding to their cognate receptors, which in turn bind to the hormone response element on the DNA. Members of the super family also enhance the transcription of mitochondrial genes.[18,21] The recognition of this function of steroid hormones provides a further explanation of how these hormones function in energy balance. Enhanced mitochondrial gene expression should result in an increased mitochondrial function—that is, enhanced activity of oxidative phosphorylation. In turn, this would result in increased ATP production, which is needed for cell function and tissue growth.

The regulation of transcription occurs at the initiation step.[19–28] The promoter region contains many *cis*-acting elements, each named for the factor that controls them. An element is a particular sequence of bases in the DNA that binds with a specific binding protein. In general, these regions are called *response elements*. Examples include the retinoic acid response element (RARE), heat shock element (HSE), and cAMP response element (CRE). The transacting factors that bind these elements are in general called *transcription factors*. They are also referred to as DNA-binding receptor proteins. They are proteins with at least two domains, DNA-binding and transcription activation. A number of nutrients bind to nuclear receptors that in turn bind to DNA, and together these nutrient-bound receptors affect transcription. Recently, it has been shown that coactivators are needed to bind transcription factors and increase transcription by both interacting with basal transcription factors and altering chromatin structure. Nutrient-dependent DNA-remodeling events (*epigenetics*) also play a critical role in the regulation of transcription. Corepressors act to decrease transcription at both the level of basal transcription factors and chromatin structure. Coactivators and corepressors are proteins.

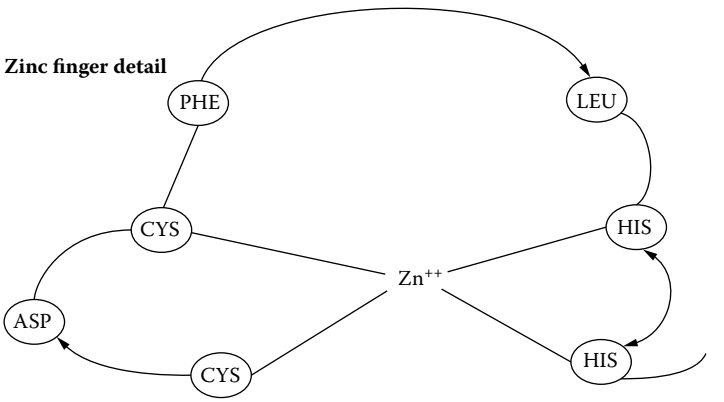


FIGURE 5.8 Synthesis of messenger RNA and its migration to the ribosomes in the cytoplasm.

The true regulation of transcription occurs by the regulation of transcription factors. Transcription factors can be regulated by:

- Their rates of synthesis or degradation
- Phosphorylation or dephosphorylation
- Ligand binding
- Cleavage of a protranscription factor
- Release of an inhibitor

There are other examples of effects of nutrients on gene transcription. It has been shown that supplementation with the vitamin biotin increases the synthesis of transcription factors Sp1 and Sp3,[20] enhancing the transcriptional activity of Sp1-/Sp3-dependent genes. Another mechanism of biotin-dependent gene expression is increased phosphorylation of "inhibitor of NF-κB" in the cytoplasm.[21] Phosphorylation of inhibitor of NF-κB causes its dissociation from NF-κB in the cytoplasm. Subsequently, NF-κB is shuttled to the cell nucleus where it binds to regulatory regions in genes, mediating transcriptional activation of these genes.

Posttranscriptional regulation of gene expression is the next stage of control.[5–11] As mentioned earlier, newly formed mRNA is edited prior to leaving the nucleus. RNA transcription can be terminated prematurely with the result of a smaller-than-expected gene product. A single mRNA can be translated into several different gene products, usually peptides. These proteins or peptides may have comparable or opposing functions, depending on the products in question. As described, messenger RNA is edited and processed such that only ~5% of this RNA leaves the nucleus.[23–27] The 95% that remains is degraded, and the purine and pyrimidine bases are reused or are subject to further degradation. The RNA that leaves the nucleus does so through pores in the nuclear membrane. This is an active process, the details of which are not well understood. Not all of the mRNA that exits the nucleus is immediately translated into protein.

All of these mechanisms serve to control transcription, a vital step in controlling gene expression. Once the bases are joined together in the nucleus to form messenger RNA, the nucleus must edit and process it. Processing it includes capping, nucleolytic, and ligation reactions that shorten it, terminal additions of nucleosides and nucleoside modifications (Figure 5.7). Through this processing, less than 5% of the original RNA migrates from the nucleus to the ribosomes, where it attaches prior to translation. The editing and processing is needed because immature RNA contains all those bases corresponding to the DNA introns. Introns are those groups of bases that are not part of the structural gene. Introns are intervening sequences that separate the exons or coding sequences of the structural gene. The removal of these segments is a cut-and-splice process whereby the intron is cut at its 5′ end, pulled out of the way, and cut again at its 3′ end; at the same time, the two exons are joined. This cut-and-splice routine is continued until all the introns are removed and the exons joined. Some editing of the RNA also occurs with base substitutions made as appropriate. Finally, there is a 3′-terminal poly A tail added.

The editing and processing step is now complete. The mature messenger RNA now leaves the nucleus and moves to the cytoplasm for translation. The nucleotides that have been removed during editing and processing are either reused or totally degraded. Of note is the fact that editing and processing also are mechanisms used to degrade the whole message unit. This serves to control the amount and half-life of this RNA. The endonucleases and exonucleases used in the cut-and-splice processing also come into play in the regulation of mRNA stability. The mRNAs have a very short half-life when compared to DNA and the ribosomal and transfer RNAs. If mRNA half-life is shortened or prolonged, gene expression is affected. Many of the very unstable mRNAs have half-lives in terms of minutes; among these are those which code for short-lived regulatory proteins such as the protooncogenes, fos, and myc. This instability is probably due to an A- and U-rich 3′-untranslated region. Stability of mRNA can be affected by steroid hormones, nutritional state, and drugs.

Some gene products are needed for only a short time. Hormones and cell signals must be short-lived and therefore the body needs to control or counterbalance their synthesis and action. One of the ways to do this is by regulating amount of mRNA (number of copies of mRNA for each gene product) that leaves the nucleus. Thus, this regulation is a key step in metabolic control.

Mitochondrial gene expression. Mitochondrial gene expression is similar to nuclear expression in several respects. Transcription is responsive to some of the same nutrients and hormones that also affect nuclear transcription. Both genomes require binding proteins, but it seems that each compartment has its preferred binding form. Unlike the nuclear genome where each gene has its own promoter region, all of the genes in the mitochondrial genome have a common promoter found in the D-loop.[3] In contrast to nuclear gene transcription and translation that involves both the nuclear and cytoplasmic compartments, mitochondrial transcription and translation occurs totally within the mitochondrial compartment. It is affected by a wide variety of nuclear-encoded transcription factors and nutrients.[3,18,22]

Epigenetic Control of Chromatin Structure and Function

Epigenetics is the study of heritable changes in gene expression that occur without a change in the sequence of the DNA. Epigentic regulation of gene expression occurs at the chromatin level—for example, DNA methylation and histone modification (see below). Chromatin comprises DNA, histones H1, H2A, H2B, H3, and H4, and nonhistone proteins.[29] DNA and histones form repetitive nucleoprotein units, the nucleosomal core particles. Each particle consists of 146 base pairs of DNA wrapped around an octamer of core histones (one H3-H3-H4-H4 tetramer and two H2A-H2B dimers). The DNA located between nucleosomal core particles is associated with histone H1 (Figure 5.9). This 11-nm histone fiber is then further packed into an irregular 30-nm chromatin fiber structure, which is coiled into even more complex structures to eventually assemble the chromosome.

The amino-terminal tails of histones protrude from the nucleosomal surface; covalent modifications of these tails affect the structure of chromatin and form the basis for the epigenetic regulation of chromatin structure and gene function.[30,31] Epigenetics is defined as the study of heritable changes of gene function that occur without a change in the nucleotide sequence of DNA. For example, amino acid residues in histone tails are modified by covalent acetylation, biotinylation, methylation, phosphorylation, and ubiquitination (Figure 5.10) to regulate gene transcription, mitotic condensation of chromatin, and DNA repair.[29–36] These modifications are deciphered by proteins containing motifs that target chromatin. For example, some transcription factors contain bromodomains with affinity of acetylated histones, increasing gene expression.[37] Modifications of distinct amino acid residues in histones have unique functions. For example, tri-methylation of lysine (K)-4 in histone

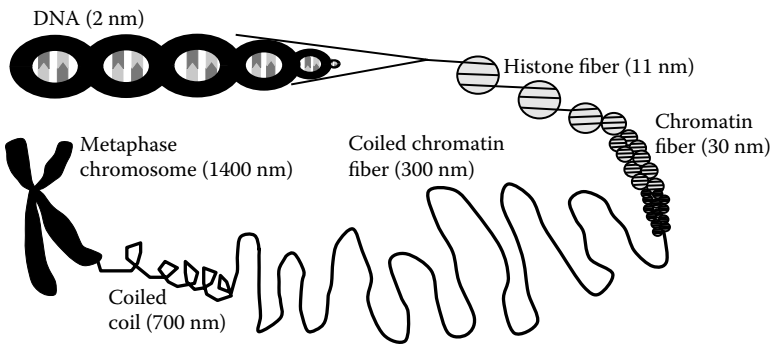


FIGURE 5.9 Chromatin structure.

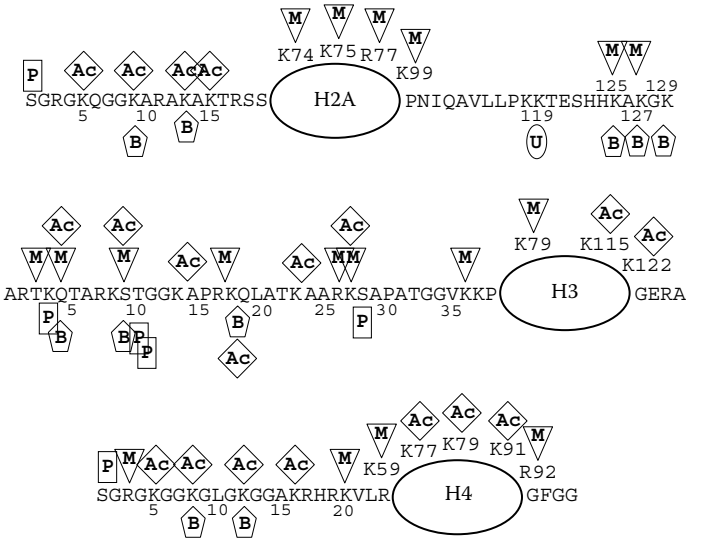


FIGURE 5.10 Modification sites in histones H2A, H3, and H4. Abbreviations: Ac, acetate; B, biotin; M, methyl; P, phosphate; U, ubiquitin.

H3 is associated with transcriptional activation of surrounding DNA, whereas dimethylation of K9 is associated with transcriptional silencing.[32,33] Covalent modifications of histones can be reversed by a large variety of enzymatic processes.[32] A series of vitamin-dependent modifications of histones have been identified: (a) binding of the vitamin biotin to lysine residues in histones (see Chapter 10, "Biotin"); (b) folate-dependent methylation of lysine residues (see Chapter 10, Folate); and niacin-dependent poly(ADP-ribosylation) of glutamate residues (see Chapter 10, Niacin). Acetylation of histones also represents a vitamin-dependant form of chromatin structure regulation, based on the fact that pantothenate-derived coenzyme A is a building block in the formation of acetyl-CoA, which is the substrate for acetylation of histones (see Chapter 10, Pantothenic Acid). However, acetylation of histones does not receive much attention from a nutrition perspective as pantothenic acid deficiency is never a practical issue. This observation notwithstanding, methylation of histones can alter acetylation patterns, and deacetylation of histones is dependant on dietary niacin status (nicotinamide adenine dinuleotide pools), so there are many opportunities for nutrient interactions.

Finally, another folate-dependent epigenetic event has captured scientific interest for decades: covalent binding of methyl groups (derived from folate) to produce 5-methylcytosine in mammalian DNA.[38] This modification of DNA also depends on a number of other nutrients, including *S*-adenosyl methionine, cobalamin (vitamin B_{12}), pyridoxine (vitamin B_6), methionine, betaine, riboflavin (vitamin B_2), zinc, and choline.[38] Methylation of cytosine residues in DNA is associated with repression of transcription (see Chapter 10, Folate).

Translation. Once the mRNA has migrated from the nucleus to the cytoplasm and attaches to ribosomes, translation is ready to begin. Translation is the synthesis of the protein using the order of the assemblage of constituent amino acids as dictated by the mRNA. All of the amino acids needed for the protein being synthesized must be present and attached to a transfer RNA (tRNA). These tRNA-amino acids dock on the mRNA again, using base pairing, and the amino acids are joined to one another via the peptide bond. The newly synthesized protein is released as it is made on the ribosome and changes to its conformation and structure occur. These changes depend on the constituent amino acids and their sequence.[39]

Translation is also influenced by specific nutrients. The translation of the ferritin gene, for example, is influenced by the amount of iron available in the cell.[40] In iron deficiency, the messenger RNA start site for ferritin translation is covered up by an iron-responsive protein. This protein binds the 3′-untranslated region (UTR) and inhibits the movement of the 40s ribosome from the cap to the translation start site. When iron status is improved, the start site is uncovered and translation can proceed. The actual site of translation is on the ribosomes; some ribosomes are located on the membrane of the endoplasmic reticulum and some are free in the cell matrix. Ribosomes consist almost entirely of ribosomal RNA and ribosomal protein. RNA is synthesized via RNA polymerase I in the cell nucleus as a large molecule; in this location, this RNA molecule is cleaved and leaves the nucleus as two subunits, a large one and a small one. The ribosome is reformed in the cytoplasm by the reassociation of the two subunits; the subunits, however, are not necessarily derived from the same precursor.

Ribosomal RNA makes up a large fraction of total cellular RNA. It serves as the "docking" point for the activated amino acids bound to the transfer RNA and the mRNA that dictates the amino acid polymerization sequence. Transfer RNA (tRNA) is used to bring an amino acid to the polysome, the site of protein synthesis. Polysomes are clusters of ribosomes that are associated with the endoplasmic reticulum. Each amino acid has at least one specific tRNA. Each tRNA molecule is thought to have a cloverleaf arrangement of nucleotides. With this arrangement of nucleotides, there is the opportunity for the maximum number of hydrogen bonds to form between base pairs. A molecule that has many hydrogen bonds is very stable. Transfer RNA also contains a triplet of bases known, in this instance, as the *anticodon*. The amino acid carried by tRNA is identified by the codon of mRNA through its anticodon; the amino acid itself is not involved in this identification.

Translation takes place in four stages, as illustrated in Figure 5.11. Each stage requires specific cofactors and enzymes. In the first stage, which occurs in the cytosol, the amino acids are activated by esterifying each one to its specific tRNA. This requires a molecule of ATP. In addition to a specific tRNA, each amino acid requires a specific enzyme for this reaction.

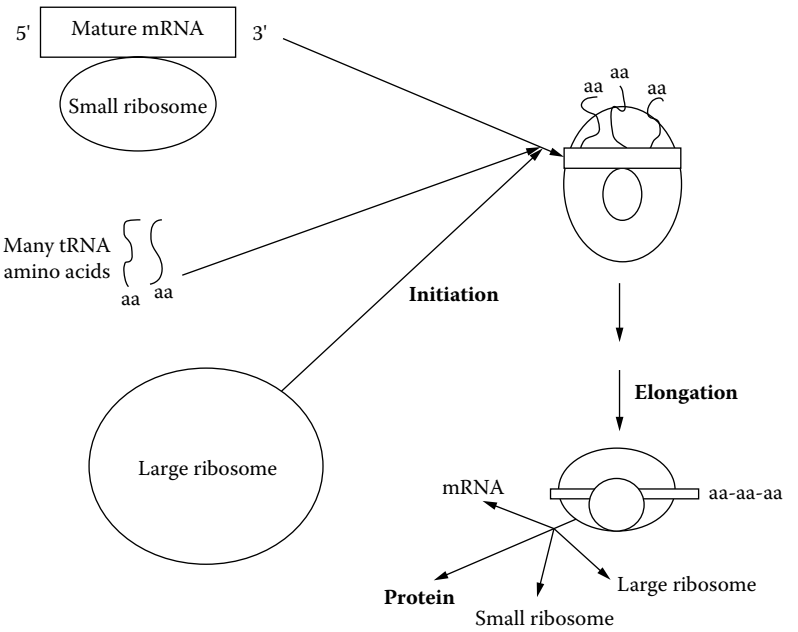


FIGURE 5.11 Overview of translation involving mRNA, tRNA-amino acids, small and large ribosomal units.

During the second stage, the initiation of the synthesis of the polypeptide chain occurs. Initiation requires that mRNA binds to the ribosome. An initiation complex is formed by the binding of mRNA cap and the first activated amino acid-tRNA complex to the small ribosomal subunit. The ribosome finds the correct reading frame on the mRNA by "scanning" for an AUG codon. The large ribosomal unit then attaches, thus forming a functional ribosome. A number of specific protein initiation factors (eIFs) are involved in this step.

In the third stage of protein synthesis, the peptide chain is elongated by the sequential addition of amino acids from the tRNA complexes. The amino acid is recognized by base-pairing of the codon of mRNA to the bases found in the anticodon of tRNA, and a peptide bond is formed between the peptide chain and the newly arrived amino acid. The ribosome then moves along the mRNA; this brings the next codon in the proper position for attachment of the next activated amino acyl-tRNA complex. The mRNA and nascent polypeptide appear to "track" through a groove in the ribosomal subunits. This protects them from attack by enzymes in the surrounding environment.

The final stage of protein synthesis is the termination of the chain. The termination is signaled by one of three special codons (*stop codons*) in the mRNA. After the carboxy terminal amino acid is attached to the peptide chain, it is still covalently attached to tRNA, which is, in turn, bonded to the ribosome. A protein-release factor promotes the hydrolysis of the ester link between the tRNA and the amino acid. Once the polypeptide chain is generated and free of the ribosome, it assumes its characteristic three-dimensional structure. At this point some posttranslational modification can occur. These modifications can include a wide variety of changes. For example, nuclear-encoded proteins needed for the mitochondrial metabolism are synthesized with a leader sequence that allows them to migrate into the mitochondria. This leader is then removed as the oxidative phosphorylation system is assembled or as this protein functions within the mitochondrial compartment. Another example is prothrombin, which is assembled with a large number of glutamic acid residues. In the presence of vitamin K these residues are carboxylated, and this posttranslational change results in a dramatic increase in the calcium-binding capacity of the resultant protein. Unless prothrombin can bind calcium, it cannot function in the clotting process. This is another example of how a nutrient can affect gene expression: in this instance the expression of functional prothrombin. The site of the nutritional effect is that of posttranslational protein modification.

If, during the course of synthesis, there is any interference in the continuity of a supply of the needed amino acids, synthesis is stopped. Because protein biosynthesis is very costly in terms of its energy requirement, synthesis is severely inhibited by starvation or energy restriction. In experimental animals, it has been shown that starvation inhibits the polymerization of mRNA units, thus significantly reducing the activity of the transcription process. Other studies have shown that animals starved and then refed "overcompensate" for this period of reduced mRNA synthesis by markedly increasing mRNA synthesis above normal during the period of realimentation after the starvation period.[41] This "starvation–refeeding"-induced increase in mRNA is manifested as an increase in the synthesis of enzymes necessary for the metabolism of the various ingredients in the diet used for realimentation. The signal(s) for the release of the starvation-induced inhibition of mRNA and enzyme synthesis include the macronutrients in the diet as well as hormones such as glucocorticoids, thyroxine, insulin, and others.[42–44]

Effects of diet on genetic diseases. Some genetic diseases can be treated by dietary choices. For example, mutations of the *biotinidase* gene in humans is associated with decreased recycling of the vitamin biotin in human tissues, leading to increased urinary excretion of biotin metabolites and, hence, biotin deficiency (see Chapter 10, Biotin).[30,39] Afflicted individuals can be treated by lifelong supplementation with pharmacological doses of biotin. A similar example is lactose intolerance, which is caused by absence of the enzyme lactase, leading to decreased tolerance to dietary lactose ("milk sugar") in afflicted individuals. Lactose intolerance (gastrointestinal discomfort, gas, and diarrhea in response to lactose feeding) can be observed in many ethnic groups that do not consume milk or milk products after weaning.[45,46] People with lactose intolerance are symptom-free as long as their diet does not contain significant amounts of milk. In fermented milk products

(e.g., yoghurt), most of the lactose has been metabolized by microbe cultures and, hence, these products are tolerated reasonably well by most people with lactose intolerance.

In contrast to biotinidase deficiency and lactose intolerance, many genetic diseases cannot be influenced by diet.[47] There are a number of reasons for this. First, some gene mutations are lethal to embryos and are associated with spontaneous abortions. For example, there is not one reported case of a living patient with a complete absence of holocarboxylase synthetase, a gene that controls crucial steps in biotin metabolism (see Chapter 10, Biotin). Second, some genes control essential steps in intermediary metabolism, and expression of these genes cannot be sufficiently modulated by diet. For example, expression of interleukin-2 receptor gamma is absolutely critical for immune function,[48] but the expression of this gene can be affected by diet only within narrow limits.[49]

Genetic Diseases of Interest to Nutrition

If there is a mutation in the genetic code for a given protein, the amino acid sequence generated for that protein will be incorrect. Whether this substitution of one amino acid for another in the protein being generated affects its functionality depends entirely on the amino acid in question and its position in the amino acid chain. Some amino acids can be replaced without affecting the secondary, tertiary, or quaternary structures of the protein (and, hence, its chemical and physical properties), whereas others cannot. In addition, genetic errors in amino acid sequence may pose no threat to the individual if the protein in question is of little importance in the maintenance of health and well-being, or it can have large effects on health if the protein is a critical one. In the synthesis of the important protein, hemoglobin, if the genetic code calls for the use of valine instead of the usual glutamic acid in the synthesis of the beta chain in the hemoglobin molecule, the resulting protein is less able to carry oxygen. This amino acid substitution not only affects the oxygen-carrying capacity of the red blood cell but also affects the solubility of the hemoglobin in red blood cells. The decreased solubility of the hemoglobin can be understood if one remembers the relative polarity of the glutamic acid and valine molecules. The glutamic acid side chain is more ionic and thus contributes more to the solubility of the protein than the nonpolar carbon chain of valine. This change in pH decreases its solubility in water, and, of course, a change in solubility leads to an increased viscosity of the blood as the red cells rupture, spilling their contents into the bloodstream.

The amino acid sequence within a given species for a given protein is usually similar. However, some individual variation does occur. An example of an "acceptable" amino acid substitution would be those that account for the species differences in the hormone insulin. As a hormone, it serves a variety of important functions in the regulation of carbohydrate, lipid, and protein metabolism. Yet, even though there are species differences in the amino acid sequence of this protein, insulin from one species can be given to another species and be functionally active. Obviously, the species differences in the amino acid sequence of this protein are not at locations in the chains that determine its biological function in promoting glucose use.

There are a number of metabolic diseases affecting nutrient metabolism that are genetically determined. Most of these are quite rare and most are recessive disorders.[47] Table 5.2 is a list of these disorders and the mutation responsible for the disorder. This is a partial list and not all of the genetic diseases are listed. For some of the disorders, there are more than one mutation associated with the disease. For example, there are a number of genetic mutations in the code for red-cell glucose-6-phosphate dehydrogenase. The code is carried as a recessive trait on the X chromosome and thus only males are affected. These mutations are usually silent. That is, the male, having a defective red-cell glucose-6-phosphate dehydrogenase, does not know he has the problem unless his cells are tested or unless he is given a drug such as quinine or one of the sulfur antibiotics that increases the oxidation of $NADPH^+H^+$. When this happens, $NADPH^+H^+$ is depleted and is not available to reduce oxidized glutathione. In turn, the red cell ruptures. In almost all cases the affected male has sufficient enzyme activity to meet the normal demands for $NADPH^+H^+$. It is only when stressed by these drugs that a problem develops.

TABLE 5.2
Genetic disorders in nutrient metabolism

Disease	Mutated gene	Characteristics
	Amino acid diseases	
"Maple syrup" urine disease	Branched-chain keto acid dehydrogenase	Elevated levels of α-ketoacids and their metabolites in blood and urine; mental retardation, ketoacidosis, early death
Homocysteinemia	Cystathionine synthase	Absence of crosslinked collagen; eye malformations, osteoporosis; mental retardation, thromboembolism and vascular occlusions
Phenylketonuria	Phenylalanine hydroxylase	Mental retardation, decreased neurotransmitter production; shortened life span; six mutations in this gene have been reported
Tyrosinemia	Tyrosine transaminase	Eye and skin lesions, mental retardation
	Fumarylacetoacetate Hydroxylase	Normocytic anemia, leukocytosis, increased serum bilirubin, increased hepatic enzymes, prolonged prothrombin time
Albinism	Tyrosinase	Lack of melanin production; sensitivity to sunlight
Alcaptonuria	Homogentisate oxidase	Elevated homogentisate levels in blood, bones, internal organs; increased susceptibility to viruses and arthritis
Histidinemia	Histidase	Elevated blood and urine levels of histidine; decreased histamine
Hyperprolinemia	Proline oxidase (two mutations)	Mental retardation; seizures
Hyperornithenia	Ornithine-δ-aminotransferase	Increased levels of ornithine in blood; progression vision loss; increased urine loss of ornithine
Urea cycle defects	Arginase Carbamyl phosphate syn'tase Ornithine transcarbamylase Arginosuccinate syn'tase Argininosuccinase	Progressive lethargy; mental retardation; urea cycle failure; symptoms of ammonia toxicity; early death
Hyperlysinemia	α-Aminoadipic semialdehyde synthase	Elevated levels of lysine in blood and urine; condition is generally benign
Hypermethionemia	Methionine adenyltransferase	Benign
Nonketotic hyperglycemia	Glycine cleavage system (three mutations)	Early death; hypoglycemia, mental retardation, seizures
Gout	Unknown	Excess uric acid production; renal uric acid stones; Uric acid crystals accumulate in joints
Lesch–Nyhan	Hypoxanthine phosphoribosyl transferase	Hyperuricemia, choreathetosis, spasticity; mental retardation; self-mutilation; gouty arthritis
	Lipid diseases other than those relating to lipemia	
Tay Sach's	Hexosaminidase A	Early death, CNS degeneration, ganglioside GM2 accumulation
Gaucher's	Glucocerebrosidase	Hepatomegaly, splenomegaly, erosion of long bones and pelvis, mental retardation, glucocerebroside accumulation
Fabry's	α-Galactosidase A	Skin rash, renal failure, pain in legs, ceramide trihexoside accumulation
Nieman–Pick	Sphinogomyelinase	Enlarged liver and spleen, mental retardation, sphigomyelin accumulation

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TABLE 5.2 (continued)
Genetic disorders in nutrient metabolism

Disease	Mutated gene	Characteristics
Krabbe's	Galactocerebroside	Mental retardation, absence of myelin
Gangliosidosis	Gandiioside: β-galactosidase	Enlarged liver, mental retardation
Sandhoff–Jatzkewitz	Hexsaminidase A and B	Same as Tay–Sach's but develops quicker
Fucosidosis	α-L-fucosidase	Cerebral degeneration, spastic muscles, thick skin
Refsum's	α-Hydroxylating enzyme	Neurological problems: deafness, blindness, cerebellar ataxia
	Carbohydrate-related disorders	
Lactose intolerance	Lactase	Chronicc or intermittent diarrhea, flatulence, nausea, vomiting, growth failure in young children
Sucrose intolerance	Sucrase	Diarrhea, flatulence, nausea, poor growth in infants
Galactose intolerance	Galactose carrier	Diarrhea, growth failure in infants, stools contain large amounts of glucose, galactose and lactic acid
Galactosemia	Three genes: Galactose-1-*P*-uridyl transferase	Increased cellular content of galactose-1-phosphate, eye cataracts, mental retardation, cell galactitol; three mutations in this gene
	Galactokinase	Cataracts,cellular accumulation of galactose and galactitol
	Galactoepimerase	No severe symptoms; two mutations in this gene
Fructosemia	Fructokinase	Fructosuria, fructosemia
	Fructose-1-*P*-aldolase	Hypoglycemia, vomiting after a fructose load, fructosemia, fructosuria In children: poor growth, jaundice, hyperbilirubinemia, albuminuria, amino aciduria
	Fructose 1,6-diphosphatase	Hypoglycemia, hepatomegaly, poor muscle tone, increased blood lactate
Pentosuria	NADP-linked xylitol dehydrogenase	Elevated levels of pentose in urine
Hemolytic anemia	Red-cell glucose-6-phosphate dehydrogenase	Low red- cell NADPH, hemolysis of red cell especially with quinine treatment
	Pyruvate kinase	Nonspherocytic anemia, accumulation of glucose metabolites in red cells Jaundice in the newborn
Type VII glycogen	Phosphofructokinase	Intolerance to exercise, elevated muscle glycogen levels, accumulation of hexose monophosphates in muscle
Storage disease		Hexose monophosphates in muscle
Von Gierke's (Type I glycogen storage)	Glucose-6-phosphatase	Hypoglycemia, hyperlipemia, Brain damage in some pts, excess liver glycogen, shortened life span, increased glycerol utilization
Amylopectinoisis (Type IV glycogenosis)	Branching enzyme, hepatic amylo (1,4→1,6) transglucosidase	Tissue accumulation of long-chain glycogen that is poorly branched
Pompe's (Type II glycoenosis)	Lysomal α-1,4-glucosidase (acid maltase)	Generalized glycogen excess in viscera, muscles, nervous system, early death, muscle weakness, hepatomegaly, enlarged heart

TABLE 5.2 (continued)
Genetic disorders in nutrient metabolism

Disease	Mutated gene	Characteristics
	(Amylo-1,6-glucosidase debranching enzyme)	Generalized glycogen excess in viscera, nervous system, muscles, hepatomegaly, enlarged heart
Forbe's (Type III glycogenosis)	Muscle phosphorylase	Tissue accumulation of highly branched glycogen, hypoglycemia, acidosis muscle weakness, enlarged heart
McArdle's (Type V glycogenosis)	Liver phosphorylase	Intolerance to exercise
Her's (Type VI glycogenosis)	Phosphorylase kinase (3 defects reported)	Hepatomegaly, increased liver glycogen content, elevated serum lipids, growth retardation, hyperglycemia
	Micronutrient-related disorders	
Porphyria	Uroporphyrinogen III cosynthase	Increased red cell porphyrin, excess excretion of δ-aminolevulin acid (urine) and porphobilinogen (stool); photosensitivity
	Ferrochelatase	Excess protoporphyrin in stool; photosensitivity
	ALA dehydratase	Neurovisceral symptoms; excess δ-aminolevulinic acid in urine
	Porphobilinogen deaminase	Neurovisceral symptoms; excess δ-aminolevulinic acid and porphobilinogen in urine
	Coporphyrinogen oxidase	Photosensitivity; neurovisceral symptoms; excess δ-aminolevulinic acid, porphobilinogen, and coproporphyrin in urine
	Protoporphyrinogen oxidase	Photosensitivity; neuron and visceral symptoms; excess δ-aminolevulinic acid, porphobilinogen and coproporphyrin in urine; coproporphyrin and protophyrin in feces
	Uroporphyrinogen decarboxylate	Photosensitivity; uroporphyrin and 7-carboxylate porphyrin in urine; isocoproporphyrin in feces (two mutations)
Wilson's	Unknown	Reduction in the rate of incorporation of copper into ceruloplasmin
		Reduction in the biliary excretion of copper; increased urinary copper; excess hepatic copper; liver disease
Menke's	Copper transport	Symptoms of copper deficiency; deficient copper-dependent enzymes
Hemochromotasis	Unknown	Excess serum iron; excess liver iron; excess serum ferritin
Molybdenum cofactor deficiency	Unknown	Mental retardation; deficient sulfite oxidase and xanthine dehydrogenase activity; neuronal loss and demyelination; early death
Rickets	$25(OH)_2$ D-hydroxylase	Vitamin D-deficient rickets
	D-receptor resistance (five mutations)	

Source: Scriver, C.R., Beaudet, A.L., Sly, W.S., and Valle, D., Eds. (1989). *The Metabolic Basis of Inherited Disease,* 6th ed. McGraw-Hill, New York, 3000 pp.

There are other genomic defects that are silent as well. For example, people unable to metabolize the pentoses found in plums and cherries are unaware of their condition. It may come to light if a nonglucose-specific screening test is used for diabetes screening just after the individual has consumed these fruits. There may appear to be an elevated level of sugar in the blood and urine, and diabetes might be thought present. If a specific assay for glucose (glucokinase) is used for the

diabetes screening rather than a nonspecific test, this mistake in diabetes diagnosis will not be made. Another example of a silent genetic disease is that of McCardle's disease.[47] In this disease the individual is intolerant of exercise. This occurs because the individual is unable to use the glycogen in their muscles for fuel. Unless forced to exercise, these people might not be aware of their metabolic defect. They may have adopted a very sedentary lifestyle by unconscious realization of their intolerance.

Unconscious food selection has been observed in children with some of the macronutrient intolerances. Children who are lactose intolerant may refuse to consume milk; those who are gluten intolerant may avoid wheat-containing products and so forth. There may be an instinctive avoidance that helps the individual enjoy their food without serious consequences.

Many of the disorders listed in Table 5.2 have no cure, and many are characterized by a shortened life span. However, for some there are nutrition strategies that may be helpful. Diseases associated with the malabsorption of carbohydrates (i.e., lactose intolerance, galactose intolerance, etc.) can be managed by the omission of these carbohydrates from the diet. Some of the amino acid disorders can be managed by the reduction of the dietary intakes of the particular amino acid in question. For example, phenylkentonuria can be managed by a reduction in the phenylalanine content of the diet. This is rather tricky because enough of this essential amino acid must be provided, but not too much, so that there is a surplus that cannot be appropriately metabolized. In addition, since phenylalanine is used to make tyrosine, this amino acid must then be provided in the diet in sufficient amounts to meet the need.

There are two genetic disorders that have assisted scientists in understanding the function and metabolism of copper (see Chapter 12, Copper). In one, Menke's syndrome, copper absorption is faulty. Intestinal cells absorb the copper but can not release it into the circulation. Parenteral copper corrects most of the condition that resembles copper deficiency, but care must be exercised in its administration. Too much can be toxic. In addition, parenterally administered copper does not reach the brain and cannot prevent the cerebral degeneration and premature death characteristic of patients with Menkes disease.

Another genetic disorder in copper status is Wilson's disease. This condition is also associated with premature death and is due to an impaired incorporation of copper into ceruloplasmin with decreased bilary excretion of copper. This results in an accumulation of copper in the liver and brain. Early signs of Wilson's disease include liver dysfunction, neurological disease, and deposits of copper in the cornea manifested as a ring that looks like a halo around the pupil. This lesion is called the Kayser–Fleischer ring. Renal stones, renal aciduria, neurological deficits, and osteoporosis also characterize Wilson's disease. Periodic bleeding, which removes some of the excess copper, can be helpful in managing Wilson's disease as can treatment with copper-chelating agents such as D-penicillamine and by increasing the intake of zinc which interferes with copper absorption.

Iron is an essential mineral for hemoglobin formation. However, there are a number of genetic reasons why excess iron and subsequent problems are encountered. These are the porphyrias and hemochromatosis. In the latter, excess iron accumulates, and in the former, there are abnormalities in porphyrin metabolism. Some of these disorders are devastating, whereas others are less so. Vitamin D and the uptake of calcium and phosphate are linked. In the absence of active vitamin D rickets develops. Some individuals are unable to activate the vitamin. That is, they do not have normal activity of renal enzyme, $25(OH)_2$ D hydrolase. As a result, these individuals develop vitamin D rickets. The bones are not appropriately mineralized because there is a deficiency of the active vitamin. This can be managed by providing the active form of the vitamin. In addition, there is also a resistance to the activity of active vitamin D. The genetic problem here is a deficiency in the receptor for the vitamin. Five different mutations have been reported that accounts for this problem. Patients with these genetic problems also develop rickets and collectively are referred to as having vitamin D-resistant rickets. Lastly, there are a couple of complex disorders that affect more pathways in addition to those of vitamin D. These include the Franconi syndrome, X-linked hypophosphatemia, and pseudohypoparathyroidism. In each of these there is a disturbed mineralization

of the skeletal system as well as disturbed regulation of calcium status, phosphorus status, and soft tissue mineralization.

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6 Protein

After energy needs are met, protein is the next most important macronutrient in demand by the body. Proteins provide the amino acids that are needed to synthesize body protein. Protein, in its many forms, is an essential and universal constituent of all living cells. As much as one-half of the dry weight of the cell is protein. The human body is, on the average, 18% protein. Besides being plentiful, proteins serve a variety of functions. They function as structural components, as biocatalysts (in the form of enzymes), as antibodies, as lubricants, as messengers (in the form of hormones), and as carriers. Proteins are composed of amino acids that must be provided in food. On the average, Americans consume about 100 g of protein per day. After digestion, the amino acids that compose food proteins are absorbed and used to synthesize body proteins. In this chapter, the chemistry and physiology of the proteins are discussed.

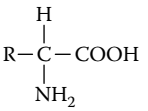
AMINO ACIDS

Chemistry

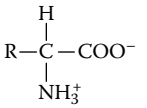
Amino acids consist of carbon, hydrogen, oxygen, nitrogen, and occasionally sulfur. All amino acids with the exception of proline have a terminal carboxyl group



and an unsubstituted amino ($-NH_2$) group attached to the α carbon. Proline has a substituted amino group and a carboxyl group. Also attached to the α carbon is a functional group identified as R; R differs for each amino acid (Table 6.1). The general structure of amino acids can be represented as



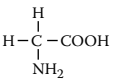
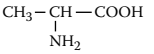
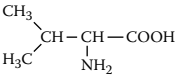
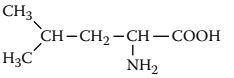
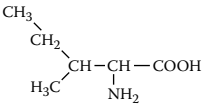
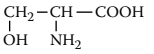
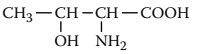
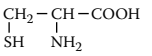
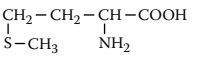
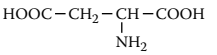
Although it is convenient to represent amino acids in this manner, in reality, amino acids exist as the dipolar ion



in the range of pH values (5.0–8.0) found within the body.

The student will find it useful to remember the basic structure of alanine, and then remember that all of the rest of the amino acids have R groups that replace the terminal methyl group in alanine. For example, in valine, the methyl group is replaced with an isopropyl group; in phenylalanine, it is replaced with a phenyl group. There are several ways to classify the amino acids.

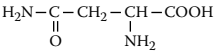
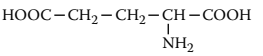
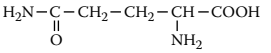
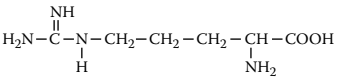
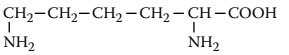
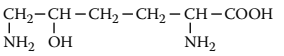
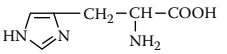
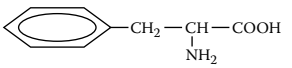
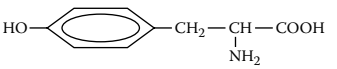
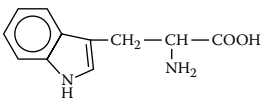
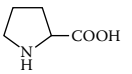
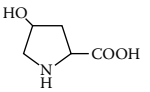
TABLE 6.1
Structures and abbreviations of the amino acids

Name	Abbreviation	Structure
Glycine	Gly, G	
Alanine	Ala, A	
Valine	Val, V	
Leucine	Leu, L	
Isoleucine	Ile, I	
Serine	Ser, S	
Threonine	Thr, T	
Cysteine (Cystein)	Cys, C	
Methionine	Met, M	
Aspartic acid	Asp, D	

Note: The single-letter abbreviations are used as shorthand in delineating large protein sequences.

Protein chemists use the polarity of the R group as the basis for their classification of the amino acids. This classification system divides the amino acids into four groups: (1) nonpolar, (2) polar but not charged, (3) positively charged at pH 6.0–7.0, and (4) negatively charged at pH 6.0–7.0. The distribution of the amino acids into these groups is shown in Table 6.2. This classification system is considered more useful than others because it relates to the functions of the amino acids in protein structures. Another classification that is frequently useful is based on the chemical nature of the amino acids. This grouping is listed in Table 6.3 Nutritionists, although interested in the physical and chemical characteristics of the individual amino acids, classify the amino acids on the basis of whether the body can synthesize them in sufficient quantities to meet its need or whether the diet must provide them. For these purposes, then, amino acids are classified as essential or nonessential. The definition of essentiality rests with the species of animal in question and its physiological need. Felines, for example, require taurine, a metabolite of L-cysteine as a component of their diets. In the adult human, arginine need not be in the diet. However, during periods of high rates of protein

TABLE 6.1 (continued)
Structures and abbreviations of the amino acids.

Asparagine	Asn, N	
Glutamic acid	Glu, E	
Glutamine	Gln, Q	
Arginine	Arg, R	
Lysine	Lys, K	
Hydroxylysine	Hyl	
Histidine	His, H	
Phenylalanine	Phe, F	
Tyrosine	Tyr, Y	
Tryptophan	Trp, W	
Proline	Pro, P	
Hydroxyproline	Hyp	

synthesis—growth, for example—not enough arginine can be synthesized. Additional supplies must then be provided in the diet. Table 6.4 lists the essential and nonessential amino acids for adults.

Occasionally, through a mutation in one or more genes that encode enzymes needed for amino acid interconversion or through a specific illness, certain of the nonessential amino acids cannot be synthesized. In these instances, the amino acid in question then becomes essential and must be provided in the diet. An example of the former is the mutation in the gene for phenylalanine hydroxylase, which results in phenylketonuria (PKU). This mutation is clinically characterized by severe mental retardation. Phenylalanine hydroxylase catalyzes the conversion of phenylalanine to tyrosine. In the patient with phenylketonuria, tyrosine cannot be synthesized and thus becomes an

TABLE 6.2
Classification of amino acids based on polarity of the functional groups

Nonpolar R groups			
Alanine	Phenylalanine	Methionine	Leucine
Valine	Tryptophan	Proline	Isoleucine
Polar uncharged R groups			
Serine	Asparagine	Cysteine	Glycine
Threonine	Glutamine	Hydroxyproline	Tyrosine
Positively charged R groups			
Lysine	Hydroxylysine		
Arginine	Histidine		
Negatively charged R groups			
Aspartic acid			
Glutamic acid			

TABLE 6.3
Amino acids classified according to chemical nature

Monoamino monocarboxylic: glycines, alanine, valine, leucine, isoleucine
Diamino monocarboxylic (basic): arginine, lysine
Monoamino dicarboxylic (acidic): glutamic acid, aspartic acid
Sulfur containing: cystine (and cysteine), methionine
Aromatic: tyrosine, phenylalanine
Heterocyclic: proline, hydroxyproline, histidine, tryptophan

TABLE 6.4
Essential and nonessential amino acids for adult mammals

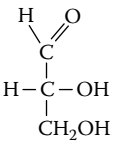
Essential	Nonessential
Valine	Hydroxyproline
Leucine	Cysteine
Isoleucine	Glycine
Threonine	Alanine
Phenylalanine	Serine
Methionine	Proline
Tryptophan	Glutamic acid
Lysine	Aspartic acid
Histidine	Glutamine
Arginine[a]	Asparagine
	Hydroxylysine
	Tyrosine

[a]Not essential for maintenance of most adult mammals.

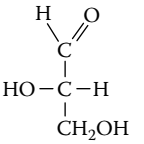
essential amino acid. Phenylalanine metabolites other than tyrosine are made and accumulated, and it is this accumulation of neurotoxic compounds that destroys cells in the brain, which in turn results in the characteristic symptom of phenylketonuria, mental retardation. Care must be taken in the above instance to provide the needed amino acids in the diet in sufficient quantities to maintain tissue protein synthesis without exceeding the body's capacity to utilize phenylalamine. If too much of the amino acid is provided to an individual unable to use it because of a genetic mutation in one or more steps in its metabolic pathway, some unusual metabolites of these amino acids may be formed and these metabolites can be toxic and destructive.

Stereochemistry

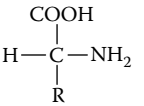
Amino acids, like the simple sugars, exist as stereoisomers. Their absolute configuration, similarly, is related to the configuration of glyceraldehyde. The Fischer projection of D-glyceraldehyde shows the hydroxyl function on the α carbon to the right. At a similar point in a D-amino acid, the amino function is to the right.



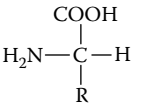
D-glyceraldehyde



L-glyceraldehyde



D-amino acid

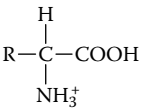


L-amino acid

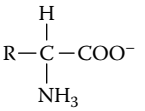
All of the amino acids except glycine (which has no asymmetric carbon atom) possess optical activity. The amino acids of nutritional importance are all L-amino acids, whereas the nutritionally important sugars are of the D-series. Species differences exist in the utilization of L- versus D-amino acids. There are a number of D-amino acids that are of use to single-cell organisms and, further, some D-amino acids combine to form potent antibiotics. Gramicidin D and actinomycin D, for example, contain D-amino acids. Their utility as antibiotics rests with the fact that mammalian cells cannot absorb them as readily as microorganisms. Pathogenic organisms incorporate them into their intracellular material, and these materials then become antimetabolites, successfully terminating the metabolic activity of the pathogen in question.

Acid-Based Properties

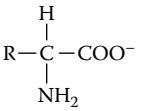
Because amino acids possess acidic carboxyl and basic amino groups, they can function as either hydrogen acceptors or donors. At low pH, amino acids can exist in the fully protonated form:



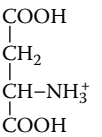
At higher pH levels, H^+ from the carboxyl function will be released and the amino acid exists as the dipolar ion,

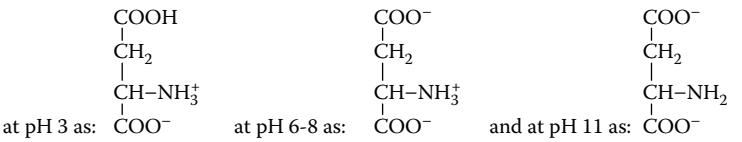


At even higher pH values, the amino function dissociates and the amino acid exists in the negatively charged form



If the amino acid has more than one amino or carboxyl group, further dissociation can occur and the range in pH over which this occurs is much broader. For example, aspartic acid exists at pH 1.0 as:





With each change in the form of the amino acid that occurs, from the lowest to the highest pH, a hydrogen ion is released. The capacity to accept or release hydrogen ions is characteristic of all amino acids; however, only a few (glutamate, aspartate, histidine, and, perhaps, arginine) serve as buffers with respect to the regulation of hydrogen ion concentration in the body. Glutamine is especially important because its metabolism yields bicarbonate, which serves in the regulation of pH. However, because free amino acids are in low concentrations relative to the other buffering systems in the body, their buffering power is much less important than that of the carbonate and phosphate buffering system.

Reactions

The amino acids undergo characteristic chemical reactions at the carboxyl group, at the α-amino group, and at the functional groups of the side chains. Such characteristic reactions are particularly useful to the biochemist, for they assist in the quantitative determination of the amino acid composition and sequence in a given protein. These reactions are summarized in Table 6.5.

Among the reactions that the functional groups on the side chains of the amino acids undergo, those that involve the thiol or sulfhydryl group of cysteine are important. This group is weakly acidic and quite reactive. It is very susceptible to oxidation by either oxygen in the presence of iron salts or by other oxidizing agents. When oxidized, cysteine is converted to cystine. In this conversion, two cysteine residues are joined together by a disulfide (–*S*–*S*–) bridge. Within the

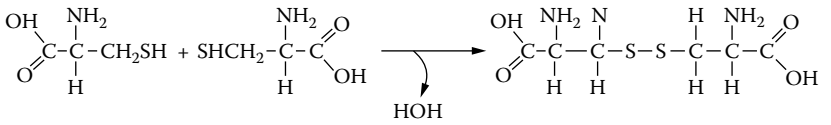
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TABLE 6.5
Characteristic chemical reactions of amino acids

Reaction name	Reagent	Use
Ninhydrin reaction	Ninhydrin	To estimate amino acids quantitatively in small amounts
Sanger reaction	1-Fluro-2,4-dinitrobenzene	To identify the amino-terminal group of a peptide
Dansyl chloride reaction	1-Dimethylamino- napthalene (also called dansylchloride)	To measure very small amounts of amino acids quantitatively
Edmann degradation	Phenylisothiocyanate	To identify the terminal NH_2 group in a protein
Schif base	Aldehydes	To measure labile intermediates in some enzymatic reactions involving α-amino acid substrates

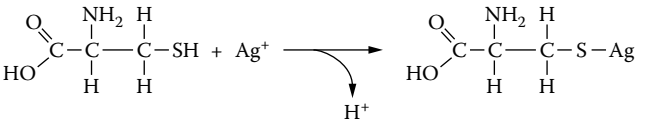
extracellular proteins, sulfhydryl groups react with one another to form disulfide bridges. These bridges stabilize the internal structure of the protein. Sulfhydryl groups also react with heavy metals to form mercaptides. This reaction is of great interest to the nutritionist since protein–mineral interactions, or more truly, mineral–sulfhydryl reactions, are important not only for an understanding of how minerals serve as cofactors in enzymatic reactions and for mineral transport into and out of cells, but also to an understanding of the mechanisms involved in heavy metal intoxication. Figure 6.1 illustrates this reaction.

No discussion of the chemical reactions of the amino acids would be complete without discussing the formation of the peptide bond (Figure 6.2). Without question, this is the most important reaction of these compounds. The formation of the peptide bond involves the removal of one molecule of water with the resultant linkage between the carbon of one amino acid to the amino group of a second amino acid. Water is formed when the hydroxyl ion of the carboxyl group of one amino acid combines with a hydrogen atom from the amino group of a second amino group. Peptide bonding is the basis for the formation of peptides, polypeptides, and proteins and is the linkage used in the primary structure of any sequence of amino acids.



Formation of disulfide bridge

(a)



Formation of a mercaptide

(b)

FIGURE 6.1 Examples of sulfhydryl group reactions.

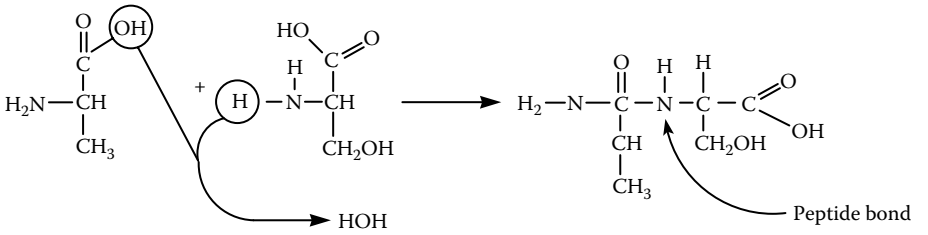


FIGURE 6.2 Formation of the peptide bond. Example: L-alanine + L-Serine → Alanyl-serine (a dipeptide).

The number of possible combinations of the 20 amino acids commonly found in proteins is almost limitless. In a dipeptide that contains two different amino acids (A and B), two combinations are available: A-B and B-A. In a tripeptide with three different amino acids, six combinations are available if all three amino acids are used and each used only one time: A-B-C, B-A-C, A-C-B, B-C-A, C-A-B, and C-B-A. The number of possible combinations of the sequential arrangement of different amino acids is determined by the expression n! (n factorial), when n is the number of different amino acids. If 20 amino acids are present in a protein, the number of possible combinations would be $20 \times 19 \times 18 \times 17 \ldots \times 1 = 2 \times 10^{18}$. The molecular weight of this molecule is about 2,400 (average molecular weight of an amino acid × number of amino acids = 120 × 20), a relatively small protein. If the molecule were larger, and if the amino acids were used more than once, the number of possible combinations is increased even more. That nature can consistently reproduce the same protein, when there are so many choices of amino acids and sequences, is due to its dependence on the codes for each of these proteins in the genetic material, DNA.

Amino Acid Derivatives

Creatine Phosphate

The energy needed to drive the energy-dependent reactions of the body is provided by the high-energy bonds of the adenine nucleotides, the guanine nucleotides, and the uridylnucleotides. The concentrations of these nucleotides are carefully regulated, and their energy is meted out as needed. Short, quick bursts of energy from these nucleotides are not possible. However, Mother Nature has devised another compound, creatine phosphate, that can do just this. Creatine is formed from glycine, arginine, and *S*-adenyl methionine in a reaction sequence as shown in Figure 6.3. The creatine circulates in the blood and can be found in measurable quantities in brain and muscle. The creatine is phosphorylated to form creatine phosphate via the enzyme creatine phosphate. Creatine can be converted to creatinine by irreversible nonenzymatic dehydration. Upon hydrolysis creatine phosphate will provide sufficient energy for muscle contraction. This hydrolysis yields creatine and inorganic phosphate. Some of the creatine can be rephosphorylated, whereas the remainder is converted to creatinine that then is excreted in the urine. Creatine phosphate has the same free energy of hydrolysis as ATP. Creatine phosphate is found primarily in the muscle, and provides the quick burst of energy needed each time a muscle contracts. Although muscle activity produces the end product, creatinine, measuring creatinine allows for the estimation of the muscle mass (see Chapter 1, Body Composition).

Because creatinine excretion is reasonably constant from day to day, researchers use the amount of creatinine in a 24-hour urine collection as an assurance of the completeness of the urine sample. Variations can occur if the usually sedentary subject has an unusually active day. A dramatic increase in muscle use will result in an increase in creatine phosphate breakdown and an increase in creatinine excretion.

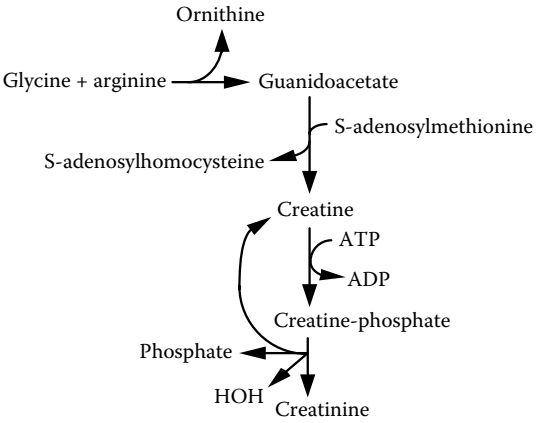


FIGURE 6.3 Formation of creatine phosphate.

Choline

Choline is a highly methylated compound synthesized from serine. Choline is an essential component of the neurotransmitter acetylcholine, as well as an essential ingredient of the phospholipid, phosphatidylcholine.

Polyamines

Certain amino acids can be decarboxylated to form the polyamines. Some polyamines are very short-lived compounds that are neurotransmitters. They are quickly broken down so as to limit their effects. The catecholamines fall into this category of polyamines. Other polyamines, putrescine and spermine, bind nucleic acids and other polyanions. They have a role in cell division.

Peptides

Two amino acids joined together form a dipeptide; three form a tripeptide, and so on. Each amino acid in a chain is referred to as an *amino acid residue*. A chain of up to 100 amino acids joined together is called a *polypeptide*. If many amino acids are involved, then the compound is called a *protein*. Proteins have been identified that have as many as 300,000 amino acid residues and molecular weights in excess of 4×10^7.

The sequence of the amino acids that a given protein comprises is represented by a sequential arrangement of abbreviations for each. For example, the polypeptide bradykinin is represented in the following manner: Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. The right-hand side of the chain represents the carboxyl terminal, whereas the left-hand side represents the amino terminal. The systematic name for bradykinin is arginyldiprolylglyclyphenylalanyl-serylprolylphenylalanylarginine. This systematic name is seldom used except when one wishes to give the amino acid sequence of this peptide. The amino acid sequence of a given peptide or protein can vary and its variation is controlled genetically. Some of the proteins of importance in nutrition have been sequenced but these are few in number compared with the vast array of proteins in nature. Even if the proteins were sequenced, their systematic names would not be used because such a name would be very cumbersome.

Protein Structure

Proteins are complex molecules having characteristic primary, secondary, tertiary, and quaternary structures. The primary structure is determined genetically as the particular sequence of amino acids in a given protein.

Under normal pH and temperature conditions, a protein is characterized not only by its amino acid sequence but also by its 3-D structure; that is, how the chain of amino acids twists and turns and what shape this long chain of amino acids assumes. This 3-D shape, unique to each protein's particular amino acid sequence, is known as the *native conformation* of the protein. This assumption of shape may be spontaneous or may be catalyzed by enzymes, and reflects the lowest energy state of the protein in its native environment. Protein conformation is usually divided into two categories: secondary and tertiary. The secondary and tertiary structures of a protein result from interactions between the reactive groups on the amino acids in the protein.

Secondary structure is the local conformation of the protein molecule. It is due to the formation of hydrogen bonds, disulfide bridges, and ionic bonds (in the case of the polar amino acids) between adjacent or nearby amino acids in an amino acid chain. As a result of these bonds, there is a regular recurring arrangement in space of the amino acids within the chain that can extend over the entire chain or only in small segments of it. Two kinds of periodic structures are found in proteins: the helix and the pleated sheet. In the helix, the amino acid chain can be viewed as wrapping itself around a long cylinder. The most common helical arrays are the α-helix and triple helix. The other periodic shape is the pleated sheet. It is essentially a linear array of the amino acid chain. All of these structures are stabilized by hydrogen bonding.

Tertiary structure is the regional conformation a protein molecule possesses; it develops after the secondary structure is established. Tertiary structure refers to how the amino acid chain bends or folds in three dimensions to form a compact or tightly folded protein. Tertiary structure results from hydrogen bonding, disulfide cross-linkages, ionic bonds between polar amino acids, and interactions between hydrophobic R groups. This last feature tends to locate the hydrophobic R groups internally in the protein structure, away from the aqueous environment. A protein that clearly demonstrates tertiary structure is hemoglobin. Some parts of the amino acid chains in this molecule can form helices; others cannot. This gives the molecule the fluidity to assume different 3-D shapes along the chain: It will bend back upon itself to accomplish the maximum number of hydrogen bonds and disulfide bridges.

As a result of the various bonds that can form within a protein molecule, essentially two kinds of proteins exist: fibrous protein and globular protein. Fibrous proteins resemble long ribbons or hairs. They tend to be insoluble in most solvents and include such tough, resilient protein structures as collagen and elastin. Fibrous protein can have either the helical or pleated-sheet structure. Collagen is an example of the triple helix; silk, a pleated-sheet structure. Where the fibrous proteins appear to have a long "stringy" shape, globular proteins are roughly spherical or elliptical. Many enzymes and antibodies have a globular structure.

The quaternary structure refers to how groups of individual amino acid chains are arranged in relation to each other within a given protein. It is the structure that results when two or more polypeptide chains combine. The chains (subunits) may be different or identical, yet each subunit still possesses its own primary, secondary, and tertiary structure. The number of subunits in a protein may vary; some proteins have only two subunits, whereas others have as many as 2,130 subunits. The protein hemoglobin, for example, consists of four subunits and is one of the few proteins whose primary, secondary, tertiary, and quaternary structures are known. It contains four separate peptide chains: two chains that contain 141 amino acid residues and two chains that contain 146 amino acid residues. To each of these is bound a heme (iron) residue in a noncovalent linkage.

Protein Denaturation

One of the most striking characteristics of proteins is the response to heat, alcohol, and other treatments that affect their quaternary, tertiary, and secondary structures. This characteristic response is called *denaturation*. Denaturation results in the unfolding of a protein molecule, thus breaking its hydrogen bonds and the associations between functional groups; as a result, the 3-D structure is lost. Denaturation affects many of the properties of the protein molecule. Its physical shape is changed, its solubility in water is decreased, and its reactivity with other proteins may be lost. When denatured, the protein loses its biological activity. Heating will denature most proteins. As little as 15°C can denature some proteins, whereas the majority of food proteins are denatured at heats in excess of 60°C. Some proteins are very heat stable (those found in thermophillic bacteria, for example), whereas others are quite labile. A very good example of protein denaturation is the coagulation of egg white when heated. Heat denaturation, unless extreme, does not affect the amino acid composition of protein and, indeed, may make these amino acids more available to the body because heating provokes the unfolding or uncoiling of the protein and exposes more of the amino acid chain to the action of the proteolytic digestive enzymes. For this reason, many cooked proteins are of higher biological value than those same proteins if consumed without heat treatment. If only mild denaturation occurs, it can be reversed. This process is called *renaturation*. If a protein is renatured, it will resume its original shape and biological activity.

Classification of Proteins

Classification by Solubility and Prosthetic Groups

In addition to the conformational classification of proteins as described above, proteins have been classified on the basis of their solubility characteristics. As more and more information has been acquired about proteins, this classification system has become outmoded. However, because the vocabulary from this system has become so firmly entrenched in discussions of protein, it is necessary to be familiar with it. In this system, proteins are classed as simple or conjugated proteins. Simple proteins are those that contain only L-amino acids or their derivatives and no prosthetic group. Such proteins as albumin, histones, and protamines are examples of simple proteins.

Conjugated proteins contain some nonprotein substances linked by a bond other than an ionic bond. Because most proteins occur in cells in combination with prosthetic groups, conjugated proteins are the ones most nutritionists will recognize. Table 6.6 and the following text describe representative conjugated proteins.

TABLE 6.6
Some conjugated proteins

Name	Prosthetic group	Example
Lipoproteins	Neutral fats, phospholipids, cholesterol	Cell membranes; Blood-lipid-carrying proteins
Nucleoproteins	Nucleic acid	Chromosomes
Phosphoproteins	Phosphate joined in ester linkage	Milk casein
Hemoproteins	Iron	Catalase, hemoglobin, the cytochromes
Flavoproteins	Flavin adenine nucleotide (FAD)	FAD-linked succinate dehydrogenase
Metalloproteins	Metals (not part of a nonprotein prosthetic group)	Ferritin
Glycoproteins	Carbohydrates	α,β,γ-Globulins
Mucoproteins	Carbohydrates, hexosamines	Mucin

Glycoproteins

A majority of the naturally occurring conjugated proteins are glycoproteins.[1] Sugar molecules are covalently bound to proteins, especially those proteins that are secreted from cells (mucin, for example) and those that compose the proteins found in the outer surface of the plasma membrane. Different types of covalent linkages have been found. The most common are the *N*-glycosidic linkages formed between asparagine amide and the sugar. Another common linkage is the *O*-glycosidic linkage between either the serine or threonine hydroxyl group and a sugar. Glycoproteins include the mucin in saliva as well as the conjugated proteins of plasma, collagen, ovalbumin (the major protein of egg white), and plant agglutinins. The glycoproteins range in size from a molecular weight of 15,000 to more than one million; the carbohydrate component of these proteins varies from 1% to 85%. Some of these proteins have amino acids and uronic acids and are called *proteoglycans*.

Only 8 of the 100 or so carbohydrates that are known to occur in nature are found in glycoproteins (Table 6.7). These carbohydrates occur in chains containing no more than 15 saccharide units. Of the 20 amino acids in these proteins, only four actually bind to the carbohydrate moiety. The carbohydrates are linked to these amino acids by a nitrogen-oxygen glucosidic bond or through an oxygen bond. Some proteins contain small amounts of carbohydrate in loose association rather than as integral and characteristic parts of their structure. An example of this association is the glycosylated hemoglobin found in the blood of poorly controlled diabetics. In diabetes, blood glucose levels may fluctuate and exceed the normal range of 80–120 mg/dL. Some of this excess of glucose may be picked up by the hemoglobin and form glycosylated hemoglobin. Levels of glycosylated hemoglobin are used as indicators of the degree of control of the diabetic state. The function of the carbohydrate moiety of glycoproteins is not well defined. Some of the glycoproteins, those located on the exterior aspect of the plasma membrane are part of the cell-recognition system. Others are essential to the immune mechanism as a component of globulin. Glycoproteins are essential components of membrane transport systems and are components of many receptors.

Lipoproteins

Lipoproteins are multicomponent complexes of lipids and protein that form distinct molecular aggregates with approximate stoichiometry between each of the components.[2] In addition to protein, they contain polar and neutral lipids, cholesterol, or cholesterol esters. The protein and lipid are held together by noncovalent forces. The protein component (apolipoprotein) is located on the outer surface of the micellular lipid structure, where it serves a hydrophilic function. Lipids, primarily hydrophobic molecules, are not easily transported through an aqueous environment such as blood. However, when they combine with proteins, the resulting combination becomes hydrophilic and can be transported in the blood to tissue that can use or store these lipids. The importance of these lipoproteins as carriers is discussed later in Chapter 8.

Membrane lipoproteins, like the glycoproteins, are essential components of membrane transport systems and, as such, are important in the overall regulation of cellular activity.

TABLE 6.7
Components of glycoproteins

Sugars found in glycoproteins		Amino acids that bind to carbohydrates in glycoproteins
Glucose	Acetylglucosamine	Asparagine
Galactose	Acetylgalactosamine	Serine
Mannose	Arabinose	Threonine
Fucose	Xylose	Hydroxylysine

Nucleoproteins

Nucleoproteins are combinations of nucleic acids and simple proteins. The protein usually consists of a large number of the basic amino acids. Nucleoproteins are ubiquitous molecules that tend to have very complex structures and numerous functional activities. All living cells contain nucleoproteins. Some cells, such as viruses, seem to be entirely composed of nucleoprotein.

Other Conjugated Proteins

The phosphoproteins and the metalloproteins are associations of proteins with phosphate groups or such ions as zinc, copper, and iron. The association of protein with phosphate may be fairly loose, as with the phosphate-carrying protein, or tight as with the phosphate in casein and the iron in ferritin.

Heme proteins sometimes are grouped with the metalloproteins because of the iron they contain. Flavoproteins are primarily enzymes and have as their prosthetic group a phosphate containing adenine nucleotide, which functions as an acceptor or donor of reducing equivalents.

Classification by Function

In addition to the system described above for the classification of proteins, the biochemist classifies these compounds on the basis of their function. Thus, proteins are classified as enzymes, storage proteins such as casein or ferritin, transport proteins such as hemoglobin, DNA-binding proteins such as the various transcription factors, contractile proteins such as myosin, immune proteins such as antibodies, toxin proteins such as the *Clostrodium botulinum* toxin, hormones such as insulin, receptor proteins such as the insulin receptor, intracellular transporters such as the mobile glucose transporters, and structural proteins such as elastin and collagen. Nutritionists might not use this system for classifying food proteins, yet will want to understand these functions as part of their knowledge about the protein nutrient class.

Classification by Nutritive Value

In nutrition, we are interested in food proteins as sources of needed amino acids.[3,4] Those proteins that contain the essential amino acids in the proportions needed by the body are referred to as *complete proteins*. They are primarily of animal origin. Eggs, cheese, milk, meat, and fish are sources of complete protein. Proteins lacking in one or more essential amino acids or having a poor balance of amino acids relative to the body's need are *incomplete or imbalanced proteins*. These proteins are usually of plant origin, although some animal proteins are incomplete. The connective tissue protein called collagen, from which gelatin is prepared, lacks tryptophan; zein, the protein in corn, is low in lysine as well as tryptophan. Table 6.8 gives the protein content of some foods in the human diet, and Table 6.9 gives the amino acid content of several food proteins. When food selection is limited and there is a shortage of high-quality protein-rich foods, incomplete proteins can be combined so that all of the essential amino acids are provided. For example, corn or wheat and soy or peanut proteins can be combined in the same meal so that all of the essential amino acids are provided. When these proteins are combined and consumed in sufficient amounts they will meet the amino acid needs of the consumer. This combination of incomplete proteins must be consumed within a relatively short time interval (less than 4 hours) to obtain the appropriate and needed amounts of amino acids. Maximum benefit is obtained when the combination is consumed at the same time. Supplementation of incomplete proteins with missing amino acids has been suggested for populations consuming diets having a single dietary item as its main protein source. This supplementation is not very practical over a long period of time due to the cost of the pure amino acid supplement. Such populations are also likely to develop other nutritional disorders when their food supply is so limited.

TABLE 6.8
Protein content of representative foods in the human diet

Food (serving size)	Protein, grams
Milk, 244 g (8 oz)	8
Cheddar cheese, 84 g (3 oz)	21.3
Egg, 50 g (1 large)	6.1
Apple, 212 g (1–3¼ in. diameter)	0.4
Banana, 74 g (1–8¾ in. long)	2
Potato, cooked, 136 g (1 potato)	2.5
Bread, white, slice, 25 g	2.1
Fish, cod, poached, 100 g (3½ oz)	20.9
Oysters, 100 g (3½ oz)	13.5
Beef, pot roast, 85 g (3 oz)	22
Liver, pan fried, 85 g (3 oz)	23
Pork chop, bone in, 87 g (3.1 oz)	23.9
Ham, boiled, 2 pieces, 114 g	20
Peanut butter, 16 g (1 T)	4.6
Pecans, 28 g (1 oz)	2.2
Snap beans, 125 g (1 C)	2.4
Carrots, sliced, 78 g (½ C)	0.8

Source: www.nal.usda.gov/fnic/foodcomp/Data/foods,82 nutrients.

Through selected plant breeding and the use of biotechnology some of these plant foods can be improved to provide a better array of amino acids in their edible portions. A corn variety containing more lysine has been developed. This high-lysine corn shows promise for populations that have corn as a major food component. Other plant species have also been improved with respect to their amino acid content. However, some of these improved varieties may have special cultural requirements that economically challenged farmers cannot meet. Some of these cultivars may require added fertilizer, a very expensive item in such a farmer's budget. Agronomists, plant scientists, and agricultural economists continue to work to improve the nutritional value of the crops raised as well as improve crop yield. With judicious planning of food choices, nonetheless, it is possible to meet the protein and amino acid needs of populations subsisting on plant foods with little food from animal sources. Vegetarians, those who abstain from consuming nonplant foods, are particularly challenged in meeting their amino acid needs. Soy protein, peanut protein, dried beans, peas, lentils, and other protein containing plant foods need to be combined to provide the appropriate amounts of the essential amino acids. For example, soy protein is low in methionine whereas corn is not. Corn is low in tryptophane and lysine. These two plant foods could be consumed in the same meal to provide all the needed amino acids.

In addition to the amino acid content, protein quality—or rather the quality of the food containing the protein—is classed according to its total protein content. Potatoes, for example, contain a very good distribution of essential and nonessential amino acids, yet because the potato contains so little protein (1.7%), it is not considered a good protein source. One would have to consume a lot of potatoes (3.18 kg or ~ 7 lb) to meet one's daily amino acid and total nitrogen requirements.

TABLE 6.9

Average amino acid content of selected foods (mg/100 g)

Food	Tryptophan	Threonine	Isoleucine	Leucine	Lysine	Methionine	Cysline	Phenylalanine	Tyrosine	Valine	Arginine	Histidine	Alanine	Aspartic acid	Glutamic acid	Glycine	Proline	Serine
Milk	90	294	407	626	496	156	57	309	325	438	233	168	220	465	1491	126	709	376
Cheddar cheese	87	237	430	622	468	166	36	342	305	458	233	208	179	372	1745	98	731	384
Whole egg	103	311	415	550	400	196	146	361	269	464	410	150	0	438	773	221	265	525
Beef	73	276	327	512	546	155	79	257	212	347	403	217	361	583	946	387	308	262
Lamb	81	286	324	484	506	150	82	254	217	308	407	174	349	576	948	365	289	250
Bacon	65	210	274	500	403	97	73	298	161	298	427	169	0	589	702	589	331	242
Chicken	76	266	330	452	549	163	84	246	220	307	395	180	0	614	1004	418	0	0
Fish	62	271	317	472	548	182	84	232	169	333	352	0	0	551	796	345	381	193
Baked beans	61	295	314	524	381	64	19	359	179	336	270	20	0	0	0	0	0	0
Pecans	78	219	312	436	245	86	122	318	178	296	668	154	0	0	0	0	0	0
White bread	61	189	288	448	151	95	134	312	163	292	228	129	180	286	1980	202	675	0
Corn meal	38	249	289	810	180	116	81	284	382	319	220	129	622	776	1103	212	522	353
Rice	64	233	279	513	235	107	81	299	272	416	343	100	0	281	815	407	288	302
Banana	95	0	0	0	289	55	0	0	162	0	0	0	0	0	0	0	0	0
Oranges	39	0	0	0	221	33	0	0	0	0	0	0	0	0	0	0	0	0
Peas	52	229	287	390	295	50	68	240	152	256	555	102	183	596	442	202	0	0
Brussels sprouts	63	218	264	276	280	66	0	210	0	274	396	150	0	0	0	0	0	0
Potatoes	67	246	274	311	333	78	60	276	112	334	308	90	292	0	625	0	208	250

Source: From Handbook #8, Amino Acid Composition of Foods, USDA, U.S. Government Printing Office, Washington, D.C.

Protein Analysis

The total protein content of food is estimated from the total nitrogen content of the food as determined by the classical Kjeldahl method. This method also determines nonprotein nitrogen as well; however, the amount of error in the method due to the inclusion of these compounds is very small. Most proteins contain about 16 percent nitrogen. To convert the nitrogen content to protein, one uses the following formula:

$$P_G = N_G \times 100/16 = N_G \times 6.25$$

where

P_G = grams of protein in 100 grams of food

N_G = grams of nitrogen in 100 grams of food

This conversion factor is an average factor. If more exact figures are required, established conversion factors for each food category are available. For example, cereals generally have less protein nitrogen and more nonprotein nitrogen, thus, the conversion factor of 5.7 is used for cereal foods. On the other hand, milk has more protein nitrogen and the factor of 6.4 can be used. Generally speaking, because humans usually consume a mixed diet, the lower and higher factors tend to average out, and the value of 6.25 is acceptable when the total protein content of a day's food is determined in this way.

The amino acid determination of a protein has two phases: qualitative identification and quantitative estimation of the residues. The peptide bond that connects the amino acid residues is cleaved by acid, base, or enzyme-catalyzed hydrolysis to give a mixture of amino acids. The free amino acids are separated from one another and identified using chromatographic or electrophoretic techniques. Once separated and identified, each amino acid present can be determined quantitatively. Several of the reactions given in Table 6.5 can be used. These assays do not establish the sequence of the amino acids or the protein's primary structure, but merely tell how much of each amino acid is present. The sequence of amino acids can be determined by cleaving (one by one) the amino acids in the chain and following this cleavage with an analysis of the individual amino acids. Usually high-performance liquid chromatography (HPLC) is used for this aspect of sequence analysis.

Biological Value of Dietary Protein (BV)

Although the total protein (nitrogen) content can be readily determined, as can the amino acid content of the food, albeit with greater difficulty, the determination of the BV of a given protein within a food is far more difficult.[3] Biological value means how well the food is digested and absorbed and how well the component amino acids meet the amino acid needs of the consumer. The BV of a food protein depends not only on its amino acid content, but also on the needs of the consumer.[5] For example, the BV of a food for a rapidly growing child is quite different from that for a nongrowing adult. Growth carries with it a demand for particular amino acids as part of the total nitrogen requirement, whereas maintenance (as in the adult, nongrowing animal) has a total nitrogen requirement with less stringent demands for specific amino acids.

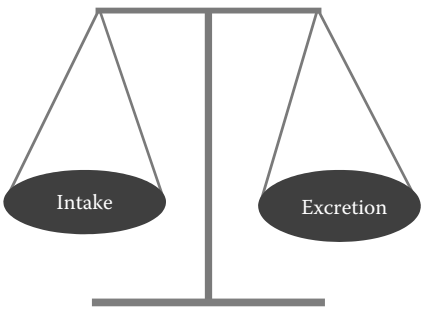
There is also a species dependence to BV.[6] Chickens, because they grow feathers, need more sulfur-containing amino acids in their diets than humans. Thus, proteins having a higher proportion of sulfur-containing amino acids will have relatively higher biological values for chickens than for other species. Rats, the usual test animals in nutrition studies, grow fur, which contains a lot of

arginine. This means that proteins rich in arginine will have a higher BV for rats than for humans. A number of methods for assessing BV have been used. Each has its advantages and disadvantages.

Using a holistic approach to assessing protein quality, H.H. Mitchell devised the nitrogen balance technique in 1924. This technique was based on Folin's definitions of endogenous and exogenous nitrogen excretion. By definition, the endogenous nitrogen comes from the nitrogen-containing excretory products synthesized in the body and not recycled. It is an unavoidable nitrogen loss. Exogenous nitrogen was defined as those nitrogen-containing products that are excreted in direct proportion to the amount of nitrogen consumed in food. These are arbitrary definitions, and there is some crossover between categories. They are, however, useful in the context of evaluating the biological usefulness of dietary protein. Endogenous nitrogen comes from the breakdown of body tissue and represents nitrogenous compounds produced as a result of one-way reactions. For example, when muscles contract, creatine phosphate breaks down to creatine and phosphate. Although both the creatine and the phosphate can be recycled, some of the creatine is converted to creatinine and excreted in the urine. Its excretion is relatively constant, reflecting the muscle mass of an individual who is a normal healthy adult following a fairly regular daily routine. Other nitrogenous compounds considered to be in the endogenous category are uric acid, allantoin, 3-methyl histidine, and ammonia. Regardless of the dietary protein intake, excretion of these compounds by normal individuals with fairly uniform daily activity levels is relatively constant.

In contrast, exogenous nitrogen fluctuates in response to the dietary protein intake. The main compound providing this nitrogen is urea. Urea results when excess amino acids are deaminated. The body converts the $-NH_3$ to urea via the urea cycle and excretes the urea in the urine. Exogenous nitrogen is also found in the feces and represents undigested food protein. Fecal nitrogen represents not only the undigested and unabsorbed food but also the nitrogen of intestinal flora, desquamated intestinal cells, and intestinal enzymes. There are species differences in these excretory patterns. Mammals excrete primarily urea, but birds excrete uric acid as their major nitrogenous excretory product. Figure 6.4 illustrates the principle of nitrogen balance.

These definitions, developed initially by Folin, were used by Thomas and Mitchell[7] when they devised the nitrogen balance technique for evaluating protein quality. The method assumes that a given protein, when fed at maintenance levels, will completely replace the protein being catabolized during the normal course of metabolic events in the body. It also assumes that all nitrogen gain and loss can be measured. Thus, for good-quality proteins, nitrogen balance (intake versus excretion) should be zero for an adult individual and for poor-quality proteins, nitrogen balance will be negative. For growing individuals, good-quality proteins result in high nitrogen retentions, whereas poor-quality proteins result in low nitrogen retentions. The amount of protein retained can be determined by analyzing the total nitrogen content of the food, the feces, and the urine.



Nitrogen balance exists when intake equals excretion

FIGURE 6.4 Nitrogen balance exists when the diet contains sufficient high-quality protein to provide amino acids for the synthesis of proteins lost through degradation.

The BV was conceived as a ratio of the nitrogen retained to that absorbed multiplied by 100. In the original method, animals were fed a nitrogen-free diet for 7–10 days, then fed a diet containing the test protein at a level commensurate with their protein maintenance requirements for the same time interval. During each of these periods, the urine and feces were collected and analyzed for total nitrogen. Knowing the nitrogen intake and the nitrogen excretion during both the nitrogen-free and test periods, the biological value was calculated as follows:

$$BV = 100 \times N \text{ retained/N absorbed} = 100 \times N_I\text{-}(N_{FT}\text{-}N_{FF}) - (N_{UT} - N_{UF})/N_I - (N_{FT} - N_{FF})$$

Where

BV = biological value
N_I = nitrogen intake
N_{FT} = fecal nitrogen during test period
N_{FF} = fecal nitrogen during nitrogen-free period
N_{UT} = urinary nitrogen during test period
N_{UF} = urinary nitrogen during nitrogen-free period

The method makes a lot of assumptions about overall protein metabolism, and these assumptions can account for some of the error associated with evaluating the quality of the test protein in this way. The method is noninvasive and very useful for work with humans. However, nitrogen can be lost via routes other than urine and feces. In hot climates or in physically active subjects, significant nitrogen losses can occur through sweat. On an adequate protein intake, it has been estimated that up to 10 g N/L can be lost. On an inadequate or low-protein diet, sweat losses can amount to 5 g N/L. Hair, nail, and menstrual losses can also contribute error to the nitrogen balance technique. Usually sweat, hair, skin, and nail losses are ignored since they are minor in comparison with the urine and fecal losses but, as mentioned, they do contribute to the error term for the method. Other errors in the method include the possibility of daily cumulative errors in the collection and analysis of the food, urine, and feces and the effects of prior poor nutritional status on the responses of the subject to this procedure. Good subject cooperation is essential to insure quantitative ingestion of the food and quantitative collection of the urine and feces.

The main disadvantage of this method is theoretical in character. The basic assumption of the "replaceability" of body protein by a food protein is valid only when comparing good-quality proteins. When poor proteins such as zein, gelatin, or gluten are evaluated, unrealistically high values result. This overestimation results from the failure of the method to account for the mobilization of body protein to meet particular amino acid needs. If there is a deficiency of one or more of the essential amino acids in the test protein, the animal will catabolize body proteins in an effort to provide the missing amino acid to support the synthesis of vital short-lived proteins such as enzymes or hormones. There appears to be a hierarchy of body proteins; those that are most essential to the survival of the animal are synthesized in preference to those, such as muscle protein, that are not as essential. Similarly, those that are most essential to survival are "protected" from catabolism in favor of those proteins less essential to life. In any event, when the body proteins are mobilized, the needed amino acids they contain are utilized and the remaining ones are deaminated and used for energy. The amino group is then converted to urea and excreted, contributing to the urinary nitrogen level. The Mitchell nitrogen balance technique does not differentiate the source of the nitrogen in the excreta and, because of this, the technique is not as valid in evaluating incomplete proteins. Source differentiation could be accomplished through the use of isotopic markers that would allow the investigator to identify body protein as distinct from food protein. Heavy isotopes incorporated into the food protein as it is grown could identify the food nitrogen that was excreted. The use of heavy isotopes, however, is expensive as well as time consuming, so this variation in the Mitchell technique might not be frequently used. In efforts to circumvent the problem of overestimating the

biological values of incomplete or imbalanced proteins, other methods—some of which are variations of the original nitrogen balance technique—have been proposed and used.

The nitrogen balance index (NBI) of Allison and coworkers relates the absorbed nitrogen to the nitrogen excretion of a separate but concurrent group of individuals fed a nitrogen-free diet. This technique requires less time than the Thomas-Mitchell method, but is subject to many of the same kinds of errors. Both methods suffer from the inaccuracies contributed by the methodological measure of the so-called endogenous nitrogen loss. Both methods assume that this loss is represented by the nitrogen excreted by the animal during the nitrogen-free period, when actually the feeding of such a diet accelerates protein loss rather than modeling the endogenous nitrogen excretion.

The dynamic state of the body proteins—that is, the constant need to synthesize proteins having a short half-life (see Chapter 4 for discussion of half-life)—must be considered. This synthesis must be accommodated by the catabolism of tissue protein, a catabolic process unlikely to occur extensively if good-quality proteins are consumed. Because this overestimation of endogenous loss is more serious with proteins of poor quality, inconsistent biological values are obtained. This is particularly true when unrefined proteins or food mixtures are evaluated.

A more accurate method for the evaluation of protein quality is one that actually measures the retention of nitrogen in the carcass from the ingested protein nitrogen. This is called the NPU-BV or NPU method. Using these methods that calculate BV from the change in carcass protein, groups of animals, usually rats, are fed diets containing graded amounts of the test protein or a nitrogen-free diet. After a period of 7–10 days, the animals are killed and the nitrogen content of the carcasses determined. Obviously, proteins of high quality will evoke a greater retention of nitrogen in the carcass than proteins of poor quality. Using this technique, biological value can be calculated as follows:

$$\text{NPU-BV} = B_F - B_K + I_K/I_F \times 100$$

Where

B_F = carcass nitrogen of animals fed test-protein diet
B_K = carcass nitrogen of animals fed nitrogen-free diet
I_K = absorbed nitrogen of animals fed nitrogen-free diet
I_F = absorbed nitrogen of animals fed test-protein diet

Although this method has the obvious advantage of actually measuring nitrogen retention, its disadvantages are also obvious. Feeding a protein-free diet induces an exaggerated body protein loss. This method is inappropriate for large animals because of the technical difficulties associated with the determination of body composition and because of the excessive cost. Obviously, too, human studies would not be possible. However, conceptually, the method has merit and several investigators have devised variations that are useful in a variety of species.

One variation that is useful in humans is to measure the changes in body composition indirectly using heavy or radioactive isotopes. The various methods for determining body composition changes (see Chapter 1, Body Composition) can be used to evaluate proteins. For example, Steffee et al. used a stable isotope of nitrogen, ^{15}N, to study the rates of total body protein synthesis and breakdown as a response to variation in amounts of dietary protein. Constant infusions of ^{15}N-glycine allowed these investigators to assess the value of given proteins in the homeostatic situation where there is constant protein synthesis and breakdown. This is a very useful technique, but it is also very expensive and requires sophisticated techniques and equipment to make the appropriate measurements.

Another variation of the carcass-retention method uses the naturally occurring radioactive isotope ^{40}K. The method measures the change in ^{40}K concentration in the adult body as a result of consuming a given protein for a period of 7–10 days. This method is based on the constancy of potassium as an intracellular ion. The concentration of potassium in the body can be directly related to the number of cells in the body and indirectly related to the protein in the body. Because a set percentage of this potassium exists as the naturally occurring radioactive isotope, measuring ^{40}K

levels is a direct measure of body cell number and an indirect measure of body protein. If a poor-quality protein is consumed, ^{40}K levels will fall. If a good-quality protein is consumed, there will be no change. Again, although this method has the advantage of its applicability to humans, its disadvantage is one of cost and availability of the whole-body counters needed to determine the presence of the isotope.

By far the easiest variation of the carcass-retention method is the protein efficiency ratio (PER). In this method, carcass composition is not determined, and it is assumed that the gain in body weight of a given animal is related to the quality of the protein fed. Thus, young growing animals are fed test protein-containing diets for a period of 28 days. The weight gain is computed and divided by the total protein intake.

The formula, PER = Weight gain (g)/Protein intake (g) is easy to use and the method requires no specialized expensive equipment. This is the method used for protein quality evaluation by most food companies and has been adopted by the regulatory agencies of the United States and Canada as their method of choice in evaluating the nutritional quality of foods. The ease and simplicity of the method, however, should not lull the reader into thinking that it is a "choice" method. PER can vary from species to species and, within a given species, from strain to strain. Examples of within-species variation in the PER of a given protein is shown in Table 6.10. Seven different strains of rats were fed the same diet for 4 weeks. PER was calculated at the end of each week. As can be seen in this table, the PER varied from week to week and between the seven strains. Variation can be introduced if levels of protein intake higher or lower than 10% by weight are used. In addition, the methods make no allowance for the maintenance requirement of the animal. Values obtained from a variety of food proteins are nonlinear. That is, a protein having a PER of 2 may not have twice the nutritional value of a protein having a PER of 1. Examples of the PER for a variety of food proteins is shown in Table 6.11.

A modification of the PER is the net protein ratio. This method attempts to account for the maintenance needs of the animal. In this method, two groups of animals are used. One is fed a nitrogen-free diet, the other the test diet. This modification is accompanied by all the pitfalls of using the nitrogen-free diets that were discussed above.

A variety of methods employing the assay of enzymes concerned with protein metabolism have been devised. The determination of the activity of transaminase, xanthine dehydrogenase, renal arginase, and others have been reported as indicators of protein quality. Unfortunately, these methods have not been rigorously tested and compared with the presently available whole-body methods.

TABLE 6.10
The PER of a test protein as calculated using the weight gain of rats from seven different strains

	PER			
Rat strain	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Holtzman	4.14	3.94	3.61	3.50
Charles River	4.29	3.64	3.54	3.60
Sprague–Dawley	4.31	3.76	3.32	3.36
Osborne–Mendel	4.20	3.52	3.24	3.39
Wistar	4.47	3.59	3.33	3.37
Wistar Lewis	3.69	3.64	3.37	3.28
SSB/PL (NIH)	3.80	3.37	2.96	2.47

TABLE 6.11
PER of various foods

Eggs	3.9–4.0
Fish	3.5
Milk	3.0–3.1
Beef	2.3
Soybeans	2.3
Beans	1.4–1.9
Nuts	1.8
Peanuts	1.7
Gluten	1.0
Rice	2.0
Corn	1.2

The holistic approach, although valuable and useful, is nonetheless time consuming and not particularly good for poor-quality proteins. It also is expensive. The approaches discussed here do have the advantage of digestibility and amino acid availability being given due consideration.

Attempts to circumvent the time and expense of whole-animal work have resulted in a number of techniques. One method, the amino acid score or chemical score is a nonbiological method and requires the amino acid analysis of the test protein. The amount of the most limiting amino acid (only the essential amino acids are considered) is related to the content of that same amino acid in a reference protein. In most cases, this reference is egg protein; however, a theoretical protein based on the amino acid requirements of the species in question could also be used.

This method is quick and does not use animals, but it makes no allowance for digestibility or availability of the constituent amino acids. This is a rather important aspect of protein nutrition. Some protein foods are poorly digestible; others contain compounds that interfere with absorption. For example, some amino acids form sugar–amino acid complexes that render the amino acids less available to the body. This occurs with the browning of baked goods such as bread; although bread is not considered a prime protein source, evaluation of its protein quality using the amino acid score would be in error due to the browning reaction.

A variation on this chemical method tries to account for digestibility.[8] In this method, test proteins are first digested in vitro using conditions resembling those in the gastrointestinal tract. The amino acid score is then determined on the products of this digestion. Another method is a variation of the amino acid-digestibility method. In this method the metabolic availability of essential amino acids is assessed. This amino acid oxidation indicator method has been used to evaluate the metabolic availability of sulfur amino acids in casein and soy protein.[4] It is likely other essential amino acids can be evaluated this way, and the method might be useful in determining the BV of a variety of dietary proteins.

Protein Use

Digestion

The daily protein intake plus that protein that appears in the gut as enzymes, sloughed epithelial gut cells, and mucins, is almost completely digested and absorbed. This is a very efficient process that ensures a continuous supply of amino acids to the whole-body amino acid pool. Less than 10% of the total protein that passes through the gastrointestinal tract appears in the feces. If the food contributes between 70 and 100 g of protein and the endogenous protein contributes another 100 g

(range: 35 to 200 g) then about 1–2 g of nitrogen might be expected to be found in the feces. This is equivalent to 6–12 g protein. Of the dietary protein, the fecal protein might include the hard to chew or digest, tough, fibrous, connective tissue of meat or nitrogen-containing indigestible kernel coats of grains or particles of nuts that are not attacked by the digestive enzymes. For example, whole peanuts have a structure that is difficult to broach by the digestive enzymes. Unless chewed very finely, much of the nutritive value of this food may be lost. Peanut butter, on the other hand, is very well digested because its preparation ensures that its particle size is very small and is thus quite digestible.

The purpose of protein digestion is to liberate the amino acids from the consumed proteins. Except for the period shortly after birth, the enterocyte cannot absorb intact proteins. Prior to gut closure, the neonate can absorb some proteins. Most of these proteins are immunoglobulins. The proteins found in the colostrum, or first milk, provide passive immunity to the newborn. After gut closure, only amino acids and small peptides can pass from the lumen of the gut into the bloodstream. Thus, the food proteins must be hydrolyzed into their component amino acids, dipeptides, and tripeptides. This is accomplished through a series of enzymes that have specific target linkages as their point of action. These enzymes are summarized in Table 6.12. The protein hydrolases, called peptidases, fall into two categories. Those that attack internal peptide bonds and liberate large peptide fragments for subsequent attack by other enzymes are called the *endopeptidases.* Those that attack the terminal peptide bonds and liberate single amino acids from the protein structure are called *exopeptidases.* The exopeptidases are further subdivided according to whether they attack at the carboxy end of the amino acid chain (carboxypeptidases) or the amino end of the chain (aminopeptidases). The initial attack on an intact protein is catalyzed by endopeptidases, and the final digestive action is catalyzed by the exopeptidases. The final products of digestion are free amino acids and some di- and tripeptides that are absorbed by the intestinal epithelial cells.

In contrast to carbohydrate and lipid digestion, which is initiated in the mouth with the salivary amylase and the lingual lipase, protein digestion does not begin until the protein reaches the stomach and the food is acidified with the gastric hydrochloric acid. The HCl serves several functions. It acidifies the ingested food, killing potential pathogenic organisms. Unfortunately, not all pathogens are killed. Some are acid resistant or are so plentiful in the food that the amount of gastric acidification is insufficient to kill all of the pathogens.

Hydrochloric acid also serves to denature the food proteins, thus making them more vulnerable to attack by pepsin, an endopeptidase. Actually, pepsin is not a single enzyme. It consists of pepsin A, which attacks peptide bonds involving phenylalanine or tyrosine, and several other enzymes that have specific attack points. The pepsins are released into the gastric cavity as pepsinogen. When

TABLE 6.12
Digestive enzymes and their target linkages

Enzyme	Location	Target
Pepsin	Stomach	Peptide bonds involving the aromatic amino acids
Trypsin	Small intestine	Peptide bonds involving arginine and lysine
Chymotrypsin	Small intestine	Peptide bonds involving tyrosine, tryptophan, phenylalanine, methionine, and leucine
Elastase	Small intestine	Peptide bonds involving alanine, serine, and glycine
Carboxypeptidase A	Small intestine	Peptide bonds involving valine, leucine, isoleucine, alanine
Carboxypeptidase B	Small intestine	Peptide bonds involving lysine and arginine
Endopeptidase, Aminopeptidase, Dipeptidase	Cells of brush border	Di- and tripeptides that enter the absorptive cells

the food entering the stomach stimulates HCl release and the pH of the gastric contents falls below 2, the pepsinogen loses a 44 amino acid sequence. The activation of the pepsins from pepsinogen occurs by one of two processes. The first, called autoactivation, occurs when the pH drops below 5. At low pH, the bond between the 44th and 45th amino acid residue falls apart and the 44 amino acid residue (from the amino terminus) is liberated. The liberated residue acts as an inhibitor of pepsin by binding to the catalytic site until pH 2 is achieved. The inhibition is relieved when this fragment is degraded, as happens at pH 2 or below or when it is attacked by pepsin. Because the fragment binds at the catalytic site of pepsin, this can happen. The other process is called *autocatalysis* and occurs when already active pepsin attacks the precursor pepsinogen. This is a self-repeating process that ensures ongoing catalysis of the resident protein. The cleavage of the 44 amino acid residue, in addition to providing activated pepsin, has another purpose. That is, it serves as a signal peptide for cholecystokinin release in the duodenum. This, then, sets the stage for the subsequent pancreatic phase of protein digestion. As described in the units on lipids and carbohydrates, cholecystokinin stimulates both the exocrine pancreas and the intestinal mucosal epithelial cell to release its digestive enzymes. The intestinal cell releases an enzyme, enteropeptidase, or enterokinase, which activates the protease, trypsin, released as trypsinogen by the exocrine pancreas. This trypsin not only acts on food proteins, it also acts on other preproteases released by the exocrine pancreas and activating them. Thus, trypsin acts as an endoprotease on chymotrypsinogen, releasing chymotrypsin; on proelastase, releasing elastase, and on procarboxypeptidase, releasing carboxypeptidase. Trypsin, chymotrypsin, and elastase are all endoproteases, each having specificity for particular peptide bonds as detailed in Table 6.13. Each of these three proteases have serine as part of their catalytic site so any compound that ties up the serine will inhibit the activity of these proteases. Such inhibitors as diisopropylphosphofluoridate react with this serine and in so doing bring a halt to protein digestion.

Through the action of pepsin, trypsin, chymotrypsin, and elastase, numerous oligopeptides are produced that then are attacked by the amino and carboxypeptidases of the pancreatic juice and those on the brush border of the absorptive cells. One by one, the amino acids are liberated from these chains and one by one, they are absorbed and appear in the portal blood.

Absorption

Although single amino acids are liberated in the intestinal contents, there is insufficient power in the enzymes of the pancreatic juice to render all of the amino acids singly for absorption. The brush border of the absorptive cell therefore not only absorbs the single amino acid but also di- and tripeptides. In the process of absorbing these small peptides, it hydrolyzes them to their amino acid constituents. There is little evidence that peptides enter the bloodstream. There are specific transport systems for each group of functionally similar amino acids, dipeptides, and tripeptides. Most of the biologically important L-amino acids are transported by an active carrier system against a concentration gradient (see Figure 6.5).[9] This active transport involves the intracellular potassium ion and the extracellular sodium ion. As the amino acid is carried into the enterocyte, sodium also enters in exchange for potassium. This sodium must be returned (in exchange for potassium) to the extracellular medium. This return uses the sodium–potassium ATP pump. In several instances, the carrier is a shared carrier. That is, the carrier will transport more than one amino acid. Such is the case with the neutral amino acids and those with short or polar side chains (serine, threonine, and alanine). The mechanism whereby these carriers participate in amino acid absorption is similar to that described for glucose uptake (see the unit on carbohydrates). This mechanism is illustrated in Figure 6.5.

TABLE 6.13
Some protein and peptide hormones and their function

Hormone	Source	Type	Function
Thyroid	Thyroid gland	Dipeptide	Regulates oxygen consumption by tissues
Thyroid-stimulating hormone (TSH)	Pituitary	Polypeptide	Stimulates synthesis and release of thyroid hormone by thyroid gland
Thyroid-releasing factor	Hypothalamus	Tripeptide	Stimulates pituitary to release thyroid-stimulating hormone
Calcitonin	Thyroid gland	Polypeptide	Stimulates bone uptake of calcium
Parathyroid hormone	Parathyroid glands	Polypeptide	Raises serum calcium levels, lowers serum phosphorus levels; increases urinary phosphorus excretion, decreases urinary calcium excretion; activates vitamin D in renal tissue
Insulin	β cells of Islets of Langerhans (pancreas)	Protein	Regulates glucose utilization; stimulates glucose uptake; influences lipid and protein synthesis
Glucagon	α cells of Islets of Langerhans (pancreas)	Polypeptide	Rapid mobilization of hepatic glucose from glycoen; mobilizes fatty acids from adipose tissue stores; enhances hepatic glucose production from amino acids and glycerol
Somatostatin	D cells of Islets of Langerhans (pancreas)	Polypeptide	Inhibits food passage along gastrointestinal system, decreases gall bladder release of bile, slows uptake of nutrients from intestinal lumen; inhibits growth hormone release
Epinephrine	Adrenal medulla	Tyrosine derivative	Stimulates lipolysis, stimulates glycogen breakdown; increases vasodilation of arterioles of skeletal muscles, vasoconstriction of arteriolesin skin and viscera
Norepinephrine	Adrenal medulla	Tyrosine derivative	Exerts an overall vasoconstriction effect on vascular system
ACTH	Pituitary	Polypeptide	Stimulates production and release of adrenal corticoid hormones
Antidiuretic hormone pituitary (ADH) (Vasopressin)	Pituitary	Polypeptide	Promotes water conservation; controls water resorption by kidney; raises blood pressure
Follicle-stimulating hormone (FSH)	Anterior pituitary	Peptide	Controls testicular function; spermatogenesis; stimulates ovum production; enhances release of estrogen
Luteotropic hormone (LH) (Prolactin)	Anterior pituitary	Protein	Controls testicular function and spermatogenesis; stimulates ovum production; enhances release of estrogen
Growth hormone	Anterior pituitary	Protein	Stimulates growth of long bones and muscles; stimulates production of somatomedin
Gastrin	Gastric glands	Polypeptide	Stimulates acid and pepsin secretion; stimulates growth of gastric mucosa
Cholecystokinin	Intestine, pancreas	Polypeptide	Stimulates gall bladder contraction; stimulates pancreatic enzyme release
Secretin	Intestine, pancreas	Polypeptide	Stimulates pancreatic secretion; augments action of cholecystokinin
Leptin	Adipose tissue	Peptide (cytokine)	Signals the satiety center of the brain; suppresses appetite; stimulates UCPs and apoptosis

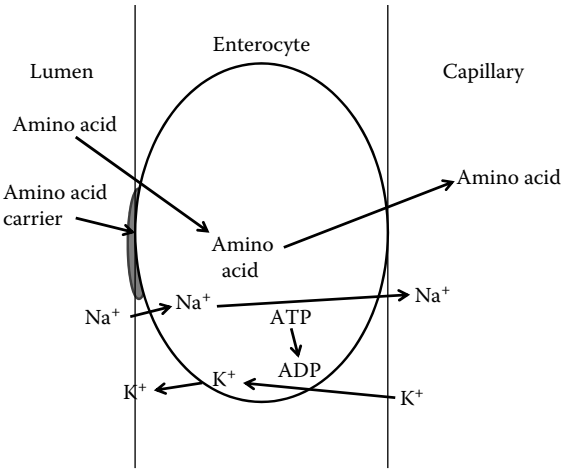


FIGURE 6.5 Carrier-mediated sodium-dependent amino acid transport. The amino acid enters the absorptive cell with sodium. The sodium is recirculated back to the lumen for reuse. The sodium is pumped out via Na^+K^+ ATPase system. As the sodium leaves the cell, potassium flows back in and the electrolyte balance is maintained.

Metabolism

The amino acids leaving the absorptive cell enter a whole-body amino acid pool from which all cells withdraw amino acids for use in the synthesis of biologically important proteins, peptides, and amino acid derivatives. The peptides may be hormones or cytokines that the various signaling systems found in the body comprise. Amino acids not used for peptide or protein synthesis can be deaminated and the carbon unit used for energy or for the synthesis of glucose or fatty acids. In humans consuming a fat-rich diet, very little fatty acid synthesis occurs. Amino acids can also be decarboxylated to form amines. Amines are quite potent compounds that act as intracellular effectors such as the neurotransmitters or as high-energy compounds such as creatin phosphate.

Hormones: Regulators of Protein Metabolism

Hormones serve as internal messengers regulating the ebb and flow of a variety of cellular functions. As internal signals, hormones regulate such processes as heartbeat, vascular contraction, salt conservation, glucose uptake and utilization, glucose production, and oxygen consumption. Hormones can be viewed as system regulators.

Hormones are substances that are released into the bloodstream by a tissue or organ and have an action distal to that tissue. Not all hormones are proteins. Some are peptides, some are steroids, some are small polypeptides, and some are amino acid derivatives. All, however, serve as coordinators of activity in specific tissues.

The actions of many of these hormones are mediated by specific interactions with specific proteins in the membrane or cytosol, or certain of the organelles within the cell. These proteins are called *receptors* (see Chapter 4). In turn, the hormones themselves are released via a cascade of sequential reactions that usually begin in the central nervous system, followed by signals generated in the hypothalamus, then the pituitary, and finally by the endocrine cell itself.[10,11] This cascaded signaling system is a means to amplify the response to a specific stimulus. For example, an individual is suddenly exposed to a dangerous situation—perhaps an explosion or an oncoming car or a charging bull. The person perceives the danger and responds to it. The response involves the central nervous system (CNS) as well as other systems that are involved in the fight-or-flight response to danger. Figure 6.6 illustrates this signal cascade system.

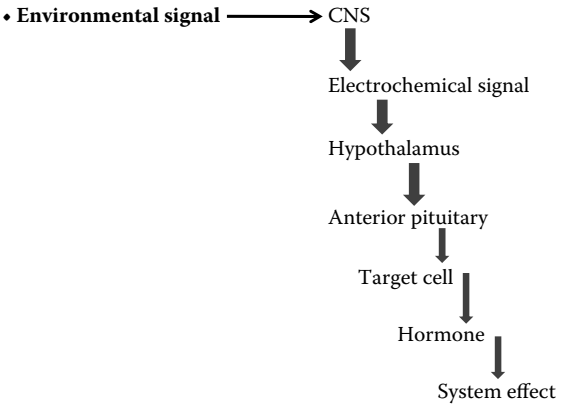


FIGURE 6.6 Signaling cascade that begins with an initial signal originating in the environment and ends with a whole-body systemic response.

Other stimuli may originate from the environment and likewise elicit a systemic response. Specific foods or nutrients can also evoke such cascades as discussed in Chapter 2 with respect to food-intake regulation. This regulation involves a variety of signals from both the CNS and the gut. In summary, stimuli from the environment elicit specific electrical or chemical signals. These are generated by the hypothalamus, which, either directly or indirectly (via releasing hormones) stimulates the anterior pituitary, which directly or indirectly signals the target endocrine cell to synthesize or release its hormone, which has the needed effect on metabolism. Not all hormone-release mechanisms use all steps in this cascade. Sometimes, short loops are used that bypass some of the initial steps in the cascade. In addition, there is some negative feedback involved that tells the target endocrine cell that enough hormone has been released.

Some of the peptide and protein hormones in the body are listed in Table 6.13. Others, notably the cytokines, are discussed in Chapters 1 and 4. The local hormones (the eicosanoids) are discussed in Chapter 8. In general, hormones function through one of several mechanisms.

1. They induce enzyme synthesis by stimulating mRNA transcription. An increase in mRNA synthesis leads to an increase in enzyme protein synthesis. Hormones acting in this way are thought to be functioning as modifiers of gene expression.
2. Hormones stimulate enzyme synthesis through enhancing the translation of messenger RNA. Growth hormone, a small protein, appears to act in this fashion.[12]
3. Hormones directly activate enzymes by either changing the phosphorylation state of the cell or one of its compartments or by changing the flux of ions as cofactors in the reactions.
4. Hormones activate membrane transport systems, which have G proteins as part of their structures.

In the cascade system illustrated in Figure 6.6, hormones or signals must emanate from one level of the cascade to the next for the system to work. The precision of the signaling system depends largely on the specificity of the signal for its target, and the specificity of the target for its signal. Some hormones are more specific than others. In general, the protein and peptide hormones are more specific than the steroid hormones. The polypeptide hormones bind to their cognate receptors lodged in the plasma membrane. These receptors generally penetrate the membrane and have a tyrosine-rich tail protruding into the cytosol (see Chapter 4). Part of the hormone-receptor binding and subsequent cellular events involve G proteins. Not all of these hormone-receptor cascades use G proteins. The G proteins serve as the signal transducer for the hormone, stimulating a subsequent

series of metabolic events that may involve the enzymes, adenyl cyclase, or phospholipase C and the calcium ion. The calcium ion plays a role in exocytosis, may be active in the exchange of metabolites across intracellular membranes, or may itself be a signal molecule.

Although the details of the synthesis of all of these peptide and protein hormones have yet to be elucidated, some pathways are well known. The synthesis of thyroxine from tyrosine by the thyroid gland (Figure 6.7), and of dopamine, epinephrine, and norepinephrine, also from tyrosine, by the adrenal medulla and CNS is established. Note in Figure 6.7 that a cascade of signals is needed for the release of the hormone. The synthesis of the pancreatic hormones and of the more complicated protein hormones is not clear. The enzymes needed for some of these synthetic pathways have not been fully described nor have the control points in the synthetic pathways been fully elucidated. This is a very active area for research in endocrinology, as is the study of the signal transduction

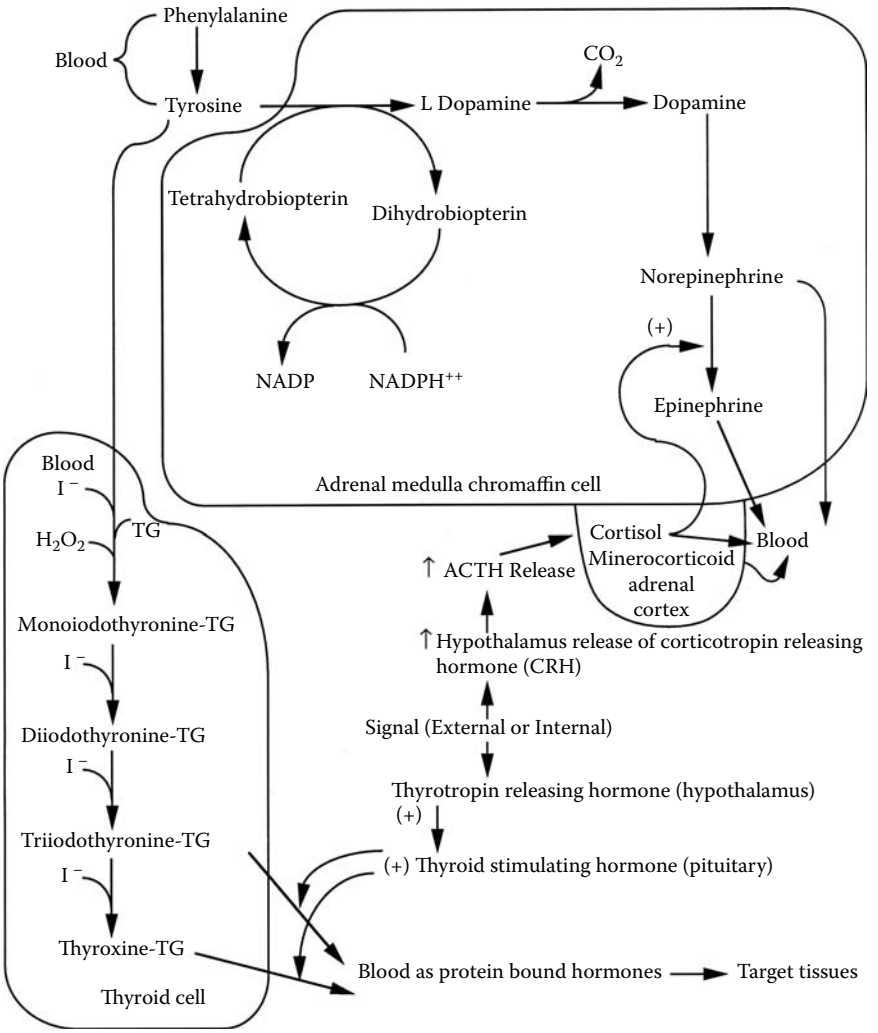


FIGURE 6.7 Tyrosine in the circulation enters specific cells of either the thyroid or adrenal glands. This tyrosine is used to make thyroid hormone and the catecholamines. These hormones are released into the circulation and are carried to their target cells, where they have their effects. Thyroxine synthesis occurs as a sequential iodination of tyrosine residues contained by the protein thyroglobulin (TG). At the target tissue, thyroxine is deiodinated to its active form, triiodothyronine.

systems that explain the actions of each of these hormones. One of the more interesting observations is that many of the peptide hormones are encoded together in a single gene and that many copies of the same message can exist in this gene. For example, one gene has been found to encode ACTH (adrenocorticotropin hormone), β-lipotropin, α-lipotropin, α-MSH (melanocyte stimulating hormone), β-MSH, CLIP (corticotropin like peptide), β-endorphin, and some of the enkephalins. This gene encodes for proopiomelanocortin as illustrated in Figure 6.8, which in turn can generate the above eight hormones.

Not all are generated by the same tissue or cell type but occur separately, generated by specific signals in specific cells. In the above example, one of the gene products, ACTH, is produced and released by the anterior pituitary under the control of the corticotrophin-releasing hormone. Enzymes are present in the corticotrophic cells that cleave the amino acid sequence of proopiomelanocortin at specific sites releasing ACTH and β-lipotropin into the circulation. These products can be cleaved further by cells of the pars intermedia to produce and release α-MSH, CLIP, α-lipotropin, and β-endorphin. CLIP is an abbreviation for corticotropin-like intermediary peptide. α-Lipotropin and β-endorphin can be split further to produce β-MSH and met-enkephalin. The reason that all these gene products arise from the same initial compound has to do with the unique location in the different cell types of specific proteases that act on specific linkages and thus release these smaller peptides into the circulation. Only a few cell types possess these specific proteases.

Other hormones are also encoded together. Just as ACTH and β-lipotrophin are components of the proopiomelanocortin, so, too, are other hormones split off from prohormone molecules. Vasopressin and neurophysin II arise from the same molecule. Oxytocin and neurophysin I also have the same parent peptide. Although the above examples are all peptide hormones, no doubt we will find that nonhormone peptides can arise in similar fashion. Amino acids in the circulation enter a vast

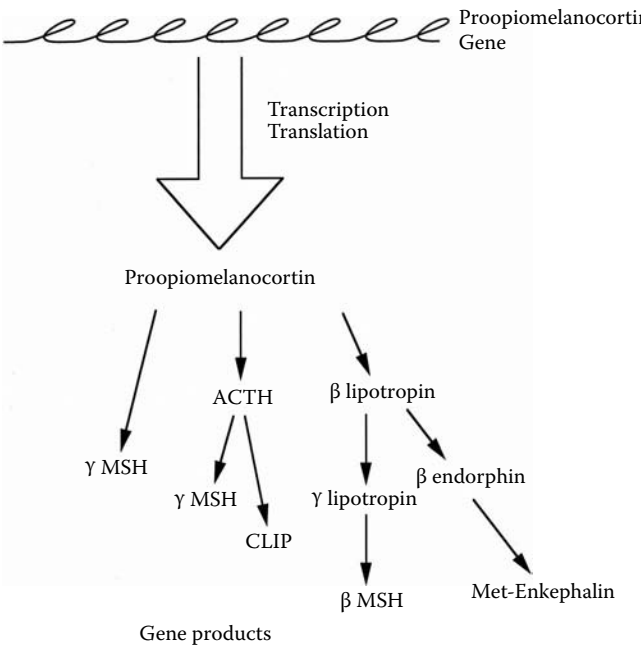


FIGURE 6.8 One gene encodes several signal peptides depending on the cell type and the signals generated to synthesize one or more of these products, ACTH and β-lipotropin gene products are under the control of corticotropin-releasing hormone (CRH), whereas αMSH, CLIP, γ-lipotropin, and β-endorphin gene products in the pituitary are under the control of dopamine. MSH = melanocyte-stimulating hormone; CLIP = corticotropin-like intermediary peptide.

array of cell types, each of which has many uses for the amino acids brought to it by the circulation. As mentioned, small, medium, and large molecules can be made. The largest of these molecules are the proteins.

Protein synthesis is described and discussed in Chapter 5.

Protein Turnover

Protein turnover consists of two processes: synthesis and degradation. Synthesis is described in Chapter 5. The proteins synthesized by the body have a finite existence. They are subject to a variety of insults and modifications. Some of these modifications, touched upon as metabolic control processes, have been discussed; a prohormone is converted to an active hormone, an enzyme is activated or inactivated with the addition or removal of a substituent, and so forth. Thus, a dynamic state within the body exists with respect to its full complement of peptides and proteins. Some proteins have very short lifetimes and very rapid turnover times; other proteins are quite stable and long-lived.[13] Their turnover time is quite long. The estimate of the life of a protein—that is, how long it will exist in the body—is its half-life (see Chapter 4). A half-life is that time interval that occurs when half of the amount of a compound synthesized at time X will have been degraded. Given the dynamic state of metabolism, some of these time estimates will be very short. Half-lives of biologically active compounds are very difficult to estimate, yet the concept is handy when one is trying to understand and quantitate the turnover of body protein. Hormones are examples of short-lived proteins. They may be released, serve their function, and be degraded within a very short period (seconds to minutes to hours) of time. The protein of the lens is an example of the latter; once synthesized, the lens protein is not degraded or recycled. Adult humans have a daily turnover of about 1–2% of their total body protein. Most of this is muscle protein because the largest group of body proteins is found in muscle. Accretion of muscle mass means that protein synthesis is greater than protein degradation.[14] The control of this balance of synthesis versus degradation (positive nitrogen balance) rests at the level of gene transcription.[14,15]Apparently the signals for growth and muscle protein accretion turn on the transcription of genes encoding the various muscle proteins.[12,14,15]

Protein Degradation

Protein degradation ultimately results in amino acids that are usually recycled. There are some exceptions to this general rule; histidine in the muscle protein is methylated and excreted as 3-methyl histidine. This 3-methyl histidine cannot be reused and thus is an indication of muscle breakdown. It is often used as an indicator of excess protein catabolism as happens in malnutrition or in such clinical states as thyrotoxicosis[16] or trauma.[17] Most of the products of protein degradation (the liberated amino acids) join the body's amino acid pool, from which the synthetic processes withdraw their needed supply. It is estimated that 75–80% of the liberated amino acids are reused.

Different proteins are degraded at different rates. The rates of degradation are determined not only by the physiological status of the individual but also by the amino acid composition of the protein in question. High rates of degradation of the structural proteins mean that considerable structural rearrangement is occurring. For example, during starvation, structural proteins are degraded at higher rates than during nonstarvation conditions. Prolonged trauma also elicits accelerated structural protein degradation.[17] Short-lived proteins such as those mentioned above as proteins, enzymes, or receptors have in common regions rich in proline, glutamate, serine, and threonine. These amino acids, when clustered, provide a target for rapid degradation.

The process of degradation first reduces the protein to peptides and then reduces these peptides to their constituent amino acids. Two major pathways are used for this process. Extracellular, membrane, and long-lived intracellular proteins are degraded in the lysosomes by an ATP independent pathway. Short-lived proteins as well as abnormal proteins are degraded in the cytosol using ATP and ubiquitin. This pathway is illustrated in Figure 6.9

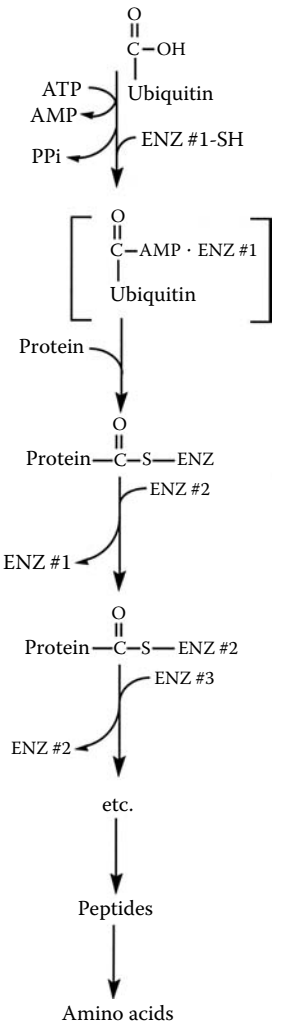


FIGURE 6.9 General pathway for intracellular protein degradation.

Protein degradation in the cytosol requires energy from ATP and the highly conserved 76 amino acid protein called ubiquitin.[18–23] Proteins that are to be degraded via the ubiquitin-dependent pathway are derivatized by several molecules of ubiquitin. The ubiquitin is attached by nonpeptide bonds between the carboxy terminus of ubiquitin and the D-amino groups of lysyl residues in the protein. This requires ATP.

Although the proteases of digestion are important to the degradation of dietary protein, they have no role in the intracellular protein degradation. Intracellular proteases hydrolyze internal peptide bonds. This is followed by the action of carboxy- and aminopeptidases, which remove single amino acids from the carboxy end or the amino end of the peptides.

Proteins in the extracellular environment are brought into the cell by endocytosis.[24,25] This is a process similar to pinocytosis, where the cell membrane engulfs and encapsulates the extracellular material. Endocytosis occurs at indentations in the plasma membrane that are internally coated with a protein called clathrin. As in pinocytosis, the extracellular protein is surrounded by the plasma membrane to form an intracellular vesicle which, in turn, fuses with a lysosome. Degradation then

occurs via calcium-dependent proteases called *calpains* or *cathepsin*. Both the Golgi and the endoplasmic reticulum are involved in providing proteases that degrade peptide fragments arising during the maturation of proteins in the secretory pathway.[25,26]

The rate of degradation varies from protein to protein, and this rate is determined by the amino acid at the amino end of the protein amino acid chain. Proteins with short half-lives have regions rich in proline, glutamate, serine, and threonine. Proteins that are degraded slowly, therefore, have slower turnover times and longer half-lives.

Amino acid catabolism is described in Chapter 3, Nutritional Biochemistry. Those amino acids in the body's amino acid pool that are neither used for peptide or protein synthesis nor to synthesize metabolically important intermediates are deaminated, and the carbon skeletons are either oxidized or used for the synthesis of glucose or fatty acids. Very little fatty acid synthesis occurs in humans consuming 20–30% of their energy as fat. There are three general reactions for the removal of NH_3 from the amino acids.

1. They can be transaminated with the amino group transferred to another carbon chain via amino transaminases.
2. They can be oxidatively deaminated to yield NH_3.
3. They can be deaminated through the activity of an amino acid oxidase.

Figure 6.10 illustrates these general reactions.

Functions of Proteins

A variety of proteins are found in the body. Each serves a specific function in the maintenance of life. Any loss in body protein, in effect, means a loss in cellular function. In contrast to lipids and carbohydrates, which have a body reserve to be used in times of need, the functioning body has no true protein reserve. Humans, when they are deprived of or insufficiently supplied with protein, will compensate for this dietary deficiency by catabolizing some, but not all, of their tissue protein

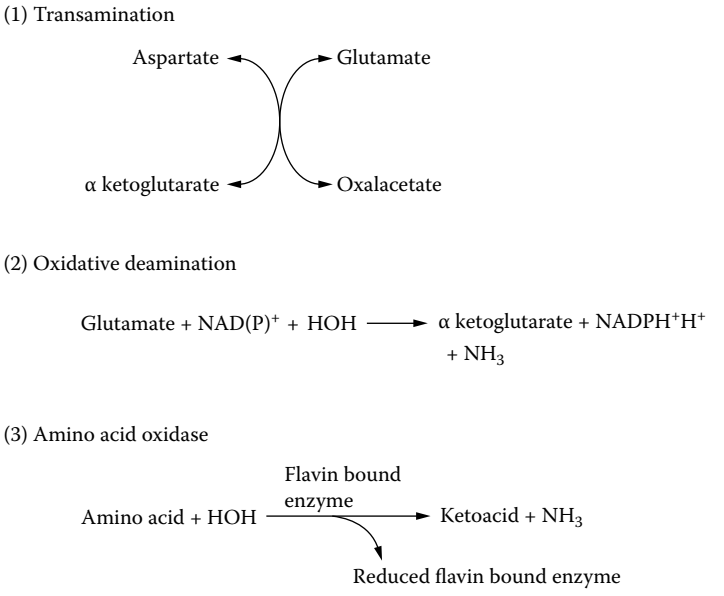


FIGURE 6.10 Amino acid reactions that result in the removal or transfer of the amino group.

with a consequent loss in tissue functionality. Cells, tissues, organs, and whole systems cannot exist without the proteins serving their various functions.

Proteins as Enzymes

From conception to death, living cells use oxygen and metabolize fuel. Cells synthesize new products, degrade others, and generally are in a state of metabolic flux. For these processes to occur, catalysts are needed to enhance each of the many thousands of reactions occurring in the cell. These catalysts, called *enzymes*, are proteins.[27] Enzymes make up the largest and most specialized class of proteins. Each enzyme is unique and catalyzes a specific reaction. In the cell, enzymes are found in all the cellular compartments (cytoplasm, nucleus, mitochondria, etc.) as well as the membranes within and around the cell. The membrane-associated enzymes are part of the protein component of the cell wall. The location of an enzyme is one of its characteristics and dictates, in part, its role in metabolism.

Enzymes consist of specific sequences of amino acids. Should the sequence deviate, alterations in enzyme activity can be anticipated, unless, of course, the change in amino acid does not affect the active site of the enzyme or its molecular shape. The importance of the amino acid sequence is obvious and is related to the availability of R groups (on the amino acids) that will complement reactive groups on the substrate, the molecule on which the enzyme exerts its catalytic action. The catalytic function of the enzyme is intimately related to its amino acid sequence. Enzymes must possess a shape that will complement the molecular shape of the substrate in much the same way as a key fits into a lock. This shape, of course, is a function of the enzyme protein's primary, secondary, tertiary, and quaternary structure. Just as enzymes must have a specific shape, substrates must also have specific shapes to be catalyzed by their respective enzymes. This is the reason that only D-sugars or only L-amino acids can be metabolized by mammalian cells. These sterioisomers conform to the shape required by the enzyme that serves as its catalyst. Although enzymes show absolute specificity, the specificity generally applies to only a portion of the substrate molecule. If the substrate is a small compound, this specificity applies to the entire molecule. If, however, the substrate is large and complex, the structural requirements are less stringent in that only that part of the substrate involved in the enzyme–substrate complex must have the appropriate molecular arrangement. The portion of the substrate not involved in the reaction (i.e., the nature of the R group) need not be the appropriate conformation. How does this specificity work? Many substrate–enzyme complexes form with a three-point attachment that leaves a fourth atom or R group free. If the attachment site can be approached from only one direction and only complementary atoms can attach, the substrate molecule can bind in only one way.

Some enzymes are specific for only one substrate; others may catalyze several related reactions. Although some are specific for a particular substrate, others are specific for certain bonds. This is called *group specificity*. For example, glycosidases act on glycosides (any glycoside), pepsin and trypsin act on peptide bonds, and esterases act on ester linkages. Within these groups, certain enzymes exhibit greater specificity than others. Chymotrypsin preferentially acts on peptide bonds in which the carboxyl group is a part of the aromatic amino acids (phenylalanine, tyrosine, or tryptophan). Enzymes such as carboxypeptidase or aminopeptidase catalyze the hydrolysis of the carboxy-terminal or amino-terminal amino acid of a polypeptide chain. This bond specificity rather than molecular specificity is useful to the animal in that it reduces the number of enzymes needed within the organism. Incidentally, the above enzymes are very useful to protein chemists in their determination of the amino acid sequence of a given protein.

Cells synthesize enzymes in much the same fashion as they synthesize other proteins, yet enzymes are relatively short-lived. Cells must continually synthesize their enzymes if they are to survive. A variety of signals serve as stimulants or inhibitors of this synthesis. One of these signals is the substrate. An excess of substrate will not only activate any preexisting enzyme in the cell, but will also serve to stimulate enzyme synthesis. The substrate may act at the level of mRNA

transcription or at the level of translation or protein assembly. Hormones such as insulin, thyroid hormone, and glucocorticoid may also serve in this way to stimulate enzyme synthesis and/or activation. Hormonal inhibition of enzyme synthesis also occurs and serves as an internal regulator of the synthetic process.

Proteins as Carriers and Receptors

A wide variety of compounds is carried in the blood between tissues and organs of the body. Some of the compounds require a specific protein for their transport. Not only is this protein necessary for the transport of compounds insoluble in blood, but it is also necessary to protect these compounds from further reactions during the transport process. Some of the membrane proteins are carriers, some are both carriers and enzymes, and still others are receptors. Both intracellular and extracellular carriers and receptors have been identified. Receptors were discussed in Chapter 4.

The plasma proteins that can have a carrier function are the albumin and the α- and ß-globulins. Perhaps the best studied of the plasma proteins are those associated with the transport of lipid, because these lipoproteins (carriers plus lipids), when elevated, appear to be related to the development of cardiovascular disease (see Chapter 8). These lipoproteins make up about 3% of the plasma proteins. They are loose associations of such lipids as phospholipids, triacylglycerols, and cholesterols, and represent an example of how proteins function as carriers.

In addition to serving as carriers of lipids, some of the globulins in the plasma can combine stoichiometrically with iron and copper as well as with other divalent cations. These combinations are called *metalloproteins*. The globulins serve to transport these cations from the gut to the tissues where they are used. The monovalent cations, sodium and potassium, do not need carriers, but many minerals do.

Many hormones and vitamins require transport or carrier proteins to take them from their point of origin to their active site. In addition, there are intracellular transport proteins, such as those in the mitochondrial membrane, that are responsible for the transfer of metabolites between the mitochondrial and cytosolic compartments. Finally, there are transport proteins that carry single molecules. The classic example is, of course, hemoglobin, the red-cell protein responsible for the transport of oxygen from the lungs to every oxygen-using cell in the body.

Proteins as Regulators of Water Balance

As substrates and solutes are transferred or exchanged across membranes, water has a tendency to follow in order to maintain equal osmotic pressure on each side of the membrane. If osmotic pressure is not maintained, the individual cells either shrink from lack of internal water or burst from too much. The balance of water between the intracellular and extracellular compartments is closely regulated.

One of the most carefully controlled points of water balance is across the capillary membrane, where a close balance is maintained between the osmotic pressure of the blood plasma, the interstitial fluids, and the cells and the hydrostatic pressure exerted by the pumping action of the heart. The total osmotic pressure of the plasma and of the intra- and extracellular fluids is the result of its content of inorganic electrolytes, its organic solutes, and its proteins. The concentrations of the electrolytes and organic solutes in plasma, interstitial fluid, and cells are substantially the same, so that the contribution to the osmotic pressure by these substances is practically equal. However, because there are more proteins in plasma than in the cells, plasma exerts an osmotic pressure on the tissue fluids. The result of this inequity of solutes is the drawing of fluids from the tissue spaces and from the cells into the blood. Opposing this force is the hydrostatic pressure exerted by the pumping action of the heart, which moves fluids from the blood into the tissue spaces and into the cells. The hydrostatic pressure is greater on the arterial side of the capillary loop than on the venous side. There is an interplay among these four kinds of pressure—blood osmotic pressure, tissue osmotic pressure, blood hydrostatic pressure, and tissue hydrostatic pressure. This interplay results in a

filtration of solutes and metabolites and the transfer of oxygen from the arterial blood into the tissues and cells it supplies and on the venous side, a resorption from the tissue space of CO_2, metabolites, and solutes back into the blood supply.

Albumin plays a more significant role in maintaining the osmotic pressure than the other blood proteins because of its size and abundance. In the blood, there are more albumin molecules than other serum proteins, and thus, albumin has considerable influence on osmotic pressure. Malnourished individuals are characterized by a reduction in serum proteins. With fewer proteins in the serum, water leaks out into the interstitial space and accumulates. The condition known as *edema* results. The edema of protein deficiency may also be the result of the body's inability to synthesize the protein hormones, particularly ADH, which plays a role in controlling water balance. Edema not involving protein deficiency can also result from other factors such as increased blood pressure or renal disease, but the edema that results in these situations is a result of factors other than the plasma proteins. The effect of protein is on the distribution of water among the various body compartments, rather than on the total body water.

Proteins as Biological Buffers

Proteins have the ability to accept or donate hydrogen ions and by so doing they serve as biological buffers. In blood, there are three important buffering systems: plasma proteins, hemoglobin, and carbonic acid–bicarbonate. The equilibrium reactions for each of these buffering systems follows:

$$\text{HProtein} \leftrightarrow H^+ + \text{Protein}$$

$$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

$$\text{HHb} \leftrightarrow H^+ + \text{Hb}^-$$

The first of these buffering systems, the plasma proteins, can function as a weak acid/salt buffer when the free carboxyl groups on the protein dissociate or as a weak base/salt buffer when the free amino groups dissociate. Although the buffering ability of the plasma protein is extremely important in maintaining blood pH, it is not as important as the other two systems.

The second buffering system, carbonic acid–bicarbonate, is extremely effective because there are reactions that follow this equilibrium that will regulate either acids or bases. The H_2CO_3 level in plasma never goes too high because it is in equilibrium with CO_2 ($H_2CO_3 \leftrightarrow CO_2 + H_2O$), which is expelled by the lungs. In blood, this equilibrium proceeds very quickly because of the presence of carbonic anhydrase, an enzyme found in red blood cells that catalyzes it. If the carbonic acid–bicarbonate reaction goes in the opposite direction, the concentration of the HCO_3^- so formed will be regulated by the kidneys.

The third important buffering system in blood results from hemoglobin. Hemoglobin has six times the buffering power of the plasma proteins. It functions well as a buffer for three reasons. First, it is present in large amounts. Second, it contains 38 histidine residues, which are good buffers because they can dissociate to H^+ and the imidazole group. Third, hemoglobin exists in blood in two forms, reduced hemoglobin and oxyhemoglobin. The imidozole groups of reduced hemoglobin dissociate less than those of oxyhemoglobin; thus, it is a weaker acid and a better buffer.

Hemoglobin's role in maintaining blood pH is extremely important and is best appreciated by understanding the transport of oxygen and carbon dioxide during respiration through the blood. Oxyhemoglobin is formed when each of the four ferrous ions in hemoglobin reversibly combines with one O_2 molecule. The reaction between oxygen and hemoglobin is written as $Hb + O_2 \rightarrow HbO_2$.

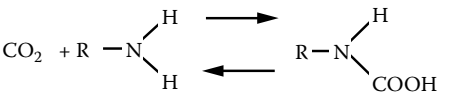
The combination is not an oxygenation reaction because iron stays in the positive 2-oxidation state. Instead, there is a loose association that exists between the oxygen and hemoglobin molecules.

At an oxygen tension of at least 100 mm Hg, hemoglobin is almost completely saturated with oxygen. This curve has a sigmoid shape rather than linear, because after the first oxygen molecule is added to the hemoglobin, the affinity for the second is increased; the second increases the affinity for the third, etc. This increase in affinity results because, as the O_2 is taken up, the two ß chains move closer together. As O_2 is given up, they move farther apart. When fully saturated, as it is at normal physiologic conditions, each gram of hemoglobin contains 1.34 mL of oxygen. In a normal 100 mL of blood, there are about 15 g of hemoglobin and, hence, 20 mL of oxygen.

In addition to the oxygen carried in the blood in association with hemoglobin, there is additional oxygen present that is dissolved in the blood. Henry's law states that this concentration is a linear function of the pressure of the oxygen. Only 0.4 mL of oxygen is in blood by being dissolved there and, thus, the major source of oxygen in the blood is hemoglobin.

As the cells consume the oxygen carried to them by hemoglobin, the oxygen tension falls, the oxyhemoglobin more readily dissociates, and the saturation of the hemoglobin falls. At about 50 mm Hg, hemoglobin rapidly "unloads" its oxygen. In the tissues, oxygen tension is about 40 mm Hg. This tension is below that necessary for unloading to occur and a gradient of high to low oxygen tension exists that facilitates the passage of oxygen to the tissues. This gradual change in oxygen content of the blood can be followed visually. Leaving the lungs, the blood is bright red. As it moves through the vascular system toward the periphery, the blood gradually darkens and, by the time it returns to the lungs, it has a bluish hue, a color characteristic of deoxygenated hemoglobin and the reason that persons lacking good respiratory exchange have a bluish tinge to their skin.

The CO_2 that is expired by the cells is transported in three forms. That which diffuses into the red blood cells is rapidly hydrated to H_2CO_3 by the presence of the carbonic anhydrase. The H_2CO_3 then dissociates into H^+ and HCO_3^-. The H^+ is neutralized primarily by hemoglobin and the bicarbonate diffuses into the plasma. This is the form in which most expired CO_2 is carried. The hemoglobin is present primarily in the blood in the reduced form by the time significant quantities of carbon dioxide are present so it can better function as a buffer. Some CO_2 that diffuses into red blood cells is not converted into bicarbonate ion but instead reacts with the amino groups of proteins, principally hemoglobin, to form carbamino compounds:



Reduced hemoglobin forms the carbamino compounds more readily than does oxyhemoglobin. The third form of CO_2 is that which is dissolved; it will exist as carbonic acid. It makes up only a small fraction of the total amount of CO_2 present. CO_2 present as carbonic acid will lower the blood pH. The CO_2 that diffuses into the plasma also exists in these three forms. The carbamino compounds will form with the plasma proteins. Much less CO_2 is hydrated to form carbonic acid in plasma than in red blood cells because the carbonic anhydrase enzyme is located only in red blood cells. And, finally, a small amount of dissolved CO_2 is present in blood.

There is a much greater rise in the HCO_3^- concentration in red blood cells than in plasma. As a result, HCO_3^- will diffuse into the plasma. This presents the problem of maintaining electrochemical neutrality. It is accompanied by a diffusion of chloride ions from the plasma to the red blood cells. This phenomenon is known as the *chloride shift*. Because CO_2 transport will occur primarily in venous blood, the chloride content of venous blood is higher than that of arterial blood. At the lungs, when venous blood becomes arterial, all these reactions are reversed. Occasionally, these reactions are perturbed and the acid–base balance is not maintained. Under normal conditions, the carbonic acid:bicarbonate ratio is maintained at 1:20. When the CO_2 levels rise, carbonic acid

levels rise and respiratory acidosis occurs. Conversely, when CO_2 levels fall, respiratory alkalosis occurs. By adjusting inspiration and expiration, respectively, small rises and falls in CO_2 levels can be adjusted, and the ratio of carbonic acid to bicarbonate remains constant. The kidney, through increasing its resorption of bicarbonate, can also assist in the regulation of this buffer system.

Large shifts in the acid–base balance that originate not in the respiratory system but rather as a result of metabolic alterations also occur. The prime example is the metabolic acidosis that occurs in the severe, uncontrolled diabetic. As a result of the lack of insulin, the metabolic fuel, glucose, cannot be used. The body then uses an alternate fuel, fatty acids. Fatty acid oxidation in the diabetic results in an accumulation of ketone bodies—acetone, acetoacetate, and ß-hydroxybutyrate. Normally, these substances are further oxidized to produce CO_2 and water. However, because so much fatty acid is being oxidized, its completion to CO_2 and water does not occur and the ketone bodies accumulate. These are acidic, and must be neutralized. All of the buffering systems are mobilized, but eventually they will not be able to compensate for this gradual increase in ketone bodies. The pH will fall, the enzymes that work at pH 7.4 will be appreciably less active, and the hemoglobin will become less able to carry oxygen. Unless treated with insulin, the patient will become comatose (lack of oxygen to the brain) and die.

Metabolic alkalosis is not nearly as common as metabolic acidosis. However, it can occur in persons having prolonged bouts of both diarrhea and vomiting. This is a result of the loss of the stomach acid, hydrochloric acid (the chloride ion), and the loss of potassium ions. Alkalosis can also occur in individuals who consume large quantities of alkali, as might occur in persons with peptic ulcers who self-medicate with antacids. In any event, the elevated blood pH may result in slow and shallow breathing, lowered serum calcium and potassium levels, and muscle tetany (prolonged contractions). Buffering activity is not limited to the blood. The carbonic acid–bicarbonate buffering system is active in interstitial fluids. Intracellular buffers include the proteins and organic phosphates.

Although the blood pH is maintained within a fairly narrow range through the systems just described, the urine pH can vary considerably depending on the metabolic state of the individual. The kidney and the urinary tract can tolerate a greater fluctuation in pH than can other tissues. Nonetheless, a number of buffer systems are also active in this tissue. In the kidney, although little buffering is done by the proteins, the bicarbonate buffer system is particularly active in the extracellular fluid. Once urine is produced, little effort is made to buffer its pH; it can vary from 4.7–8.0. The bladder and urinary tract are constructed so that this broad pH range is tolerated with little effect on the metabolism that occurs in these tissues.

Proteins as Structural Elements and Structural Units

The lipid component of the membrane is discussed in Chapter 8. Analysis of the liver cell membrane has shown that this membrane contains 50–60% protein, 35% lipid, and 5% carbohydrate. The carbohydrate present is found primarily in the membrane glycoproteins. These glycoproteins serve as receptors as well as cell-recognition proteins. The protein portion of the membrane is so oriented that its hydrophilic aspects are also in proximity to the intracellular and extracellular fluids. The protein molecules are interspersed within the lipids and lend both structural stability and fluidity to the membrane.

Membrane function depends on how the proteins are placed in the membrane and on the fluidity that results from the combination of proteins in a lipid mixture. As indicated earlier, the lipid portion of the membrane needs to be fairly unsaturated. If saturated, a more rigid crystalline structure will form. By being fluid and less rigid, these lipids can allow the proteins to change their shape in response to ionic changes, and thus these proteins can function as enzymes, carriers, or receptors, for the large variety of materials binding, entering, or leaving the cell.

How can these proteins serve in this fashion? Recall the protein conformation. The R groups of the amino acids project both in and out from the conformation and interact to form hydrogen bonds and disulfide bridges, however briefly, with compounds in proximity to them. Proteins that

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react with materials external to the cell may change their conformation and, in so doing, change their position within the membrane structure so that the reactive site changes from facing outward to the extracellular environment to facing inward toward the intracellular environment. Once facing inward, the material temporarily attracted to the membrane protein might be more attracted to another protein within the cell and "let go" of the membrane protein. Of course, this explanation is very simplistic. Transport into and out of cells probably involves many other reactions, a source of energy, and cations and/or anions for exchange. Yet this simplified explanation may be sufficient to explain the passage of a number of materials through the cell membrane. The reactive groups of the amino acid side chains, the R groups of the "structural" membrane proteins may also serve as identifiers or facilitators of reactive sites. Proteins serve as receptors for materials, such as insulin, that do not usually enter the cell. Insulin, also a protein, has reactive side chains protruding from it just as reactive side chains protrude from the proteins in the membrane. These two reactive groups interact and the binding of the insulin to the receptor occurs. Again, this is a very simplistic explanation of receptor action. In the instance of insulin, the receptor is probably a glycoprotein.

In addition to the function of proteins in the cell membrane as a structural element and as functional units, proteins are important intracellular structural units. Muscle is 20% protein, 75% water, and 5% inorganic material, glycogen, and other organic compounds. The major proteins in muscle are myosin, a large globular protein, and actin, a smaller globular protein. These two proteins plus the filamentous tropomyosin and troponin are the structural proteins of the muscles. The muscle proteins are characterized by their elasticity, which in turn contributes to the contractile power of this tissue.

Perhaps one of the most important structural functions of protein is that related to skin and connective tissue. The skin is composed of epithelial tissue that covers not only the exterior of the body but lines the gastrointestinal tract, the respiratory tract, and the urinary tract. One of the proteins found in the skin is melanin. Melanin is a tyrosine derivative and provides the pigmentation or color to the skin. Persons unable to form this pigment are albinos, and their disease is called *albinism*. Three separate genetic errors have been identified as causes for albinism.

Another of the proteins found in skin is keratin, which is the protein that forms hair, nails, hooves, feathers, or horns. Each of these structures is slightly different from the others, but all contain keratin. This protein is insoluble in water and is resistant to most digestive enzymes. It has a high percentage of cystine.

Connective tissue holds all of the various cells and tissues together. It contains two distinct types of proteins: collagen and elastin. Collagen is the principal solid substance in white connective tissue.[28] It contains a high percentage of proline, hydroxyproline, and glycine. It is difficult to degrade this protein and, like hair, it is relatively inert metabolically. Even in protein-deficient states, the body will synthesize collagen and elastin, and these proteins will not be catabolized for needed amino acids. Incidentally, although the body does not readily catabolize collagen, this protein can be degraded to a limited degree by boiling in acid. It is then converted to gelatin. The collagen of bone, skin, cartilage, and ligaments differs in chemical composition from that of the white fibrous tissue that holds individual cells together within muscle, liver, and other organs. Elastin and chondroalbumoid are two other proteins in the connective tissue. They are present in small amounts and serve as part of the structural protein. Finally, bones and teeth belong to the structural protein class because they start out with a matrix protein ("ground substance") into which various amounts of minerals are deposited.

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Proteins as Lubricants

The mucus of the respiratory tract, the oral cavity, the vaginal tract, and the rectal cavity reduces the irritation that might be caused by materials moving through these passages. This mucus is a mucoprotein, a conjugated protein that contains hexosamine. Proteins as lubricants also surround the joints and facilitate their movement. Should these lubricants be absent, or present but with

substantial decreases in their fluidity through the deposition of minerals, skeletal movement may be difficult and painful.

Proteins in the Immune System

Proteins such as γ-globulin serve to protect the body against foreign cells. The immunoglobins produced by lymphocytes are large polypeptides having more than one basic monomeric unit. These proteins differ in their amino acid structure, which affects their secondary, tertiary, and quaternary structures. Just as the amino acid sequence of an enzyme determines substrate specificity, the amino acid sequence of the immunoprotein assures antigen–antibody specificity. The synthesis of particular immunoglobulins has been much studied. It is now well accepted that initiation of synthesis by the lymphocyte requires the binding of an antigen (a foreign protein) to the cell surface at particular locations called *antigen receptors*. As with other receptors on other cells, there must be a good conformational "fit" between the site and the antigen. Once the immunoglobulin is synthesized, it will bind with the foreign protein, immobilizing it, and the complex antigen–antibody will be formed.

Proteins as Signaling Molecules

Already described are the hormones that are released as signals to other tissues. However, proteins and peptides also serve as part of a variety of signaling systems. The G proteins, for example, are intimately involved in signals perceived extracellularly and transmitted intracellularly; they are involved in the signal transmission process. G proteins are intracellular proteins that bind either GDP or GTP as part of their function.[29] Peptides (cytokines that have been discussed in Chapters 1, 2, and 4) serve as signals sent from one tissue to another. Amino acid amines can also serve as signals. Histamine, for example, is involved in allergic reactions, headache, hypotension, cardiac arrhythmia, and the group of intense allergic reactions by sensitive people to certain allergins.[30,31] Glutamine, although not truly a signaling compound as it is the most abundant α amino acid in the body,[32] seems to signal cell protein synthesis.[33,34] Glutamine is essential for the optimal growth of cells and seems to be an important component for the normal recovery from illness and injury. Lastly, glutathione, a tripeptide, serves an important role in the response to oxidative stress, detoxification reactions, cell proliferation and in some instances in the response to hormones. In these functions it may indeed be a signaling peptide.[35]

Protein Intake Recommendations

Human protein and amino acid requirements have been studied for well over 100 years using a variety of techniques. Nutrition scientists have collected data on the quantity of protein foods consumed versus health, growth, and weight gain of various populations. The assumption was made that whatever "healthy" people ate was probably what kept them healthy and should, therefore, be used as a standard of comparison for other diets. These standards, with respect to protein, were invariably high for populations having an abundance of meat, milk, poultry, and fish in their diets. Voit and Atwater, around the turn of the century, found intakes of 118 and 125 g of protein per day, respectively, for adult men and termed these intakes as desirable.

As nutrition developed as a science, more accurate methods for assessing nutrient needs were developed. Among these methods were those for assessing the intake and excretion of nitrogen compounds. The Kjeldahl method described in an earlier section, as well as methods for determining the nitrogenous end products of metabolism, were devised. These methods made possible the development of the concepts that today's scientists use to determine the nutrient requirements of humans as well as other species. In protein nutrition, it was realized that the body consists of two pools of protein—one with a short half-life that must be constantly renewed and one that is long-lived, slowly broken down, and replaced. If one assumes that over a short period of time the pool

having the long half-life contributes almost nothing to the nitrogenous metabolic end products, then a measure of the amount of nitrogen excreted will reflect only the turnover of the short-lived proteins. These proteins must be replaced by proteins newly synthesized from the amino acids provided by the diet. Hence, the term *protein requirement* (or the requirement for any nutrient) means that amount of protein that must be consumed to provide the amino acids for the synthesis of those body proteins irreversibly catabolized in the course of the body's metabolism. The intake of nitrogen from protein must be sufficient to balance that excreted; this basic concept is called *nitrogen balance* and has been discussed in the section that deals with the biological value of protein. Although this concept is useful in understanding why we have a minimal need for protein in the diet, its application is fraught with difficulty. In the section on the biological value of protein, the possible sources of error in the nitrogen balance technique were discussed in terms of their contribution to the accuracy and precision of the methods available for comparing various food proteins. These same sources of errors also apply when using the nitrogen balance technique for assessing protein and amino acid requirements. Finally, the need for dietary protein is influenced by age, environmental temperature, energy intake, gender, micronutrient intake, infection, activity, previous diet, trauma, pregnancy, and lactation.

Age

A protein intake in excess of maintenance needs is required when new tissue is being formed. Certain age periods (such as rapid growth) require more dietary protein than other periods. Age differences in protein turnover (protein flux) as well as protein synthesis explain some of the effects of age on protein need. Table 6.14 gives figures for humans at different ages. Premature infants (those infants born before their 10-lunar-month gestation time) grow at a very rapid rate and require between 2.5–5 g/protein/kg/d if they are to survive.

Studies of full-term infants have indicated that a protein intake of 2–2.5 g/kg/d resulted in a satisfactory weight gain and that further increases in protein intake did not measurably improve growth. Older infants and children whose growth rate is not as rapid as the premature or newborn infant require considerably less protein (~1.25 g/kg/d). As growth rate increases during adolescence, the need for protein increases. Again, this can be related to the demands for dietary amino acids to support the growth process. As humans complete their growth, the need for protein decreases until it arrives at what is called the *maintenance level*. It is at this level that the concept of body protein replacement by dietary protein applies. During the growth period, it is very difficult to separate the requirements for maintenance from those of growth. The impulse for growth is so strong that it will occur in many instances at the expense of the maintenance of body tissues. For example, protein-malnourished children will continue to grow taller even though their muscles, as well as other tissues, show evidence of wastage due to dietary protein deficiency.

TABLE 6.14
Age effects on nitrogen flux that reflects protein flux and protein synthesis rate per day

Age	Protein (N) flux mg (N/kg/h)	Total body protein synthesis (g/kg/d)
Newborn	124 ± 46	17.4 ± 9.9
Infant	65 ± 7	6.9 ± 1.1
Young Adult	26 ± 2	3.0 ± 0.2
Elderly	19 ± 2	1.9 ± 0.15

Growth carries with it not only a total nitrogen requirement but also a particular amino acid requirement. Maintenance, on the other hand, appears to have only a total protein requirement. Adults can make a number of short-term adjustments in their protein metabolism that can compensate for possible inequities or imbalances in amino acid intake as long as the total protein requirement is met. The young growing animal is not that flexible. The essential amino acid requirements are age-dependent. Histidine, although it can be synthesized in sufficient quantities by the adult to meet maintenance needs, is not synthesized in great enough amounts to support growth. Thus, histidine is an essential amino acid for the infant and growing child. This is due to the nature of the growth process.

At the other end of the life span, there are effects on protein needs and metabolism. In part, this is due to the gradual accumulation of errors in the genetic code of the aging individual. Studies of humans from newborn to 80 years of age have shown that there is a graduate increase in the number of base deletions in the mitochondrial genome. This may be due to the wide variety of genomic insults that occur over a life span. Exposure to ultraviolet light, noxious chemicals, drugs, or changes in the food supply can all affect the fidelity of the genome. These insults inflict their damage randomly so no one instance is responsible for the age effect, yet altogether there can be subtle and cumulative effects on gene expression and these, in turn, can affect the efficiency and fidelity of the gene products. Thus, metabolic processing of food materials loses its efficiency. The elderly person, although synthesizing less protein than the young growing person, may not synthesize this protein accurately. This, in turn, means a decline in function. Does this mean that the elderly may have an increase in protein need? We don't know. Until recently, this portion of the population had not been well studied vis à vis protein need.

Environmental Temperature

As environmental temperatures rise or fall above or below the range of thermic neutrality, animals begin to increase their energy expenditure to maintain their body temperature. In environments that are too warm, vasodilation occurs along with sweating and increased respiration. All of these mechanisms are designed to cool the body and all require an increase in the basal energy requirement as expressed per unit of body surface area. In cool environments, vasoconstriction and shivering occur in an effort to warm the body and prevent undue heat loss. Again, an increase in basal energy requirement is observed. Smuts, in 1934, found that nitrogen requirements were related to basal energy requirements. Through the study of a large number of species, he concluded that 2 mg nitrogen was required for every basal kilocalorie required when the energy requirement was expressed on a surface area basis. Thus, any increase in basal energy needs due to change in environmental temperature will, because of the relationship between protein and energy, be accompanied by an increase in the protein requirement for maintenance. In addition, profuse sweating, as it occurs in very warm environments, carries with it a nitrogen loss that must be accounted for in the determination of minimal protein needs.

Previous Diet

The effects of previous diet on the determination of protein requirements may be rather profound. If, for example, the subjects selected for studies on protein needs have been poorly nourished prior to the initiation of the study, their retention of the protein during the study will be greater than would be observed in subjects who have been well nourished prior to the initiation of the study. In other words, malnourished subjects have a higher protein requirement than well-nourished subjects. This, of course, raises the issue of whether there are body protein reserves. Many investigators have observed that animals fed a protein-free diet exhibit a "lag" before their nitrogen excretion level is minimized; during this phase, the animal is metabolizing his protein reserve. Other investigators maintain that there is no such thing as a protein reserve or store. These investigators maintain that

every protein in the body has a function and if some of these proteins are lost, there is a loss in body function. Support for this concept is seen in the reduced ability of protein-depleted animals to fight infection or respond to the metabolic effects of trauma. Whether one believes that there is such an entity as a protein reserve may depend upon whether one perceives a difference between an optimal protein intake and a minimum protein intake. This difference may relate more to a personal opinion on how nutrient requirements should be defined. Some nutritionists believe in stating the absolute minimum requirement to sustain life and then adding on increments for each body function above mere survival; this is known as the *particulate approach*. Other nutritionists believe that one cannot separate and quantitate the individual requirements of each function beyond survival. They advocate a protein intake sufficient for optimal function of the animal; this is known as the integrative approach. The particulate and integrative approaches each have their merits when argued intellectually. However, because humans do not merely exist, many human nutritionists tend to take the integrative approach to human nutrition requirements in their determination of protein needs.

Physical Activity

Research on protein needs for muscular work had its beginning in 1863 when Von Leiberg postulated that muscle as protein was destroyed with each contraction of the muscle. On this basis, he recommended that heavy muscular work required a heavy protein diet. This theory has been amply disproved, yet even today, many believe that a protein-rich diet will contribute to athletic prowess. Today, we know that muscle contraction does not result in destruction of the muscle. It does, however, require energy in the form of ATP, glucose, and fatty acids, and does result in the breakdown of creatine-phosphate to creatine, some of which is then converted to creatinine, a nitrogenous waste product excreted in the urine.

As the energy requirement is increased to support the increase in muscular activity, so, too, is the protein requirement in much the same manner as described above for the effects of temperature. In a number of studies, the athletic performance of subjects could not be directly related to the quantity of protein consumed above that determined to be the requirement for those subjects. When subjects were fed less than their respective protein requirements, their muscular efficiencies were reduced unless a vigorous training program was included as part of the experiment's protocol. Because most of the studies were of short duration and because muscle protein has a relatively long half-life, the lack of any demonstrable effect of protein intake on muscle performance (aside from the energy/protein relationship) is not surprising.

Other factors such as sex, pregnancy, lactation, and trauma affecting the protein requirement have been studied. As can be anticipated, males, due to their greater physical activity and larger body size, have a larger protein requirement than females; pregnancy, lactation, and trauma all increase the protein requirement.

Dietary Reference Intakes (DRI)[36]

For many years, the Recommended Dietary Allowance (RDA) for protein was set at 1 g/kg body weight for the average adult male. He was assumed to weigh 70 kg (about 155 lb) so the RDA was 70 g/d. With an ever-increasing database that the Nutrition Board of the National Research Council can use for its recommendations, the RDA for protein has been adjusted downward about every 5 years. At present, protein intake recommendations are given as a Dietary Reference Intake (DRI) rather than RDA. This is a set of recommendations for the protein intake by various age and life stage groups. The intake is given as grams protein per kilogram body weight per day. This is a different expression than that used for the RDA. It makes allowances for differences in body size. It presumes that the dietary protein is coming from a mixed diet containing a reasonable amount of good-quality proteins. For persons subsisting on mixtures of poor-quality proteins, this recommendation may not be adequate. Table 6.15 gives the current DRI for protein for different age groups.

TABLE 6.15
Dietary reference intakes (DRI) for protein

Life-stage group	Intake recommendation
	g/kg/d
Infants, 7–12 months	1.0
Children, 1–3 years	0.87
Children, 4–8 years	0.76
Males, 9–13 years	0.76
14–18 years	0.73
19–30 years	0.66
31–50 years	0.66
51–70 years	0.66
>70 years	0.66
Females, 9–13 years	0.76
14–18 years	0.71
19–30 years	0.66
31–50 years	0.66
51–70 years	0.66
>70 years	0.66
Pregnancy, 14–18 years	0.88
19–30 years	0.88
31–50 years	0.88
Lactation, 14–18 years	1.05
19–30 years	1.05
31–50 years	1.05

Source: www.nap.edu

Protein Deficiency

One of the most common nutritional disorders in the world today is the deficiency of protein. Both adults and children are affected as the populations in the less-developed nations of the world exceed their food supply. Instances of deficiency in the United States have also been observed. Due to the ubiquitous nature of protein and its role in bodily function, protein deficiency is characterized by a number of symptoms. In many situations, not only is protein lacking in the diet but the energy intake is also insufficient. For this reason, it is difficult to segregate symptoms due solely to protein deficiency from those due solely to energy deficit. In children, one can observe the different symptoms and visualize them all as parts of a continuum called *protein-energy* or *protein-calorie malnutrition* (PEM or PCM) rather than distinctly different nutritional disorders. Kwashiorkor, a disease initially observed in Africa, was first regarded as a dietary state where only protein was deficient, not energy. Marasmus, on the other hand, was regarded as a dietary state where both protein and energy were deficient. Today, as mild and moderate cases of these two diseases are treated, it has become apparent that the symptoms of one may intermingle with the other so that a clear-cut diagnosis is impossible. For every severe case of either kwashiorkor or marasmus that is identified, treated, and cured, there are probably another 99 people who are not diagnosed and treated and who will, if they survive, experience lifelong effects from their early nutritional deprivation.

Kwashiorkor

Kwashiorkor usually affects young children after they weaned from their mothers' breast. The children are usually between 1 and 3 years old; they are weaned because their mothers have given birth to another child or are pregnant and cannot support both children. If the children have no teeth, they are given a thin gruel. This may be a fruit, vegetable, or cereal product mixed with water; it is not usually a good protein source. Cultural food practices or taboos may further limit the kinds and amounts of protein given to the child. Concurrent infections, parasites, seasonal food shortages, and poor distribution of food among the family members may also contribute to the development of kwashiorkor. The deficiency develops not only because of inadequate intake but also because, at this age, the growth demands for protein and energy are high.

Growth failure is the single most outstanding feature of protein malnutrition. Affected children's height and weight for their age will be less than that of their well-nourished peers. Tissue wastage is present but may not be apparent if edema is also present. The edema begins with the feet and legs and gradually presents itself in the hands, face, and body. If edema is advanced, the child may not appear underweight but many appear "plump." This plumpness can be ascertained as edema by feel. If a thumb were to be pressed on the surface of the foot or ankle and then removed, the depression would remain for a short time. This is edema. It is thought to result from insufficient ADH production and insufficient serum and tissue proteins needed to maintain water balance.

Protein-deficient children are usually apathetic, have little interest in their surroundings, and are listless and dull. These children are usually "fussy" and irritable when moved. Mental retardation may or may not result. Hair changes are frequently observed. Texture, color, and strength are affected. Black, curly hair may become silkier, lusterless, and brown or reddish-brown in color. Lesions of the skin are not always present, but if present, they give the appearance of old flaky paint. Depigmentation or darkly pigmented areas may develop, with a tendency for these areas to appear in places of body friction such as the backs of legs, groins, elbows. Diarrhea is almost always present. The diarrhea may be a result of the inability of the body to synthesize the needed digestive enzymes so that the food that is consumed can be utilized, or it may be the result of concurrent infections and parasites. In rats fed protein-free diets, significantly less intestinal enzyme activity has been measured. Anemia due to an inability to synthesize hemoglobin as well as red blood cells is invariably present. Hepatomegaly (enlarged liver) is usually observed.

In children consuming energy-sufficient–protein-insufficient diets, the enlarged liver is usually fatty because the child is unable to synthesize the proteins needed to make the transport proteins, which, in turn, are needed to transport the lipids out of the liver. Studies with rats and chickens have shown that protein deficiency also results in decreases in a variety of hepatic enzymes, a decrease in hepatic RNA and DNA content, a reduction in spleen size, a decrease in antibody formation, a decrease in urea cycle activity and urea production, and a decrease in the levels of plasma amino acids. All of the above symptoms can be related to the various functions of proteins as discussed in the earlier section.

Marasmus

Although children of all ages and adults can suffer from a deficiency of both energy and protein, the marasmic child is usually less than one year old. In developing countries, a common cause for marasmus is a cessation of breast feeding. Milk production by the mother may have stopped because of the mother's poor health, or the mother may have died, or, there may be a desire on the part of the mother to bottle-feed her infant rather than breast feed. This decision to bottle-feed may be made for a variety of reasons. The mother may view bottle feeding as a status symbol, or she may be forced to work to earn a living and may be unable to have her baby with her, or she may not be able to lactate. Although under optimal conditions of economics and sanitation, the bottle-fed child may be well fed, in emerging nations this is not always true. The mother may not be able to buy the

milk formula in sufficient quantities to adequately nourish the child, she may overdilute the milk, or she may use unsafe water and unsanitary conditions to prepare the formula for the child. This, plus the insufficient nutrient content, often precipitously leads to the development of marasmus, a form of starvation characterized by growth failure with prominent ribs, a characteristic monkey-like face, and matchstick limbs with little muscle or adipose-tissue development; tissue wastage but not edema is present. Whereas the kwashiorkor child has a poor appetite, the marasmus child is eager to eat. The child is mentally alert but not irritable. Anemia and diarrhea are present for the same reasons as in kwashiorkor. The skin and hair appear to be of normal color.

The treatment of both kwashiorkor and marasmic children must be approached with due care and caution. Because their enzymes for digestion and their protein absorption and transport systems are less active, feeding these children with large quantities of good-quality protein would be harmful. Their diets must be gradually enriched with these proteins to allow their bodies sufficient time to develop the appropriate metabolic pathways to handle a better diet. Giving these children solutions of either predigested proteins or solutions of amino acids may be of benefit initially, but these solutions, too, must be used with care. If the amino acids in excess of immediate use are deaminated, and if the pathway for synthesizing urea is not fully functional, ammonia can accumulate in the child and become lethal. Schimke has shown that, in the rat, up to 3 days are needed to increase the activities of the urea cycle enzymes. The rat has a much faster metabolic rate than the human, so one would anticipate that a much longer period of time would be necessary for a similar induction in humans.

Not only must one be concerned about the enzymes of the malnourished child, protein-depleted children are also unable to synthesize adequate amounts of the protein hormones that regulate and coordinate their use of dietary nutrients. In addition, protein deprivation affects the structures of the cell and hormone receptors, further dampening the effectiveness of those hormones produced. Children with marasmus or kwashiorkor have been shown to have decreased blood sugar levels, decreased serum insulin and growth hormone levels, and, in marasmus, decreased thyroid hormone levels. Additional hormonal changes have been observed, but their relevance to treatment has not been ascertained. Most likely these changes in the levels of the protein-, peptide-, or amino acid-derived hormones are reflective of reduced synthesis of them as a result of a shortage of incoming amino acids. Changes in the steroid hormones probably reflect the response of the child to the stress of deprivation.

Protein and Amino Acid Interactions with Other Nutrients

Recently, it has come to the attention of nutrition scientists that there can exist nutrient interactions that can have either negative or positive effects on the nutrients concerned.[37] For example, the availability of iron from food depends on its source. Soybean protein contains an inhibitor of iron uptake. Diets such as those in Asia contain numerous soybean products and iron absorption is adversely affected by this soybean inhibitor. Tannins, phytates, certain fibers (not cellulose), carbonates, phosphates, and low-protein diets also adversely affect the apparent absorption of iron. In contrast, ascorbic acid, fructose, citric acid, high-protein foods, lysine, histidine, cysteine, methionine, and natural chelates (e.g., heme) all enhance the apparent absorption of iron.

Two types of iron are present in the food; namely, heme iron, which is found principally in animal products and nonheme iron, which is inorganic iron bound to various proteins in plants. Most of the iron in the diet, usually greater than 85%, is present in the nonheme form. The absorption of nonheme iron is strongly influenced by its solubility in the upper part of the intestine. Absorption of nonheme iron depends on the composition of the meal and is subject to enhancers of absorption such as animal protein and by reducing agents such as vitamin C. On the other hand, heme iron is absorbed more efficiently. It is not subject to these enhancers. Although heme iron accounts for a smaller proportion of iron in the diet, it provides quantitatively more iron to the body than dietary nonheme iron.

Zinc will form complexes with phosphate groups (PO_4^-), chloride (Cl^-), and carbonate groups (HCO_3^-) as well as with cysteine and histidine. Zinc can sometimes be displaced on the zinc fingers of DNA-binding proteins (receptors) by other divalent metals.[38] Iron, for example, has been used to displace zinc on the DNA binding protein that also binds estrogen. This protein binds to the estrogen response element of the DNA promoter region encoding estrogen responsive gene products. When this occurs in the presence of H_2O_2 and ascorbic acid, damage to the proximate DNA, the estrogen response element, occurs. It has been suggested that in this circumstance of an iron-substituted zinc finger, free radicals are more readily generated with the consequence of genomic damage. This suggestion has been offered as an explanation of how excess iron (iron toxicity) could instigate the cellular changes that occur in carcinogenesis.

As ions, minerals react with charged amino acid residues of intact proteins and peptides. Table 6.16 provides a list of minerals and the amino acids with which they react. Depending on their valence state these electrovalent bonds can be very strong or very weak associations or anything in between. The marginally charged ion (either an electron acceptor or an electron donor) will be less strongly attracted to its opposite number than will an ion with a strong charge.

The formation of mineral–organic compound bonds is also seen when one examines the roles of minerals in gene expression. Almost every mineral is involved in one or more ways. Zinc-cysteine or histidine linkages form the zinc fingers that bond with certain base sequences of DNA, thereby affecting transcription. Mineral-protein complexes serve as cis- or *trans*-acting elements that enhance or inhibit promoter activity and/or RNA polymerase II activity. Minerals can bond either by themselves or in complexes with proteins to inhibit or enhance translation.

There are also interactions and competitions between dietary amino acids in the small intestine. The amino acid carriers (carriers that transport amino acids from the lumen into the absorptive cell) will transport more than one amino acid.[9] Neutral amino acids share a common carrier as do those with short or polar side chains (serine, threonine, and alanine). There is also a carrier for phenylalanine and methinonie and one specific for proline and hydroxyproline. Similarly, there are carriers that transport amino acids across the blood–brain barrier, and there is some competition among amino acids for these carriers. For example, tryptophan competes with tyrosine for entry through the blood–brain barrier. Both tryptophan and the aromatic amino acids (phenylalanine and tyrosine) serve as precursors of neurotransmitters and a competition between them for transport may have a role in the regulation of the balance of these transmitters in the body. Sulfur containing amino acids are spared by each other (e.g., methionine) is spared by cysteine.

In intermediary metabolism there appears to be an interaction between leucine, isoleucine, and valine. All are branched-chain amino acids and share the same enzymes for their metabolism (see Chapter 3). All are needed to support protein synthesis. Should one be in short supply, the rate of protein synthesis will fall.

TABLE 6.16
Mineral–amino acid interactions

Minerals	Amino acid
Calcium	Serine, carboxylated glutamic acid (GLA)
Magnesium	Tyrosine, sulfur containing amino acids
Copper	Histidine
Selenium	Methionine, cysteine
Zinc	Cysteine, histidine

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Vegetarian Diets

Vegetarianism is rapidly growing in popularity. Technically defined, vegetarians are individuals who do not eat any meat, poultry, or seafood.[38] Estimates on the number of vegetarians in the United States vary greatly according to the definition of vegetarianism provided in the survey. True vegetarians make up about 2.8% of the population, representing approximately 5.7 million adults. A higher percentage of teenagers than adults follow a vegetarian diet.

Many people consider themselves to be vegetarian when they eat meals consisting of nonflesh foods several days a week. Others will claim to be vegetarians when they consume fish or poultry but not red meat such as beef, pork, or lamb. Table 6.17 lists the types of vegetarian diets and describes what foods are excluded. Some are more restrictive than others. The more restrictive the diet, the more likely it will be deficient in essential micronutrients.

Most of the nutrition-health risks associated with a vegetarianism are really those associated with the strict vegetarian diet (veganism). The more liberal forms of this diet choice (lacto-vegetarians, ovo-vegetarians, or lacto–ovo vegetarians) usually are less risky. The health risks are not unique to vegetarians, however, as they can be found in people following an imbalanced omnivorous diet. Typical vegetarian diets are rich in many beneficial nonnutritive factors such as dietary fiber and phytochemicals.

A common misconception about a vegetarian diet concerns protein. Individuals following a lacto–ovo vegetarian diet rarely have to worry about protein. Even adult vegans eating a reasonably balanced diet with adequate calories can meet their protein needs. It is much more likely that a dietary deficiency of a micronutrient would occur, rather than a protein deficiency. Deficiencies in vitamin B_{12} have been reported as well as deficient intakes of calcium and other micronutrients.[38] Children are particularly vulnerable with respect to malnutrition when required by parents or caregivers to follow a vegan diet. The more liberal diets do not pose as much risk with respect to

TABLE 6.17
Types of vegetarian diets

Vegan	Consumes nuts, fruits, grains, legumes, and vegetables. Does not consume animal-based food products, including eggs, dairy products, red meats, poultry, or seafood. Some vegetarians may avoid foods with animal processing (honey, sugar, vinegar, wine, beer).
Lacto–vegetarian	Consumes milk and other dairy products, nuts, fruits, grains, legumes, and vegetables. Does not consume eggs, red meats, poultry, or seafood.
Ovo–vegetarian	Consumes eggs, nuts, fruits, grains, legumes, and vegetables. Does not consume milk or dairy, red meats, poultry, or seafood.
Lacto–ovo vegetarian	Consumes milk and other dairy products, eggs, nuts, fruits, grains, legumes and vegetables. Does not consume red meats, poultry, or seafood.
Pollo–vegetarian[a]	Not technically considered a vegetarian type of diet, although often referred to as "vegetarian" in popular culture. Consumes milk and other dairy products, eggs, nuts, fruits, grains, legumes, vegetables, and poultry.
Peche–vegetarian also called pesco- and pecto-vegetarian[a]	Not technically considered a vegetarian type of diet, although often referred to as "vegetarian" in popular culture. Consumes milk and other dairy products, eggs, nuts, fruits, grains, legumes, vegetables, and seafood.

[a]*This is not technically a vegetarian diet although it is often referred to as such.*

Source: Plaisted Fernandez, C. and Adams, K.M. (2007). Vegetarian diets in health promotion and disease prevention. In *Handbook of Nutrition and Food*, 2nd ed. (Berdanier, C.D., Feldman, E.B., and Dwyer, J., Eds.). CRC Press, Boca Raton, FL. pp. 389–416.

children; nonetheless, care must be taken to assure adequate intakes of the essential nutrients by all who follow these food intake patterns.

Renal Disease

The kidneys play a vital role in the maintenance of normal blood volume and pressure, water balance, and acid-base balance. Approximately one-fourth of the cardiac output is filtered through the kidneys each minute. They play important roles in red cell synthesis as well as bone mineralization. They are a major excretory organ in that they are important to the urinary excretion of the waste products of absorption and metabolism. Waste products include ammonia, urea, creatinine, phosphorus, water, sodium, and potassium. Their role in bone mineralization has to do with the activation of vitamin D which, in turn, regulates calcium and phosphorus deposition in the bone. The kidneys produce the hormone erythropoietin. Deficiency of this hormone results in profound anemia. Should the kidneys fail a plethora of problems develop that if untreated will result in death.

The role of the kidneys as an excretory organ is important to protein nutrition. Urea as well as other nitrogenous end products are excreted by the kidneys in the urine. If this excretion does not take place, these end products of protein metabolism accumulate and become toxic.

Renal disease is most often a gradual process and may be secondary to other diseases such as cardiovascular disease and diabetes mellitus. There are two major types of renal disease. The acute, inflammatory disease, nephritis, may be sudden and secondary to pathogen invasion. In this instance renal failure is abrupt. Immediate attention is needed. Frequently the patient is placed on dialysis until the pathogenic condition can be corrected. Once corrected, the kidneys may begin to function once again. However, sometimes this does not occur.

The chronic condition, nephropathy, may develop gradually sometimes secondary to diabetes mellitus and/or cardiovascular disease. Again this can be either acute or chronic. Nephrotic syndrome is a dysfunction of the glomerular capillaries. Symptoms include urine losses of plasma proteins, low serum albumin, edema, and elevated blood lipids. In acute renal failure, the nephrons lose function and the glomerular filtration rate (GFR) drops. Symptoms include increased blood urea nitrogen, catabolism, negative nitrogen balance, elevated electrolytes, acidosis, increased blood pressure, and fluid overload. Nephrotic syndrome and acute renal failure are usually reversible conditions. In chronic renal failure the GFR declines gradually. In early stages compensation occurs by enlarging the remaining nephrons. Following a low-protein diet that reduces the workload of the kidneys assists the patient. Symptoms similar to those in acute renal failure appear when the kidney is at 75% of normal function. When the GFR is 10% or less of the normal rate, the patient is considered to be in end-stage renal disease. Dialysis is started to replace diminished kidney function. Electrolytes, fluids, anemia, and diet are monitored monthly. Some patients in end-stage renal failure receive a kidney transplant. This restores kidney function and the patient is able to return to a more liberal diet. These terms are listed and defined in Table 6.18.

Patients with acute or chronic renal disease may become malnourished. Protein energy imbalances worsen this prognosis regardless of disease stage. There may be a significant depletion in visceral protein stores and weight loss. Common manifestations include edema, uremia, hypertension, anemia, and metabolic acidosis. The medical nutrition therapy for kidney failure becomes increasingly complex as the renal disease advances. The diet prescription is matched to the stage of renal failure in order to keep the diet as liberal as possible.

Integration of the Metabolic Features of Protein Nutrition

In the preceding section, protein malnutrition and protein-energy malnutrition or starvation and protein nutrition problems associated with renal disease have been characterized. Throughout this chapter, the chemical and biochemical nature of proteins has been discussed in detail. But how does the body "know" when to synthesize a new protein or degrade a resident one? What messages are

TABLE 6.18
Definition of terms used in renal disease

Term	Explanation
Acute renal failure	Sudden onset secondary to shock, trauma, hypertension, exposure to nephrotoxic substances of bacteria. Reversible in many cases.
Azotemia	Elevated concentrations of nitrogenous wastes in blood serum.
Chronic renal failure (CRF) or insufficiency (CRI)	Gradual progression terminating with end-stage renal disease requiring dialysis or transplant. Causes include obstructive disease of the urinary tract like congenital birth defects, systemic diseases such as diabetes mellitus or systemic lupus erythematosis, glomerular disease, and overdosing on analgesic medications. Patients follow an increasingly restricted diet as renal failure progresses.
Hemodialysis	Removal from blood of the waste products of metabolism by use of a semipermeable membrane and a dialysis treatment machine. This process takes 3–6 hours three times per week in an outpatient clinic or hospital. Some patients, with appropriate training and assistance, can dialyze at home. Hemodialysis patients follow a diet restricted in sodium, potassium, phosphorous, and fluids.
Nephrotic syndrome	Failure of the glomerular basement membrane to filter waste products appropriately. Large amounts of protein are found in the urine. Patients frequently have edema secondary to hypoalbumenemia.
Peritoneal dialysis	Removal of the waste products of metabolism by perfusion of a sterile dialysate solution throughout the peritoneal cavity. This method of dialysis is done at home. Dialysate exchanges are performed several times per day or continuously at night with the aid of a peritoneal dialysis machine. Peritoneal dialysis patients follow a liberalized diet because dialysis is daily. The diet is normally a low- sodium diet with diabetes mellitus restrictions as necessary.
Uremia	A toxic-systemic syndrome caused by retention of high levels of urea.

Source: Thomas, L. and Poole, R. (2007). Renal nutrition. In *Handbook of Nutrition and Food*, 2nd ed. (Berdanier, C.D., Feldman, E.B., and Dwyer, J., Eds.). CRC Press Boca Raton, FL, pp. 815–832.

sent and received that integrate these anabolic and catabolic processes? How does the body cope with its ever-changing environment—of which nutrition is but a part?

Recall that some of the amino acids that are contained by the dietary protein can be decarboxylated and converted to amines. These amines are potent neurotransmitters. That is, they are capable of eliciting system responses via activation of certain neurons or neuronal pathways. Some of these systemic responses include the release of hormones that have positive effects on protein synthesis. An example might be the signals to the pituitary to release growth hormone which, when bound to its cognate membrane receptor, elicits a cascade of intracellular signals which, in turn, migrate to the nucleus and serve as instigators of protein synthesis. Providing that sufficient ATP and amino acids are available, protein synthesis will be stimulated. Another example might be the chronic ingestion of a high-sugar diet that stimulates insulin release. The insulin plus the glucose plus several other factors instigates the synthesis of enzyme proteins needed to metabolize this sugar load. Initiation and cessation of feeding is an example of a system response to changing levels of the neurotransmitter serotonin. Likely, other neurotransmitters are involved as well (see Chapter 2). Through the action of neurotransmitters, other systems can be activated or suppressed and these systems might include sleep or voluntary activity or other such whole-body actions. These responses will have effects on the need for energy and, because the energy need is tightly linked to the protein need, effects on the latter should be expected.

Dietary protein, once consumed, stimulates the release of a variety of gut hormones. These hormones likewise elicit systemic responses as outlined in the section on digestion and absorption.

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Further, once amino acids are liberated through the action of the digestive enzymes, these amino acids, as substrates for enterocyte carriers (see section on absorption) stimulate not only the carrier activity (substrate activation) but also are involved in the synthesis of the carrier itself by the enterocyte. This is not an uncommon phenomenon. A number of high-turnover proteins (i.e., enzymes, carriers, and hormones) have their synthesis dictated by rising levels of the substrates upon which they act. Hence, rising levels of cytosolic citrate in the cell might be expected to instigate the transcription of the messenger RNA for ATP citrate lyase, the enzyme that catalyzes the formation of acetyl CoA and oxalacetate from citrate and CoA with a concomitant hydrolysis of ATP to ADP and phosphate. So, too, might one expect to find in the enterocyte an increase in the mRNAs for those proteins that are responsible for the transport of the amino acids into the enterocyte via carrier-mediated mechanisms. Thus, we see multiple roles for the amino acids found in the dietary proteins, and these roles are not restricted to just the enterocyte. Other cells, tissues, and organs also are affected. The enzymes that are responsible for amino acid metabolism are likewise synthesized or degraded in response to the levels of those amino acids on which they work. The expression of the genes that encode these enzymes may be unique to a given cell type, tissue, or organ, or may be universal. Uniqueness of gene expression means that factors other than the substrate are operative in the regulation of that expression. For example, muscle cells cannot complete the oxidation of histidine, which is liberated as muscle protein is degraded during muscle contraction and relaxation. Even though the muscle cell contains the same DNA as every other cell type, the expression of those genes responsible for the complete deamination and oxidation of histidine (see Figure 4.23) does not occur despite the rising levels of histidine. Instead, this histidine is methylated and excreted in the urine. In few other cell types does this methylation occur; histidine is usually metabolized. However, the expression of the gene for the enzyme responsible for histidine methylation is "turned on" by its substrate, histidine, and its affinity for this substrate as well as its activity as a catalyst exceeds that of any other enzyme in the muscle cell that might use histidine. As the muscles are increased in activity and size, the amount of methylated histidine found in the urine also increases. Here, then, is a complete loop. The histidine in the food is transported to the muscle that uses it to synthesize myosin and actin, and when that muscle is actively working, myosin and actin are degraded, with 3-methyl histidine appearing in the urine. Histidine has acted as a signal for the expression of genes coded for its transport, for its incorporation into muscle protein, and for its methylation and excretion. It has also served as a substrate for all of these processes plus those outlined in Chapter 3.

Each of the amino acids, as well as every other nutrient consumed as part of the diet, likewise has multiple roles. In and of themselves, amino acids serve as signals of metabolic processes, or they can serve as substrates for the synthesis of proteins that act as carriers, receptors, enzymes, hormones, or structural materials.

The complexity of these interacting roles of amino acids as neurotransmitters, as enzyme activators, as inducers of gene expression, and as substrates for a multitude of synthetic and degradative reactions is enormous. Yet the brain and other vital organs signal each other such that integration of function occurs and a comprehensive metabolic pattern emerges. When the body is without sufficient nutrient intake to sustain normal body function, it has a hierarchy in place that controls amino acid use so that more important functions are maintained at the expense of less important ones. Hence, in the protein-malnourished child, the symptoms of weight loss, skin lesions, and hair changes are observed, whereas the activity of metabolic enzymes and hormones is conserved.

Protein synthesis is energetically very expensive as well as being dependent on amino acid availability. Thus, protein synthesis is also suppressed in a hierarchical manner. Skin cells and hair cells are not replaced, due to this decreased protein synthesis, as rapidly as in the well-nourished individual. Hence, the skin lesions and hair changes that typify protein malnutrition. Energy conservation and protein conservation similarly are preserved. The synthesis and release of hormones that accelerate protein breakdown and use are suppressed via effects of diet deprivation on the brain, peptides that signal food-seeking behavior are maintained, gut motility is suppressed so as to retard the passage of food from mouth to anus and extract as much nourishment as possible from

that food, body activity is diminished to reduce energy expenditure (the symptom of lethargy), and sleep time is increased for the same reason. All of these defenses against death due to starvation are directed by the CNS and executed by the secretions of the endocrine organs. In turn, these defenses are activated when the body senses, via the gut cells, that insufficient food has been consumed.

Some of these defenses can be compromised by additional problems in the environment. If the drinking water is contaminated, the reduction in gut passage time as a defense is negated by pathogen-induced diarrhea. This results in a loss of gut contents and abrasion of the cells lining the intestinal tract. In this instance, the person is less able to cope with an inadequate food supply. The coordination of the defense against death due to starvation is disrupted by the stress response (also coordinated by hormones) to the invading pathogens. Such stress elicits a signal from the pituitary that stimulates an outpouring of the catecholamines and steroidal hormones by the adrenals. These hormones mobilize muscle protein, body fat stores, and glycogen stores needed for the synthesis of antibodies to the pathogens as well as for the synthesis of replacement enterocytes and the conservation of electrolytes and water. Because such syntheses require energy, amino acids, and a number of micronutrients—all of which may be in short supply—it is easy to understand why malnourished individuals are more vulnerable to environmental contaminants and why the resultant disease is far more severe.

The interaction of disease and nutritional state can have lasting effects on body function. Hence, it is not uncommon to observe short stature in populations whose food supply is inadequate. Growth, a reflection of protein synthesis that in turn is dependent on nutrient intake, is suppressed because growth is lower on the hierarchical scale of body functions that are preserved in times of need. When the food supply changes and becomes reliably abundant with a variety of foods, including sources of good-quality protein, then the genetic potential for body size is fully realized. The average height of the population increases from one generation to the next and the body fat stores are maintained at capacity. The latter feature may not be deemed to be desirable.

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7 Carbohydrates

Carbohydrates provide as much as 60% of the daily energy intake. The percentage of the diet that is carbohydrate varies inversely with economic conditions and with the percentage of the diet that is fat and protein. As a general rule, carbohydrate-rich foods are less expensive than fat- and protein-rich foods. Hence, as people have more disposable income, they tend to buy and consume fewer cereals, breads, fruits, and vegetables and buy more meat, butter (or margarine), milk, and eggs. This is not always true, however. Over the last decade, education about the possible health risks of high intakes of fatty foods and the health benefits of high fiber intakes, as well as the benefits of consuming fruits and vegetables, has altered the eating habits of many consumers. This, in turn, has had an impact on the percent of intake provided by this macronutrient class.

The term carbohydrate originated in the late 1800s with the idea that there existed naturally occurring compounds composed of carbon, hydrogen, and oxygen, which could be represented as hydrates of carbon. For instance, glucose ($C_6H_{12}O_6$), sucrose ($C_{12}H_{22}O_{11}$), and starch $(C_6H_{10}O_5)$n could all be represented by the general formula $Cx(H_2O)y$. This definition was too rigid, however, because it excluded such common compounds that could be classed as carbohydrates such as deoxyribose, a component of DNA, ($C_5H_{10}O_4$), and ascorbic acid, a vitamin, ($C_6H_8O_6$). This definition included the compound acetic acid ($C_2H_4O_2$), which, strictly interpreted, is not a carbohydrate. To overcome these exclusions, a better definition was developed, and that is that carbohydrates are polyhydroxy aldehydes or ketones and their derivatives.

CLASSIFICATION

Carbohydrates are divided into three major classes: monosaccharides, oligosaccharides, and polysaccharides. A monosaccharide consists of a single polyhydroxy aldehyde or ketone unit. An oligosaccharide contains two to ten monosaccharide units. The upper limit on the number of monosaccharides in an oligosaccharide is not rigorously defined. Disaccharides, composed of two monosaccharides, are the most common oligosaccharides. A polysaccharide contains many monosaccharides.

STRUCTURE AND NOMENCLATURE

Monosaccharides

Monosaccharides, called *simple sugars*, have the empirical formula (CH_2O)n, where n is 3 or more. Although monosaccharides may have as few as three or as many as nine carbon atoms, those of interest to the nutritionist have five or six. The carbon skeleton is unbranched, and each carbon atom, except one, has a hydroxyl group and a hydrogen atom. At the remaining carbon atom, there is a carbonyl group. If the carbonyl function is on the last carbon atom, the compound is an aldehyde and is called an *aldose*; if it occurs at any other carbon, the compound is a ketone and is called a *ketose*. These structures are illustrated in Figure 7.1.

The simplest monosaccharide is the 3-carbon aldehyde or ketone, *triose*. Glyceraldehyde is an aldotriose; dihydroxyacetone is a *ketotriose*. Successive chain elongation of trioses yields *tetroses, pentoses, hexoses, heptoses*, and *octoses*. In the aldo-series. these are called *aldotriose, aldotetrose, aldopentose, aldohexose*, etc.; in the keto-series, they are *ketotriose, ketotetrose, ketopentose, ketohexose*, etc. Figure 7.2 shows the D-aldose monosaccharides that have three to six carbons.

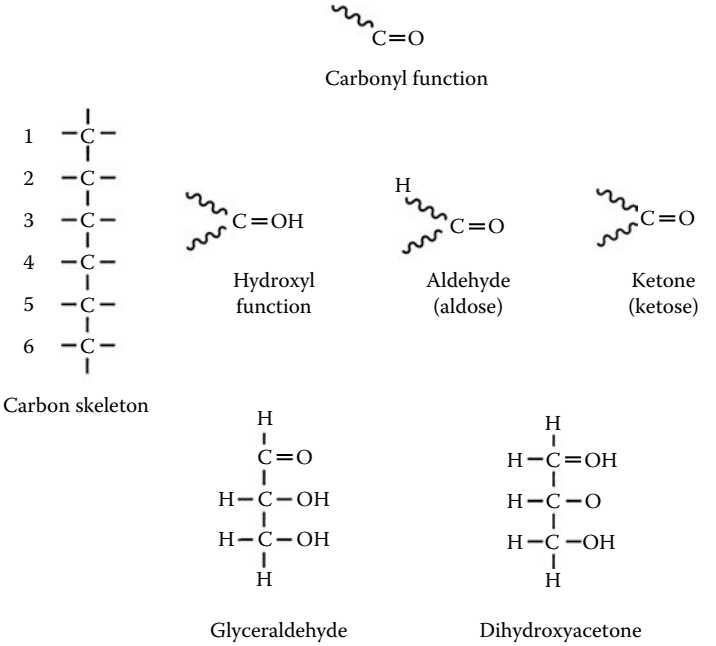


FIGURE 7.1 Structures of monosaccharides.

Figure 7.3 illustrates the corresponding D-ketoses. Within a given series of the same number of carbon atoms, these molecules differ only in the arrangement of the hydrogen atom and the hydroxyl group about the carbon atoms. Compare, for example, glucose and mannose—the configuration of the hydrogen and hydroxyl group about each of the carbons is the same except for carbon two. In glucose, the hydroxyl function is to the right; in mannose, it is to the left. Two sugars that differ only in the configuration about one carbon atom are referred to as *epimers*. D-glucose and D-mannose are epimers with respect to the number-two carbon atom; D-mannose and D-talose are epimers with respect to the number-four carbon atom.

Configuration and conformation are two frequently used terms that are easily confused. *Configuration* is the arrangement in space of atoms or groups of atoms of a molecule that can be changed only by breaking and making bonds. *Conformation* is the arrangement in space of atoms or groups of atoms of a molecule that can arise by rotation about a single bond and that are capable of a finite existence.

The common monosaccharides are white crystalline compounds, freely soluble in water but insoluble in such nonpolar solvents as benzene and hexane. Most of them have a sweet taste and are by far the most abundant of the simple sugars. Of these, glucose, fructose, and galactose are most often found in foods, frequently as members of disaccharides or polysaccharides. Mannose is occasionally found, but only in complexes that are poorly digested. Aldopentoses are important components of DNA and RNA; derivatives of triose and heptose are intermediates in carbohydrate metabolism.

Stereoisomeric Forms

All of the monosaccharides, except dihydroacetone, contain at least one asymmetric carbon atom, which, in the simplest sense, is one to which four different constituents are attached. Molecules that possess an asymmetric center can exist in stereoisomeric forms.

Stereoisomers have the same structural framework but differ in the spatial arrangement of the various substituent groups. Stereoisomers include *cis* and *trans* forms, (chair and boat forms) and epimers. They are different from structural isomers. Structural isomers have different structural

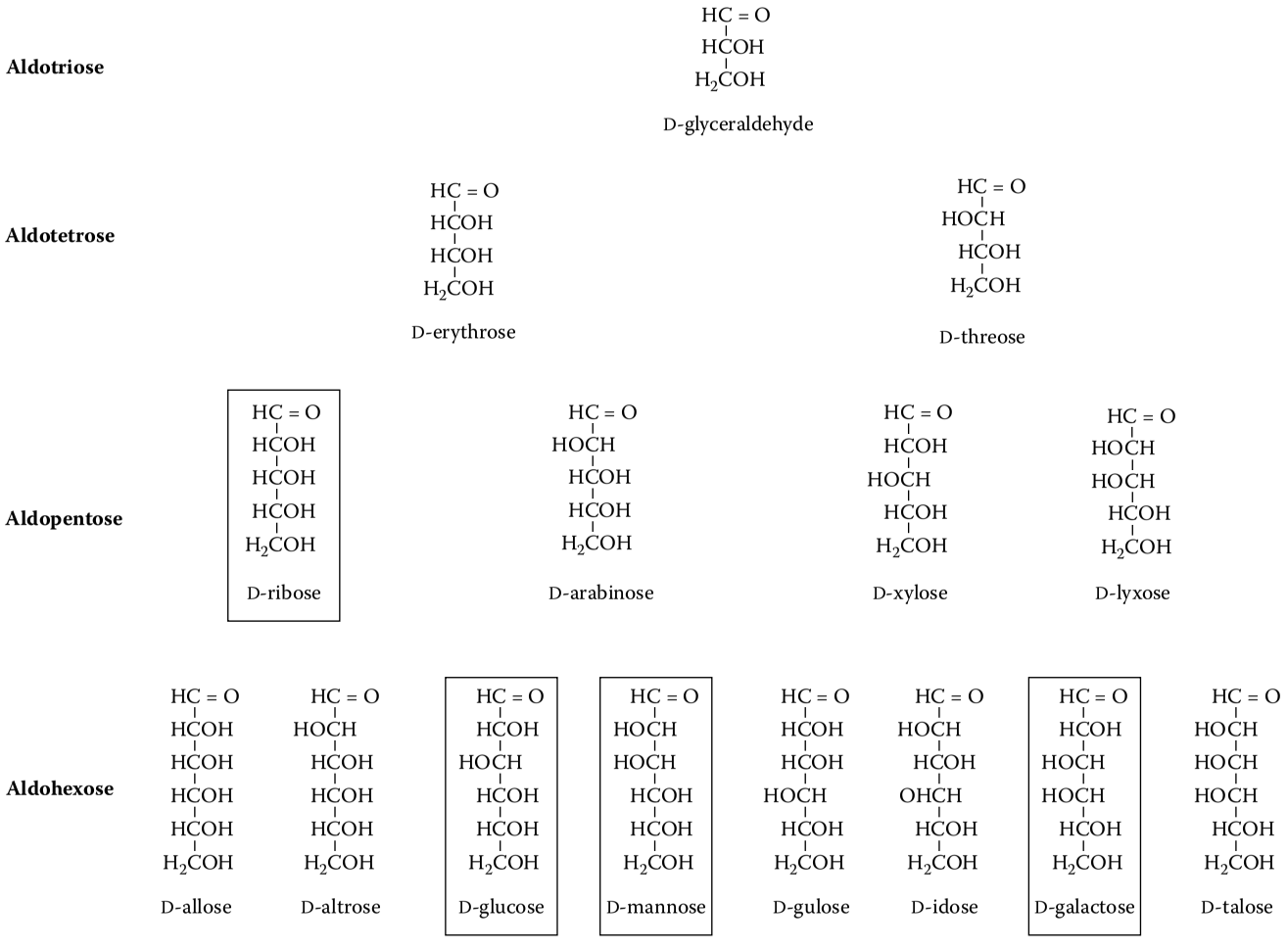


FIGURE 7.2 D-aldoses having from three to six carbon atoms. Those of the greatest biological significance are enclosed in boxes.

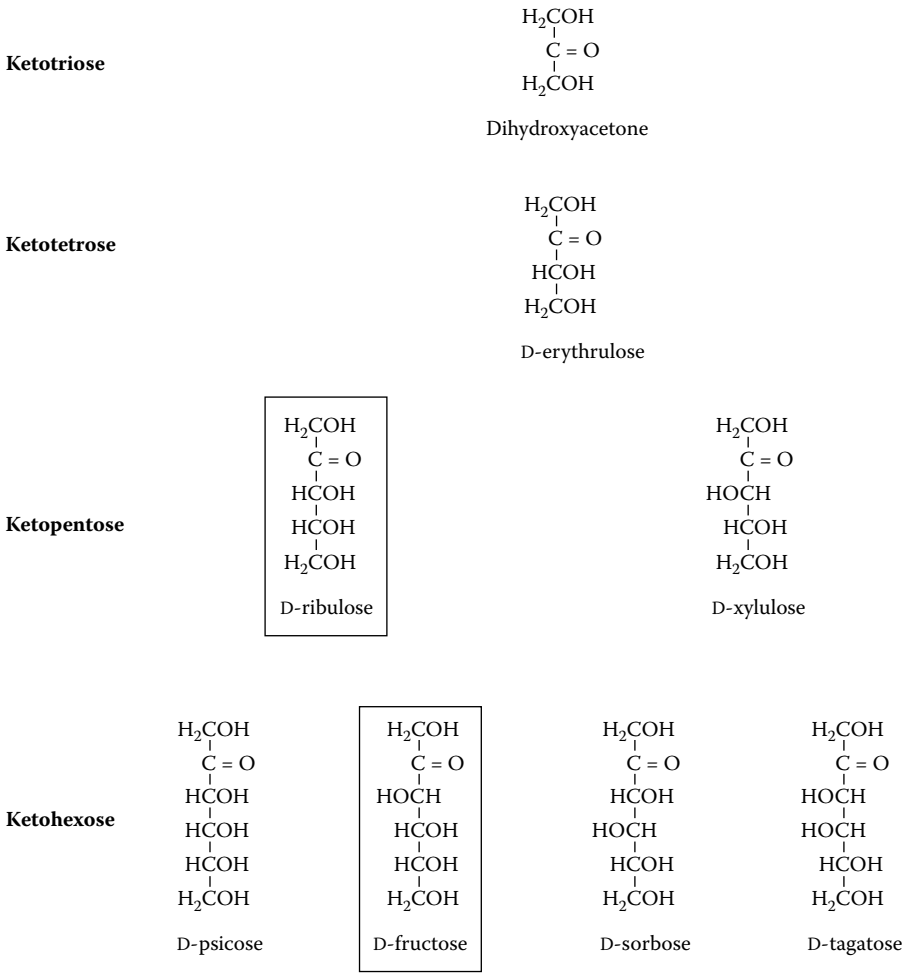


FIGURE 7.3 D-ketoses having from three to six carbon atoms. Those of the greatest biological significance are enclosed in boxes. Ketoses are sometimes named by inserting an "-ul" into the name of the corresponding aldose. D-ribulose is the ketopentose corresponding to the aldopentose D-ribose.

frameworks; the bonding arrangements for the component atoms are different. C4H8 is the molecular formula for the four structural isomers in Figure 7.3.

For example, glyceraldehyde may occur in either the D or the L stereoisomer. The two stereoisomers bear the same relationship to each other as one's right and left hands; if both hands are laid flat on a table and the right hand slid on top of the left hand, the right hand does not cover the left (note that thumbs stick out in opposite directions). When the chemical structure for each of these molecules is written as projection formulas, their differences are obvious: The hydroxyl group and the hydrogen atom on the asymmetric carbon reverse positions; these two compounds are mirror images of each other. Mirror-image forms of the same compound are called *enantiomers.* Pairs of enantiomers (the D and L stereoisomers of a molecule) possess the same chemical and physical properties except for rotating plane-polarized light in opposite but equal directions; that is, the values of their specific rotation have opposite signs. A note of caution, however; a D or an L used in the name of a sugar molecule does not refer to the sign of its optical rotation. These letters specify the absolute configuration of the molecule. If one desires to include the sign of optical rotation in the molecular name, a (+) for dextrorotary, rotation to the right, or (–) for levorotary, rotation to the left,

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is used. For example, the specific rotation of the D-glyceraldehyde is 14°; hence, its name is D-(+)-glyceraldehyde; D-lactic acid has a specific rotation of –3.8, and is called D-(–)-lactic acid.

By convention, a carbohydrate belongs to the D series if, when it is written in the projection formula with the aldehyde or hydroxyketone group at the top, the hydroxyl group on the next to the last carbon is to the right; conversely, if the molecule is written exactly as just described except for the fact that the hydroxyl group on the next to the last carbon is written to the left, it belongs to the L-series. A molecule which possesses n asymmetric carbons has 2n as the upper limit of the number of stereoisomers. The aldohexose series has four asymmetric carbon atoms; thus, it has 16 stereoisomers or eight pairs of enantiomers. One of these pairs is α-D-glucose and α-L-glucose.

In nature, D-monosaccharides are much more abundant than L. Most mammalian cells require D sugar because they are unable to metabolize the L form. However, a few L-monosaccharides can be found; among the most important are L-rhamnose and L-sorbose. Glucose is often called "dextrose" because the stereoisomer almost always rotates plane-polarized light to the right; it is dextrorotary. Similarly, fructose is often called "levulose" because its most common natural form is levorotary.

Anomeric Forms

In the section on stereoisomeric forms, glucose was referred to as α-D-glucose instead of D-glucose. The presence of the Greek letter alpha (α) adds another dimension to the structure of carbohydrates. This feature can be more readily understood if one realizes that aldehydes and ketones can add hydroxyl groups at the carbonyl function. If water is added, the unstable hydrate of the aldehyde is formed. If alcohol is added, a hemiacetal is formed. If alcohol is added to the hemiacetal, a full acetal (or simple acetal) is formed by the elimination of water. Ketones undergo similar reactions but form hemiketals or ketals. The hemiketal, having four different constituents, is an asymmetric carbon atom; it exists in one of two stereoisomeric forms.

In the case of carbohydrates, the same hemiacetal formation can occur but it will be an intramolecular reaction and a ring is produced. A six-membered ring, containing five carbon atoms and one oxygen atom, is called pyranose (after pyran). A five-membered ring, containing four carbons and one oxygen, is called a *furanose* (after "furan"). Figure 7.4 illustrates these two structures. As with aldehydes, this addition reaction in a carbohydrate produces an asymmetric carbon atom; it is referred to as the *anomeric carbon*. We now have two monosaccharides that differ only in their configuration about the anomeric carbon; they are called *anomers* and are referred to as α and β forms. In the α form, the hydroxyl group is below the plane of the ring to which it is attached. In the β form, it is above. This ring closure is illustrated for D-glucose and D-fructose in Figure 7.5.

The monosaccharides in the body undergo a number of reactions, described in Chapter 3 (Nutritional Biochemistry) in the section on carbohydrate metabolism. These reactions produce five general groups of products as shown in Table 7.1.

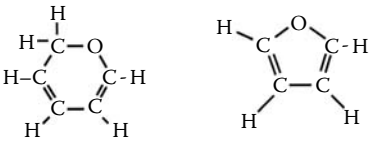


FIGURE 7.4 Structural representations of pyran and furan rings.

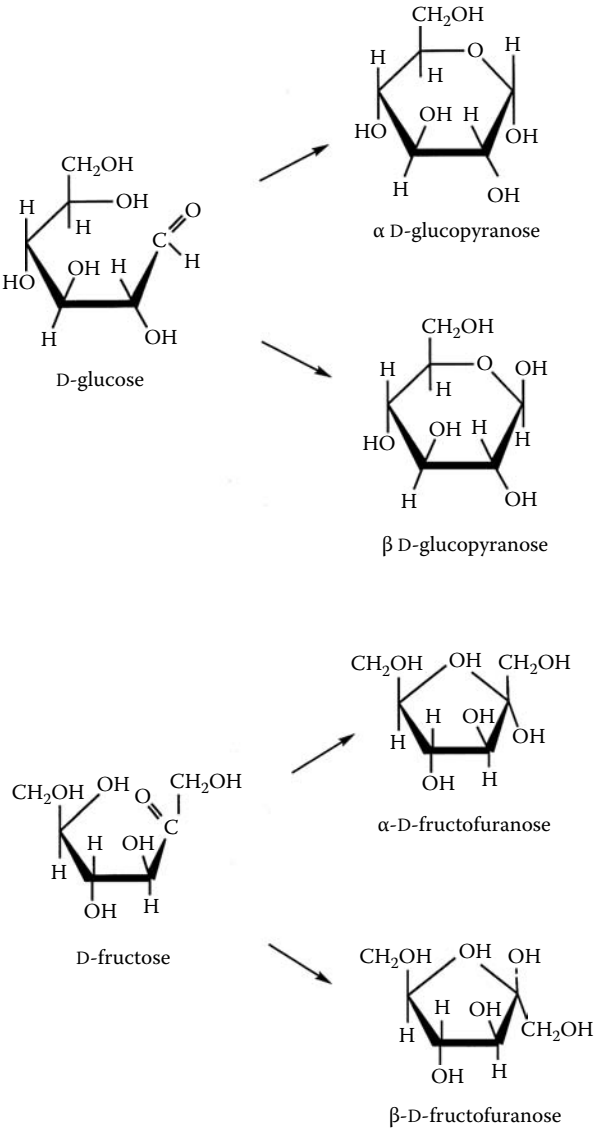


FIGURE 7.5 Ring closure of D-glucose and D-fructose to make the pyranose and furanose forms.

Oligosaccharides

Oligosaccharides consist of two to ten monosaccharides joined with a glycosidic bond. The bond is formed between the anomeric carbon of one sugar and any hydroxyl function of another. If two monosaccharides are bonded in this manner, the resulting molecule is a disaccharide; if three, a trisaccharide; if four, a tetrasaccharide, etc.

Disaccharides

Of the oligosaccharides, by far the most prevalent in nature are the disaccharides. Of dietary significance are lactose, maltose, and sucrose.

TABLE 7.1
Reaction products of monosaccharides

Product	Example
Phosphoric acid esters	Glucose-6-phosphate
Polyhydroxy alcohols	Sorbitol
Deoxy sugars	Deoxyribose
Sugar acids	Gluconic acid
Amino sugars	Glucosamine

Lactose, the sugar found in the milk of most mammals (it is lacking in the milk of the whale and the hippopotamus), consists of a D-galactose and D-glucose joined with a glycosidic linkage at carbon one of galactose and carbon four of glucose. Lactose is a reducing sugar. The glycosidic linkage between galactose and glucose is symbolized by α (1→4). An important characteristic of lactose is its ability to promote the growth of certain beneficial lactic acid-producing bacteria in the intestinal tract. These bacteria have a possible role in the displacement of undesirable putrefactive forms of bacteria. Lactose also enhances the absorption of calcium.

Maltose, also known as malt sugar, contain two glucose residues. The glycosidic linkage is α (1→4). Celliobiose, the repeating disaccharide unit of cellulose, and gentiobiose are two other disaccharides that have as their repeating units D-glucose. In celliobiose, the glycosidic linkage is β (1→4); in gentiobiose it is β (1→6). Because all have free anomeric carbons, they are reducing sugars. The β linkage cannot be broken by the intestinal enzymes but are approachable by the intestinal flora.

Sucrose, also known as table sugar, cane sugar, beet sugar, or grape sugar, is a disaccharide with glucose and fructose linked through the anomeric carbon of each monosaccharide. Because neither anomeric carbon is free, sucrose is a nonreducing sugar. It is not a hemiacetal and does not undergo mutarotation. In dilute acid or in the presence of the enzyme invertase, sucrose hydrolyzes into its constituent monosaccharides. The hydrolysis of sucrose to D-glucose and D-fructose is called *inversion* because it is accomplished by a change in the sign of specific rotation from dextro (+) to levo (–) as the equimolar mixture of glucose and fructose is formed; this mixture is called *invert sugar.* The development of an enzyme that will isomerize D-glucose to D-fructose has proven to be commercially valuable. It provides a method of obtaining a product known as *high-fructose corn syrup*, similar to invert sugar and containing no sucrose.

Invert sugar syrups are available commercially at varied levels of inversion. They are sweeter than sucrose at comparable concentrations. This greater sweetness reflects the D-fructose component of the syrup and is an important and critical feature. When sucrose is used in the preparation of acidic foods, some inversion invariably takes place. For instance, if it is used to sweeten fruit drinks, it is completely inverted within a few hours. In soft drinks, there is also a considerable amount of inversion. Honey is largely invert sugar. Bees collect sucrose from flowers, and they have an enzyme that inverts the sucrose. Honey, however, is not pure glucose and fructose; it also contains sucrose, water, and small quantities of flavor extract peculiar to the flower from which the sucrose is obtained.

In general, the disaccharides, although of importance as dietary sources of carbohydrates, have few metabolic functions. They are hydrolyzed into their component monosaccharides by enzymes located in the brush border of the enterocyte of the small intestine. These monosaccharides are then converted to glucose, the body's primary metabolic fuel (see Chapter 3, Nutritional Biochemistry). Some of the hydrolysis takes place in the lumen of the small intestine whereas most takes place within the absorptive cell.

Two oligosaccharides are of nutritional interest. These are stachyose and raffinose. Stachyose, a tetrasaccharide, is composed of two molecules of D-galactose, one of D-glucose, and one of D-fructose. It is found in certain foods, particularly those of legume origin. It is usually found with raffinose (fructose, glucose, and galactose) and sucrose. The human digestive tract does not possess an enzyme that can hydrolyze either stachyose or raffinose. Evidently, however, these sugars are fermented in the lower intestinal tract by the intestinal flora to some short-chain fatty acids and some other metabolites. The fermentation of these oligosaccharides is thought to be responsible for the unwanted flatus (gas produced and released by the intestinal tract) that frequently follows the ingestion of foods containing these compounds. Dried legumes (peas and beans) are good sources of raffinose and stachyose.

Polysaccharides

Polysaccharides (also known as glycans) are compounds consisting of large numbers of monosaccharides linked by glycosidic bonds; they are analogous in structure to oligosaccharides. Some have as few as 30 monosaccharides. However, most of the polysaccharides found in nature have a high molecular weight; they may contain several hundreds or even thousands of monosaccharide units. Polysaccharides differ from one another in the nature of their repeating monosaccharide units, in the number of such units in their chain, and in the degree of branching. The polysaccharides that contain only a single kind of monosaccharide or monosaccharide derivative are called homopolysaccharides; those with two or more different monomeric units are called heteropolysaccharides. Often homopolysaccharides are given names that indicate the nature of the building blocks: for example, those that contain mannose units are *mannans*; those that contain fructose units are called *fructans*. The important biological polysaccharides are the storage polysaccharides, the structural polysaccharides, and the mucopolysaccharides.

Storage Polysaccharides

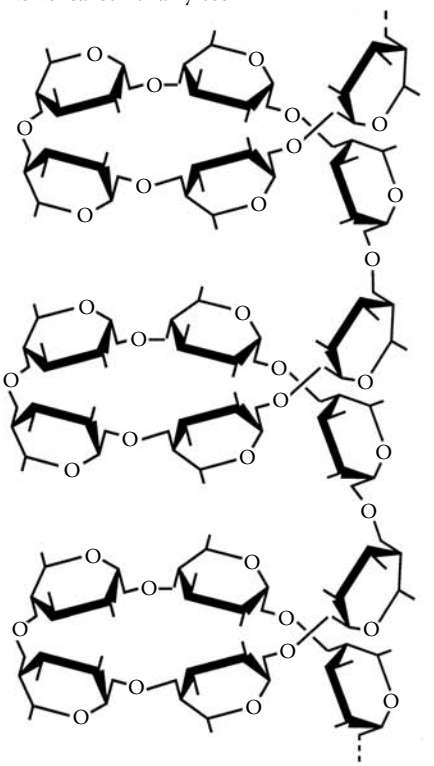
Among plants, the most abundant storage polysaccharide is starch. It is deposited abundantly in grains, fruits, and tubers in the form of large granules in the cytoplasm of cells; each plant deposits a starch characteristic of its species. Starch exists in two forms: α-amylose and amylopectin. α-Amylose makes up 20–30% of most starches and consists of 250–300 unbranched glucose residues bonded by α (1→4) linkages. The chains vary in molecular weight from a few thousand to 500,000. The molecule is twisted into a helical coil. Amylopectin, which the remainder of the starch in a plant comprises, is highly branched. Its backbone consists of glucose residues with α (1→4) glycosidic linkages; its branch points are α (1→6) glycosidic bonds. Although the structure of amylopectin is shown in Figure 7.6 as being linear, it too exists as a helical coil.

When amylose is broken down in successive stages by either the enzyme amylase or by the action of dry heat, as in toasting, the resulting polysaccharides of intermediate chain length are called *dextrins*. Amylopectin, when broken down by the same methods, does not cleave at its branch points. Its end product is a large, highly branched product called *limit dextrin*.

Other homopolysaccharides are found in plants, bacteria, yeast, and mold as storage polysaccharides. Dextrans, found in yeast and bacteria, are branched polysaccharides of D-glucose with their major backbone linkage α (1→6). Inulin, found in artichokes, consists of D-fructose monomers with β (2→1) glycosidic linkages. Mannans are composed of mannose residues and are found in bacteria, yeasts, mold, and higher plants.

Among animals, the storage polysaccharide is glycogen. Glycogen is stored primarily in the liver and muscles. Like amylopectin, glycogen is a branched polysaccharide of D-glucose with a backbone glycosidic linkage of α (1→4) and branch points of α (1→6). However, its branches occur every 8 to 10 residues, as compared with every 12 for amylopectin. Glycogen is of no importance

The helical coil of amylose



An α (1→6) branch point in amylopectin

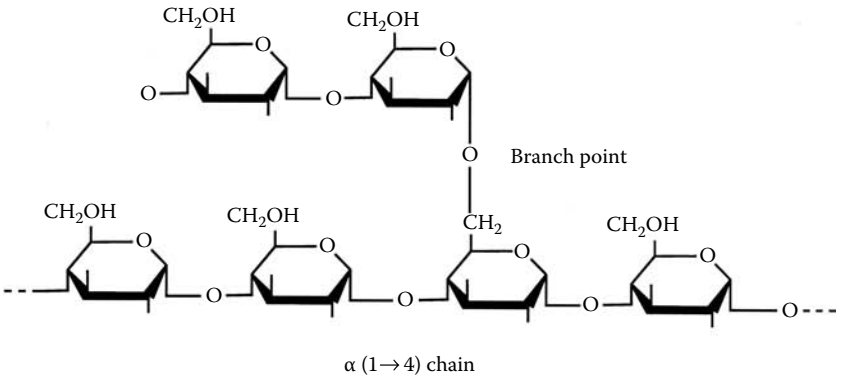


FIGURE 7.6 Structures of storage polysaccharides.

as a dietary source of carbohydrate. The small amount of glycogen in an animal's body when it is slaughtered is quickly degraded during the postmortem period.

Structural Polysaccharides

Cellulose is the most abundant structural polysaccharide in the plant world. Fifty percent of the carbon in vegetables is cellulose; wood is about one-half cellulose and cotton is nearly pure cellulose.

It is a straight-chain polymer of D-glucose with β(1→4) glycosidic linkages between the monosaccharides. Cellobiose, a disaccharide, is obtained on partial hydrolysis of cellulose. The molecular weight of cellulose has been estimated to range from 50,000 to 500,000 (equivalent to 300 to 3000 glucose residues). Cellulose molecules are organized in bundles of parallel chains, called *fibrils*. These fibrils are crosslinked by hydrogen bonding; the chains of glucose units are relatively rigid and are cemented together with hemicelluloses, pectin, and lignin.

Hemicellulose bears no relation structurally to cellulose. It is composed of polymers of D-xylose having β(1→4) glycosidic linkages with side chains of arabinose and other sugars. Pectin is a polymer of methyl D-galacturonate. Pectin is found in fruit and is the substance needed to make jelly out of cooked fruit. The juice plus sucrose plus the pectin form a gel that is stable for many months at room temperature. Pectin is a nonabsorbable carbohydrate that has pharmacological use as well. It is a key component, together with kaolin (clay), of an antidiarrheal remedy.

There are other structural polysaccharides found in nature. Chitin is a polysaccharide that forms the hard skeleton of insects and crustaceans and is a homopolymer of *N*-acetyl-D-glucosamine. Agar, derived from sea algae, contains D- and L-galactose residues, some esterified with sulfuric acid, primarily with 1→3 bonds; alginic acid, derived from algae and kelp, contains monomers of D-mannuronic acid; and vegetable gum (gum guar) contains D-galactose, D-glucuronic acid, rhamnose, and arabinose. These are used as food stabilizers by the food-processing industry. Algin derivatives, for example, are used to stabilize the emulsions of salad dressings; gum guar is frequently used to stabilize processed cheese products. It acts to retard the separation of the solids from the fluid component in such products.

Mucopolysaccharides

The mucopolysaccharides are heteropolysaccharides that are components of the structural polysaccharides found at various places in the body. Mucopolysaccharides consist of disaccharide units in which glucuronic acid is bound to acetylated or sulfurated amino sugars with glycosidic β(1→3) linkages. Each disaccharide unit is bound to the next by a β(1→4) glycosidic linkage. Thus, they are linear polymers with alternating β(1→3) and β(1→4) linkages. Hyaluronic acid is the most abundant mucopolysaccharide. It is the principal component of the ground substance of connective tissue and is also abundant in the synovial fluid in joints and the vitreous humor of the eye. The repeating unit of hyaluronic acid is a disaccharide composed of D-glucuronic acid and *N*-acetyl-D-glucosamine; it has alternating β(1→3) and β(1→4) glycosidic linkages. The molecular weight is several million.

Another mucopolysaccharide that forms part of the structure of connective tissue is chondroitin. It differs from hyaluronic acid only in that it contains *N*-acetyl-D-galactosamine residues rather than *N*-acetyl-D-glucosamine ones. The sulfate ester derivatives of chondroitin, chondroitin sulfate A and chondroitin sulfate C, are major structural components of cartilage, bone, cornea, and other connective tissue. Types A and C have the same structure as chondroitin, except for a sulfate ester at carbon atom 4 of the *N*-acetyl-D-galactosamine residue on type A and one at carbon atom 6 of type C.

Heparin is a mucopolysaccharide that is similar in structure to hyaluronic acid, in that it contains residues of D-glucuronic acid. However, in heparin, these residues contain varying portions of both sulfate and acetyl groups. Its structure is not entirely known. It is a blood anticoagulant.

SOURCES OF CARBOHYDRATE

Carbohydrates are constituents of all living cells. As such, one could anticipate finding one or more carbohydrates in almost any food of importance to man. Practically speaking, however, foods from animal sources contain few carbohydrates. Milk, with its high lactose content, is the only significant animal source of carbohydrate. Cow's milk contains 4.8% lactose; human milk, 7%. Eggs, scallops,

and oysters contain small amounts of carbohydrate. Foods from plant sources, on the other hand, contain large amounts of carbohydrates. Oranges, bananas, and apples are good sources of fructose. Potatoes and cereal grains are good sources of starch. The percentage of carbohydrate in several common foods can be seen in Table 7.2.

The sugar content of several fresh fruits and vegetables is given in Table 7.3. The starch content is not shown. Canned and frozen fruits often contain sugar in addition to that which occurs naturally. This sugar is used in processing the fruit to preserve the structure, color, and flavor of the fruit. Thus, canned and frozen fruits may have more sugar in them than when eaten fresh and unprocessed. The high-fructose corn syrup solutions are also used in soft drinks to add body without affecting or masking flavors. Canners and preservers choose these syrups because they penetrate the fruit easily and preserve its natural form, flavor, and color.

In addition to the sugars and starches, plants contain cellulose, hemicellulose, pectin, and lignin (a noncarbohydrate). These carbohydrates provide fiber, or indigestible residue. Table 7.4 gives the amounts of crude fiber in many common foods. By definition, crude fiber is the residue of plant food left after extraction by dilute acid and alkali. The exact amount of fiber that a food can provide is the subject of some dispute.[3–6] In the past, chemical analyses of foods have provided values for the amount of crude fiber in specific foods. However, the term *crude fiber* is not the same as the undigested carbohydrate that appears in the feces. Although cellulose, plant fibers, and other so-called nondigestible carbohydrates are not digestible by the enzymes located in the upper portion of the intestine, the intestine contains flora that can partially degrade some of these food components. This degradation provides fatty acids and other useful compounds that are then absorbed by the lower small intestine and colon. Several methods of analysis for dietary fiber are available but none truly assess and quantitate the amount of fiber that is not used. As mentioned, several of the nondigestible carbohydrates provide useful substrates to the colon flora and thus should not be considered as nondigestible carbohydrate.

TABLE 7.2
Carbohydrates in common foods

Item	Percentage
Fruits and vegetables	5–20
Milk	5 (depending on species)
Shellfish	<1
Fish	<1
Lean pork	<1
Ice cream, cake, pie	40–50
Lunch meat	<5
Cheese, roast beef	<1
Peanut butter, bacon	<10
Nuts	<10
Butter, margarine	0
Salad oil	0
Sugar	100

Source: http://www.nal.usda.gov/fnic/foodcomp/Data/foods.82nutrients

TABLE 7.3
Free sugar content (gram/100 gram fresh weight) in fruits and vegetables

Item	Glucose	Fructose	Sucrose	Maltose	Raffinose	Stachyose
Apple	1.17	6.04	3.78	Trace	—	—
Beans, lima	0.04	0.08	2.59	—	0.20	0.59
Beans, snap	0.48	1.30	0.28	—	0.26	—
Broccoli	0.73	0.67	4.24	—	—	—
Peach	0.91	1.18	6.92	0.12	—	—
Strawberry	2.09	2.40	1.03	0.07	—	—

Source: http://www.nal.usda.gov/fnic/foodcomp/Data/food.82nutrients

TABLE 7.4
Fiber content in common foods

Item	Fiber (g/100 g)
Almonds	2.6
Apples	0.9
Beans, lima	1.8
Beans, string	1.0
Broccoli	1.5
Carrots	1.0
Flour, whole wheat	2.3
Flour, white wheat	0.3
Noodles, dry	0.4
Oat flakes	1.4
Pears	1.5
Pecans	2.3
Popcorn	2.2
Strawberries	1.3
Walnuts	2.1
Wheat germ	2.5

Source: Tables of Food Composition, Scientific Tables, K. Diem and C. Letner, Eds., 7th ed., Ardsley, New York: CIBA-Geigy, 1974.

DIGESTION AND ABSORPTION

Once a carbohydrate-rich food is consumed, digestion begins. As the food is chewed, it is mixed with saliva, which contains α amylase. This amylase begins the digestion of starch by attacking the internal α 1,4-glucosidic bonds. It will not attack the branch points having α-1,4,- or α-1,6-glucosidic bonds, hence the salivary α amylase will produce molecules of glucose, maltose, α-limit dextrin, and maltotriose. The α-amylase in saliva has an isozyme with the same function in the pancreatic juice. The salivary α-amylase is denatured in the stomach as the food is mixed and acidified with the gastric hydrochloric acid. As the stomach contents move into the duodenum, it is called *chyme*. The movement of chyme into the duodenum stimulates pancreazymin release. This gut hormone acts on the exocrine pancreas, stimulating it to release pancreatic juice into the

duodenum. Pancreazymin has another name: *cholecystokinin*. The two names were given before it was realized that the two different functions—the stimulation of the release of pancreatic juice from the exocrine pancreas and bile from the gall bladder— were performed by the same hormone. This hormone is secreted by the epithelial endocrine cells of the small intestine, particularly the duodenum. Its release is stimulated by amino acids in the lumen and by the acid pH of the stomach contents as it passes into the duodenum. The low pH of the chyme also stimulates the release of secretin, which, in turn, stimulates the exocrine pancreas to release bicarbonate and water so as to neutralize the acidity of the stomach contents as they are moved into the small intestine. This neutralization then optimizes the environment for the activity of the digestive enzymes located on the surface of the luminal cell.

The disaccharides in the diet are hydrolyzed to their component monosaccharides. Lactose is hydrolyzed to glucose and galactose by lactase; sucrose is hydrolyzed to fructose and glucose, and maltose is hydrolyzed to two molecules of glucose. Table 7.5 lists these enzymes together with their substrates and products. One of these enzymes, lactase, declines in activity after weaning. In rodents, this decline is regulated at the level of transcription by factors in addition to the cessation of milk consumption.[7–9] This may also occur in the human; however, the identity of these regulatory substances has not been fully elucidated. Because the majority of humans in the world are lactose intolerant at adulthood and because milk contains so many essential nutrients in addition to lactose, it would be useful to understand the regulation of lactase gene expression. If one could prevent the age-related decline in lactase activity, milk and milk products could be used by more people to improve their nutritional status. Therapy for lactose-intolerant individuals consists simply of restricting lactose intakes. Some individuals tolerate fermented products such as yogurt and cheese fairly well, whereas varying amounts of fresh milk induce the typical symptoms of diarrhea and flatulence.

As mentioned, starch digestion begins in the mouth with salivary amylase. It pauses in the stomach as the stomach contents are acidified, but resumes when the chyme enters the duodenum and the pH is raised. The amylase of the pancreatic juice is the same as that of the saliva. It attacks the same bonds in the same locations and produces the same products: maltose, maltotriose, and the small polysaccharides (average of eight glucose molecules) the limit dextrins. The limit dextrins are further hydrolyzed by α-glucosidases on the surface of the luminal cells. The carbohydrates whose bonds are not attacked by α-amylase or α-glucosidase, or the disaccharidases maltase, lactase, or sucrase are then passed to the lower part of the intestine where they are attacked by the enzymes of the intestinal flora. Most of the products of this digestion are used by the flora themselves; however, the microbial metabolic products may be of use. The flora can produce useful amounts of short-chain fatty acids and lactate as well as methane gas, carbon dioxide, water, and hydrogen gas. The carbohydrates of legumes typify the substrates these flora use. Raffinose, which is an α-galactose 1→6 glucose 1→2 β-fructose and trehalose, and α-glucose 1→1 α-glucose are the typical substrates from legumes for these flora. The flora will also attack portions of the fibers and celluloses that are the structural elements of fruits and vegetables. Again, some useful metabolic products may be pro-

TABLE 7.5
Enzymes of importance to carbohydrate digestion

Enzyme	Substrate	Products
α-Amylase	Starch, amylopectin, glycogen	Glucose, maltose, maltotriose, limit dextrins
α-Glucosidase	Limit dextrin	Glucose
Lactase	Lactose	Galactose, glucose
Maltase	Maltose	Glucose
Sucrase	Sucrose	Glucose, fructose

duced, but the bulk of these complex polysaccharides have undigestible β linkages and are largely untouched by both intestinal and bacterial enzymes. These undigested, unavailable carbohydrates serve very useful functions:

1. They provide bulk to the diet, which in turn helps to regulate the rate of food passage from mouth to anus.
2. They act as adsorbants of noxious or potentially noxious materials in the food.
3. They assist in the excretion of cholesterol and several minerals, thereby protecting the body from overload.

Populations consuming high-fiber diets have a lower incidence of colon cancer, fewer problems with constipation, and, as a general rule, lower serum cholesterol levels.

Once the monosaccharides are released through the action of the above enzymes, they are absorbed by one of several mechanisms. Glucose and galactose are absorbed by an energy-dependent, sodium-dependent, carrier-mediated mechanism.[10,11] This mechanism is termed *active transport* because glucose is transported against a concentration gradient. Because the transport is against a concentration gradient, energy is required to "push" the movement of glucose into the enterocyte. This transport is diagrammed in Figure 7.7. Glucose and galactose appear to compete for the same active transport system. They also compete for a secondary transporter, a sodium-independent transporter (the glucose transporter, GLUT 2) found in the basolateral surface of the enterocyte membrane. The two transporters differ in molecular weight. The sodium-dependent transporter has a molecular weight of 75 kDa, whereas the sodium-independent transporter weighs 57 kDa. This sodium-independent transporter is a member of a group of transporters called GLUT 1, 2, 3, or 4.[10–16] Each of these transporters is specific to certain tissues (Table 7.6). They are sometimes called *mobile glucose transporters* because, when not in use, they reside in the endoplasmic reticulum. Under the appropriate conditions they move from the endoplasmic reticulum to plasma membrane, where they fuse with the plasma membrane and bind glucose. Upon binding, the transporter and its

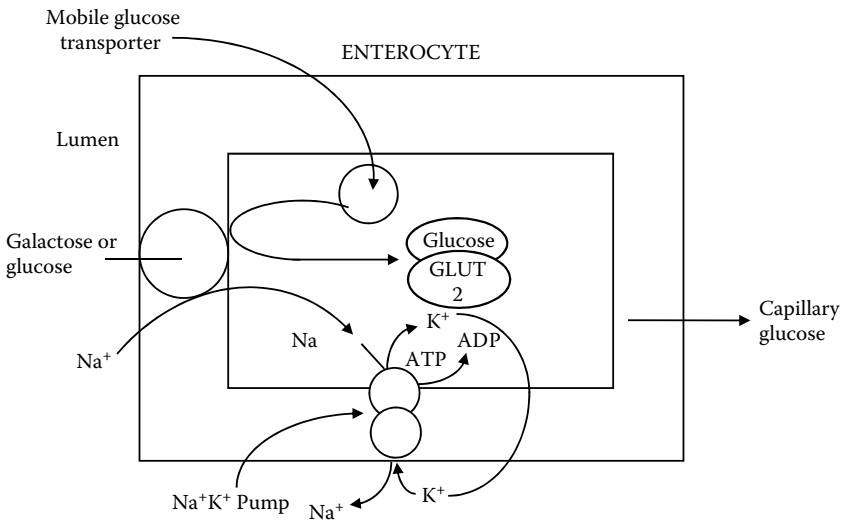


FIGURE 7.7 Two systems are used for glucose uptake by the enterocyte. One, the active transport of glucose from the lumen into the enterocyte uses the energy-dependent Na^+K^+ exchange to facilitate the movement of glucose across the cell membrane. The other uses a mobile glucose transporter (GLUT2) to move the glucose through the cell to the capillary side of the enterocyte.

TABLE 7.6
Location of the glucose transporters

Transporter	Location
GLUT 1	Ubiquitous but found mainly in brain, erythrocytes, placenta, and cultured cells. Is not particularly responsive to insulin regulation
GLUT 2	Liver, β cells of pancreas, kidney, intestine
GLUT 3	Ubiquitous in human tissue, central nervous system
GLUT 4	Adipose tissue, heart, skeletal muscle

associated glucose are released from the membrane. Through this mechanism, the glucose enters the intestinal absorptive cell.

Fructose is not absorbed via an energy-dependent, active-transport system, but by facilitated diffusion.[16,17] This process is independent of the sodium ion and is specific for fructose. Once in the enterocyte, it, too, is transported by a carrier. In the enterocyte, much of the absorbed fructose is metabolized so that little fructose can be found in the portal blood even if the animal is given an intraluminal infusion of this sugar. On the other hand, an infusion of sucrose will sometimes result in measurable blood levels of fructose. The reason that this occurs may be due to the location of sucrase on the enterocyte. Rather than extending out into the lumen as do the other disaccharidases that are anchored to the enterocyte by a glycoprotein, sucrase is more intimately anchored. The sucrose molecule then is closely embraced by the enzyme, which, in turn, facilitates both the hydrolysis of sucrose and the subsequent transport of its constituent monosaccharides. Thus, both monosaccharides enter the enterocyte simultaneously. If the diet is particularly rich in sucrose, the rise in both glucose and fructose in the portal blood will be measurable. This has some interesting consequences, as will be discussed later in the section on metabolism.

Of the other monosaccharides present in the lumen, passive diffusion is the means for their entry into the enterocyte. Pentoses, such as those found in plums or cherries and other minor carbohydrates, will find their way into the system only to be passed out of the body via the urine if the carbohydrate cannot be used.

METABOLISM

Once absorbed by the intestinal cell, glucose passes into the portal blood and circulates first to the liver and then throughout the body.[18–22] Glucose is the universal, and, in many cases, the preferred fuel for almost all cells. Even though humans consume almost as much energy from carbohydrate as from fat, the body prefers to oxidize the carbohydrate and store the fat. However, because humans are not constantly consuming food, these fuel choices by individual tissues are not always possible. The body can protect itself from a lack of food energy by using stored energy. Some of this store (less than a day's need) can be provided by glycogen, a polymer of glucose. Hence, glucose in excess of immediate oxidative need is used to synthesize glycogen in the muscle and liver. Once the glycogen stores are filled, surplus glucose from the diet can be converted to fatty acids and stored as triacylglycerols in the adipose fat depots. In times of need, this fat can be oxidized, as can the stored glycogen. For those cells having an absolute requirement for glucose (certain brain cells), this important fuel can be provided through glycogenolysis or synthesized via gluconeogenesis in the liver and kidney. All of these processes (see Figure 7.8), including food intake regulation (see Chapter 2), glucose oxidation, lipid oxidation, fatty acid synthesis glycogenesis, glycogenolysis, and gluconeogenesis are under genetic, dietary, and hormonal control. This control is carefully integrated so that normal blood glucose levels are maintained at 100 ± 20 mg/dL (4–6 mmol/L). Excursions below or above this range may occur. If the excursion is of short duration, there is no

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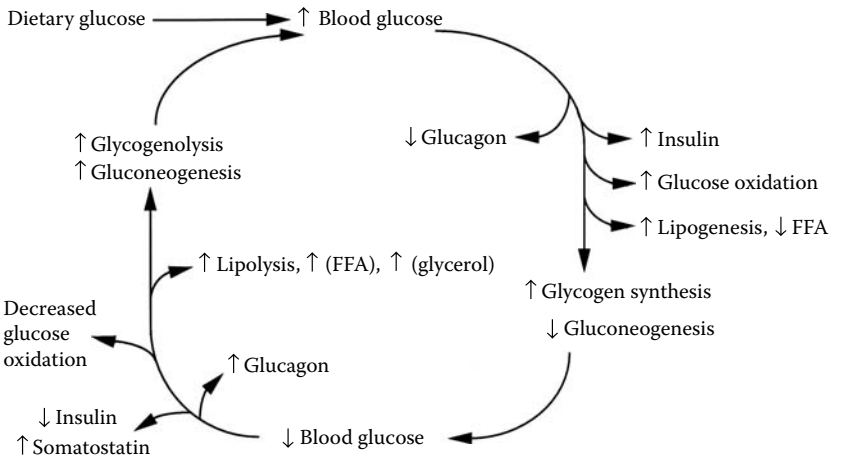


FIGURE 7.8 Overview of glucose metabolism. As blood glucose levels rise due to the influx of dietary glucose, blood insulin levels rise and glucagon levels fall. Glucose oxidation increases, as does glycogenesis and lipogenesis. Gluconeogenesis falls. As the blood glucose levels fall, these processes reverse. Glucagon levels rise, insulin falls; glycogenolysis and gluconeogenesis rise, and peripheral glucose oxidation decreases.

cause for alarm. However, if these excursions are of more than an hour, then, depending on the reason for these excursions, medical assistance may be required. Before a discussion of the regulation of glucose homeostasis can be entered, the reader is directed to Chapter 3 where a review of the pathways of glucose intermediary metabolism can be found.

Glucose Homeostasis

In Chapter 3, each of the metabolic pathways involved in the use and production of glucose was described with the goal of providing a framework for understanding how the blood glucose level is maintained within very close limits. Soon after food is consumed, digested, and absorbed, the blood glucose levels rise. Glucose is a hydrophillic compound and can circulate freely in the bloodstream without the need for a special carrier. However, as a hydrophillic compound, it cannot penetrate the plasma membrane surrounding the cells of the body without help. The mechanism for glucose entry is dependent on the hormone insulin and on proteins called glucose transporters. As was discussed in the absorption of glucose by the enterocyte, there are at least four of these proteins.[10–16] GLUT 1 has a Km that ranges from 16.9–26.2 mM. Under the same conditions, GLUT 2 has a low affinity for glucose and a Km between 3.9 and 5.6 mM. GLUT 3 has a Km of 10.6 and GLUT 4 has a Km of 1.8–4.8 mM. The GLUT 2 transporter can also transport fructose. Its activity is regulated by glucose concentration and by triiodothyronine. Some cell types have only one of these proteins, whereas others have more than one.

The transporters differ slightly in their structure yet have large areas of homology. As mentioned, they also differ in their affinity for glucose. GLUT 3 and 4 have a higher affinity for glucose than does GLUT 1. These higher affinities assure that glucose transport will be maximal in tissues containing these isoforms even when the substrate levels are relatively low. This is particularly important to the CNS as glucose is its main energy source. GLUT 2 has the lowest affinity for glucose and the rate of transport of glucose through this carrier is directly proportional to the change in glucose concentration. This has importance in the absorption of glucose by the enterocyte, as described in the section on digestion and absorption. It means that the intestinal cell has a fail-safe glucose-uptake system: one that is energy dependent and another that is not. In addition, when the

body is in the postprandial state (after a meal has been consumed) and blood glucose levels are high, there is a net flux of glucose into the hepatocytes and islet cells of the pancreas because of the presence of the GLUT 2 transporter. It is not particularly active unless blood glucose levels are elevated. In contrast, when the body is starving, intracellular glucose levels rise as a result of increased glucose production via gluconeogenesis and glycogenolysis. When the intracellular glucose levels rise, GLUT 2 can work in the opposite direction transporting glucose out of the liver cell for transport to cells lacking significant glucose production capacity. Table 7.6 lists the transporters and their locations. As mentioned in the section on absorption, the glucose transporter is referred to as a mobile transporter because, when it is not in use, it is sequestered in an intracellular pool. When needed, it leaves its storage site, moves to the interior aspect of the plasma membrane, forms a loose bond with the membrane, picks up the glucose molecule, and moves it through the membrane into the cytosol, whereupon the glucose can be phosphorylated and metabolized. Figure 7.9 is a cartoon of how these transporters work when the recruitment of the transporter is responsive to insulin signaling.

The action of insulin is initiated when it binds to its plasma membrane receptor. In the binding process, the transport form of insulin, proinsulin, is cleaved with the release of the C-peptide. The insulin receptor extends through the plasma membrane and has an intrinsic protein kinase as part of its structure. When insulin binds to the receptor, almost instantly, autophosphorylation of the receptor occurs with phosphate groups from ATP attached to the exposed tyrosine residues. Phosphorylation of the tyrosine residues requires the movement of the calcium ion from its storage site on the endoplasmic reticulum. This mobilization of calcium occurs when phosphatidyl inositol triphosphate and diacylglycerol are produced from the membrane phospholipid, phosphatidylinositol. In turn, the diacylglycerol stimulates the temporary binding of Ca^{++} to the kinase to facilitate

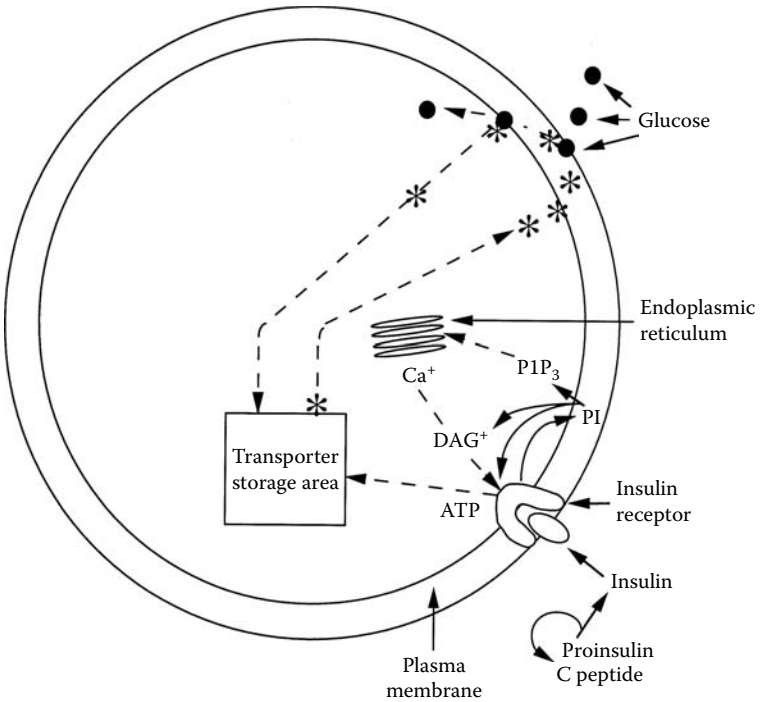


FIGURE 7.9 As blood glucose levels rise, the β cell of the pancreas releases insulin, which binds to its receptor on the surface of the target cell and signals the release of the mobile glucose transporter from its storage site. The transporter migrates to the plasma membrane, picks up the glucose, transports it to the cytosol, and releases it.

the phosphorylation of the tyrosine residues. All of these reactions result in a change in the phosphorylation state of the transporter in its storage site facilitating its release and migration to the cell surface. This, then, accomplishes the goal of moving the glucose from the bloodstream into the cell for its appropriate disposal.

Not all cells mobilize their glucose transporters under the influence of insulin. Some use a different mechanism. Brain cells are an example, as are the β cells in the islets of Langerhans in the pancreas. These cell types have stringent requirements for glucose as their principal metabolic fuel, yet their use and transport of glucose is independent of insulin bound to a plasma membrane receptor. The presence of glucose transporters in a wide variety of tissues has been ascertained by a number of scientists, yet the details of their recruitment and the signals necessary for this recruitment are not fully known.

As mentioned, insulin bound to its receptor on the plasma membrane is essential to the entry of glucose into insulin-dependent cells. When a person (or animal) consumes a glucose-rich food the blood glucose rises.[23,24] This glucose stimulates the pancreatic β cells in the islets of Langerhans to release insulin. How the glucose transporter is recruited is not known but when glucose enters the cytosol of the β cell via GLUT 2 and is phosphorylated to glucose-6-phosphate by the enzyme glucokinase, it is thought that this phosphorylation step provides the signal to the β cell to release insulin from its insulin store. Aberrations in pancreatic glucokinase result in impaired insulin release and subsequent impairment in the use of glucose. Again, exactly how this signal is generated is not known. The phosphatidyl inositol phosphate (PIP) cycle is involved, as is tyrosine kinase and the calcium ion, but information is lacking on the specifics of the mechanism. The transport of glucose and subsequent release of insulin via the Golgi complex also involves calcium, sodium, and potassium ions and ATP.

As the blood glucose levels fall because of the insulin-stimulated use of glucose, less glucose is transported into the β cell and thus less insulin is released. In turn, all of the various cells that use glucose will have less glucose to use as well as less insulin bound to receptor signaling glucose use. In the fully fed state, insulin is the key to normal glucose oxidation or conversion to glycogen or fatty acids.[18] However, in the absence of continuous feeding, the body must adjust its metabolism to ensure a continuous fuel supply.[25] Other hormones now play key roles as the blood glucose falls. These hormones switch metabolism from glucose disposal to blood glucose maintenance. First, they exert an anti-insulin action at insulin target cells by interfering with insulin-stimulated glucose uptake. In so doing, they promote insulin resistance. That is, the cells are resisting the positive effects of insulin on glucose uptake and oxidation via glycolysis. Second, these hormones provide signals that enhance glycogenolysis and gluconeogenesis to provide glucose to those cells that need it. The enhancement of glycogenolysis in the liver precedes that of gluconeogenesis as glycogen is more readily available than are the substrates for gluconeogenesis. In addition, glycogenolysis is energetically less expensive than is gluconeogenesis. Third, lipolysis and fatty acid oxidation are enhanced to provide energy and glycerol for glucose synthesis. All of these inhibitions and enhancements are coordinated so that the blood glucose level remains within the normal range of 80–110 mg/dL (4–6 mmol/L). Excursions above and below might occur briefly, but, as soon as they occur, mechanisms are activated that restore overall normoglycemia.

Abnormalities in the Regulation of Glucose Homeostasis: Diabetes Mellitus

Just as we have learned about the details of the synthesis and use of gluconeogenesis, pentose shunt, glycolysis, and glycogen through the study of genetic anomalies in these pathways, so too have we learned about the regulation of glucose homeostasis through the study of a large number of genetic errors that result in the disease, diabetes mellitus. According to the American Diabetes Association (ADA), one person in 14 (~7%) in the United States either has diabetes mellitus or will develop it during his or her lifetime. Of this population, 80–90% will develop the Type 2 or the noninsulin-dependent diabetes mellitus form; 0.1–9% will develop mitochondrial diabetes, and the rest will

develop the Type 1 or insulin-dependent form. The risk of developing the secondary complications varies with the particular genetic mutation that has caused the disease.

Prevalence of Diabetes

The number of Americans having diabetes is growing.[26–30] The prevalence has increased by 42% in the last decade. In part, this is due to better care and management of the disease, thus prolonging the lives of those with the disease. In part, it is also due to earlier diagnosis of the disease and to the fact that as the population with diabetes grows and reproduces, the genetic traits carried by these people are passed on to their progeny. In addition, some of the cases of diabetes are a companion to obesity. Obesity is a growing problem in the United States. In some instances the obesity precedes the diabetes and in some, the two conditions develop simultaneously.

The prevalence of diabetes in male and female adults as a percent of the total population is shown in Figure 7.10.

The overall distribution of people with diabetes within the total U.S. population is shown in Figure 7.11.

It has been estimated that 10,240,000 Americans have physician-diagnosed diabetes. The American Diabetes Association estimates that perhaps that many people have the disease but are undiagnosed.[31] Approximately 798,000 new cases are diagnosed each year. Death due to diabetes

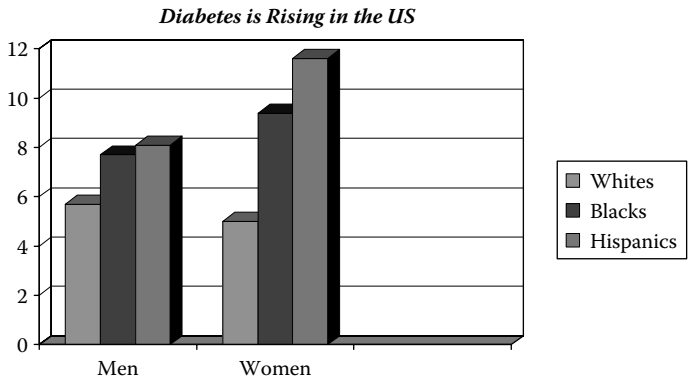


FIGURE 7.10 Estimated age-adjusted prevalence of physician-diagnosed diabetes in adults 20+ years of age, segregated by gender and race. (From NHANES III, 1988–94. *Diabetes Care* 21: S1–144.)

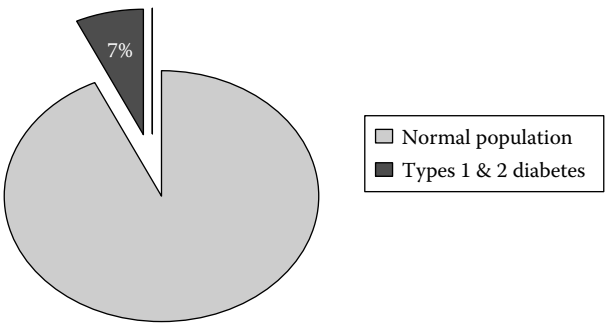


FIGURE 7.11 The percentage of the population with diabetes is rising as the population becomes overly fat. Some racial/ethnic groups are more at risk than others.

ranks seventh in the top ten causes of death in the United States. Diabetes as a contributory cause of death is rising. Diabetes death rates were 24.2 per 100,000 population for white males, 47.0 for black males, 19.5 for white females, and 48.8 for black females. Two thirds of people with diabetes die of some form of vascular disease. This includes heart disease and stroke. More than 60% of people with end-stage renal disease are people with diabetes. Within the total population in the United States, there are groups that have an even higher prevalence rate of diabetes than those shown above for black and Caucasian populations. The Pima Indians in Arizona have a prevalence of 60% of the adult population. Hispanic Americans aged 50 years or older have a prevalence of 25–30%. Diabetes is two to three times more common in Mexican-American and Puerto Rican adults as in non-Hispanic Caucasian adults.[26]

The prevalence of diabetes depends on the diagnostic criteria used for identification. The newest criteria are those of the American Diabetes Association.[31] These are modifications of those previously recommended by the NDDG[29] or WHO.[30] These criteria are shown in Table 7.7. The ADA expert committee recognized that there may be deviants in people who fail to meet these criteria yet may not be considered normal. This group is defined as having fasting blood glucose levels ≥110 mg/dL (6.1 mmol/L) but ≤126 mg/dL (7.0 mmol/L) or 2-hour values in the oral glucose tolerance test of ≥140 mg/dL (7.8 mmol/L) but ≤200 mg/dL (11.1 mmol/L). People who fall into this category have impaired glucose tolerance but are not yet considered to have diabetes. They may progress to diabetes or may remain impaired and never progress to diabetes.

The Glucose Tolerance Test[31]

Although diabetes is a collection of diseases arising for a variety of reasons, its diagnosis is based on the results of a glucose tolerance test. Glucose tolerance in persons suspected of having diabetes mellitus is tested by giving the person a large dose (usually 1 gram/kilogram body weight) of glucose and monitoring the blood glucose level before and at 30-minute intervals after the glucose bolus. Variations in the procedure have been developed for screening purposes. A single fasting blood sample may be examined for its glucose content, or a fasting plus a 2-hour postmeal blood sample may be examined, or the test may be 5 hours in duration rather than the usual 2 hours. The type of test is usually determined by the patient's symptoms and the family history. In normal nondiabetic fasted individuals, administration of a bolus of glucose elicits a typical rise then fall in blood glucose levels. In contrast, diabetic individuals may have an elevated fasting blood glucose level and/or may have a failure to appropriately reduce the glucose level after the test dose.

Abnormal glucose tolerance is defined in several ways: there may be a departure from the normal fasting blood glucose level (80–110 mg/dL, 4–6 mmol/L) and/or post challenge values may be excessively high (exceeding 250 mg/dL) and/or fail to return to the prechallenge blood glucose level by 120 minutes after the challenge. Blood insulin values may exceed normal in some individuals, and this is interpreted as a sign of target tissue insulin resistance. These individuals are referred

TABLE 7.7
Criteria for the diagnosis of diabetes mellitus

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dL (11.1 mmol/L). Casual is defined as any time of day without regard to time since the last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss. OR
2. Fasting blood glucose ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no food intake for at least 8 h.[a] OR
3. 2-h plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test.[a] The test should be performed as described by WHO using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

[a]These measures should be repeated on a different day to confirm diagnosis

Source: American Diabetes Association Clinical Practice Recommendations 2007. *Diabetes Care* 30: S1-103.

to as *hyperinsulinemic*. They may be hyperinsulinemic yet have normal blood glucose levels and normal glucose tolerance. There are several variations in response to a glucose challenge, and these will be discussed subsequently.

Definition of Different Types of Diabetes Based on Management

Figures 7.10 and 7.11 show population figures with all causes and types of diabetes lumped together. Figure 7.12 shows the distribution of the different types of diabetes within the population with this disease. Not included are people with gestational diabetes and people for whom the diabetes is secondary to another disorder. Clinicians divide the population with diabetes based on the management of the disease. Clinically, the disease is divided into two major subgroups: Type 1 diabetes and Type 2 diabetes mellitus. The division is based on the therapy needed upon diagnosis; however, there is some blurring of this separation. Patients requiring hormone replacement upon disease recognition are Type 1 patients, whereas those whose disease can be managed with diet, exercise, and/or oral medications are Type 2 patients. In children, Type 1 diabetes is of rapid onset and hormone replacement plus careful management of diet and activity is essential to the survival of the child. In adults, the division between Type 1 and Type 2 patients is blurry because Type 2 patients might not be diagnosed early in their disease development. Type 2 patients that are poorly managed frequently progress to islet cell exhaustion and these patients must then have daily insulin replacement for survival. If their disease is not recognized in its early stages, they will be classified as a Type 1 diabetic at diagnosis rather than a Type 2. Type 1 diabetes can occur in the adult, however, it is difficult to separate this patient from the Type 2 patient who has progressed to needing hormone replacement. The distribution of the various types of diabetes is shown in Figure 7.12.

Of the total population with diabetes mellitus, about 10% have the disease as a result of pancreatic insulin production failure, whereas 81% develop the disease as a response to one or more failures in the target tissues: liver, muscle, and/or adipose tissue. An additional 9% develop the disease as a consequence of mitochondrial failure. This estimate of the number of people with mitochondrial diabetes is a very ”soft” figure because very few population studies have been conducted to document its prevalence. Although the number of people with Type 1 diabetes has remained fairly constant since insulin replacement therapy was made possible, the number of people with Type 2 diabetes is rapidly rising. In part, this is due to the rising number of people with excess fat stores and in part due to more active population screening.

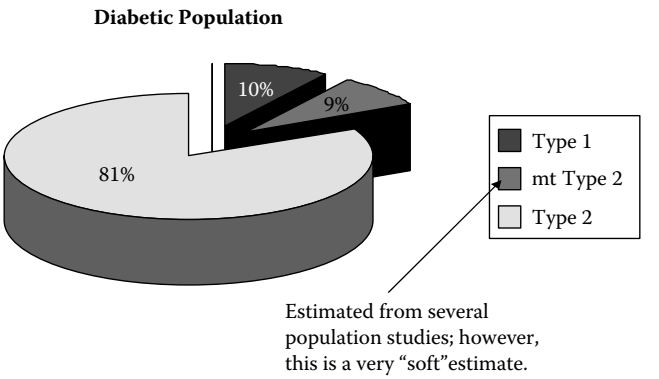


FIGURE 7.12 Distribution of the types of diabetes within the diabetic population.

Genetics and Diabetes

Currently, the number of genetic mutations thought to be responsible for the development of this disease are in excess of 300, depending on the definitions used for the disease. Type 2 diabetes, although more prevalent, has a diverse genetic origin as shown in Table 7.8. The number of genetic mutations thought to be responsible is in excess of 200 depending on the definitions used for the disease. A computer search of the Online Mendelian Inheritance in Man (OMIM, accessed Feb 14, 2007) revealed 338 entries of mutations associated with diabetes. Some of these mutations phenotype as other diseases that have diabetes as a secondary feature. A number of these are relatively rare diseases. Some of the reports relate to mutations associated with type 1 diabetes. Quite a bit of information suggests that the phenotypic expression of these mutations can be modified by environmental factors. These environmental influences may explain the discrepancy between the frequencies of the genotypes and phenotypes. That is, far more people have these mutations in their

TABLE 7.8
Mutations that associate with type 1 and type 2 diabetes mellitus

Gene	Comments
Nuclear DNA	
Hepatic nuclear transcription factor 4α (MODY 1)	Subjects have impaired insulin secretion; 44 different mutations in this gene have been reported.[32,33]
Glucokinase (MODY 2)	Subjects are generally nonobese[34–40]
Hepatic transcription factor 1α (MODY 3)	Hypertension and type 2 are found in this genotype, which is polymorphic[41–43]
Glycogen synthase	Activation of the enzyme is impaired
Glucagon receptor	Susceptibility to type 2 is variable among different population groups[44,45]
Insulin	Several mutations reported[52–54]
Insulin receptor	Associated with peripheral insulin resistance in some type 2 subjects[46–51]
Insulin receptor substrate (IRS-1)	Results in a signal transmission defect associated with insulin resistance[55]
Mitochondrial α-glycerol 3 phosphate dehydrogenase	Associated with type 2 and with impaired mitochondrial function[56]
Glucose transporters (GLUT 1-4)	Associated with both obesity and type 2 diabetes[10–16, 57–60]
Mitochondrial DNA[a]	
$tRNA^{leu}$	7 mutations have been found that associate with diabetes[57]
ND 1	11 mutations have been reported
ND 2, ND 3, ND 4	1 mutation in each of these genes has been reported
$tRNA^{Cys, Ser, Lys}$	1 mutation in each of these genes has been reported
$tRNA^{thr}$	4 mutations have been found
D-loop[b]	3 mutations have been reported
COX II	2 mutations have been reported
ATPase 6,8	4 mutations have been reported

[a]Mutations in the mitochondrial genome that associate with diabetes are heteroplasmic; that is, there is mixture of normal and abnormal DNA in each cell. The percentage of the abnormal determines the degree to which the function of that cell is impaired.

[b]Promoter regions for the 13 structural genes found in the mitochondrial genome are found here.

genome than the number of people who actually develop the disease, diabetes mellitus. However, this is changing. This relates to the association of the obesity genotype with the diabetes genotype. Several of these genotypes are one and the same. That is, both the obesity and the diabetes are due to the same mutation. If the food supply is abundant and people with these genotypes consume excess food that in turn results in excess body fat stores, they will also become diabetic. This means that the discordance between genotype and phenotype for these mutations will disappear. Similarly, there are other genotypes that could be affected by lifestyle choices that affect phenotypic expression. Choosing an active lifestyle is an example. People with a diabetes genotype that choose to incorporate exercise in their daily routine will be less likely to develop diabetes than those having the same genotype who do not exercise. Here is an example of the interaction of diet and exercise with one or more diabetes-associated gene mutations. Here, too, is a scenario that might be manipulated. Through weight management (maintaining energy balance such that the individual does not have excess fat stores) and through maintenance of an active lifestyle, the person with the diabetes genotype might never express the diabetes phenotype. In addition, there may be certain dietary constituents that may be particularly active with respect to enhancing or suppressing the phenotypic expression of the diabetes genotype. Studies in rodents have suggested that this may occur and these results may be applicable to the human.

There are additional groupings (not shown in Figure 7.12) that refer to specific circumstances. One is the diabetes that develops during pregnancy (gestational diabetes) that either disappears after pregnancy or goes on as Type 2 diabetes. The other is impaired glucose tolerance (described above) that may or may not proceed into Type 2 diabetes. In each of these instances, the patients are managed as though they were diabetic. Careful attention is paid to the diet as well as to physical activity. In the pregnant woman, as gestation proceeds, some women may require insulin supplements but the physician will monitor this very carefully. Most obstetricians will observe the growth and development of the fetus and terminate the pregnancy as soon as it is apparent that the fetus can be delivered (by caesarian usually) and will do well. By paying careful attention to this problem, it is hoped that the unusually large baby typical of gestational diabetes can be avoided, as well as the problem of neonatal hypoglycemia. Neonatal hypoglycemia is a response to the mother's inability to fully use glucose; the fetal islet cells respond to this by producing insulin in amounts larger than needed by the fetus. When the child is born this insulin production must be downregulated to avoid the potentially dangerous hypoglycemia that could develop.

Type 1 Diabetes (Autoimmune Diabetes)

Insulin deficiency characterized by high blood glucose levels (hyperglycemia) results in the excessive thirst (polydipsia), excessive urine production (polyuria), and rapid weight loss so typical of severe diabetes. The body fat stores are raided, but fatty acid oxidation is incomplete. As a result, acetone, β-hydroxybutyrate, and acetoacetate, products of incomplete fatty acid oxidation, accumulate. These are the ketone bodies.

Elevated blood levels of these ketones (ketonemia) are observed, as is an elevated urinary excretion (ketonuria). Rising levels of ketones increase the need for buffering power because they tend to lower pH. Acidosis is a characteristic feature of diabetes. Not only are the fat stores raided, but so is the body protein. Proteolysis (body protein breakdown) is enhanced, and the amino acids thus liberated are used for energy or as substrates for intracellular glucose synthesis. The ammonia released as a product of the deamination of these amino acids assists in the buffering of the accumulating ketones. However, this ammonia is in itself cytotoxic, so the body must increase its capacity to convert it to urea. Humans with uncontrolled diabetes thus are characterized by a loss in body protein, an increase in blood and urine levels of ammonia, an increase in urea synthesis, a negative nitrogen balance, a loss in fat store, elevated blood and urine levels of glucose, and elevated levels of fatty acid oxidation products. Some of these metabolic products are also excreted via the lungs

in the expired air. The breath of an uncontrolled diabetic has the aroma of the ketones—somewhat like the aroma of fingernail-polish remover.

Pancreatic Islet β Cell Failure

Several explanations for pancreatic insulin-production failure have been offered. The two most generally accepted are: (1) failure due to autoimmune disease and (2) failure due to viral destruction of the islet β cells. In each of these instances, the genetic heritage of the individual plays a role. In both, the immune system is involved. In autoimmune disease, the insulin-producing pancreatic islet cell is destroyed because the immune system has sensed the presence of an antigen that it recognizes, not as a self-made protein, but as a foreign protein. It then destroys this protein. The interleukins are involved in the autoimmune process and so, too, is apoptosis.

Autoimmune Disease

Autoimmune diabetes can be accompanied by a number of other diseases; multiple endocrine failure is not uncommon. This means that autoimmune diabetes can be found in a person who also develops psoriasis, rheumatoid arthritis, or thyroiditis (inflammation of the thyroid gland). In autoimmune diabetes, there may be an immune tolerance that is particularly difficult to break. This is because the body may lack antibody specificity. Antibodies can be raised that will react to a group of closely related compounds in an instance of "mistaken identity." Usually, an antibody reacts to a single antigen rather than to a group of closely related antigens. In autoimmune disease, the body tolerates a range of exposures to these antigens before it reacts. Thus, the term, *immune tolerance* means that the individual will tolerate a wide range of exposures to an antigen or group of antigens before a systemic immunologic reaction is elicited. This is not the same thing as food allergies or skin allergies or localized reactions to allergens. Neither should this term *immune tolerance* be confused with diseases of the immune system per se.

Autoimmunity is characterized by an increase in the number of T cells. The T cell originates in the thymus, and is a type of cell that is an essential component of the immune system. The B cell originates in the bone marrow and produces antibodies in response to an antigen. T cells interact with B cells in order to initiate antibody production. In some instances, the recognition is nonspecific. That is, antibodies are produced that react to a group of related antigens. In autoimmune disease, this is what happens. There is a loss in specificity resulting in a progressive autoimmunologically mediated destruction of the islet cells. Immunosuppression can interfere with this destruction, but the use of such drugs has its own set of problems, such as kidney disease or increased susceptibility to infectious disease. The immunologic response is initiated when the T cell receptor recognizes an antigen on the correct histocompatability complex on the surface of the antigen-presenting cell. Transmission of this signal to the nucleus of the cell involves the calcium ion, a complex of related proteins, and the phosphatidylinositol cycle, which is responsible for the movement of the calcium ion from the intracellular store to where it is needed. Antigens are then produced.

Autoimmune disease can result when mutations in the genes that encode the various components of the immune system occur and the body's own protein is recognized as foreign. The body then develops antibodies that effectively destroy that protein. Several cell surface proteins have been found to stimulate antibody production in susceptible individuals. Several of these are listed in Table 7.9. In autoimmune diabetes, the destruction of the β cell in the islets of Langerhans appears to be associated with one or more mutations in the genes that encode the major histocompatibility complex (MHC). More than 100 genes are involved. These genes have been mapped to the short arm of chromosome 6 and comprise approximately 2×10^6 nucleotides. Mutations in these genes have been divided into two classes (I and II) and are further identified by letter designations. There are so many genes that it would be impossible to list them all in this text. Suffice it to say that the class II HLA genes are more closely linked to autoimmune diabetes than are the class I HLA genes.

TABLE 7.9
Autoantigens that elicit islet cell reactive antibodies in humans with autoimmune type 1 diabetes mellitus

Autoantigen	Comments
Glutamic acid decarboxylase (GAD)	Antibodies present before clinical state develops
Insulin (58,000 kDa, 51 amino acids)	Antibodies found after diagnosis
Insulin receptor	Antibodies found after diagnosis
38,000 Mr	Antibodies develop in patients with insulinoma
RIN polar	Antibodies are related to those formed in response to certain gangliosides
52,000 Mr	Antibodies present before clinical state develops. Likely the same as GAD
Carboxypeptidase H	Cell surface autoantigen and a major protein in insulin secretory granules
ICA 12/ICA 512	Putative islet cell antigens
PM-1 60,000 Mr	A 24-amino acid peptide that may be a polyclonal activator of autoreactive T cells
IAAb (antialgAb)	Antiimmoglobulin antibodies that appear prior to IDDM[a]
C-peptide	Antibodies found after IDDM diagnosis
ICA 69	Antibodies found prior to IDDM development

[a]IDDM = Type 1 insulin-dependent diabetes mellitus.

In rodents (NOD mice and BB rats), several mutations in the MHC region have also been identified that express themselves as autoimmune diabetes mellitus. These rodents have been studied extensively and have helped scientists understand the process of the disease.

The development of autoimmune diabetes is associated with an inflammation of the pancreatic islet cells (insulitis). In insulitis, CD4 and CD8 T cells, B cells, macrophages, and killer T cells have all been found. The presence of these marker cells indicates that an immunologic reaction is taking place. In humans with autoimmune diabetes, islet-cell antibodies to cytoplasmic antigens were reported in 1974. Following this initial report, hundreds of antibodies have been reported in tissues from humans. Glutamic acid decarboxylase (GAD), an enzyme found in β cells that catalyzes the synthesis of the neurotransmitter gamma amino butyric acid (GABA), has been shown to be an antigen. Islet β cells, like neuronal cells, possess a mechanism for hormone release that depends on a specific trophic stimulus. GABA and GAD are components of this secretion signaling system, as is the glucose transporter, the enzyme, glucokinase, and the mitochondrial oxidative phosphorylation system that produces ATP. ATP and the calcium ion are major players in the insulin-release mechanism.

Autoantibodies to GAD have been observed prior to the development of the clinical state, and the presence of these antibodies has been suggested as an early indication of the disease. The gene for GAD has been cloned, characterized, and mapped to chromosome 10. Two isoforms (a 65 kDa and a 67 kDa form) have been isolated and a single gene encodes each. Although antibodies to GAD have been found in patients with autoimmune diabetes, the GAD itself is not abnormal nor has its gene been found to be abnormal. Thus, the GAD genes are not candidate genes in this disease.

Although GAD antibodies can indicate incipient Type 1 diabetes, these antibodies are also elevated in other autoimmune diseases in the absence of diabetic symptoms; yet, in each disease, there are distinct differences in epitope recognition. This suggests that the autoantigen is being presented to the T cells and the B cells by different mechanisms. However, so many have been reported that it is difficult to assign causality to many of them. For example, islet-cell antibodies to plasma insulin C-peptide have been reported in adults having diabetes for at least 10 years. Antibodies to insulin have been found in newly diagnosed children but not in their high-risk relatives. This suggests that these antibodies are a result, not a cause, of the disease. As mentioned, GAD antibodies have been

reported to occur prior to the development of diabetic symptoms, so it is possible that they may serve as an early marker of autoimmune disease.

Diabetes Secondary to Viral Infections

Epidemiologists have noted that, following epidemics of diseases such as flu (influenza), there are upsurges in the numbers of people with newly diagnosed Type 1 diabetes.[61–65] Not all people who develop flu develop diabetes, nor have all people who develop Type 1 diabetes had their disease preceded by flu. Other infections (mumps, rubella) can also precede diabetes. Infections due to cytomegalo virus, the Epstein–Barr virus, the picornaviruses, (which include several strains of encephalomyocarditis virus, the foot-and-mouth disease virus), and a number of the Coxsackie B viruses can precede diabetes in susceptible individuals. These persistent infections can cause damage in addition to the typical lytic effect that occurs during the acute phase. Damage can take the form of producing (or inducing) small changes in cell proteins that change the function of the protein or change the recognition of this protein as a normal cell constituent. These slightly modified self proteins then are recognized as foreign, and an antibody is produced to destroy it. One such protein is the islet-cell surface protein (ICS). Antibodies to ICS have been found in virally infected humans. Viruses also induce the production of cytokines such as interferon, the interleukins, and tumor necrosis factor. These substances alter the immune response by regulating the expression of β-cell antigens and thus contribute to the development of insulitis. The mechanisms whereby viruses induce diabetes have been the subject of considerable speculation.

There appears to be a genetic determination of susceptibility to these infections that probably involves genes that encode the various components of the immune system. This explains why some humans may develop diabetes secondary to a viral infection, whereas others do not. Notkins and coworkers have demonstrated that susceptibility is compatible with a single gene acting in an autosomal recessive manner.[64] The mode of action of such a gene would be to control specific virus receptors on the β-cell membrane, or perhaps to control the membrane permeability to such viruses, thus facilitating the incorporation of the viral DNA into the β-cell DNA and altering its function. Fluorescein labeling of viral antibody has confirmed the entry of the coxsackie B virus into β cells, as well as changes in β-cell mRNA. Here is another example of an interaction between the environment and genetics. If the genetic material is such that the immune system is somewhat incompetent in its response to a particular virus, then the viruses will inflict serious damage on the β cell. In persons with a fully competent immune system, such damage does not occur. Evidence of virus-induced diabetes in man has been gathered through postmortem studies of pancreatic tissue excised from children with fatal viral infections.[61] Of the 250 children studied, seven had a Coxsackie B infection, and of these, four had significant β cell destruction and evidence of acute or chronic inflammation in the islet tissue. Whether immunization against these viruses is possible and will prevent this disorder is a significant research problem.

Insulin Release

Although rare, persons with mutations in the gene for the pancreatic-glucose-sensing enzyme glucokinase have provided insights into how insulin is released by the pancreatic beta cell.[34–40] The β cell glucokinase plays a pivotal role in the cascade of events leading to the release of the insulin molecule by the β cell (see subsequent discussion of MODY). There are several important players: The glucose transporter (GLUT 2), the glucokinase (GK) enzyme, and the mitochondrial system (OXPHOS) that produces the energy (ATP) needed for both insulin synthesis and release, for the movement of the sodium (Na^+), potassium (K^+), and calcium (Ca^{++}) ions, and for the Na^+K^+ATPase.[23] Shown in Figure 7.13 is a proposed mechanism whereby the islet cell is stimulated to release insulin.

Rising levels of glucose in the blood promote the uptake of this glucose by the β cell. The glucose transporter (GLUT 2) facilitates its entry and the ATP-dependent glucokinase facilitates the

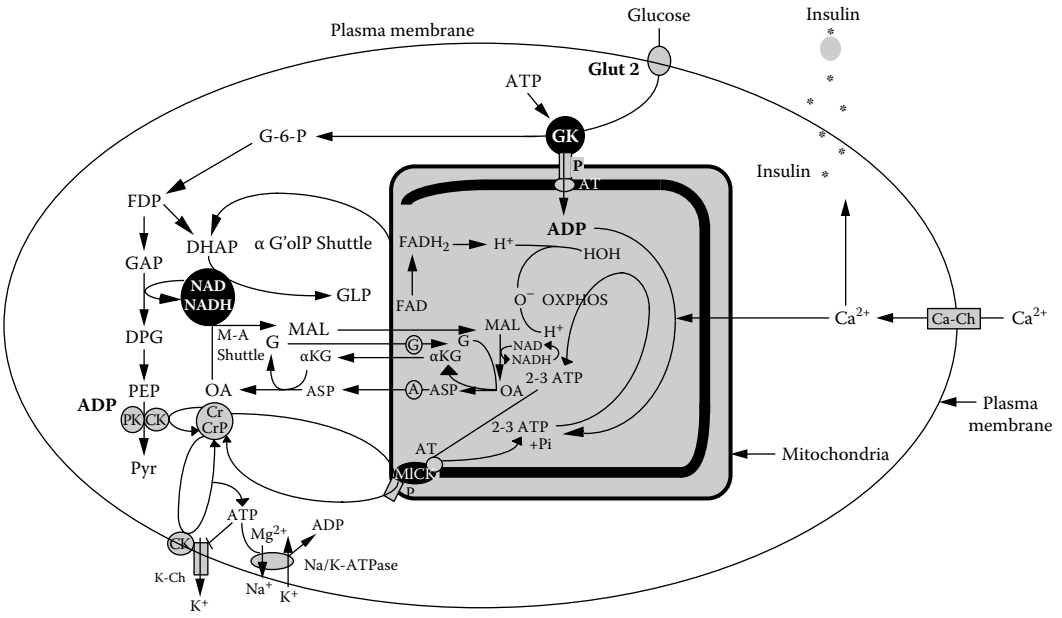


FIGURE 7.13 Proposed mechanism for the release of insulin by the pancreatic βcell. Abbreviations used are those for glucose metabolism: Glut2, mobile glucose transporter 2; ATP, adenine triphosphate; G6P, glucose-6-phosphate; FDP, fructose diphosphate; GAP, glyceraldehyde phosphate; PEP, phosphoenopyruvate; DHAP-α G'olP shuttle, glycerol phosphate shuttle; MAL, malate; OXPHOS, oxidative phosphorylation. The inner compartment shown here is the mitochondrial compartment. The interdigitation of the two compartments is discussed in Chapter 3.

phosphorylation of this glucose. If either the glucose transporter or the glucokinase is abnormal due to a mutation in the genes that encode either of these proteins, then the glucose-sensing system that is part of the insulin release process will not work appropriately and the βcell will not respond appropriately to the glucose signal. Actually, numerous mutations have been reported for the glucokinase gene, yet altogether these many mutations are thought to account for less than 0.1% of the population with diabetes mellitus. Indeed, there is some argument as to whether these people should be included in the Type 1 category rather than the Type 2. Usually they are thought to have Type 2 diabetes because although abnormal, the islets do respond to some secretogogues such as amino acids (leucine, glutamine, arginine) and fatty acids (palmitate) as well as some metabolites (inosine, and membrane permeant analogs of pyruvate and succinate) and do produce and release insulin.[23,24] Again, lifestyle choice determines whether hormone supplements are required for the successful management of the disease.

Maturity-Onset Diabetes of the Young (MODY)

Within the category of Type 2 diabetes (Table 7.8) is a subgroup called maturity-onset diabetes of the young (MODY). People with this form of diabetes have a problem with the responsiveness of the pancreas to changes in blood glucose level. The islet cells do not adequately sense blood glucose change and therefore do not release appropriate amounts of insulin in response. There are three major groups of mutations that phenotype as MODY. One group (MODY1) has mutations in the gene for hepatic nuclear factor 4α.[32,33] A second group has mutations in the gene for pancreatic glucokinase (MODY2),[34–40] and a third group (MODY3) has mutations in the gene for hepatic nuclear factor 1α.[41,43] The hepatic nuclear factors are thought to serve as DNA transcription enhancers in

the islet cell and other cells as well, and are abbreviated HNF 1 or 4α. As can be seen in Table 7.8, MODY is associated with a large number of mutations. Theoretically, people with the MODY gene mutations could, through appropriate food choice, avoid diabetic symptoms. With a problem in glucose sensing, these people would benefit from diets that do not challenge the islet cells with excessive loads of glucose. Using low glucose (as well as other simple sugars that can be converted to glucose) diets, these patients could reduce the metabolic problems associated with the sensing of blood glucose by the islet cell. A diet with glucose coming from complex carbohydrates would be a benefit to these people because it would provide a leveling of glucose influx from the diet. As well, a diet where the major energy sources are proteins and fats might be useful because these foods also do not stress the islet cell. There are some amino acids and some metabolites as well as fatty acid analogs that stimulate insulin release but these substances do this using sensing systems that do not involve the glucokinase reaction.

All of the MODY mutations are inherited as an autosomal dominant traits. Although all MODY patients have an islet cell secretory defect, MODY 1 and MODY 3 patients also manifest a defective responsiveness to the glucose-lowering effect of insulin. Thus, they are classed as insulin resistant. If given a dose of insulin, this insulin is less effective in lowering blood glucose than a person without these genetic defects. This is because the hepatic transcription factors 1α and 4α affect the transcription of several of the genes that encode the enzymes of intermediary metabolism. These enzymes are critical to the appropriate oxidation of glucose, and if less than optimally active, glucose will not be as easily oxidized, and hence the apparent reduction in glucose uptake and use by the target tissues. Indeed, some of the common hepatic nuclear facto 4α variants are associated with high serum lipids and metabolic syndrome.[32]

Genetic Errors in Insulin Structure

In addition to failure of the islet cell to release insulin in response to a glucose signal, the possibility exists that the islet cell may not be producing insulin in the appropriate amino acid sequence to have full biological activity. Insulin gene mutations have been documented in both humans and mouse. These mutations result in a variety of amino acid substitutions and have a variety of effects on insulin action. These mutations are very rare. Steiner et al.,[54] for example, studied a number of families with aberrant insulin genes. Ten families were studied that had single-point mutations that resulted in amino acid substitutions in the proinsulin molecule. Six of these substitutions resulted in the secretion of defective insulin molecules due to changes within the A or B chains. These changes resulted in molecules that were immunoreactive but did not bind adequately to the plasma membrane insulin receptor on the adipocyte. Four additional families were found to have insulin gene mutations that prevented the recognition of the C peptide-A chain dibasic cleavage site and its removal. These families had high levels of proinsulin in the blood because the C-peptide was not removed prior to the release of the insulin from the islet cell. Proinsulin is a large insulin molecule that may be released by the pancreas when this mutation is present. When insulin is ready for release, a fraction of this large molecule is split off. This fraction is called the C-chain or C-peptide. Proinsulin is 1/40th to 1/60th as active as insulin. The aberrant proinsulin molecule may have the appropriate β cell processing protease but not the appropriate C-peptide recognition site.

The variability of the insulin gene in a group of phenotypically normal individuals has been studied. Although several variants have been found, none were considered mutants. That is, the base pair substitutions that coded for specific amino acids in the insulin molecules were in places that did not affect the conformation of the molecule or the active site of the insulin molecule, nor did they affect the cleavage of proinsulin to active insulin. The variety of base pair substitutions and subsequent amino acid substitutions in the insulin molecule appears to be large, yet the impact of these aberrations (if they can be called aberrations) is very small or nonexistent. However, should substitutions occur that affect proinsulin cleavage, or reactive sites in the insulin molecule as described

above, then diabetes would develop. Whether the diabetes is Type 1 or Type 2, would, as mentioned, depend on the nature of the defect.

Mitochondrial Diabetes

There is a third form of the disease that does not follow Mendelian genetics; it is the diabetes that occurs due to a mutation in the mitochondrial genome.[57–60] Included in this group of errors are those due to mutation in the mitochondrial genome (listed in Table 7.8). Mutation in this genome results in an impaired production of ATP. ATP is essential to the synthesis of insulin in the islet cell and also is essential to the activity of the glucose-sensing enzyme, glucokinase, and to the release of insulin in response to the glucose signal (Figure 7.13). ATP is also essential to intermediary metabolism. In patients with these mutations, diabetes may be secondary to more serious neuromuscular disease or to neuronal disease that occurs due to a shortfall in ATP for the neuronal pathways. These pathways are exquisitely dependent on adequate ATP supplies. The central nervous system is the largest consumer of ATP, followed by pancreatic islet cells and renal cells. Diabetes occurs in patients whose mutation burden is not high enough to manifest the severe neuronal or neuromuscular diseases. Recognition of diabetes as a phenotype for mitochondrial DNA mutation has been fairly recent so the numbers of people with diabetes due to this cause are poor estimates at best. Furthermore, diabetes itself can cause mitochondrial DNA mutations[59] and it is difficult to assign causality to the mutation without familial studies that show that the appearance of the mutation appears before the appearance of the signs and symptoms of diabetes. Current estimates of the number of people with diabetes due to a mitochondrial mutation vary from 0.1% to 10% of the population with diabetes (Figure 7.12).

Peripheral Insulin Resistance

Insulin resistance due to a mutations in the genes for the insulin receptors in fat cells, muscle cells, and liver cells, have been found. Insulin receptors have been isolated and their structures analyzed and sequenced. It is generally agreed that the receptor is synthesized as a single polypeptide precursor of 1382 amino acids, which contains a signal peptide of 27 amino acids: the α subunit of 735 amino acids (including four basic amino acids of the processing site) and the β subunit of 620 amino acids. After glycosylation, processing, and disulfide bonding, it is expressed as a heterotetramer composed of two α-subunits with a molecular weight of 95,000. The α subunit is entirely located on the exterior aspect of the plasma membrane, whereas the β subunit extends through the plasma membrane into the cytoplasm. The human insulin receptor gene is located on chromosome 19. It consists of 22 exons. The α subunit is encoded by the first 120 kb and includes a signal peptide, an insulin binding region, and a cysteine-rich region. The β subunit is encoded by the last 30 kb. As mentioned, this subunit is the portion of the receptor molecule that crosses the plasma membrane. It has a proreceptor processing site and the final portion of the unit of the receptor molecule consists of tyrosine kinase. When insulin binds to the α subunit, autophosphorylation occurs, resulting in activation of tyrosine kinase. If the DNA coding for the receptor protein has mutated such that lysine, an important amino acid for ATP binding and receptor activity, is replaced by arginine, alanine, or methionine, the receptor is no longer able to mediate insulin action. Thus, lysine is a critical component of the ATP binding site. Clusters of tyrosine residues are also critical to the autophosphorylation process. If tyrosine is replaced by phenylalanine, again, receptor activity is compromised. Other mutations in the code for the receptor protein that affect its amino acid sequence have been reported.[46–51] These mutations (depending on their location) likewise can affect the activity of the receptor and, in turn, can explain insulin resistance.

Insulin resistance can be observed in muscle as well as adipose tissue. By definition, insulin resistance means that it takes abnormally large amounts of insulin to regulate or control blood glucose within normal limits. A person with insulin resistance may not develop abnormal

glucose tolerance if (a) the individual is physically active or/and (b) the pancreas is able to sustain an abnormally high insulin output and (c) the person maintains low body fat. In this circumstance, the individual may be insulin resistant and hyperinsulinemic, but not hyperglycemic or have abnormal glucose tolerance. Eventually, the islet β cells may not be able to sustain this high insulin output and the insulin resistance will then progress to abnormal glucose tolerance and subsequently to the diabetic hyperglycemic state. Physical activity stimulates the noninsulin-dependent entry of glucose into the exercising muscle. This reduces the level of circulating glucose and thus assists in maintaining normal glucose homeostasis. In addition, the very active individual will be less likely to accumulate excess fat stores and this, too, contributes to the maintenance of glucose homeostasis. The American Diabetes Association recommends that people with diabetes exercise 2.5 hours/week with an exercise that raises the heart rate to 50–70% of capacity. Many activities can do this, not just the traditional jogging or cycling activity.[66]

One feature of Type 2 diabetes is excess fat stores. With enlarged fat cells there is the resistance of the cell to the effects of insulin.[67] When the fat cell becomes enlarged, it loses its responsiveness to the action of insulin in facilitating the entry of glucose into that fat cell. For decades it has been known that overly fat people who are insulin resistant can reverse their condition by restricting their energy intake, thereby reducing their excess fat store. When the adipocyte returns to its normal size it becomes normally responsive to the action of insulin. For many years it was thought that the enlarged fat cell receptor defect was simply due to a distortion of the plasma membrane insulin receptor brought about by the increased fat store. In some instances this may be true. However, it is now thought that the resistance of the enlarged fat cell favors its smaller neighbors that bind insulin normally.

In addition to mutations in the genes for the insulin receptor, mutations in the downstream signaling (the INS-1 system) system have been found.[55] These mutations result in impaired signal transduction from the receptor to the downstream reactions that metabolize glucose in the cell. Mutations in the genes for this system result in impaired glucose use. Signals generated when insulin binds to its receptor on the plasma membrane are needed for the recruitment of the glucose transporters from their storage sites on the endoplasmic reticulum to the plasma membrane and thus transport glucose into the cell and present it to the various enzymes for metabolism. As mentioned above, aberrations in the genes for the glucose transporters likewise can associate with diabetes, particularly that associated with obesity. Finally, mutations in the mitochondrial genome can affect the amount of ATP available for glucose metabolism. Again, glucose metabolism will be aberrant, and this aberration fits the description of diabetes.

The information provided in Table 7.8 is useful in understanding the genetic basis of diabetes, but it is altogether possible that the mutations listed here are merely characteristic of the disorder, not causal. Some of these mutations have been identified as being associated with the diabetic state, and some of the mutations cause other metabolic problems that in turn elicit abnormalities in glucose metabolism. In other words, not all of the mutations listed here are truly causal. The American Diabetes Association[31] reminds diabetologists that there are numerous reasons why diabetes develops, and only some of these reasons are genetic in nature. Endocrine diseases such as Cushing's disease (adrenal steroid hypersecretion) has, as its secondary characteristic, diabetes. When the primary disease is managed, the diabetes disappears. There are other instances as well of diabetes appearing as a secondary consequence of a primary disease. In fact, most of the mutations in the mitochondrial genome could be classified in this way.[57–60] Mutations in this genome when mutation load is high elicit some very serious diseases involving the central nervous system, the neuromuscular system, and the sensory system, as well as intermediary metabolism. When the mutation load is high these primary diseases receive the clinician's attention. That diabetes also develops is a secondary concern. Lastly, those mutations that phenotype primarily as obesity may also have diabetes as a secondary condition. If the obesity could be managed (fat stores reduced), then the diabetes might be mitigated. However, most of the cases of genetic obesity are extremely difficult to manage if the mutations have occurred in the genes that encode components of the food intake regulation system. The Prader–Willi syndrome is a prime example of this type of genetic disorder. Persons

with this disorder have insatiable appetites and become exceedingly obese. Diabetes is a secondary consequence of this massive obesity that shortens the life span of the affected individual.

However, there are mutations that elicit both obesity or excessive fat stores and diabetes. Examples here are the mutations in the genes that encode the intracellular glucose transporters (GLUT 1-4). Mutations in one or more of these genes can phenotype as both excessive fatness and diabetes. It is a condition that could be viewed not as the primary/secondary related condition but as a coequal syndrome. Both diabetes and excess fat stores develop at similar times. If there is insulin resistance, there will be a reduction in the insulin effect on the mobilization of the glucose transporters.[16,68] Similarly, if the transporters gene(s) is aberrant, although there is sufficient insulin bound to the receptor site, glucose is inadequately transported into the cell due to the GLUT gene mutation and hence is inadequately metabolized. Furthermore, if there is a defect in glucose transporter, there is an increase in intramyocellular lipid metabolism as well as a reduction in muscle glycogen synthesis.[69] The fatty metabolites lead to a defect in IRS-1 signaling through serine/threonine phosphorylation. This results in muscle insulin resistance and the appearance of diabetes. A similar mechanism is proposed for liver, where an aberration of the hepatic GLUT gene could lead to a fatty liver and reduced mitochondrial function mostly attributed to a reduction in mitochondrial density.

Animal Models for Humans with Type 2 Diabetes

The range of disorders in humans that fit the description for Type 2 diabetes is far greater than that for Type 1 diabetes. Much of what we have learned about diabetes has come from studies of animals that develop the disease. Type 1 diabetes can be mimicked through chemical ablation of the islet cells using either surgical ablation of the pancreas or the administration of either alloxan or streptozotocin. There is one mouse model that is resistant to alloxan.[70] Alloxan is a powerful oxidizing chemical. It works by attacking the β cells of the pancreas. Alloxan resistant mice have an equally powerful antioxidant system that suppresses the oxidant action of alloxan. This suggests that there may be humans that are diabetes resistant for the same reason. They may possess sufficient antioxidant power to suppress the oxidant actions on the pancreas of a variety of insults from intermittent anoxia, viral attack, or certain chemicals in the environment. There are also animals that become diabetic through a spontaneous mutation in their genome. These animal models are listed in Table 7.10.

Animals that are genetically obese usually overeat, and studies of the neuroendocrine influence on food intake have suggested that, in part, the genetic error in these animals may reside in the satiety-signaling system in the brain.[72] In the obese ob/ob mouse the mutation involves the production of the satiety-signaling cytokine, leptin. In the db/db mouse and the fa/fa rat, the mutation is that of the gene for the leptin receptor. In all of the animal models for obesity described to date that have hyperphagia as a part of it character, the satiety signal leptin is either missing or ineffective due to an aberrant leptin receptor.

There could be other aberrant signals and receptors as well. Adiponectin is a candidate for its role in satiety signaling. If in low amount, fatty acid oxidation is faulty. Adiponectin seems to play a role in stimulating metabolism and metabolic turnover. Low levels of adiponectin are associated with obesity and insulin resistance as well as with a condition known as polycystic ovary disease (PCOS).[73–77] Variants in the adiponectin gene and in the adiponectin receptor gene are associated with overall adiposity especially in the abdomen.[78]

Lastly, animal models of Type 2 diabetes have been produced through genetic engineering.[79,80] In these animals, an aberrant gene is inserted into a fertilized egg. Transgenic mice have been produced harboring aberrant genes for the insulin receptor, the insulin receptor substrate (IRS-1), hexokinase, GLUT 1 or 4, PEPCK (the rate-limiting enzyme in gluconeogenesis, phosphoenolpyruvate carboxykinase), and one of the G proteins (the protein that inhibits adenylcyclase). No doubt other transgenic mice have been constructed as well allowing researchers to learn how single mutations can phenotype as diabetes and whether environmental manipulations such as dietary variation

TABLE 7.10
Animal models for type 2 diabetes mellitus and characteristics

1. Low-dose chemical (streptozotocin and alloxan) treatment that reduce the capacity of the insulin-producing cells of the pancreas
2. C57BL/KS db/db mouse; initially hyperinsulinemic and hyperphagic; obese; hyperglycemic; insulin resistant. When beta cells collapse and necrotize, mice are hypoinsulinemic,hyperglycemic, ketotic, and have a shortened life span
3. BL/6J ob/ob mouse; defective leptin gene; obese, insulin resistant, hyperinsulinemic, when the ob gene is placed on the BL/Ks background, the features are not as severe
4. Agouti or yellow obese mouse; obese trait is a dominant trait and only heterozygotes exist. Homozygosity is lethal. Hyperglycemic, hyperphagic, enlarged adrenals
5. KK mouse; hyperphagia, moderate obesity, hyperglycemic
6. PBB/Ld mouse; latent obesity and hyperglycemia, insulin resistance, hyperlipidemia
7. NZO mouse; obese; mild hyperglycemia and hyperinsulinemia
8. Zucker fa/fa rat; hyperphagic, hyperglycemic, insulin resistant
9. BBZ/Wor rat; cross between Zucker and autoimmune diabetic BB rat; divergent progeny segregate into lean and obese phenotypes
10. SHR/N-cp rat; hypertensive, hyperglycemic, obese
11. BHE/Cdb rats; hyperglycemic at maturity; early hyperinsulinemia that subsides to hypoinsulinemia; models the human with diabetes due to mitochondrial mutation
12. Spiny mice (Acomys cahirinus); obese, beta cell hypertrophy; with age, beta cells collapse with onset of hypoinsulinemia, ketosis, hyperinsulinemia, early death
13. *Psammonys obesus* (sand rat); obese, insulin resistant, hyperinsulinemia, at midlife islets are depleted and symptoms of diabetes appear, followed by early death.
14. Chinese hampster (Cricetulus griseus); four gene mutations seem to be involved; hyperphagic, obese, hyperglycemic, insulin resistant
15. Djungarian hampster (Phodopus sungorus) and South African hampster (Mystromys albicaudatus) are spontaneously diabetic but have not been genetically characterized

Note: Shafrir, E. (1992), *Diabetes/Metab Rev* 8: 179–208.

can affect phenotypic expression. Knockout mice have also been produced allowing researchers to learn the function of some of the more obscure proteins involved in pancreatic function and glucose homeostasis. In these models, a specific gene is removed from the fertilized egg that encodes a specific protein. Knockout models for the glucose transporters as well as some of the signaling molecules such as IRS-1 have been produced. Recently, there was a knockout mouse in which the gene for resistin was reported.[81] Resistin is an adipocyte-secreted protein that circulates at increased levels in obesity. Acute administration of resistin to normal mice results in an impaired glucose tolerance. This was found to be due to an effect of resistin on glucose production via an increased expression of the gene for PEPCK.

Population Surveys Relating Diet to Diabetes

West and Kalbfleisch[82] were among the first to suggest that diet played a role in the development of diabetes. Sixteen countries were surveyed, examining adults over the age of 30. They found a positive association between the prevalence of diabetes and the intake of both fats and sugars. There was a negative association of diabetes with total carbohydrate intake and correlations between diabetes and serum cholesterol and with body fat. This was one of the first papers to point out the relationship between nutritional status and diabetes.

Following the NHANES observations of rises in both obesity and Type 2 diabetes, a number of surveys of different population groups were conducted. Werfalt et al.[83] examined the relationship between food patterns and five components of the metabolic syndrome in Sweden. They reported that consumption of high sugar, high bread, cheese, cake, and alcoholic beverages were associated with increased development of metabolic syndrome. In women, increased dairy food consumption provided a protective effect with respect to Type 2 diabetes. They concluded that there may be gender differences in how different foods affect metabolic consequences with respect to diabetes. The studies of van Dam et al.[84] asked a somewhat similar question. They found that following a prudent diet reduced the risk for developing Type 2 diabetes and that following a "nonprudent" diet together with inactivity had the reverse effect especially in men. Mennen et al.[85] reported that increased bread and/or dairy product consumption might be related to an increased risk of developing metabolic syndrome. In contrast, Alvarez-Leon et al.[8] reported on an inverse relationship between dairy food intake and hypertension, stroke, and colorectal cancer. Mensink[86] followed this with a summary of the epidemiological evidence supporting a negative association of dairy food consumption and the development of Type 2 diabetes. Those populations consuming dairy products were at less risk to develop Type 2 diabetes as well as metabolic syndrome than populations consuming a low dairy food diet. Meyer et al.[87] reported that there was an inverse relationship between the incidence of Type 2 diabetes and the intake of vegetable fat as well as with the substitution of polyunsaturated fat for saturated fat in the daily diet of older women in Iowa. Lopez-Ridaura et al.[88] reported that, after adjusting for age, body mass index, physical activity, family history, and hypercholesterolemia at base line, the relative risk of developing Type 2 diabetes in women was 0.66, whereas for men it was 0.67, comparing the highest to the lowest of magnesium intakes. These findings suggested that there was a negative association between magnesium intake and diabetes risk. That is, at higher magnesium intake the risk of developing diabetes falls. Son et al.[89,90] and Ma et al.[91] reached a similar conclusion through estimating magnesium intakes and measuring insulin levels or examining the data from the Insulin Resistance Atherosclerosis Study (IRAS). In another population study using 12,700 participants without evidence of diabetes, serum magnesium levels were inversely correlated with fasting serum insulin, plasma glucose, plasma high density lipoproteins and diastolic and systolic blood pressure. These findings were similar to those reported by Humphries et al.[92] and Rosolova,[93] suggesting that, because dairy foods provide so much magnesium (~16% of the daily magnesium intake), these foods would be beneficial with respect to insulin action and glucose homeostasis. It should be noted that one of the secondary effects of diabetes is a weakening or demineralization of the bones.[94–97] Some of this bone demineralization can be reversed with good insulin treatment and good glucose control as well as a regular exercise program. Exercise on its own can assist in glucoregulation.[98–100]

In the CARDIA study, young (18–30 years of age) adults were examined over 10 years to determine associations between the consumption of dairy products, obesity, and the development of insulin resistance.[101] They found that the consumption of dairy products was inversely related to the development of insulin resistance. In a continuing survey of the nutrient contributions of dairy products Weinberg et al.[102] reported that the consumption of these foods was associated with higher intakes of micronutrients without any adverse effects on either the fat intake of these people or their cholesterol intake.

Berkey et al.[103] studied adolescents with respect to dairy intake, dietary calcium intake, and body weight gain. Those subjects consuming the most dairy food gained the least weight. Multivariant analysis of milk, dairy fat, calcium, and total energy intake suggested that energy intake was the most important predictor of weight gain by these adolescents. Liu et al.[104] examined the food intakes of calcium and vitamin D of 10,066 middle-aged and older women and the prevalence of metabolic syndrome. Dietary vitamin D was inversely associated with metabolic syndrome but this effect was not independent of the calcium intake. A similar relationship was reported for the consumption of dairy foods and metabolic syndrome. The report was seconded by Azadbakht et al.[105] in Iranian adults. Those consuming more dairy food were less likely to develop metabolic syndrome. In men,

Rajpathak et al[106] reported on the long-term (12-year) weight gain by men. When the data were adjusted for potential confounders there was no association between a change in calcium intake or dairy food intake and weight gain. These data did not support the hypothesis that an increase in calcium intake or dairy consumption could result in a lower long-term weight gain in men.

Prospective Studies Linking Food Intake to a Reduction in Type 2 Diabetes

Salmeron et al.[107] prospectively followed 84,204 women aged 34–59 with no history of diabetes, cardiovascular disease, or cancer. They were particularly interested in the role of fat (both type and amount) in the development of diabetes. They found no association between total fat, monounsaturated fatty acids, and saturated fatty acids and Type 2 diabetes. They did note that trans fatty acids increase and polyunsaturated fatty acids reduce the risk for diabetes. Song et al.[90] asked a similar question with respect to red meat intake and Type 2 diabetes. An increase in red meat consumption was related to an increase in risk for Type 2 diabetes. Liu et al.[108] and also Choi et al.[109] conducted a prospective study of dairy intake and the risk of Type 2 diabetes. They found an inverse relationship between the two.

Dietary Intervention in Diabetes

As pointed out above, diabetes is often associated with obesity. Thus, it should come as no surprise that intervention strategies that target obesity will have an effect on the time course of diabetes development. Marshall et al.[110] studied 134 subjects with impaired glucose tolerance. Those subjects consuming 40.6% of their intake energy as fat were less likely to proceed onto Type 2 diabetes than those subjects consuming 43.4% of their daily energy intake as fat. These authors concluded that the level of fat consumption significantly affected diabetes risk even after correcting for obesity and markers for abnormal glucose metabolism. The implication is that the fat intake can significantly affect the time course for diabetes development.

King and Dowd[111] as well as Stern,[112] Melander,[113] and Kumar et al.[114] have reviewed the literature on the prevention of Type 2 diabetes. Table 7.11 provides a summary of some of these studies.

From the diet perspective, reduction in the conversion of impaired glucose tolerance to Type 2 diabetes was reduced from 29% to 13% in Swedish subjects through the limitation of energy intake from carbohydrates and fats.[115] Similar results were obtained in another study also conducted in Sweden.[116] In this second study, subjects were instructed in diet choices as well as in an exercise protocol. Annual assessments of the subjects showed that over the 6 years of the study, the rate of conversion from glucose intolerance to Type 2 diabetes was reduced 50%. A number of studies all directed toward increasing physical activity and reducing body fatness have shown that it is possible to intervene with the process of diabetes development where it is associated with excess fat stores or obesity.

The research to date suggests that not only should clinicians focus on the reduction in body fat stores but also pay attention to the various risk factors for cardiovascular disease. As noted in the introduction on the prevalence of diabetes, people with diabetes have five times the risk of developing cardiovascular disease as people without diabetes. Hence, intervention strategies should also include those tactics that will reduce the likelihood of a cardiovascular event. In view of the observation that more obesity is being observed in children and adolescents, it would appear that these age groups should be studied to determine if the interventions found successful in adults would be successful in children as well.

Several intervention trials have been conducted that addressed cardiovascular risk factors in children. The CATCH Multicenter Trial was a school-based research study that had as its objective the effectiveness of changes in school lunches, physical education, smoking policy curricula, and family activities.[117] The percent saturated fat and total fat was reduced in the diets of those subjects in the intervention group. Physical activity was also increased. This trial lasted 3 years and involved

TABLE 7.11
Intervention studies

Investigators	Subjects/origin	Study length	Intervention/outcome
O'Dea[118]	Australian aborigines	7 weeks	Reversion to traditional lifestyle resulted in an improvement in GTT
Sartor et al.[115]	Swedish adults	10 years	Reduced energy intake/Reduced conversion to diabetes
Ericsson et al.[119]	Swedish adults	6 years	Reduced energy intake, increased exercise/reduced conversion to diabetes
Viswanathan et al.[120]	Offspring of diabetic parents	4 years	Weight control and exercise: diabetes onset delayed
Ericsson et al.[116]	Overweight adults	1 year	Weight control, lifestyle advice/ weight loss: no effect on diabetes
Pan et al.[120]	Chinese adults	6 years	Diet advice and/or exercise/weight loss and diabetes delayed
Knowler et al.[121]	3234 adults	2.8 years	Placebo or metformin or weight loss and exercise: delay in diabetes

5016 third-grade students. Whereas the objectives of reducing fat intake and increasing physical activity were achieved, there were no significant differences between the control and intervention groups in blood pressure, body size, or blood cholesterol. In another study, the Heart Start Study, children in the third grade were again used, and again the goal was to reduce diet fat intake and increase physical activity.[113] This was achieved, and again few differences in cardiovascular risk factors were noted in these children. As with the CATCH Trial, diabetes was not assessed but the hoped-for outcome was that in modifying diet and physical activity in the child one could have an effect on that child as he or she matures. Habits adopted as a child might have a long-term effect on subsequent adult health behavior such that a delay in the development of heart disease and also diabetes might occur. Intervention studies per se in children with respect to Type 2 diabetes have not been conducted, primarily because this has not been a child health problem until recently. Now with the early onset of obesity these studies are needed.

Are there other strategies that could modify the phenotypic expression of a diabetes genotype? In BHE/Cdb rats having a diabetes genotype in the mitochondrial genome, feeding these rats an egg-based diet resulted in a delay in the onset of impaired glucose tolerance.[122] Rats of this strain fed a 10% fat diet where the fat was beef tallow lived longer and had a delay in the development of diabetic renal disease as compared to those animals fed a 10% corn oil or a 9% fish oil-1% corn oil diet. Those fed the fish oil diet had the shortest life spans and early onset of renal lesions.[123] Even though extra vitamin E was provided to these animals, free-radical production was increased in the fish oil and corn oil fed rats. Oxidative capacity clearly had a role in the lesions that developed in these rats that, in turn, shortened their life spans. Longevity studies in humans with this kind of strict dietary regimens have not been conducted, yet it is known that oxidative capacity as well as lipotoxicity does occur. Some have suggested that free-radical damage to the mitochondrial DNA could explain some of the secondary symptoms occurring in people with diabetes.[124] Lee[59] has proposed that diabetes itself damages DNA through an increase in free-radical production and that this damage could be part of the downward spiral of tissue damage that occurs in people with diabetes.

In humans with defects in insulin receptors, the strategy logically would be that of reducing the glucose-stimulated need for insulin bound to the fat cell, liver cell, or muscle cell. Diets low in

simple sugars and diets that maintain (or attain) ideal body weight should be used. This strategy would also be useful in those with mutations in the gene for glycogen synthesis. The objective would be to decrease the need to synthesize glycogen by keeping the carbohydrate intake relatively unrefined and low in amount. In persons having mutations in the glucose transporters and mutations in the mitochondrial genome, the strategy would be to increase the fluidity of the plasma and intracellular membranes. Such a strategy would maximize the activity of the insulin receptors through inducing a more fluid membrane. The receptors are membrane bound, and their activity is dependent on the fluidity of the lipid in which they are embedded. Several studies have been conducted showing that the dietary fat type will have this effect. In diabetes-prone BHE/Cdb rats fed fish oil, insulin sensitivity was increased via an effect on glucose uptake by the fat cell.[125,126] The type of fat in the diet fed these rats affected glucose homeostasis, insulin receptor number and binding activity, and glucose uptake and oxidation.[127,128] Ryan et al.[129] showed that humans provided a Mediterranean diet had an improvement in their insulin sensitivity, which these investigators attributed to a diet-induced change in membrane composition that, in turn, influenced membrane fluidity and function. The Mediterranean diet is an oleic-acid-rich diet. Oleic acid is a monounsaturated fatty acid (18:1). This fatty acid is one of the most common fatty acids found in dairy fat. Thus, if a Mediterranean diet could have this effect, there is no doubt that ingestion of dairy fat would have the same effect. The mol% of oleic acid in dairy fat is about ~26.5%. The only fatty acid in greater concentration in dairy fat is palmitic acid (16:0) at ~29 mol% .[130]

Where there are mutations in the gluconeogenic pathway, the strategy would be to avoid prolonged periods without food, necessitating the upregulation of gluconeogenesis that in turn contributes glucose to the circulation in excess. In this scenario the problem is not in the gluconeogenic pathway itself but in the factors that control it. Upregulation is a common feature in diabetes, and part of the problem in glucose homeostasis management is the downregulation of this pathway. Several of the oral hypoglycemic drugs recently developed have this as their pharmaceutic objective.

For those patients with diabetes due to mutations in the MODY genes, the diet prescriptions might be different (see discussion on MODY). These people might need to increase their fat and protein intake to gain better control of their glucose metabolism. This may also be true for those who develop diabetes due to an error in the mitochondrial genome.

Increasing physical activity in most of the people with genotypes that phenotype as diabetes would potentiate the diet strategies because it would not only increase energy expenditure but also would increase the noninsulin dependent use of glucose by the muscles. This is not a good strategy with respect to those afflicted with mutations in the mitochondrial genome. These persons usually are unable to sustain significant muscle activity because the mutations result in malfunctioning mitochondria that are so needed for muscular activity.

Other Intervention Studies

There have been a few reports of studies where dairy foods have been used as the experimental variable with respect to the development of Type 2 diabetes in humans. Nonetheless, there are studies where this variable was used to modulate adiposity. Indirectly, then, because adiposity correlates with Type 2 diabetes, one could suppose that dairy foods, wisely selected, could reduce the risk of developing Type 2 diabetes.

Melanson et al.[131] studied the effect of low- and high-calcium dairy-based diets on macronutrient oxidation in humans. They reported that under energy balance conditions there was no effect of dairy food ingestion on the respiratory quotient or on 24-hour macronutrient oxidation. Under energy deficit conditions, 24-hour fat oxidation was significantly increased in those subjects consuming the dairy-rich diet. No measures of glucose metabolism or measurements of diabetes status were made.

Tricon et al.[132] reported that dairy products naturally enriched with *cis*-9, *trans*-11 conjugated linoleic acid had no effect on serum lipid profiles, inflammatory markers, insulin, or blood glucose.

Conjugated (*trans*-10, *cis*-12) linoleic acid (CLA), but not *cis*-9, *trans*-11 conjugated linoleic acid, caused a drop in human adipocyte lipid content, a decrease in insulin-stimulated glucose uptake (suppression of GLUT 4 transporter), fatty acid uptake, incorporation into lipid, and oxidation compared to control adipocyte cultures.

Zemel[133–136] has suggested mechanisms for dairy modulation effect. He proposes that the calcium in the dairy product plays a pivotal role in the regulation of energy metabolism. He has reported that high-calcium diets can attenuate adipose lipid accretion and weight gain during periods of high-energy intake and that the calcium also increases lipolysis and well as preserves the thermogenic response to feeding. These are energy-wasting processes. In addition, he suggests that low calcium diets stimulate calcitriol production. Calcitriol is an active vitamin D analog. It plays a role in gene expression in cell types that possess a cytosolic vitamin-D-binding protein. Not all cells possess this receptor protein. The vitamin also may have a role in metabolic regulation vis-à-vis intermediary metabolism. Using agouti transgenic mice, Sun and Zemel[135] showed that calcium and dairy products inhibited weight and fat regain during ad libitum consumption following food restriction.

Calcium attenuates obesity-related oxidative stress and inflammation.[136] When added to cultures of human adipocytes it inhibits calcitriol action in stimulating ROS production and inflammatory cytokine production. Calcitriol (a vitamin D_3 analog) also increased the activities of macrophage inhibitory protein (MIF) and macrophage surface-specific protein (CD14) expression in cultured adipocytes. These responses could explain the beneficial effect of calcium in weight loss and reduction of obesity-related inflammation,[135] as well as obesity-related increases in the rennin–angiotensin system.[136] Whether these actions could explain a beneficial effect of dairy products on Type 2 diabetes development is subject to investigation. In cultured 3T3-L1 preadipocytes, additional vitamin D upregulates vitamin D receptor gene expression[137] so it is possible that there may be a vitamin D effect. Yet, one must remember that in vivo there are no vitamin D receptors in the mature adipocyte and that what may be found in an in vitro cell-culture system may not be replicated in the adult in a whole-body situation.

Overall Management of Both Type 1 and Type 2 Diabetes

The American Diabetes Association[31] maintains that the most effective nutrition management approach is an individualized one. The most effective diet plan is one that has been individualized to meet the needs of the patient, taking into account that patient's personal likes and dislikes as well as the eating plan that best maintains that particular person's blood glucose within normal limits. The strategy includes balancing food intake against exercise, and the maintenance of blood glucose within normal limits. As the patient is adjusting their food plan and activity schedule they are asked to monitor their blood glucose levels. They soon come to realize what foods they can tolerate well with little effect on overall glucose control. They also learn what foods are not well tolerated. Patients should keep a diary to help them remember what works and what does not work. Because it is not possible to detect the genetic reason for their diabetes, this kind of trial-and-error food plan development will be the next best plan for their disease management.

The general recommendations for Americans is that of the food pyramid. With this as the backbone, the ADA recommends the spacing of meals throughout the day avoiding binge eating or eating one or two large meals. A three-meal pattern plus a small snack is preferred to the two-meal pattern followed in many households (no breakfast, small lunch, large dinner). The distribution of energy from fats, carbohydrates, and proteins is important; 10–20% of the energy intake should be from protein with the remainder coming from fats and carbohydrates. Contrary to the historical position of the ADA on the food management of the person with diabetes, there is no prohibition of certain foods. Rather, there is the recommendation that the diet be designed to satisfy the patient yet keep the energy intake at a level commensurate with ideal body weight. In some instances this means energy intake restriction needed to reduce the body fat stores of the patient. The ADA recommends that the person with diabetes should try to keep their saturated fat intake to 10% of total

calories. For those patients with diabetes due to mutations in the MODY genes, the diet prescriptions might be different. These people might need to increase their fat intake to gain better control of the glucose metabolism. This may also be true for those who develop diabetes due to an error in the mitochondrial genome. Again, individualization of the food management plan will allow for such adjustments. At this time there are no studies that show that certain foods are more efficacious in the management of diabetes in humans than other foods.

Other Health Concerns in Carbohydrate Nutrition

There are a number of genetic errors in glycolysis, shunt-activity, gluconeogenesis, and glycogen turnover that phenotype as disorders in carbohydrate metabolism (see Chapter 5). Most of these are very rare.

Fiber

A number of years ago, several scientists noticed that populations whose traditional diets were rich in fibrous food had low incidences of colon cancer as well as heart disease and diabetes mellitus. They compared these diets to those typically consumed by people in the United States and Europe and suggested that the health status of these populations was related to their high-fiber diets. With the consumption of high-fiber diets, the feces were bulkier, moister, and more frequent than when low-fiber diets were consumed. These reports attracted a lot of attention and a plethora of high-fiber foods appeared in the market place. People began to consume these products in the hope of having similar benefits conferred. It is not clear that such benefits have been collected.

Dietary fiber refers to those carbohydrates that are indigestible and unabsorbed. The component glucose moieties are joined by β linkages rather than α linkages. They may also contain additional substituents, but their chief characteristic is that of nondigestibility by the α-amylases of the mammalian gastrointestinal system. These nondigestible carbohydrates are plant products and fall into five major categories: celluloses, hemicelluloses, lignins, pectins, and gums. The first three provide bulk to the gastrointestinal contents due to their property of absorbing water. The increased bulkiness of the gut contents stimulates peristalsis and results in shorter passage time and more frequent defecation. In addition to the water-holding property, fibers of the lignin type adsorb cholesterol, aiding in its excretion in the feces. Pectins and gums also influence gastric emptying but in the opposite direction. These fiber types form gels that slow gastric emptying and interfere with the absorption of sugars, starches, and also fats. The fibers, collectively, help to lower the level of serum cholesterol while increasing its excretion. It is for these reasons that nutritionists encourage the consumption of fiber-rich plant foods. Fruits are good sources of pectin, whereas cereal grains and the woody parts of vegetables are good sources of the celluloses, hemicelluloses, and lignins. Dried beans and oats are good sources of gums. The inclusion of foods containing all of these fiber types will no doubt be of benefit with respect to intestinal transit time and may also result in a small decrease in cholesterol absorption. This may have some impact on cholesterol balance. If fiber increases cholesterol excretion and reduces cholesterol recirculation, a small decrease in serum cholesterol level may occur. However, because cholesterol synthesis may rise to compensate for decreased absorption and reabsorption, the net effect with respect to cardiovascular disease development may be minor indeed.

The increased transit speed of high-fiber diets may be of benefit to those people susceptible to colon cancer. The fiber not only adsorbs cholesterol and hastens its excretion, but also adsorbs potentially noxious components of the ingesta, hastening their excretion as well. Thus, carcinogenic compounds have less time for exposure to colon cells and thus less opportunity to convert a normal cell to a cancer cell.

Finally, as with all comparisons of population groups, one must be careful in the interpretation of the data. Yes, a certain primitive group has less degenerative disease than we do in the United

States and, yes, the diet contains more fiber. Is this a cause-and-effect scenario? No, it is not. The picture is incomplete without observations on the incidence of other diseases, the average life span, the availability of clean water, immunizations, medical care, and observations on other aspects of lifestyle that can affect disease development.

Ethanol

Alcoholic beverages have been consumed by humans since the dawn of history. They have been used to ease anxiety, to promote social interaction, and as a vehicle to dominate others. Ethanol, the alcohol in beverages, is the quantitative end product of yeast glycolysis. Small amounts can be synthesized in mammalian cells. Thus, ethanol is a drug, a food, and a metabolite. It has been estimated that upward of 90 million Americans consume alcoholic beverages every day, and that about 10% of these people are addicted to its consumption. This affliction is called alcoholism.

There are genetic components of alcoholism, and it may well be that alcoholism is truly a disease that is a result of a diet–gene interaction.[138–140] The dietary ingredient in this instance is alcohol; the gene is as yet unidentified. However, there is ample evidence in the literature that supports the concept that the tendency toward alcoholism is inherited. Studies of twins reared by adoptive parents as well as multigeneration studies of families give support to this idea. An alcoholic is more likely than a nonalcoholic to have an alcoholic relative. At least 33% of alcoholics have an alcoholic parent. This has been observed in adopted individuals where the biological parent was unknown to the alcoholic and so the parent's proclivities were not taught. Studies of monozygotic (identical) and dizygotic (fraternal) twins indicated a high degree of concordance for alcoholism. If one twin became an alcoholic, the other twin also became one if that twin chose to consume alcohol. The concordance was greater in the identical twins than in the fraternal twins. Although scientists agree on the heritable nature of alcoholism, no one gene or group of genes has been identified and found culpable for the disorder.

Ethanol, once consumed, is rapidly absorbed by simple diffusion. The diffusion is affected by the amount of alcohol consumed, the regional blood flow, the surface area, and the presence of other foods. The different segments of the gastrointestinal tract absorb ethanol at different rates. Absorption is fastest in the duodenum and jejunum, slower in the stomach, ileum, and colon, and slowest in the mouth and esophagus. The rate of absorption by the duodenum depends on gastric emptying time, which, in turn, depends on the kinds and amounts of foods consumed with the ethanol. Certain drugs may also influence gastric-emptying time and thus influence absorption. Complete absorption may vary from 2 to 6 hours. The type of beverage can influence ethanol absorption. Ethanol from beer is absorbed slower than that found in whisky, which is slower than gin, and red wine. Of course, pure ethanol is absorbed the fastest of all.

Once absorbed, ethanol is rapidly distributed between the intracellular and extracellular compartments. This is because ethanol is completely miscible in water and thus freely travels any place water travels. The uptake of ethanol by the fat depots is minimal. Ethanol crosses the plasma membranes but, in so doing, changes them. When ethanol is in contact with a protein, it denatures it. Thus, large and frequent ethanol exposures results in damage to proteins both within and around the cells. The most damaged tissue is the liver because ethanol is carried directly to this tissue via the portal blood. Gut cells are also damaged, but these cells have such a rapid turnover time (less than 7 days) that such damage due to intermittent ethanol consumption is not as long-lasting as the damage that happens in the liver. Liver cells, in contrast, have a longer half-life and, once damaged, do not repair as readily. Alcoholic liver disease is a major cause of death among those who drink heavily.

Ethanol diffuses from the blood to the alveolar air so the ethanol content of expired air bears a constant relationship to pulmonary arterial blood ethanol levels. The partition coefficient is 2100:1. This means that 2100 mL of expired air contains the same amount of ethanol as 1 mL of blood. If the blood contains 100 mg of ethanol, the expired air will contain 232 ppm. This is the basis for the

"breathalyzer" tests for intoxication. Intoxication occurs at 150 mg/100 mL of blood but most states prosecute drivers having blood levels exceeding 100 mg/dL.

Of the ethanol consumed, 90–98% is oxidized to carbon dioxide and water. The rest is excreted as ethanol in the breath or in the urine. The metabolism of ethanol is shown in Figure 7.14. The rate of oxidation is fairly constant at about 10–20 mg/mL. This indicates that the first rate-limiting reaction catalyzed by alcohol dehydrogenase is saturated at this level. This is a zero-order reaction. The average rate at which alcohol can be metabolized is about 10 mL/h (or 7 g/h). The ethanol in 4 oz of whisky requires 5–6 h to metabolize completely to CO_2 and water. One mole of ethanol requires 16 mol of ATP for its conversion to CO_2 and water.

Ethanol is distributed throughout the body, but the liver is the chief site for its oxidation.[141–144] As mentioned, the first-rate-limiting reaction is catalyzed by alcohol dehydrogenase and converts ethanol to acetaldehyde. Acetaldehyde is quite damaging to cellular proteins and part of the hepatic injury found in alcoholics is due to this metabolite. It binds covalently to protein, impairs the microtubular assembly and the mitochondrial respiratory chain, depletes pyridoxine supplies, stimulates inappropriate collagen synthesis, inhibits DNA repair, and downregulates the transcription of the gene for microsomal triglyceride transfer.[140–144] Ethanol interferes with the cell's signaling systems associated with the phospholipases C and D. Acetaldehyde is also produced when ethanol is metabolized by the microsomal ethanol oxidizing system (MEOS). This system uses peroxide (H_2O_2) and produces a molecule of water as well as the acetaldehyde. The acetaldehyde is converted to acetate, which can either be joined with a CoA or released to the circulation. If too much acetate is released, acidosis develops.

Acetyl CoA can either be used for fatty acid synthesis or be shuttled into the mitochondria via carnitine to be oxidized as through the citric acid cycle. A fatty liver typifies the alcoholic. The

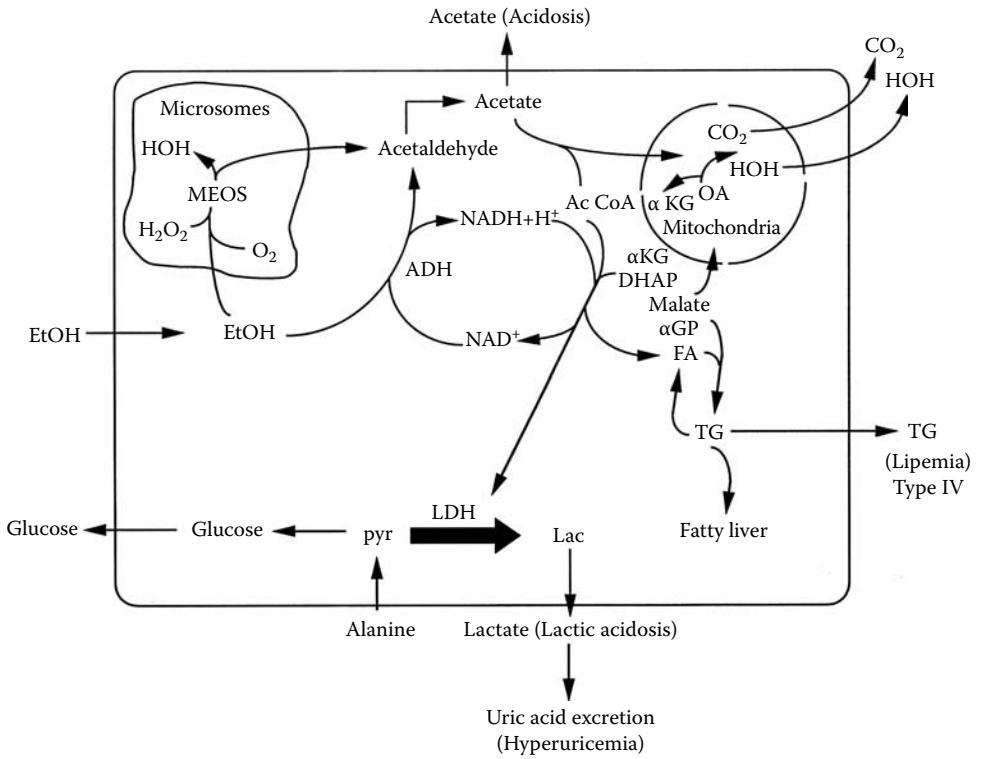


FIGURE 7.14 Metabolism of ethanol by the hepatocyte.

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fatty liver may progress to alcoholic hepatitis, to cirrhosis, liver failure, and death. The fatty liver is due to accelerated hepatic fatty acid synthesis, as well as due to an ethanol-induced impairment in hepatic lipid output. If the hepatocyte accumulates too much lipid, the cell will burst and die. Areas of dead tissue within the liver are known as *cirrhosis*. When too much tissue dies, the liver may cease to function, and the alcoholic dies.

In addition to the direct effects of ethanol on cell function, there are a number of auxiliary health concerns related to ethanol consumption. People who consume large quantities of ethanol find that their needs for thiamin, niacin, pyridoxine, and pantothenic acid increase dramatically. The alcoholic frequently manifests symptoms of beriberi, pellegra, and other deficiency diseases. In part, this is due to the increased need for these vitamins when ethanol is metabolized and, in part, because alcoholics may choose to consume alcoholic beverages in preference to nourishing food. Those alcoholics who continue to eat nourishing food in addition to consuming ethanol do not develop overt deficiency diseases as frequently. Nonetheless, the nutritionist should be aware of ethanol-induced increases in needs for the B vitamins.

Finally, there is another concern with respect to ethanol consumption. That is the development of fetal malformations in women who drink ethanol during pregnancy.[145] In 1973, eight cases of unrelated children were described having similar congenital defects. Particularly noticeable were the facial malformations involving eye placement, and nose and mouth development. All of these children had mothers who were alcoholic. In a subsequent report, it was noted that alcoholism in mothers was associated with an increased incidence of spontaneous abortions, premature delivery of fetuses who were poorly developed for their gestational age, and infants born with respiratory distress syndrome. Many of these children failed to grow and develop normally with full intellectual capacity. Various learning disabilities (partial hearing or visual loss) also characterized these children. How the ethanol affects fetal development, particularly the development of the central nervous system, is not known. Yet awareness of the potential damage of ethanol to the developing embryo and fetus should dictate abstinence prior to and during the gestational period.

Carbohydrate Needs

Carbohydrates are not considered essential nutrients aside from their use as providers of energy. As discussed in the section on gluconeogenesis, the body can usually synthesize sufficient glucose to sustain its absolute need for this fuel. Those cells that require other monosaccharides likewise can synthesize them. The mammary gland, for example, can convert glucose to galactose and use it (joined to glucose) to make the milk sugar lactose. The seminal vesicles likewise can isomerize glucose to fructose to meet the need for this particular monosaccharide. Thus, the traditional definition of nutrient essentiality is not met. Neither the normal human nor members of other animal species need to consume glucose in any set amount if synthesis is sufficiently active to meet need and if the diet provides sufficient gluconeogenic precursors. It has been estimated that the average human uses about 125 g of glucose per day to sustain neural activity. If this glucose is supplied by gluconeogenesis, 40% will come from lipid precursors and 60% will come from protein components. As mentioned, this synthesis is very expensive with respect to the energy needed and, more important, with respect to the amount of dietary protein that must be consumed in order to support glucose synthesis. If no dietary carbohydrate is consumed, more than 155 g of protein (2–3 times the usual protein need) must be consumed to provide the 75 g of the 125 g glucose per day that is needed.

Are there circumstances where this is not true? Are there times when glucose serves a vital function that is in addition to its role as an energy provider? The answer to these questions is yes. Just as the growing child needs dietary arginine, whereas the adult does not, the traumatized or septic patient needs glucose, when otherwise this need could be met by gluconeogenesis.

In trauma or sepsis, the energy requirement is greatly increased because these conditions elicit a stress response. This response means an increase in levels of the catabolic hormones, which are anti-insulin hormones as well as antiinflammatory hormones. The stress hormones mobilize body

protein and fat stores to provide the means for repair of injured tissues. When this catabolic response is prolonged (days to weeks), it must be reversed or patients could die if their protein and energy stores are insufficient to sustain the prolonged mobilization. If sufficient glucose is provided in a hypertonic solution via a central vein such as the subclavian, it can reverse this catabolic response. This treatment is called parenteral nutrition. This will minimize the loss in body muscle mass. The effect of this hypertonic solution of glucose is to overcome (through mass action) the anti-insulin effects of the catabolic hormones and thereby stimulate glycolysis and glycogenesis, and inhibit proteolysis and lipolysis.

The questions are, how much carbohydrate (glucose) is needed to have this effect, and how long should glucose be provided at this high level? The estimates vary. If the liver is functioning normally, 50–80% of the energy intake should have the desired anticatabolic effect. This would mean a 5–30% increase over the usual carbohydrate intake of 45–55% of the total energy intake, presuming that the patient can and will eat. This is not always possible. The patient may be in a coma, have a broken jaw, or the injury or sepsis may involve the gastrointestinal tract. In such instances, all needed nutrients, including glucose, must be supplied by the parenteral route. This creates other kinds of problems with respect to our knowledge about micronutrient needs in the absence of the protective role of the gastrointestinal system. Micromineral needs are especially difficult to manage under these circumstances. Humans need small amounts of most of these nutrients. If supplied in excess, a toxic state can develop.

With respect to the absolute amount of glucose that must be provided to the traumatized or septic patient, the estimates vary from 4 g/kg body weight (280 g/d/70 kg man) to 7 g/kg body weight (490 g/d/70 kg man). This would provide between 1120 to 1960 kcal (4686–8200 kJ) from glucose per day. The period of administration of this high a level of glucose to achieve anticatabolism has been estimated to be from 4 days to 2 weeks. After this period, a more normal distribution of macronutrients may be more appropriate.

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8 Lipids

The third macronutrient group consists of the lipids. They are energetically more dense than the carbohydrates, having more than twice the energy value of carbohydrate per gram. Americans consume approximately 32–42% of their total energy intake as lipid. The lipids make up a group of compounds which are, in general, insoluble in water and soluble in such solvents as diethyl ether, carbon tetrachloride, hot alcohol, chloroform, and benzene. They are present in various amounts in all living mammalian cells. Nerve cells and adipose cells are rich in lipid; muscle cells and epithelial cells have considerably less.

In addition to being a very important source of energy, lipids serve a variety of other needs. They perform a basic role in the structure and function of biological membranes. In the body, they are the precursors of a variety of hormones and some important cellular signals are lipids.

CLASSIFICATION

Lipids are relatively insoluble in water and relatively soluble in such solvents as ether, chloroform, benzene, and some alcohols. Additionally, some lipids are saponifiable; others are not. Saponifiable lipids, when treated with alkali, undergo hydrolysis at the ester linkage, resulting in the formation of an alcohol and a soap (fatty acids combined with hydroxide). Triacylglycerol (triglyceride), for example, when treated with sodium hydroxide, is hydrolyzed yielding a mixture of soaps and free glycerol. Traditionally, saponifiable lipids have been classified into three groups (A,B,C), each with subgroups:

A. Simple lipids: esters of fatty acids with various alcohols
    1. Fats: esters of fatty acids with glycerol (acylglycerols)
    2. Waxes: esters of fatty acids with long-chain alcohols
    3. Cholesterol esters
B. Compound lipids: esters of fatty acids that contain substituent groups in addition to fatty acids and alcohol
    1. Phospholipids: esters of fatty acids, alcohol, a phosphoric acid residue, and usually an amino alcohol, sugar, or other substituent
    2. Glycolipids: esters of fatty acids that contain carbohydrates and nitrogen (but not phosphoric acid), in addition to fatty acids and alcohol
    3. Lipoproteins: loose combinations of lipids and proteins
C. Derived lipids: substances derived by saponification from the above groups.

Two other terms are frequently used in the classification of lipids but are not a division of the system just described. These are the neutral and polar lipids. Neutral lipids are uncharged lipids and include triacylglycerols (TGs), cholesterol, and cholesterol esters. Polar lipids have positive and negative charges on certain atoms of the molecule. Examples of these are the phospholipids that are the lipids in the cell membrane. Phosphatidyl inositol, phosphatidyl choline, and phosphatidyl ethanolamine, for example, are membrane phospholipids and are polar.

STRUCTURE AND NOMENCLATURE

Simple Lipids

Fatty Acids

Fatty acids are carboxylic acids. They have a polar group (the carboxyl group) at one end and a methyl group at the other. A hydrocarbon chain is in the middle. Fatty acids can have few carbon atoms or more than 20; however, chain lengths of 16 and 18 carbons are the most prevalent. There is usually an even number of carbon atoms (with no branching) in the chain. The chain may be saturated (containing no double bonds) or unsaturated (containing one or more double bonds). Monounsaturated acids have one double bond; polyunsaturated acids have two or more.

The nomenclature of fatty acids is frequently confusing because the same fatty acid can have more than one name: its common (or trivial) name and its systematic name. The common name is rarely, if ever, related to the structure of the molecule. It is sometimes derived from the plant or animal source from which the acid was first isolated. The student has no choice but to memorize these names. Common names for nutritionally important fatty acids are given in Table 8.1.

The systematic method of naming fatty acids is based on a modification of the name of the straight-chain hydrocarbon having the same number of carbon atoms. The final "-e" from the hydrocarbon name is removed and -oic added, followed by the word "acid." To name the salt of the acid, the "-e" from the hydrocarbon base is replaced with "-ate." For example, the saturated, 18-carbon hydrocarbon, $C_{18}H_{38}$, has as its systematic name *n*-octadecane (the n means normal or no branching); its saturated fatty acid counterpart is *n*-octadecanoic acid (its common name is stearic acid); the salt is *n*-octadecanate. The monounsaturated, 18-carbon is 9-octadecene; one form of the unsaturated acid is *cis*-9-octadecenoic acid (common name, oleic acid). The salt is *cis*-9-octadecenate.

In the systematic name it is possible to address each carbon atom as was done in the last example just cited. There are two ways of doing this: with lowercase Greek letters or with Arabic numerals. With Greek letters, the carbon adjacent to the carboxyl group is α (alpha); the next one is β (beta); the last in the molecule is ω (omega). The carboxyl carbon is not given a designation. With Arabic numerals, the carboxyl carbon is 1; the next carbon, 2; the next, 3; and so on. The number 9 in the above example means that a double bond exists between carbons 9 and 10.

Other conventions are also used to indicate the position of the double bond. In one of them, Δ 9 indicates a double bond between carbons 9 and 10. In another, the number of carbon atoms, the number of double bonds, and the position of each double bond are all clearly shown. For example, oleic acid is 18:1 Δ 9 or 18 carbons with one double bond between the ninth and tenth carbons. Similarly, the 18-carbon acid with two double bonds is linoleic acid; in this system it would be referred to as 18:2 Δ 9, 12. In yet another convention, the position of the double bond from the terminal carbon (the methyl group) is indicated. The number of carbon atoms and the number of double bonds are shown as in the previous convention. The position of the double bond from the terminal carbon is then shown. For instance, oleic acid is 18:1ω9; linoleic acid is 18:2ω6. This convention is particularly useful in discussions about the interconversion of fatty acids; because carbon atoms are added between this double bond and the carboxyl group, it remains the terminal carbon.

As mentioned, fatty acids are either saturated or unsaturated. These two forms of fatty acids differ significantly in their structural considerations. In the saturated fatty acid, the hydrocarbon tail can exist in an infinite number of conformations because each carbon atom can rotate freely.

Unsaturated fatty acids, on the other hand, have a rigid feature to their structure because the carbons in the double bonds are not free to rotate; they exist as the *cis* or *trans* geometrical isomers. The *cis* configuration produces a bond in the hydrocarbon chain so that it resembles the shape of the letter U. Each *cis* configuration in the tail will add another bend. The *trans* configuration has nearly the same shape as the extended conformation of the saturated fatty acid. These structural features of the unsaturated fatty acids have a biological significance, as will be discussed in later sections.

TABLE 8.1
Structure and names of fatty acids found in food

Structure	# Carbons: double bonds	Systematic name	Trivial name	Source
Saturated fatty acids				
$CH_3(CH_2)_2COOH$	4:0	*n-Butanoic*	Butyric	Butter
$CH_3(CH_2)_4COOH$	6:0	*n-Hexanoic*	Caproic	Butter
$CH_3(CH_2)_6COOH$	8:0	*n-Octanoic*	Caprylic	Coconut oil
$CH_3(CH_2)_8COOH$	10:0	*n-Decanoic*	Capric	Palm oil
$CH_3(CH_2)_{10}COOH$	12:0	*n-Dodecanoic*	Lauric	Coconut oil, nutmeg, butter
$CH_3(CH_2)_{12}COOH$	14:0	*n-Tetradecanoic*	Myristic	Coconut oil
$CH_3(CH_2)_{14}COOH$	16:0	*n-Hexadecanoic*	Palmitic	Most fats and oils
$CH_3(CH_2)_{16}COOH$	18:0	*n-Octadecanoic*	Stearic	Most fats and oils
$CH_3(CH_2)_{18}COOH$	20:0	*n-Eicosanoic*	Arachidic	Peanut oil, lard
Unsaturated fatty acids				
$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	16:1	9-Hexadecenoic	Palmitoleic oils	Butter and seed
$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	18:1	9-Octadecenoic	Oleic	Most fats and oils
$CH_3(CH_2)_5CH{=}CH(CH_2)_9COOH$	20:1	11-Octadecenoic	*trans-Vaccenic*	Hydrogenated vegetable oils
$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	18:2	9,12-Octadecadienoic	Linoleic	Linseed oil, corn oil, cottonseed oil
$CH_3(CH{=}CHCH_2)_3(CH_2)_7COOH$	18:3	9,12,15-Octadecatrienoic	Linolenic	Soybean oil, marine oils
$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$	20:4	5,8,11,14-Eicosatetraenoic	Arachidonic	Cottonseed oil

In polyunsaturated fatty acids, the double bonds are not conjugated; that is, the double bonds will be separated by more than one single bond as in: $–CH_2–CH{=}CH–CH_2–CH{=}CH–CH_2–$, The unsaturated fatty acids of higher plants and animals are usually palmitoleic, oleic, linoleic, and linolenic acids; the first double bond will be between atoms 9 and 10. Others, if present, will be at carbon 12 or greater. Most of these unsaturated fatty acids will exist in the *cis* configuration. When vegetable oils are solidified to make margarine, some of the double bonds will be converted to single bonds through the addition of hydrogen. The process is called *hydrogenation* and the oils must be heated before hydrogenation will take place. When this occurs, some of the residual unsaturated fatty acids will change from the *cis* to the *trans* residual form. Margarine and vegetable shortening are thus sources of the trans fatty acids in the human diet. Some fatty acids exist naturally in the *trans* configuration. Eight percent of the unsaturated fatty acids in cows' milk is in the *trans* form. In this instance, the presence of trans fatty acids is due to their production by the rumen flora. Unlike the trans fatty acids produced when unsaturated fatty acids are hydrogenated industrially, the trans fatty acids in milk do not have atherogenic potential.[1]

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Fatty acids with an odd number of carbon atoms are found in limited amounts in natural products. Less than 0.4% of the total fatty acids in olive oil and 0.8% of those in lard contain an odd number of carbon atoms. In contrast, 60% of olive oil and 40% of lard consists of the even-numbered monounsaturated fatty acid, oleic acid. Few oils or waxes contain significant amounts of odd-numbered fatty acids. Odd-numbered fatty acids can be found in vitamin B_{12}-deficient individuals because B_{12} is an essential coenzyme in the conversion of methylmalonyl CoA to succinyl CoA.[2,3] When this conversion is impaired, methylmalonic acid accumulates, and some of this is used to synthesize odd-numbered fatty acids.

Of the polyunsaturated fatty acids, linoleic and linolenic are designated as the essential fatty acids (EFA).[4–6] Linoleic is an 18-carbon fatty acid with two double bonds (ω6,18:2, $\Delta^{9,12}$). Linolenic acid is also an 18-carbon fatty acid but with three double bonds (ω3, 18:3, $\Delta^{9,12,15}$). Both are in the *cis* configuration. Food oils of plant origin are good sources of linoleic acid, whereas the marine oils are good sources of linolenic acid. A few oils of plant origin contain linolenic acid (i.e., primrose oil). Most mammals require these fatty acids and cannot synthesize them. Felines, in addition, cannot convert linoleic acid to arachidonic acid (ω6, 20:4, $\Delta^{5,8,11,14}$). Hence, for these animals, arachidonic acid is an essential fatty acid. It is estimated that 1–2% of the lipid intake as essential fatty acids should meet the needs for normal individuals. The Food and Nutrition Board of the National Academy of Science has provided figures for an acceptable macronutrient intake for the essential fatty acids as a percent of the energy intake. For children age 1–3 years the range for the *n*-6 polyunsaturated fatty acids is 5–10% with an energy intake from fat of 30–40%. For children age 4–18, the range for polyunsaturated *n*-6 fatty acids is the same but the energy intake from fat is 25–35%. For adults the range for polyunsaturated *n*-6 fatty acids likewise is unchanged but the recommended fat intake as a percent of energy is 20–35%. The suggested range for the *n*-3 polyunsaturated fatty acids for these population groups is 0.6–1.2% of energy.

Triacylglycerols (Fats or Triglycerides)

Fats are formed when one or more fatty acids react with the hydroxyl group(s) of glycerol to make an ester. They are called *neutral fats, glycerides,* or *acylglycerols.* The fat most frequently found in nature has all three hydroxyl positions esterified; it is known as triacylglycerol. Triacylglycerols that contain the same fatty acid residue on all three carbons are simple triacylglycerols. An example would be tripalmitin. In nature, the triacylglycerols usually contain more than one fatty acid, and thus are called *mixed triacylglycerols* or *triglycerides.* Most fats in nature are complex mixtures of simple and mixed triacylglycerols. Many oils and fats contain between six and ten different saturated and unsaturated fatty acids. The number of possible combinations with this many fatty acid residues is very large.

The ratio of unsaturated to saturated fatty acids is defined as the P/S ratio. A high ratio of 3:1 means that there are three molecules of unsaturated fatty acids to every saturated fatty acid present. Animal fats and the foods rich in saturated fats usually have a low P/S ratio. Most vegetable oils have a high P/S ratio. However, there are some exceptions to this. The tropical plant oils (palm and coconut) provide a solid fat with a low P/S ratio. These fats, although called "oils" are rich in medium-chain (chain lengths of 8–14 carbons) saturated fatty acids. By convention, these lipids are called "oils" because they are fluid at much lower temperatures (the ambient temperature of the tropics) than are the fats of animal origin. Thus, we have coconut oil, palm oil, and palm kernel oil as solids at temperatures of 18–22°C but liquid at 26–30°C. The P/S ratio of the fat in the adipose tissue of humans reflects the P/S ratio of the food consumed. In order to assess the P/S ratio of the fat intake of populations, only a small sample of adipose tissue would need to be obtained and analyzed for its P/S ratio. Because the body metabolizes the fat it consumes, both lengthening and shortening the food fatty acids, an exact copy of the food lipid fatty acids will not be obtained, but some general trends will be observed.

SOURCES OF LIPIDS

When discussing food sources of lipids, the food triacylglycerols are primarily meant because they are the lipids that occur in foods in large quantities. Lipids are found in almost every natural food. Fruits and vegetables have small amounts of lipid, whereas meat, whole milk, cheese, eggs, and table spreads have larger amounts. A few plant foods—olives and avocados—contain as much as 20% (by weight) fat. Nuts are rich sources of fat: pecans are 71% fat, and walnuts are 60% fat. Table 8.2 shows the total fat content of several foods and also gives some values for the saturated fatty acid content and the amount of two common unsaturated fatty acids, oleic and linoleic.

More than one-half of dietary fat is contributed from invisible sources: the fat in whole milk and eggs, whole grain cereals, baked products, and convenience foods. The other fraction of dietary fat is visible. The marbling in meat, butter on bread or vegetables, and salad oil on lettuce are examples of the latter. The fatty acid composition of several fats and oils is given in Table 8.2. Of the saturated

TABLE 8.2
Percent fat in 100 g portions of common foods

Food	Total fat % by weight	Total saturated fat of total % fatty acids	Unsaturated fatty acids	
			Oleic	Linoleic
Animal				
Bacon	53	20	27	7
Butter	81	45	27	3
Chicken, broiled	3.5	1	1	1
Egg, whole	12	4	5	Trace
Hamburger	12	6	4.7	Trace
Ice cream, 10% fat	10	6	4	Trace
Lamb, leg, roasted	19	10	7	Trace
Milk, whole	3.5	2	1.2	Trace
Milk, 2%	2	1.2	0.8	Trace
Milk, skim	Trace	Trace	Trace	Trace
Pork chops	21	8	9	2
Salmon, pink	6	1	1	Trace
Vegetable				
Avocados	13	2.3	6	1.8
Bread, whole wheat	2.6	0.4	1.3	0.4
Bread, white enriched	3.3	0.7	1.8	0.4
Broccoli	0.6	Trace	Trace	Trace
Cereal, 40% bran	2.8	Trace	Trace	Trace
Cereal, oatmeal	0.8	Trace	Trace	Trace
Orange juice, fresh	0.4	Trace	Trace	Trace
Peanut butter	50	12.5	25	12.5
Pecans	71	4.6	44	14
Potato, baked	Trace	Trace	Trace	Trace
Strawberries, raw	0.7	Trace	Trace	Trace
Walnuts	60	3	24	33

Source: www.nal.usda.gov/fnic/foodcomp/Data/food.82nutrients; accessed 8/22/07.

fatty acids, palmitic (16:0) and stearic (18:0) are the most prevalent in nature. Of the unsaturated fatty acids, oleic (18:1) and linoleic (18:2) are the most abundant. Together, these two fatty acids make up 90% of the unsaturated fatty acids in the American diet.

As mentioned earlier, fats of plant origin tend to be less saturated than fats of animal origin. Fats from marine creatures are usually more unsaturated than either plant fats or land animal fats. Beef fat contains a lot of stearic acid. Mutton fat and cocoa butter contain a similar array of fatty acids but not in the same amounts. Mutton fat has as much as 27% saturated fatty acids by weight, whereas cocoa butter has 2.5%. This accounts for the marked difference in the physical properties of these two fats. Milk fat is similar to lard (pig fat) because it contains large amounts of palmitic and oleic acid. Fish, on the other hand, tend to have much more polyunsaturated fatty acid in its glycerides. Freshwater fish contain more unsaturated C18 and less unsaturated C20 and C22 than do marine fish. Fatty marine fish such as mackerel contain long-chain polyunsaturated fatty acids of the omega ω-3 or n-3 type.

Cholesterol, cholesterol esters, free fatty acids, phospholipids, and sphingolipids are also present in the foods humans consume. These compounds represent only a small fraction of the total fat consumed. Because of the population studies linking heart disease to cholesterol levels in the blood, nutritionists are frequently interested in the levels of this lipid in the food. Listed in Table 8.3 are some human foods and their percentage of fat and cholesterol content. Greater detail on the composition of the tremendous variety of foods that humans consume may be found in the USDA Web site: www.nal.usda.gov/fnic/foodcomp/data/food.82nutrients. These food-composition data can be obtained on disk. Other handbooks and computer programs have been developed to assess the nutrient intakes of not only man, but other species as well. The data provided in these sources are averages of several different analyses, but are not absolute. Variation in the source of the food, how it is prepared, and how much fat is removed prior to preparation and consumption contributes to the uncertainty associated with these values.

Interest in the sphingolipids in food has emerged as reports have appeared, suggesting that these lipids might have an anticancer or antilipemia function.[7] Sphingolipids are complex lipids having a sphingosin backbone. There are more than 60 variants of these compounds. There are various alkyl chain lengths (4–22 carbons) and varying degrees of saturation, as well as varying positions for their component double bonds, chain branching, and presence of a hydroxyl group. The amino group of the sphingoid base can be substituted with a long-chain fatty acid to produce a ceramide. There are so many variants of these compounds that this group is the largest group of complex dietary lipids, yet their nutritional significance has not been well studied.

DIGESTION AND ABSORPTION

The digestion and absorption of the various food lipids involves the mouth, stomach, and intestine (Figure 8.1). The digestion of lipid is begun in the mouth with the mastication of food and the mixing with the acid-stable lingual lipase. Digestion can proceed only when the large particles of food are made smaller through chewing. The action of the tongue and teeth mixes the food with the lingual lipase. Later, the churning action of the stomach mixes the food particles with hydrochloric acid. These actions separate the lipid particles, exposing more surface area for enzyme action and emulsion formation. The changes in physical state, illustrated in Figure 8.2, are essential steps that precede absorption. In the stomach, lipid–protein complexes are cleaved, releasing lipid when the proteins of these complexes are denatured by gastric hydrochloric acid and attacked by the proteases (pepsin, parapepsin I, and parapepsin II) of the gastric juice. The remaining lipid components of the diet are mixed with other diet ingredients by the churning action of the stomach. Little degradation of fat occurs in this organ except that catalyzed by lingual lipase, which is thought to originate from glands in the back of the mouth and under the tongue. This lipase is active in the acid environment of the stomach. However, because of the tendency of lipid to coalesce and form a separate phase, lingual lipase has limited opportunity to attack the triacylglycerols. Those that

TABLE 8.3
Total lipid and cholesterol content of 100 g portions of several common foods

Food	% Fat	% Cholesterol
Codfish	0.67	0.043
Halibut	2.94	0.041
Mackerel	6.30	0.076
Salmon	3.45	0.052
Beef steak, cooked	31.8	0.36
Hamburger, cooked	12.8	0.10
Stew meat	18.8	1
Lamb chops, cooked	36.0	1.11
Pork chops, cooked	21.1	1.03
Pork sausage	46.2	1.35
Bologna	28.6	1.04
Chicken, white meat	3.5	0.64
Eggs, whole	12	4.2
Egg white	<3	—
Whole milk	3.3	0.32
Skim milk	Trace	Trace
Cheddar cheese	32	1.0
Cottage cheese (4%)	4.29	0.21
Mozzarella cheese, skim milk	17.9	4.5
Butter	81.4	2.4
Margarine	81.4	—
Fruits, all kinds	<1	—
Avocado	13	—
Leafy vegetables	<1	—
Legumes (except peanuts)	1	—
Root vegetables	<1	—
Cereals and grains	1–2	0
Crackers	1	0
Bread, white, enriched	4	<0.1

Note: Foods were selected from the vast array of foods found in the USDA data bank: www.nal.usda.gov/fnic/foodcomp/Data/food.82nutrients.

are attacked release a single fatty acid, usually a short- or medium-chain one. The remaining diacylglycerol is subsequently hydrolyzed in the duodenum. In adults consuming a mixed diet, lingual lipase is relatively unimportant. However, in infants having an immature duodenal lipase, lingual lipase is quite important. In addition, this lipase has its greatest activity on the triacylglycerols commonly present in whole milk. Milk fat has more short and medium-chain fatty acids than fats from other food sources.

Although the action of lingual lipase is slow relative to lipases found in the duodenum, its action to release diacylglycerol and short- and medium-chain fatty acids serves another function: these fatty acids act as surfactants. Surfactants spontaneously adsorb to the water–lipid interface, stabilizing emulsions as they form. Other dietary surfactants are the lecithins and the phospholipids.

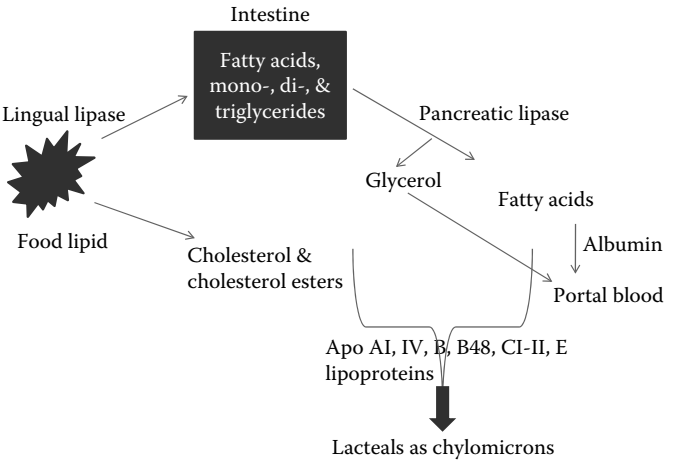


FIGURE 8.1 Overview of digestion and absorption of food lipid.

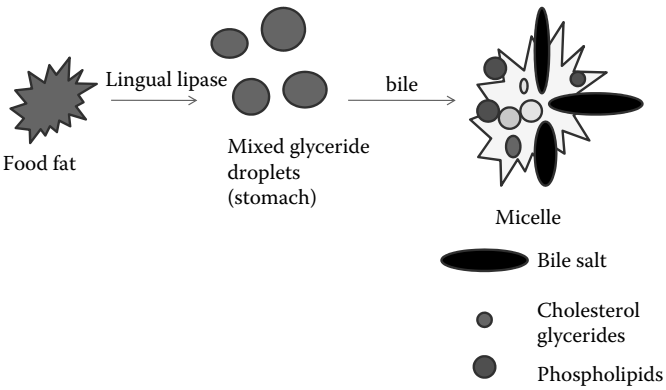


FIGURE 8.2 Changes in the physical state of food lipid as it is prepared for absorption.

Altogether, these surfactants, plus the churning action of the stomach, produce an emulsion that is then expelled into the duodenum as chyme.

The chyme's entry into the duodenum stimulates the release of the gut hormones pancreozymin and cholecystokinin into the bloodstream. Cholecystokinin stimulates the gall bladder to contract and release bile. Bile salts serve as emulsifying agents and to further disperse the lipid droplets at the lipid–aqueous interface facilitating the hydrolysis of the glycerides by the pancreatic lipases. The bile salts impart a negative charge to the lipids, which in turn attracts the pancreatic enzyme colipase.

Pancreozymin (secretin) stimulates the exocrine pancreas to release pancreatic juice, which contains three lipases (lipase, lipid esterase, and colipase). These lipases act at the water–lipid interface of the emulsion particles. One lipase acts on the fatty acids esterified at positions 1 and 3 of the glycerol backbone, leaving a fatty acid esterified at carbon 2. This 2-monoacylglyceride can isomerize, and the remaining fatty acid can move to carbon 1 or 3. The pancreatic juice contains another less specific lipase (called a *lipid esterase*), which cleaves fatty acids from cholesterol esters, monoglycerides, or esters such as vitamin A ester. Its action requires the presence of the bile salts. The lipase that is specific for the ester linkage at carbons 1 and 3 of the triacylglycerols does not

have a requirement for the bile salts and, in fact, is inhibited by them. The inhibition of pancreatic lipase by the bile salts is relieved by the third pancreatic enzyme, colipase.

Colipase is a small protein (mol. wt. 12,000 Da) that binds to both the water–lipid interface and to lipase, thereby anchoring and activating the lipase. The products of the lipase-catalyzed reaction, a reaction that favors the release of fatty acids having 10 or more carbons, are these fatty acids and mono-acylglyceride. The products of the lipid esterase-catalyzed reaction are cholesterol, vitamins, fatty acids, and glycerol. The phospholipids present in food are attacked by phospholipases specific to each of the phospholipids. The pancreatic juice contains these lipases as prephospholipases, which are activated by the enzyme trypsin.

As mentioned, the release of bile from the gall bladder is essential to the digestion of dietary fat. Bile contains the bile acids cholic acid and chenodeoxycholic acid. These are biological detergents or emulsifying agents. At physiological pH, these acids are present as anions, so they are frequently referred to as bile salts. At pH values above the physiological range, they form aggregates with the fats at concentrations above 2–5 mM. These aggregates are called micelles. The micelles are much smaller in size than the emulsified lipid droplets. Micelle sizes vary depending on the ratio of lipids to bile acids, but typically range from 40 to 600 Å.

Micelles are structured such that the hydrophobic portions (triglycererols, cholesterol esters, etc.) are toward the center of the structure, whereas the hydrophilic portions (phospholipids, short-chain fatty acids, bile salts) surround this center. The micelles contain many different lipids. Mixed micelles have a disk-like shape wherein the lipids form a bilayer and the bile acids occupy edge positions, rendering the edge of the disc hydrophilic. During the process of lipase and esterase digestion of the lipids in the chyme, the water-insoluble lipids are rendered soluble and transferred from the lipid emulsion of the chyme to the micelle. In turn, these micelles transfer the products of digestion (free fatty acids, glycerol, cholesterol, etc.) from the intestinal lumen to the surface of the epithelial cells, where absorption takes place. The micellar fluid layer next to this cell surface is homogenous, yet the products of lipid digestion are presented to the cell surface and, by passive diffusion, these products are transported into the absorptive cell. Thus, the degree to which dietary lipid, once digested, is absorbed, depends largely on the amount of lipid to be absorbed relative to the amount of bile acid available to make the micelle. This, in turn, is dependent on the rate of bile acid synthesis by the liver and bile release by the gall bladder. People who have had their gall bladder removed still have their bile acids. Instead of stockpiling them in the gall bladder to be released upon the cholecystokinin signal, surgeons simply remove the gall bladder and make a direct connection between the liver and the duodenum. Once the fat has been absorbed, the bile acids pass on through the intestine, where they are either reabsorbed or conjugated and excreted in the feces.

The primary bile acids, cholic and chenodeoxycholic acids, are produced from cholesterol by the liver. They are secreted into the intestine, where the intestinal flora convert these acids to their conjugated forms by dehydroxylating carbon 7. Further metabolism occurs at the far end of the intestinal tract where lithocholate is sulfated. Although the dehydroxylated acids can be reabsorbed and sent back to the liver via the portal blood, the sulfated lithocholate is not. It appears in the feces. All four of the bile acids, the primary and dehydroxylated forms, are recirculated via the enterohepatic system so that very little of the bile acid is lost. It has been estimated that the bile acid lost in the feces (~0.8 g/day) equals that newly synthesized by the liver, so that the total pool remains between 3–5 g. The amount secreted per day is on the order of 16–70 g. Because the pool size is only 3–5 g, this means that these acids are recirculated as often as 14 times a day.

The function of the bile acids is thus quite similar to that of enzymes. Neither are "used up" by the processes they participate in and facilitate. In the instance of fat absorption, the bile acids facilitate the formation of micelles, which, in turn, facilitate the uptake of the dietary fatty acids, monoglycerides, sterols, phospholipids, and other fat-soluble nutrients by the enterocyte of the small intestine. Not only do these bile acids recirculate, so too does cholesterol. Gallstones develop when the resecreted material is supersaturated with cholesterol, and this cholesterol-laden bile is stored in the gall bladder. With time, the cholesterol precipitates out, providing a crystalline structure for the

stone. Because the bile also contains a variety of minerals, these minerals form salts with the bile acids and are deposited within and around the cholesterol matrix. Eventually, these stones irritate the lining of the gall bladder or may lodge themselves in the duct connecting the bladder to the duodenum. When this happens, the bladder becomes inflamed, the duct may be blocked, and the patient becomes unable to tolerate food. In some cases, treatment consists of reducing the irritation and inflammation through drugs, but often the patient has the gall bladder and its offending stones removed. This surgery is called a *cholecystectomy.*

Virtually all of the fatty acids, as part of the mono, di, and triacylglyceride, and glycerol are absorbed by the enterocyte. Only 30–40% of the dietary cholesterol is absorbed. The percent cholesterol absorbed depends on a number of factors, including the fiber content of the diet, the gut passage time, and the total amount of cholesterol present for absorption. At higher intake levels, less is absorbed and vice versa at lower intake levels. Compared with fatty acids and the acylglycerides, the rate of cholesterol absorption is very slow. It is estimated that the half-life of cholesterol in the enterocyte is 12 hours. With high fiber intakes, less cholesterol is absorbed because the fiber acts as an adsorbent, reducing cholesterol availability. Chapter 7 discusses the different fibers and their biological activity. Cellulose and lignens are good adsorbants of cholesterol, whereas transit through the intestine can be hastened by cellulose and hemicellulose. Pectins and gums increase transit time, yet they lower serum cholesterol levels by creating a gel-like consistency of the chyme, rendering the cholesterol in the chyme less available for absorption. High-fiber diets reduce gut passage time, which, in turn, results in less time for cholesterol absorption.

The fate of the absorbed fatty acids depends on chain length. Those fatty acids having 10 or fewer carbons are quickly passed into the portal bloodstream without further modification. They are carried to the liver bound to albumin in concentrations varying between 0.1 to 2.0 meq/ml. Those fatty acids remaining are bound to a fatty-acid-binding protein and transported through the cytosol to the endoplasmic reticulum, whereupon they are converted to their CoA derivatives and reesterified to glycerol or residual monoacylglycerides to reform triacylglycerides. These reformed triacylglycerides adhere to phospholipids and fat-transporting proteins that are members of the lipoprotein family of proteins. This relatively large lipid–protein complex migrates to the Golgi complex in the basolateral basement membrane of the enterocyte. The lipid-rich vesicles fuse with the Golgi surface membrane, whereupon the lipid–protein complex is exocytosed or secreted into the intercellular space which, in turn, drains into the lymphatic system. The lymphatic system contributes these lipids to the circulation as the thoracic duct enters the jugular vein prior to its entry into the heart.

TRANSPORT

Blood lipid values vary depending on the age, sex, lifestyle, genetics, and diet of the population. Typical values for the different lipids are shown in Table 8.4. After a meal, the blood lipids rise. The time it takes for the peak value to appear depends on a variety of factors but, most notably, on the proximate composition of the meal. A high-fat meal leaves the stomach at a slower rate than does a low-fat meal. A high-fat meal will result in more total lipid entering the blood than a low-fat meal, but this lipid will enter the blood at a slower rate.

As fat digestion and absorption proceed, TG and cholesterol are processed in the intestinal epithelial cells into lipid-rich particles containing about 1% protein. The lipid associates with amphipathic protein known as *lipoprotein*. Lipoproteins carry not only the absorbed food lipids, but also the lipids synthesized or mobilized from organs and fat depots. Nine different lipid-carrying proteins have been identified, and each plays a specific role in the lipid-transport process. In addition, there are several minor proteins that may be involved in some aspects of lipid cycling and uptake. The proteins involved in lipid transport are listed in Table 8.5, and their characteristics are listed in Table 8.6. Mutations in the genes that encode these proteins can lead to aberrant lipid transport, and the individual may have either abnormally high or low blood lipid values.[9–13] The hepatic and intestinal apolipoproteins can be distinguished using electrophoresis, a technique of separating proteins

TABLE 8.4
Average blood lipid levels for normal fasting humans

Fraction	Range of values[a]	
	mmol/L	mg/dL
Cholesterol	3.95–5.56	152–214 males
	4.08–5.88	157–226 females
Phospholipids	9–16 mg/dL as lipid P	
Triacyglycerides	0.77–1.30	68–115 males
	0.72–1.24	64–110 females
Free Fatty Acids	6–16 mg/dL	

[a] Selected from a table of usual clinical findings in Feldman, E.B. and Cooper, G.R. (2007). In *Handbook of Nutrition and Food* (Berdanier, C.D., Dwyer, J., and Feldman, E.B. Eds.). CRC Press, Boca Raton, FL. pp. 683–692.

based on their electrophoretic mobility. Typically, these lipid-carrying proteins contain protein, triacylglycerols, phospholipids, and cholesterol. When separated, they have a distinct and characteristic lipid content, as shown in Table 8.5.

The intestinal cell has three apolipoproteins, called A-1, A-IV, and B-48. Apolipoprotein B-48 is unique to the enterocyte and is essential for chylomicron release by the intestinal cell. A-1 is synthesized in the liver. Apo B-48 is actually an edited version of the hepatic apo B-100. It is the result of an apo B mRNA editing process that converts codon 2153 to a translational stop codon. Apo B-48 is, thus, an edited form of apo B-100, and this editing is unique to the intestinal cell. Failure to appropriately edit the apo B 100 gene in the intestinal cell will result in a disorder called *familial hypobetalipoproteinemia*. In this disorder, there will be a total or partial (depending on the genetic mutation) absence of lipoproteins in the blood. Hypobetalipoproteinemia can also develop should there be base substitutions in the gene for the apo B 100 protein. In addition to very low blood lipids, patients with this disorder also have fat malabsorption (steatorrhea). The feces contain an abnormally large amount of fat and have a characteristic peculiar odor. In this disorder, not only is the triacylglyceride absorption affected but so too are the fat-soluble vitamins. Without the ability to absorb these energy-rich food components and the vital fat-soluble vitamins, the patient does not thrive and survive. Fortunately, this genetic mutation is not very common. It is inherited as an autosomal recessive trait.

The particles of absorbed lipid and transport proteins are called *chylomicrons*. They are present in the blood of feeding animals but are usually absent in starving animals. As the chylomicrons circulate, they acquire an additional protein, apo C-II. This additional protein is an essential cofactor for the recognition and hydrolysis of the chylomicron by the capillary endothelial enzyme, lipoprotein lipase (LPL). The LPL hydrolyzes most of the core triglycerides in the chylomicron leaving a remnant that is rich in cholesterol and cholesterol esters. During the LPL catalyzed hydrolytic process, the excess surface compounds (i.e., the phospholipids and apolipoproteins B, A-I, and A-IV) are transferred to high-density lipoproteins and, in exchange, apo-E is transferred from the HDL to the cholesterol ester-rich chylomicron remnant. These remnants are then cleared from the blood by the liver. On the hepatocyte is a lipoprotein receptor that recognizes Apo-E, and this receptor plays an important role in remnant clearance. Figure 8.3 illustrates the process of exogenous lipid absorption, transport, and clearance.

The chylomicron is a relatively stable way of ensuring the movement, in an aqueous medium (blood), of hydrophobic molecules such as cholesterol and triacylglycerols from their point of origin, the intestine, to their point of use or storage. As mentioned, there are several unique proteins that facilitate this movement. These lipid-transporting proteins determine which cells of the body receive which lipids. At the target cell, the particles lose their lipid through hydrolysis facilitated by an interstitial lipoprotein lipase, which is found in the capillary beds of muscle, fat cells, and other

TABLE 8.5
Proteins involved in lipid transport

Protein	Function
apo A-II	Transport protein in HDL
apo B-48	Transport protein for chylomicrons; synthesized in the enterocyte in the human
apo B-100	Ligand for LDL receptor; synthesized in the liver and secreted into the circulation as part of the VLDL
High density lipoprotein binding protein (HDLBP)	Binds HDl and functions in the removal of excess cellular cholesterol
apo D	Transport protein similar to retinol-binding protein
apo (a)	Abnormal transport protein for LDL
apo A-I	Transport protein for chylomicrons and HDL; synthesized in the liver and its synthesis is induced by retinoic acid; antiatherogenic
apo C-III	Transport protein for VLDL; impairs TAG hydrolysis by inhibiting LPL
apo A-IV	Transport protein for chylomicrons; antiatherogenic
apo A-V	Reduces plasma TG by lowering VLDL production and enhancing lipolysis
CETP	Participates in the transport of cholesterol from peripheral tissue to liver; reduces HDL size
LCAT	Synthesized in the liver and is secreted into the plasma where it resides on the HDL. Participates in the reverse transport of cholesterol from peripheral tissues to the liver; esterifies the HDL cholesterol
apo E	Mediates high-affinity-binding of LDLs to LDL receptor and the putative chylomicron receptor. Required for clearance of chylomicron remnant. Synthesized primarily in the liver
apo C-I	Blocks apo E binding to receptors; transport protein for VLDL
apo C-II	Chylomicron transport protein required cofactor for LPL activity
apo H	Antiatherogenic; ligand for LDL receptor
Lipoprotein lipase	Catalyzes the hydrolysis of plasma triglycerides into free fatty acids
Hepatic lipase	Catalyzes the hydrolysis of triglycerides and phospholipids of the LDL and HDL. It is bound to the surfaces of both hepatic and non hepatic tissues
Diacylglycerol acyl transferase (DGAT 1 and 2)	Acyl transferase required for synthesis of TAG molecule
Lecithin-cholesterol acyl transferase	Catalyzes the esterification of free cholesterol to cholesterol ester on the lipoproteins
AcylCoA:cholesterol acyl transferase	Catalyzes cholesterol esterification

Source: Feldman, E.B. and Cooper, G.R. (2007). In *Handbook of Nutrition and Food* (Berdanier, C.D., Dwyer, J., and Feldman, E.B. Eds.). CRC Press, Boca Raton, FL. pp. 683–692.

tissues using lipid as a fuel. Lipoprotein lipase is synthesized by these target cells but is anchored on the outside of the cells by a polysaccharide chain on the endothelial wall of the surrounding capillaries. Should this lipoprotein lipase be missing or genetically aberrant (Type I lipemia or chylomicronemia) so that the chylomicrons cannot be hydrolyzed, these chylomicrons accumulate and the individual develops a lipemia characterized by elevated levels of triacylglyceride and cholesterol-containing chylomicons. Also characteristic of this condition is considerable abdominal discomfort, the presence of an enlarged liver and spleen, and of subcutaneous xanthomas (clusters of hard, saturated fatty acid and cholesterol-rich nodules). Like familial hypolipoproteinemia, this condition is

TABLE 8.6
Characteristics of various lipoproteins

			% Lipid as:		
Fraction	**% Protein**	**Density**	**Triacylglycerol**	**Phospholipid**	**Cholesterol**
Chylomicrons	1.5–2.5	<0.95	84–89	7–9	1–5
VLDL	5–10	0.95–1.006	50–65	15–20	5–15
LDL[a]	20–25	1.006–1.019	7–10	15–20	35–401
LDL[b]	20–25	1.019–1.063	7–10	15–20	7–102
HDL	40–55	1.068–1.210	5	20–35	4–12

[a] Primarily esterified cholesterol.
[b] Primarily unesterified cholesterol.

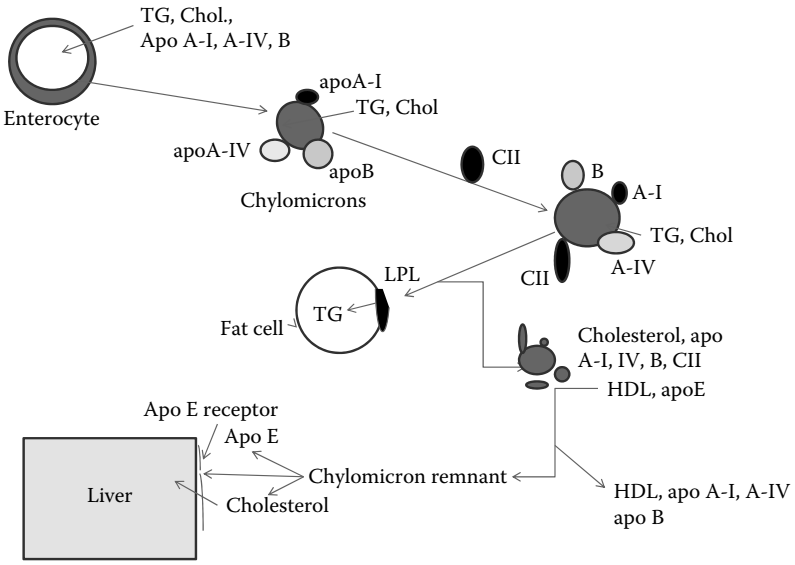


FIGURE 8.3 Schematic representation of the uptake, transport, and disposal of dietary lipids. TG: triacylglycerides; CHOL: cholesterol and cholesterol esters; apo A-I, apo B, Apo E: polypeptide lipid carriers; HDL: high-density lipoprotein.

rare. Of interest is the observation that, despite the very high blood lipid levels of these people, few die of coronary-vessel disease. Their shortened life span is due to an inappropriate lipid deposition in all of the vital organs which, in turn, has a negative effect on organ function and life span.

Endogenous Lipid Transport

Fatty acids, TG, cholesterol, cholesterol esters, and phospholipids are synthesized in the body and are transported from sites of synthesis to sites of use and storage. Although the transport of these lipids is, in many instances, similar to that of the dietary lipids, there are differences in the processing and in some of the proteins involved. Endogenous fat transport (Figure 8.4) involves the production and secretion of very-low-density lipoproteins (VLDL) by the liver. These lipid–protein complexes are rich in TG but also contain cholesterol. The polypeptides that transport these lipids

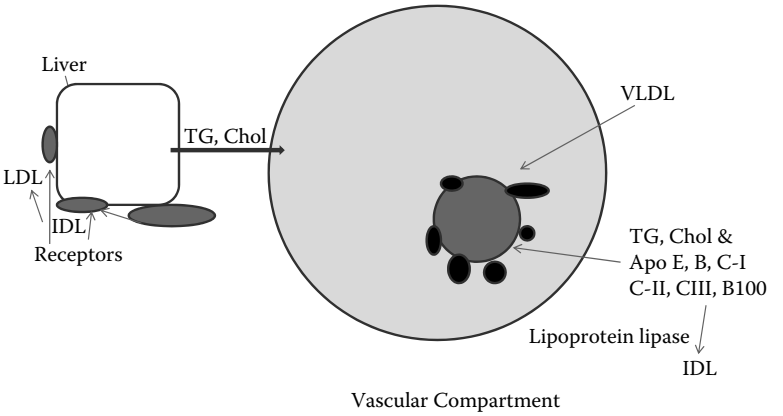


FIGURE 8.4 Lipid recycling: endogenous lipid transport and disposal. Very-low-density lipoproteins (VLDL) are secreted by the liver and circulated back to it, where various receptors (shown as a half-moon on the cell surface) bind and removes the lipid in a stepwise sequence. TG: triacylglycerides; CHOL: cholesterol and cholesterol esters; apo A E, apo B, apo B-100, apo C-III, apo C-II, apo C-I: polypeptide lipid carriers; IDL: intermediate-density lipoproteins; LDL: low-density lipoproteins.

make up approximately 10% of the weight of the VLDL. They include the polypeptides apo-B, B-100, apo C-I, apo C-II, apo C-III, and apo E. As mentioned, several of these polypeptides are also involved in exogenous lipid transport. Once the VLDLs are released by the hepatocyte, they are hydrolyzed by the interstitial lipoprotein lipase, and intermediate-density lipoproteins (IDL) are formed. These are cleared from the circulation as they are recognized and bound to hepatic IDL receptors. The hepatic receptors recognize the apo E that is part of the IDL. Any of the IDL that escapes hydrolysis at this step is available for hydrolysis by the hepatic lipoprotein lipase. This hydrolysis leaves a cholesterol-rich particle of low density lipoprotein (LDL). The LDL has apo B-100 as its polypeptide carrier and both hepatic and extrahepatic cells have receptors that recognize this polypeptide. Normally, about 70% of LDL is cleared by the LDL receptors, and most of this is cleared by the liver. The endogenous fat transport and disposal system is diagrammed in Figure 8.4. From the foregoing, it is apparent that considerable lipid recycling occurs in the liver. The VLDLs originate in the liver, which is the primary site for LDL disposal. Other organs and tissues also participate in disposal, but their participation is minor compared with that of the liver.

Genetic Basis for Lipoproteinemia

As mentioned, there are a number of proteins involved in the uptake, synthesis, and disposal of lipid. The genes for the transport proteins, apo A-I, apo A-II, apo A-IV, apo (a), apo B, apo D, apo C-I, apo C-II, apo C-III, apo D, and apo E have been identified, as have mutations that result in lipid transport abnormalities (Table 8.7). In addition to these transport proteins, we have the proteins that are the important rate-limiting enzymes and receptors that are involved in lipoprotein processing; the peripheral lipoprotein lipase and the hepatic lipoprotein lipase; the lipoprotein receptors on the plasma membrane of the cells that receive and oxidize or store the transported lipids; and the rate-limiting enzymes of lipid synthesis and use.[9] Included in this last category are the fatty acid synthase complex, acetyl CoA carboxylase, HMG CoA reductase, HMG CoA synthase, cholesterol ester transfer protein (CETP), fatty acid-binding protein (FABP), lecithin-cholesterol acyltransferase (LCAT), cholesterol 7-hydroxylase, and the high-density lipoprotein-binding protein (HDLBP). Many of these proteins have been isolated and studied in detail. Several of their cognate genes have been identified and mapped.[13] Listed in Table 8.7 are the chromosomal locations and mutation

TABLE 8.7
Location of genes involved in lipoprotein metabolism

Gene	Chromosome location	Characteristics of mutation	Frequency of mutation
apo A-II	1	Transport protein in HDL	?
apo B 48	2 p 23–24	Hypobetalipoproteinemia	1:1,000,000
High-density lipoprotein-binding protein (HDLBP)	2 q 37	—	?
apo D	3	Transport protein similar to retinol-binding protein	?
apo (a)	6	Abnormal transport protein for LDL	?
LPL	8 p 22	Defective chylomicron clearance	1:1,000,00040 known mutations
apo A-I	11	Defective HDL production (Tangier's disease)	1:1,000,000
apo C-III	11	—	?
apo A-IV	11	—	?
Hepatic LPL (HTGL)	15 q 21	Defective IDL clearance	?
CETP	16 q 22.1	—	?
LCAT	16 q 22.1	Familial lecithin:cholesterol transferase deficiency. 2 Types	Rare
LDL receptor	19	Familial hypercholesterolemia	1:500
apo B 100	2	Familial defective apo B 100	1:500-1:10003500 mutations
apo E	19	Type III hyperlipoproteinemia	1:5000
apo C-I	19	Transport protein for VLDL	?
apo C-II	19	Defective chylomicron clearance	1:1,000,000

frequencies of these genes. With this many genes involved in the uptake, synthesis, transport, and degradation of the circulating lipids, it is not surprising to find mutations that phenotype as either lipemia or fat malabsorption. Not all of these mutations in the gene products needed for lipid transport are associated with atherosclerosis.

Defects in exogenous fat transport are manifested in several ways. As described, defective chylomicron formation due to mutations in either the apo B gene or its editing leads to and is characterized by fat malabsorption. This includes the malabsorption of fat-soluble vitamins as well. Many different mutations have been identified in the gene for the apo B protein.[14–20] These mutations are inherited via an autosomal recessive mode. Those that affect fat absorption are characterized not only by fat malabsorption, but also by acanthocytes, retinitis pigmentosa, and muscular neuropathies. Some of the symptoms can be attributed to a relative deficiency of the fat-soluble vitamins because the absorption of fat-soluble vitamins is dependent on normal fat absorption. Some of the apo B mutations phenotype as lipemia where there is a defective binding to the LDL receptor. Approximately 400 mutations have been reported in five classes of the LDL gene, and these mutations phenotype as familial hypercholesterolemia. Although a defect in the apo B gene can account for defective fat absorption in some cases, there may be another mutation in the microsomal triglyceride transfer protein that also results in fat malabsorption.[21,22] This transfer protein is essential for apo B translocation and subsequent synthesis of chylomicrons. Defects in this transfer protein would impair apo B availability and chylomicron formation. In these defects, very low levels of

chylomicrons are found in the blood. Persons with this disorder are rare (one in a million). In this circumstance, the severity of the disease is related to the size of the mutated gene product and whether it can associate with the lipids it must carry. The size of the truncated apo B can vary from apo B-9 (41 residues) to apo B-89 (4487 residues). Except in the case of the apo B-25, the result of a deletion of the entire exon 21, all the truncated forms reported to date are C-T transitions or base deletions.[21] These deletions can involve misaligned pairing deletion mechanisms.[19] Frameshift mutation can be compensated by a reading frame restoration of the apo B gene.[20]

Familial hyperchylomicronemia is characterized by elevations in chylomicrons having both triglycerides and cholesterol. Hyperchylomicronemia was found to be due to mutations in the genes that encode the enzyme lipoprotein lipase needed for the hydrolysis and clearance of the chylomicrons from the blood.[23] This enzyme is a glycoprotein having an apparent monomeric molecular weight of about 60,000 daltons on SDS gel electrophoresis and 48,300 daltons by sedimentation-equilibrium ultracentrifugation. The enzyme is linked to the endothelial cells of the capillary system. Lipoprotein lipase is quite similar to hepatic triglyceride lipase, an enzyme found in the hepatic sinusoids. The main difference between the two lipases is that the interstitial lipase has a requirement for the lipid-carrying protein, apo C-II, for full activity whereas hepatic LPL does not. Mutations in the gene for apo C-II can result in aberrant lipase activity because apo C-II serves as a cofactor in the lipoprotein lipase-catalyzed reaction.[24] Hepatic triglyceride lipase has no such requirement. Aberrations in the hepatic triglyceride lipase result in an accumulation of VLDL rather than accumulations of chylomicrons. Mutations in the genes for LPL and apo C-II are very rare, occurring at a frequency of one in a million. The gene for LPL has been mapped to chromosome 8, whereas that for apo C-II has been mapped to chromosome 19, and the hepatic LPL to chromosome 15.[25–30] Other features of these disorders include an inflammation of the exocrine pancreas and eruptive xanthomas. Chylomicronemia does not appear to be atherogenic. The mutations in the LPL gene appear to be insertions or deletions or due to aberrant splicing, whereas those in the apo C-II gene seem to be due to splice site mutations or small deletions. Twenty-two mutations in the apo C-II gene have been reported. With respect to the aberrant splicing of the LPL gene in three unrelated humans, Holzl et al.[31] reported a C→A mutation in position 3 of the acceptor splice site of intron 6, which caused aberrant splicing. The major transcript showed a deletion of exons 6 through 9 and amounted to about 3% of normal. Trace amounts of both a normally spliced LPL mRNA and a second aberrant transcript devoid of exon 7 were found. In one of these patients, Holzl et al.[31] found a 3′ splice mutation on one allele whereas on the other allele they found a missense mutation resulting in Gly 188→ Glu substitution. All three subjects were classed as hyperchylomicronemic due to LPL deficiency.

The absence of LPL activity in certain tissues or in certain individuals can be attributed to mutations in lipoprotein lipase promoter region. Studies of tissue-specific expression of LPL in a variety of murine tissues by Gimble et al.[11] showed that *cis*-acting elements located within the −1824-bp of the 5′ flanking region were required for the expression of LPL. These include nuclear factors recognizing both the CCAAT box and the octamer sequence immediately flanking the transcriptional start site. Those tissues that have no LPL activity lack this promoter region. Because humans and mice have identical CCAAT and octamer sequences, one could suppose that humans having an intact LPL gene of normal sequence but lacking LPL activity might have a deficient or mutated promoter region. Although such has been suggested by Sparkes et al.,[25] proof that this might occur is presently lacking.

Mutations in the gene for hepatic lipoprotein lipase result in elevated blood levels of triglycerides and cholesterol, and these elevations are related to an increased risk for atherosclerosis. Hepatic LPL must be secreted by the hepatocyte into the sinusoids to function as a catalyst for the hydrolysis of core TG and surface phospholipids of chylomicron remnants, HDL, and IDL. Through its activity, it augments the uptake of HDL cholesterol by the liver (reverse cholesterol transport) and is involved in the reduction of HDL size from HDL2 to HDL3. Hepatic LPL aids in the clearance of chylomicron remnants by exposing the apo E epitopes for enzymatic action. Missense mutations in the hepatic LPL gene include substitutions of serine for phenylalanine at amino acid position 267,

threonine for methionine at position 383, and asparagine for serine at position 291.[31,32] These mutations result in poorly secreted enzyme and thus the phenotypic expression of the mutation is low hepatic lipase activity. The frequency of the Asn 291 Ser mutation in a population having premature CVD has been reported as 5.2%.

Defects in chylomicron remnant clearance are much more common than any of the mutations discussed.[33–35] Defective clearance due to mutations in the apo E gene results in a lipemia known as Type III hyperlipoproteinemia. It is associated with premature atherosclerosis, and patients with these defects have high serum triglyceride levels as well as high serum cholesterol levels. Xanthomas are found in nearly three-quarters of the population with these defects. The lipemia is responsive to energy restriction using diets that have 40% of energy from carbohydrate, 40% from fat, and 20% from protein. Weight loss is efficacious for most people with this defect. The apo E gene codes for the protein on the surface of the chylomicron remnant that is the ligand for receptor-mediated clearance of this particle.

A number of mutations in the apo E gene have been reported, and the phenotypes of these mutations are grouped into three general groups labeled E2, E3, and E4.[40] Those of the E2 groups fail to bind the particles to the cell surface receptor for the chylomicron remnant. Those of the E3 and E4 groups have generally low remnant clearance rates. The apo E allele and phenotype frequency varies. The E2 frequency is about 8%, the E3 about 77%, and the E4 about 15% of the total population with an apo E mutation. The incidence of apo E gene mutations is about 1% of the population. Because apo E is involved in both endogenous and exogenous lipid transport and clearance, a faulty apo E gene is devastating. Mature human apo E is a 299 amino acid polypeptide. Apo E, as well as other apolipoproteins, contains 11 or 22 amino acid repeated sequences as one of their key features. These appear to encode largely amphipathic helices, which are needed for lipid binding. There is a high degree of conservation among species of nucleotide sequences in the gene fragment that encodes the amino acid repeats. The gene for apo E has been mapped to chromosome 19, as have the genes for apo C-I, apo C-II, and LDLR. There appears to be a tight linkage among these genes that coordinates their expression. Among the common mutations are amino acid substitutions at positions 112 and 158, and less-frequent substitutions occur at other positions in the polypeptide chain. Several of these involve the exchange of neutral amino acids for acidic amino acids with the net result of alterations in polypeptide charge and subsequent inadequate binding to the appropriate cell surface receptors.

In a rare form of the disorder, the mutation is such that no useful apo E is formed. Transgenic mice have been constructed with an apo E mutation that mimics apo E deficiency in humans.[36–39] These mice, like humans, develop hypercholesterolemia and increased susceptibility to atherosclerosis. When these mice were fed low- (5%) or high-fat (16%) diets, a differential serum cholesterol pattern was observed: those fed the high-fat diet had significantly higher levels of cholesterol and VLDL and LDL than those fed a low-fat or stock diet. The transgenic mice, even when fed the stock diet, had significantly higher levels of cholesterol and VLDL and LDL than the normal control mice. There was a gender difference as well. Male transgenic mice were less diet responsive in terms of their cholesterol levels than female transgenic mice. As mentioned, there is some sequence homology between humans and mice in the apo E DNA and it could be inferred that these responses to dietary fat intake in mice could be observed in humans as well.

Although the above transgenic approach used the gene-knockout paradigm (an extreme in the variants of apo E mutants), it nonetheless suggests that variation in the apo E genes could determine the responsiveness of humans with apo E defects to dietary manipulation. Indeed, such nutrient–gene interactions have been reported.[14,38–41] The dietary fat clearance in normal subjects appears to be regulated by the genetic variance in apo E sequence and this, in turn, is related to fat intake. Not only is triacylglycerol clearance affected, but so too is cholesterol clearance. One study reported that the apo E genotype declares the response to cholesterol intake with respect to blood cholesterol levels, and that this genotype influences cholesterol synthesis. Those subjects who respond poorly to an oral cholesterol challenge vis-à-vis blood cholesterol clearance had higher rates of cholesterol

synthesis than those who could rapidly clear their blood of cholesterol after an oral challenge. One of the more interesting variants of the apo E is called the *Milano variant*. In this variant, blood lipid levels are elevated, but these elevations are not associated with an increased risk of atherosclerosis.

Cholesterol traffic is also controlled by the LDL receptor and the transport protein apo B-10. Mutations in the gene for the LDL receptor or in the gene for apo B-100, the ligand for the LDL receptor results in high serum levels of cholesterol. The former results in the disorder called familial hypercholesterolemia and occur with a frequency of about one in 500. Familial hypercholesterolemia is associated with early death from atherosclerosis in man and related primates. Dietary fat saturation affects transcription of LDL receptor mRNA, in that feeding a diet containing saturated fat results in decreased LDL receptor mRNA, compared with feeding an unsaturated-fat diet.[42] These results suggest that unsaturated fatty acids may interact with proteins that in turn serve as either *cis*- or *trans*-acting elements for this gene in much the same way as polyunsaturated fatty acids affect fatty acid synthetase gene expression.[43]

Familial defective apo B-100 hypercholesterolemia is due to a mutation in the coding sequence of the apo B gene at bp 3500 that changes the base sequence such that glutamine is substituted for arginine. This is in the LDL receptor-binding region of the apo B protein and results in a binding affinity of less than 4% of normal. Polymorphic variation in the genes for both the LDL receptor and the apo B-100 have been reported for mice, and this variation has provided the opportunity to identify the genetic and molecular constraints of lipoprotein gene expression.

Both apo B and apo E serve as ligands for the LDL receptor. In contrast to apo E, apo B has little homology with the other apolipoproteins. Apo B in mice is quite variable, and this variation imparts or confers a diet-responsive characteristic in inbred mouse strains vis-à-vis polypeptide sequence and activity. That is, some mouse strains have reduced levels of plasma apo B when fed a high-fat diet, compared with controls fed a stock diet, whereas other mouse strains are unresponsive to diet vis à vis their plasma apo B-100 levels.

Such polymorphism also exists in humans. Apo B has been mapped to chromosome 2 and produces two gene products, apo B-100 and B-48. In the intestine, the apo B primary transcript is co- or posttranscriptionally modified. This modification converts codon 2153 from a glutamine (CAA) to an in-frame, premature termination codon (UAA), thereby causing translation to terminate after amino acid 2152. This mRNA editing thus explains the difference in size of these two proteins. If more of the apo B gene is deleted, hypocholesterolemia is observed. This is because apo B-48 is required for the transport of the chylomicrons from the intestine. If lacking, chylomicron formation is impaired and low serum cholesterol levels are observed. Both familial defective apo B-100 and familial hypercholesterolemia are characterized by high levels of LDL. Both are associated with CVD, but only the familial hypercholesterolemia is characterized by tendon xanthomas. Both are inherited as autosomal dominant disorders. Collectively, these mutations have a cumulative frequency of 1 in 250. However, because polymorphism in the apo B gene can and does occur, there is the possibility that, collectively, the frequency is much greater than this. Perhaps it is as high as one in five. If this is the case, then the population variation in plasma cholesterol levels could be explained on the basis of these genetic differences alone, apart from those mutations that are associated with the rest of the genes that encode components of the lipid transport system.

Genetically determined abnormalities in LDL metabolism may also be due to mutation in the large glycoprotein, apo (a). Apo (a) is a highly variable disulfide protein bonded to the apo B-100. It is thought to resemble plasminogen. In fact, the genes that encode Lp(a) and plasminogen are very close to each other on the long arm of chromosome 6. In general, LDLs containing apo (a) do not bind well to the LPL receptor, and people having significant amounts of apo (a) have a two- to threefold increase in CVD risk. Many individuals have little or no apo (a), and it has been suggested that those who have it are abnormal with respect to LPL activity.

Several mutations in the genes that encode the endogenous lipid transport have been reported (Skretting et al., 1992; Kushwaha et al., 1994). The reverse cholesterol transport pathway is part of this endogenous lipid-transport system. It involves the movement of cholesterol from peripheral

tissues to the liver. The peripheral tissues cannot oxidize cholesterol and so must send it to the liver, where it is prepared for excretion, via cholesterol 7 α hydroxylase, as bile acids. This pathway uses the HDL to shuttle the cholesterol in this direction. HDL consists primarily of apo A-1 and cholesterol, which is esterified by the enzyme lecithin:cholesterol acyl transferase (LCAT). Mutations in the LCAT gene have been reported and result in one of two diseases: familial LCAT deficiency and fish-eye disease. Thirteen different mutations have been identified and in one, a single T→A transversion in codon 252 in exon 6, converting met (ATG) to Lys (AAG), was observed. Three unrelated families were found to have this mutation; however, the severity of their disease varied. In these families, no other mutation in LCAT was observed. Of the remaining 12 LCAT mutations, ten were point mutations, three were frameshifts, and one consisted of a three-base insertion that maintained its reading frame. For fish-eye disease, three mutations have been reported. This disorder is less serious than familial LCAT deficiency and is characterized by dyslipoproteinemia and corneal opacity. LCAT activity is 15% of normal, and there is a reduced level of HDL in the plasma. In contrast, familial LCAT deficiency is characterized by a variety of symptoms, including lipoprotein abnormalities, renal failure, premature atherosclerosis, reduced levels of plasma cholesterol esters, and high plasma levels of cholesterol and lecithin.

The LCAT enzyme requires apo A-I as a cofactor. If there is a mutation in the gene for apo A-I, defective HDL production results. This is a rare mutation and its frequency is estimated as one in a million. Individuals with this defect have premature CVD, corneal clouding, and very low HDL levels. Plasma apo A-I has a variety of charge isoforms with similar antigenicity and amino acid composition. Humans, baboons, African green monkeys, and cynomolgus monkeys have been studied, and there are species differences in hepatic and intestinal apo A-I production. In all instances, the differences in apo A-I were reported between intestine and liver. In the liver, there was a twofold higher level of apo A-I mRNA than in the intestine, and the abundance of this mRNA was species specific. The apo A-I gene is regulated at the level of transcription and a portion of the species-specific difference in apo A-I gene expression is due to a sequence divergence in the 5′ regulatory region, including the exon/intron 1 of the apo A-I gene. The capacity to produce HDLs is both genetically controlled and tissue specific, and probably explains why some genotypes respond normally to a high-fat diet by producing more HDL, whereas other genotypes become hypercholesterolemic under these same conditions. Attempts to create a transgenic mouse expressing a human apo A-I gene have not been fully successful, but have provided additional information about the relationship of apo A-I to HDL size. The human apo A-I gene was inserted into the mouse, and in these mice, both the mouse and the human genes were expressed. This dual expression suggests a species difference in the control of this expression. In other words, the control points differed, and this resulted in a broader spectrum of HDL particles.

Defective HDL metabolism due to a mutation in the apo A-I gene results in a rare autosomal-dominant disorder described in a small group of villagers in Italy. Affected individuals have reduced levels of HDL cholesterol and apo A-I levels, but have no increased risk of CVD. The disorder, named Apo A-I Milano is due to a point mutation in the apo A-I gene, changing codon 173 so that cysteine is used instead of arginine. Normal apo A-I has no cysteine, so this change has an effect on the apo A-I structure.

Defective lipoprotein processing has already been discussed with respect to LDP, LCAP, and apo C-II deficiencies. A deficient cholesterol ester transport protein (CETP) has been reported due to a mutation of the gene for this protein located on chromosome 16. A mutation in this gene has been used to explain the atherogenicity of high-fat diets in primates, but, to date, no evidence of such a mutation in humans has been put forward.

The majority of the cholesterol in the circulation is synthesized de novo by the body. This synthesis can be suppressed pharmacologically. The statin drugs are very effective cholesterol-lowering agents because they specifically target the HMG CoA reductase enzyme inhibiting it. The fibrate drugs do about the same but are less effective. There are a few people (one in 20,000) who cannot make cholesterol. They have a genetic disorder known as the Smith-Lemli-Opitz syndrome,

abbreviated as SLO disease.[44,45] This disease is sometimes called the RSH syndrome. It is an autosomal recessive disorder characterized by an accumulation of 7-dehydrocholesterol due to a mutation in the gene that encodes Δ7 cholesterol reductase. This enzyme catalyzes an intermediate step in the synthesis of cholesterol. This metabolite accumulates in many tissues, especially the eye. In doing so, it causes cataracts to form. Children with this disorder fail to grow and develop normally (failure to thrive) because cholesterol is a vital ingredient to so many important biological structures. This is especially true with respect to the cell membranes and the lipids in the central nervous system. Some investigators have tried to provide dietary supplements of cholesterol to these children in an effort to improve their development; however, another feature of these children is their great number of food intolerances. Many are intolerant of cholesterol-rich foods such as eggs.

Nutrient–Gene Interactions in Lipid Transport

The previously described observations of diet and genetic factors suggest that CVD could develop as a result of a nutrient–gene interaction. There are a number of genes involved in the regulation of blood lipid levels. Further, the diet influence involves not only the amount of fat consumed but also the type of fat and the amount and type of carbohydrate.[14,41] For example, the editing of the apo B gene is enhanced by dietary carbohydrate. A number of genes have carbohydrate-response elements, and it is possible that this gene has this element in its promoter region. A carbohydrate response element has been identified in the apo E gene. Carbohydrate influences mRNA stability and RNA processing of this gene. Similarly, the gene that encodes the seven-enzyme complex of mammalian fatty acid synthetase has a fatty acid response unit in its promoter. The expression of this gene is downregulated by dietary polyunsaturated fatty acids. The lipid transport genes might also have a lipid-response element.

Overtranscription of the fatty acid synthase gene (which has a lipid response element) has been shown to occur in genetically obese Zucker rats. Mouse apo A-IV gene expression in C57BL/6 mice, which were shown by Paigen et al.[39] to develop atherosclerosis, is induced by high-fat feeding. In normal mice, this does not occur. The apo A-IV gene consists of three exons and two introns. The introns separate the evolutionarily conserved and functional polypeptide domains. Intron 1 divides most of the apo A-IV signal peptide from the amino terminus of the mature plasma protein. The second intron separates a highly conserved variant amphipathic peptide repeat from the remainder of the mature apo A-IV protein. The 5′ flanking region has several features: variant TATA- and CAT-box sequences (TTTAAA and CCAACG); five G-rich direct repeats of 10 nucleotides and a short inverted repeat. The variants found in this region may determine whether dietary fat can induce apo A-IV gene expression. Some may constitute a lipid-response element, whereas other sequence variants may not be lipid responsive. Further studies showed that the genetic control of apo A-IV mRNA levels involves both *cis*-acting elements and genetically distinct trans-acting factors. The *cis*- acting elements (nucleic acid sequences) and the trans-acting factors (specific proteins) determine both the rate of mRNA transcription and its turnover. Transcription requires the recognition of promoter sequences by protein transcription factors. These factors may bind lipid and be part of the lipid response unit or the lipid response unit may be entirely separate. This has not been determined, merely speculated.

Dietary lipids, even in the absence of direct effects on transcription and translation, influence the phenotypic expression of specific genotypes either because of overconsumption or because they have effects on certain of the hormones, that is, insulin or the steroid hormones, or the catabolic hormones that regulate or influence lipid synthesis, oxidation, and storage. For example, Chen et al.[10] showed that, in diabetic mice, insulin regulated the transcription of the apo C-III gene and that this regulation in turn affected serum triglyceride levels. Triglyceride levels fell in the insulin-treated diabetic mice subsequent to an insulin-induced increase in the apo C-III mRNA level. In turn, Chen et al. showed (in mice) that this mRNA increase was due to a specific effect of insulin on apo C-III gene transcription.

The level of cholesterol in the blood depends on the diet consumed and how much cholesterol is being synthesized. The cholesterol content of the gut LDL of a person on a low-cholesterol diet might run as low as 7–10% of the total lipid in the lipoprotein, whereas the hepatic LPL of this same individual might be as high as 58% of total lipid. People consuming a low-cholesterol, low-saturated-fat diet may reduce the contribution of the diet to the blood cholesterol while increasing the hepatic de novo cholesterol synthesis. Persons having an LPL receptor deficiency are characterized by high serum cholesterol levels and, in some cases, by high serum triacylglycerides. The reason these blood lipids are elevated is that the individuals cannot utilize the lipids carried by the LPL due to the errors in the receptor molecule. Further, because these circulating lipids do not enter into the adipose and hepatic cell in normal amounts, the synthesis of triacylglycerides and cholesterol is not appropriately downregulated. Hence, this individual has elevated serum lipids not only because the LPL lipid is not appropriately cleared from the blood, but also because of high rates of endogenous lipid synthesis. Individuals with this disorder have lipid deposits in unusual places such as immediately under the skin, around the eyes, on the tendons, and in the vascular tree. It is this last feature that probably accounts for the shortened life span of these people, with the cause of death being cardiovascular disease (CVD). As can be seen from the metabolic characteristics of this disorder, low-cholesterol diets are probably useless in reducing serum cholesterol levels because de novo synthesis of cholesterol from nonlipid precursors can and does occur.[46,47] Treatment with lipid adsorbents (high-fiber diets, and drugs such as cholestyramine that block lipid absorption) will help reduce the cholesterol (but not triacylglycerides) coming from the intestine, and there are drugs (the statins) that can safely lower de novo cholesterol synthesis as well as increase intracellular lipid oxidation. All of these therapies may help reduce the serum lipid levels, but even doing this only treats the symptoms, not the genetic disorder. Gene therapy is needed to correct the genetic disorder that is the basic underlying cause of the symptoms.

Heart disease in its various forms is also associated with elevated levels of the VLDL. A specific genetic error has not been identified; however, the disorders have been subdivided into three general categories. In one, Type III lipemia, the patients are characterized by elevated serum cholesterol, phospholipid, and triacylglyceride levels, elevated VLDL levels (and sometimes LDL levels), fatty deposits on the tendons and in areas on the arms just under the skin, vascular atheromas, and ischemic heart disease. This type of lipemia is inherited as an autosomal dominant trait in one person in 5000. Another lipemia (Type IV) having a normal cholesterol level but an elevated triacylglyceride level and elevated VLDL levels is also associated with ischemic heart disease and premature atherosclerosis. It is frequently seen in obese patients with Type 2 diabetes. People with diabetes mellitus have five to six times the risk of normal people of developing premature atherosclerosis and its associated coronary events. A combination of CVD that is followed by renal disease is the leading cause of death for people with diabetes mellitus.

Those people with Type 1 diabetes mellitus are more likely to develop a lipemia (Type V lipemia) that is slightly different from the aforementioned Type 2 diabetes-related lipemia. Whereas the incidence of both is two in 1000, those with the latter problem inherited their trait in an autosomal recessive manner, and those with the Type 2-related lipemia inherited their trait as an autosomal dominant trait. Elevated chylomicron and VLDL levels and reduced dietary fat tolerance characterize this type of lipemia. Patients with this disorder are usually of normal body weight.

FUNCTION

Once the dietary lipids are transferred to the target tissues, one of several processes occurs. The fatty acids of the triacylglycerols are oxidized for energy, stored as energy in the form of resynthesized triacylglycerols, converted to eicosanoids or are incorporated into the membranes within and around the cells. An overview of these processes is shown in Figure 8.5. Certain of the fatty acids, the essential fatty acids, linoleic and linolenic, have a special role as precursors for the 20-carbon compounds called *eicosanoids*. Diet can affect all of these processes and, as well, stimulate lipid

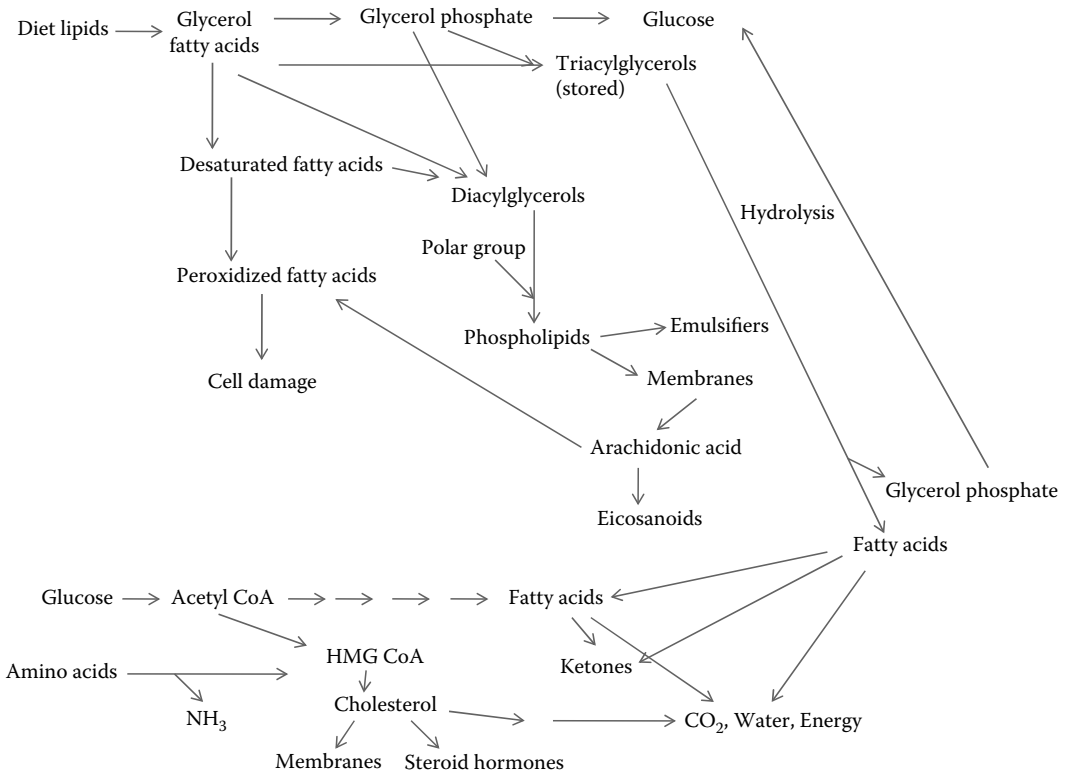


FIGURE 8.5 Overview of lipid metabolism.

synthesis. Chapter 3 reviews fatty acid and cholesterol synthesis, fatty acid interconversion, fatty acid and cholesterol catabolism, and the synthesis of the phospholipids that are incorporated into the membranes within and around the cells. In this section, the essentiality of certain of the fatty acids, the synthesis of eicosanoids from the 20-carbon fatty acids, the auto oxidation of fatty acids and free-radical formation, and the roles these two processes have in the inflammatory response are described. Membrane function as a process involving lipid is also addressed.

Essential Fatty Acids

Essential fatty acids are deemed essential because the body cannot synthesize them. They are important precursors to longer fatty acids, especially arachidonic acid that, in turn, serves as precursor to the eicosanoids. They are also important components of membrane phospholipids contributing to the maintenance of appropriate fluidity. Mammals except felines can synthesize arachidonic acid from linoleic acid. Because felines cannot do this, arachidonic acid is also an essential fatty acid that must be provided in the diet. The essential fatty acids are shown in Figure 8.6. Although linolenic and linolenic are the stipulated essential fatty acids, γ-linolenic is included in Figure 8.6 because it can be found in certain plant oils such as primrose oil.

Animals fed diets deficient in these essential fatty acids exhibit a variety of symptoms as listed in Table 8.8. Detailed studies of cells from deficient rats have revealed diet-induced changes in energetic efficiency that include a partial loss of the ability of mitochondria to trap energy in the high-energy bond of ATP. Myocardial arrhythmias are more frequent in deficient rats, and this may be due to the deficiency symptom of dysfunctional mitochondria. Essential fatty acid deficiency also results in an impairment in the glucose transporter activity in selected cells and an alteration

Linoleic acid [18:2 (9, 12)]

$CH_3(CH_2)_3CH{=}CH\ CH_2CH{=}CH(CH_2)_7COOH$

18 12 9

γLinolenic acid [18:3 (6, 9, 12)]

$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_4COOH$

18 12 9 6

Linolenic acid [18:3 (9, 12, 15)]

$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

18 15 12 9

FIGURE 8.6 Essential fatty acids showing the positions of the double bonds.

TABLE 8.8
Major effects of EFA deficiency in the rat

Skin	Scaly, dry skin
Weight	Decrease
Circulation	Heart enlargement; decreased capillary resistance (lower blood pressure at periphery); increased permeability
Kidney	Enlargement; intertubular hemorrhage
Lung	Cholesterol accumulation
Endocrine glands	(a) Adrenals: weight decreased in females and increased in males
	(b) Thyroid: reduced weight
Reproduction	(a) Females: irregular estrus and impaired reproduction and lactation
	(b) Males: degeneration of semini-ferrous tubules
Metabolism	(a) Changes in fatty acid composition of most organs
	(b) Increase in cholesterol levels in liver, adrenals, and skin
	(c) Decrease in plasma cholesterol
	(d) Changes in swelling of heart and liver mitochondria and uncoupling of oxidative phosphorylation
	(e) Increased triglyceride synthesis and release by the liver

Source: From Burr, G.O. and Burr, M.M. (1929). A new deficiency disease produced by the rigid exclusion of fat from the diet. *J. Biol. Chem*. 82: 345–367.

in insulin receptor number. Although none of these effects are especially dramatic, collectively they help explain why animals fed such a deficient diet are growth impaired and have poor food efficiency. Some of the effects shown in Table 8.8 can be due to an effect on membranes per se, whereas other effects are attributable to a lack of arachidonic acid to serve as a precursor for eicosanoid synthesis.

Dietary fat composition can affect both membrane fatty acid composition as well as the fatty acid distribution of the stored lipids.[48] Shown in Table 8.9 are some data giving the phospholipid fatty acid composition of livers from rats fed either a 6% corn oil or hydrogenated coconut oil diet.[49] Recall that coconut oil is devoid of essential fatty acids, whereas corn oil is a good source

TABLE 8.9
Effect of 6% corn or coconut oil diets on the phospholipid fatty acid composition of rat liver

	Diet	
Fatty acids[a] (% of the total)	**Corn oil**	**Coconut oil**
14:0	0.09 ± 0.03	0.30 ± 0.03[b]
14:1	0.10 ± 0.02	0.05 ± 0.01
15:0	0.17 ± 0.03	0.20 ± 0.03
16:0	15.2 ± 0.03	15.4 ± 0.03
16:1	0.70± 0.08	2.26 ± 0.07[b]
17:0	0.38 ± 0.04	0.13 ± 0.02[b]
17:1	0.09 ± 0.02	0.11 ± 0.02
18:0	20.8 ± 0.82	2.0 ± 0.4
18:1	7.17 ± 0.61	2.0 ± 0.5[b]
18:2	14.4 ± 0.4	7.98 ± 0.27[b]
20:0	0.01 ± 0.01	0.16 ± 0.04[b]
18:3	0.41 ± 0.06	0.35 ± 0.04
20:1	ND	0.18 ± 0.04
20:2	0.89 ± 0.17	6.44 ± 0.50[b]
20:3	0.82 ± 0.16	2.20 ± 0.13[b]
20:4	29.4 ± 0.5	18.0 ± 0.7[b]
22:1	0.34 ± 0.02	0.35 ± 0.03
22:2	0.14 ± 0.02	0.48 ± 0.03[b]
24:0	0.96 ± 0.01	0.95 ± 0.03
22:4	1.37 ± 0.09	0.54 ± 0.02[b]
22:5	2.14 ± 0.25	2.24 ± 0.12
22:6	3.53 ± 0.31	6.64 ± 0.30[b]
20:5	ND	0.41 ± 0.04

[a] Designations used: number of carbons in chain followed by number of double bonds.

[b] Indicates a significantly different value from that of the corn-oil-fed rats.

Notes: Hydrogenated coconut oil is deficient in essential fatty acid but is a good source for 8, 10, 12, and 14 carbon fatty acids; ND indicates nondetectable.

Source: Berdanier, C.D. (1988). *Nutrition* 4: 295–299.

of the unsaturated fatty acids, linoleic, oleic, and palmitoleic, and of the saturated fatty acid, palmitic acid. Note that the corn-oil-fed rats had a phospholipid fatty acid profile that reflected their corn-oil intake. Note, too, that rats fed the hydrogenated coconut oil, which has no linoleic or arachidonic acid, likewise had less of these fatty acids in their profile. These rats tried to compensate for their essential fatty acid-deficient diet by increasing their desaturase and elongase activity so as to preserve (as much as possible) the fluidity of their plasma and mitochondrial membranes. Thus, compared with the corn-oil-fed rats, increases in unsaturated fatty acids (16:1, 18:1, 20:2, 20:3, 22:2, 22:6) were found. Hydrogenated coconut oil contains the medium-chain saturated fatty acids, and these account for the fact that hydrogenated coconut oil is a solid at room temperature (20°C). It would be logical to assume that animals consuming this fat would have more rigid (less fluid) membranes, but Mother Nature has designed a fairly competent compensatory system that can partially

overcome the diet fat effect on the membrane. Thus, the increases in the unsaturated fatty acids as noted above. When the fatty acid unsaturation to saturation ratios (P:S) in these two groups of rats were calculated, these ratios were nearly the same. Despite this compensation, consumption of a diet lacking the essential fatty acids resulted in some profound effects on metabolism and bodily function. Their mitochondrial OXPHOS system was compromised, as was the use of glucose by both hepatocytes and adipocytes. The animals had other symptoms as well: poor food efficiency, poor coats, and so forth.[50–54] Needless to say, a fatty-acid-deficient diet (the hydrogenated coconut-oil diet) lacked sufficient linoleic acid for conversion to arachidonic acid that is then used for the synthesis of the eicosanoids.

Eicosanoid Synthesis

Eicosanoids are derived primarily from plasma membrane arachidonic acid but other 20-carbon polyunsaturated acids can also serve as substrates (Figure 8.7). Arachidonic acid is released from the plasma membrane phospholipids through the action of phospholipase A_2.[55] Membrane phospholipid arachidonate release is merely part of the constant remodeling process that membranes undergo.[56] Eicosanoids serve to mediate inflammation and immune cell function.[57] Eicosanoids include thromboxanes (TBXs), leukotrienes (LTs) and prostaglandins (PGs). Specific eicosanoids have specific effects and frequently one will oppose the effect of another. Prostaglandin E_2 (PGE_2) induces cyclooxygenase 2 (COX-2) in fibroblasts and upregulates its own synthesis. It also induces the production of IL-6 by macrophages.

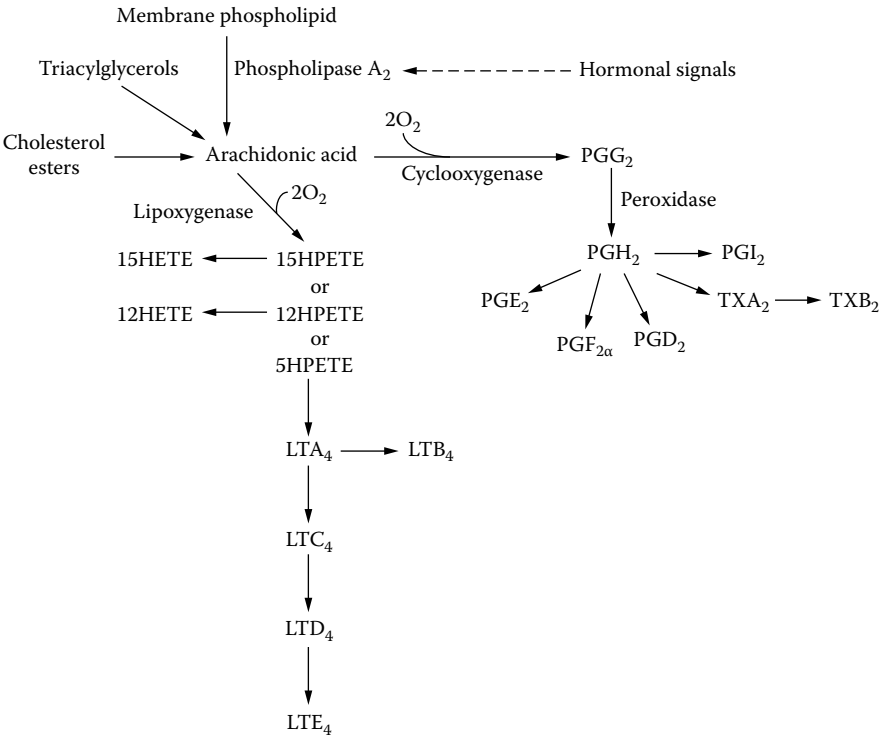


FIGURE 8.7 Overall pathway for the synthesis of the major eicosanoids.

TABLE 8.10
Functions of eicosanoids

Eicosanoids	Function
PGG_2	Precursor of PGH_2
PGH_2	Precursor of PGD_2, PGE_2, PGI_2, and $PGF_2\alpha$
PGD_2	Promotes sleeping behavior; precursor of PGF_2
PGE_2	Enhances perception of pain when histamine or bradykinin is given
	Induces signs of inflammation. Promotes wakefulness
	Precursor of $PGF_2\alpha$. Reduces gastric acid secretion
	Vasoconstrictor in some tissues. Vasodilator in other tissues
	Maintains the patency of the ductus arteriosis prior to birth
$PGF_2\alpha$	Induces partuition. Bronchial constrictor. Vasoconstrictor especially in coronary vasculature. Increases sperm motility; stimulates steroidogenesis corpus luteum; induces luteolysis
PGI_2	Inhibits platelet aggregation
PGE_1	Inhibits motility of nonpregnant uterus; Increases motility of pregnant uterus. Bronchial dilator
TXA_2	Stimulates platelet aggregation. Potent vasoconstrictor
LTB_4	Potent chemotaxic agent

The eicosanoids fall into three general groups of compounds:

1. The prostaglandins (compounds of the PG series)
2. The thromboxanes (compounds of the TBX series)
3. Leukotrienes (compounds of the LKT series)

All of these compounds arise from a 20-carbon polyunsaturated fatty acid. This fatty acid is usually arachidonic acid (20 carbons, 4 double bonds at 5, 8, 11, 14). However, in instances where the diet is rich in omega-3 (*n*-3) fatty acids, the precursor may be a 20-carbon-5 double bond fatty acid, eicosapentaenoic acid (double bonds at 5, 8, 11, 14, 17). Other eicosanoids can be synthesized from a 20-carbon fatty acid, dihomo-γ-linoleic acid, which has 3 double bonds at carbons 8, 11, and 14. Each of these precursors yields a particular set of eicosanoids. The function of the various eicosanoids are listed in Table 8.10.

The 20-carbon fatty acids are the substrates for eicosanoid synthesis, which involves the use of oxygen and cyclization (Figure 8.8). Dihomo γ linoleic acid is the precursor of prostaglandin E_1 (PGE_1) and prostaglandin $E_1\alpha$($PGE_1\alpha$) and subsequent prostaglandins. Arachidonic acid is the precursor of prostaglandins of the 2 series (PGE_2, $PGF_2\alpha$, etc.) and eicosapentaenoic acid is the precursor of prostaglandins of the 3 series (PGE_3, $PGF_3\alpha$, etc.).

The cyclization of these 20-carbon fatty acids is accomplished by a complex of enzymes called the *prostaglandin synthesis complex*. The first step is the cyclooxygenase step, which involves the cyclization of C-9–C-12 of the precursor to form the cyclic 9-11 endoperoxide 15-hydroperoxide (PGG_2) shown in Figure 8.8. PGG_2 is then used to form prostaglandin H_2 (PGH_2) through the removal of one oxygen from the carbonyl group at carbon 15. Glutathione peroxidase and prostaglandin H synthase catalyze the reaction shown in Figure 8.9.[58] Prostaglandin H synthase is a very unstable, short-lived enzyme with a messenger RNA that is one of the shortest-lived species so far found in mammalian cells. The expression of genes for this enzyme is under the control of polypeptide growth factors such as interleukin 1α, and colony-stimulating factor 1. Interferon α and β inhibit expression and prostanoid production by the macrophages. Glutathione peroxidase is a selenium-containing enzyme (see Chapter 12). In animals fed a high-polyunsaturated-fat diet,

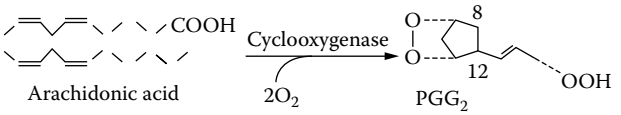


FIGURE 8.8 Cyclization and oxygenation of arachidonic acid.

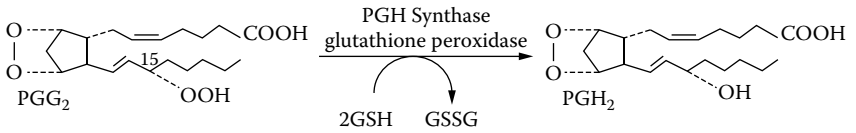


FIGURE 8.9 Formation of PGH_2.

one might expect to see a higher than normal requirement for selenium in the diet to accommodate the need for this enzyme in its role for eicosanoid synthesis. However, such an expectation is without merit. Studies of rats fed a high-polyunsaturated-fat diet such as a marine oil-rich diet show a greater need for vitamin E to accommodate the increased need to support the antioxidation system, but not an increased need for selenium.[59] The need to make the eicosanoids is quite low compared with the need to suppress the formation of free radicals. This probably explains why the selenium requirement is not increased under these dietary conditions.

PGH_2 is then converted through the action of a variety of isomerases to PGD_2 or PGE_2 or prostacylin I_2 (PGI_2) or prostaglandin $F_2\alpha$ ($PGF_2\alpha$). These are the primary precursors of the prostaglandins of the D, E, and F series and PGI or thromboxane. The conversion to subsequent prostaglandins is mediated by enzymes that are specific to a specific cell or tissue types. Not all of these subsequent compounds are formed in all tissues. Thus, PGE_2 and $PGF_{2\alpha}$ are produced in the kidney and spleen. $PGF_{2\alpha}$ and PGE are also produced in the uterus only when signals from the pituitary induce their production and so stimulate parturition. PGI_2 is primarily produced by endothelial cells lining the blood vessels. This prostaglandin inhibits platelet aggregation and thus is important to maintaining a blood flow free of clots. It is counteracted by thromboxane A_2, which is produced by the platelets when these cells contact a foreign surface. PGE_2, $PGF_2\alpha$, and PGI_2 are formed by the heart in about equal amounts. All of these prostaglandins have a very short half-life. No sooner are they released than they are inactivated. The thromboxanes are highly active metabolites of the prostaglandins. As mentioned, they are formed when PGH_2 has its cyclopentane ring replaced by a six-membered oxane ring, shown in Figure 8.9. Imidazole is a potent inhibitor of thromboxane A synthase and is used to block TXA2 production and platelet aggregation.

Thromboxane A_2 has a role in clot formation and the name *thromboxane* comes from this function (*thrombus* means clot). The half-life of TXA_2 is less than one minute. TXB_2 is its metabolic end product and has little biological activity. Measuring TXB_2 levels in blood and tissue can give an indication of how much TXA_2 has been produced. PGD_2 and PGE_2 are involved in the regulation of sleep–wake cycles in a variety of species.[60]

The cyclooxygenase reaction illustrated in Figure 8.10 can be inhibited by certain antiinflammatory drugs such as aspirin, indomethacin, and phenylbutazone. These are the nonsteroidal antiinflammatory drugs that are commonly available in pharmacies. These drugs block the action of cyclooxygenase by acetylating the enzyme. Use of these drugs for the occasional injury or headache is harmless, but long-term chronic use can result in untoward effects. Long-term chronic use of aspirin, for example, can affect vascular competence and blood clotting. People consuming large amounts of aspirin over long periods of time may find an increase in bruises (subcutaneous hemorrhages). Small contact injuries that normally would not result in a bruise will do so in these people.

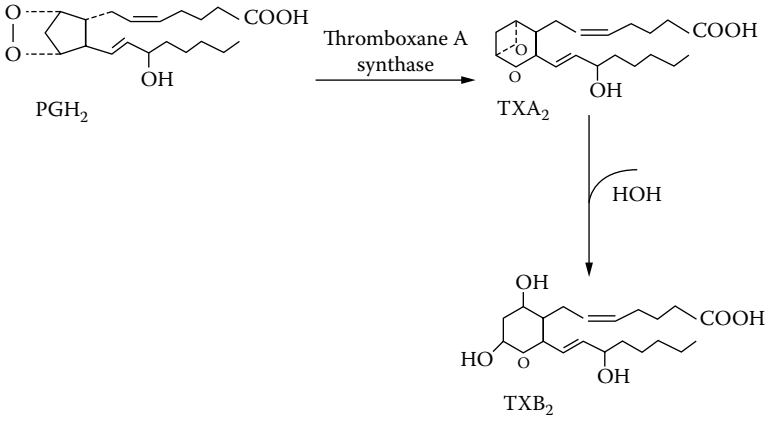


FIGURE 8.10 Reaction sequence that produces TXA_2 and TXB_2.

Gastric bleeding is another possible complication with long-term chronic aspirin ingestion. Aplastic anemia can result from long-term phenylbutazone therapy. Again, the occasional use of these drugs is not likely to have these effects.

A second group of antiinflammatory agents are the steroids: hydrocortisone, prednisone, and other similar compounds.[61] These drugs are prescription drugs that act by inhibiting the enzyme phospholipase A_2. Phospholipase A_2 stimulates the release of arachidonic acid from the membrane phospholipids. Hence, inhibition of this reaction will result in a decreased supply of arachidonic acid for eicosanoid synthesis. When the conversion of arachidonic acid to eicosanoid is inhibited as above or when either of the other 20-carbon fatty acids are abundantly available, a different series of prostaglandins and leukotrienes are produced. Eicosapentaenoic acid is not as good a substrate for cyclooxygenation as is arachidonic acid. As a result, less of the arachidonic-acid-related eicosanoids (even-numbered PGs and TBXs) and more of the odd-numbered prostaglandins and leukotrienes are produced.

Although the cyclooxygenase pathway is quite important in the production of eicosanoids, equally important is the lipoxygenase pathway. This pathway is catalyzed by a family of enzymes called the *lipoxygenase enzymes*. These enzymes differ from the cyclooxygenase enzymes in the catalytic site for oxygen addition to the unsaturated fatty acid. One lipoxygenase is active at the double bond at carbon 5, whereas a second is active at carbon 11 and a third is active at carbon 15. The products of these reactions are monohydroperoxy-eicosatetraenoic acids (HPETEs) and are numbered according to the location of the double bond to which the oxygen is added: 5-HPETE is the major lipoxygenase product in basophils, polymorphonuclear leukocytes, macrophages, mast cells, and any organ undergoing an inflammatory response; 12-HPETE is the major product in platelets, pancreatic endocrine cells, vascular smooth muscle, and glomerular cells. The 15-HPETE predominates in reticulocytes, eosinophils, T lymphocytes, and tracheal epithelial cells. The HPETEs are not in themselves active hormones; rather, they serve as precursors for the leuko-trienes. The leukotrienes are the metabolic end products of the lipoxygenase reaction. These compounds contain at least three conjugated double bonds. The unstable 5-HPETE is converted to either an analogous alcohol (hydroxy fatty acid) or is reduced by a peroxide or converted to leukotriene. The peroxidative reduction of 5′-HPETE to the stable 5-HETE (5 hydroxyeicosatetraenoic acid) is similar to that of 12-HPETE to 12-HETE and of 15-HPETE to 15-HETE. In each instance, the carbon–carbon double bonds are unconjugated, and the geometry of the double bonds is *trans*, *cis*, and *cis*, respectively. In contrast to the active thromboxanes, which have very short half-lives, the leukotrienes can persist as long as 4 hours. These compounds make up a group of substances known as the slow-acting anaphylaxis substances. They cause slowly evolving but protracted contractions of smooth muscles in the airways and gastrointestinal tract. Leukotriene C4 is rapidly converted to

LTD4, which, in turn, is slowly converted to LTE4. Enzymes in the plasma are responsible for these conversions.

The products of the lipoxygenase pathway are potent mediators of the response to allergens, tissue damage (inflammation), hormone secretion, cell movement, cell growth, and calcium flux. Within minutes of stimulation, lipoxygenase products are produced. In an allergy attack, for example, an allergen can instigate the release of leukotrienes, which are the immediate mediators of response. The leukotrienes are more potent than histamine in stimulating the contraction of the bronchial nonvascular smooth muscles. In addition, LTD4 increases the permeability of the microvasculature. The mono HETEs and LTB4 stimulate the movement of eosinophils and neutrophils, making them the first line of defense in injury resulting in inflammation.

As mentioned, when dihomo γ linoleic acid or eicosapentaenoic acid serve as substrates for eicosanoid production, the products are either of the 1 series or 3 series. The products they form may be less active than those formed from arachidonic acid, and this decrease in activity can be of therapeutic value. Hence, ingestion of omega-3 fatty acids leads to the decreased production of prostaglandin E_2 and its metabolites, a decrease in the production of thromboxane A_2, a potent platelet aggregator and vasoconstrictor; and a decrease in leukotriene B_4, a potent inflammatory hormone and a powerful inducer of leukocyte hemotaxis and adherence. Counteracting these decreases are an increase in thromboxane A_3 (TXA_3), a weak platelet aggregator and vasoconstrictor; an increase in the production of PGI_3 without an increase in PGI_2, which stimulates vasodilation and inhibits platelet aggregation; and an increase in leukotriene B_5, which is a weak inducer of inflammation and a weak chemotoxic agent. Marine oils, rich in omega-3 unsaturated fatty acids, affect (decrease) platelet aggregation because they stimulate the synthesis of thromboxane A_3. Thromboxane A_3 does not have the platelet-aggregating property of the other eicosanoids. In addition, eicosapentaenoic acid is used to make the antiaggregating prostaglandin PGI_3. Animals fed omega-3-rich oils produce significantly more of the eicosanoids of the LTB_5 series. LTB_4 is an important inflammatory mediator, whereas LTB_5 is not. Fish oil consumption results in an increased neutrophil LTB_5 production, with a concomitant decrease in LTB_4 production. This diet-influenced change in LTB_4 and LTB_5 production seems to be related to a reduced incidence of autoimmune inflammatory disorders such as asthma, psoriasis, and rheumatoid arthritis in populations consuming omega-3 fatty acids routinely. Thus, eicosanoid synthesis can be used as an explanation of the beneficial effects of fish oil ingestion on rheumatoid arthritis. In arthritics, the joints are inflamed and painful. The prostaglandins PGE_2 and leukotriene are both produced from arachidonate. PGE_2 induces the signs of inflammation, which include redness and heat due to arteriolar vasodilation, swelling, and localized edema resulting from increased capillary permeability. Leukotriene prevents platelet aggregation. If there is less arachidonate available for the synthesis of these prostaglandins, then the inflammation is inhibited.

Tumorigenesis likewise can be influenced by the relative amounts of the various eicosanoids. Prostaglandin G of the 2-series acts as a tumor promoter. It downregulates macrophage tumoricidal activities and inhibits interleukin-2 production. Increased PGE_2 levels (from omega-6 fatty acids) have been associated with aggressive growth patterns of both basal and squamous cell skin carcinomas in humans. Vegetable oils are rich in these fatty acids. Products of the lipoxygenase pathway (stimulated by the omega-3 fatty acids) have the reverse effect. Although the various eicosanoids have different (and sometimes conflicting) effects on inflammatory processes and on tumor promotion, it might be anticipated that susceptibility to pathogenic organisms would be similarly affected. Studies with mice exposed to a variety of pathogens and fed either a fish oil or a control diet showed no diet-fat-related differences in susceptibility to these organisms.

FATTY ACID AUTOOXIDATION

Fatty acids, particularly the unsaturated fatty acids can autoxidize and form reactive compounds called *free radicals*.[62] Free radicals (reactive oxygen species, ROS) can form when the oxygen atom

is excited by a variety of drugs and contaminants and by ultraviolet light. Inflammation stimulates ROS formation as does heavy exercise, ischemia-reperfusion, xanthine oxidase, arachidonic acid metabolism, transition metals (iron for example), smoking, environmental pollutants, UV light, ozone, and certain environmental chemicals. The excited oxygen atom is called singlet oxygen ($O_2^{-\cdot}$). Pollutants such as the oxides of nitrogen or carbon tetrachloride can provoke this reaction. The unsaturated fatty acids are not the only compounds that can be radicalized, yet these are the ones that are most often discussed.[63]

In food, autooxidation is responsible for the deterioration of food quality. The discoloration of red meat upon exposure to air at room temperature is an indication of the autooxidation process. The off odor that accompanies this discoloration is the result of the autooxidation of the fatty acids and some of the amino acids in the meat. In living systems, the process of autooxidation is suppressed to a large extent. This is essential because the products of this oxidation, amino acid and fatty acid peroxides, can be very damaging. Peroxides denature proteins, rendering them inactive, and attack the DNA in the nucleus and mitochondria, resulting in base-pair deletions or breaks in the DNA which, in turn, result in mutations or errors. In the nucleus, these breaks or deletions can be repaired or misrepaired. In the aging animal, the repair mechanism loses its efficiency and one of the characteristics of aged cells is the loss of its DNA-repair ability.[64] To prevent widespread damage to cellular proteins and DNA by these radicals, there is a potent antioxidation system (see Chapter 12). This system includes the selenium-containing enzyme, glutathione peroxidase, catalase, and superoxide dismutase. These enzymes are found in the peroxisomes.[65] All of these components serve to suppress free-radical formation.

Under circumstances where mitochondrial OXPHOS efficiency is downregulated, as under stress with the release of epinephrine, or the release of uncoupling proteins, more heat is released and less ATP is produced. Under this circumstance, there is a relative excess of oxygen, which then can radicalize. It is for this reason the mitochondria possess a particularly potent peroxide suppressor, superoxide dismutase or SOD. SOD in the mitochondria requires the manganese ion as a cofactor. The cytosol also has SOD, but this enzyme requires the copper and zinc ions. Both forms of the enzyme catalyze the reaction $O_2^{-\cdot} + O_2^{-\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$. Two superoxides and two hydrogen ions are joined to form one molecule of hydrogen peroxide and a molecule of oxygen. In turn, the peroxide can be converted to water through the action of the enzyme catalase. Peroxides can also be "neutralized" through the action of glutathione-*S*-transferase. This reaction requires 2 mol of reduced glutathione and produces 2 molecules of oxidized glutathione and 2 molecules of water. Fatty acid radicals can also be neutralized by glutathione peroxidase, producing a molecule of an alcohol with the same chain length as the fatty acid. Glutathione-*S*-transferase can duplicate the action of glutathione peroxidase.

With infection, partial mitochondrial uncoupling occurs, and the heat so produced (fever) combats invading pathogens, which are more sensitive to heat than normal body cells. Under these conditions, the peroxides that are formed have pathogen-killing capability. This is an important body defense system.

Food components that downregulate or upregulate free-radical formation are important considerations in inflammation and oxidative stress. Szeto et al.[66] compared plasma biomarkers of antioxidant status, oxidative stress, inflammation, and risk for coronary artery disease (CVD) in long-term vegetarians compared to nonvegetarians (omnivores). Vegetarians had lower blood levels of triacyglycerols, uric acid, and C-reactive protein as well as lower levels of vitamin E (α-tocopherol). Sebekova et al.[67] also studied vegetarians and omnivores and reported that vegetarians had lower levels of markers of oxidative stress that included advanced glycation end products. They had higher levels of advanced oxidation proteins (including C-reactive protein) than omnivores. Surprisingly, vegetarians appeared to consume less of the antioxidant vitamins, vitamins A and C. Bowen and Borthakur[68] reported that a mild prooxidative state accompanies meal ingestion, which results in an increase in biomarkers of inflammation, adhesion, and endothelial dysfunction,

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all of which are factors in the development of CVD. After a meal there is an abundance of energy-containing substrates that could provide excess substrate for superoxide formation. This may be a dominant factor in postprandial oxidative stress and a decrease in nitric oxide. It may also be related to the observation that more heart attacks occur in the postprandial condition than in the fed or starved condition. As mentioned above, fatty acids, especially the polyunsaturated fatty acids are excellent substrates for the production of ROS. If, in fact, the number of fatty acid ROS is a marker of oxidative stress. Oxidative stress has been linked to inflammation and to a variety of degenerative conditions such as atherosclerosis.

On the other hand, saturated but not unsaturated fatty acids induce apoptosis of human coronary artery endothelial cells via NFκB activation.[69] NFκB is nuclear factor-κB, a nuclear gene signaling factor that appears to be involved in the inflammatory response. Endothelial apoptosis contributes to atherothrombosis (blood clot in a coronary vessel).

Fatty acids and various fatty acid-derived metabolites (the eicosanoids) can affect gene expression via binding to and activating the various PPARs (α,δ,γ). All three of these PPARs (peroxisome proliferating factors) act as transcription agents that in turn affect cellular differentiation and functional properties. PPARα is expressed in the T cells and the B cells, whereas γ dominates in cells of myeloid lineage (monocytes and macrophages). PUFAs bind to these PPARs and probably have their effects on the expression of some of the genes of the inflammatory cascade in this way in addition to their direct effects on the fatty acid radical formation.[70] There are other food constituents that can form or suppress free-radical formation. The mode of action of these components is described as follows:

1. *Amino acids*: Cysteine and arginine can be converted to free radicals and when this happens there is an increase in C-reactive protein. Cysteine will form homocysteine and hyperhomocysteinemia is associated with CVD. It is a strong independent risk factor for new cardiovascular events; however, the mechanism of its action is unclear. A proinflammatory state has been found to exist in the elderly that is associated with hyperhomocysteinemia.[71] A study of 586 men and 734 elderly women revealed that hyperhomocysteinemia was associated with elevated levels of plasma IL-1 receptor antagonist and IL-6. The sedentary state, intakes of vitamins B_6 and folacin, and serum levels of folacin, vitamin B_{12}, vitamin B_6 and α-tocopherol were significant and independent correlates of homocysteine levels.
2. *Folacin* supplementation suppresses homocysteine formation and resultant C-reactive protein. Folacin also reduces adhesion molecules VCAM-1 expression in the aortic endothelium.[72] Hyperhomocysteinemia increases the expression of intracellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1). It also increases NFκB activation and consequently increases the expression of inflammatory factors in vivo. Folacin supplementation reverses these effects.[73,74]
3. *Vitamin E* reduces fatty acid free-radical formation. It is a potent antioxidant with anti-inflammatory properties. Vitamin E supplementation results in decreases in lipid peroxidation and superoxide (O_2^-)production by impairing the assembly of NADP (reduced) oxidase as well as decreasing the expression of scavenger receptors (SR-A and CD 36), particularly important to the formation of foam cells. α-Tocopherol therapy at high doses decreases the release of proinflammatory cytokines, chemokine IL-8, plasminogen activator inhibitor-1 (PAL-1), and decreases the adhesion of monocytes to the endothelium. It also decreases the release of C-reactive protein. The mechanisms that account for the non-antioxidant function of α-tocopherol include the inhibition of protein kinase C, 5-lipoxygenase, tyrosine-kinase and cycloxygenase-2.[75] Although diabetes can elicit an increase in free radicals, vitamin E supplementation has no effect on the risk of diabetes nor does it have any beneficial effect on any of the diabetes-related complications.[76]
4. *Vitamin D* modulates the production of inflammation markers and oxidative stress. Vitamin D exerts effects on the immune system.[77] These effects are exerted on multiple

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immune cell types and are predominately suppressive at pharmacological levels yet are potent enough to have therapeutic potential in the management of normal and abnormal immune diseases.

Receptors for active vitamin D (1α25-dihydroxycholecalciferol) have been found in human thymus cells, peripheral blood leukocytes, dendritic cells, monocytes macrophages and in T lymphocytes. The monocytes are viewed as precursors of macrophages that are phagocytic cells capable of secreting an array of proinflammatory products and intimately involved in the host defense against bacterial infection. The dendritic cells serve to initiate T-cell-mediated immune response and when fully mature, express high levels of class II MHC, CD80, CD86, CD40 and an array of other accessory ligands and immunostimulatory products.[78,79] Through binding to its cognate receptor, vitamin D inhibits T-cell proliferation, IL-2 secretion and cell cycle progression from G_{1a} to G_{1b}. Thus, vitamin D serves as an antiinflammatory nutrient. Vitamin D also serves to suppress B-cell proliferation as well as the proliferation of natural killer cells. It inhibits the production of mitogen or antigen-stimulated immunoglobulin (IgM and IgG). The evidence for this role is not as convincing as for its role in T-cell activity.

These actions of vitamin D are probably due to its role in the expression of genes encoding elements of the immune system (see Chapter 5). Nuclear vitamin D receptor proteins have been identified and bind to specific elements in the promoter regions of a number of genes. Actually, the vitamin plays a part in the NF-κB signaling pathway that consists of a number of related proteins that regulate gene expression when translocated to the nucleus as heterodimers or homodimers. This pathway is central to differentiation, maturation, activation and survival of antigen-presenting cells (APCs), lymphocytes and other cells of the immune system. Vitamin D also modulates signaling for the nuclear factor of activated T cells (NFAT), the phosphatidylinositol 3-kinase, and the mitogen-activated protein kinase (MAPK) pathway.

The extensive literature documenting these effects on elements of the immune system provides strong and convincing evidence of the role of this vitamin/hormone in normal immune function. In summary, it enhances innate immunity through increased macrophage recruitment and differentiation; it prevents autoimmunity through inhibition of the ability of dendritic cells to induce Th-1-type cellular immune responses; it promotes self-tolerance through permissive effects on the generation of Th-2 or T_{reg}-type cells.

5. *Conjugated (trans*-10,*cis*-12*) linoleic acid* (CLA) but not *cis*-9,*trans*-11 conjugated linoleic acid is effective in reducing fat mass.[80] It causes a drop in adipocyte lipid content and a decrease in insulin-stimulated glucose uptake (suppression of GLUT 4 transporter), fatty acid uptake, and incorporation into lipid and oxidation compared to control adipocyte cultures. In parallel, gene expression of PPARγ and many of its downstream targets were also reduced. CLA caused a robust and sustained activation of mitogen-activated protein kinase kinase/extracellular signal-related kinase (MERK/ERK) signaling. This was linked to hypersecretion of IL-6, IL-8, and NFκB. The NFκB increase preceded that of IL-6 and IL-8, which in turn preceded PPARγ activation. This promotes adipocyte insulin resistance, and increases the IL-6 and 8 production response to an increased mitogen-activated kinase. These sequential responses of adipocytes to this particular conjugated fatty acid elucidate the sequence of events (at least in adipocytes) that occur in the inflammatory process.[81,82] This work was extended to include the study of nonadipocyte cells in culture. Using the endotoxin lipopolysaccharide Chung et al.[83] showed that LPS induced the mRNA for the proinflammatory cytokines (IL-6, TNF-α, IL1b) and chemokines (IL-8, monocytes chemoattractant protein-1) and that the inflammatory response of the fat pad was due primarily to that of the preadipocyte component. In mice, feeding of this isomer had the reverse effect: there was a rapid induction of inflammatory factors such as TNF-α and IL-6 gene expression in adipose tissue, whereas no effect was observed on serum levels

of these cytokines. There was also a dramatic increase in macrophage infiltration and gene expression that appeared to promote the inflammatory response. In turn this was interpreted as contributing to adipose tissue insulin resistance.[84] Elevated intakes of trans fatty acids in general is associated with markers of inflammation in women, and there may be implications of this relationship to chronic disease; however, this has not been proven.[85]

6. *Iron*: Iron is one of the transition metals that, in excess, enhances the formation of ROS. Excess iron in the circulation is generally thought to be a genetic disease called *hemochromatosis* (see Chapter 12). Iron is absorbed, and because there is little that can be excreted, it remains in the circulation causing a variety of metabolic disturbances; among these is the excess formation of ROS. Iron may play a role in vascular inflammation as elevated levels of ferritin result in increased levels of C-reactive protein. NHANES data have been analyzed to determine whether ferritin, total cholesterol, LDL, HDL, and ferritin–lipid combinations were related to C-reactive protein.[86] Neither elevated ferritin nor elevated LDL was associated with elevated C-reactive protein. However, subjects with elevated ferritin and LDL were more likely to have elevated C-reactive protein; subjects with high ferritin and low LDL were also likely to have elevated C-reactive protein. There may be a role for each in the inflammatory process.
7. *Copper*: Excess copper can also be absorbed again due to a genetic error in absorption. It, too, can create metabolic problems and, like iron, can result in excess ROS formation (see Chapter 12).
8. *Vitamin A*: The carotenoids, vitamin A precursors, are potent antioxidants in foods. Foods rich in these compounds are excellent foods to reduce oxidative stress (see Chapter 9).
9. *Flavonoids, polyphenols*: The flavonoids are a diverse group of polyphenolic compounds widely distributed in the plant kingdom. There are more than 6400 known compounds that contribute flavor and pigmentation to the fruits and vegetables in the human diet. They are divided into eight classes based on their molecular structure. The biological effects of these flavonoids are contingent upon their interaction with proteins. Some foods such as cocoa products are sources of flavan-3-ols. Intake of flavonoid-rich foods has been shown to reduce plasma levels of proinflammatory cysteinyl leukotrienes, inhibit human 5-lipoxygenase (a key enzyme in leukotriene synthesis), and increase the level of nitric oxide (a vasorelaxing compound). They are also associated with a reduction in the "stickiness" of platelets (platelet aggregation). A suppression of mediators of inflammation and peroxide formation has been observed.[87–92] These changes were associated with a reduction in risk for CVD. Studies of diabetic LDL-receptor-deficient mice showed that polyphenols stimulate AMP-activated protein kinase, lowered blood lipids, and inhibited the accelerated atherosclerosis that is characteristic of these mice.[93]

Foods that contain the above nutrients have tweaked the curiosity of people interested in the relationship between diet and health. A few studies have been reported that compare the milk protein, casein, with soy protein containing isoflavones. Crouse et al.[94] reported that soy protein lowered plasma concentrations of total and LDL cholesterol by 4 and 6%, respectively. There were no diet effects on triglyceride levels and HDL cholesterol. Nilausen and Meinertz[95] reported that casein lowered lipoprotein(a). They concluded that soy protein may have potentially damaging effects on atherogenesis because it appeared to raise the levels of the atherogenic Lp(a). In contrast, Tonstad, Smerud, and Hoie[96] reported that adding soy protein or casein to a lipid-lowering diet significantly reduced LDL cholesterol concentrations as well as homocysteine levels without having the effect of Lp(a). Campbell et al.[97] have reported that there were no differences between soy protein and casein with respect to copper-induced LDL oxidation, serum C-reactive protein, serum IL-6, serum fibrinogen or total cholesterol, HDL cholesterol, LDL cholesterol or triglycerides. Lastly, Oner et al.[98] showed that feeding whey protein suppressed markers of oxidative stress that were part of the whole-body response to burn injury.

In addition to the reactions that counteract the in vivo formation of oxygen radicals or fatty acid peroxides, certain of the vitamins have this role as well. Ascorbic acid has an antioxidant function as it can donate reducing equivalents to a peroxide, converting it to an alcohol. β-carotene can quench singlet oxygen and thus convert it into O_2. Vitamin E is perhaps the best known antioxidant vitamin, and its action is similar to that of ascorbic acid. It donates reducing equivalents to a peroxide, converting it to an alcohol.

Although the foregoing has emphasized the negative aspects of the partial reduction products of oxygen, there is some evidence that peroxide formation has some benefit. For example, leukocytes produce peroxides as a means of killing invading bacteria. Peroxide formation is a part of the body's response to inflammation or injury.[99] It is a complex and coordinated response and has been described as a progression from acute to chronic to healing and then reconstruction. The overall function of the inflammatory response is to bring humoral and cellular defense and repair components together.

NUTRITION, INFLAMMATION, AND CHRONIC DISEASE

The initial event causing an inflammatory response can be a major external event and noticeable. It is typified by redness, swelling, heat, and pain. However, an initial event can also occur internally and not be noticeable until sufficient time has elapsed for the symptoms of a chronic disease caused by the immunologic response to this injury is diagnosed. Examples of external and internal events include:

Physical injury—for example, cuts, abrasions, burns, multiple bruises, bone fractures, organ injuries, and so forth—can serve as an initial event. Internal initial events can be as subtle as a sudden stop of a forward moving body that causes the blood to collide with the endothelial lining of the blood vessel. (It should be remembered that blood does not move in a direct line but with considerable turbulence within blood vessel walls.) If there is a weakness in the wall or where the vessel bifurcates, injury to the endothelial lining of the vessel can occur. This will initiate an inflammation cascade. Although a localized temporary anoxia could also be classed as a chemical injury, it is classed here as a physical injury because of the nature of the local event that elicits a local anoxia. This anoxia can result in oxidative stress that in turn results in a number of free-radical compounds that in turn can injure tissue.

Chemical injury—for example, tissue injuring chemicals and toxins—are also capable of initiating an injury cascade. As described above, free radicals can inflict damage to cells and cell components. Injury can also occur with the ingestion of food-borne toxins or by exposure (via the lungs or through the skin) to some other toxin. Pathogen invasion is another event eliciting an inflammatory response. It can be either a viral, fungal, or bacterial invasion. The response to these invasions may vary, depending on the pathogen and the entry site.

The Injury Cycle

Following an injury there is an increased blood flow and an increased permeability of membranes. Leukocytes are mobilized and migrate to the site of injury. Chemoattractants are released by the injured tissue and attract the leukocytes to the injury site. Adhesion molecules migrate upward from the underlying tissue and the leukocytes adhere to them. This activates the leukocytes, which then release eicosanoids, inflammatory cytokines, platelet activating factor, and reactive oxygen species. Chemoattractants attract the activated leukocytes back to the endothelium and the inflammatory cycle is complete (see Figure 8.11).

As noted, injury elicits a number of almost simultaneous reactions. Included is the movement of complement, antibodies (if the injury is exogenous and involves one or more antigens) and cytokines: IL1β, IL-6, IL-8, TNFα, NFκB. The adhesion molecules include granulocytes, monocytes, macrophages, and lymphocytes.

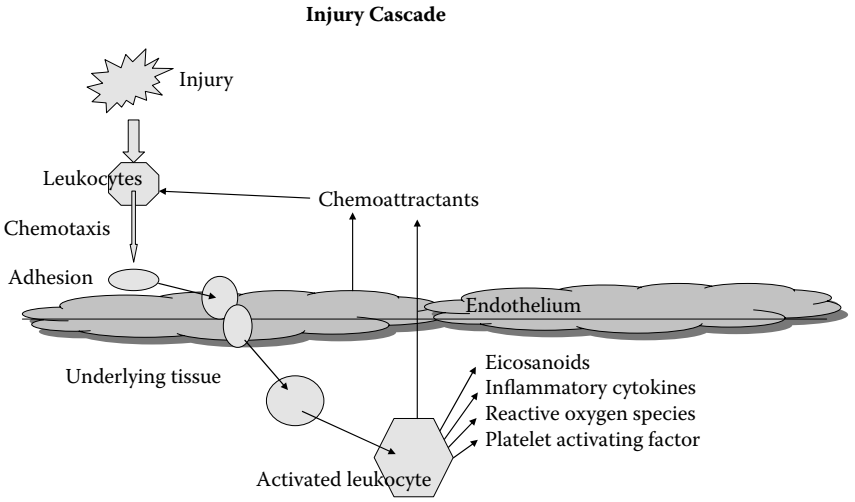


FIGURE 8.11 The injury cascade.

The typical immunologic response to an external injury is a result of increased blood flow, increased permeability across blood capillaries that permits large molecules (complement, antibodies, and cytokines) to leave the bloodstream and cross the endothelial wall. This increase in blood flow and permeability also increases the movement of leukocytes from the bloodstream into the surrounding tissue. Inflammation begins the immunologic process of eliminating invading pathogens and toxins, and marks the beginning of the repair of the injured tissue. The movement of cells to the injury site is induced by the up regulation of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin on the surface of endothelial cells. This allows leukocytes to bind with the result of subsequent diapedesis. (The passage of blood or any of its components through the intact walls of the blood vessels.) The earliest cells to arrive at the injury site are granulocytes followed by monocytes, macrophages, and then lymphocytes. Granulocytes, monocytes, and macrophages are involved in pathogen killing and in clearing up the cellular and tissue debris. The activity of these cells is triggered by the presence of bacterial toxins (lipopolysaccharides) that are components of the cell wall of Gram-negative bacteria. This trigger induces the macrophages and monocytes to release cytokines such as tumor necrosis factor α (TNFα); interleukins IL-1β, IL-6, and IL-8; eicosanoids; PGE_2; nitric oxide (NO); matrix metalloproteinases; and other mediators. Endotoxin also induces adhesion molecule expression on the surfaces of endothelial cells and on leukocytes. One of the more interesting aspects of this cascade is that there are genetically determined variations in the response to injury. Shen et al.[100] have shown that an individual's susceptibility to the development of metabolic syndrome is related to polymorphisms in IL1β.

Pathogens serving as antigens elicit this cascade of immunologic events because the immune system has evolved primarily to provide a rapid and effective protection against such an invasion. It has an array of potent mechanisms that can destroy invading pathogens. It is also carefully regulated to prevent self-injury (autoimmunity).

The immune system defenses are divided into two general groups: The innate group is one which is nonantigen specific; the cognate group is antigen specific. The former group is immediate in action, and members of this group rapidly appear at the site of pathogen invasion. The responses are based on a fixed pattern recognition and are amplified predominantly by the production of soluble (proinflammatory) products that stimulate or attract additional cell populations. Innate immunity is defined as that which is not qualitatively and quantitatively affected by repeated contact

with the same immunologic stimulus. Innate immunity is mediated by the parenchymal cells of all organs and tissues, as well as by the mobile cells with specialized functions (macrophages and natural killer cells).

Cognate immunity is mediated by cells specialized for antigen presentation (dendritic cells) and antigen recognition (T-lymphocytes and B-lymphocytes, abbreviated as T-cells and B-cells). Both cell types are capable of a number of different response patterns that ultimately dictate the nature and duration of immune activity.[101] The two arms of the immune system are coordinately regulated such that invading pathogens are efficiently eliminated. With respect to nutrition, oxidative stress, and inflammatory disease, it should be noted that the endothelium lining the vasculature of the body is a key target for infectious agents.[102] A number of pathogens have been identified that target the endothelium.

Involved in the inflammatory response are the cytokines. These are protein-based regulators of inflammation and immune function.[103] The cytokines regulate the whole-body response to infection and injury, and serve to amplify the initial inflammatory signal. They are opposed by antiinflammatory cytokines such as IL-10 and by receptor antagonists such as IL-1 receptor antagonist. The cytokines of importance to the inflammatory process are: nuclear factor kappa B (NFκB); the interleukins 1β, 6, and 8 (IL-1β, IL-6, IL-8); and tumor necrosis factor α (TNFα). The release of NFκB precedes the release of the interleukins and TNFα. The link between polyunsaturated fatty acids (PUFAs) and inflammation is the eicosanoids which are mediators and regulators of the inflammatory process.[104]

A fourth player in the inflammation process, especially regarding the inflammatory processes occurring in the vascular system, is LDL cholesterol. When oxidized, it can injure the endothelial lining of the vessel, eliciting an inflammatory response (Figure 8.12). Increased levels of oxidized LDL cholesterol results in the formation of minimally modified LDL (MM-LDL) in the subendothelial space. This stimulates the production of monocytes chemotaxic protein-1 that promotes monocytes chemotaxis. MM-LDL also stimulates production of monocytes colony-stimulating factor (M-CSF). M-CSF promotes the differentiation and proliferation of monocytes into macrophages. MM-LDL uptake by the scavenger receptor pathway of the macrophages occurs because the normal receptor for LDL does not recognize it once it is oxidized. Macrophage LDL uptake

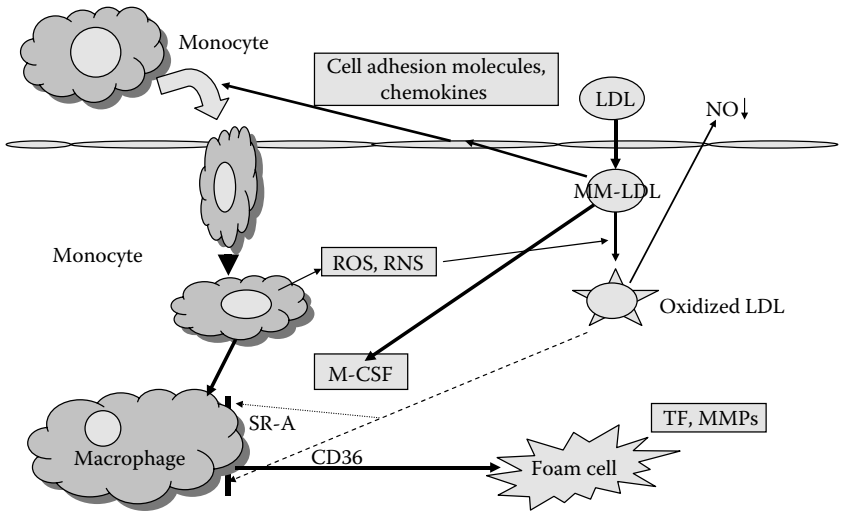


FIGURE 8.12 Oxidized LDL and injury to the vascular tissue. Scheme for the role of oxidized LDL in atherosclerosis. NO, nitric oxide (a vasodilator); LDL, low density lipoprotein; ROS, reactive oxygen species; M-CSF, monocytes stimulating factor.

leads to appreciable cholesterol ester accumulation and foam cell formation. This is at the heart of plaque formation.

After a cholesterol-rich deposit on the endothelium develops, calcium ions are deposited within this plaque. This solidifies the plaque and, if it is rough, can attract platelets and a clot can form. The presence of a plaque narrows the passage for blood and potentiates the possibility of an outright blockage to the vessel.

C-reactive protein and low density lipoprotein are both are markers of CVD risk. However, the use of C-reactive protein as a marker for CVD is no better than using LDL cholesterol as a predictor. The assay for C-reactive protein is more expensive and more complicated. Actually, any inflammation will elicit a rise in C-reactive protein, so if the person has a sore throat, an increase in C-reactive protein value might be obtained that has nothing to do with the development of CVD.

Relationship of Inflammation to Chronic Disease

In tissues from obese individuals there are increases in the markers of inflammation. Several studies have reported on the association of low-grade inflammation with metabolic syndrome. This has been reported in adolescents as well as adults.[105,106] A state of chronic inflammation has been observed in obesity and in Type 2 diabetes[107] in metabolically relevant sites such as the liver, muscle, and adipose tissue. Interference with this inflammation improves or alleviates insulin resistance. In obesity TNFα production is elevated, and when this production is blocked there is an improvement in insulin sensitivity. TNFα regulation of insulin sensitivity is, in turn, regulated by c-jun N-terminal kinase and by I kappa beta kinase. Because obesity and diabetes frequently are associated, a study was performed to determine whether caloric restriction of streptozotocin rats would affect oxidative stress and inflammation markers. Ugochukwu et al.[108] found that it did. Caloric restriction ameliorated the oxidative and inflammatory effects of diabetes.

Type 2 diabetes mellitus is characterized by a chronic inflammatory state associated with insulin resistance.[109,110] Hyperglycemia has been shown to induce proinflammatory cytokines and chemokine genes in monocyte cells. TNFα induces insulin resistance and has a role in obesity development as well (see prior mention). IL-6 alters insulin sensitivity. Hyperglycemia results in increases in the glycated hemoglobin, increases in the polyol pathway and the hexosamine pathway, and activates protein kinase C activity.[111] Acidosis and nonketotic hyperglycemia induce the release of inflammatory cytokines, and this can be reversed once the glycemia is normalized.[112] Acidosis and nonketotic hyperglycemia are associated with an elevation in proinflammatory cytokines, ROS and cardiovascular risk in the absence of obvious infection or cardiovascular pathology. Mastorilou et al.[113] reported that people with Type 2 diabetes have a defect in the metabolism of oxidized phospholipid by HDL. Park et al.[114] have shown that oxidative stress and chronic inflammation are closely related to insulin resistance, obesity, CVD, and Type 2 diabetes. In these people there appears to be an overexpression of the gene for glucose-6-phosphate dehydrogenase in adipocytes, and this over expression is linked to the expression of prooxidative enzymes and the activation of NFκB. Fridlyand and Phillipson[115] have shown that glucose-dependent insulin release itself causes oxidative stress in the pancreatic islet cells. This suggests that dietary antioxidant measures might be of benefit to everyone at genetic risk of developing diabetes. Natali et al.[116] reported that Type 2 diabetes clusters with inflammatory markers (cytokines), fibrinolytic markers PAI-1 and tPA, central obesity, vascular dysfunction/damage, impaired fibrinolysis, and low-grade infection.

In CVD, markers of inflammation appear to precede disease development.[117] Atherosclerosis has elements of an inflammatory disease. The process is characterized by an accumulation of cholesterol-containing LDLs in the intima of the blood vessels. Leukocyte adhesion molecules and chemokines promote the recruitment of monocytes and T cells. The monocytes differentiate into macrophages and upregulate pattern recognition receptors, including scavenger receptors and toll-like receptors. The scavenger receptors internalize the LDLs that in turn lead to foam cell formation. Toll-like receptors transmit activating signals that lead to the release of cytokines,

proteases, and vasoactive molecules. T cells recognize local antigens and mount T helper-1 responses with the secretion of proinflammatory cytokines that contribute to local inflammation and plaque growth. Local inflammation may lead to proteolysis, plaque rupture, and thrombus (clot) formation. This causes ischemia (reduced oxygen supply to the tissue being served by the blocked vessel) and infarction.

Likewise, in renal disease, markers of oxidative stress accompany and may even precede disease diagnosis. Kaysen and Elserich[118] studied the role of oxidative-stress-altered lipoprotein structure and function, and microinflammation on cardiovascular risk in patients with minor renal dysfunction. As renal function declined, many patients became malnourished and were more likely to have deficiencies of nutrients that were antiinflammatory. In addition, these people were also likely to have localized anoxic events that furthered the oxidative stress state and the inflammatory state. These patients have increased C-reactive protein levels as well as increased levels of IL-6. As renal function declines, hepatic apo A-I synthesis decreases and HDL levels fall. Inflammation causes a further structural and functional abnormal state with respect to the lipoproteins: Apolipoprotein C III, a competitive inhibitor of lipoprotein lipase increases. Intermediate lipoprotein lipase increases with increases in serum triglycerides. This intermediate lipoprotein consists of VLDL and chylomicron remnants. All of these changes affect vascular relaxation. As well there is an activation of the angiotensin axis and a loss in renal mass. This in turn leads to an increase in free-radical formation and a decrease in activity of the free-radical suppression system. Leukocyte-derived myeloperoxidase functions as an nitric acid oxidase in the inflamed vasculature and contributes to decreased nitric acid bioavailability and compromised vascular reactivity. All of these events are associated with hypertension and increased cardiovascular risk.[119,120]

Aging may be related to an accumulation of oxidative stress events.[64] It is also associated with a reduced ability to cope with physiological challenges. This includes a lowered ability to suppress the formation of free radicals and to suppress the process of oxidative stress. In turn, this is reflected in an increase in inflammatory markers,[121] damage to important macromolecules, and impaired function. Heat shock proteins play a role in the preservation of the normal state. The heat shock proteins form a highly conserved system responsible for the preservation of the correct DNA sequence and subsequent correct protein structure. Acetyl carnitine may be another material of importance to maintaining normal metabolism.[122] It plays a role in maintaining appropriate relationship of reducing equivalents between the cytosol and mitochondria, thereby contributing to the maintenance of normal mitochondrial activity.

Lastly, there may also be some genetic influences with respect to free-radical formation and suppression as well as the inflammatory process. Some nutrients mediate the expression of genes encoding components of the inflammation process. These include the unsaturated fatty acids. There is accumulating evidence in both humans and animal models that indicates that PUFAs of the omega-3 family can affect gene expression (see Chapter 5). They regulate two groups of transcription factors: sterol-regulatory element binding proteins and peroxisome-proliferator-activated receptors (PPARs).[123] There may also be polymorphisms in the codes for components of the inflammatory process that are related to the development of chronic disease, and the expression of these polymorphisms may be nutrient related

Milk and dairy foods have a number of components that taken separately (as discussed above) could have effects on inflammatory disease as well as oxidative stress. Unfortunately, studies using whole foods are lacking in the literature. Milk fat for example contains ~66% of its fat as saturated fat, ~2% as linoleic, ~2% α-linolenic, and about 30% as monounsaturated fat in addition to ~0.5% cholesterol. Milk fat also contains a small amount of trans fat that appears in the fat as a result of biohydrogenation of pasture and feed linoleic and linolenic acids by the rumen microorganisms. The trans fat is trans-11-18:1 conjugated linoleic acid as found in milk fat. This trans fat seems to have little relationship to any of the degenerative diseases usually associated with the consumption of trans fat found in hydrogenated plant fats such as margarine.[1] Milk is also an excellent source of essential vitamins and minerals and these micronutrients play roles as described above in

suppressing free-radical formation and the inflammatory response. Data are lacking as to their specific roles in these processes as components of whole foods (i.e., dairy foods). Lastly, as described earlier, milk contains some valuable proteins that have important nutritional functions. Calcium absorption, for example, is facilitated by milk proteins. Integrating the functions of all of these components with respect to chronic disease that is related to oxidative stress and inflammation has been difficult and is constantly changing.

Aging and Free Radicals

Aging is characterized by a progressive decline in the biochemical and physiological functions of various organs in an individual. It has been proposed that the process of aging is related, in part, to the accumulated effects of free-radical damage to cell membranes and to DNA. Although cells have developed various enzymatic and nonenzymatic systems to protect them from damage by these free radicals, a fraction of these may escape the cellular defense mechanisms and cause permanent or transient damage to proteins, lipids, and nucleic acids. The free-radical attack of mitochondrial DNA (mtDNA) could be involved in these pathological conditions because mitochondria are the major source of free radicals in cells. There are several reasons this occurs. The mtDNA is naked (has no protective histone coat), it is compact, and is preferentially attached to the mitochondrial inner membrane, which is the major site of lipid peroxidation in the mitochondria.

Free-radical damage can result in large-scale deletions in the mtDNA of various tissues. A study by Wei[124] of aged humans revealed various deletion mutations in this genome. Base substitutions in this genome were found as well. With age, mitochondrial respiratory function declines in a variety of tissues coincident with rising levels of lipid peroxides and evidence of cumulative mtDNA damage. Although the amount of mutated DNA is quite small, these data do support the notion that free-radical damage could be involved in the aging process. Of course, there are so many mitochondria in each cell (200–20,000, depending on cell type), and each mitochondrion has 8–10 copies of its genome, that the damage reported in this aging study could be rather trivial. In contrast, free-radical damage to the nuclear DNA, if unrepaired, could be more serious.

Marine Oils and Health Concerns

A number of years ago, a group of Danish scientists compared the food intake and health status of Danes and Greenland Eskimos.[125–126] Both populations consumed high-protein, high-fat diets; however, whereas the Danish diet included a variety of milk and meat products, the Eskimo diet included primarily fish and marine creatures such as whale, walrus, seal, and so forth. These marine foods contain fat that is rich in the omega-3 fatty acids such as linolenic, eicosapentanoic, and docosahexanoic acids. The P:S ratio of the Greenland Eskimo diet was 0.84 compared with 0.24 for the Danes. The Eskimo diet contained more fatty acids with a single double bond than did the Danish diet. The Eskimo diet also contained significant amounts of long-chain fatty acids having 5 or 6 double bonds.

The Danish investigators observed these diet differences between Danes and Eskimos and also noticed the differences in blood lipid profiles, as well as differences in the incidence of CVD. Although CVD was one of the leading causes of death among the Danes, the leading cause of death in Eskimos was cerebral hemorrhage (stroke). The Eskimos had prolonged bleeding times, and a nosebleed was a serious problem. The prolonged clotting time was probably due to the omega-3 fatty acid effect on those eicosanoids involved in clot formation. These death statistics considered age-matched groups, but were not age-adjusted. That is, the investigators compared the two populations using age-matched groups without correcting for total population longevity or for early death due to communicable diseases, malnutrition (in infants particularly), or the hazards of daily life. For the Eskimo, these factors could have been quite important. Furthermore, one must realize that all causes must add up to 100%. Thus, if fewer die of CVD, more may have died from communicable

disease or something else. Nonetheless, the findings of the Danes regarding omega-3 fatty acid intake and CVD set off a whole flurry of animal and human studies directed toward understanding how the omega-3 fatty acids affected metabolism.

The observations on marine oils and CVD have stimulated considerable research on the responses of man and animals to the inclusion of marine oils in the diet.[127] Rats, mice, swine, monkeys, and rabbits have been studied in addition to normal humans and humans having a variety of diseases thought to benefit from the consumption of these oils.[128] Whether the inclusion of omega-3 fatty acids in the diets of hyperlipidemic subjects was of benefit was tested by Phillipson et al.[129] Twenty hyperlipemic subjects were studied. Ten of these subjects had type IIb lipemia (cholesterol range: 238–411 mg/dL, triglyceride range: 198–720 mg/dL) and 10 had type V lipemia (cholesterol range: 274–840 mg/dL, triglyceride range: 896–5775 mg/dL). The subjects consumed either a corn-oil-based or a fish-oil-based diet or a control diet for 4 weeks each. In the type IIb group, fish oil consumption resulted in a 27% decrease in plasma cholesterol and a 64% decrease in triglyceride. In the type V group, the reductions were 45% and 79%, respectively. In the type V group, the consumption of the corn-oil diet resulted in a significant rise in plasma triglyceride levels. These and other investigators reported that fish oil consumption resulted in a decrease in VLDL levels in the blood.

Not only has there been interest in the relationship of dietary omega-3 fatty acids to CVD, there has also been interest in its relationship to diabetes. People with diabetes mellitus have five to six times the risk of people without diabetes of having CVD. Diabetes and heart disease are closely related.

With respect to marine oils and diabetes, Feldman et al.[130] studied two groups of Alaskan Eskimos. One group consumed a typical western diet, whereas the other consumed the traditional Alaskan Eskimo diet which, like the Greenland Eskimo diet, is high in marine foods and low in total carbohydrates as well as sugar. As found in the Greenland Eskimos, serum lipids were very low and glucose tolerance normal, when the traditional diet was consumed. When the Eskimos consumed western diets, which are higher in carbohydrate, particularly sugar, they had significantly higher free fatty acid and triglyceride levels, as well as minor abnormalities in their glucose tolerance. Although Alaskan Eskimos, in general, have a very low prevalence of diabetes, these findings suggest that as these Eskimos begin to include significant amounts of carbohydrate, particularly sweets, in their diet, the prevalence of diabetes may change. In addition, because the Eskimos studied by Feldman consumed significant quantities of marine foods, it might also be inferred that the development of glucose intolerance was genetically controlled and unaffected by the presence of omega-3 fatty acids in the diet.

All of the observations of the differential effects of different fatty acids on metabolism prompted nutrition scientists to investigate the possible benefits of consuming foods rich in omega-3 fatty acids. Such foods include ocean fish, marine mammals, and some plant foods. Some vegetable oils such as primrose, canola, wheat germ, linseed, and walnut also contain significant amounts of these fatty acids. Small amounts are also found in a number of other foods such as spinach, certain margarines, broccoli, and lettuce. Hens fed omega-3 fatty acid-rich fats will lay eggs containing these fatty acids in the yolks.

Fish oil in the diet easily oxidizes and forms peroxides. These peroxides are what gives these oils their peculiar and often objectionable odor. Diets containing large amounts of these oils are not as well liked by experimental animals and appetite is suppressed. Food intake may be decreased by as much as 20%. Even when the scientist takes stringent care of the diet by preventing autooxidation, the food intake of fat-rich diets is reduced. Despite a reduction in food intake, animals utilize their diet very well, and a marine-oil diet usually is characterized by an increase in feed efficiency. That is, the animal will gain more weight per unit of food consumed than an animal consuming a diet containing some other fat source. This is not always true, however. Genetically obese rats and mice gain less weight when fed a marine-oil diet than when fed a corn oil or tallow or safflower-oil diet.

At any rate, a decrease in food consumption despite an increase in feed efficiency means that there is less food for the liver to metabolize and convert to VLDL for transport to the peripheral fat depots for storage, and, in part, this explains the effect of the marine oils on serum lipid levels.

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Dietary marine oils have two other equally important effects that can also explain their serum-lipid-lowering action. The first of these is an inhibition of hepatic fatty acid, phospholipid, and cholesterol synthesis. In part, this attenuation of lipid synthesis by marine oils occurs at the level of specific genes that encode for the lipogenic enzymes. The transcription of genes encoded for enzymes necessary for lipogenesis is suppressed in hepatic tissue from rats fed the marine oils. Although we realize that enzymes are never fully active in vivo and that the amount of enzyme may not fully predict the amount of product (in this case lipid) produced, these findings do contribute to our understanding of how dietary marine oils can lower serum lipid levels.

Added to the decrease in lipogenic enzyme amount and activity, and decreased rates of lipid synthesis observed in animals fed marine oils, is the observation that marine-oil-fed animals also have a reduced hepatic lipid output. This means that lipids formed in the liver are not as readily exported, and the liver of the animal fed the marine oils contains more fat than the liver of the rat fed a control diet. Reduced hepatic lipid output by humans consuming fish oil supplements has been reported. This, too, results in a lowering of serum triglycerides and serum cholesterol. Accumulated lipids in the liver may be an additional reason that lipid synthesis is downregulated. Product inhibition—in this case newly synthesized lipid—is a well-recognized metabolic-control mechanism.

Consumption of omega-3 fatty acids nonetheless does result in lower blood lipid levels, and this effect is presumed to explain the lower incidence of CVD in the Eskimos. Whether this presumption is correct cannot be taken for granted. CVD has a complicated pathophysiology that is not well understood. Genetic factors as well as lifestyle choices can influence its development and can determine whether it is a life-threatening condition.

LIPIDS AND MEMBRANE FUNCTION

Biological membranes contain a large number of lipids, proteins, lipid–protein complexes, glycolipids, and glycoproteins. The arrangement of these many compounds within the membrane structure has been studied extensively.

The membranes exist as a lipid bilayer because the phospholipids have amphipathic characteristics. They have both polar (the phosphorylated substituent at carbon 3) and nonpolar (the fatty acids) regions. The polar region is hydrophilic and is positioned such that it is in contact with the aqueous media around and within the cells. The nonpolar or fatty acid region is oriented toward the center of the bilayer so that it is protected from contact with the contents of the cell and the fluids that surround it. Figure 8.13 shows the arrangements of the polar phospholipids that are oriented as described with the membrane proteins embedded in this bilayer. Some proteins sit on the exterior aspect of the bilayer, other proteins rest on the interior aspect, whereas additional proteins extend through the bilayer.

The ability of these amphipathic compounds to self-assemble into a bilayer can be demonstrated in vitro. Lipid vesicles can be made by the addition of these phospholipids to water. A lipid bilayer

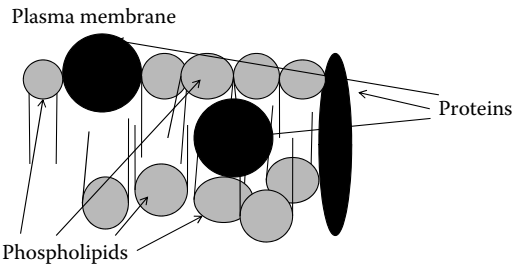


FIGURE 8.13 A schematic view of a plasma membrane.

will form just as described above. This feature of the phospholipids is consistent with one of the many roles a membrane serves. It is a permeability barrier for the cells and cell compartments. The lipid bilayer forms the matrix into which specific proteins are placed. Each of the individual phospholipids and the cholesterol provide specific regional characteristics that satisfy the insertion requirements of each of the many membrane proteins. The lipid bilayer serves as a seal around these membrane proteins and thus prevents nonspecific leakage. These lipids also serve to maintain the proteins in their most appropriate functional conformations. The polar position of the phospholipids satisfies the requirements for the electrostatic charge that is needed for the surface associations of specific cell surface proteins. All of these characteristics are needed and are critical to normal cell function. For example, an intact permeability barrier to sodium, potassium, calcium, and hydrogen ions is needed so that electrochemical gradients, which in turn drive other membrane transport processes, are maintained.

Cell membranes usually work best when their lipids are in the liquid crystal state. This means that there are regional differences in the physical state of the lipid. Some portions may be fairly fluid, whereas others may be fairly solid. The localized difference in physical state or fluidity has to do with the chain length and saturation of the fatty acids attached at carbons 1 and 2 of the phospholipid; it is this portion of the molecule that extends into the center of the bilayer. Membranes whose phospholipid fatty acids are saturated are less fluid than those membranes containing polyunsaturated fatty acids in their phospholipids. Even within a membrane there can be regional differences in fluidity due to the nature of the fatty acids in the phospholipids of that region. Differences in fluidity are due not only to the ratio of saturated fatty acids to unsaturated fatty acids, but also to the ratio of cholesterol to fatty acids. This ratio varies according to the location of the membrane. Plasma membranes, for example, contain more cholesterol than do mitochondrial membranes.

Membrane Phospholipid Composition

There are three major classes of lipids in membranes: glycolipids, cholesterol, and phospholipids. The glycolipids have a role in the cell surface-associated antigens, whereas the cholesterol serves to regulate fluidity. The phospholipids have fatty acids attached at carbons 1 and 2. It is usual to find a saturated fatty acid attached at carbon 1 and an unsaturated fatty acid at carbon 2. In addition, phosphatidyl ethanolamine and phosphatidyl serine usually have fatty acids that are more unsaturated than phosphatidylinositol and phosphatidylcholine. Less than 10% of the membrane phospholipid is phosphatidylinositol. Plasma membranes have no cardiolipin and the mitochondrial membranes have very little phosphatidylserine. Several of these phospholipids have important roles in the signal transduction processes that mediate the action of a variety of hormones. Phosphatidylinositol and its role in the phosphatidylinositol cycle (PIP cycle) is one of the most important.[131,132] Phosphatidylcholine and phosphatidylethanolamine also play roles in these systems. The PIP cycle is shown in Chapter 10 (Water-Soluble Vitamins, Inositol). Its importance relates to the action of inositol-1,4,5-phosphate in moving the calcium ion from its intracellular store to where it can stimulate protein kinase C. Phosphatidylinositol also serves to anchor glycoproteins to the membrane. Glycoproteins are tethered to the external aspect of the plasma membrane and play a role in the cell recognition process. Pathogens and foreign proteins are recognized by these structures.

Disease Effects on Membrane Lipids

There are numerous reviews on the effects of diet and disease on the fatty acid content of membrane fatty acids.[48] Whereas the primary focus has been on the abnormalities of the proteins in the membrane lipid bilayer, there is a role for the lipid in some of these disorders. Muscular dystrophy and multiple sclerosis are characterized by changes in the lipid structure of the membrane. In the former, the change consists of an increase in the amount of lysophosphatidyl choline and cardiolipin. In multiple sclerosis there is a degeneration of the myelin of both central and peripheral nerves. The

myelin is 75% lipid and 25% protein. Although the disease could be attributed to a specific abnormality in either component, there are reports that the lipid contains about 27% fatty acid (palmitate and oleate) in a covalent linkage, and that multiple sclerosis is associated with a derangement in this association and a reduction in the amount of phosphatidylserine. There have been many reports of these lipid changes or a lack of change in myelin; however, it is generally agreed that the disease is not primary to the lipid portion of the membrane. Rather, it is the specific myelin protein that somehow becomes abnormal. Such other diseases as renal disease, hepatic disease, ethanol intoxication, spur cell anemia, and diabetes likewise result in secondary effects on the membrane lipids. These changes can, however, make the disease worse by compromising the functionality of the membrane and its role in metabolic regulation. In humans with cirrhotic liver disease, the erythrocyte membrane fatty acids change. In cirrhotic subjects, the membranes contain less phosphatidyl ethanolamine and more phosphatidyl choline than membranes from normal subjects. In these subjects, the membrane cholesterol and fatty acid content remains unchanged, but the ratio of cholesterol to phospholipid increases.

Hormonal Effects on Membrane Lipids

The hormone insulin can affect the fatty acid profile of the membrane phospholipids through its effect on glucose conversion to fatty acids and through its effect on the desaturases. Other hormones have an influence on this profile as well. Examples of this influence are shown in Table 8.11. Daily injections of the synthetic glucocorticoid, dexamethasone, resulted in an increase in the mole percent of linoleic (18:2) acid and a decrease in arachidonic acid (20:4). Thyroidectomy resulted in a small increase in rat liver mitochondrial levels of 18:2 and 20:4. This is probably due to the reduction in fatty acid turnover that occurs in the absence of the thyroid gland. Hypophysectomy, which causes a decrease in growth hormone levels, resulted in an increase in 18:2 and a decrease in 20:4 in hepatic mitochondria. Studies on the influence of all the many hormones that affect fatty acid synthesis, phospholipid synthesis, and membrane phospholipid fatty acid levels are not as readily available as are reports on the dietary-fat effects on these parameters. However, they are of interest because these hormones may also have a considerable influence on the function of the protein components that are embedded in the various membranes. These hormones may act directly on the synthesis and activation of these proteins, which in turn may affect their conformation and hence their activity.

TABLE 8.11
Effects of glucocorticoid or thyroid deficiency or diabetes on fatty acid profiles of liver or isolated liver mitochondria

Treatment	Fatty acids (mol %)						Tissue
	16:0	18:0	18:1	18:2	20:4	22:6	
1 mg GC[a]/day	14.6	22.7	6.6	28.5	19.0	3.5	Liver
Control	17.0	20.3	7.61	5.12	7.4	1.5	Liver
Thyroidectomy	12.5	21	8.6	19.5	19.0	8.1	RLM
Control	13.0	20	9.0	17.4	15.7	7.0	RLM
Hypophysectomy	27.1	21.5	13.5	17.0	14.5	2.0	RLM
Control	27.4	22	12.2	12.1	17.4	2.8	RLM
Diabetes[b]	24.5	23.3	7.02	2.4	15.2	4.2	RLM
Control	16.9	23.2	9.3	22.7	22.2	3.1	RLM

[a] GC, synthetic glucocorticoid, dexamethasone; RLM, rat liver mitochondria.
[b] Streptozotocin-induced diabetes.

Thyroxine, for example, negatively affects the activity of the Δ9 desaturase, which, because it is less active, results in fewer unsaturated fatty acids in the membrane. In turn, microsomal cytochrome b_5 activity is less dependent on the lipid environment. That is, it must be surrounded by a very fluid lipid, and this lipid must have a number of unsaturated fatty acids in it. Just as thyroidectomy results in a decrease in membrane phospholipid fatty acid unsaturation, hyperthyroidism has the reverse effect. The thyroid hormones also affect fatty-acid elongation by inducing an increase in the activity of the microsomal fatty-acid elongation system, although having little effect on mitochondrial elongation. It has been reported that the incorporation of labeled choline into brain and liver phosphatidylcholine was less in thyrotoxic rats than in normal rats.

Age Effects on Membrane Lipids

As animals age, their hormonal status changes, as does the lipid component of their membranes. With age, there is a decrease in growth-hormone production, an increase followed by a decrease in the hormones for reproduction, and, as the animal ages, larger fat stores. Larger fat cells are resistant to insulin and insulin levels may rise as a result of increased fat cell size (insulin resistance). As mentioned in the preceding section, these hormones can affect the lipid portion of the membranes within and around the cells and hence affect how these cells regulate their metabolism. With age, the degree of unsaturation of the membrane fatty acids decreases and the cholesterol level rises. There is also an increase in the number of superoxide radicals. This increase may be responsible for the degradation of the membrane lipids, which, in turn, might explain the age-related changes in membrane function. Membranes from aging animals are less fluid and have reduced transport capacities. As animals age, there is a decline in hepatic mitochondrial respiratory rate and a decrease in the respiratory control ratio and the ADP/O ratio. In addition, there are reports of an age-related decrease in membrane fatty acid unsaturation coupled with a decrease in membrane fluidity and a decrease in the exchange of ATP for ADP across the mitochondrial membrane; a decrease in ATP synthesis; and an amelioration of these age-related decreases in mitochondrial function by restricted feeding.

Membrane Function

In the previous section, the importance of diet, age, and hormonal status was described in terms of their influence on the composition of the membrane lipids. Although not emphasized, these compositional differences have important effects on metabolic regulation. This regulation consists of the control of the flux of nutrients, substrates, and/or products into, out of, and between the various compartments of the cell.

The cellular membranes serve as the "gatekeepers" of the cells and their compartments. They regulate the influx and efflux of nutrients, substrates, hormones, and metabolic products produced or used by the cell or compartment in the course of its metabolic activity. For example, the mitochondrial membrane, through its transport of 2- and 4-carbon intermediates and through its exchange of ADP for ATP, regulates the activity of the respiratory chain and ATP synthesis. If too little ADP enters the mitochondria because of decreased ADP transport across the mitochondrial membrane, respiratory chain activity will decrease, less ATP will be synthesized, and there may be a decrease in other mitochondrial reactions that are either driven by ADP influx or dependent on ATP availability. Through its export of citrate from the matrix of the mitochondria, ATP regulates the availability of citrate to the cytosol for cleavage into oxaloacetate and acetyl CoA, the beginning of fatty acid synthesis. If more citrate is exported from the mitochondria than can be split to oxaloacetate and acetyl CoA, this citrate will feed back onto the phosphofructokinase reaction, and glycolysis will be inhibited. Thus, the activity of the mitochondrial membrane tricarboxylate transporter has a role in the control of cytosolic metabolism.

Other transporters such as the dicarboxylate transporter or the adenine nucleotide translocase have similar responsibilities vis à vis the control of cytosolic and mitochondrial metabolic activity. In the plasma membrane, receptors embedded in the membrane have a similar function. That is, they control the entry of nutrients or hormones into the cell. Further, the plasma membrane hormone receptor may bind a given hormone and, with binding, elicit a cascade of reactions characteristic of the hormone effect without permitting the entry of the hormone itself into the cystosolic compartment. An example here is the hormone insulin. Insulin binds to its receptor and, in so doing, elicits the cascade of events that include the transport and metabolism of glucose by the cell. The insulin, bound to the receptor site, is inactivated and is brought into the cell by pinocytosis for further degradation. Other hormones, notably the nonprotein steroids and the low-molecular-weight hormones such as epinephrine and thyroxine, pass through the plasma membrane and attach to receptors in the cytosol or nuclear membrane or on the endoplasmic reticulum. Once attached to their respective binding sites, they also elicit a metabolic response.

In the membranes are a variety of closely packed proteins and lipids. The membrane-bound proteins have extensive hydrophobic regions and usually require lipids for the maintenance of their activity. Adenylate cyclase, cytochrome b_5, and cytochrome c oxidase have all been shown to have phospholipid affinities. Cytochrome c oxidase from mitochondria has tightly bound aldehyde lipid that cannot be removed without destroying its activity as the enzyme that transfers electrons to molecular oxygen in the final step of respiration. A number of other membrane proteins have tightly bound fatty acids as part of their structures. These fatty acids are covalently bound to their proteins as a posttranslational event and act to direct, insert, and anchor the proteins in the cell membranes. Other lipids are bound differently to membrane proteins. Some are acylated with fatty acids during their passage from their site of synthesis on the rough endoplasmic reticulum to the membrane, whereas others acquire their lipid component during their placement in the membrane. β-Hydroxybutyrate dehydrogenase, for example, requires the choline head of phosphatidylcholine for its activity. If hepatocytes are caused to increase their synthesis of phosphatidyl-methylethanolamine, which substitutes for phosphatidylcholine in the membrane, β-hydroxybutyrate dehydrogenase activity is reduced.

All of these examples illustrate the importance of the cellular and intracellular membranes in the regulation of metabolism. They illustrate the fact that the gate-keeping property of the membrane is vested in the structure and function of the various transporters and receptors or binding proteins embedded in the membrane. Whereas the genetic heritage of an individual determines the amino acid sequence of the proteins and hence their function, this function can be modified by the lipid milieu in which they exist. Diet, hormonal state, and genetics in turn control the lipid milieu in terms of the kinds and amounts of the different lipids that are synthesized within the cell and incorporated into the membrane. Membranes differ in the amount of cholesterol they contain; mitochondrial membranes contain very little whereas plasma membranes contain somewhat more.

CANCER AND DIETARY FAT

The many different forms of cancer account for the second-largest number of deaths due to disease in developed nations. It, too, is probably influenced by the fat intake but in these disorders, it is not the saturated fat that is culpable.[132] Studies on the development of mammary tumors in rats and mice have shown that high intakes of polyunsaturated fat containing the omega-6 fatty acids promote the development of cancer. Carcinogenesis is thought to occur in two steps. The first is the initiation step. This concerns the conversion of normal DNA to the DNA typical of a cancer cell. Large segments of the gene code are deleted or so changed that the translation products are either not present or are not normal in function. This conversion can be instigated by chemicals such as 7,12-dimethylbenz(*a*)anthracene (DMBA), by viruses, radiation, and perhaps oxidized (free radical) food components. By itself, this initiation step will not progress to the metastatic cell unless the next step, the promotion step, also occurs. Cells whose DNA has been injured will and can repair this DNA. However, if the "right" environment is provided, this does not occur and a metastatic cell is

produced and reproduced. Dietary corn oil has been shown to be a very good promoter of DMBA-induced breast cancer in rats and mice, whereas dietary beef tallow is not. How corn oil has this effect is not known and, of course, cause-and-effect studies cannot be conducted in humans. Just as epidemiological studies cannot provide definitive proof of the role of diet in CVD development, the same is true for cancer. Both the level of intake and the type of fat have been implicated but strong proof is lacking. As with CVD and/or diabetes and/or obesity, the genetic influence is quite strong.

OTHER DISEASES

No discussion of the role of dietary fat in human disease would be complete without the mention of obesity (see Chapter 1). As discussed in the chapter on energy, this disorder has a multiplicity of causes of which the total energy intake is but a part. The excess energy consumed could easily be provided by the dietary fat because it has twice the energy value per gram as does dietary carbohydrate and protein. However, intake alone does not fully explain the obese state. Genetic factors that control energy intake, storage, and use are also important.

Gall bladder disease is frequently associated with excess body fatness (or its loss) and, as explained in the section on absorption, may be attributable to the precipitation of cholesterol from the bile while in the gall bladder. Other genetic diseases in addition to those previously described with respect to lipid transport, due to mutations in genes for specific enzymes exist. Many are associated with mental retardation and early death. None seem to be related to nutrition or dietary fat intake.

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9 Fat-Soluble Vitamins

There are four vitamins that are soluble only in fat solvents and not in water. As such, these vitamins are found in the lipid extracts of tissues and foods. They were named alphabetically in the order of their discovery. The National Academy of Sciences Food and Nutrition Board has developed Daily Dietary Recommended Intakes (DRIs) for vitamins A and E, shown in Table 9.1, and has proposed adequate intakes (AI*) for vitamins D and K. The vitamin A DRI is given as retinol equivalents (RAE). One RAE equals 1 μg retinol, 12 μg β-carotene, 24 μg α-carotene or 24 μg β-cryptoxanthin. The DRI for vitamin E is as α-tocopherol which includes RRR-α-tocopherol and the 2R-stereoisomeric forms of α-tocopherol that occurs in fortified foods. There are recommended adequate intakes (but no DRIs) for infants because there are not enough data to support a DRI recommendation. This is also true for vitamins D and K. In Table 9.1 it can be seen that only adequate intake recommendations are provided for these vitamins. Again, this is because the Food and Nutrition Board felt that there were insufficient data to support a DRI but that there were enough to make an adequate intake recommendation.

VITAMIN A

Vitamin A was the first vitamin identified as an essential micronutrient needed by humans.[1,2] Although it has been recognized as a chemical entity for about 70 years, foods rich in this vitamin have long been prescribed as treatment for night blindness. Ancient Egyptian physicians recommended the consumption of ox or chicken liver for people unable to see at night. In India it was recognized that inadequate diets were related to night blindness and, in France, that diets consisting of sugar, starch, olive oil, and wheat gluten fed to animals resulted in ulcerated corneas. Mori, in Japan, reported on the curative power of cod-liver oil in the treatment of conjunctivitis and, later, Hopkins, in the United States, reported on the importance of whole milk in such treatments. During the second decade of the 20th century, Osborne and Mendel at Yale and McCollum's group in Wisconsin identified the substance in cod-liver oil, egg yolk, and butterfat that cured night blindness and that was essential for normal growth. They called this substance "fat soluble A."

Structure and Nomenclature

Vitamin A is not a single compound. It exists in several forms and is found in a variety of foods such as liver and highly colored vegetables.[1] The IUPAC-IUB Commission on Biochemical Nomenclature has proposed the following rules for naming the compounds having vitamin A activity. The parent substance, all-*trans* vitamin A alcohol, is designated "all-*trans* retinol." Derivatives of this compound are named accordingly. In Table 9.2 are listed the major vitamin A compounds.

In foods of animal origin the vitamin usually occurs as the alcohol (retinol). However, it can also occur as an aldehyde (retinal) or as an acid (retinoic acid). In foods of plant origin, the vitamin is associated with the plant pigments and is a member of the carotene family of compounds. These compounds can be converted to vitamin A in the animal body and are known as *provitamins*. Of the carotenes, β-carotene is the most potent. Figure 9.1 gives the structure of vitamin A as all-*trans* retinol. Some of the biologically important compounds having vitamin A activity are shown in Figure 9.2.

TABLE 9.1
Daily dietary recommended intakes (DRI) and adequate intake recommendations for fat-soluble vitamins

Group	Vitamin A (μg)	Vitamin E (mg)	Vitamin D (μg)	Vitamin K (μg)
Infants				
0–6 months	400[a]	4[a]	5[a]	2[a]
7–12 months	500[a]	5[a]	5[a]	2.5[a]
Children				
1–3 years	**210**	**5**	5[a]	30[a]
4–8 years	**275**	**6**	5[a]	55[a]
Males				
9–13 years	**445**	**9**	5[a]	60[a]
14–18 years	**630**	**12**	5[a]	75[a]
19–30 years	**625**	**12**	5[a]	120[a]
31–50 years	**625**	**12**	5[a]	120[a]
51–70 years	**625**	**12**	10[a]	120[a]
70+ years	**625**	**12**	15[a]	120[a]
Females				
9–13 years	**420**	**9**	5[a]	60[a]
14–18 years	**485**	**12**	5[a]	75[a]
19–30 years	**500**	**12**	5[a]	90[a]
31–50 years	**500**	**12**	5[a]	90[a]
51–70 years	**500**	**12**	10[a]	90[a]
70+ years	**500**	**12**	15[a]	90[a]
Pregnancy				
14–18 years	**530**	**12**	5[a]	75[a]
19–30 years	**550**	**12**	5[a]	90[a]
31–50 years	**550**	**12**	5[a]	90[a]
Lactation				
14–18 years	**885**	**16**	5[a]	75[a]
19–30 years	**900**	**16**	5[a]	90[a]
31–50 years	900	16	5[a]	90[a]

Note: Figures in bold represent the DRI.
[a] Adequate intakes.
Source: www.nap.edu, accessed 8/22/07.

Note that all of these compounds have a β-ionone ring to which an isoprenoid chain is attached. This structure is essential if a compound is to have vitamin activity. If any substitutions to the chain or ring occur, then the activity of the compound as vitamin A is reduced. For example, the substitution of methyl groups for the hydrogen on carbon 15 of the side chain results in a derivative that has no vitamin activity. However, the preparation of a methyl ester or other esters at carbon 15 results in a very stable compound with full vitamin activity. In addition to improving the chemical stability of the compound, the ester forms confer an improved solubility in food oils. These vitamin ester forms are frequently used in food products for vitamin enrichment.

TABLE 9.2
Nomenclature of major compounds in the vitamin A group[1]

Recommended name	Synonyms
Retinol	Vitamin A alcohol, axerophthol
Retinal	Vitamin A aldehyde, retinene, retinaldehyde
Retinoic acid	Vitamin A acid
3-Dehydroretinal	Vitamin A_2 (alcohol)
3-Dehydroretinol	Vitamin A_2 aldehyde; retinene
3-Dehydroretinoic acid	Vitamin A_2 acid
Anhydroretinol	Anhydro vitamin A
retro Retinol	Rehydrovitamin A
5,6-Epoxyretinol	5,6-Epoxyvitamin A alcohol
Retinyl palmitate	Vitamin A palmitate
Retinyl acetate	Vitamin A acetate
Retinyl β-glucuronide	Vitamin A acid β-glucuronide
11-*cis*-Retinaldehyde	11-*cis* or Neo b vitamin A aldehyde
4-Ketoretinol	4-Keto vitamin A alcohol
Retinyl phosphate	Vitamin A phosphate
β-Carotene	Provitamin A
α-Carotene	Provitamin A
γ-Carotene	Provitamin A

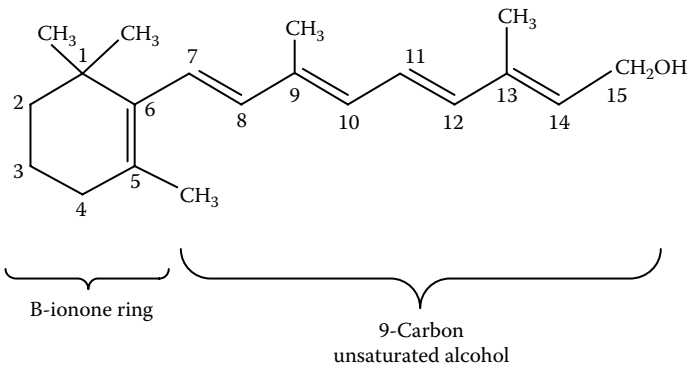
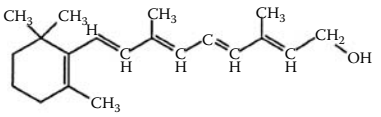
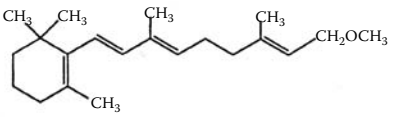
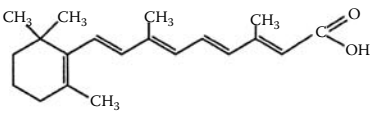


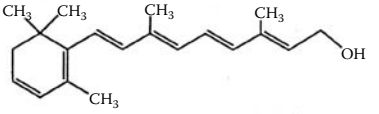
FIGURE 9.1 Structure of vitamin A.

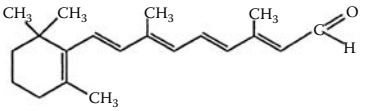
If the side chain is lengthened or shortened, vitamin activity is lost. Activity is also reduced if the unsaturations are converted to saturated bonds or if the side chain is isomerized. Oxidation of the β-ionone ring and/or removal of its methyl groups likewise reduces its vitamin activity. Some of these substituted or isomerized forms are potent therapeutic agents. For example, 13-*cis*-retinoic acid has been used in the treatment of certain kinds of cancer. Other analogs, notably the fluro and chloro derivatives, have been synthesized with the hope of providing chemotherapeutic agents for the treatment of certain skin diseases and cancer.

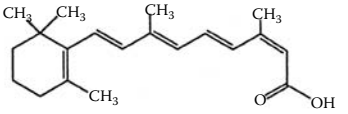

All-*trans* Retinol

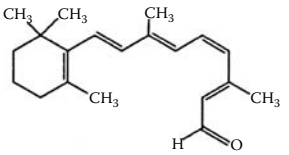

Retinyl methyl ether

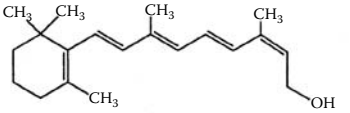

All-*trans* Retinoic acid


All-*trans* 3-Dehydroretinol


All-*trans* Retinal


13-*cis*-Retinoic acid


11-*cis* Retinal


13-*cis* Retinol

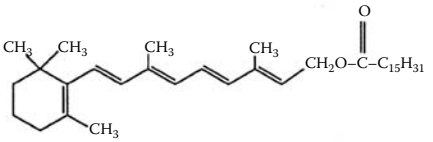

Retinyl Palmitate

FIGURE 9.2 Structures of the various vitamin A compounds.

The provitamin A group consists of members of the carotene family.[3] In plants, these compounds serve as photoprotectors of photosynthesis.[4] Shown in Figure 9.3 are the structures of some of these compounds. Also shown are structures of related compounds that, although highly colored, have little potential as a precursor of retinol. More than 600 members of the carotenoid family of pigments exist. However, only 50 or about 10%[5] can be converted (or degraded) into components that have vitamin activity.[6] All these compounds have many conjugated double bonds and thus each can form a variety of geometric isomers. β-carotene, for example, can assume a *cis* or a *trans* configuration at each of its double bonds and in theory could have 272 isomeric forms. The asymmetric carotene, α-carotene, can in theory appear in 512 forms. The vitamin A activity of the provitamin members of the carotene family is variable. Theoretically, β-carotene should provide two molecules of retinol. However, in living systems this does not always happen. The β-carotene

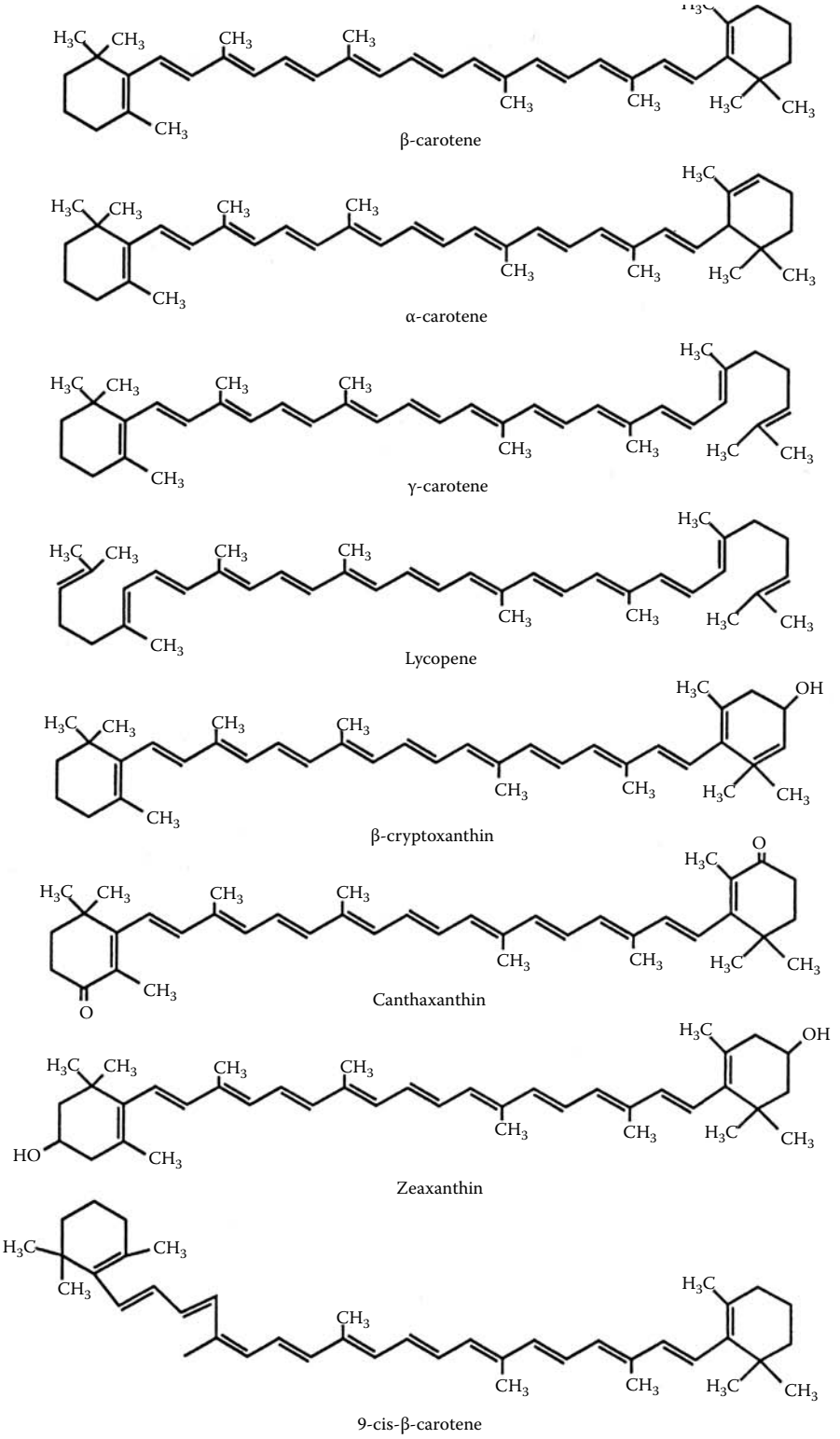


FIGURE 9.3 Structures of carotenes having vitamin A activity.

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content of food varies with the growing conditions and the postharvest storage of the food. In addition, the digestibility of the food affects the availability of the vitamin. Even when fully available, β-carotene and other provitamin A compounds may not be absorbed efficiently and, further, the enzyme responsible for cleaving the β-carotene into two equal parts may not be active. In general, the β-carotene molecule will provide about 50% of its quantity as vitamin A.[7] During its cleavage by the enzyme, β-carotenoid-15,15′-dioxygenase, there is some oxidative conversion of the cleavage product to retinal and some oxidation to retinoic acid. This retinoic acid is rapidly excreted in the urine. Other carotenes are less potent than β-carotene, due not only to a decrease in their absorption, but also due to their chemical structures, which do not meet the requirements described above for vitamin activity. These compounds are listed in Table 9.3. Note that some of these compounds (i.e., xanthophylls and lycopenes) have no vitamin activity even though they are highly colored and are related chemically to β-carotene. They may have other functional properties, however, and may have some usefulness in the human diet.

Chemical Properties

Through the careful work of Karrer and his associates, the structures of both β-carotene and all-*trans* retinol were determined. It was only after this work was completed that it was realized that β-carotene was a precursor for retinol.[1]

With the structures known, the next step was the crystallization of the compounds. This was accomplished for vitamin A from the lipid fraction of fish liver by Holmes and Corbet. Ten years later, in 1947, Arens and van Drop and also Isler et al. were able to synthesize pure all-*trans* retinol. The chemical synthesis of β-carotene was achieved shortly thereafter. With the crystallization, structure identification, and synthesis came the understanding of the physical and chemical properties of these compounds and the development of techniques to measure their presence in food. All-*trans* retinol is a nearly colorless oil and is soluble in such fat solvents as ether, ethanol, chloroform, and methanol. Although fairly stable to the moderate heat needed to cook foods, it is unstable to very high heat, to light, and to oxidation by oxidizing agents. Alpha-tocopherol or its acetate (vitamin E) through its role as an antioxidant, if present, can prevent some of this destruction of retinol.

The above properties of vitamin A allow for the removal of the vitamin from foods by fat solvent extraction and its subsequent determination using agents such as antimony trichloride (the Carr–Price reaction) that produce a blue color. The intensity of the color is directly proportional to the amount of retinol in the material being analyzed. More recently, the development of high resolution, high pressure chromatography (HPLC), the separation and quantification of each of the

TABLE 9.3
Carotenoids with vitamin A activity

Compound	Relative potency
β–Carotene[1]	100
α-Carotene	53
γ-Carotene	43
Cryptoxanthin	57
Lycopene	0
Zeaxanthin	0
Xanthophyll	0

Note: Reference compound for subsequent compounds. Observe that β-carotene compared to all-*trans* retinol is only half as active.

vitamers A has been made possible. β-Carotene is also soluble in such fat solvents as acetone or ethanol. It is bright yellow in color and it, too, is stable to moderate heat but unstable to light or oxidation. The members of the large carotenoid family can also be separated and quantified using high pressure liquid chromatography. Several isomers and derivatives (Figure 9.2) of retinol have been identified. The isomerization of all-*trans* retinol to a mixture of its isomers is enhanced by high temperatures, ultraviolet light and iodine or iron. Although the isomerization of all-*trans* retinol to 11-*cis* retinol is an important in vivo reaction for the maintenance of the visual cycle, the production of isomers outside of the body through the application of high heat, light and oxidizing agents results in a loss in biological activity of the original compounds. For example, retinyl acetate, if irradiated in hexane, forms a nonsymmetrical dimer (kitol) which has less than 1% of its original activity. This dimer has been isolated from whale liver oil, and some investigators have suggested that, in this species, the kitol could serve as a storage form of the vitamin. However, whether the kitol found in the whale liver was produced enzymatically or was consumed by the whale as a component of its food supply has not been determined. In any event, for most species, vitamin A is stored in the liver not as a kitol but as an ester with a fatty acid (usually palmitate) in a lipid–protein complex.

Retinal, the aldehyde, combines with various amines to form Schiff bases. This reaction is important to the formation of rhodopsin within the visual cycle. If mineral oil is present with retinal and the amine, the retinal may first form a hemiacetyl which then complexes with a variety of amines to form the Schiff base. All-*trans* retinal also reacts with both the sulfhydryl and amino groups of cysteine or the amino group of tryptophane to form a 5-membered thiazolidine ring. The formation of the thiazolidine ring is enhanced if formaldehyde is present. The resulting compound has a red color that is bleached upon exposure to light. This reaction, similar to the bleaching of rhodopsin (the visual pigment in the eye) with light exposure, does not utilize the same amino acid that is used in rhodopsin. In the latter, retinal binds to the epsilon amino group of lysine rather than the amino group of tryptophane or cysteine. Bleaching occurs when the energy transmitted by the light causes a shift in the electrons within the *N*-retinyl lysine. The same principle is involved in the loss of color with light exposure of *N*-retinyl cysteine and *N*-retinyl tryptophane. Interestingly, the many retinyl-amino acid complexes possible in the laboratory apparently do not form in the body. This is probably due to the limited amounts of both the free vitamin and the free amino acids. Both are usually bound or complexed to some other compound and are not readily available for this reaction.

In nature, vitamin A and carotene are bound to proteins, particularly the lipoproteins. Indeed, the vitamin is stored as an ester in those tissues (notably the liver) that synthesize the lipoproteins. Usually retinol is covalently bound to palmitic acid as well as being associated with a protein. The vitamin also complexes with other proteins as part of its mechanism of action at the subcellular level.

Retinol and its derivatives are soluble in nonpolar solvents, but not in water. However, retinol or retinyl esters can be made miscible in water with the use of detergents such as Tween 80 (polyoxyethylene sorbitan monoleate). The resulting aqueous micellar suspension allows for the preparation of aqueous multivitamin supplements, which are then useful for infants, small children, or persons unable to swallow a capsule. Studies of the absorption of vitamin A in this form, compared to the absorption of vitamins dissolved in an oil, have shown that this form of the vitamin is readily absorbed. This is particularly important for those persons having a fat absorption problem as in pancreatitis, celiac disease, idiopathic steatorrhea, bilary disease or short gut syndrome.

Aqueous preparations are also useful to the food processor wishing to fortify or enrich a particular food product. Some food formulations would not be compatible with an oil-based vitamin A preparation whereas an aqueous preparation combines very well with the ingredients in the food.

Biopotency

The varying potencies of the different vitamin A compounds have, in the past, contributed confusion to the literature. This has been resolved by the establishment of a standard definition for vitamin A. One international unit (IU) of vitamin A is defined as the activity of 0.3 μg of all-*trans* retinol. Because β-carotene must be converted to retinol to be active and because this conversion

is not 100% efficient, 1 μg of all-*trans* β-carotene is equivalent to 0.167 μg of all-*trans* retinol. For other members of the carotene family, appropriate (Tables 9.2 and 9.3) correction factors must be applied to determine the vitamin A activity in terms of retinol equivalents (RE). In general, however, 1 mg of retinol is roughly equivalent to 6 mg β-carotene and 12 mg of mixed dietary carotenes (Table 9.4). The use of the term retinol equivalents allows for the calculation of the vitamin A activity found in a variety of foods, each containing one or more vitamin A compounds. For example, if 100 g of a given food contained 100 mg of α-carotene that has 53% of the potency of β-carotene, the retinol equivalence would be 100 × .53 × 0.167 or 8.851. Thus, this food would provide 8.851 retinol equivalents or 29.5 IU of vitamin A (8.851/0.3) per 100 g. Most tables of food composition give the vitamin A content in terms of IU; however, the reader should be aware of how these units are derived and realize that corrections must be made to allow for availability and efficiency of conversion of the provitamin to the active vitamin form, all-*trans* retinol. Table 9.4 lists the retinol equivalents (REs) for major A vitamin sources.

Sources

Vitamin A and its carotene precursors are present in a variety of foods. Red meat, liver, whole milk, cheese, butter, and fortified margarine are but a few of the foods containing retinol. Carotene-rich foods include the highly colored fruits and vegetables such as squash, carrots, rich green vegetables (peas, beans, etc.), yellow fruits (peaches, apricots), and vegetable oils.

Metabolism of Vitamin A

Absorption. The availability of the vitamin depends largely on the form ingested and on the presence and activity of the system responsible for its uptake. Figure 9.4 schematically shows the route of retinol absorption. It is generally accepted that all-*trans* retinol is the preferred form for absorption. Retinal and retinoic acid are less well absorbed, although both will disappear from the intestinal contents at a rate commensurate to that of an active transport system. Absorption requires the presence of food lipid and bile salts, and the vitamin appears in the lymphatic system along with the absorbed lipids.[8] Carotenoids are absorbed less efficiently, via diffusion. There is no active carrier

TABLE 9.4
Retinol equivalents (RE) for humans[a]

Compound	μg/IU	IU/μg	RE/μg
All-*trans* retinol	0.300	3.33	1.00
All-*trans* retinyl acetate	0.344	2.91	—
All-*trans* retinyl palmitate	0.549	1.82	—
All-*trans* β-carotene	1.800	0.56	0.167
Mixed carotenoids	3.600	0.28	0.083

[a] Source: Olson J.A. (1991) In: *Handbook of Vitamins* (L.J. Machlin, ed.) Marcel Dekker, New York, pp. 1–57.

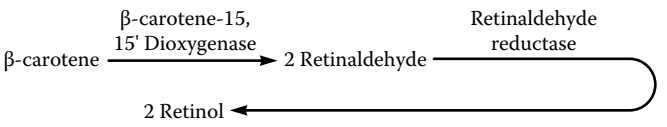


FIGURE 9.4 β-carotene conversion to retinol.

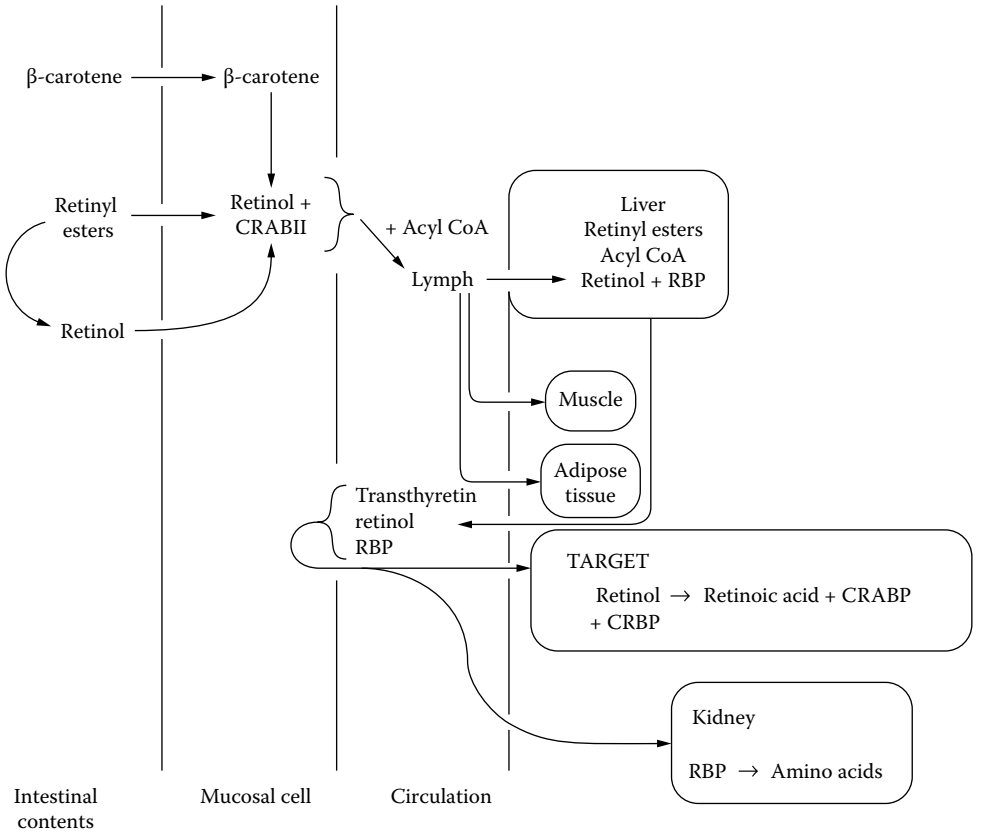


FIGURE 9.5 Uptake and transport of retinol and β-carotene.

for carotene within the luminal cell. Carotene is oxidatively cleaved to retinal and to small amounts of longer chain β-apocarotenoids. The major cleavage products are retinol and retinoic acid. Both central and excentric cleavage is possible. The cleavage enzymes are very unstable. They are rapidly inactivated. Two enzymes are involved in the cleavage: The first, β-carotene-15,15′-dioxygenase, catalyzes the cleavage of the central double bond to yield two molecules of retinaldehyde. The second, retinaldehyde reductase, catalyzes the reduction of retinaldehyde to retinol. This reaction is shown in Figure 9.5. The conversion of β-carotene to Vitamin A is not 100% efficient; thus, 2 moles of retinol are not obtained from one mole of β-carotene. In order for the first enzyme to work, the β-carotene must be solubilized. This means that bile salts and some lipid must be present. Oxygen is also required because this is a dioxygenase type of reaction. Carotene dioxygenase is present in the soluble cell fraction and has been isolated from rat, hog, and rabbit intestinal tissue and characterized. The enzyme of the second reaction, retinaldehyde reductase, is also a soluble mucosal cell enzyme. It requires the presence of either $NADPH^+$ or $NADH^+$ as donors of reducing equivalents. It is not particularly specific for the resulting retinaldehyde for it will catalyze the reduction of several short- and medium-chain aliphatic aldehydes, in addition to retinaldehyde. There are two pathways for retinol esterification. In the first, an acyl-CoA-independent reaction is used. This involves a complex between retinol and Type II cellular-retinol-binding protein (CRBP II). As retinol intake increases there is a corresponding increase in the activity of the enzyme (acyl-CoA retinol acyl transferase), which catalyzes the formation of this complex. Cellular retinol-binding protein (CRBP

II) is found only in the cytosol of the intestinal mucosal cell and, interestingly, its synthesis is influenced by the intake of particular fatty acids. Suruga et al. have shown that unsaturated fatty acids, particularly linoleic and α-linolenic acid, enhance CRBP II mRNA transcription. These fatty acids also enhance S14 and retinoic acid receptor (RAR) transcription in the adipocyte. Thus, it would appear that vitamin A uptake by the mucosal cell is dependent on dietary fat not only because of its influence on its absorption but also because of its role in enhancing the synthesis of the mucosal cell CRBP II.

The second pathway is an acyl-CoA-dependent pathway whereby retinol is bound to any protein, not just the specific CRBP mentioned above. In both pathways, the retinol is protein-bound prior to ester formation. As mentioned in the section on vitamin A structure, the esterification of retinol results in a more lipophilic material readily soluble in the lipids of the cell. This, in turn, facilitates its movement into the cell and its metabolic function as well as its storage.

Retinoic acid, like retinal and retinol, is rapidly absorbed by the mucosal cells. However, it is also rapidly excreted (rather than stored as are the other vitamin forms) in the urine; thus, this form is not as useful as the alcohol or aldehyde forms.

In food, all-*trans* retinol is usually esterified to long-chain fatty acids. Palmitic acid is the most common of these fatty acids. The ester linkage is cleaved in the intestine by a specific esterase which, in some species, is activated by the bile salt, sodium taurocholate. After the ester has been hydrolyzed, the resulting retinol is actively transported (carrier mediated) into the mucosal cell where it is then reesterified to palmitic acid and incorporated into lymph chylomicrons. The absorption of vitamin A thus follows the same route as the long-chain fatty acids of the dietary triacylglycerides, cholesterol and cholesterol esters. The chylomicrons are absorbed by the lacteals of the lymphatic system and enter the circulation when the thoracic duct joins the circulatory system at the vena cava. Once in the vascular compartment, the triacylglyceride of the retinol containing chylomicron is removed, leaving the protein carrier, retinol, and cholesterol. Whereas the triacylglycerides are removed from the chylomicrons primarily by the extrahepatic tissues, retinol is removed primarily by the liver. In the liver, hydrolysis and reesterification occur once again as the vitamin enters the hepatocyte and is stored within the cell associated with droplets of lipid. Interestingly, retinoic acid is absorbed not via the lymphatic system but by the portal system and does not accumulate in the liver. Retinoic acid, because it is not stored, represents only a very small percentage of the body's vitamin A content.

As mentioned previously, β-carotene is converted to retinol in the mucosal cells of the intestine. Dietary retinal may also be converted to retinol and all forms of the vitamin are transported to the liver as part of the chylomicrons.

Transport. It has become apparent that a specific protein is needed for subsequent transport of retinol from the liver to the peripheral target tissues.[9–11] This protein, called the retinol-binding protein (RBP), was first isolated by Kanai et al. in 1968. RBP is synthesized in the liver (Figure 9.5). It is a single polypeptide chain (MW 21000) and possesses a single binding site for retinol. The mobilization of vitamin A from the liver requires this protein. In plasma, vitamin A circulates bound to the retinol-binding protein, which in turn forms a protein-protein complex with transthyretin, a tetramer that also binds thyroxine in a 1:1 complex. Because this complex contains the vitamin and thyroxine, there is an association of thyroid status and vitamin A status.

The RBP of the serum has been isolated from a number of different species including man. It is a single polypeptide chain with a molecular weight of approximately 21000 daltons. It binds, on a one-to-one basis, to one molecule of retinol. The usual level of binding protein in plasma is about 40–50 μg/mL. However, the level of this protein is responsive to nutritional status. In protein malnourished children it is depressed, whereas in vitamin-A-deficient individuals it is elevated. Patients with renal disease have elevated levels of retinol-binding protein[12,13] and may be at risk of developing vitamin A toxicity if vitamin A intake is above normal. In the patient with renal disease, the increase in the binding protein is probably due to the decreased capacity of the kidney to remove the protein. The kidney is the main catabolic site for retinol-binding protein. When protein intakes are

low, binding protein levels are also low, thus explaining the simultaneous observations of symptoms of protein and retinol deficiency in malnourished children. This association is due to the inability of the liver in the protein-malnourished child to synthesize the retinol-binding protein, thus the child is unable to utilize the hepatic vitamin stores. Once protein is restored to the diet, symptoms of both deficiency syndromes will disappear. If dietary retinol (or its precursors) is lacking, serum-retinol-binding protein levels will fall, whereas hepatic levels rise. Within minutes after retinol is given to a deficient individual, these changes are reversed: Serum levels rise, whereas hepatic levels fall. These observations thus provide clear evidence of the importance of this binding protein in the utilization of retinol. Compounds that influence the levels of this binding protein influence the mobilization and excretion of retinol. Estrogens increase the levels of this protein, whereas cadmium poisoning, because it increases excretion, reduces the level of this protein.

Once the retinol-binding protein complex arrives at the target tissue, it must then bind to its receptor site on the cell membrane. Receptors (retinol-binding proteins) for retinol and retinoic acid have been identified on the cell membranes of a variety of cells. The retinol is then released from the serum-binding protein and transferred into the cell where it is then bound to intracellular binding proteins. Of interest are the observations that these binding proteins, although similar to the binding protein in the serum, are highly specific for the different vitamin A forms. The cytosolic retinol-binding protein (CRBP) is the cellular retinol-binding protein (molecular weight 14,600), whereas the cytosolic retinoic-acid-binding protein (CRABP) is the cellular retinoic-acid-binding protein (molecular weight 14,600), cytosolic retinal-binding protein (CRLBP) is the retinal-binding protein (molecular weight 33,000), and interstitial retinol-binding protein (IRBP) is the interphotoreceptor or interstitial retinol-binding protein (molecular weight 144,000). The latter is found only in the extracellular space of the retina. As described earlier, CRBP (II) is the retinol-binding protein found in the mucosal cells of the small intestine. The presence of these binding proteins in different tissues is highly variable. Shown in Table 9.5 are the features and functions of these binding proteins.

The bulk transport of the various vitamers A occurs via the chylomicrons that are released into the lymph, but there seems to be no specific protein within the chylomicron that has a special affinity for this vitamin. However, once the chylomicron remnants (the remains of the chylomicrons which have lost some of its lipid to the muscle and the adipose tissue) are taken up by the liver, the special binding proteins have their effects. The chylomicron remnant retains most of its original vitamin A content as vitamin A esters. These esters are stored in the hepatocyte or are released following hydrolysis into the circulation bound to RBP.

Functions

Protein synthesis: Some of the earliest reports of retinol deficiency included observations on the changes in epithelial cells of animals fed vitamin-A-deficient diets. Normal columnar epithelial cells were replaced by squamous keratinizing epithelium. These changes were reversed when vitamin A was restored to the diet. Epithelial cells, particularly those lining the gastrointestinal tract, have very short half-lives (in the order of 3–7 days) and as such are replaced frequently. Thus, in vitamin A deficiency, changes in these cells, as well as other cells having a rapid turnover time, indicate that the vitamin functions at the level of protein synthesis, cellular differentiation, and turnover. Studies of protein synthesis by mucosal cells from deficient and control animals indicated that the vitamin is involved directly in protein synthesis both at the transcriptional and translational level. A variety of gene products are subject to retinoic acid influence.[14] Such a role for retinol is supported by observations of an alteration in messenger RNA synthesis in vitamin-A-deficient animals. Table 9.6 lists a number of genes for cellular proteins whose expression are regulated by retinoic acid. Retinol is converted to retinoic acid, which regulates cell function by binding to intracellular retinoic acid receptors (RRS). Two distinct families of DNA-binding proteins (retinoic acid receptors RAR and RXR) have been identified, and three genes for each of these receptors have been found.[15–26] Each gene has several isoforms that arise from either different promoter usage or

TABLE 9.5
Retinol-binding proteins

Acronym	Protein	Molecular weight	Location	Function
RBP	Retinol-binding protein	21,000	Plasma	Transports all-*trans* retinol from intestinal absorption site to target tissues
CRBP	Cellular retinol-binding protein	14,600	Cells of target tissue	Transports all-*trans* retinol from plasma membrane to organelles within the cell
CRBP II	Cellular retinol-binding protein Type II	16,000	Absorptive cells of small intestine	Transports all-*trans* retinol from absorptive sites on plasma membrane of mucosal cells
CRABP	Cellular retinoic-acid-binding protein	14,600	Cells of target tissue	Transports all-*trans* retinoic acid to the nucleus
CRBP	Cellular retinal-binding protein	33,000	Specific cells in the eye	Transports 11-*cis* retinal and 11-*cis* retinol as part of the visual cycle
IRBP	Interphotoreceptor or interstitial retinol-binding protein	144,000	Retina	Transports all-*trans* retinol and 11-*cis* retinal in the retina extracellular space
RAR	DNA-binding-protein (retinoic acid receptor, 3 main forms: α, β, γ)		All cells α—liver β—brain γ—liver, kidney, lung	Binds retinoic acid and regions of DNA
RXR	DNA-binding protein (retinoic acid receptors, multiple forms)	—	—	Binds retinoic acid and DNA

alternative splicing.[21–33] The differences between the receptors occur at the n-terminal region. In addition they have different expression patterns in the embryo and adult. Their synthesis and activation are regulated differently by vitamin A.[27,28]

The RAR and RXR are structurally similar to the receptor that binds the steroid and thyroid hormones. The RARs are activated by the all-trans or 9-*cis* isomers of retinoic acid, whereas the RXRs are activated only by the 9-*cis* retinoic acid. These receptors function as ligand-activated transcription factors (see Table 9.6) that regulate mRNA transcription. Both nuclear and mitochondrial DNA are responsive to retinoic acid. Each compartment has its preferred form. The mitochondrial DNA has RAR γ 1 and 2 as its preferred form, whereas the nucleus has RARα as its preferred form.[34] Both compartments will use the β form. In some instances, these factors stimulate transcription, and in other instances they suppress the process. These receptors bind to DNA as dimers, which is reflected in the paired nature of the DNA response elements terms RARE and RXRE, respectively, for RAR and RXR. The nucleotide sequence archetype upon which the RARE and RXREs are based is in accordance with a direct repeat (DR) 1-to-5 rule. This pattern, suggested by Umesono et al.,[32] has a direct repeat of the sequence AGGTCA separated by between 3 and 5 nucleotides. The original 3-4-5 rule was extended to include a separation of one or two nucleotides, depending on whether the element was for RAR or RXR.: RAREs were DR2 and DR5, the RXREs, DR1 and the response elements for vitamin D and thyroid hormone were DR 3 and 4, respectively. The guideline worked well to describe several of the strongest RA-response elements, including the DR5 type that is the most prevalent of the simple direct repeats. The DR1-5 rule is only a framework, however, and

TABLE 9.6
Retinoic-acid-responsive proteins

I. Proteins that have their synthesis increased due to RA-receptor effect on the transcription of their mRNA

Growth hormone	Neuronal cell
Transforming growth factor β2	Calcium-binding protein
Transglutaminase	Calbindin DZ8K
Phosphoenolpyruvate carboxykinase	Ornithine decarboxylase
Gsα	Osteocalcin
Alcohol dehydrogenase	Glycerophosphate dehydrogenase
t-plasminogen activator	

II. Retinoic acid proteins that function in mRNA transcription

$1,25\text{-}(OH)_2D_3$ receptors	MSH receptors
Interleukin-6 receptors[a]	Retinoic acid receptors-α
cfos[a]	Interleukin-2 receptors
Progesterone receptors[a]	EGF receptors (corneal epithelium)[a]
Zif 268 transcription factor	EGF receptors (corneal endothelium)
Peroxisomal-proliferator-activated receptors	AP-2 transcription factor

[a] The activity of these proteins are suppressed when the RA-receptor is bound to it.

a series of additional factors also influences the effectiveness of each response element. More than 40 genes contain these elements as part of their promoter regions.

The differences between the RAR and RXR response elements goes beyond simple binding preference or ligand specificity. They have separate functions. The RARs are ligand-regulated receptors that function similarly to the vitamin D and thyroid hormone receptors; once ligand-bound, they become transcription activators. The RXRs can also do this but they have another function as well. They serve as accessory factors for many members of the nuclear family of super receptors, the so-called steroid super family of receptors. The RXRs can heterodimerize with these receptors without having 9-*cis* retinoic acid as a ligand.

There is also an interaction of vitamin A with other vitamins. For example, the synthesis of the calcium-binding protein, calbindin, is usually regulated by vitamin D. This protein, found in the intestinal mucosal cells and the kidney, is also found in the brain. In the brain its synthesis Is regulated by retinoic acid rather than vitamin D. In vitamin-A-deficient brain cells, additions of retinoic acids increased the mRNA for calbindin and calbindin synthesis. Additions of vitamin D were without effect. The retinoic acid receptors contain zinc finger protein sequence motifs, which mediate its binding to DNA. The carboxyl terminal end of the receptor functions in this ligand binding. Retinoic-acid-binding to nuclear receptors sets in motion a sequence of events that culminate in a change in transcription of the cis-linked gene. That is, proteins are synthesized, and these proteins bind to regions of the promoter adjacent to the start site of the DNA that is to be transcribed. Such binding either activates or suppresses transcription and, as a result, there are corresponding increases or decreases in the mRNA coding for specific proteins which, in turn, lead to changes in cell function. Table 9.7 lists a number of enzymes that have been reported to be affected by the deficient state. In each of these instances it could be assumed that the reason for the change in activity could be explained by the effect of vitamin A on the synthesis of these enzymes. Where there is an increase, it is likely that transcription and, hence, synthesis is usually suppressed (or kept within normal bounds) when vitamin A intake is adequate.

TABLE 9.7
Enzymes that are affected by vitamin A deficiency

Enzyme	Reaction	Effect
ATPase	ATP ↔ADP + Pi	Increase
Arginase	L-arginine → ornithine + urea	Increase
Xanthine oxidase	Hypoxanthine → → uric acid	Increase
Alanine amino transferase	L-alanine →α-ketoglutarate→pyruvate + L-glutamate	No change
Aspartate amino transferase	L-aspartate + α-ketoglutarate → oxaloacetate + L-glutamate	No change
Vitamin A palmitate hydrolase	Vitamin A palmitate → vitamin A + palmitic acid	No change
Vitamin A ester synthetase	Vitamin A + fatty acid →ester	No change
?5,3 ?-hydroxysteroid dehydrogenase 11?-steroid hydroxylase	Progesterone, glucocorticoid, estrogen, testosterone. Removal of H synthesis of steroid hormones.	Decrease
ATP sulfurylase sulfotransferase	ATP + SO_4 → adenyl sulfate + PPi-^{3} transfers sulfuryl groups to –O and –N of suitable groups. Synthesis of mucopolysaccharides.	Decrease
L -γ-gulonolactone oxidase	L-gulonolactone → L-ascorbic acid	Decrease
p-hydroxyphenol pyruvate oxidase	*p*-hydroxyphenyl pyruvate → homogentisic acid	Decrease

There is another aspect of vitamin A nutriture that is of importance when the function of this vitamin is considered. This concerns the structure and function of the nuclear retinoic-acid-binding protein, the RA receptor. Should this receptor not be synthesized as can occur in the absence of retinoic acid, the whole cascade of events dependent on the binding of the RA-receptor complex will not occur. Further, should the receptor itself be aberrant in amino acid sequence, both its capacity to bind retinoic acid and its affinity for specific regions of the DNA will be affected. In this instance it is easy to understand how cellular differentiation would be affected. Indeed, several investigators have suggested that this could explain the occurrence of congenital defects in a number of species where early embryonic development of the spinal column and the heart might be due to abnormal RA-receptor binding with the subsequent result of defective differentiation, organ formation, and organ function. This role for vitamin A, although lacking biochemical mechanistic detail, was among the first functions recognized by early investigators. Several excellent papers on retinoid signaling and the generation of cellular diversity in the embryonic mouse spinal cord have recently been published, as has a paper on retinoid signaling in the developing heart.[18,24–29,33,34] In addition, there is a need for retinoic acid by the insulin-secreting cells of the pancreas.[16] The need probably relates to its function in stimulating mitochondria DNA transcription. The islet cell requires large quantities of ATP for both the synthesis of insulin (and other hormones) and its release. In fact, the pancrease, brain, and kidney requires more ATP than other tissues and organs in the body. Retinoic acid bound to its receptor binds to the promoter of the mitochondrial DNA to stimulate the synthesis of the 13 essential OXPHOS proteins.[31,35] This is turn stimulates oxidative phosphorylation and ATP production without which the islet cell can not synthesize or release sufficient insulin to meet need.

Reproduction and Growth: The role of vitamin A in the growth process is related to its function in RNA synthesis as described above. Animals fed vitamin-A-deficient diets do not eat well, and their poor growth may stem from their inadequate intakes of the other essential nutrients. As mentioned previously, vitamin A is responsible for the maintenance of the integrity of the epithelial tissues. Because the taste buds are specialized epithelial tissues, feeding a deficient diet probably results in a change in the structure and function of these taste buds, resulting in a loss in appetite. As well, other epithelial cells are also affected, particularly those cells which secrete lubricating

and digestive fluids in the mouth, stomach, and intestinal tract. The lack of lubrication due to atrophy of these important cells would certainly affect food intake and, hence, result in poor growth. In addition, reduction in food intake itself imposes a stress on the growing animal, and stress, with its attendant hormonal responses, such as an increase in thyroid hormone release and an increase in adrenal activity, would have profound influences on protein turnover and energy utilization and, of course, growth.

As mentioned, the role of the vitamin in reproduction relates to its role in RNA and protein synthesis. Ornithine decarboxylase (ODC), an enzyme that closely correlates to cell division and tissue growth, has recently been identified as a protooncogene. ODC is the first rate-limiting enzyme in the biosynthesis of polyamines, which are essential for cell growth. Retinoic acid suppresses ODC and mRNA, and by doing so serves as a brake on uncontrolled cell growth. This function of retinoic acid on ODC transcription is counterbalanced by retinoic-acid–estrogen-receptor binding. The latter enhances ODC mRNA transcription. Not only would the growth of a fertilized ova be affected in this manner, but also, through its effects on protein synthesis, vitamin A could affect the synthesis of enzymes needed to produce the steroid hormones which regulate and orchestrate the reproduction process. Several of the enzymes listed in Table 9.7 are involved in the synthesis of these hormones. Of the other enzymes listed, three (ATPase, arginase, and xanthine oxidase) relate primarily to energy or protein wastage, as would be expected in a deficient animal, and which are increased in the deficient animal. The transferase and the enzymes of retinol metabolism are unchanged, whereas enzymes for the synthesis of mucopolysaccharides are decreased. Observations of increases in the phospholipid content of a variety of cellular and subcellular membranes in vitamin-A-deficient rats suggest that enzymes of lipid metabolism are also affected. Cholesterol absorption is increased in the deficient rat, and this increase may in turn affect phospholipid synthesis and membrane phospholipid content because mammalian membranes consist largely of cholesterol and phospholipids. Changes in membrane composition conceivably could explain the increased susceptibility of deficient animals to infection but equally likely is the reduction in the protective effect of a normal intact epithelium, which acts as a physical barrier to the pathogenic organisms and the reduction in the synthesis of antibodies and antibody-forming cells in the spleen of vitamin-A-deficient animals.

The secondary characteristics of vitamin A deficiency again are probably related to the role of the vitamin in protein synthesis as described. The primary characteristics of decreased dark adaptation, poor growth, reduced reproductive capacity, xerophthalmia, kerotomalacia, and anemia are all related.

Vision: Of the various functions vitamin A serves, its role in the maintenance of dark adaptation was the first to be fully described on a molecular basis.[36] When animals are deprived of vitamin A, the amount of rhodopsin declines. This is followed by a decrease in the amount of the protein opsin. Rhodopsin is present in the rod cells of the retina of most animals. The synthesis of rhodopsin and its subsequent bleaching has been elucidated primarily through the work of Wald and others (Figure 9.6). All-trans retinol is transported to the retina cell, transferred into the cell, and converted to all-trans retinal. All-trans retinal is isomerized to the active vitamin 11-*cis* retinal, which combines with opsin to form rhodopsin. Rhodopsin is an asymmetric protein with a molecular weight of about 38,000. It has both a hydrophilic and a hydrophobic region with a folded length of about 70 Å. It spans the membrane of the retina via seven helical segments that cross back and forth, and comprises about 60% of the membrane protein. The light-sensitive portion of the molecule resides in its hydrophobic region. When rhodopsin is exposed to light it changes its shape. The primary photochemical event is the very rapid isomerization of 11-*cis* retinal to a highly strained transold form, bathorhodopsin. Note that the alcohol (retinol) and the aldehyde (retinal) are interchangeable with respect to the maintenance of the visual function. Retinoic acid is ineffective primarily because there are no enzymes in the eye to convert the retinoic acid to the active 11-*cis* retinal needed for the formation of rhodopsin. Also, 11-*cis* retinol is involved in the formation of iodopsin in the cones and the photochemical isomerization of the 11-*cis* isomer triggers the visual process. In the

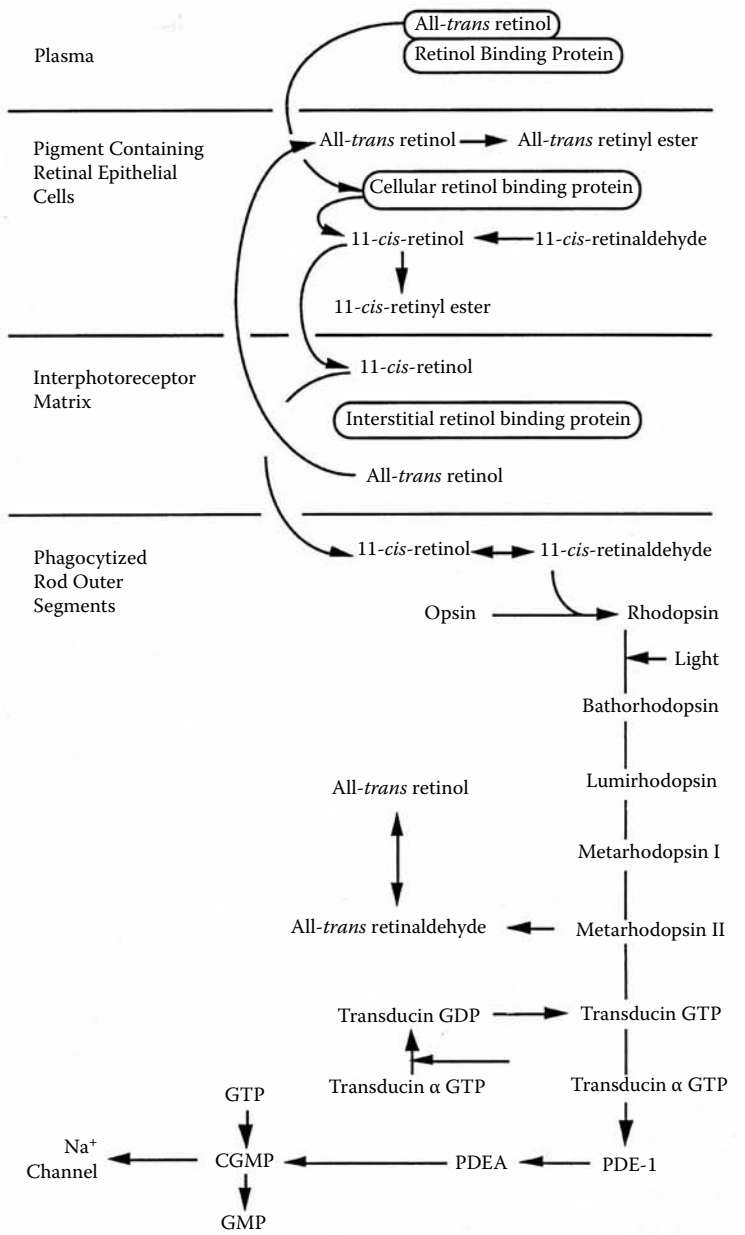


FIGURE 9.6 Retinoic acid and the visual cycle.

rhodopsin breakdown process, an electrical potential arises and generates an electrical impulse that is transmitted via the optic nerve to the brain. That 11-*cis* retinal is also involved in color vision (the responsibility of the cones) has been suggested; however, the mechanism of this involvement has not been fully explored. Three major cone pigments have been identified having absorption maxima of 450, 525, and 550 nm, respectively. Whether these are single pigments or a mixture and whether one or more contain 11-*cis* retinal has not been determined.

Hypervitaminosis A

Because vitamin A is stored in the liver, it is possible to develop a toxic condition when very high (10 times normal intake) levels of the vitamin are consumed.[1,37] As early as 1934, reports appeared in the literature of vitamin A intoxication in humans, rats, and chicks. In chicks, the most obvious clinical signs are a reduced growth rate, an encrustation of the eyelids, and a reddening of the corners of the mouth. In rats, bone fractures are observed. These bone fractures may be related to the unusual brittleness of the bone in hypervitaminosis. In humans, hypervitaminosis A is characterized by increased intracranial pressure resulting in headaches and blurring vision, and in young children, a bulging fontanel. Hair loss and skin lesions, anorexia, weight loss, nausea, vomiting, vague abdominal pain, and irritability are common symptoms. In experimental animals, excess vitamin A intake during gestation results in congenital malformations in the young. Because of the limitation in the conversion of β-carotene to retinol, vitamin A intoxication is less likely with large intakes of carotene; however, reports of yellowing of the skin of persons consuming large amounts of carrot juice have appeared. This yellowing is likely due to the deposition of carotene in the subcutaneous fat.

Vitamin Degradation and Excretion

The early Nobel-Prize-winning work of Wald et al., which showed that retinol must be converted to retinal before the vitamin can function in vision, led the way for other workers to investigate the further metabolism of the various active forms. Because vitamin A not only maintains the visual cycle but is also necessary for growth, epithelial cell differentiation, skeletal tissue development, spermatogenesis, and the development and maintenance of the placenta, and because each of these functions requires a specific form of the vitamin, it appears that each of these tissues has specific structural requirements. Retinal for vision has been demonstrated and it appears that retinol but not retinal or retinoic acid is required for the support of reproduction, and that all of the three forms will support growth and cell differentiation. That the three forms are interchangeable in the latter function suggests that retinoic acid is the active form for this function. Both retinol and retinal can be converted to retinoic acid, but the reverse reactions are not possible, and only in specific tissues such as the retina's are retinol and retinal interconvertible. From studies using radioactively labeled retinal, retinol, and retinoic acid, it is apparent that retinoic acid is the common metabolic intermediate of the vitamin A group. Once retinol is mobilized from hepatic stores, transported to its target tissue via the retinol-binding protein, and transferred to its intracellular active site via the intracellular retinol-binding proteins, it is then utilized either as retinol, retinal, or converted to retinoic acid. Studies of the excretion patterns of labeled retinol and retinoic acid revealed that retinol was used more slowly than retinoic acid. Label from retinoic acid was almost completely recovered within 48 hours of administration, whereas more than 7 days were required to recover even half of the label from the retinol.

The use of retinoic acid labeled in several different locations allowed for the determination of the metabolic pathway of retinoic acid. The labeled retinoic acid was recovered as $^{14}CO_2$ from the expired air as well as from ^{14}C-labeled decarboxylated metabolites and the β-ionene ring lacking part of its isoprenoid side chain. The structures of all the metabolites that appear in the urine and feces are not known.

Need

Adult requirements for retinol have been estimated using a variety of techniques. Individuals vary in their needs for the vitamin. Reports in the literature indicate that requirements may be increased by fever, infection, cold, hyperthyroidism, chemical toxicants, and excessive exposure to sunlight. Excess alcohol consumption likewise compromises vitamin A status and it has been suggested that fetal alcohol syndrome may be due to this alcohol effect.[38] People with renal disease especially

those in acute renal failure release of hepatic stores of retinol is increased, and this affects vitamin A status.[12,13] Ultimately, this means that their vitamin need is increased because serum retinol is a positive feedback mechanism that more is needed by the peripheral tissues. Malnutrition not just due to renal disease and other diseases but also due to inadequate food supply leads to multiple-nutrient deficiency. Vitamin A deficiency among children in developing nations remains the leading cause of impaired vision and blindness. It is a significant contributor to infectious disease in these children.[18] Undernutrition and malnutrition affects an estimated 254 million children and is a leading concern of the World Health Organization. It is preventable through food supplementation programs that include vitamin supplementation as well as protein and energy supplements. In addition to an inadequate food supply, there are some individuals whose genetic heritage introduces an additional measure of variability in the need for vitamin A. This variability is not easily identified but the DRIs allow for such in the recommended intakes for vitamin A.

To determine an individual's vitamin A requirement, that individual must first be deprived of all dietary sources of the vitamin until the first signs of deficiency appear. Because the liver stores the vitamin, this depletion period may be as long as two years for the human. Once depleted, graded amounts of the vitamin are restored to the diet until the signs of deficiency disappear. When a steady state is achieved, that level of intake is the requirement. Obviously such as procedure is not practical for large numbers of people. Because of the time and expense involved in determining requirements, daily dietary recommended intakes (DRIs) are used. These are shown in Table 9.1. These are estimates of the needs of large population groups based on detailed studies (as per above) of a small group of subjects. The recommendation is usually twice the determined requirement so as to allow for variability in need. As more data are collected on human subjects, the safety factor may be reduced and the recommended intake changed. The dietary intake recommendations for humans in retinol equivalents are given as retinol equivalents due to the fact that the many forms of the vitamin are interconvertible.

VITAMIN D

Just as night blindness has been recognized for centuries as a disease treatable by diet (vitamin A), the classical disease of vitamin D deficiency, rickets, has been evident since ancient times. Historians do not agree as to when the first symptoms of vitamin D deficiency were evident. Some suggest that the stooped appearance of the Neanderthal man (circa 50,000 B.C.) was due to an inadequate vitamin D intake rather than being characteristic of a low evolutionary status. Evidence of rickets in skeletons from humans of the Neolithic age, the first settlers of Greenland, the ancient Egyptians, Greeks, and Romans has been reported.

The first detailed descriptions of the disease are found in the writings of Dr. Daniel Whistler of Leiden, the Netherlands, and Prof. Francis Glisson in the mid-1600s. Beyond these descriptions and the acceptance of rickets as a disease entity, little progress was made until the late 1800s when it was suggested that the lack of sunlight and perhaps a poor diet were related to the appearance of bone malformation. It was frequently reported that infants born in the spring and dying the following winter did not have any symptoms of rickets, whereas infants born in the fall and dying the next spring had rickets. Funk, in 1914, suggested that rickets was a nutrient deficiency disorder. This was verified by the brilliant work of Sir Edward Mellanby. Mellanby constructed a grain diet that produced rickets in puppies. When he gave cod-liver oil, the disease did not develop. At that time, Mellanby did not know that there were two fat soluble vitamins (A and D) in cod-liver oil, and he thought that he was studying the antirachitic properties of vitamin A. Not until the two vitamins were separated and identified was it realized that Mellanby's antirachitic factor was vitamin D. The recognition of vitamin D as a separate entity from vitamin A came from the work of McCollum and associates in 1922. In a landmark paper, McCollum reported the results of his work on the characterization of vitamin A. He described the vulnerability of the vitamin to oxidation and the

fact that the antirachitic factor remained even after the cod-liver oil was aerated and heated and the antixerophthalmic factor (vitamin A) was destroyed.

Although the importance of sunlight had long been recognized in the prevention and treatment of rickets, the relationship of ultraviolet light to the dietary intake of vitamin D was not appreciated until Steenbeck and also Goldblatt et al. demonstrated that ultraviolet light gave antirachitic properties to sterol-containing foods if these foods were incorporated into diets previously shown to produce rickets. From this point on, the research concerning vitamin D, as it was so named by McCollum and associates, became largely chemical in nature. It has only been within the last decade or so that work on vitamin D has elucidated its mechanism of action.

Structure and Nomenclature

Like vitamin A, vitamin D is not a single compound. The D vitamins listed in Table 9.8 are a family of 9,10-secosteroids that differ only in the structures of their side chains. Figure 9.7 shows some of these different structures. There is no D_1 because when the vitamins were originally isolated and identified, the compound identified as D_1 turned out to be a mixture of the other D vitamins rather than a separate entity.

Because the other D vitamins were already described and named, the D_1 designation was deleted from the list. All the D vitamin forms are related structurally to the four-ring compounds cyclo-pentano-perhydrophenanthrenes from which they were derived by a photochemical reaction. The official nomenclature proposed for vitamin D by IUPAC-IUB Commission on Biochemical Nomenclature relates the vitamin to its steroid nucleus. Each carbon is numbered using the same system as is used for other sterols such as cholesterol. This is illustrated in Figure 9.8. The numbering system of the four-ring structure is retained even though the compound loses its B ring during its conversion to the vitamin.

The chief structural prerequisite of compounds serving as D provitamins is the sterol structure, which has an opened B ring that contains a $D_{5,6}$ conjugated double bond. No vitamin activity is possessed by the compound until the B ring is opened. This occurs as a result of exposure to ultraviolet light. In addition, vitamin activity is dependent on the presence of a hydroxyl group at carbon 3 and upon the presence of conjugated double bonds at the 10-19, 5-6, 7-8 positions. If the location of these double bonds is shifted, vitamin activity is substantially reduced. A side chain of a length at least equivalent to that of cholesterol is also a prerequisite for vitamin activity. If the side chain is replaced by a hydroxyl group, for example, the vitamin activity is lost. The potency of the various D vitamins is determined by the side chain. D_5, for example, with its branched 10-carbon side chain, is much less active with respect to the calcification of bone cartilage, than is D_3 with its nine-membered side chain.

TABLE 9.8
D vitamers produced from provitamin forms when these precursor forms are exposed to ultraviolet light[a]

Precursor	D vitamer
Ergosterol	D_2 (ergocalciferol)
7-Dehydrocholesterol	D_3 (cholecalciferol)
22,23 Dihydroergosterol	D_4
7-Dehyrositosterol	D_5
7-Dehydrostigmasterol	D_6
7-Dehydrocompesterol	D_7

[a] These are not active until they are hydroxylated at carbons 1 and 25.

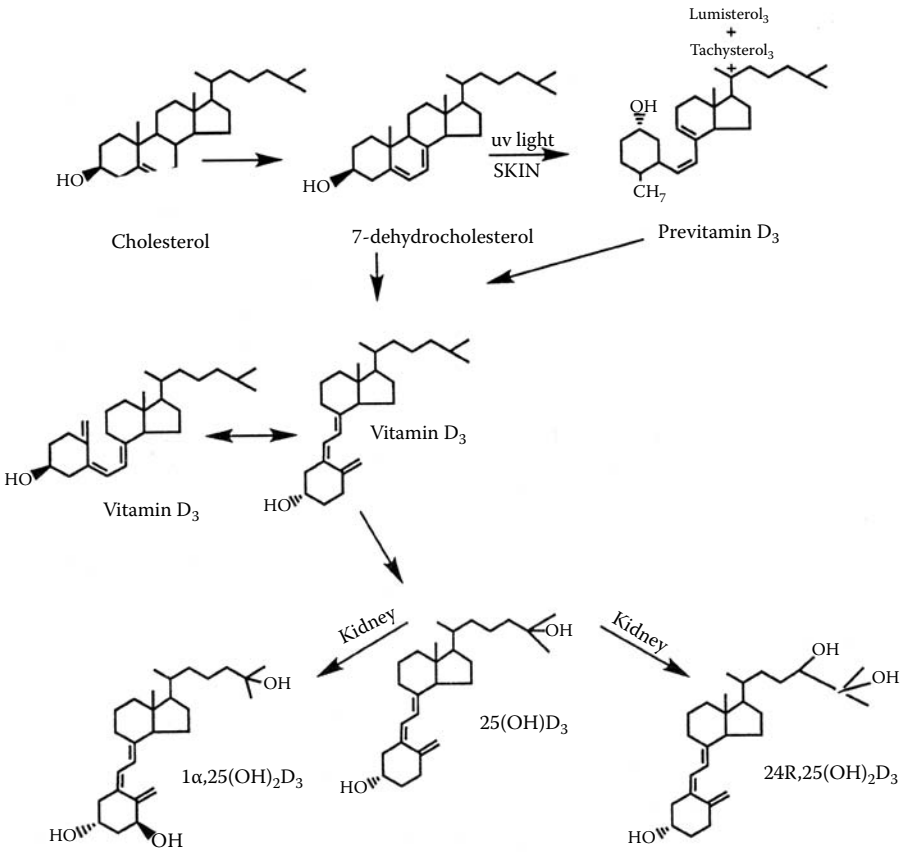


FIGURE 9.7 Compounds that have vitamin D activity. Not all of these compounds have identical activity.

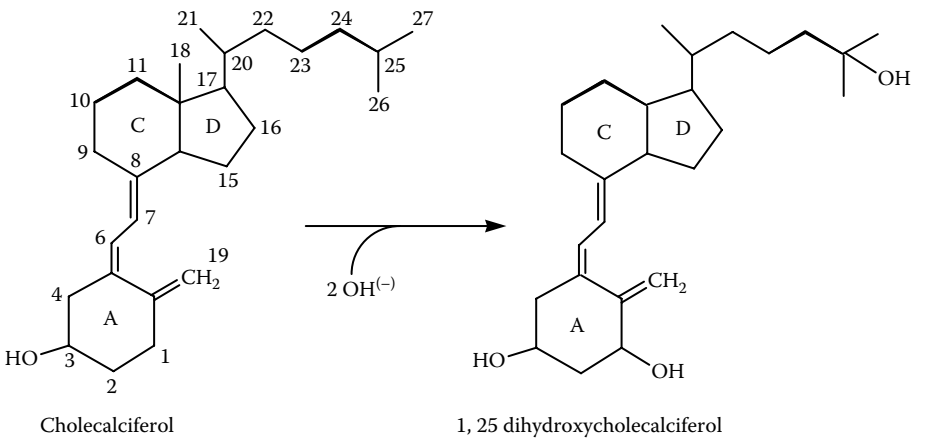


FIGURE 9.8 Conversion of cholecalciferol to 1,25-dihydroxycholecalciferol. The carbons in the structures are numbered and the rings are given letter designations. When activated a hydroxyl group is added to carbon 25.

Of the compounds shown in Figure 9.7, the most common form is that of D_2, ergocalciferol, so called because its parent compound is ergosterol. Ergosterol can readily be prepared from plant materials and, thus, serves as a commercially important source of the vitamin. Vitamin D_3, cholecalciferol, is the most important member of the D family because it is the only form which can be generated in vivo. Cholesterol, from which cholecalciferol takes its name, serves as the precursor. The 7-dehydrocholesterol at the skin's surface is acted upon by ultraviolet light and is converted to vitamin D_3. Here, then, is the connection between diet, sunshine, and rickets sought many years ago when rickets was prevalent in young children. In the absence of sunshine this conversion does not take place. Recall the dress patterns of the people of the 18th and 19th century. Children (as well as adults) wore many layers of clothing that shielded the skin from ultraviolet light. This practice severely restricted vitamin D synthesis.

Although most mammals can convert D_2 to D_3 and use it to make the active principle (1,25-dihydroxycholecalciferol) responsible for Ds biological function, birds seem unable to make this conversion; the Ds birds produce is not effectively utilized. The conversion product is either rapidly degraded and/or excreted. Thus, birds must be supplied with D_3 rather than D_2 as the vitamin of choice. It has been estimated that for birds, D_2 has only one-tenth the biological activity of D_3 on a molar basis.

Physical/Chemical Properties

The history of vitamin D would not be complete without mentioning the careful work of a Frenchman, Charles Tanret, who isolated and characterized a sterol from fungus-infected rye which he called *ergosterol*. The melting point, optical rotation and elemental composition of ergosterol reported by Tanret in 1889 were identical to those reported by Windaus more than 30 years later. Windaus and his associates were able to elucidate the structure of ergosterol and ergocalciferol. However, its complete structure was not verified until the techniques of x-ray analysis and infrared spectroscopy were developed.

The precursors and the vitamins are sterols, which are members of the nonsaponifiable lipid class. At room temperature, they are white to yellowish solids with relatively low melting points. The various structural and physical characteristics of D_2 and D_3 are listed in Table 9.9. Under normal conditions, D_3 is more stable than D_2; however, both compounds undergo oxidation when exposed to air for periods of 24–72 hours. When protected from air and moisture and stored under refrigeration, oxidation of the vitamin can be minimized. In acid solutions, the D vitamins are unstable. However, in alkaline solutions they are stable. All the D vitamins are moderately soluble in fats, oils, and ethanol, and very soluble in fat solvents such as chloroform, methanol, and ether. All of the vitamers are unstable to light. In the dry form the vitamers are more stable than when in solution. Stability in solution can be enhanced by the presence of such antioxidants as α-tocopherol and vitamin A.

Although the D vitamins are not soluble in water, they, like the A vitamins, can be made miscible with water through the use of detergents or surfactants. However, because of the vitamins' vulnerability to oxidation, such solutions are very unstable. This is due to the wide dispersion of the vitamin molecules in water which has oxygen dissolved in it. Thus, each vitamin molecule is available to the dissolved oxygen for oxidation with the resultant loss in vitamin activity. Some protection against this oxidation can be provided if α tocopherol (vitamin E) is added to the solution. In addition to the loss of vitamin activity through oxidation, other chemical alterations can result in decreased vitamin potency. Saturation of any of the double bonds or the substitution of a chloride, bromide, or mercaptan residue for the hydroxyl group attached to carbon three results in a loss of vitamin activity.

TABLE 9.9
Physical characteristics of vitamins D_2 and D_3

Vitamin	Number double bonds	Melting point	UV absorption maximum	Molar extinction coefficient	Optical rotation
D_2	4	121¡C	265 mm	19,400	$\alpha\ ^{20}_{D} + 106°C$
D_3	3	83-85¡C	264–265	18,300	$\alpha\ ^{20}_{D} + 84.8°C$

Biopotency

The comparative potency of the D vitamers depends on several factors, including the species consuming the vitamers and the particular function assessed. With respect to species specificity, in mammalian species both the D_2 and D_3 are equivalent, and both would be given a value of 100 if rickets prevention was used as the functional criteria. However, should these two vitamers be compared in chicks as preventers of rickets, D_2 would be given a value of perhaps 10, whereas D_3 would be 100. In this instance it is clear that species differ in their use of these two vitamers. A related sterol, dihydroachysterol, a product of irradiated ergosterol would have only 5–10% of the activity of ergocalciferol. In contrast, the activated forms of D_3 (25-hydroxy and 1,25-dihydroxy cholecalciferol) are far more potent (2-5Xs and 5-10Xs, respectively) than their parent vitamer, D_3. The synthetic analog of D_3, 1α-hydroxycholecalciferol likewise has 5–10 times the potency of cholecalciferol. There are other vitamin D analogs that have selective biological activity and may have use as therapeutic agents. The analog 3-deoxy-1,25-dihydroxycholecalciferol is far more active as an agent to promote intestinal calcium uptake than as an agent to promote bone calcium mobilization. This is also true for the analog, 25-hydroxy-5,6-cholecalciferol.

The reverse effects—increased bone calcium mobilization rather than increased intestinal calcium absorption—have been shown for analogs having a longer carbon chain at carbon 20 and/or having a fluorine attached at carbon 3 (see Figure 9.8). Cell differentiation, another vitamin D function, is markedly enhanced by the addition of a hydroxyl group at carbon 3, a unsaturation between carbons 16 and 17 and triple bond between carbons 22 and 23. This analog has greater activity with respect to cell differentiation than for intestinal calcium uptake and bone calcium mobilization.

Methods of Assay

Because mammals require so little vitamin D and because so few foods contain the vitamin, methods for its determination have to be sensitive, reliable, and accurate. A wide variety of assays have been developed that are capable of quantifying fairly well the amount of vitamin D in a test substance. These assays can be divided roughly into two groups: chemical and biological. Biological assays with few exceptions are usually more sensitive than chemical assays because so little of the vitamin is required by animals. The least amount of vitamin detectable by the biological methods is 120 ng or 0.3 nmoles, whereas, with the chemical methods, the least amount detectable is approximately nine times that of the bioassay of 2.6 nmol. The exception to this comparison is the technique that utilizes high pressure liquid chromatography followed by ultraviolet absorption analysis. This technique can measure as little as 5 ng or 1/24th that of the bioassay techniques. Gas chromatography is also very sensitive, especially if the chromatograph is equipped with an electron capture detector. Using this technique, as little as 50 pg of the vitamin can be detected. This degree of sensitivity is needed for the detection of tissue vitamin levels, for aside from vitamin B_{12}, vitamin

D is the most potent of the vitamins. Only small amounts are needed and so only small amounts will be found in those tissues requiring the vitamin. Table 9.10 summarizes the main methods that have been used for the detection of biological levels of vitamin D. Under the chemical assay techniques, note that a variety of color reactions can be used in vitamin D quantification. These color reactions are possible because the vitamin contains several rings which can react with a variety of compounds in solution and produce a color. The intensity of the color is directly related to the quantity of the vitamin in solution.

Although these colorimetric methods are relatively easy to perform, they have several drawbacks. First, and most important, the color reaction is possible because of the ring structure; other sterols have this same ring structure but have little vitamin activity. Thus, colorimetric methods are

TABLE 9.10
Summary of methods used in determining vitamin D content of tissues and foods

Method	Sensitivity[a] nmol	Usual working range nmol	Comments
Chemical methods			
Colorimetric[b]			
Aniline-HCl	No values given	No values given	Devised in 1925
Antimony chloride	3.2	3.2–6.5	Used primarily to assess pharmaceutical preparations
Trifluoroacetic	2.6	1–80	
Ultraviolet absorption	2.6	2.6–52	A solution with 5.47 nmol of vitamin D will have an absorbance of 0.10 at 264 nm
Ultraviolet fluorescence	2.6	2.6–26	Based on the property of acetic anhydride–sulfuric acid-induced fluorescence of the vitamin
Gas chromatography	0.1 pmole	0.01–10	Based on use of electron capture detector
High pressure liquid chromatography	0.01	0.05–100	
Gas chromatography–mass spectrometry	0.01	0.01–50	Equipment not widely available due to expense. Can separate and quantify individual vitamers in a mixture
Biological methods			
Rat line test	0.03	0.03–0.07	Time consuming—requires 7 days of feeding after rats become rachitic
Chick test	0.13	0.006–15	Requires 21 days of feeding
Intestinal Ca^{++} absorption	In vivo 0.33	0.125–25 µg	Requires 1 day
	In vitro 0.66	250–1000	Requires 1 day
Bone Ca^{++} mobilization	0.32	0.125-25 µg	Requires 1 day
Body growth	0.06	50–1250	Requires 21–28 days
Immunoassay of calcium-binding protein	0.0025	1 ng	Requires 1 day

[a] Defined as the least amount of vitamin detectable by the method.
[b] Other color reagents have also been used.

not specific enough to permit true vitamin quantification. The second drawback is that there must be sufficient vitamin in the test substance to react with the color reagent to produce a measurable color change. This requires instrumentation that is able to measure these changes. In general, this degree of sensitivity is missing in most instruments designed to measure colorimetric changes. Thus, the colorimetric methods lack sensitivity as well as specificity.

The ring structure of vitamin D, although common to many different sterols, can be utilized very well in assay techniques where the sterols are first separated and then assayed. The vitamin D sterol ring structure has a characteristic ultraviolet absorption spectra. At 264–265 nm, the intensity of the light absorbed is directly proportional to the quantity of vitamin D present. Sterols can be separated from the lipid component of a sample by saponification. The nonsaponifiable lipids (the sterols) can be further separated by digitonin precipitation. Vitamin D and its related sterols will not precipitate out, whereas cholesterol and the other four-ring sterols will. If the remaining supernatant containing the vitamin D components is then fractionated using chromatographic techniques, the resulting fractions can be assayed according to the amount of ultraviolet light absorbed.

The ultraviolet light absorption characteristic can also be used to determine the extent of conversion of provitamin D to D_2 or D_3. Ergosterol or cholesterol can be irradiated until the resultant compound exhibits the typical absorption spectra characteristic of the vitamin. Of course, just as ultraviolet light is needed for this conversion, one must remember that light in excess will destroy the vitamin and its usefulness will be lost.

Just as there are a number of chemical assay techniques useful for the determination of vitamin D activity in biological samples, there are also a number of biological assay techniques that can be used. The advantages of the bioassay are those of sensitivity and specificity. The disadvantages are those of time, expense, and accuracy. The basis of the bioassay is that the physiological effect is quantifiable and, theoretically, the magnitude of this effect is in direct proportion to the amount of vitamin D in the test substance. For many years, the standard bioassay for vitamin D was the rat line test first devised by McCollum in 1922. This test consisted of preparing rats and then treating them with graded amounts of the test substance for one week. The rats are then killed; the bones of the forepaws are excised and cleaned of adhering tissue, sliced longitudinally, and placed in a solution of silver nitrate. The silver nitrate is absorbed by the areas of the bone where calcium has recently been deposited. These regions will turn black upon exposure to light. The resultant black line is then measured and compared to lines obtained from rats fed known amounts of vitamin D. Thus, the line test is based on the activity of the vitamin in promoting calcium uptake by the bone. Although this is probably a good method to estimate vitamin activity in a given substance, it has the pitfall of not distinguishing between the various D forms. The user of this method also must assume that bone calcium deposition is the vitamin's most important function; this is not always true.

Use of the line test to assess vitamin content of human foods is probably acceptable because rats and humans can use all forms of D similarly. However, use of the rat line test to assess the D content of feeds for chickens would present problems due to the fact that the chickens do not use D_2 and D_3 equally well. As pointed out earlier, chickens need D_3 in their diets rather than D_2. Other bioassays listed in Table 9.9 also are based on a physiological/metabolic parameter that assumes the quantity of the vitamin in a test substance is proportional to the activity of the system being assessed. Tests such as the intestinal calcium uptake test, the bone calcium mobilization test, the body growth test, and the calcium-binding protein assay all have been devised and published. They all have the advantage of assessing the biological potency of the vitamin D contained in the test substance and all are sensitive to quantities of D likely to be present in biological (as opposed to pharmacological) preparations.

International Units (IU)

It was from the use of the bioassay techniques that the definition of the international unit (IU) was developed. One IU was defined as the least amount of vitamin required to elicit a physiological

response (i.e., the calcification of bone). As it was used in this context, with the rat as the reference animal, one milligram of vitamin D was equivalent to 40,000 IU of the vitamin.

In 1931, the World Health Organization of the League of Nations adopted as their international standard of reference for vitamin D the activity of 1 mg of a reference solution of irradiated ergosterol. At the time this definition was developed, researchers were not aware of the different D vitamins nor were they aware of the species specificity for the vitamin form. Later, as knowledge about the vitamin increased, the definition was changed such that the present definition developed by Nelson in 1949 uses cholecalciferol, D_3, as the reference standard where 1.0 g of a cottonseed oil solution of D_3 contains 10 mg of the vitamin or 400 USP units. Thus, the potency of the different D vitamins are related to the most important biologic compounds in the D family of compounds.

Metabolism

Absorption

Prior to the understanding and elucidation of the conversion of cholesterol to ergosterol and then to its activation as 1,25-dihydroxy cholecalciferol, considerable attention was given to the mechanisms for the intestinal absorption of vitamin D. It was found that dietary vitamin D was absorbed with the food fats and was dependent on the presence of the bile salts. Any disease which resulted in an impairment of fat absorption likewise resulted in an impairment of vitamin D absorption. Absorption of the vitamin is a passive process that is influenced by the composition of the gut contents. Vitamin D is absorbed with the long-chain fatty acids and is present in the chylomicrons of the lymphatic system. Absorption takes place primarily in the jejunum and ileum. This has a protective effect on vitamin D stores because the bile, released into the duodenum, is the chief excretory pathway of the vitamin; reabsorption in times of vitamin need can protect the body from undue loss. However, in times of vitamin excess, this reabsorptive mechanism may be a detriment rather than a benefit. The vitamin is absorbed in either the hydroxylated or the unhydroxylated form.

Although many of the other essential nutrients are absorbed via an active transport system, there is little reason to believe that absorption of vitamin D is by any mechanism other than by passive diffusion. The body, if exposed to sunlight, can convert the 7-dehydrocholesterol at the skin's surface to cholecalciferol, and this compound is then further metabolized in the kidney, producing the active principle, 1,25-dihydroxy cholecalciferol. Because the body, under the right conditions, can synthesize *in toto* its vitamin needs, and because it needs so little of the vitamin, there appears to be little reason for the body to develop an active transport system for its absorption. However, in the person with renal disease or in persons using excessive amounts of sun blocker, the synthesis of 1,25-dihydroxy cholecalciferol is impaired and in this individual intestinal uptake of the active form is quite important. Oral supplements can be used to ensure adequate vitamin D status.

Because the body can completely synthesize dehydrocholesterol and ergosterol, convert it to D_2 or D_3, and then hydroxylate it to form the active form, an argument against its essentiality as a nutrient can be developed. In point of fact, because the active form is synthesized in the kidney and from there distributed by the blood to all parts of the body, this active form meets the definition of a hormone and the kidney, its site of synthesis meets the definition of an endocrine organ. Thus, whether vitamin D is a nutrient or a hormone is dependent on the degree of exposure to ultraviolet light. Lacking exposure, vitamin D must be provided in the diet and thus is an essential nutrient.

Transport

Once absorbed, vitamin D is transported in nonesterified form bound to a specific vitamin-D- binding protein. This protein (DBP) is nearly identical to the α-2 globulins and albumins with respect to its electrophoretic mobility. All of the forms of vitamin D (25-hydroxy D_3, 24,25- dihydroxy D_3, and 1,25-dihydroxy D_3 are carried by this protein, which is a globulin with a molecular weight of 58,000 daltons. Its binding affinity varies with the vitamin form. DNA sequence analysis of DBP

shows homology with a fetoprotein and serum albumin. DBP also has a high affinity for actin but the physiological significance for this cross-reactivity is unknown.

Metabolism

Once absorbed or synthesized at the body surface, vitamin D is transported via DBP to the liver. Here, it is hydroxylated via the enzyme vitamin D hydroxylase at carbon 25 to form 25-hydroxycholecalciferal. Figure 9.9 illustrates the pathway from cholesterol to 1,25-dihydroxy-cholecalciferol. With the first hydroxylation a number of products are formed but the most important of these is 25-hydroxycholecalciferol. The biological function of each of these metabolites is not known completely. As mentioned, the hydroxylation occurs in the liver and is catalyzed by a cytochrome P-450-dependent mixed-function monooxygenase. This enzyme has been found in both mitochondrial and microsomal compartments. It is a two-component system that involves flavoprotein and a cytochrome P-450, and is regulated by the concentration of ionized calcium in serum. The hydroxylation reaction can be inhibited if D_3 analogs having modified side chains are infused into the animal fed a rachitic diet and a D_3 supplement.

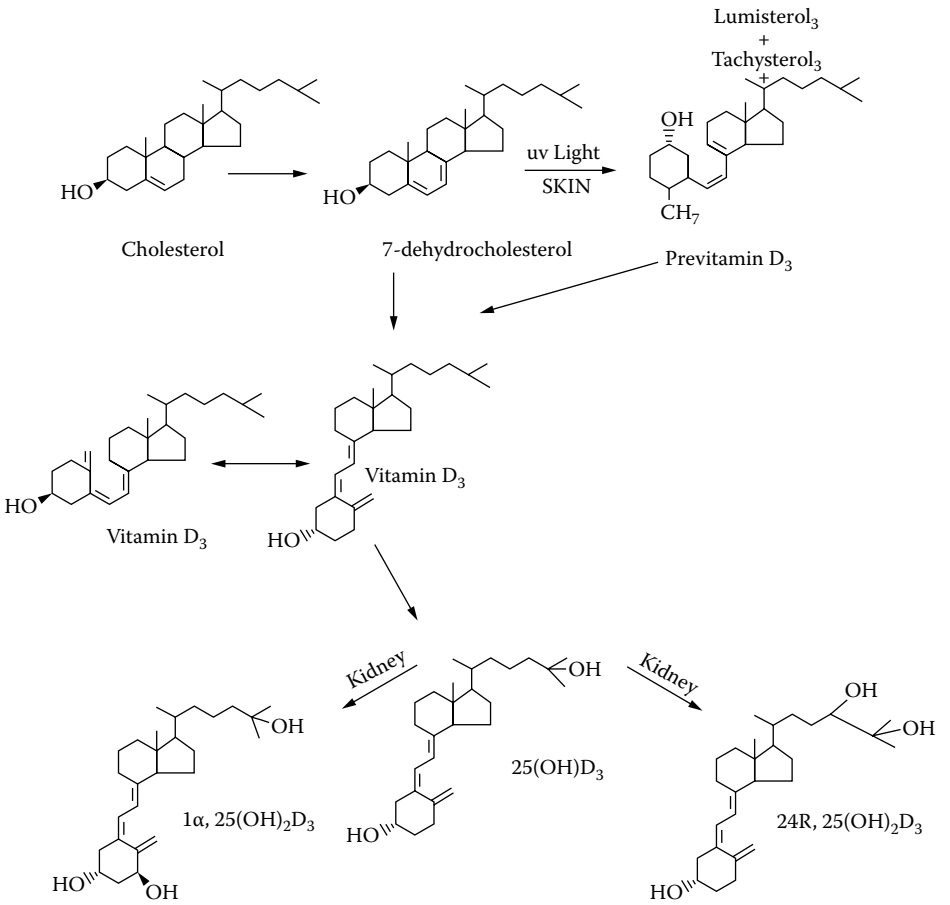


FIGURE 9.9 Synthesis of active 1,25-dihydroxycholecalciferol using cholesterol as the initial substrate. Several isomers can result when previtamin D or 7-dehydrocholesterol is converted to D_3, cholecalciferol.

The 25-hydroxy D_3 is then bound to DBP and transported from the liver to the kidney where a second hydroxyl group is added at carbon 1. This hydroxylation occurs in the kidney proximal tubule mitochondria catalyzed by the enzyme 25-OH-D_3-1α-hydroxylase. This enzyme has been characterized as a three-component enzyme involving cytochrome P-450, an iron sulfur protein (ferredoxin) and ferredoxin reductase. The reductant is $NADPH_2$. Considerable evidence has shown that 1,25-dihydroxy cholecalciferol is the active principle that stimulates bone mineralization, intestinal calcium uptake, and calcium mobilization.[39–48] Because this product is so active in the regulation of calcium homeostasis its synthesis must be closely regulated. Indeed, product feedback regulation not only on the activity of this enzyme but also at the level of its transcription has been shown. In both instances, the level of 1,25-dihydroxy cholecalciferol negatively affects 1α-hydroxylase activity and suppress mRNA transcription of the hydroxylase gene product. In addition, control of the hydroxylase enzyme is also exerted by the parathyroid hormone, PTH. When plasma calcium levels fall, PTH is released, and this hormone stimulates 1-hydroxylase activity while decreasing the activity of 25-hydroxylase. In turn, PTH release is downregulated by rising levels of 1,25-dihydroxy cholecalciferol and its analog, 24,25-dihydroxy cholecalciferol. Insulin, growth hormone estrogen and prolactin are additional hormones that stimulate the activity of the 1α-hydroxylase. The mechanisms that explain these stimulatory effects are less well known and are probably related to their effects on bone mineralization as well as on other calcium using processes.

Just as several metabolites are formed in the 25-hydroxylase reaction a number of products result with the second hydroxylation. Some of all of these transformations is shown in Figure 9.10. Also shown are the degradative products found primarily in the feces.

D_3 is subject to other metabolic reactions as well (Figure 9.10). Whether these metabolites have specific functions with respect to mineral metabolism is not fully understood. Instead of 1,25-dihydroxy D_3, 24,25-dihydroxy D_3 may be formed and may serve to enhance bone mineralization, embryonic development, and suppress parathyroid hormone release. Then, 24,25-dihydroxy D_3 arises by hydroxylation of 25 hydroxy D_3. When 25-OH D_3 1α-hydroxylase activity is suppressed, 24,25-hydroxylation is stimulated. This hydroxylase is substrate inducible through the mechanism of increased enzyme protein synthesis and has been found in kidney, intestine, and cartilage. 24,25-dihydroxy D_3 may represent a "spillover" metabolite of D_3. That is, a metabolite is formed when excess 25-hydroxy D_3 is present in the body. Other D_3 metabolites such as 25-hydroxy D_3 26,23-lactose also can be regarded as spillover metabolites because measurable quantities are observed under conditions of excess intake. Although 24,25-dihydroxy D_3 does function in the bone mineralization process, it is not as active in this respect as is 1,25-dihydroxy D_3.

The question of whether the hydroxy group at carbon 25 is a requirement for vitamin activity has been posed because several D_3 metabolites, as per Table 9.2, lack this structural element. Studies utilizing fluro-substituted D_3 showed conclusively that although maximal activity is shown by the 1,25-D_3, activity can also be shown by compounds lacking this structure. In part, the structural requisite for vitamin activity may relate to the role the 25-hydroxy substituents play in determining the molecular shape of the compound. This shape must conform to the receptor shape of the cellular membranes in order for the D_3 to be utilized. Specific intracellular receptors for 1,25-dihydroxy D_3 have been found in parathyroid, pancreatic, pituitary, and placental tissues All these tissues have been shown to require D_3 for the regulation of their function. For example, in D_3 deficiency, pancreatic release of insulin is impaired. Insulin release is a calcium-dependent process.

When discussing vitamin D metabolism, it is important to recognize that 7 hydroxycholesterol irradiated by ultraviolet light becomes a cholecalciferol, which is transported to liver, converted to 25-hydroxycholecalciferol, transported to the kidney, and converted to 1,25-dihydroxy cholecalciferol, which is, in fact, the active principle. This compound is then distributed throughout the body exerting its effect on a variety of tissues. Because the body can, indeed, synthesize the active compound, and because this synthesis takes place in an organ distal to the tissues upon which it acts, this compound is more truly a hormone rather than an essential nutrient. Of course, if irradiation

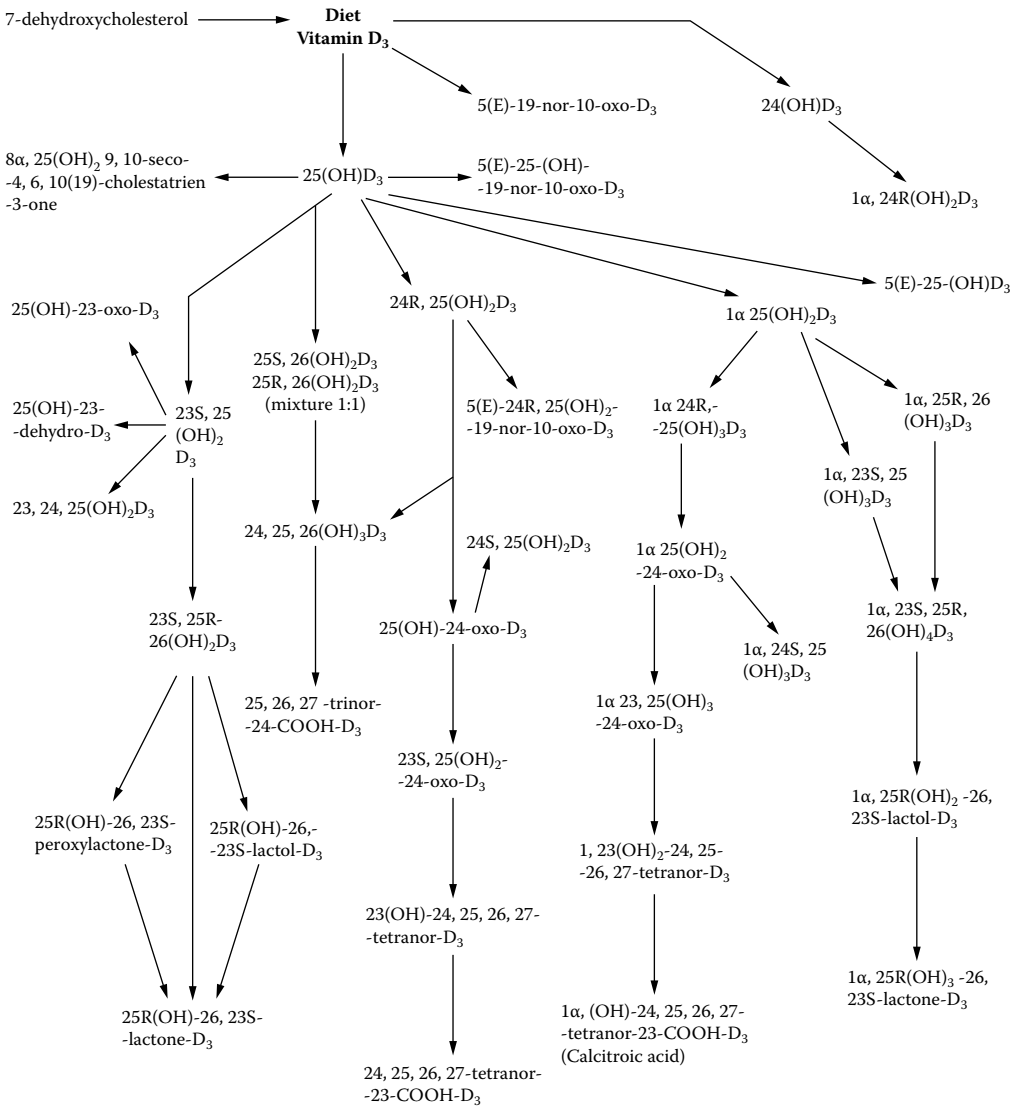


FIGURE 9.10 Pathways for vitamin D_3 synthesis and degradation.

does not or cannot occur sufficiently to effect the conversion of 7-hydroxycholesterol, then cholecalciferol must be provided in the diet and then, by definition, becomes a required nutrient.

As can be seen in Figure 9.10, several pathways exist for the degradation of the active 1,25- dihydroxycholecalciferol. These include oxidative removal of the side chain, additional hydroxylation at carbon 24, the formation of a lactone (1,25 OH_2 D_2-26,23-lactone) and additional hydroxylation at carbon 26. Although 25-hydroxycholecalciferol can accumulate in the heart, lungs, kidneys, and liver, 1,25-dihydroxycholecalciferol does not accumulate. The active form is not stored appreciably but is found in almost every cell and tissue type.

Function

Until the recognition of the central role of the calcium ion in cellular metabolic regulation, it was thought that vitamin D's only function was to facilitate the deposition of calcium and phosphorus

in bone.[42] This concept developed when it was recognized that the bowed legs of rickets was due to inadequate mineralization in the absence of adequate vitamin D intake or exposure to sunlight.[43] We now know that vitamin D plays a key role in the regulation of the glyoxylate cycle in the liver,[44] stimulates cell proliferation in the adipose tissue via reactive oxygen species,[42] and modulates adipocyte glucocorticoid function.[43]

Studies of in vivo calcium absorption by the intestine revealed that D deficient rats absorb less calcium than D sufficient rats and that rats fed very high levels (10,000 IU/day) absorbed more calcium than did normally fed rats. These observations of the vitamin effects on calcium uptake lead to work designed to determine the mechanism of this effect. It was soon discovered that vitamin D (1,25-dihydroxy D_3) served to stimulate the synthesis of a specific protein in the gut cells that was responsible for calcium uptake.[44] This protein, called the calcium-binding protein (calbindin), was isolated from the intestine and later from brain, bone, kidney, uterus, parotid gland, parathyroid glands, and skin. Several different calcium binding proteins have been found but not all of these binding proteins are vitamin D dependent. That is, once formed, their activity with respect to calcium binding was unaffected by vitamin deficiency. Most, however, are dependent on vitamin D for their synthesis. Thus, these calcium-binding proteins are molecular expressions of the hormonal action of the vitamin.

As animals age, the levels of calcium-binding protein fall.[39] Yet, when calcium intake levels fall, the synthesis and activity of the binding proteins rise. This mechanism explains how individuals can adapt to low calcium diets. Interestingly, calcium deprivation stimulates the conversion of cholecalciferol to 25-hydroxycholecalciferol in the liver and to 1,25-dihydroxy cholecalciferol in the kidney. Aging, however, seems to affect this regulatory mechanism. As humans age, they are less able to absorb calcium and may develop osteoporosis, a condition analogous to rickets in children and characterized by demineralization of the bone. In osteoporotic patients, intestinal absorption of calcium is decreased but when 1,25-dihydroxy D_3 is administered, calcium absorption is increased. It would appear, therefore, that one of the consequences of aging is an impaired conversion of 25-hydroxy D_3 to 1,25-dihydroxy D_3, and because less of the latter is available, less calcium-binding protein is synthesized. Measures of calcium-binding protein in aging rats, using an immunoassay technique, have shown that this is indeed the case.

Vitamin D also increases intestinal absorption of calcium by mechanisms apart from the synthesis of calcium binding protein. It does this as part of its general tropic effect as a steroid on a variety of cellular reactions. Vitamin D effects a change in membrane permeability to calcium at the brush border, perhaps through a change in the lipid (fatty acid) component of the membrane. It stimulates the $Ca^{++}Mg^{++}$ ATPase on the membrane of the cell wall, increases the conversion of ATP to cAMP, and increases the activity of the alkaline phosphatase enzyme. All these effects in the intestinal cell are independent of the vitamin's effect on calcium binding protein synthesis.

In addition to its role in calcium absorption, vitamin D serves to induce the uptake of phosphate and magnesium by the brush border of the intestine. The effect on phosphate uptake is independent of its effect on calcium absorption and is due to an effect of the vitamin on the synthesis of a sodium-dependent membrane carrier for phosphate. The effect of vitamin D on magnesium absorption is incidental to its effect on calcium absorption because the calcium binding protein has a weak affinity for magnesium. Thus, if synthesis of the calcium binding protein results in an increase in calcium uptake, it also results in a significant increase in magnesium uptake.

Regulation of Serum Calcium Levels

Serum calcium levels are closely regulated in the body so as to maintain optimal muscle contractility and cellular function.[45] Several hormones are involved in this regulation: 1,25- dihydroxy D_3, produced by the kidney, parathyroid hormone released by the parathyroid gland, and thyrocalcitonin released by the thyroid C cells. Each has a specific function with respect to serum calcium levels, and all three are interdependent. Vitamin D_3 increases blood calcium by increasing intestinal

calcium uptake and decreases blood calcium by increasing calcium deposition in the bone. In the relative absence of vitamin D, parathyroid hormone increases serum calcium levels by increasing the activity of the kidney 1α-25-hydroxylase with the result of increasing blood levels of 1,25-dihydroxy D_3 and through enhancing bone mineral mobilization and phosphate diuresis. Parathyroid hormone in the presence of vitamin D has the reverse action on bone. When both hormones (parathormone and 1,25-D_3) are present, bone mineralization is stimulated. Even though parathyroid hormone stimulates the production of 1,25-dihydroxy D_3, D_3 does not stimulate parathyroid hormone release. Thyrocalcitonin serves to lower blood calcium levels through stimulating bone calcium uptake, and its effect is independent of parathyroid hormone yet is dependent on the availability of calcium from the intestine. If serum calcium levels are elevated through a calcium infusion, thyrocalcitonin will be released and stimulate bone calcium uptake even in animals lacking both parathyroid hormone and D_3.

Mode of action at the genomic level: The process of vitamin D (1,25-dihydroxy cholecalciferol) receptor binding to specific DNA sequences follows the classic model for steroid hormone action. Like vitamin A, vitamin D binds to a receptor protein in the nucleus.[48–50] The receptor protein then acquires an affinity for specific DNA sequences located upstream from the promoter sequence of the target gene.[50] These specific DNA sequences are called *response elements* and consist of a structure in which two zinc atoms are coordinated in two finger-like domains. The N-terminal finger confers specificity to the binding, whereas the second finger stabilizes the complex. When bound, transcription of the cognate protein mRNA is activated. Several response elements have been identified, with each being specific for a specific gene product. As shown in Figure 9.11, the response elements have in common imperfect direct repeats of six base-pair half elements separated by a three base-pair spacer. Affinity of the nuclear receptor protein for vitamin D is modified by phosphorylation. Two sites of phosphorylation have been found both on serines. One site located in the DNA-binding region at serine 51 between two zinc finger DNA-binding motifs appears to reduce DNA binding when phosphorylated by protein kinase C (or a related enzyme). The second site is located in the N-terminal region of the hormone-binding region at Ser 208. It has the opposite effect. When phosphorylated, probably by casein kinase II or a related enzyme, transcription is activated.

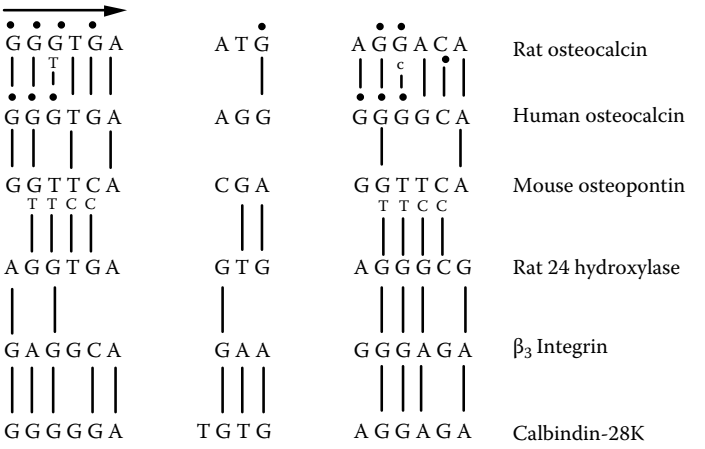


FIGURE 9.11 Vitamin D response elements for osteocalcin, osteropontin, D_3-24-hydroxylase, β_2-integrin, and calbindin 28K. Circles above bases indicate guanine residues that have been shown by methylation interference experiments to be protected upon protein binding to the vitamin D responsive element. The small letters below the large ones indicate points at which base substitutions have been found and which abolish responsiveness to vitamin D.

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The vitamin–protein receptor complex that binds to the DNA consists of three distinct elements: 1,25-dihydroxy cholecalciferol (the hormone ligand), the vitamin receptor, and one of the retinoid X receptors (RxRα). Here is an instance where, once again, a vitamin D–vitamin A interaction can occur.[46] It has been reported that 9-*cis* retinoic acid can attenuate transcriptional activation by the vitamin D receptor binding to the vitamin-D-responsive element. Perhaps 9-*cis* retinoic acid has this effect because, when it binds to the retinoid X receptor, it blocks or partially occludes the binding site of the vitamin D protein for DNA. Figure 9.12 illustrates the mode of vitamin D action at the genomic level. To date, the nuclear vitamin D receptor has been found in 34 different cell types, and it is quite likely that it is a universal nuclear component.[53] Probably this is because every cell has a need to move calcium into or out of its various compartments as part of its metabolic control systems. Thus, calcium binding proteins whose synthesis is vitamin-D-dependent are needed. Among the proteins thus far identified are osteocalcin, vitamin-D binding protein, osteoportin, 24 hydroxylase β-3 interferon, calbindin, prepro PTH, calcitonin Type II collagen, fibronectin, bone matrix GLA protein, interleukin 2 and interleukin-γ, transcription factors (GM-CSF, c-myc, c-fos, c-fms), vitamin D receptors, calbindin D_{28x} and $_{9x}$, and prolactin. The transcription of the genes for each of these proteins are affected or regulated by vitamin D.

Vitamin D Deficiency

As discussed earlier, bone deformities are the hallmarks of the vitamin-D-deficient child, whereas porous brittle bones are indications of the deficiency in the adult.[52] Rickets was very common in the United States prior to the enrichment of milk and other food products with vitamins $D_{2\&3}$. Prior to the discovery of the role of light in vitamin D activation, vitamin-D-deficiency disorders developed in part because of the custom of wearing heavy clothing which shielded the skin from the ultraviolet rays of the sun. Ultraviolet light is essential for the in vivo synthesis of the active vitamin. Today, with the heavy emphasis on the use of sunscreen to prevent skin cancer, there is concern that the people who follow this recommendation may be at greater risk for developing vitamin D deficiency

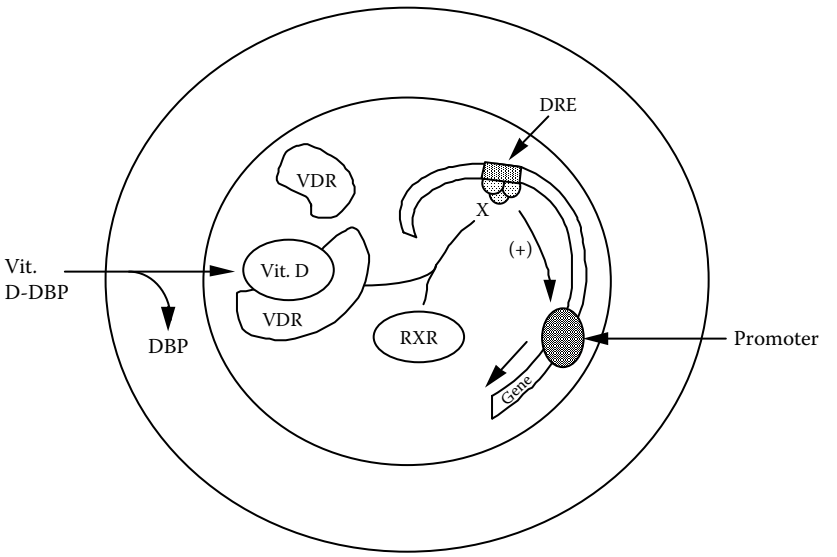


FIGURE 9.12 Schematic representation showing vitamin D bound to vitamin-D-binding protein (DBP) entering the nucleus, binding to receptor (VDR), complexing with retinoid receptor (RXR), and then binding to the vitamin-D-responsive elements (DRE) as a transacting factor enhancing the transcription of a variety of calcium-binding proteins.

disorders.[51–53] Lacking ultraviolet light exposure, the only source is food. Very few foods contain significant quantities of the vitamin unless they are enriched. Without this source of dietary vitamin, a deficiency state could develop. A recent population study of children in the northeastern part of the United States revealed low serum levels of 25(OH)D, and these low levels were associated with reduced bone mass.[53] Nutrition scientists are currently discussing whether the recommended intake for the vitamin should be increased so as to prevent vitamin D deficiency.

Today, osteoporosis develops not only because of the above reasons but also may be due to disease or damage to either the liver or kidney. As pointed out in the metabolism section, both these organs are essential for the conversion of cholecalciferol to 1,25-dihydroxy D_3. If either organ is nonfunctional in this respect, the deficient state will develop. On rare occasions, the deficient state will develop not because of any lack of dietary D or sunlight or because of kidney or liver damage, but because through a genetic error, the 1,25-hydroxylase enzyme is missing and the 1,25-dihydroxy D_3 cannot be synthesized. In individuals so afflicted, 1,25-dihydroxy D_3 must be supplied to prevent the deficiency state from developing.

Also, 1,25-dihydroxy D_3 must be provided to the anephritic patient, as these patients cannot synthesize this hormone. Until the realization that the kidney served as the endocrine organ for 1,25-dihydroxy D_3 synthesis, renal disease was almost always accompanied by a disturbed calcium balance and osteoporosis.

Hypervitaminosis D

Because pre-vitamin D is fat soluble it, like vitamin A, can be stored. The storage capacity of the liver for the D precursor is much less than its capacity for A, and toxic conditions can develop if large amounts of D (in excess of need and storage capacity) are consumed over extended periods of time. Because D's main function is to facilitate calcium uptake from the intestine and tissue calcium deposition, excess D in the toxic range (1000 to 10,000 IU/d) will result in excess calcification not only of bone but of soft tissues as well. Hathcock et al.[53] has suggested that upper tolerable intake limit for this vitamin is ≥ 250 μg/d (10,000 IU vitamin D_3). This value is higher than that suggested by the Food and Nutrition Board of the National Academy of Medicine. Symptoms of excess (toxic) intakes include: renal stones, calcification of the heart, major vessels, muscles, and other tissues. These have been observed in experimental animals including primates, as well as in humans. A series of patients who were unusually sensitive to vitamin D either in utero or in infancy have been described. These patients had multiple abnormalities in soft tissues and bones and were mentally retarded. Whether excess D intakes provoke mild, moderate, or severe abnormalities is related not only to the individual's genetic background but also to his or her calcium, magnesium, and phosphorus intake. If any of these are consumed in excess of the others, vitamin D intoxication becomes more apparent.

Need

Because the active D_3 hormone can be synthesized in the body, an absolute requirement is difficult to determine. Few foods naturally contain sufficient preformed vitamin D. In the absence of in vivo synthesis, preformed vitamin D must be added to the diet. This is done almost to excess in the United States. Milk is fortified with 10 μg/quart and other dairy products such as margarine are also fortified with the vitamin. Due to this fortification, infantile rickets is almost unknown today. The level of supplementation for milk was selected based on the concept that the growing child should drink one quart of milk a day and if he/she does do so, he or she will receive the recommended daily intake for growth. Adults usually do not require vitamin supplementation unless they are either pregnant or lactating. Pregnant or lactating females are also recommended to consume 10 μg vitamin D per day. There is considerable discussion today about these recommendations. Because

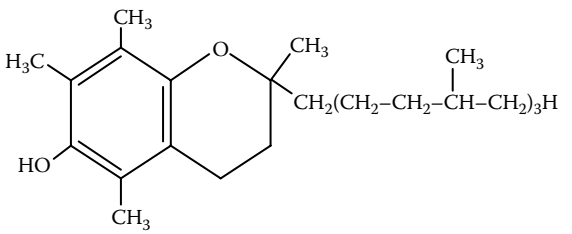
of the fear of skin cancer, more people are using sunscreen to block the harmful actions of ultraviolet light. In blocking ultraviolet light these people are also inadvertently blocking the synthesis of active vitamin D. There is a thought that dietary intake recommendations should be increased for such people.

VITAMIN E

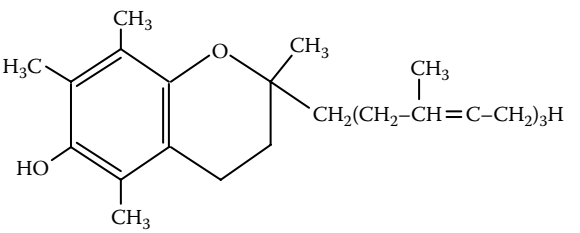
Although much of the excitement of the "vitamin discovery era" was devoted to the work on vitamins A and D, Evans and Bishop in 1922 discovered an unidentified factor in vegetable oil that, if lacking from the diet, resulted in reproductive failure. The term *tocopherol* was proposed by Emerson for this factor because of its role in reproduction. Rats fed diets lacking this vitamin failed to reproduce. The name *tocopherol* comes from the Greek work *tokos*, meaning childbirth, *pherein*, meaning to bring forth, and *ol*, the chemical suffix meaning alcohol. Vitamin E, the name suggested by Evans and Bishop, is commonly used for that group of tocol and tocotrienol derivatives having vitamin E activity. The most active of these is α-tocopherol (Figure 9.13). Much of the earlier work was devoted to tocopherol's role in reproduction such that its function as an antioxidant was ignored. However, in the last 30 years, considerable attention has been given to this role in metabolism. It is recognized that vitamin E is an essential nutrient for many animal species. The level of vitamin E in plasma lipoproteins and in the phospholipids of the membrane around and within the cell are dependent on vitamin E intake as well as on the intake of other antioxidant nutrients and on the level of dietary polyunsaturated fatty acids.

Structure and Nomenclature

The most active, naturally occurring form of vitamin E is *d*-α-tocopherol. Other tocopherols have been isolated having varying degrees of vitamin activity. Figure 9.13 shows the molecular



α-Tocopherol (5, 7, 8 Trimethyltocol)



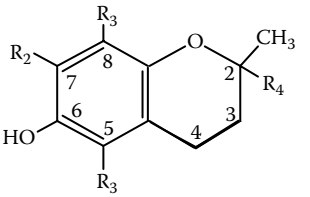
α-Tocotrienol (5, 7, 8 Trimethyltrienol)

FIGURE 9.13 Basic structures of vitamin E.

structures of α-tocopherol and α-tocotrienol. Figure 9.14 shows the other tocopherols and tocotrienols and their relationship to α-tocopherol. To have vitamin activity, the compound must have the double ring structure (chromane nucleus) as shown in Figures 9.13 and 9.14, and must also have a side chain attached at carbon 2 and methyl groups attached at carbons 5, 7, or 8. α-Tocopherols have methyl groups attached at all three positions and represent the most active vitamin compounds. β-Tocopherols have methyl groups attached to carbons 5 and 8; γ- tocopherols have methyl groups attached at carbons 7 and 8, and δ-tocopherols have only one methyl group attached at carbon 8. If the side chain attached to carbon 2 is saturated, then the compound is a member of the tocol family of compounds; if unsaturated, it belongs to the tocotrienol family. All forms have a hydroxyl group at carbon 6 and a methyl group at carbon 2. Other forms, ε, ζ, and η, have their methyl groups at carbon 5, or 5 and 7, or 7, respectively. Naturally occurring vitamin E is in the D form whereas synthetic vitamin E preparations are mixtures of the D and L forms. Both tocols and trienols occur as a variety of isomers. There are commercially available products usually marketed as acetate or succinate esters. The ester form does not usually occur in nature. Table 9.11 lists some of these commercially available forms.

International Units and Methods of Analysis

The international unit (IU) of vitamin E activity uses the activity of 1 mg of *dl*-α-tocopherol acetate (all-rac) in the rat fetal absorption assay as its reference standard.



$R_1R_2R_3 = CH_3$ or H

$R_4 = CH_2(CH_2CH_2\underset{\displaystyle CH_3}{CH}CH_2)_3H$ (Tocols)

or $CH_2(CH_2CH = \underset{\displaystyle CH_3}{C}\ CH_2)_3H$ (Tocotrienols)

α Tocol or Tocotrienol have $R_1R_2R_3 = CH_3$

β Tocol or Tocotrienol have $R_1R_3 - CH_3$, R = H

γ Tocol or Tocotrienol have $R_2R_3 = CH_3$, R_1 = H

δ Tocol or Tocotrienol have $R_3 = CH_3$, R_1R_2 = H

ε Tocol or Tocotrienol have $R_1 = CH_3$ R_2 R_3 = H

ζ Tocol or Tocotrienol have R_1,$R_2 = CH_3$ R_3 = H

η Tocol or Tocotrienol have $R_2 = C+_3$, R_1R_3 = H

FIGURE 9.14 Structures of naturally occurring compounds having vitamin E activity.

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TABLE 9.11
Commercially available products having vitamin E activity

Form	Units of activity/mg
dl-α-tocopheryl acetate (all-rac)	1.00
dl-α-tocopherol (all-rac)	1.10
D, α-tocopheryl acetate (RRR)[a]	1.36
D, α-tocopheryl acid succinate (all-rac)	1.49
dl-α-tocopheryl acid succinate (all-rac)	0.89
D, α-tocopheryl acid succinate (RRR)[a]	1.21

[a] RRR—only naturally occurring stereoisomers bear this designation.

Even though d α-tocopherol is 36% more active than the dl form, the latter was selected as the reference substance because it is more readily available as a standard of comparison. This choice may be a poor one because of the lack of validation of the fetal rat absorption assay as a true test of vitamin E potency. The fetal resorption test uses female vitamin-E-depleted virgin rats. These rats are mated to normal males and given the test substance. After 21 days the number of live fetuses and the number of dead and resorbed ones are counted. The potency of the test material is compared to a known amount of dl, α-tocopherol. Other tests have also been devised and are based on other functions of vitamin E. These tests may yield more reliable comparisons and may result in a redefinition of the IU. Tests of biopotency include the red-cell hemolysis test and tests designed to evaluate the potency of the test substance in preventing or curing muscular dystrophy. Using *dl*-α-tocopherol as the standard with a value of 100, β-tocopherol has a value of 25–40 on the fetal resorption test, 15–27 on the hemolysis test, and 12 on the preventative muscular dystrophy test using the chicken as the test animal. Even less potent is γ-tocopherol with values of 1 to 11, 3 to 20, and 5 for the same tests, respectively. The other tocopherols and tocotrienols are even less potent by comparison. Burton and Traber have reviewed the biopotency of vitamin E isomers as antioxidants.

Biochemical methods that utilize changes in enzyme activity rather than functional tests such as the rat fetal resorption test allow for the comparison of the different E vitamin forms. For example, plasma pyruvate kinase, hepatic glutathione peroxidase, and muscle cyclooxygenase activity are reduced in vitamin-E-deficient rats. However, a true dose response curve showing intake versus changes in enzyme activity patterns, which in turn precede tissue changes, does not clearly provide a basis for biopotency. These enzyme activity studies are not as sensitive with respect to vitamin intake and potency as one would like.

Chemical analyses using thin layer chromatography, gas-liquid chromatograph and high performance liquid chromatography (HPLC) are now available. These methods are very sensitive and can separate and quantify the various isomers in food, plasma, blood cells, and tissues. The HPLC method is the one of choice because sample preparation is minimal. Amounts of the isomers in the nanogram range can be detected and quantified.

Chemical and Physical Properties

The tocopherols are slightly viscous oils that are stable to heat and alkali. They are slowly oxidized by atmospheric oxygen and rapidly oxidized by iron or silver salts. The addition of acetate or succinate to the molecule adds stability towards oxidation. The tocopherols are insoluble in water but soluble in the usual fat solvents. Ultraviolet light destroys its vitamin activity. Table 9.12 gives the properties of four of the most potent tocopherols.

TABLE 9.12
Characteristics of the major tocopherols

Compound	Color	Boiling point (°C)	Molecular weight	Absorption maxima (nm)	Extraction (ethanol)
dl-α-tocopherol	Colorless to pale yellow	200–220	430.69	292–294	71–76
d, α- tocopherol	Colorless to pale yellow	—	430.69	292–294	72–76
dl-α-tocopherol acetate	Colorless to pale yellow	224	472.73	285.5	40–44
d, α -tocopheryl acetate	Colorless to pale yellow	——	472.73	285.5	40–44

Sources

The tocopherols have been isolated from a number of foods. Almost all are from the plant kingdom with wheat germ oil being the richest source. European wheat germ oil contains mostly β-tocopherols, whereas American wheat germ oil contains mostly α-tocopherols. Corn oil contains α-tocopherols, and soybean oil δ-tocopherols. Olive and peanut oil are poor sources of the vitamin. Some animal products such as egg yolk, liver, and milk contain tocopherols but, in general, foods of animal origin are relatively poor sources of the vitamin. Table 9.13 provides values of tocopherols in a variety of foods. Vegetable oils vary from 100 μg/g (olive oil) to nearly 1200 μg/g (wheat germ oil). Some of the foods shown in this table have a range of values given because of seasonal variations due to differences in intakes and needs of the animal from which these foods come.

Metabolism

Absorption and Transport

Because of its lipophilicity, vitamin E like the other fat soluble vitamins, is absorbed via the formation of chylomicrons and their uptake by the lymphatic system. The tocopherols are transported as part of the lipoprotein complex.[54] Absorption is relatively poor, and it is unlikely to involve a protein-carrier-mediated process. In man, studies of labeled tocopherol absorption have shown that less than half of the labeled material appears in the lymph and up to 50% of the ingested vitamin may appear in the feces.[55] Efficiency of absorption is enhanced by the presence of food fat in the intestine. Within this macronutrient class, efficiency of absorption is enhanced by saturated fat and decreased by the presence of polyunsaturated fatty acids. The use of water miscible preparations enhances absorption efficiency, particularly in those individuals whose fat absorption is impaired (i.e., persons with cystic fibrosis or biliary disease). The commercially prepared tocopherol acetate or palmitate loses the acetate or palmitate through the action of a bile-dependent mucosal cell esterase prior to absorption. The pancreatic lipases, bile acids, and mucosal cell esterases are all important components of the digestion and absorption of vitamin E from food sources. The same processes required for the digestion and absorption of food fat apply here for the tocopherols. Absorption through penetration of the apical plasma membrane of the enterocytes of the brush border is maximal in the jejunum. There are some species differences in the process, in that mammals absorb the vitamin as part of a lipoprotein complex (chylomicrons) into the lymph whereas birds have the vitamin transported directly into the portal blood. In addition, there are gender differences in absorption efficiency: females are more efficient than males. Unlike the food fats (cholesterol and the acylglycerides) hydrolysis is not followed by reesterification in the absorption process.

It appears that the tocopherols are bound to all of the lipid-carrying proteins in the blood and lymph. An excellent antioxidant, vitamin E serves this function very well as it is being transported (from enterocyte to target tissue) with those lipids that could be peroxidized and thus require protection.[55,56] Some cardiovascular researchers have suggested that one important function of vitamin E

TABLE 9.13
Vitamin E content of a variety of foods.

Food	α-tocopherol[1]
	µg/g
Corn oil	159
Olive oil	100
Peanut oil	189
Wheat germ oil	1194
Palm oil	211
Soft margarine	139
Milk	0.2–1.1
Butter	10–33
Lard	2–38
Eggs	8–12
Fish	4–33
Beef	5–8
Pork	4–6
Chicken	2–4
Almonds	270
Peanuts	72
Oatmeal	17
Rice	1–7
Wheat germ	117
Apple	3
Peach	13
Asparagus	16
Spinach	25
Carrots	4

Source: http://www.nal.usda.gov/fnic/foodcomp/Data/food.82nutrients

is to prevent the peroxidation of lipids in the blood, which would in turn suppress possible endothelial damage to the vascular tree and thus suppress some of the early events in plaque formation.[57–64] Whether this hypothesis about the role of vitamin E in preventing such degenerative disease is true remains to be proven.

Intracellular Transport and Storage

Although no specific transport protein has been found for the tocopherols in blood and lymph, there appears to be such a protein within the cells.[63] A 30-kDA α-tocopherol-binding protein has been found in the hepatic cytosol, and another 14.2-kDa one in heart and liver that specifically binds to α-tocopherol and transfers it from liposomes to mitochondria. No doubt we will also find that either this or another low molecular weight protein transfers the vitamin to the nucleus. Having the vitamin in these two organelles protects them from free-radical damage. One of the targets of free radicals is the genetic material DNA whereas the other is the membrane phospholipid. In either instance, damage to these vital components could be devastating. The smaller of the two binding proteins is similar in size to the intracellular fatty-acid-binding protein, FABP. This protein also

binds some of the eicosanoids but not α-tocopherol. The other tocopherols (β, γ, etc.) are not bound to the tocopherol-binding proteins to the same extent as α-tocopherol nor are these isomers retained as well. Tocopherols are found in all of the cells in the body, with adrenal cells, pituitary cells, platelets, and testicular cells having the most per cell. Adipose tissue, muscle, and liver serve as reservoirs, and these tissues will become depleted should intake levels be inadequate to meet need. The rate of depletion with dietary inadequacy varies considerably. Because its main function is as one of several antioxidants, other nutrients that also serve in this capacity can affect vitamin depletion. The intake of β-carotene and ascorbic acid and also the level of polyunsaturated fatty acid intake can markedly affect the rate of use of α-tocopherol as an antioxidant.[63,66-68] Increased intakes of β-carotene and ascorbic acid protect the α-tocopherol against depletion, whereas increased intakes of polyunsaturated fatty acids drive up the need for antioxidants. A further consideration is the intake of selenium.[67] This mineral is an integral part of the glutathione peroxidase system that suppresses free-radical production. In selenium deficient animals the need for α-tocopherol is increased and vice versa. The α-tocopherol-deficient animal has a greater need for selenium. In addition, α-tocopherol protects against iron toxicity in another instance of a mineral vitamin interaction.[65] In this instance high levels of iron drive up the potential for free-radical formation, and this can be overcome with increases in vitamin E intake.

Catabolism and Excretion

Upon entry into the cell very little degradation occurs. Usually less than 1% of the ingested vitamin (or its metabolite) appears in the urine (Figure 9.15). Compounds called Simon's metabolites appear in the urine. These are glucuronates of the parent compound. The major excretory route is via the intestine.

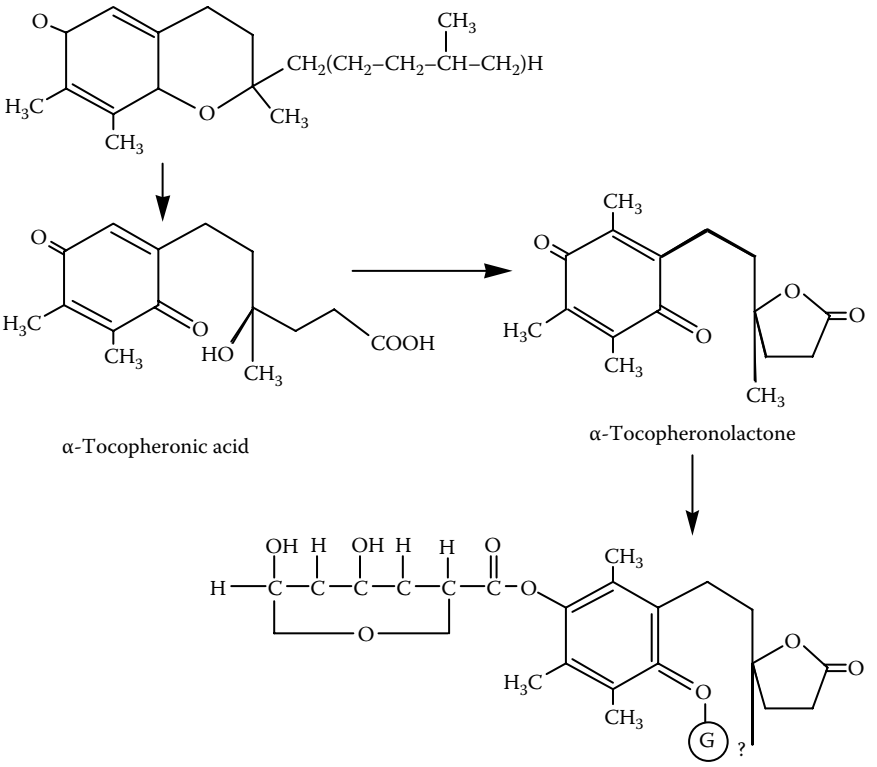


FIGURE 9.15 Excretory pathway for the tocopherols. These compounds are found in the feces.

Function

As mentioned, the main function of vitamin E is as an antioxidant. This function is shared by β-carotene, ascorbic acid, the selenium-dependent glutathione peroxidase and the copper-manganese and magnesium-dependent superoxide dismutase.

Lipid peroxidation is one of the major mechanisms responsible for biological damage to cells and tissues. Proteins and amino acids can also form free radicals and damage cells and tissues. The phospholipids of the membranes within and around the cells are the most vulnerable to this peroxidation because they are less saturated than the stored triacylglycerols within the cell. Phospholipids usually contain an unsaturated fatty acid (arachidonate) at carbon 2, a saturated fatty acid at carbon 1, and a phosphate intermediate at carbon 3. In erythrocytes, the enzyme glutathione peroxidase protects hemoglobin and the cell membrane from oxidation by maintaining glutathione levels, thus regulating the redox state of the cells. This enzyme protects hemoglobin and the cell membrane by detoxifying lipid hydroperoxides to less toxic fatty acids and by preventing the initial free radical attack on the membrane lipids. Although this is a selenoenzyme, vitamin E potentiates its action by serving as a free radical scavenger to prevent lipid hydroperoxide formation.

Glutathione peroxidase has been found in cells other than red blood cells. It is present in adipose tissue, liver, and muscle and glandular tissue, and its activity is complementary to that of catalase, another enzyme which uses peroxide as a substrate. Together these enzymes and vitamin E protect the integrity of the membranes by preventing the degradation, through oxidation, of the membrane lipids. This function of vitamin E is seen more clearly in animals fed high levels of polyunsaturated fatty acids.[66,68] As the intake of these acids increase, they are increasingly incorporated into the membrane lipids, which in turn become more vulnerable to oxidation. Unless protected against oxidation, the functionality of the membranes will be impaired and if uncorrected, the cell will die.[69] In addition there is DNA damage by the increase in the intake of polyunsaturated fatty acid that was ameliorated by vitamin E supplementation.[68] Disturbances in the transport of materials across membranes has been shown in liver with respect to cation flux. Liver slices from E deficient animals lost the ability to regulate sodium/potassium exchange and calcium flux. Investigators have shown a decline in mitochondrial respiration in vitamin E deficient rats. Such a decline probably represents a decline in the flux of ADP or calcium into the mitochondria to stimulate oxygen uptake by the respiratory chain. Such a decline would permit more oxygen to remain in the cytosol to serve in the oxidation of lipids. In addition to peroxidative damage to the membrane, there is also damage to the DNA with the possible result of aberrant gene products.[69] Thus, a whole cascade of responses to vitamin E insufficiency can be envisioned. Interestingly, in diseases manifested by an increased hemolysis of the red cells and a decreased ability of the hemoglobin to carry oxygen, red cell vitamin E levels are low. This has been shown in patients with sickle cell anemia and in patients with cystic fibrosis. In patients with sickle cell anemia, the low vitamin E level in the erythrocytes is accompanied by an increased level of glutathione peroxidase activity. It has been suggested that the increase in enzyme activity was compensatory to the decrease in vitamin E content.

Vitamin E and zinc have been found to have interacting effects in the protection of skin lipids. In zinc deficient chicks, supplementation with vitamin E decreased the severity of the zinc deficiency state suggesting that zinc also may have antioxidant properties or that there may be an interacting effect of zinc with the vitamin.

In addition to the above main function of vitamin E, there are other roles for this substance. One involves prostaglandin synthesis. Thromboxane (TXA_2), a platelet aggregating factor, is synthesized from arachidonic acid (20:4) via a free radical mediated reaction.[70] This synthesis is greater in a deficient animal than in an adequately nourished one. Vitamin E enhances prostacyclin formation and inhibits the lipooxygenase and phospholipase reactions. This effect is secondary to the vitamin's role as antioxidant. As mentioned, phospholipase A_2 is stimulated by lipid peroxides. Other secondary functions also are related to its antioxidant function. Oxidant damage to DNA in bone marrow could explain red blood cell deformation as well as explain the fragility (due to

membrane damage) of these cells.[71] In turn, this would explain why enhanced red-cell fragility is a characteristic of the deficient state.

Steroid hormone synthesis as well as spermatogenesis, both processes that are impaired in the deficient animal, could be explained by the damaging effects of free radicals on DNA, which are corrected by the provision of this antioxidant vitamin.

Hypervitaminosis E

Even though vitamin E is a fat soluble vitamin like A and D, there is little evidence that high intakes will result in toxicity in man. Excess is excreted in feces; however, E toxicity has been produced in chickens. It is characterized by growth failure, poor bone calcification, depressed hematocrit and increased prothrombin times. These symptoms suggest that the excess E interfered with the absorption and/or use of the other fat soluble vitamins as these symptoms are those of the A, D, and K deficiency states. This suggests that advocates of megadoses of vitamin E as treatment for heart disease, muscular dystrophy, and infertility (among other ailments) may unwittingly advocate the development of additional problems associated with an imbalance in fat soluble vitamin intake due to these large E intakes.

Deficiency

One of the first deficiency symptoms recorded for the tocopherols was infertility, followed by the discovery that the white muscle disease or a peculiar muscle dystrophy could be reversed if vitamin E was provided. Later it was recognized that selenium also played a role in the muscle symptom. Listed in Table 9.14 are the many symptoms attributed to inadequate vitamin E intake. All of these symptoms are related either primarily to the level of peroxides in the tissue or to peroxide damage to either the membranes and/or DNA.

Need

Because of the interacting effects of vitamin E with selenium and other antioxidants, the requirement for the vitamin has been difficult to ascertain. It has been estimated that the average adult consumes approximately 15 mg/d but the range of intake is very large. The daily dietary recommended intakes are shown in Table 9.1.

VITAMIN K

Even though vitamin K was the last fat soluble vitamins discovered, its existence was suspected as early as 1929. In that year, Henrik Dam was studying cholesterol biosynthesis and observed that chickens fed a semi-synthetic sterol-free diet had numerous subcutaneous hemorrhages. Hemorrhages were observed in other tissues as well, and when blood was withdrawn from these birds, it had a prolonged clotting time. At first it was thought that these were symptoms of scurvy in birds, but the addition of vitamin C did not cure the disorder. It was then thought that the hemorrhages characterized the bird's response to a dietary toxin. This hypothesis was also disproved. Finally, it was shown that the inclusion of plant sterols prevented the disease, and thus the disease was shown to be a nutrient deficiency.

Because the condition was characterized by a delayed blood clotting time and because it could be cured or prevented by the inclusion of the nonsaponifiable sterol fraction of a lipid extract of alfalfa, it was named the antihemorrhagic factor. Dam proposed that it be called vitamin K. The letter K was chosen from the German word *koagulation*.

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TABLE 9.14
Vitamin E deficiency disorders.

Disorder	Species affected	Tissue affected
Reproductive failure		
Female	Rodents, birds	Embryonic vascular tissue
Male	Rodents, dog, birds, monkey, rabbit	Male gonads
Hepatic necrosis[b]	Rat, pig	Liver
Fibrosis[b]	Chicken, mouse	Pancreas
Hemolysis[a,c]	Rat, chick, premature infant	Erythrocytes
Anemia	Monkey	Bone marrow
Encephalomalacia[a,c]	Chick	Cerebellum
Exudative diathesis[b]	Birds	Vascular system
Kidney degeneration[a,b]	Rodents, monkey, mink	Kidney tabular epithelium
Steatitis[a,c]	Mink, pig, chick	Adipose tissue
Nutritional myopathies		
Type A muscular dystrophy	Rodents, monkey, duck, mink	Skeletal muscle
Type B white muscle disease[b]	Lamb, calf, kid	Skeletal and heart muscle
Type C myopathy[b]	Turkey	Gizzard, heart
Type D myopathy	Chicken	Skeletal muscle

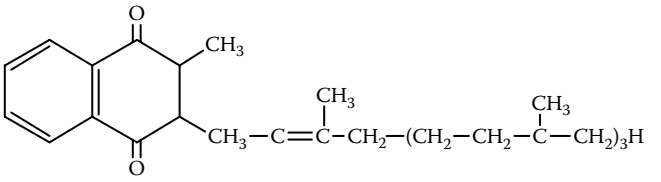
[a] Increased intake of polyunsaturated acids potentiate deficiency.
[b] Can be reversed by addition of selenium to the diet.
[c] Antioxidants can be substituted for vitamin E to cure condition.

Structure and Nomenclature

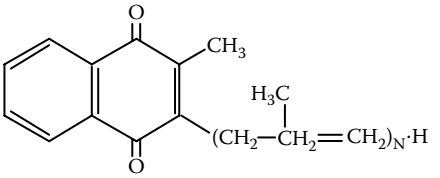
Subsequent to its recognition as an essential micronutrient, vitamin K was isolated from alfalfa and from fish meal. The compounds isolated from these two sources were not identical, so one was named K_1 and the other K_2. Almquist was the first to show that the vitamin could be synthesized by bacteria. He discovered that putrefied fish meal contained more of the vitamin than nonputrefied fish meal. It was also learned that bacteria in the intestine of both the rat and the chicken synthesized the vitamin, thus ensuring a good supply of the vitamin if coprophagy (eating feces) were permitted.

These early studies thus provided the reason to suspect that there was more than one form of the vitamin. A large number of compounds, all related to a 2-methyl-1,4-napthaquinone possess vitamin K activity (Figure 9.16). Compounds isolated from plants have a phytyl radical at position 3 and are members of the K_1 family of compounds. Phylloquinone (2-methyl-3-phytyl-1,4-napthaquinone [II]) is the most important member of this family. The K vitamins are identified by their family and by the length of the side chain attached at position 3. The shorthand designation uses the letter K with a subscript to indicate family and a superscript to indicate the side chain length. Thus, K_2^{20} indicates a member of the family of compounds isolated from animal sources having a 20-carbon side chain. The character of the side chain determines whether a compound is a member of the K_1 or K_2 family. K_1 compounds have a saturated side chain, whereas K_2 compounds have an unsaturated side chain. Chain lengths of the K_1 and K_2 vitamins can vary from 5 to 35 carbons.

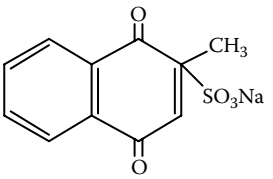
A third family of compounds is the K_3 family. These compounds lack the side chain at carbon 3. Menadione is the parent compound name, and it is a solid crystalline material (a salt) as menadione sodium bisulfite, shown in Figure 9.16. Other salts are also available. These salts are water soluble and thus have great use in diet formulations or mixed animal feeds. Clinically useful is menadiol sodium diphosphate. The use of this must be very carefully monitored as overdoses can result in hyperbilirubinemia and jaundice. These K_3 compounds can be synthesized in the laboratory. When



Phylloquinone



Menaquinone



Menadione sodium bisulfite

FIGURE 9.16 Structures of vitamin K_1 (phylloquinone), K_2 (menaquinone) and K_3 (menadione), a synthetic precursor that is converted to K_2 by the intestinal flora.

consumed as a dietary ingredient the quinone structure is converted by the intestinal flora to a member of the K_2 family.

There are several structural requirements for vitamin activity: there must be a methyl group at carbon 2 and a side chain at carbon 3. The benzene ring must be unsubstituted. The chain length can vary; however, optimal activity is observed in compounds having a 20-carbon side chain. K_1 and K_2 compounds with similar chains have similar vitamin activities. The vitamin can exist in either the *cis* or *trans* configuration. All-*trans* phylloquinone is the naturally occurring form whereas synthetic phylloquinone is a mixture of the *cis* and *trans* forms.

Biopotency

The various compounds with vitamin activity are not equivalent with respect to potency as a vitamin. The most potent compound of the phylloquinone series is the one with a 20-member side chain. Compounds having fewer or greater numbers of carbons are less active. Table 9.15 provides this comparison. The most potent compound in the menaquinone series is the one with a 25-member unsaturated side chain.

Chemical and Physical Properties

Phylloquinone (K_1^{20}) is a yellow viscous oil. The physical state of menaquinone (K_2^{20}) depends on its side chain length. If the side chain is 5 or 10 carbons long, it is an oil; if longer, it is a solid.

TABLE 9.15
Comparative potency[a] of various members of the phylloquinone and menaquinone families of vitamin K

	Family	
Side chain length (# carbons)	**Phylloquinone**	**Menaquinone**
10	10	15
15	30	40
20	100	100
25	80	120
30	50	100
35	—	70

[a] Phylloquinone, K_1^{20} is given a value of 100 and the remaining compounds are compared to this compound with reference to its biological function in promoting clot formation.

Menadione (K_3) is a solid. All three families of compounds are soluble in fat solvents. Menadione can be made water soluble by converting it to a sodium salt. All the vitamin K compounds are stable to air and moisture but unstable to ultraviolet light. They are also stable in acid solutions but are destroyed by alkali and reducing agents. These compounds possess a distinctive absorption spectra because of the presence of napthoquinone ring system.

Chemical Assays

Many different assays have been proposed for vitamin K, but they all face the same problem: Vitamin K is normally present in very low concentrations, and numerous interfering substances, such as quinones, chlorophyll, and carotenoid pigments, are usually present.

Several colorimetric methods have been developed. One of the first, known as the Dam–Karrer reaction, is the measurement of the reddish-brown color that forms when sodium ethylate reacts with vitamin K. In another method, menadione, as the sodium sulfite, is reduced and then titrated to a green endpoint with ceric sulfate. In yet another, menadione is converted to its 2,4-dinitrophenylhydrozone when heated with 2,4-dinitrophenylhydrozine in ethanol. An excess of ammonia causes the solution to become blue-green with an absorption maxima at 635 nm.

As mentioned earlier, the K vitamins have a characteristic ultraviolet absorption spectrum. If the material is sufficiently pure, it can be identified and quantitated. Vitamin K has also been quantitated by a thin-layer and gas chromatographic technique. The menadione content of foodstuffs as been determined with high performance liquid chromatography (HPLC). This technique has been used to determine the blood and tissue levels of the vitamin in humans. As little as 0.5 mmol/L has been detected in the blood of newborns and adults. Regardless of the assay method used, the analytical procedure should include protection of the samples from light. All of the vitamers are sensitive to ultraviolet light and will decompose if exposed. Care also should be exercised with respect to pH. The K vitamers are sensitive to alkali but are relatively stable to oxidants and heat. They can be safely extracted using vacuum distillation.

Bioassays

One of the earliest biotechniques for measuring vitamin K content of foods uses the chick. This method is sensitive to 0.1 μg phylloquinone/g diet. In this assay, newly hatched chicks are fed a K-free diet for 10 days and thus made deficient. They are then fed a supplement containing the assay

food. The prothrombin level of the blood is then compared with a standard curve resulting from the feeding of known amounts of phylloquinone.

Instead of measuring prothrombin concentration, plasma prothrombin times may be measured. Prothrombin time is an indirect and inverse measurement of the amount of prothrombin in the blood: an increase in prothrombin time signifies a decrease in prothrombin concentration. The technique commonly used is a modification of the one-stage method developed by A. J. Quick. Blood removed from a patient or animal is immediately oxalated; oxalate binds the calcium and prevents prothrombin from changing into thrombin. Later, an excess of thromboplastic substance (obtained from rabbit or rat brain) and calcium are added to the plasma, and the clotting time of the plasma is noted; this time is the prothrombin time. The normal prothrombin time is approximately 12 seconds; however, the actual time depends to a large extent on the exact procedure employed. To be valid, the prothrombin time of a vitamin-K-deficient animal must be compared to that of a normal individual.

Biosynthesis

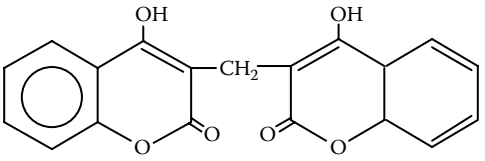
Although not too much is known about the biosynthesis of phylloquinone in plants, apparently the synthesis occurs at the same time as that of chlorophyll. The vitamin concentration is richest in that part of the plant that is photosynthetically active. Carrot tops are a good source but not the root. Peas sprouted in light contain more than peas sprouted in the dark, and the inner leaves of cabbage have about one-fourth less vitamin than the outer leaves.

Menaquinone is synthesized by intestinal bacteria in the distal small intestine and in the colon by intestinal bacteria. Martius and Esser found that chicks fed a vitamin-K-deficient diet and then given menadione (K_3) for long periods of time will synthesize menaquinone (K_2^{20}). Apparently, this synthesis is more complete at small physiological doses than at high doses, such as are used when vitamin K is given as an antidote to dicumarol. Because of the intestinal biosynthesis, experimental animals used in K deficiency studies should be protected from coprophagy.

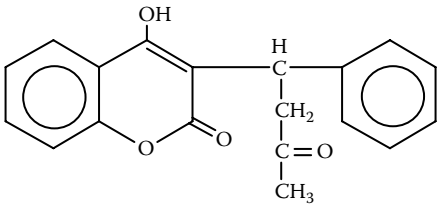
All of the natural forms of the K vitamins can be stored in the liver. Menadione is not stored as such but is stored as its conversion product, menaquinone. Menadione metabolism by the liver occurs at the expense of the redox state. When menadione is metabolized by Ca^{++} loaded mitochondria, there is a rapid oxidation and loss of pyridine nucleotides and a decrease in ATP level. The effects of menadione on Ca^{++} homeostasis are probably initiated by NAD(P)H: (Quinone-acceptor) oxido reductase. Large amounts of menadione have been shown to alter the surface structure and reduce the thiol content of the liver cell. Because of these changes menadione is cytotoxic in large quantities. This may explain the induction of jaundice in the newborn of mothers given large doses of menadione just prior to delivery. This once-popular obstetric practice has been discontinued. Using labeled vitamin, it was found that rats stored the vitamin in the liver. Fifty percent of the vitamin was found bound to the endoplasmic reticulum. When *cis* and *trans* forms were compared, the biologically active *trans* isomer was found in the rough membrane fraction (endoplasmic reticulum), whereas the inactive *cis* isomer was found in the mitochondria.

Antagonists, Antivitamins

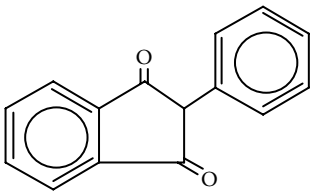
Blood coagulation can be inhibited by a variety of agents that are clinically useful. Oxalates, heparin, and sodium citrate are but a few of these useful anticoagulants. These compounds work by binding one or more of the essential ingredients for clot formation. One group of anticoagulants are those which act by antagonizing vitamin K in its role in prothrombin carboxylation. These compounds, shown in Figure 9.17, are members of a quinone family of compounds. The first of these is 3,3′-methyl-*bis*-(4-hydroxycoumarin) called *dicumarol*. It was isolated from spoiled sweet clover and was shown to cause a hemorrhagic disease in cattle. Dicumarol has been found very



Dicumarol (Coumarin)



Warfarin



2 phenyl-1, 3-indandione

FIGURE 9.17 Structures of several compounds that are potent vitamin K antagonists.

useful in the clinical setting as an anticoagulant in people at risk for coronary events. It is marketed as coumarin. Another use is as a rodenticide, marketed as warfarin. The structure of warfarin is also shown in Figure 9.17. A third group of antivitamin K compounds are the 2-substituted 1,3-indandiones. These are useful as rodenticides but because they can cause liver damage they are not used in the clinical setting.

Absorption

Under normal physiologic conditions, most nutrients are absorbed before they reach the colon. In large measure, this is true of the K vitamins. However, vitamin K can be absorbed very well by the colon. This is an advantage to the individual because it ensures the uptake of K that is synthesized in lower intestinal tract by the glut flora. The mechanism by which the K vitamins are absorbed and the rate at which this occurs is species dependent. Species such as the chicken, which have a rapid gut passage time absorb the vitamin more rapidly than do species such as the rat which have a long gut passage time. The absorption of K_1 and K_2 analogs is generally thought to occur via an active, energy dependent, transport process, whereas K_3 (menadione) analogs are absorbed by passive diffusion.

The absorption of K_1 and K_2 requires a protein carrier and again species differ in the saturability of the carrier. In rats, the carrier is saturated at far lower vitamin concentrations than occurs in chickens. These differences in carrier saturability led early investigators to suggest that vitamin

K absorption was a passive process. Subsequent studies using in vitro techniques or using labeled vitamin given in vivo showed that absorption was indeed an active process.

In contrast to the absorption of phylloquinone and the menaquinones, menadione appears to be absorbed primarily in the large intestine where the gut bacteria have converted it to a form with a side chain. Without the side chain the absorption of K_3 is a passive process. Biosynthesis by the gut flora is an adequate source of biologically active vitamin K under normal conditions, thus making it difficult to obtain a K-deficiency in humans and experimental animals. However, under conditions of stress, such as hypoprothrombinemia induced by coumarin-type anticoagulants, intestinal synthesis does not produce enough of the vitamin to overcome the effects of the drug. Proof of the importance of colonic absorption is seen in the improvement in prothrombin times in chicks and infants when given the vitamin rectally. Excessive intakes of menadione can be harmful due to their quinone structure. They can be uncouplers of oxidative phosphorylation.

Absorption of the K vitamins is dependent on the presence of lipid, which stimulates the release of bile and pancreatic lipases. As lipids are absorbed into the lymphatic system, so, too, are the K vitamers. If there is any impairment in the lipid absorption process, less vitamin K will be absorbed. For example, patients with biliary obstruction have been shown to absorb substantially less vitamin K than normal subjects.

Phylloquinone absorption shows a diurnal rhythm. In rats, the highest rate of absorption is at midnight; the lowest is at 6 am.[72] This coincides with the rats' eating pattern. The rat is a nocturnal feeder consuming most of his food between 8 pm and midnight. Estrogen enhances the absorption of phylloquinone for both intact and castrated males. Castrated rats are more susceptible to uncontrolled hemorrhage due to coumarin than are intact female rats. Female rats also synthesize more prothrombin than do male rats.

Metabolism and Function

Historically, vitamin K has been regarded as a vitamin with a single function: the coagulation of blood. Although the concept of this function is true, we now know that it serves as an essential cosubstrate in the posttranslational oxidative carboxylation of glutamic acid residues in a small group of specific proteins, most of which are proteins involved in blood coagulation. These proteins are the blood clotting factor II, VII, IX, and X, a calcium-binding bone protein, osteocalcin, and plasma proteins C and S.

Blood coagulation is not a single one-step phenomenon. Rather, it involves several phases, which must interdigitate if a clot is to be formed. Four phases have been identified: (1) the formation of thromboplastin, (2) the activation of thromboplastin, (3) the formation of thrombin, and (4) the formation of fibrin. Following injury, a blood clot is formed when the blood protein fibrinogen is transformed into an insoluble network of fibers (fibrin) by the reaction cascade illustrated in Figure 9.18. The change of fibrinogen to fibrin (phase 4) is catalyzed by thrombin, which itself must arise from prothrombin. The synthesis of thrombin from prothrombin (phase 3) is catalyzed by prothrombinase, active preaccelerin, and the phospholipid, dephalin. These factors in turn are activated by a combination of blood and tissue convertins that represent or reflect the synthesis and activation of the thromboplastin complex (phases 1 and 2). The synthesis and activation of the thromboplastin complex requires several factors, which have been named and identified. These factors, studied by different groups were given different names, which has lent considerable confusion to the understanding of the coagulation process. To clarify the literature, it was decided by the International Committee for Standardization of the Nomenclature of Blood Clotting Factors to recommend the use of a numerical system for designating the various factors. This numbering system is given in Table 9.16 along with the other names in use and the function of each in the coagulation process. Note that four of the factors are proteins whose synthesis is dependent on vitamin K. These proteins, prothrombin (factor II), proconvertin (factor VII), the Christmas factor (factor IX) and the Stuart–Prower factor (factor X) are also calcium dependent. That is, calcium ions must be present

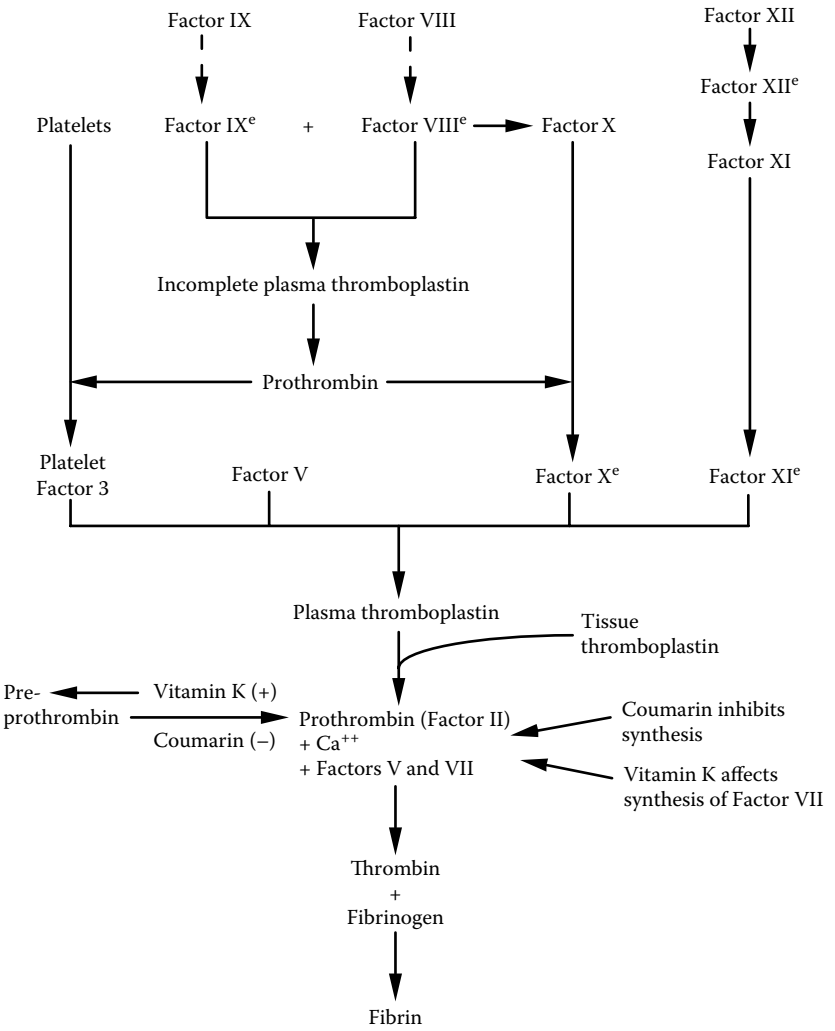


FIGURE 9.18 Formation of a clot.

for their activation and participation in the coagulation cascade (Figure 9.18). Note, too, that the four proteins that are vitamin K dependent are synthesized in the liver, hence the reason why the liver concentrates and stores this vitamin. One of these clotting factors, Factor XIIIa, is also active in the nervous system where it may play a role in the healing of injured tissue.[73]

The function of vitamin K is as a cosubstrate in the posttranslational oxidative carboxylation of proteins at selected glutamic acid residues.[75–77] This is a cyclic process (Figure 9.19). As the vitamin serves as a cosubstrate it is metabolized by the hepatic microsomes. Its metabolism is dependent on adequate intakes of niacin and riboflavin, which are components of the redox systems important in the transfer of reducing equivalents. Vitamin K is reduced to the hydroquinone (vitamin KH_2) with NADH as the coenzyme (NADH is a niacin containing coenzyme). There are two pathways for vitamin K reduction: (1) The reduction pathway—Warfarin sensitive, irreversible by coumarin and (2) DT diaphorase and microsomal dehydrogenase. This pathway is important to counteract coumarin toxicity. Upon reduction, it is then oxidized to form an epoxide. The epoxide is converted back to the quinone form by an epoxide reductase. This enzyme is a two-component

TABLE 9.16
Blood-clotting factors

Factor	Name	Function	Remarks
I	Fibrinogen	Provides the structural network upon which clot is formed	
II	Prothrombin	Precursor of thrombin, a proteolytic enzyme which causes fibrinogen to lose one or more peptides and polymerize	Vitamin K dependent, synthesized in liver
III	Thromboplastin	Serves to stimulate prothrombin conversion to thrombin	Not a single compound. Includes factors IX, XI, XII, X, V
IV	Calcium	Combines with prothrombin in presence of agents	Oxalate acts as an anticoagulant by binding Ca^{++}
V	Labile factor proaccelerin accelerator (Ac) globule	Has thromboplastic activity. Released by collagen and not ADP; proceeds normally when prostaglandin synthesis is inhibited	Disappears when plasma is heated or stored with oxalate. In the activated form, may function as the Ka receptor on the platelet membrane
VII	Proconvertin serum prothrombin conversion accelerator (SPCA) cothromboplastin, autoprothrombin I	Has thromboplastic activity	Vitamin K synthesized in liver (?)
VIII	Anti hemophilic factor, antihemophilic globulin(AHG)	Activates factor X; associated with platelet membranes. May have a role in the adherence of platelets to subendothelium	Deficiency of this factor is the cause of the classic form of hemophilia A
IX	Plasma thromboplastic component (PTC) Christmas factor	Stimulates conversion of prothrombin to thrombin	Vitamin-K-dependent deficiency results in hemophilia or Christmas disease named after first patient to have disease
X	Stuart-Prower factor	Stimulates conversion of prothrombin to thrombin	Vitamin-K-dependent glycoprotein. Gene for human factor X is essentially identical with that for factor IX and Protein C[162a]
XI	Plasma thromboplastin antecedent (PTA)	Has thromboplastic activity	Patients with PTA are mild hemophiliacs
XII	Hageman factor	Has thromboplastic activity	Deficiency does not result in hemophilia
XIII	Laki-Lorand factor (LLF) Fibrin stabilizing factor	Stabilizes fibrin network; is a proenzyme in the plasma activated by thromblin and which catalyzes the formation of covalent lysyl bonds between & and chains of fibrin monomers	
	Platelet thromboplastic factor (thromboplastino-fenase)	Catalyzes formation of platelet factor	Appears when platelets disintegrate

Source: Walsh, P.N. (1981). *Fed. Proc.* 40: 2086–2092.

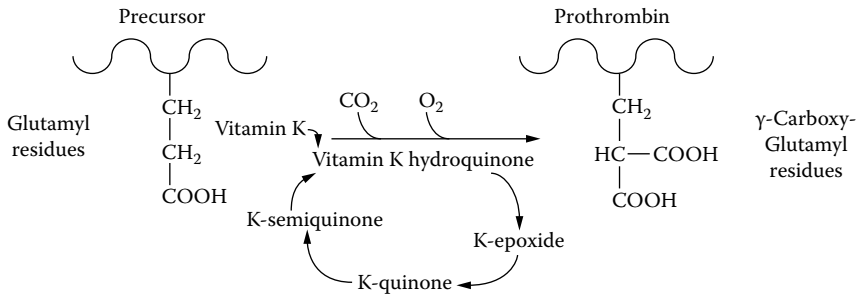


FIGURE 9.19 Posttranslational carboxylation of proteins at selected glutamic acid residues.

cytosolic enzyme that catalyzes the reduction of the vitamin K epoxide using dithiothreitol as either a primary or secondary source of reducing equivalents. Warfarin, an antivitamin, interferes with both the reduction of vitamin K to the hydroquinone and the conversion of the epoxide back to the original compound. Warfarin is bound to a protein (likely the epoxidase and the reductase). When fed to deficient rats, the epoxide has an activity similar to that of the vitamin in inducing protein carboxylation and prothrombin synthesis. This similarity is probably due to the conversion of the epoxide to the hydroquinone. The epoxidation reaction is coupled to the carboxylation of the glutamic acid residues protruding from peptides having clusters of this amino acid. Aspartyl residues can also be carboxylated. The epoxidation reaction can be coupled to the oxidation of other peptides as well; however, only those having the clusters of glutamic acid will be oxidatively carboxylated. Unless the proteins are carboxylated they are unable to bind calcium. For example, the precursor of prothrombin, acarboxyprothrombin, has within the first 33 amino acids at the amino terminal end, 10 tightly clustered glutamic acid residues and binds less than 1 mol of calcium per mole of protein. When carboxylated to prothrombin, the glutamic acid residues are converted to a carboxyglutamic acid residue and now each mole of the protein can bind 10 to 12 mol of calcium. The carboxylated glutamic acid rich region serves an important function in clot formation. Prior to activation by the protease factor Xa, both the prothrombin and the protease are absorbed onto the phospholipids of the damaged cells by way of calcium bridges. Without carboxylation, these bridges will not form and the adherence or absorption of the prothrombin to the phospholipids of the injured cell walls does not take place. The phospholipids are not only important to the binding of prothrombin to the injured cell wall but are also important determinants of carboxylase activity. Phosphatidyl choline has been found to be an essential component of the carboxylase enzyme system. When depleted of phospholipid the enzyme loses activity; when repleted, its activity is restored.

Proteins other than those involved in the coagulation process have also been shown to be vitamin K dependent. Carboxylated proteins have been found in bone matrix. Up to 20% of the noncollagenous proteins (or 1–2% of the total bone protein) is a carboxylated protein called *osteocalcin*. Another is called bone Gla protein (BGP). Osteocalcin is synthesized in bone tissue. Bone microsomes are responsible for the posttranslational vitamin-K-dependent oxidative carboxylation reaction. The synthesis of osteocalcin is highest during rapid growth periods and coincides with detectable bone mineralization.[78] The osteocalcin appears prior to mineralization and, like the blood coagulation proteins, shows a remarkable avidity for calcium. It will also bind (in order of preference after calcium) magnesium, strontium, barium, and lanthanide. In addition to its ability to bind divalent ions, osteocalcin also binds strongly to hydroxyapatite, the major calcium phosphate salt of the bone. Whether osteocalcin has a direct role in bone mineralization has not been conclusively shown; however, studies of embryonic chicks treated with warfarin showed a decrease in both osteocalcin and mineralization. Warfarin-treated animals, in contrast, do not show this same effect; osteocalcin

levels are low but bone mineralization is normal. Because the overwhelming effects of vitamin K deficiency induced either through diet or warfarin treatment results in lethal bleeding, this secondary effect on bone mineralization is difficult to study except in systems utilizing embryonic tissues. Evidence of warfarin injury has been reported in humans. Mothers consuming warfarin during their first trimester of pregnancy, have given birth to infants having defective bone development such as stippled epiphyses, "saddle" nose, punctate calcifications, and frontal bossing.

Bone Gla protein is a 49 residue, vitamin-K-dependent protein involved in the regulation of bone calcium homeostasis rather than bone formation. It is secreted by osteosarcoma cells having an osteoblastic phenotype and appears in calcifying tissues 1 to 2 weeks after mineral deposition, and at the approximate time that the maturation of bone mineral to hydroxyapatite is thought to occur. The synthesis and secretion of BGP from osteosarcoma cells are regulated by vitamin D. The renal cortex also contains a microsomal vitamin-K-dependent oxidative carboxylation system. Its function is to produce a carboxy glutamic acid rich protein that serves to bind calcium. It is located in the renal tubule and may function importantly in the conservation of calcium. Unfortunately, these proteins may be entirely too active in calcium uptake, and kidney stones may result. Renal stones have been found to contain a unique carboxylated peptide in addition to calcium oxalate. Whether this peptide was responsible for the stone accumulation or was attracted to it because of its high calcium content is not known.

In summary then, vitamin K functions as a cosubstrate in the production of unique calcium binding proteins in the blood, bone and kidney. Further research may reveal the presence of other K dependent proteins which serve to translocate the calcium ion from one cellular compartment to another. However, evidence of their existence is not now in hand.

In addition to its function as described above, vitamin K serves a role in the inhibition of the carcinogenic properties of benzopyrene. Menadione inhibits aryl hydrocarbon hydroxylase, thus reducing the levels of carcinogenic and mutagenic metabolites in the cell with resultant reduction in tumor formation. Vitamin K_1 and K_2 have the opposite effect. The different effects of these vitamin forms probably relate to the previously described effects of these forms on redox state, calcium ion status, and ATP production, all of which are involved in tumorogenesis.

Deficiency

Due to the fact that intestinal synthesis of vitamin K usually provides sufficient amounts of the vitamin to the body, primary vitamin K deficiency is rare. However, secondary deficiency states can develop as a result of biliary disease which results in an impaired absorption of the vitamin. Deficiency can also occur as a result of long-term broad-spectrum antibiotic therapy, which may kill the vitamin K synthesizing intestinal flora, or as a result of anticoagulant therapy using coumadin (warfarin) which, as shown in the previous section, interferes with the metabolism and function of the vitamin. The primary characteristic of the deficiency state is a delayed or prolonged clotting time. Deficient individuals may have numerous bruises indicative of subcutaneous hemorrhaging in response to injury. Studies of human populations have shown that low intakes of vitamin K (dihydrophlloquinone) is associated with low bone mineral density.[79] Newborn infants, because they have not yet established their K-synthesizing intestinal flora, have delayed coagulation times. For many years, it was common practice to give the mother an injection of menadione just prior to delivery in order to rectify the newborn's problem. However, it then became apparent that this prophylactic therapy was having no effect on the infant with respect to prothrombin time and, in addition, some infants became jaundiced with this treatment. As a result, vitamin K administration to women just prior to delivery is no longer a routine practice in obstetrics.

Sources

Phylloquinone serves as the major source of dietary vitamin K in humans. In general, studies of the vitamin K content of common foods, as determined by the chick bioassay method, reveal that green leafy vegetables contain large quantities of vitamin K; meats and dairy products, intermediate quantities; and fruits and cereals small quantities. For animals, alfalfa has long been recognized as a rich source of vitamin K.

Need

Healthy humans can usually synthesize sufficient vitamin to meet their needs. Nonetheless, there is a recommendation for vitamin K in the DRI table (see Table 9.1).

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10 Water-Soluble Vitamins

Water-soluble vitamins comprise a large group of fairly small organic molecules that, for the most part, can be dissolved in water. As such, the body does not store them extensively. Compared to the fat-soluble vitamins, it is relatively easy to develop a deficiency state. It is therefore very important to consume each of the nutrients every day. The Food and Nutrition Board of the National Academy of Science has published the Daily Dietary Recommended Intakes (DRI) and Acceptable Intakes (AI) for as many of the water- soluble vitamins as there are basic data to support these recommended intakes. These are shown in Table 10.1.

ASCORBIC ACID

Historically, ascorbic acid or vitamin C, has been recognized as a needed nutrient for centuries.[1] Although the chemical identity of vitamin C was unknown, foods rich in this micronutrient have long been used as treatment for the symptoms of scurvy. Ancient Egyptians, Greeks, and Romans referred to scurvy as a plague that interfered with victory in military campaigns. Accounts of the scourge of scurvy have appeared in writings of the 16th, 17th, and 18th century.

Perhaps the best known treatise on scurvy is that written by a Scottish surgeon, James Lind. Dr. Lind demonstrated that the inclusion of limes, oranges, and lemons in sailors' diets would successfully prevent the development of scurvy. Other reports similar to Lind's were published, but his remains the classic paper in this field. Even though the value of citrus fruits was amply demonstrated by Lind, it took 30 years for his recommendations to be adopted. Most nutrition historians credit Captain James Cook, discoverer of the Hawaiian Islands, as the first sea captain to include citrus fruits as part of his ship's stores and part of the sailors' diets. He thus was able to demonstrate that long ocean voyages need not result in scurvy for the crew.

Despite the knowledge that scurvy could be avoided, many sailors and explorers continued to die from scurvy. Even Captain Scott and his team were killed by scurvy in 1912 as they explored the polar regions of the Southern hemisphere.

Although the knowledge that certain foods prevented or cured scurvy was available in the 18th century, the isolation and synthesis of the antiscorbutic factor did not occur until the early 20th century. The isolation of ascorbic acid was accomplished by two independent groups of chemists in 1928. Szent-Gyorgy isolated a compound which he called hexuronic acid from orange juice, cabbage, and adrenal glands. King likewise isolated the vitamin from lemon juice and showed that it was identical to the compound isolated by Szent–Gyorgy. The structure of the vitamin was accomplished by Haworth and its synthesis by Reichstein. While this exciting chemistry was being pursued, an interesting fact became known, and that was that few species required ascorbic acid in their diets. The guinea pig, the primates (including man), the fruit bat and some fishes and birds were identified as being unable to synthesize the vitamin in vivo. All other species examined were found to be able to synthesize it from glucose (Figure 10.1). The reason why ascorbic-acid-dependent species can not synthesize ascorbic acid is because they lack the enzyme L-gulonolactone oxidase.

Structure, Physical, and Chemical Properties

Ascorbic acid and dehydroascorbic acid (the oxidized form) are the trivial names for vitamin C. The chemical name is 2,3-didehydro-L-threo-hexano-1,4-lactone. The compound can readily donate or

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TABLE 10.1
Daily dietary reference intakes (DRIs) and acceptable intakes for various age groups

Group	Ascorbic acid (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin[b] (mg)	Vitamin B[6] (mg)	Folacin (ug)	Pantholinic acid (mg)	Vitamin B12 (ug)	Biotin (ug)	Choline (mg)
Infants										
0–6 mo	—	—	0.3[a]	2[a]	0.1[a]	65[a]	1.7[a]	0.4[a]	5[a]	125[a]
7–12 mo	—	—	0.4[a]	4[a]	0.3[a]	80[a]	1.8[a]	0.5[a]	6[a]	150[a]
Children										
1–3 years	**13**	**0.4**	**0.5**	**6**	**0.5**	**150**	2[a]	**0.9**	8[a]	200[a]
4–8 years	**22**	**0.5**	**0.6**	**8**	**0.6**	**200**	3[a]	**1.2**	12[a]	250[a]
Males										
9–13 years	**39**	**0.7**	**0.9**	**12**	**1.0**	**300**	4[a]	**1.8**	20[a]	375[a]
14–18 years	**63**	**1.0**	**1.3**	**16**	**1.3**	**400**	5[a]	**2.4**	25[a]	550[a]
19–30 years	**75**	**1.0**	**1.3**	**16**	**1.3**	**400**	5[a]	**2.4**	30[a]	550[a]
31–50 years	**75**	**1.0**	**1.3**	**16**	**1.3**	**400**	5[a]	**2.4**	30[a]	550[a]
51–70 years	**75**	**1.0**	**1.3**	**16**	**1.7**	**400**	5[a]	**2.4**[d]	30[a]	550[a]
70+ years	**75**	**1.0**	**1.3**	**16**	**1.7**	**400**	5[a]	**2.4** [d]	30[a]	550[a]
Females										
9–13 years	**39**	**0.7**	**0.9**	**12**	**1.0**	**300**	4[a]	**1.8**	20[a]	375[a]
14–18 years	**56**	**0.9**	**1.0**	**14**	**1.2**	**400**	5[a]	**2.4**	25[a]	400[a]
19–30 years	**60**	**0.9**	**1.1**	**14**	**1.3**	**400**	5[a]	**2.4**	30[a]	425[a]
31–50 years	**60**	**0.9**	**1.1**	**14**	**1.3**	**400**	5[a]	**2.4**	30[a]	425[a]
51–70 years	**60**	**0.9**	**1.1**	**14**	**1.5**	**400**	5[a]	**2.4** [d]	30[a]	425[a]
70+ years	**60**	**0.9**	**1.1**	**14**	**1.5**	**400**	5[a]	**2.4** [d]	30[a]	425*
Pregnancy										
14–18 years	**66**	**1.2**	**1.4**	**18**	**1.9**	**600 c**	6[a]	**2.6**	30[a]	450[a]
19–30 years	**70**	**1.2**	**1.4**	**18**	**1.9**	**600 c**	6[a]	**2.6**	30[a]	450[a]
31–50 years	**70**	**1.2**	**1.4**	**18**	**1.9**	**600**	6[a]	**2.6**	30[a]	450[a]

Lactation										
14–18 years	**96**	**1.2**	**1.6**	**17**	**2.0**	**500**	7a	**2.8**	35[a]	550[a]
19–30 years	**100**	**1.2**	**1.6**	**17**	**2.0**	**500**	7a	**2.8**	35[a]	550[a]
31–50 years	**100**	**1.2**	**1.6**	**17**	**2.0**	**500**	7a	**2.8**	35	550[a]

Note: Figures in bold for DRIs.

[a] Acceptable intakes (AIs).

[b] Niacin and niacin equivalents assuming a conversion of tryptophan to niacin, 60:1.

[c] In view of the data linking birth defects to inadequate folacin intake early in pregnancy, it is recommended that any woman of an age to become pregnant consume 400 µg folate/d from fortified foods and supplements.

[d] Because 10–30% of older people may malabsorb this vitamin it is advisable for those older than 50 years of age to meet their vitamin requirement through consuming foods rich in this vitamin and through use of a vitamin supplement.

Source: www.nap.edu

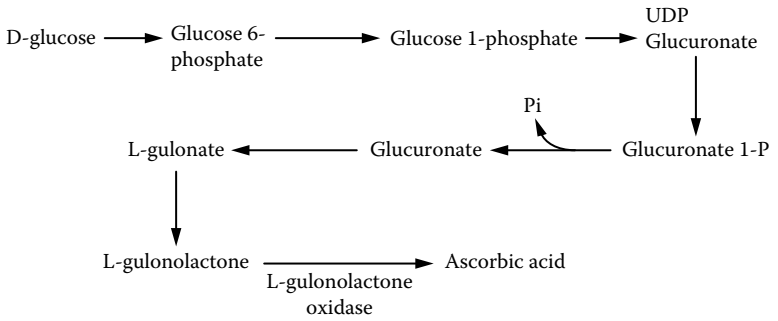


FIGURE 10.1 Biosynthesis of ascorbic acid.

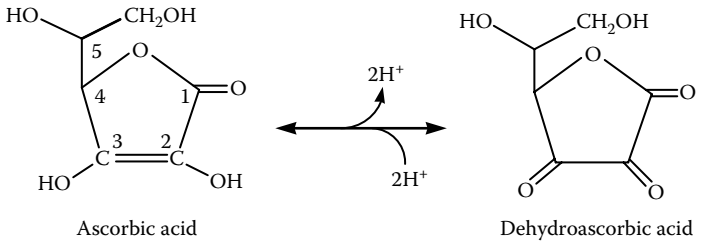


FIGURE 10.2 Structures of ascorbic acid and dehydroascorbic acid.

accept hydrogen ions and thus exists in either state as shown in Figure 10.2. In order for the compound to have vitamin activity it must have a 2,3-endiol structure and be a 6-carbon lactone.

L-ascorbic acid (Figure 10.2) is a rather simple compound chemically related to the monosaccharide, glucose, with an empiric formula of $C_6H_8O_6$. It is a white crystalline solid with a molecular weight of 176. It is soluble in water, glycerol and ethanol but insoluble in fat solvents such as chloroform and ether. It exists in both D and L forms, but the L form is the biologically active form. This is in contrast to its related monosaccharide glucose which is biologically active as the D form. The vitamin is stable in the dry form but once dissolved in water is easily oxidized. It is relatively stable in solutions with a pH below 4.0; as the pH rises the vitamin becomes less stable. Ascorbic acid is easily oxidized by metals such as iron or copper. Ascorbic acid is readily oxidized, but it is less perishable in food, although it is still oxidized in alkaline environments, especially when heated, exposed to air, or in contact with iron or copper salts. Fortunately, those foods rich in ascorbic acid are relatively acid, and lack iron and copper. If the food is not cooked quickly with a minimum of water, significant losses will occur that will decrease the value of the food as a source of the vitamin.

Ascorbic acid is easily and reversibly oxidized to form dehydroascorbate (Figure 10.2). The ease with which this interconversion occurs is the basis for its biological function with respect to its role as an acceptor or donor of reducing equivalents. Further oxidation results in the formation of diketogulonic acid which is biologically inactive. The conversion of ascorbate to dehydroascorbate is aided by sulfhydryl compounds such as glutathione. The strong reducing power of ascorbate can be used to good advantage in its assay. Ascorbate will react with a variety of cyclic compounds to form a color which can be measured spectrophotometrically. Dyes such as dichlorophenolindophenol and 2,4-dinitrophenylhydrazine are the most commonly used compounds in the assay for vitamin C. Chromatographic techniques are also available for use. An excellent enzymatic assay has been

designed using the enzyme ascorbate oxidase. This assay is both sensitive and specific. However, because of the need for ascorbic acid and the fact that it is prevalent in relatively large amounts in those foods containing the vitamin, the spectrophotometric methods are usually satisfactory. For the assay of the vitamin content of tissues such as liver or blood cells, the more sensitive thin layer chromatographic technique is preferred. In animal and plant tissues vitamin C is in mg amounts. Human plasma, for example, contains about 10 mg/L.

Sources

Ascorbic acid is provided mostly by citrus fruits, strawberries, melons; some vitamin can be found in raw cabbage and related vegetables.

Absorption and Metabolism

The metabolic fate of ascorbic acid depends on a number of factors including animal species, route of ingestion, quantity of material, and nutritional status. In species requiring dietary ascorbate, ascorbate is absorbed in the small intestine, primarily the ileum, by an active transport system that is sodium and energy dependent.[2] Studies in vitro have demonstrated clearly that the vitamin moves from the mucosal to the serosal sides of the lumen against a concentration gradient. The influx of the vitamin at the brush border follows saturation kinetics and is specific for the L isomer. Influx can be inhibited by D-isoascorbate, a naturally occurring analog. If sodium is absent, influx does not occur.

The mechanism of absorption involves a carrier protein to which both Na^+ and ascorbate are attached. The carrier then translocates these two to the cytosol, whereupon the sodium must be pumped out. The sodium disassociates from the carrier mechanism at the inner side of the mucosal membrane, and the vitamin moves into the cytosol.

The carrier then resumes its original position in the membrane and is available to repeat the process. Although this process is similar to that envisioned for glucose and alanine, these compounds do not compete with ascorbate for absorption via the above described carrier. Those species able to synthesize sufficient ascorbic acid do not possess this active transport system, and dietary ascorbate is absorbed via passive diffusion. These species differences in transport phenomena lend further evidence of a bifurcation in the evolutionary process that separates guinea pigs, primates, and other ascorbate-requiring species from those species that do not require this vitamin in their diet.

Once absorbed, there appears to be a central pathway for metabolism common to all species. Any excess vitamin consumed beyond need is excreted. In primates the excess is excreted primarily as ascorbic acid in the urine. There is a very efficient reabsorption mechanism in the kidneys, which serves to conserve ascorbic acid in times of need. In the guinea pig, the excess is oxidized to CO_2.[3] In humans, very little if any oxidation of ascorbate to CO_2 occurs. The excretory route is the urine, where it appears primarily as ascorbic acid, dehydroascorbic acid, or oxalate. A minor excretory product, ascorbate-2-sulfate, has been found in the urine of several species and, at first, was thought to be the functional form of the vitamin in the body. However, this thought had to be discarded when it was learned that ascorbate-2-sulfate would not alleviate the symptoms of scurvy in guinea pigs because the guinea pig excreted the compound very rapidly. In vivo, there is some exchange of ascorbate and ascorbate sulfate in the monkey. The significance of this exchange remains to be elucidated.

Distribution

One of the earliest investigations of ascorbic acid function included studies of the distribution of the vitamin throughout the body. Table 10.2 presents some of the findings made in the human and rat. Ascorbic acid is also found in the brain uniformly distributed, where it serves as a cofactor for an enzyme that converts dopamine to norepinephrine.

TABLE 10.2
Distribution of ascorbate in human and rat tissues

Tissue	Human	Rat
	mg/100 g tissue, wet weight	
Adrenals	30–40	280–400
Pituitary	40–50	100–130
Liver	10–16	25–40
Spleen	10–15	40–50
Lungs	7	20–40
Kidneys	5–15	15–20
Testes	3	25–30
Thyroid	2	22
Heart	5–15	5–10
Plasma	0.4–1.0	1.6

Ascorbic acid pool sizes and turnover have been estimated using isotopically labeled vitamin. In depleted humans consuming a vitamin-C-free diet, about 3% of the total existing pool of ascorbic acid is degraded daily. When the depleted subjects were given doses of vitamin C, this vitamin did not appear in the urine until the body pool approached the size of about 1500 mg. Body pool sizes of more than 1500 mg have not been observed even when megadoses of the vitamin are consumed. These observations indicate that mega intakes are not useful with respect to the body's vitamin C content. The first signs of scurvy incidentally were observed in humans having pool sizes of 300–400 mg, and these signs did not disappear until the pool size increased to 1000 mg.[4] Vitamin C turnover has likewise been estimated for normally nourished humans to be 60 mg/day.[5] Smokers, incidentally, have higher turnover rates and require more ascorbic acid to maintain their pool sizes.[6] Turnover is estimated by measuring the intake rate, the excretion rate, and the total body pool size. This can be accomplished by giving a dose of radioactively labeled vitamin and measuring its distribution and excretion.

In contrast to many vitamins, ascorbic acid does not need a carrier for its transport within the body. Like glucose, it is readily carried in the blood in its free form and likewise, freely crosses the blood–brain barrier.

Ascorbic acid and its metabolites are excreted in the urine. Over 50 metabolites of ascorbate have been identified. Most of these are excreted in minor amounts. The main metabolites in the urine are ascorbate-2-sulfate, oxalic acid, ascorbate, dehydroascorbate, and 2,3-diketogulonic acid.

Function

Although we had information about the chemistry of this vitamin, its metabolic function remained elusive for many years. Because it readily converts between the free and dehydro form, it functions in hydrogen ion transfer systems and aids in the regulation of redox states in the cells. As it is a powerful water-soluble antioxidant, it helps to protect other naturally occurring antioxidants that may or may not be water soluble.[7,8] For example, polyunsaturated fatty acids and vitamin E are protected from peroxidation by ascorbic acid. Ascorbic acid protects the lipids of the plasma membranes from oxidative damage and other labile components of the cell.[9] Ascorbic acid protects certain proteins from oxidative damage and, in addition to its role as an antioxidant, it serves to maintain the unsaturation:saturation ratio of fatty acids. Ascorbic acid aids in the conversion of folic acid to folinic acid and facilitates the absorption of iron by maintaining it in the ferrous state. Ascorbic acid plays a role

TABLE 10.3
Enzymes using ascorbate as a coenzyme

Cytochrome P450 oxidases (several)
Dopamine-β-monooxygenase
Peptidyl glycine α amidating monooxygenase
Cholesterol 7α-hydroxylase
4-hydroxyphenylpyruvate oxidase
Homogentisate 1,2-dioxygenase
Proline hydroxylase
Procollagen-proline 2 oxoglutarate 3-dioxygenase
Lysine hydroxylase
γ-Butyrobetaine, 2-oxoglutarate 4-dioxygenase
Trimethyllysine-2-oxoglutarate dioxygenase

in the detoxification reactions in the microsomes by virtue of its role as a cofactor in hydroxylation reactions. Table 10.3 provides a list of those enzymes in which ascorbate is a coenzyme. Many of these are dioxygenases. Again, this is due to the ascorbate-dehydroascorbate interconversion. A number of these enzymes are involved in collagen synthesis. This explains the poor wound healing found in deficient subjects.

The hydroxylation of proline to hydroxyproline, an important amino acid in the synthesis of collagen is one example of the function of ascorbic acid. The vitamin has been shown to be needed for the incorporation of iron into ferritin. Another is the role this vitamin plays in maintaining either iron or copper in the reduced state so that the metal can perform its function as part of a hydroxylation reaction. The hydroxylation of tryptophan to 5-hydroxytryptophan and the conversion of 3,4-dehydroxyphenylethylamine (DOPA) to norepinephrine are further examples. These roles may well explain some of the features of scurvy (Table 10.3). The poor wound healing is related to the need to form collagen to seal the wound; the anemia may be related to the inability of iron and copper to remain in the reduced state in hemoglobin. Ascorbic acid serves as an important cofactor in hydroxylation reactions. Although these reactions will occur in the absence of the vitamin, they will occur at a very slow rate.

Deficiency (Scurvy)

As with other nutrients there are large differences among individuals in their needs for ascorbic acid. As well, then, there are large differences in the duration of time needed to develop scurvy when consuming a vitamin-C-deficient diet. Hodges et al.[4] fed volunteers a diet devoid of vitamin C and described their symptoms as they developed (Table 10.4). He noted an increased fatigability, especially in the lower limbs and a mild general malaise as the symptoms of scurvy became apparent. Mental and emotional changes occurred after 30 days of treatment with symptoms of depression and suicidal tendencies developing. After 112 days on the ascorbic-acid-free diet, some subjects complained of vertigo (feeling of faintness), inappropriate temperature sensing, and profuse sweating. After 26 days of depletion, small petechial hemorrhages on the skin were observed and after 84–91 days small ocular hemorrhages were present. Gingival hemorrhages and swelling in various degrees appeared at different times in the subjects. As early as 42 days for one subject and as late as 76 days in another. Hyperkeratosis was observed after two months of depletion. All of these symptoms were reversed when the subjects were repleted.

TABLE 10.4
Signs of ascorbic acid deficiency

Hyperkeratosis
Congestion of follicles
Petechial and other skin hemorrhages
Conjunctival lesions
Sublingual hemorrhages
Gum swelling, congestion
Bleeding gums
Papillary swelling
Peripheral neuropathy with hemorrhages into nerve sheaths
Pain; bone endings are tender
Epiphyseal separations occur with subsequent bone (chest) deformities

Toxicity

Although vitamin C is a water-soluble vitamin and is not usually stored, toxic states can develop if large, long-term intakes are maintained. As mentioned earlier, oxalate is an end product of ascorbate metabolism and is excreted. As the dose consumed increases, the urinary oxalate does not increase, and some investigators have suggested that megadoses of vitamin C may be a risk factor in renal oxalate stones. Megadoses of vitamin C have been advocated for the treatment of cancer. However, studies of cancer patients have revealed that such treatment was of little benefit.[10] Massive doses of vitamin C have been shown to reduce serum vitamin B_{12} levels.[11] In part this may be due to an effect of ascorbic acid on vitamin B_{12} in food. Ascorbic acid destroys B_{12} in food. Ascorbic acid also inhibits the utilization of β-carotene.[12,13]

Need

Over the years, different countries have had widely different recommended daily allowances for ascorbic acid intake. This was due primarily to the differing standards of adequate nutritional status. In Canada, the absence of scorbutic symptoms was used as the indication of adequate nutrient intake. In the United States, adequate intake has been defined as the saturation of the white blood cell with ascorbic acid. For many years these different definitions have meant that there was a twofold difference in the two countries' recommendations. Now, however, there is similarity in the recommendations for intake. The DRI's for ascorbic acid are shown in Table 10.1. Lactating and pregnant women should consume more (+40 and +20, respectively) and children less depending on age. Vitamin C requirements may be higher in stressed or traumatized persons. In rats, administration of ACTH or cortisone has been shown to lower plasma and hepatic levels of the vitamin. In addition, women taking contraceptive steroids may absorb less vitamin or may metabolize it more quickly, and thus may require more than 60 mg/day.[14–16] Diabetes may also affect vitamin C requirements.[17] Requirements by these groups of people have not been established as yet.

THIAMIN

The discovery of the chemical structure and synthesis of thiamin marked the end of a difficult search spanning continents to identify the substance in rice polishings responsible for the cure of the disease, beri beri.[18–20] One of the earliest recorded accounts of the disease was by Jacobus Bonitus, a Dutch physician. He wrote, in 1630, "A certain troublesome affliction which attacks men

is called by the inhabitants [of Java] beri beri. I believe those whom this disease attacks with their knees shaking and legs raised up, walk like sheep. It is a kind of paralysis or rather tremor: for it penetrates the motion and sensation of the hands and feet, indeed, sometimes the whole body ... "

In 1894, Takaki, a surgeon in the Japanese navy suggested that the disease was diet related. By adding milk and meat to the navy diet, he was able to decrease the incidence of the disease. He thought the problem was one of a lack of dietary protein. About the same time (1890) a Dutch physician in Java named Eijkman observed a beri-beri-like condition (polyneuritis) in chickens fed a polished rice diet. He was able to cure the condition by adding rice polishings. Eijkman suggested that polished rice contained a toxin that was neutralized by the rice polishings.

In 1901, another Dutch physician named Grigens gave the first correct explanation for the cure of beri beri by rice polishings. He theorized that natural foodstuffs contained an unknown factor absent in polished rice that prevented the development of the disease. Jansen and Donath in 1906 and Funk in 1912 reported the isolation of material from rice polishings that cured beri beri. Funk called the material "vita amine" or "vitamine."

In 1926 Jansen and Donath isolated a crystalline material which cured polyneuritis in birds. Jansen gave the material the trivial name "aneurine." This name was used extensively in the European literature. It is now considered an obsolete term, as are the terms vitamin B_1, oryzamin, torulin, polyneuramin, vitamin F, antineuritic vitamin, and antiberiberi vitamin. All these terms arose as early nutrition scientists identified diseases associated with thiamin deficiency that were reversed when the active principle now known as thiamin was provided.

In 1934 Williams et al. isolated enough of the material to make structure elucidation possible. In 1936 thiamin was synthesized by this same group. With synthesis demonstrated, the stage was set for the commercial preparation of thiamin followed by an outburst of publications on its function and metabolism.

Structure

Thiamin is a relatively simple compound of a pyrimidine and a thiazole ring (Figure 10.3). It exists in cells as thiamin pyrophosphate (TPP). TPP used to be called "cocarboxylase." The name *thiamin* comes from the fact that the compound contains both a sulfur group (the thiol group) and nitrogen in its structure. Its biological function depends on the conjoined pyrimidine and thiazole rings, on the presence of an amino group on carbon 4 of the pyrimidine ring and on the presence of a quaternary nitrogen, an open carbon at position 2 and a phosphorylatable alkyl group at carbon 5 of the thiazole ring. In its free form it is unstable. For this reason, it is available commercially as either a hydrochloride or a mononitrate salt. The HCl form is a white crystalline material that is readily soluble (1 g/mL water) in water, fairly soluble in ethanol, but relatively insoluble in other solvents. The chemical name for the HCl form is 3-(4′ amino-2′-methyl pyrimidine-5′-yl) methyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride hydrochloride. It is stable to acids up to 120°C but

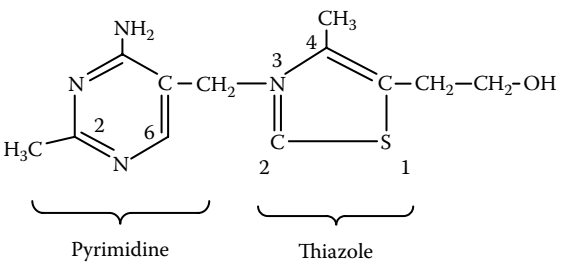


FIGURE 10.3 Structure of thiamin.

readily decomposes in alkaline solutions, especially when heated. It can be split by nitrite or sulfite at the bridge between the pyrimidine and thiazole rings. The mononitrate form is a white crystalline substance that is more stable to heat than is the hydrochloride form. This form is used more often for food processing than the HCl form. Other forms are also available. These include thiamin allyl disulfide, thiamin prophyldisulfide, thiamin tetrahydrofurfuryldisulfide, and *o*-benzoyl thiamin disulfide. The molecular weight of the disulfide form is 562.7 with a melting point of 177°C. Whereas the hydrochloride form has a molecular weight of 337.3, the mononitrate form is 327.4. The latter two are white crystalline material, whereas the disulfide form is a yellow crystal. Thiamin exhibits characteristic absorption maxima at 235 and 267 mm corresponding to the pyrimidine and thiazole moieties, respectively.

When oxidized the bridge is attacked and thiamin converted to thiochrome. Thiochrome is biologically inactive. This structure is shown in Figure 10.4.

Thiamin antagonists include pyrithiamin, a compound with the thiazole ring replaced by a pyridine, and oxythiamin, an analog having the C-4 amino group replaced by a hydroxyl group.

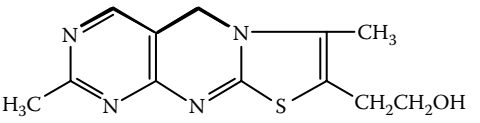
Thiamin Antagonists

The two most commonly used antagonists to thiamin are oxythiamine and pyrithiamine. A pyridine ring is substituted for the thiazole ring of thiamin in pyrithiamine. A hydroxyl group is substituted for the amino group of the pyrimidine moiety of the thiamin molecule is oxythiamine. It appears that thiamin activity is decreased when the number 2 position of the pyridine ring is changed.

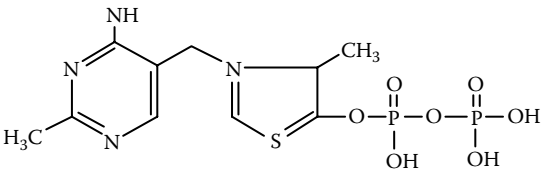
Both the molecules are potent thiamin antagonists but differ in the mechanism by which this is accomplished. Oxythiamine is readily converted to the pyrophosphate and competes with thiamin for its place in the TPP-enzyme systems. Pyrithiamine prevents the conversion of thiamin to TPP by interfering with the activity of thiamin kinase.

Oxythiamine depresses appetite, growth, and weight gain, and produces bradycardia, heart enlargement, and an increase in blood pyruvate but it does not produce neurological symptoms. Pyrithiamine results in a loss of thiamin from tissues, bradycardia, and heart enlargement but does not produce an increase in blood pyruvate.

A natural antagonist is an enzyme called *thiaminase*. It was discovered by accident when raw fish was incorporated into a commercially available feed for foxes. Foxes fed this diet developed symptoms of thiamin deficiency. When heated, this enzyme is denatured and thus no longer capable of destroying thiamin. The enzyme has several forms and has been found in fish, shellfish, ferns,



Thiochrome



Thiamin pyrophosphate (TPP)

FIGURE 10.4 Structure of thiochrome.

betel nuts, and a variety of vegetables. Also found in tea and other plant foods are antithiamin substances that inactivate the vitamin by forming adducts. Tannic acid is one such substance; another is 3,4-dihydroxycinnamic acid (caffeic acid). Some of the flavinoids and some of the dihydroxy derivatives of tyrosine have antithiamin activity.

Assays for Thiamin

There are various chemical, microbiological, and animal assays available for thiamin. In animal tissues, thiamin occurs principally as a phosphate ester, whereas in plants it appears in the free form. Both forms are protein bound.

The thiochrome method is the most widely used chemical assay for thiamin. It depends upon the alkaline oxidation of thiamin to thiochrome. Thiochrome in turn exhibits an intense blue fluorescence, which can be measured fluorimetrically. Other chemical tests for thiamin are the formaldehyde–diazotized-sulfanilic-acid method, the diazotized-sulfanilic-acid method, the diazotized-*p*-aminsacetophenone method, and the bromothymel blue method. All of these assays must be preceded by extraction and removal of protein.

Lactobacillus viridescens is the microorganism most widely used to measure thiamin concentrations. It requires the intact thiamin molecule for growth. Other organisms are available, but they are less useful.

Animal assays are used for determining the availability of thiamin in a food source. The rat is the preferred animal to use. The material being tested measures the curative effect of the food source on rats that have been made thiamin deficient, and compares it to the curative effect of pure synthetic thiamin hydrochloride. The most sensitive assays are the chromatographic ones. Both HPLC (high performance liquid chromatography) and thin layer chromatography yield excellent results.

Sources

Thiamin is widely distributed in the food supply. Pork is the richest source, whereas highly refined foods have virtually no thiamin. Polished rice, fats, oils, refined sugar, and unenriched flours are in this group. Many products are made with enriched flour and so provide thiamin to the consumer. Enrichment means that the flour (or other food ingredient) has had thiamin added to it to the level that was there prior to processing. Peas and other legumes are good sources; the amount of thiamin increases with the maturity of the seed. Cereal products contain nutritionally significant amounts of thiamin. Whole grain cereals contain the greater part of their thiamin in their outer husks, the part that is removed during milling. Thus, milled grains are enriched with thiamin. Dried brewer's yeast and wheat germ are both rich in thiamin.

Absorption and Metabolism

Thiamin is absorbed by a specific active transport mechanism.[21] In humans and rats, absorption is most rapid in the proximal small intestine. Studies in vivo on intact loops of rat small intestine revealed saturation kinetics for thiamin over the concentration range of 0.06–1.5 μM. At higher concentrations (2–560 μM) absorption was linearly related to the luminal B_1 concentration. In vitro studies using inverted jejunum sacs indicated an active transport mechanism that is energy and Na^+ dependent and carrier mediated.

Thiamin undergoes phosphorylation either in the intestinal lumen or within the intestinal cells. This phosphorylation is closely related to uptake, indicating that the carrier may be the enzyme thiamine pyrophosphokinase. There is some argument about this, however. Figure 10.5 illustrates TPP synthesis. Whereas thiamin can accumulate in all cells of the body, there is no single storage site per se. The body does not store the vitamin, and thus a daily supply is needed. Thiamin in excess of need is excreted in the urine. More than 20 metabolites have been identified in urine.

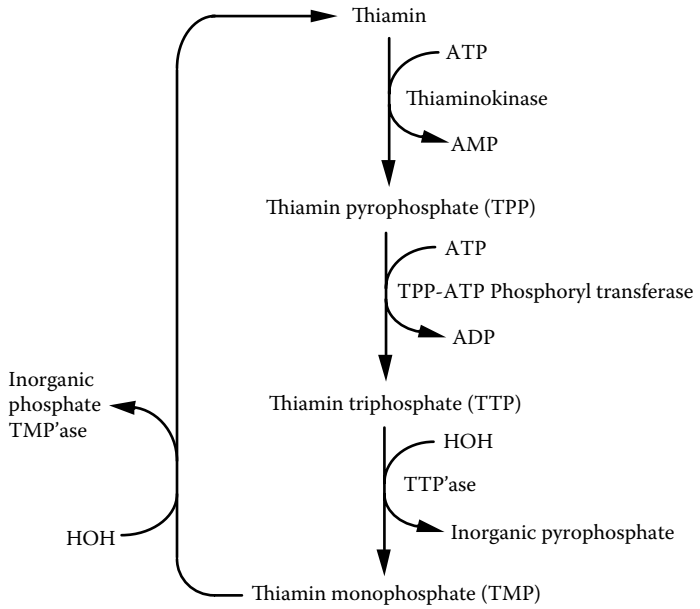


FIGURE 10.5 Formation of thiamin pyrophosphate through the phosphorylation of thiamin. About 80% of thiamin exists as TPP, 10% as TTP, and the remainder as TMP.

Biological Function

Thiamin is a part of the coenzyme thiamin pyrophosphate (TPP) (thiamin with two molecules of phosphate attached to it), also known as cocarboxylase, that is required in the metabolism of carbohydrates.[22] Figure 10.6 illustrates this function. The driving force for reactions with thiamin results because of the resonance possible in the thiazolium ring. The thiazolium dipslo ion, known as ylid, will form. Because of the formation of the ylid, the thiazole ring of TPP can serve as a transient carrier of a covalently bound "active" aldehyde group. Mg^{++} is required as a cofactor for these reactions.

The metabolism of carbohydrates has three stages at which the absence of thiamin as part of a coenzyme (TPP) leads to a slowing or complete blocking of the reactions. There are two oxidative decarboxylation reactions of α ketoacids: the formation or degradation of α ketols and the decarboxylation of pyruvic acid to acetyl CoA as it is about to enter the citric acid cycle. This reaction is catalyzed by the pyruvate dehydrogenase complex, an organized assembly of three kinds of enzymes. The mechanism of this action is quite complex. TPP, lipoamide, and FAD serve as catalytic cofactors; NAD and CoA serve as stoichiometric cofactors.[23]

As a consequence of impairment of this reaction in thiamin deficiency, the level of pyruvate will rise. When thiamin is withheld from the diet, the ability of tissues to utilize pyruvate does not decline uniformly, indicating that there are tissue differences in the retention of TPP. This role of thiamin arose from the discovery that thiamin alone promotes nonenzymatic decarboxylation of pyruvate to yield acetaldehyde and CO_2. Studies of this model revealed that the H at C-2 of the thiazole ring ionizes to yield a carbanion that reacts with the carbonyl atom of pyruvate to yield CO_2 and a hydroxyethyl (HE) derivative of the thiazole. The HE may then undergo hydrolysis to yield acetaldehyde or become oxidized to yield an acyl group. Figures 10.7 and 10.8 illustrate pyruvate metabolism and show where thiamin plays a role.

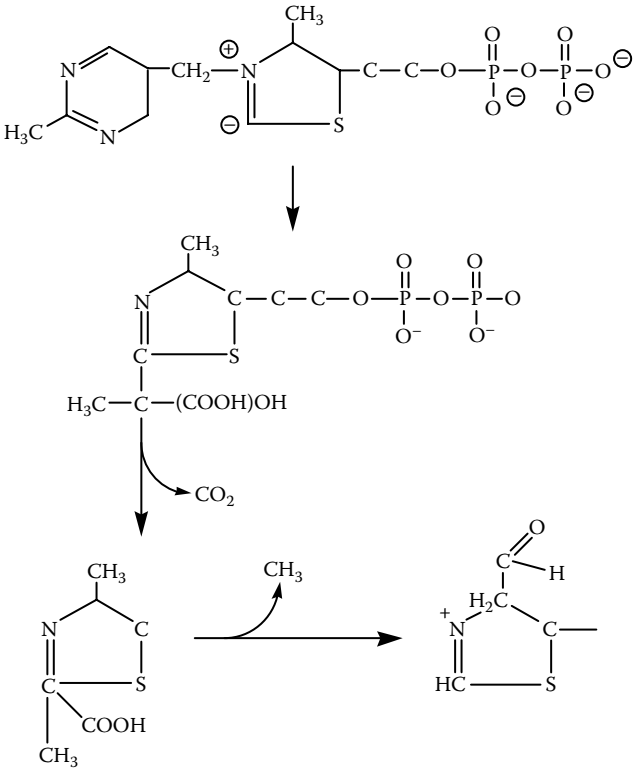


FIGURE 10.6 Structure of the coenzyme thiamin pyrophosphate.

Thiamin is also active in the decarboxylation of α-ketoglutaric acid to succinyl CoA in the citric acid cycle. The mechanism of action is similar to that described above for pyruvate.

Step I is similar to nonoxidative decarboxylation of pyruvate in alcohol fermentation.

Step II involves the hydroxyethyl group. It is dehydrogenated, and the remaining acetyl group is transferred to the sulfur atom at C-6 (or C-8) of lipoic acid. This constitutes a covalently bound prosthetic group of the second enzyme of the complex, lipoate acetyl transferase. The transfer of H^+ to the disulfide bond of lipoic acid converts the latter to its reduced or dithiol form, dihydrolipic acid.

Step III involves the enzymatic transfer of the acetyl group to the thiol group of coenzyme A and a second H^+ is added to form the dihydrolipoyl transacetylase. The Ac CoA so formed leaves the enzyme complex in the free form.

Lastly, Step IV occurs when the dithiol form of lipoic acid is reoxidized to its disulfide form by the transfer of H^+ and associated electrons to the third enzyme of the complex, dihydrolipoyl (lipoamide) dihydrogenase, whose reducible prosthetic group is FAD, $FADH_2$, which remains bound to the enzyme and transfers its electron to NAD^+ to form NADH.

These steps are illustrated in Figure 10.7.

The oxidation of αKG to succinyl CoA is energetically irreversible and is carried out by the αKG DH complex.

$$\alpha KG + NAD^+ + CoA \Leftrightarrow \text{Succinyl CoA} + CO_2 + NADH_2$$

$$\Delta \bar{G} = 8.0 \text{ kcal nol}^{-1}$$

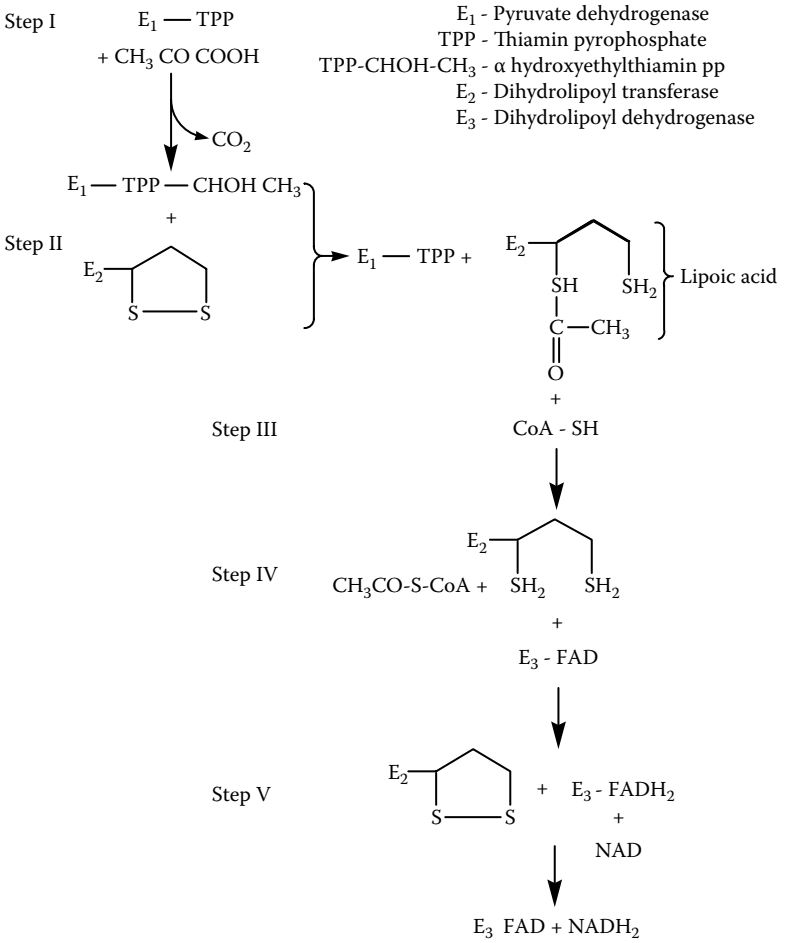


FIGURE 10.7 Oxidation of the pyruvate in the mitochondrial matrix.

Reaction is analogous to the oxidation of pyruvate to acetyl CoA and CO_2 and occurs by the same mechanism with TPP, lipoic acid, CoA, FAD, and NAD participating as coenzymes.

The metabolism of ethanol also requires thiamin. The same pyruvate dehydrogenase complex that converts pyruvate to acetyl CoA will also metabolize acetaldehyde (the first product in the metabolism of ethanol) to acetyl CoA. This system probably accounts for only a small part of ethanol degradation.

TPP also participates in the transfer of a glycoaldehyde group from D-xylulose to D-ribose 5P to yield D-sedoheptulose 7P and glyceraldehyde 3P, an intermediate of glycolysis. Transketolase contains tightly bound thiamin pyrophosphate. In this reaction, the glycoaldehyde group (CH_2OH CO) is first transferred from D-xylulose 5P to enzyme bound TPP to form the $\alpha\,\beta$-dehydroxyethyl derivative of the latter, which is analogous to the α hydroxyethyl derivative formed during the action of PDH. The TPP acts as an intermediate carrier of this glycoaldehyde group which is transferred to the acceptor molecule D ribose 5P. This is a reaction in the hexosemonophosphate shunt.

From the involvement of vitamin-containing coenzymes in the oxidation of alcohol (see Figure 10.9), it follows that vitamin deficiency could impair the rate of alcohol oxidation and thus

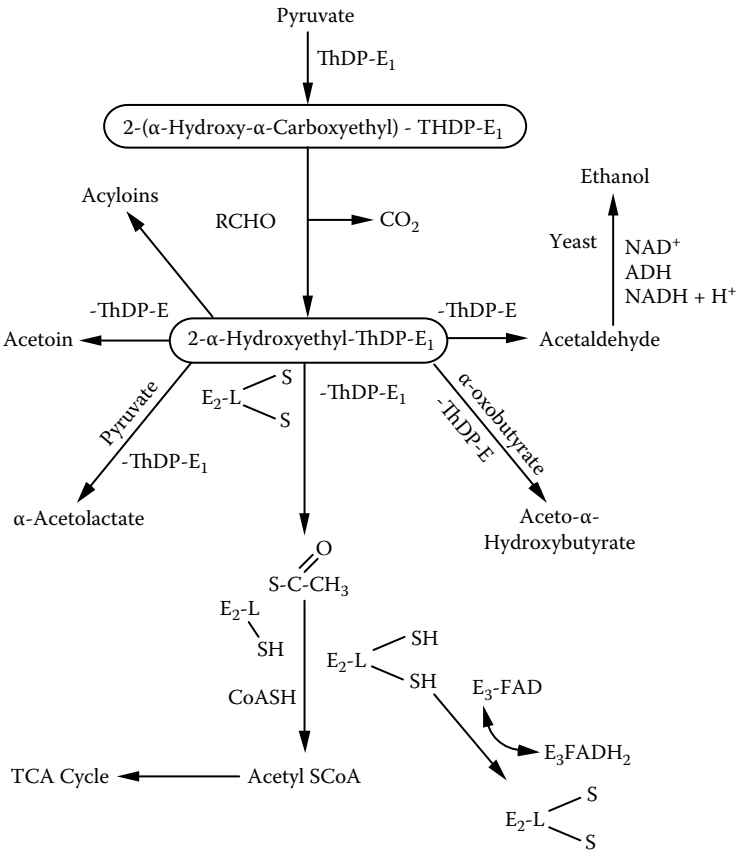


FIGURE 10.8 Summary of the metabolic pathways for pyruvate.

increase the retention of alcohol in the blood of malnourished chronic alcoholic subjects. Actually, there is little evidence for this assumption in animals or man. The rate-limiting step appears to be not the level of vitamin concentration but the amount of alcohol dehydrogenase present.

Large dietary intakes of carbohydrates will require that more thiamin be included in the diet. Ingestion of lipids, on the other hand, is considered to be thiamin-sparing. This is a consequence of the fact that thiamin is required in the metabolism of lipids in fewer places than it is in the metabolism of carbohydrates.

In addition to its role as a coenzyme, it is speculated that thiamin has an independent role in nervous tissue because it has been shown that stimulation of nerve fibers results in release of free thiamin and thiamin monophosphate. If a neurophysiologically active form of thiamin exists, it is as thiamin triphosphate. However, thiamin's role in the central nervous system (CNS) at present is viewed as an intriguing enigma.

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Deficiency

The major symptoms of thiamin deficiency are loss of appetite (anorexia), weight loss, convulsions, slowing of the heart rate (bradycardia), and lowering of the body temperature. Loss of muscle tone and lesions of the nervous system may also develop. Because the heart muscle can be weakened, there may be a cardiac failure resulting in peripheral edema and ascites in the

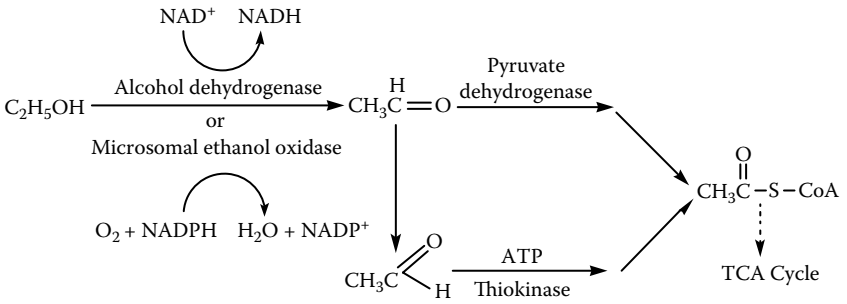


FIGURE 10.9 Metabolism of ethanol.

extremities. The urine of rats with a thiamin deficit contains a higher pyruvate:lactate ratio than that of normal animals. Thiamin-deficient rats also exhibit a reduced erythrocyte transketolase activity.[24,25] Administration of thiamin to rats brings about a remarkable reversal of deficiency symptoms in less than 24 hours.

The classical pathological condition arising from thiamin deficiency in man is beri beri. This disease was quite common in the Orient prior to the discovery of its cause. Thiamin deficiency can arise not only from poor intake of food but also from its faulty utilization or absorption.

Beri beri is classified into several types: acute, mixed, wet or dry (Table 10.5). The acute, mixed type is characterized by neural and cardiac symptoms producing neuritis and heart failure. In wet beri beri the edema of heart failure is the most striking sign; digestive disorders and emaciation are additional symptoms. In dry beri beri loss of functions of the lower extremities or paralysis predominates; it is often called polyneuritis.

Thiamin deficiency is the most common vitamin deficiency seen in chronic alcoholics in the United States. Clinical manifestations of the deficiency vary depending upon the severity of the deprivation. However, in all degrees of deficiency they involve muscle and/or nerve tissue. The most serious form of thiamin deficiency in alcoholics is Wernicke's syndrome. It is characterized by ophthalmoplegia, 6th nerve palsy, nystagmus, ptosia, ataxia, confusion, and coma, which may terminate in death. Often, the confusional state persists after treatment of the acute thiamin deficiency. This is known as Korsakoff's psychosis.

A most serious level of thiamin deficiency is seen in malnourished alcoholics. It is known as alcoholic beri beri. It is seen in those individuals who consume alcohol in preference to food and thus have a minimal intake of thiamin. It is characterized by symmetrical foot and wrist drop associated with a great deal of muscle tenderness. It may also affect cardiac muscle metabolism and may result in congestive heart failure.

The mildest and most common form of thiamin deficiency is the polyneuropathy affecting only the lower extremities of the chronic alcoholic. The signs and symptoms of thiamin deficiency are listed in Table 10.5. All forms of thiamin deficiency respond to thiamin treatment unless the pathology is irreversible, a situation not infrequently found with pathological changes in the nerve tracts (polyneuropathy).

Red-cell transketolase activity seems to be a sensitive index of thiamin nutritional status. Transketolase catalyzes two reactions of the pentose phosphate shunt. One takes ribulose-5- phosphate and ribose-5-phosphate, and makes sedoheputulose-7-phosphate plus glyceraldehyde-3-phosphate. The other takes xyulose-5-phosphate and erythrose-4-phosphate and makes fructose-6-phosphate and glyceraldehyde-3-phoshate. Both reactions require TPP as a coenzyme. Brin et al.[24] have suggested an in vitro test to differentiate between the enzymatic lesions caused by thiamin deficiency and those due to nonspecific causes. This test consists of the stimulation of red cell transketolase

TABLE 10.5
Clinical features of thiamin deficiency

Wet and dry beri beri	Malaise
	Heaviness and weakness of legs
	Calf muscle tenderness
	"Pins and needles" and numbness in legs
	Anesthesia of skin
	Increased pulse rate and heart palpitations
Wet beri beri	Edema of legs, face, trunk; serous cavities
	Tense calf muscles
	Fast pulse
	Distended neck veins
	High blood pressure
	Decreased urine volume
Dry beri beri	Polyneuritis
	Difficulty walking
	Wernicke–Korsakof syndrome
	Encephalopathy; disorientation; short-term memory loss
	Jerky movements of eyes
	Staggering gait

activity using glyceradehyde-3-phosphate as the substrate in the presence of saturating amounts of thiamin pyrophosphate (TPP). This is coupled to an NADH indicator reaction. The reaction results in the so-called TPP-effect (TPPE) and is claimed to be a good indicator of thiamin nutritional status.

In addition to the enzymatic test, a measure of urinary thiamin in relation to dietary intake has been the basis for balance studies to assess the adequacy of intake. When thiamin excretion is low, a larger portion of the test dose is retained, indicating a tissue need for thiamin. A high excretion indicates tissue saturation. On low intakes, excretion drops to zero.

Need

Thiamin needs of an individual are influenced by many factors including age, caloric intake, carbohydrate intake, and body weight. The presence of infection may drive up the need for thiamin.[26] Table 10.1 gives the DRIs for humans.

Toxicity

Although thiamin produces a variety of pharmacological effects when administered in large doses, the dose required is thousands of times greater than that required for optimal nutrition. Generally, toxic effects are reported on subcutaneous, intramuscular, intraspinal, or intravenous injections but not oral administration. In rare cases thiamin has caused reactions resembling anaphylactic shock in humans. However, because these usually develop only in individuals who have been given large intravenous injections, they apparently appear only after hypersensitivity has developed.

RIBOFLAVIN

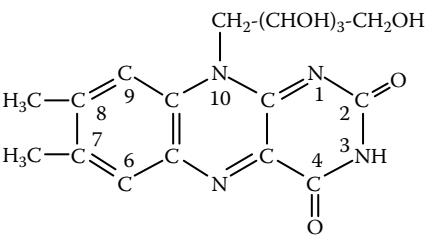
While thiamin and niacin were being recognized as the causative factors in beri beri and pellagra, respectively, riboflavin, the second B vitamin, was ignored. A food fraction isolation by McCollum and Kennedy in 1916 was shown by Emmett and Luros, and later by Goldberger and Lillie to have several functions: one as an anti beri beri curative, one as a pellagra curative, and a third whose function was not known.[18] These fractions were all water soluble but differed in their stability towards heat and light. In 1932 Warburg and Christian isolated and described a flavoprotein that was recognized by others as having vitamin properties. This, then, became the vitamin riboflavin. Because it was first isolated with thiamin and niacin, it was given a letter designation in England as vitamin B_2 and in the United States as vitamin G. As our knowledge of vitamins increased, this nomenclature was dropped in favor of the name riboflavin.

Structure, Chemical, and Physical Properties

Riboflavin (Figure 10.10) is a yellow-orange crystalline substance frequently associated with flavoproteins. As a solid it is red-orange in color. In solution, the color changes to a greenish yellow. It was first synthesized by Kuhn and also by Karrer et al. in 1935 as a needle-like crystal with limited solubility in pure water or in acid solutions. Solubility increases as the pH of the solvent increases; however, as the pH of the solution rises, riboflavin's stability to heat and light decreases. Milk loses 33% of its riboflavin activity in 1 hour of sunlight. In solution, riboflavin is easily destroyed by light and must be protected at all times from exposure. Biochemists working with riboflavin take such precautions as using deep red glassware and darkened work areas to ensure maximal recovery or assessment of vitamin activity. Because riboflavin fluoresces due to a shifting of bonds in the isoalloxazine ring, this property can be used as a basis of its determination by spectrophotometric or photofluorometric techniques. The fluorescence is due to the presence of a free 3-imino group. If substitutions are made for this group, there is no fluorescence. Fluorescence can be measured before and after reduction by such compounds as sodium hydrosulfite. Fluorescence is pH dependent and is best measured between pH 4–8; maximal fluorescence occurs at 556 nm.

The oxidized forms of different flavoenzymes are intensely colored. They are characteristically yellow, red, or green due to strong absorption bands in the visible range. Upon reduction, they undergo bleaching with a characteristic change in the absorption spectrum as illustrated in Figure 10.11.

In order to have vitamin activity, positions 8 and 7 must be substituted with more than just a hydrogen, and the amine group in position three must be unattached. There must be a ribityl group



7, 8 dimethyl 10 (1′D-ribityl) isoalloxazine
or
6, 7 dimethyl 9 (D-1′ribityl) isolloxazine
if only the carbons are numbered.

FIGURE 10.10 Structure of riboflavin.

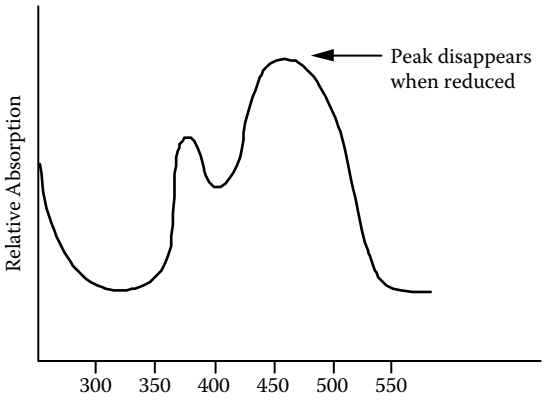


FIGURE 10.11 Absorption spectra of riboflavin.

on position 10. If the ribityl group is lost then vitamin activity is lost as shown in Figure 10.12. There are some antivitamins that interfere with riboflavin's usefulness. These compounds compete for the prosthetic groups or competitively inhibit its phosphorylation to form the coenzymes FMN (flavin mononucleotide) or FAD (flavin adenine nucleotide). The structures of these coenzymes are shown in Figure 10.13.[22]

Sources

The best sources are foods of animal origin: milk, meat, eggs. Wheat germ is also a good source.

Assay

The most sensitive procedure for the determination of riboflavin is that which uses HPLC. As mentioned, the vitamin must be protected from light and acid. The coenzyme forms must be separated from the free form. This can be done by differential solubility. The free form is soluble in benzyl alcohol, whereas the coenzymes are not.

Absorption and Metabolism

Absorption occurs by way of an active carrier and is energy and sodium dependent.[27,28] Maximum absorption occurs in the proximal segment (the jejunum) of the small intestine with significant uptake by the duodenum and ileum.

After a load dose, peak values in the plasma appear within two hours. The phosphorylated forms (coenzyme forms) are dephosphorylated prior to absorption through the action of nonspecific hydrolases from the brush border membrane of the duodenum and jejunum. There is a pyrophosphatase that cleaves FAD and FMN, and an alkaline phosphatase that liberates the vitamin from its coenzyme form. Bile salts appear to facilitate uptake and a small amount of the vitamin circulates via the enterohepatic system. Prior to entry into the portal blood, some of the vitamin is rephosphorylated to reform FAD and FMN. After absorption, the vitamin circulates in the blood bound to plasma proteins notably albumin. Specific riboflavin-binding proteins have been isolated and identified in several species. These proteins are of hepatic origin.[29] There is active uptake of the free vitamins by all of the vital organs.[30] For example, isolated liver cells will accumulate up to five times the amount of the vitamin in the fluids that surround them. Although cells will accumulate the vitamin against a concentration gradient, these cells also use the vitamin quite rapidly so there is

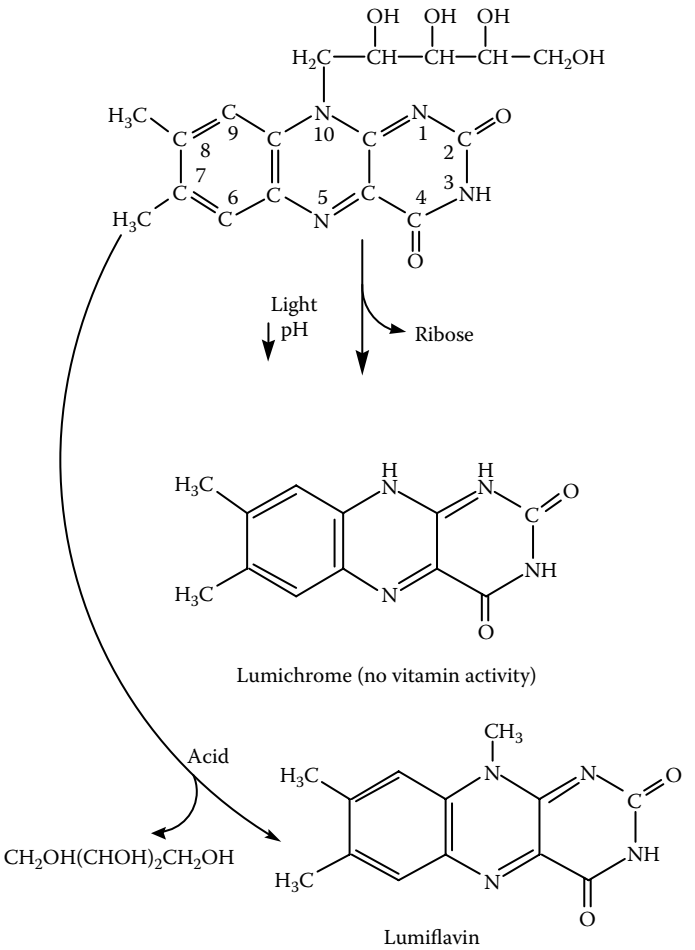


FIGURE 10.12 Degradation of riboflavin tolumichrome and lumiflavin.

little net storage. The usual blood levels of riboflavin are in the range of 20–50 mg/L, whereas 500–900 μg/day are excreted in the urine. Excretion products include 7- and 8-hydroxymethyl riboflavin 8 α-sulfonylriboflavin, riboflavin peptide esters, 10-hydroxyethylflavin, lumiflavin, 10-formylmethylflavin, 10-carboxymethylflavin, lumichrome and, of course, free riboflavin. Very small amounts of riboflavin and its metabolites can be found in the feces. Upon entry into the cell, riboflavin is reconverted to FMN and FAD as shown in Figure 10.14.[31] The initial phosphorylation reaction is zinc dependent. FMN and FAD synthesis is responsive to thyroid status. Hyperthyroidism is associated with increased synthesis whereas hypothyroidism is associated with decreased synthesis. FAD is linked to a variety of proteins via hydrogen bonding and also with purines, phenols, and indoles. Covalent bonding with certain enzymes also occurs and involves the riboflavin 8-methyl group that forms a methylene bridge to the peptide histidyl imidazole function or to the thioether function of a former cysteinyl residue. When bound to these proteins, these coenzymes are protected from degradation. However, flavins in excess of that which are protein bound are rapidly degraded and excreted in the urine. Degradation begins with hydroxylation at positions 7 and 8 of the isoalloxazine ring by hepatic microsomal cytochrome P450 enzymes. The methyl groups at these positions are removed, and the compound loses its activity as a vitamin. Because degradation and excretion

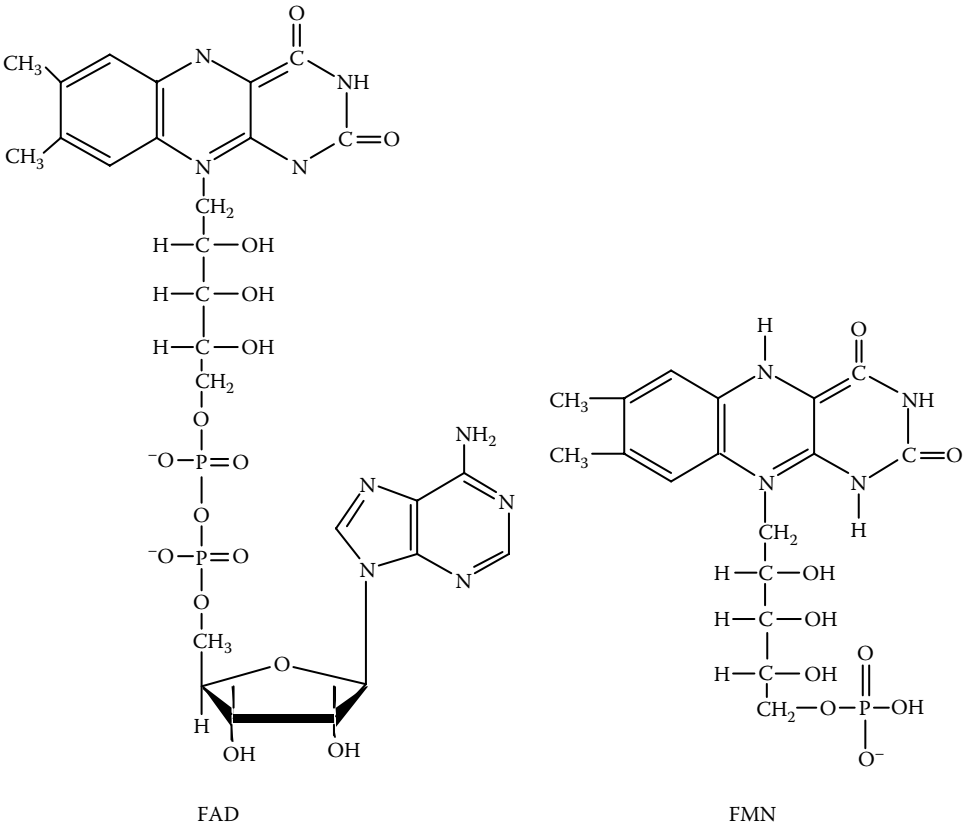


FIGURE 10.13 Structure of flavin adenine dinucleotide (FAD and flavin mononucleotide [FMN]).

occur at a fairly rapid rate, the rate of riboflavin degradation determines the requirement for the vitamin rather than the need for the vitamin in its function as a coenzyme, that is, the rate of FMN and FAD synthesis.

Function

FAD and its precursor FMN are coenzymes for reactions that involve oxidation-reduction.[22,23] Thus, riboflavin is an important component of intermediary metabolism. The respiratory chain in the mitochondria and reactions in numerous pathways that utilize either FAD or FMN as coenzymes require riboflavin.[32–34] Shown in Table 10.6 is a list of some of these enzymes. They include reactions where reducing equivalents are transferred between cellular compartments as part of a shuttle arrangement, as well as reactions that are in a mitochondrial or cytosolic sequence.

In most glutathione reductase flavoenzymes, the flavin nucleotide is tightly but noncovalently bound to the protein; an exception is succinate dehydrogenase in which the flavin nucleotide, FAD, is covalently bound to a histidine residue of the polypeptide chain.[35] The metalloflavoproteins contain one or more metals as additional cofactors. Flavin nucleotides undergo reversible reduction of the isolloxazine ring in the catalytic cycle of flavoproteins to yield the reduced nucleotides $FMNH_2$ and $FADH_2$. The enzymes that have a riboflavin-containing coenzyme are of three general types:

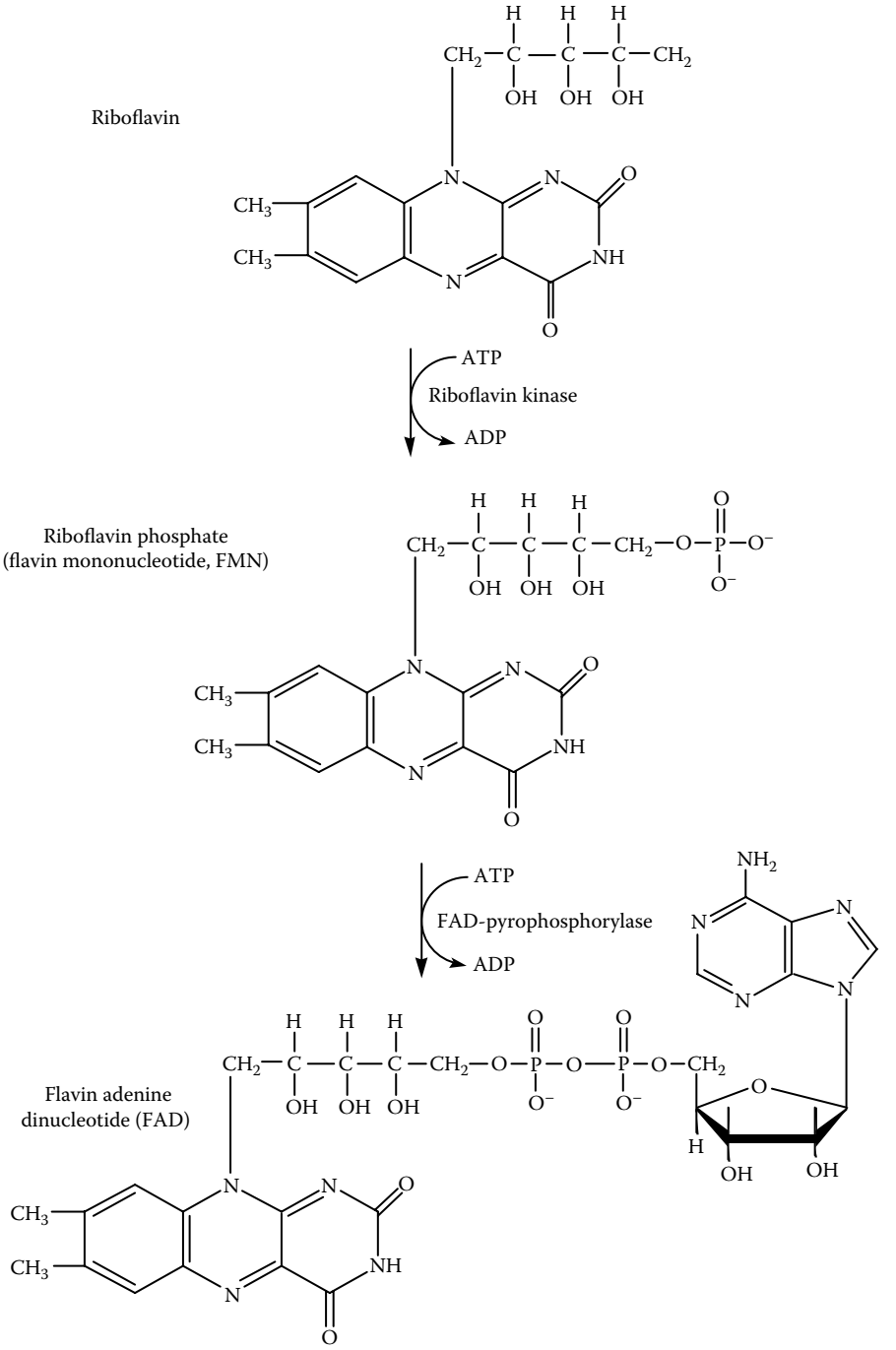


FIGURE 10.14 Synthesis of FMN and FAD.

TABLE 10.6
Reactions using FAD or FMN

FAD-linked enzymes	
Ubiquinone reductase	Xanthine oxidase
Monoamine oxidase	Cytochrome reductase
NADH-cytochrome P450 reductase	Succinate dehydrogenase
D-amino acid oxidase	α glycerophosphate dehydrogenase
Acyl-CoA dehydrogenase	Electron transport respiratory chain
Dihydrolipoyl dehydrogenase (component of PDH and α- KGDH)	Glutathione reductase
FMN-linked enzymes	
NADH dehydrogenase (respiratory chain)	Lactate dehydrogenase
	NADH cytochrome P450 reductase
L-amino acid oxidase	

a. Enzymes whose substrates are a reduced pyridine nucleotide and the acceptor is either a member of the cytochrome or another acceptor
b. Enzymes that accept electrons directly from the substrate and can pass them to one of the cytochromes or directly to oxygen
c. Enzymes that accept electrons from substrate and pass them directly to oxygen (true oxidases)

The mechanism of action is shown in Figure 10.15.

Each of the steps in this sequence is fully reversible, allowing the flavoprotein to accept or donate reducing equivalents, which in turn can be joined to oxygen. Many of the flavoproteins, proteins linked with FAD or FMN also contain a metal such as iron, molybdenum, or zinc, and the combination of these metals and the flavin structure allows for its easy and rapid transition between single and double electron donors.

Note in Table 10.6 that a number of enzymes are members of the oxidase family of enzymes. The oxidases transfer hydrogen directly to oxygen to form hydrogen peroxide. Xanthine oxidase uses a variety of purines as its substrate converting hypoxanthine to xanthine, which is then converted to uric acid. Xanthine oxidase also catalyzes the conversion of retinal to retinoic acid (see Chapter 9). Among the important enzymes shown in Table 10.6 are those that are essential to mitochondrial respiration and ATP synthesis, as well as the mitochondrial citric acid cycle. Succinate dehydrogenase is one of these, and its activity has been used as a biomarker of riboflavin intake sufficiency. The acyl-CoA dehydrogenases catalyze another of the essential pathways, fatty acid oxidation. These are FAD linked. Fatty acid synthesis requires the presence of FMN-linked enzymes. Although the list of enzymes shown in Table 10.6 is by no means complete, it gives evidence of the intimate and essential need for riboflavin in the regulation of metabolism. In its absence, profound impairments can be expected and death should follow in a short time once all of the FAD and FMN is used up. In humans, clinical signs of deficiency appear in less than six weeks on intakes less than 0.6 mg/day.

Deficiency

Despite our knowledge about riboflavin's function a coenzyme, there are a few symptoms that are specific to riboflavin deficiency.[36] Poor growth, poor appetite, and certain skin lesions (cracks at the corners of the mouth, dermatitis on the scrotum) have been observed. However, these symptoms

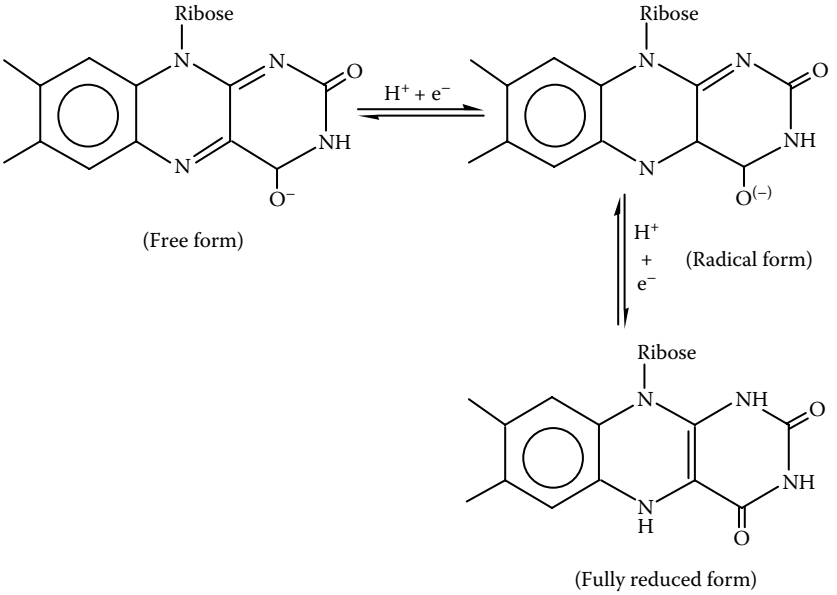


FIGURE 10.15 Mechanism of action of the riboflavin portion of the coenzyme.

can also occur for reasons apart from inadequate riboflavin intake. This lack of direct correlation of symptoms to intake is due to the almost universal need for FAD and FMN as coenzymes in such a wide variety of the reactions of intermediary metabolism. Thus, it is impossible to pinpoint a specific deficiency symptom. Nutrition assessment of adequate riboflavin intake relies upon a few reactions in readily available cells (i.e., blood cells) that can predict intake adequacy. Erythrocyte FAD-linked glutathione reductase is one of these. Low enzyme activity is associated with inadequate intakes. Succinate dehydrogenase is another enzyme frequently used in nutrition assessment.

Need

As mentioned, there is almost no riboflavin reserve. Thus, a daily intake of riboflavin is essential. The DRIs for humans are shown in Table 10.1.

NIACIN, NICOTINIC ACID

Few vitamins have as tortured a history of discovery as niacin, otherwise known as vitamin B_3 or nicotinic acid, niacin, or nicotinamide.[18] The synthesis of nicotinic acid was accomplished long before it was discovered to be a vitamin. Some 50 years elapsed before it was connected to the disease pellagra. Pellagra was described in the mid-1800s and called "mal de la rosa." Its development was associated with the consumption of low-protein–high-corn diets. The disease was more prevalent in very poor populations and again associated with the consumption of corn. At one time it was thought to be due to a toxin found in corn; however, as descriptions of pellagra arose in the literature from populations that did not consume corn, this idea was discarded. Some years later, Goldberger demonstrated that pellagra was a nutrient deficiency disease and that the nutrient in question was niacin or nicotinic acid. The term *niacin* is a generic term that includes both the acid and amide forms.

Structure, Physical, and Chemical Properties

Niacin occurs in two forms as shown in Figure 10.16. Nicotinic acid and nicotinamide are widely distributed in nature.[37] Nicotinic acid is the primary constituent of the coenzymes, NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide dinucleotide phosphate).[22] The synthesis of these pyridine nucleotides is shown in Figure 10.16.

The molecular weight of nicotinic acid is 123.1 and that of nicotinamide, 122.1. Nicotinamide is far more soluble in water than is nicotinic acid. Both are white crystals with an absorption maxima of 263 nm. The melting point of the acid form is 237°C, whereas that of the amide is 128–131°C. In order to have vitamin activity there must be a pyridine ring substituted with a β-carboxylic acid or corresponding amide and there must be open sites at pyridine carbons 2 through 6.

Nicotinic acid is amphoteric and forms salts with acids and bases. Its carboxyl group can form esters and anhydrides, and can be reduced. Both the acid and amide forms are very stable in the dry form but when the amide form is in solution, it is readily hydrolyzed to the acid form.

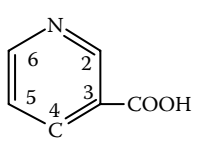
Several substituted pyridines can antagonize the biological activity of niacin. These include pyridine-3-sulfonic acid, 3-acetylpyridine, isonicotinic acid hydrazide, and 6-aminonicotinamide. HPLC is the analytical method of choice for this vitamin, which does not occur in large amounts as the free form. Most often it occurs as the coenzyme NAD^+ or $NADP^+$. Chemical analysis using the Koenig reaction (which opens up the pyridine ring with cyanogen bromide followed by reaction with an aromatic amine to form a colored product) is used. The most widely used method employs a chromophore-generating base, *p*-methylaminophenol sulfate, sulfanilic acid, or barbituric acid. The color intensity so developed is dependent on the concentration of the vitamin.

Sources

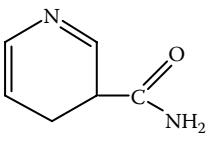
This vitamin is widely distributed in the human food supply. Especially good sources are whole grain cereals and breads, milk, eggs, meats, and vegetables that are richly colored.

Absorption and Metabolism

In contrast to thiamin and riboflavin, niacin is not absorbed via an active process.[38] Rather, both nicotinic acid and nicotinamide cross the intestinal cell by way of simple diffusion and facilitated diffusion. There are species differences in the mechanism of absorption. In the bullfrog, absorption is via active transport. In the rat, there is evidence of a transporter that is saturable, sodium dependent, and carrier mediated. This suggests facilitated diffusion. After absorption the vitamin circulates in the blood in its free form as shown. That which is not converted to NAD^+ or $NADP^+$ is metabolized further and excreted in the urine. The excretory metabolites are *N*′-methylnicotinamide, nicotinuric acid, nicotinamide-*N*′-oxide, *N*′-methylnicotinamide-*N*′-oxide, *N*′-methylnicotinamide-*N*′ oxide, *N*′-methyl-4-pyridone-3-carboxamide and *N*′-methyl-2-pyridone-5-carboxamide. Niacin can be synthesized from tryptophan in a ratio of 60 molecules of tryptophan to 1 of nicotinic acid.[39] The



Nicotinic acid



Nicotinamide

FIGURE 10.16 Structures of nicotinic acid (niacin) and nicotinamide.

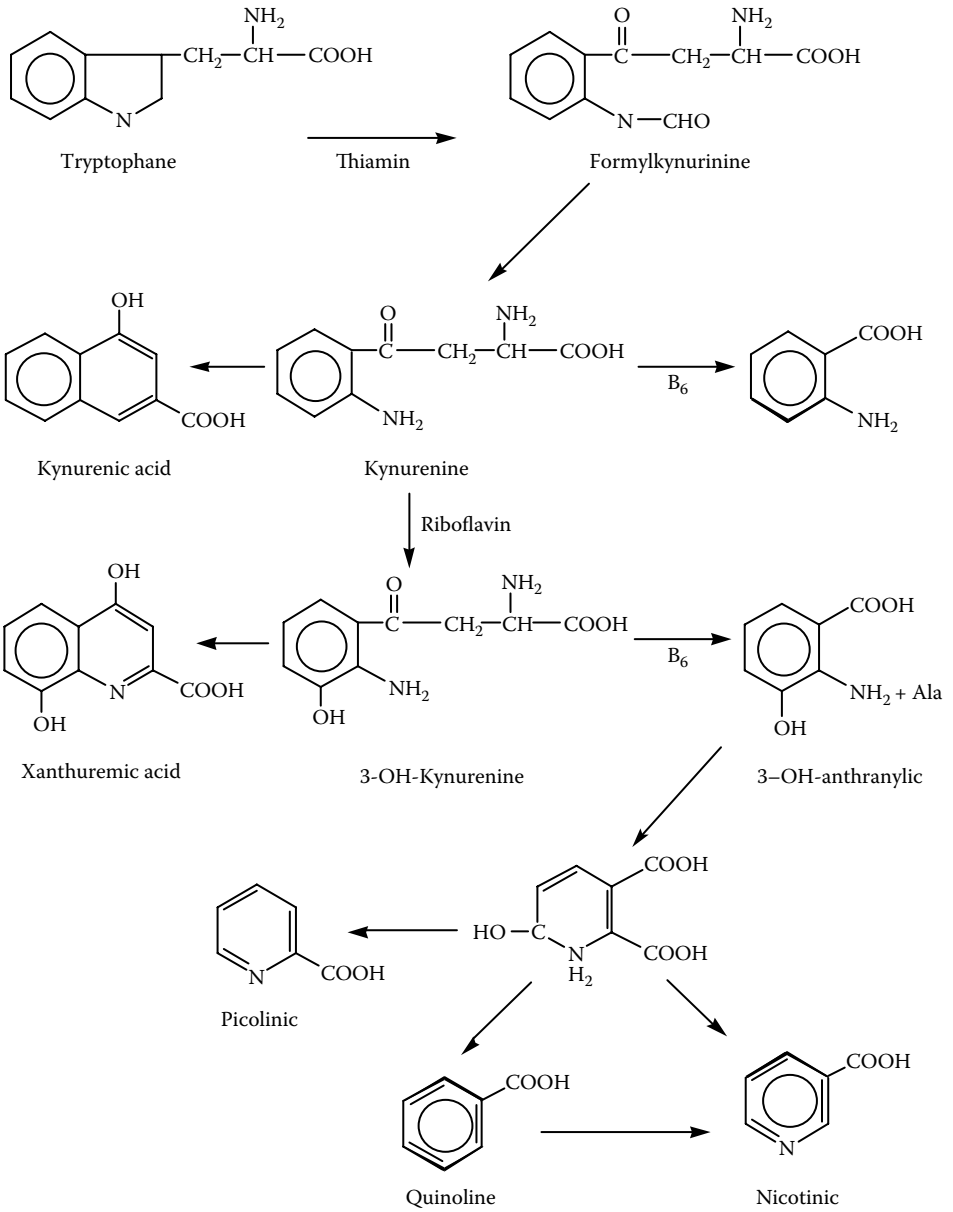


FIGURE 10.17 Synthesis of niacin from tryptophane.

pathway for conversion is shown in Figure 10.17. Note the involvement of thiamin, pyridoxine, and riboflavin in this conversion.

Function

The main function of this vitamin is that of the coenzymes NAD^+ and $NADP^+$.[22] Both function in the maintenance of the redox state of the cell. These coenzymes are bound to the dehydrogenase enzyme protein relatively loosely during the catalytic cycle and therefore serve more as substrates

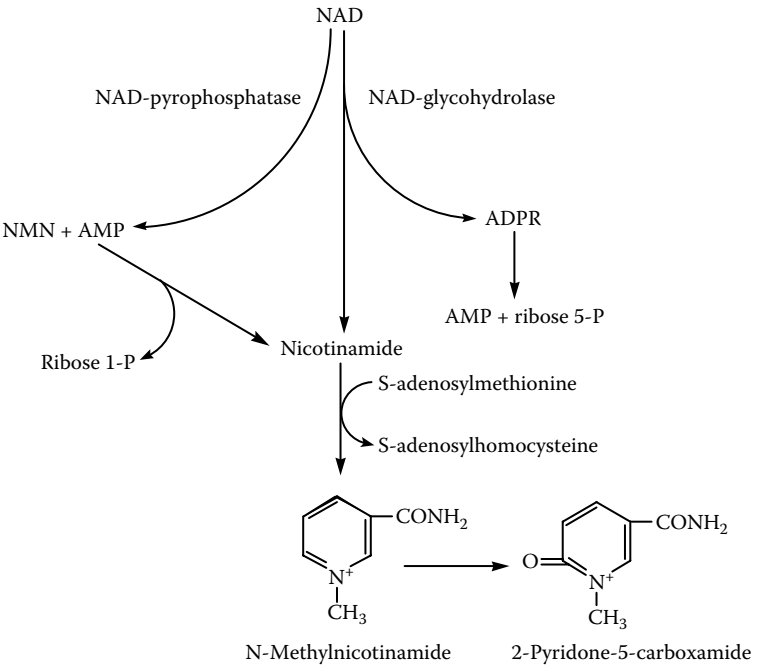


FIGURE 10.18 Degradation of NAD.

than as prosthetic groups. They act as electron acceptors during the enzymatic removal of hydrogen atoms from specific substrate molecules. One hydrogen atom of the substrate is transferred as a hydride ion to the nicotinamide portion of the oxidized NAD^+ or $NADP^+$ forms of these coenzymes to yield the reduced forms. The other hydrogen ion remains loosely associated. Thus, the reduced coenzyme is represented as $NADH^+H^+$ or $NADPH^+H^+$. Most enzymes are specific for NAD or NADP, and these enzymes are members of the oxidoreductase family of enzymes. Some will use either (e.g., glutamate dehydrogenase).

Most of the NAD- or NADP-linked enzymes are involved in catabolic pathways (e.g., glycolysis or the pentose phosphate shunt). NAD turns over quite rapidly in the cell. Its degradation is shown in Figure 10.18. However, $NADH^+H^+$ also plays an important role in covalent modifications of histones (DNA-binding proteins) as described in Chapter 5. $NADH^+H^+$ provides the substrate that leads to poly(ADP-ribosylation) of glutamate residues in histones, mediated by poly (ADP-ribose) polymerase. Poly(ADP-ribosylation) is an important event in the cellular response to DNA damage, initiating repair processes or cell death, depending on the severity of the DNA damage. Niacin-deficient rats give evidence of DNA strand breaks and reduced ply (ADP-ribose) polymerase activity, and this speaks to the role of this vitamin in the maintenance of normal cell cycle.[40]

Beyond its use in biological systems as a precursor of NAD^+ or $NADP^+$, nicotinic acid has a pharmacological use. When consumed in pharmacological doses, it is degraded as though it was benzoic acid. Nicotinic acid, the drug, is used as a lipid-lowering drug. Large intakes (1 g/d) lower serum cholesterol. However, large doses also result in flushing due to its effect on vascular tone. Nicotinic acid elicits a fibrinolytic activation of very short duration. Both nicotinic acid and nicotinamide can be toxic if administered at levels greater than 10 μmol/kg.[41] Chronic administration of 3 g/d to humans results in a variety of symptoms including headache, heartburn, nausea, hives, fatigue, sore throat, dry hair, inability to focus the eyes, and skin tautness. In experimental animals, nicotinic acid supplements result in a reduction in adipocyte free fatty acid release by

streptozotocin-diabetic rats, an inhibition of adipocyte adenylate cyclase activity in normal hamsters, and degenerative changes in the heart muscle of normal rats.[42] All of these responses were those that characterize a defense against a toxic exposure to nicotinic acid, rather than a response to a normal intake level.

Deficiency

Pellagra has been well described as the niacin deficiency disease. It is characterized by skin lesions that are blackened and rough especially in areas exposed to sunlight and abraded by clothing. The typical skin lesions of pellagra are accompanied by insomnia, loss of appetite, weight loss, soreness of mouth and tongue, indigestion, diarrhea, abdominal pain, burning sensations in various parts of the body, vertigo, headache, numbness, nervousness, apprehension, mental confusion, and forgetfulness. Many of these symptoms can be related to niacin-deficiency-induced deficits in the metabolism of the CNS. This system has, as its choice metabolic fuel, glucose. Glycolysis with its attendant need for NAD as a coenzyme is appreciably less active. As the deficient state progresses, numbness followed by a paralysis of the extremities occurs. The more advanced cases are characterized by tremor and a spastic or ataxic movement that is associated with peripheral nerve inflammation. Death from pellagra ensues if the patient remains untreated.

Early indications of niacin deficiency include reductions in the levels of urinary niacin metabolites especially those that are methylated (*N´*-methyl-nicotinamide and *N´*-methyl-2-pyridone-5-carboxamide).[43] Since the discovery early in this century of the curative power of nicotinic acid and nicotinamide, pellagra has become very rare. The exception is in the alcoholic population. This population frequently substitutes alcoholic beverages for food and thereby is at risk for multiple nutrient deficiencies including pellagra. The metabolism of ethanol is NAD dependent. This dependency drives up the need for niacin in the face of inadequate intake, setting the stage for alcoholic pellagra. In part, the CNS symptoms of alcoholism are those of pellagra as described.

There is another very small population at risk for developing niacin deficiency. This group carries a mutation in the gene for tryptophan transport. This results in a disease called Hartnup's disease. Its symptoms, apart from the effects of tryptophan inadequacy on protein synthesis, are very similar to those of niacin deficiency. This is because of the use of tryptophan as a precursor of nicotinic acid. If niacin supplements are given to people with Hartnup's disease, these pellagra-like symptoms disappear.

Need

Age, gender, and protein intake affect the DRI for niacin. Low protein diets would increase the need for niacin in the diet as there would be little excess tryptophan available for conversion to niacin. Because tryptophan can be converted to nicotinic acid, the DRI is stated in terms of niacin equivalents. A niacin equivalent is equal to 1 mg niacin or 60 mg of tryptophan. The need for niacin is related to energy intake as well, particularly the carbohydrate intake. However, the DRI takes into account varying diet composition as well as individual differences in nutrient need. The DRI for niacin is given in Table 10.1.

PYRIDOXINE

Of all the B vitamins whose nomenclature has been changed to trivial names, one vitamin remains known by its letter designation: vitamin B_6.[44,45] The vitamin was first defined by Gyorgy in 1934 as "that part of the vitamin B complex responsible for the cure of a specific dermatitis developed by rats on a vitamin-free diet supplemented with thiamin and riboflavin." The dermatitis is unlike that seen with other deficiencies of the B complex. There is a characteristic scaliness about the paws and mouth of rats in addition to a loss of hair from the body. The dermatitis is called rat acrodynia.

The vitamin was crystallized in 1938 by three different groups of researchers and was subsequently characterized and synthesized. Even though the vitamin was identified, crystallized, and synthesized in the late 1930s it was not realized until 1945 "that there were three distinct forms of the vitamin." Pyridoxine was isolated primarily from plant sources, whereas pyridoxal and pyridoxamine were isolated from animal tissues. The latter two are more potent growth factors for bacteria and are more potent precursors for the coenzymes pyridoxal phosphate and pyridoxamine phosphate. When commercially prepared (synthesized) it is commonly available as pyridoxine hydrochloride.

Structure, Physical, and Chemical Properties

Vitamin B_6 occurs in nature in three different forms which are interconvertible. It can be an aldehyde (pyridoxal), an alcohol (pyridoxine), or an amine (pyridoxamine). These three forms are shown in Figure 10.19. Vitamin B_6 is the generic descriptor for all 2-methyl-3,5-dihydroxymethyl pyridine derivatives. To have vitamin activity it must be phosphorylatable at the 5-hydroxymethyl group, and the substituent at carbon 4 must be convertible to the aldehyde form. Pyridoxine hydrochloride is the commercially available vitamin and is also shown in Figure 10.19. The molecular weight of pyridoxal is 167.2, and pyridoxine HCl has a molecular weight of 205.6. Both occur as a white crystal that is readily soluble in water. Pyridoxine HCl is stable to light and heat in acid solutions. In neutral or alkaline solutions it is unstable to light and heat. This feature was recognized in an unfortunate way: Infant formulas that were autoclaved lost their vitamin content and the infants became deficient.[46] The aldehyde form (pyridoxal) is much less stable. Its instability to heat is a major concern in food processing because foods that are rich in the vitamin are neutral to slightly alkaline. When heat treated, as is necessary to kill food-borne pathogens, and prevent spoilage, vitamin activity may be lost. This is particularly true for foods that are autoclaved (e.g., infant formulas).

Pyridoxine-al-amine can be assayed in a variety of techniques. Pyridoxal has an absorption maxima of 293, whereas pyridoxine HCl has absorption maximas of 255 and 326. Microbiological, colorimetric/spectrophotometric, and chromatographic techniques are available. The method of choice is HPLC.

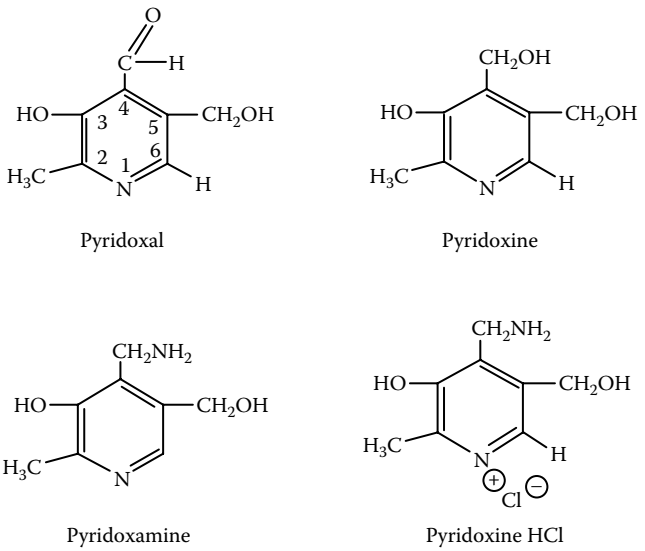


FIGURE 10.19 Structures of naturally occurring vitamin B_6.

Sources

Vitamin B_6 is widely distributed throughout foods from the plant and animal kingdoms. Meats, cereals, legumes, lentils, nuts, fruits, and vegetables all contain the vitamin. Thus, persons consuming a diet containing a variety of raw and cooked foods likely will not develop a deficiency of the vitamin.

Absorption and Metabolism

Work with rat and hamster small intestine both in vivo and in vitro has provided no evidence of an active transport mechanism for the vitamin. Uptake into everted sacs of rat jejunum over a concentration range of 0.01 M to 10 M of pyridoxine did not show saturation kinetics nor was uptake inhibited by anoxia, DNP, lack of sodium, ouabain, or the presence of a structural analog 4-deoxypyridoxine. Thus, pyridoxine uptake is by passive diffusion rather than by active transport (Figure 10.20). Once absorbed, it is carried by the erythrocyte to all cells in the body. Significant amounts of the vitamin may be found in liver, brain, spleen, kidney, and heart but like the other water-soluble vitamins, there is no appreciable store and this vitamin must be present in the daily diet. It is carried in the blood tightly bound to proteins, primarily hemoglobin and albumin. The vitamin binds via the amino group of the *N*-terminal valine residue of the hemoglobin α-chain, and this binding has twice the strength of its binding to albumin. Pyridoxal is converted via a saturable process to pyridoxial phosphate (PPS). The reaction shown in Figure 10.21 is catalyzed by pyridoxal kinase, an enzyme present in the cytoplasm of the mucosal cell. When pyridoxal is phosphorylated, transmural absorption decreases whereas uptake is unaffected. Phosphorylation thus serves as a means of control of the cellular PP level. Pyridoxal phosphate is essential to the absorption of other nutrients.

A major metabolite is 4-pyridoxic acid. It accounts for 50% of B_6 excreted in the urine. The reaction sequence is shown in Figure 10.21. Other metabolites have been found in the urine in addition to the three forms of the vitamin. Amphetamines, chlorpromazine, oral contraceptives, reserpine—all increase B_6 loss. Oral contraceptives increase tryptophane use and thus increase B_6 use.

Function

Pyridoxal phosphate serves as a coenzyme in reactions whose substrates contain nitrogen.[44] Well over 100 reactions are known that involve pyridoxal phosphate. About 50% of these are transaminase reactions. Reactions such as transamination, racemization, decarboxylation, cleavage, synthesis, dehydration, and desulfhydration have been shown to be dependent on pyridoxal phosphate. In transamination, the α-amino of amino acids such as alanine, arginine, asparagine, aspartic acid, cysteine, isoleucine, lysine, phenylalanine, tryptophane, tyrosine, and valine is removed and transferred to a carbon chain such as α-ketoglutarate, which in turn can transfer the amino group to the

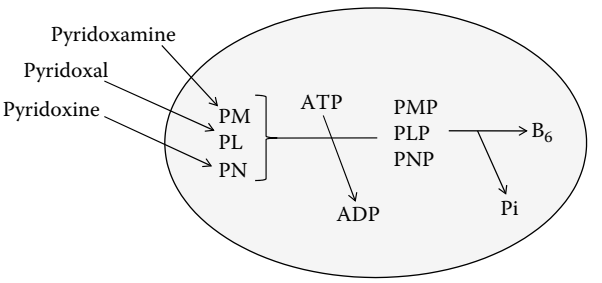


FIGURE 10.20 Absorption of the B_6 vitamins.

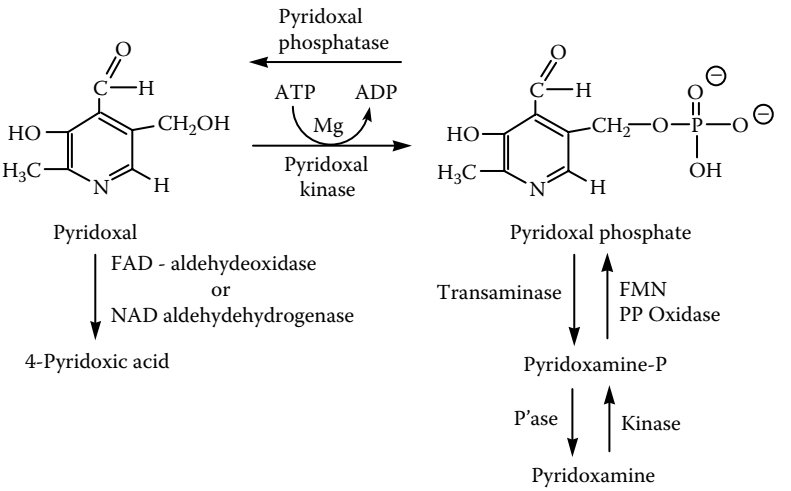


FIGURE 10.21 Phosphorylation of pyridoxal.

urea cycle for urea synthesis. Pyridoxal phosphate functions in transaminations in a Schiff-base mechanism as shown in Figure 10.22. The binding of pyridoxal phosphate to its apo enzyme is shown in Figure 10.23. The active coenzyme forms of the vitamin B_6 are pyridoxal phosphate and pyridoxamine phosphate.

Our present understanding of the role of these coenzymes came from the work of Snell et al., who found that pyridoxal will react nonenzymatically at 100°C with glutamic acid to yield pyridoxamine and α ketogluric acid. This led to the proposal that pyridoxal phosphate functions as a coenzyme by virtue of the ability of its aldehyde group to react with the α-amino group to yield a Schiff's base between the enzyme-bound pyridoxal phosphate and the amino acid, converting it to the α-keto acid; the resulting bound pyridoxamine phosphate enzyme then reacts with another α-keto acid, called an *amino acid acceptor*, in a reverse reaction to yield a new amino acid and pyridoxal phosphate.[47] The linkage of pyridoxal phosphate to the enzyme is a noncovalent bonding, presumably through the charged ring containing the nitrogen atom and the lysyl residues of the transaminase enzyme proteins. In transamination, the unprotonated amino group of the amino donor is covalently bound to the carbon atom of the aldehyde group of enzyme-bound pyridoxal phosphate, with the elimination of water, to form an aldimine which tautomerizes to the corresponding ketomine. The step involves the movement of an electron pair from the amino acid to the pyridine ring of the prosthetic group followed by tautomerization to the ketomine.

Addition of water leads to the formation of a free α-keto acid and Enz-PP complex. By oscillating between the aldehyde and amino groups, the PP acts as an amino acid carrier. Thus, the transamination reaction is an example of a double displacement reaction.

Pyridoxal phosphate also acts with cystathionine lyase to catalyze the cleavage of cystathionine to yield free enzyme, free cysteine with α ketobutyrate, and NH_3 as other products. Pyridoxal phosphate is important to the synthesis of the neurotransmitters γ-aminobutyric acid (GABA), serotonin, dopamine, norepinephrine, and epinephrine. Pyridoxine excess is also associated with sensory neuropathies[48] that may be related to its function in the synthesis of the above listed neurotransmitters. These reactions are outlined in Figure 10.24. This role of vitamin B_6 explains the CNS symptoms associated with the deficient state. Convulsions are a common symptom together with other derangements in metabolism, and anemia. The symptom of anemia arises from the role of pyridoxal phosphate in hemoglobin synthesis is outlined in Figure 10.25.

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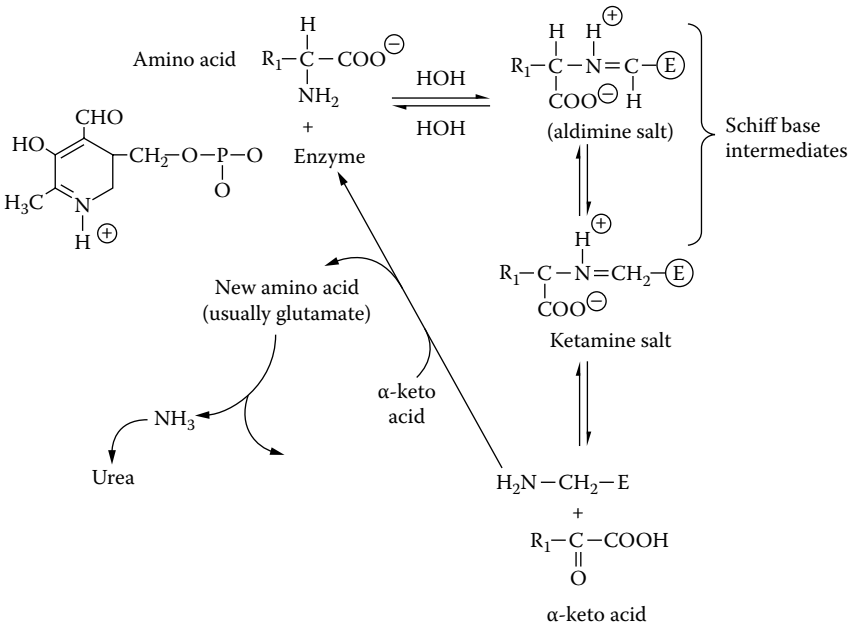


FIGURE 10.22 Pyridoxal phosphate functions in transamination reactions using a Shiff-base mechanism. Symbols used are R_1, amino acid; E, apoenzyme.

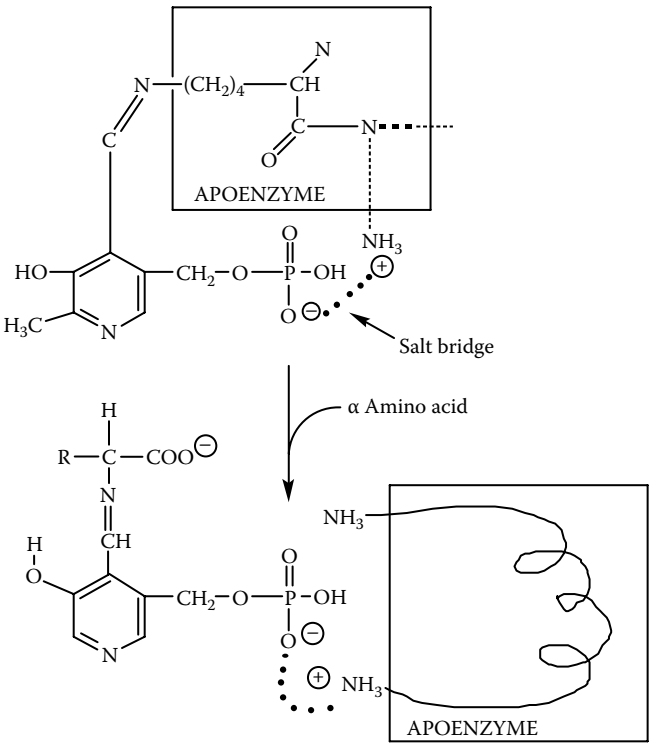


FIGURE 10.23 Binding of pyridoxal phosphate to its apoenzyme. When an α-amino acid enters, it displaces the ε-amino group of the lysyl residue of the apoenzyme.

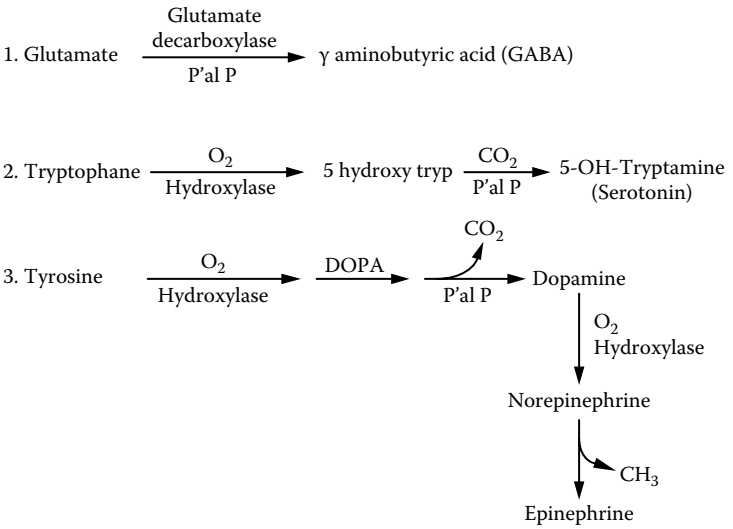


FIGURE 10.24 B_6 and the synthesis of transmitters.

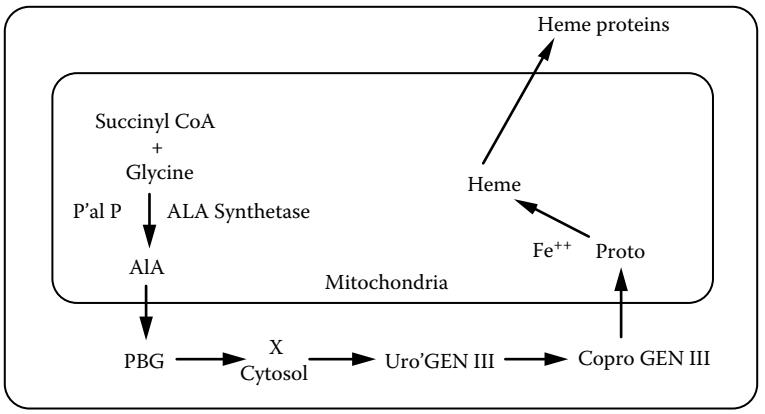


FIGURE 10.25 Role of pyridoxal phosphate (PLP) in heme synthesis. Abbreviations used: ALA = aminolevulinic acid; PBG = porphobilinogen; URO GEN III = uroporphyrinogen III; Copro Gen III = coproporphyrinogen III; Proto = protoporphyrin IX; X = intermediates. Enzymes catalyzing heme biosynthesis omitted except ALA synthetase.

More recently we have come to understand a role of vitamin B_6 in steroid-hormone-induced protein synthesis. New studies have shown that B_6 has an important role as a physiological mediator of steroid hormone function. In this role, B_6 binds to steroid hormone receptor and in so doing inhibits the binding of the steroid hormone-receptor complex to specific DNA sites.[49,50] In this way, B_6 acts as a negative control of steroid hormone action. Progesterone, glucocorticoids, estrogen and testosterone effects on RNA polymerase II and RNA transcription have been shown to be inhibited by the presence of pyridoxal phosphate. In each of these instances, the pyridoxal phosphate binds to the receptor protein and in so doing has a negative effect on hormone-receptor binding to DNA.

This role for B_6 is in addition to its role as a coenzyme in a wide variety of enzymes involved in cell growth and cell division. One of these is ornithine decarboxylase, an enzyme that plays an important role in cell division. In rapidly growing tumor cells, B_6 levels are much lower than in normal cells and some of the major chemotherapies for cancer are based on the need for B_6 by these cells.[51,52] Anti-vitamin B_6 compounds are important chemotherapeutic agents in this setting.

Deficiency

In laboratory animals, dermatitis (acrodynia) is the chief symptom. Lesions occur on paws, ears, nose, chin, head, and upper thorax. This skin disorder resembles EFA deficiency. A high-fat diet protects somewhat against a B_6 deficiency. Other symptoms include poor growth; muscular weakness; fatty livers; convulsive seizures; anemia; reproductive impairment; edema; nerve degeneration; enlarged adrenal glands; increased excretion of xanthurenic acid, urea, and oxalate; decreased transaminase activity; synthesis of ribosomal RNA, mRNA, and DNA; and impaired immune response. High protein intakes accelerate the development of the deficiency.

In humans the deficiency syndrome is ill defined. It is characterized by weakness, irritability, and nervousness, insomnia, and difficulty in walking. Cheilosis (cracks at the corners of the mouth) that is not responsive to biotin or riboflavin may also be a sign of deficiency. Infants consuming B_6 deficient milk formula have convulsive seizures that can be corrected almost immediately with intravenously administered vitamin. There is a deranged tryptophan metabolism and evidence of increased excretion of xanthurenic acid. In B_6 deficiency, the conversion of tryptophan to niacin is impaired, and thus skin lesions develop that resemble those of pellagra and riboflavin deficiency. Behavioral changes have been described and include depression and irritability.

Hypochromic, sideroblastic anemia is a common finding and is due to the role B_6 plays in hemoglobin synthesis. Although B_6 is found in a wide variety of foods, B_6 deficiency can be observed when antivitamin drugs are used. For example, isoniazid, a drug used in the treatment of tuberculosis, results in excessive B_6 loss. Penicillamine, a drug used in the treatment of Wilson's disease, has antivitamin activity. Lastly, higher-than-normal doses of B_6 have been prescribed for the treatment of skin disease and for neuromuscular and neurological diseases. Whether this prescription has a positive effect on the pathophysiology of these diseases remains under discussion.

There are several congenital diseases of importance to B_6 status. Homocysteinuria due to a defect in the enzyme cystathione β-synthase is characterized by dislocation of the lenses in the eyes, thromboses, malformation of skeletal and connective tissue, and mental retardation. Pyridoxal phosphate is a coenzyme for this synthase. Cystathionuria, which occurs due to a defect in cystathione γ-lyase, is characterized by mental retardation, and also drives up the need for B_6. GABA deficiency due to mutation in glutamate decarboxylase is manifested by a variety of neuropathies, and sideroblastic anemia due to a mutation in *o*-aminolevulinate synthetase is characterized by anemia, cystathionuria, and xanthurenic aciduria. All of these genetic disorders can be ameliorated somewhat by massive doses of the vitamin. Why this works is not known, but patients with these disorders do not have any symptoms of B_6 deficiency.

Need

The need for B_6 depends on the composition of the diet, and on the age and gender of the individual. The B_6 DRIs are shown in Table 10.1. Aging and diabetes both may have effects on vitamin need. In both instances an increase in intake is warranted.[53,54]

PANTOTHENIC ACID

Pantothenic acid was isolated and synthesized in the late 1940s and recognized as an essential growth factor for yeast. Its essentiality for mammalian species was not recognized until it was

shown to prevent or cure chick dermatitis. It was subsequently regarded as essential for the rat, mouse, monkey, pig, dog, fox, turkey, fish, hamster, and man. Pantothenic acid is synthesized by plant tissues but not by mammalian tissues. It is found in a variety of tissues in the bound form. In 1946, it was discovered to be an essential part of enzyme A.

Structure, Chemical, and Physical Properties

Pantothenic acid is the trivial name for the compound dihydroxy-β, β-dimethyl butyryl-β-alanine.[55] It has two metabolically active forms: coenzyme A (CoA) and as part of acyl carrier protein. Pantothenic acid exists as a free acid (molecular weight 219.2) or as a calcium salt (molecular weight 476.5). It is the condensation product of β-alanine, and a hydroxyl- and methyl-substituted butyric acid, pentoic acid, and its structure is shown in Figure 10.26. It is an unstable, pale-yellow oil, commercially available as a white, stable, crystalline calcium or sodium salt. When dry, the salt is stable to air and light but is hygroscopic. The salt is soluble in water and glacial acetic acid. The vitamin is stable in neutral solution but is readily destroyed by heat and either alkaline or acid pH. When heated, there is hydrolytic cleavage of the molecule yielding β-alanine and 2,4-dihydroxy-3,3-dimethyl butyrate. Pantothenic acid may be assayed colorimetrically following reaction with 1,2-naphthaquinone 4-sulphonate or ninhydrin. Radioimmunoassay also is used as are microbiological methods. The method of choice is HPLC.

Sources

Pantothenic acid is widely distributed in nature. Excellent food sources are organ meats, mushrooms, avocados, broccoli, and whole grains.

Absorption and Metabolism

Absorption occurs via facilitated diffusion and travels in the blood within the erythrocytes as well as in the plasma.[56] Large doses of pantothenic acid are rapidly excreted in the urine, indicating no storage (except for a limited amount in fat cells) and little metabolism/degradation.

Function

Pantothenic acid has as its main function a role in fatty acid metabolism as a component of coenzyme A. Pantothenic acid does not comprise the functional unit of CoA; instead, it provides the backbone for its derivative, pantotheine, whose SH group forms the reactive site. The structure of CoA is shown in Figure 10.27 and its synthesis shown in Figure 10.28.

The function of CoA is to serve as a carrier of acyl groups in enzymatic reactions involving fatty acid oxidation, fatty acid synthesis, pyruvate oxidation, and biologic acetylations. It cannot cross the cell membrane, and must therefore be synthesized in cells. Acetyl CoA (active acetate) is formed during the oxidation of pyruvate or fatty acids. It may also be generated from free acetate in

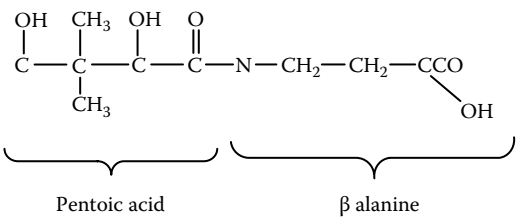


FIGURE 10.26 Structure of pantothenic acid.

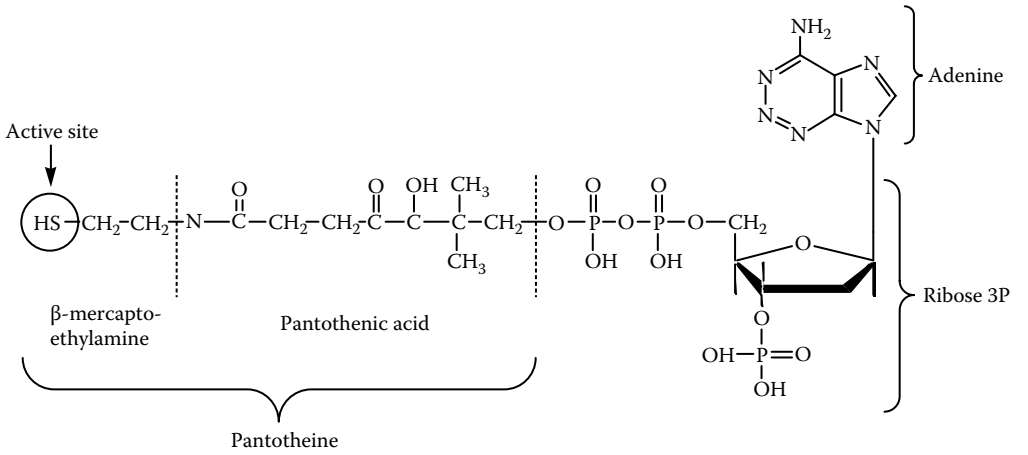


FIGURE 10.27 Structure of coenzyme A.

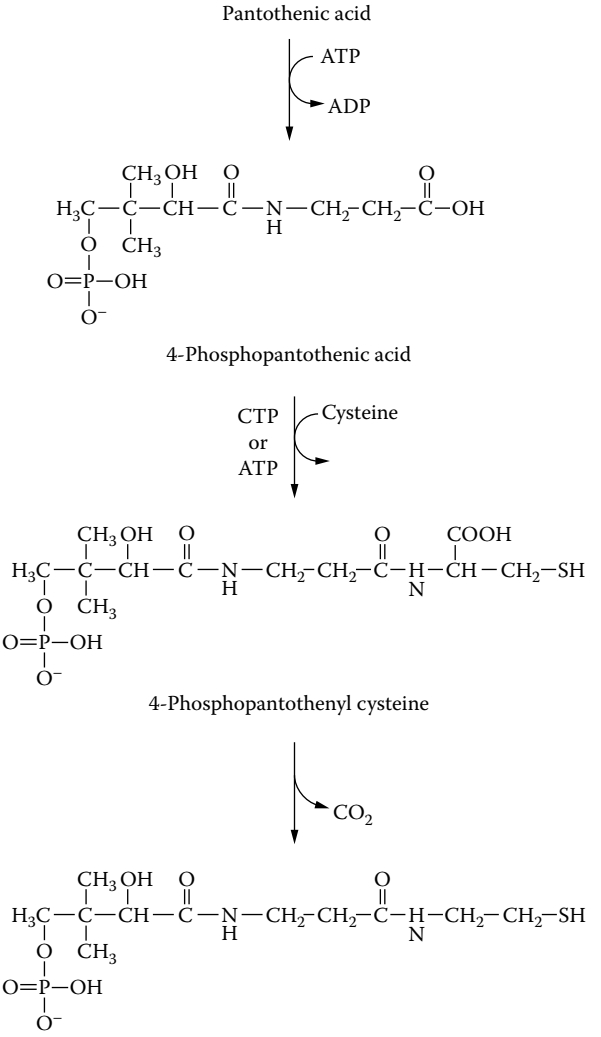
the presence of the enzyme acetyl CoA synthetase. Acetyl CoA may then react with an acyl group acceptor such as choline to yield acetylcholine or oxaloacetate for citrate (Figure 10.29). Acetyl CoA is also an important donor of acetyl groups in the acetylation of histones (DNA-binding proteins), as described in Chapter 5. Acetylation of histones at gene promoters is an important event in the transcriptional activation of genes. The sulfhydryl group of the β-mercaptoethylamine is the site at which acyl groups are linked for transport by the coenzyme. The ability of the CoASH to form thioesters with carboxylic acids is responsible for the vital role of the coenzyme in numerous metabolic processes.

All known acyl derivatives of CoA are thiol esters. These acyl derivatives of CoA may participate in a number of metabolic reactions: condensation, addition, acyl group interchanges, and nucleophilic attack.

These reactions fall into three general categories:

a. Acetylation of choline and certain aromatic amines such as sulfonamides.
b. Oxidation of fatty acids, pyruvate, α ketoglutarate, and acetalehyde.
c. Synthesis of fatty acids, cholesterol, sphingosine, citrate, acetoacetate, porphyrin, and sterols.

Thus, CoA serves not only as an acetyl donor/acceptor but also as an acyl donor/acceptor. CoA a central integrator of intermediary metabolism as illustrated in Figure 10.29. Fatty acid synthesis in the cytoplasm involves an additional role of pantothenic acid in the form of a cofactor 4-phosphopantetheine. This factor is bound to a protein commonly called acyl carrier protein (ACP). ACP plus 4-phosphopantetheine appears to be involved in all fatty acid synthesis. Its structure is shown in Figure 10.30. The acyl intermediates formed during fatty acid synthesis are esterified to the SH group. Phosphopantetheine is a cofactor bound to the GTP-dependent acyl-CoA synthetase. Thus, 4-phosphopantotheine serves in a capacity analogous to CoA during fatty acid oxidation. Carnitine reacts with fatty acyl-CoA esters to form carnitine esters capable of crossing the mitochondrial membrane. CoA does not travel across membranes and thus must be synthesized within each cell as the need for it arises.



(A)

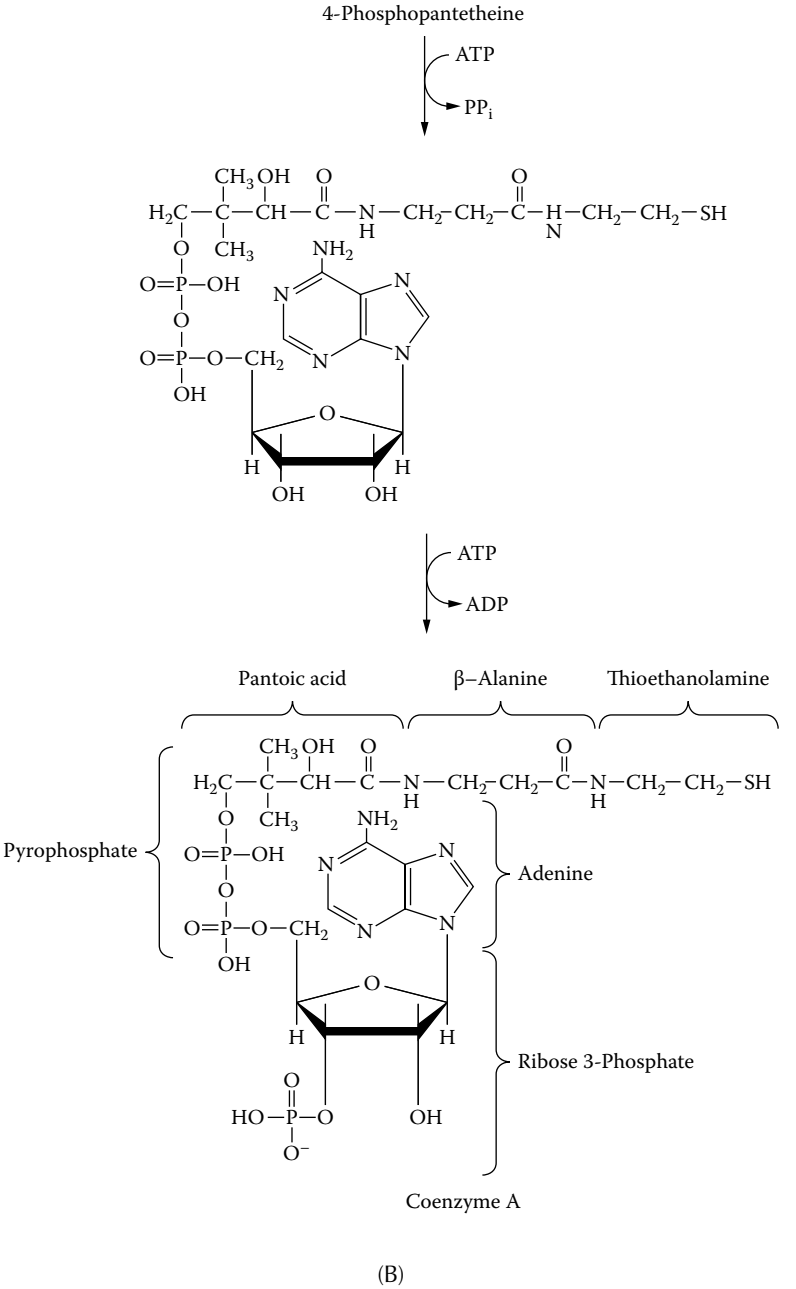
FIGURE 10.28 The intracellular synthesis of coenzyme A from pantothenic acid.

Deficiency Symptoms

Deficiency symptoms are species specific (Table 10.7). Pantothenic acid deficiency has not been described in humans as a single entity. If it occurs, it is accompanied by other deficiency disorders as well. The exception to this is in patients treated with the pantothenic acid antagonist, ω-methyl-pantothenic acid. In these patients neurological symptoms (parasthesia of toes and feet) depression, fatigue, insomnia, vomiting, and muscle weakness have been reported. Changes in glucose tolerance, increased sensitivity to insulin, and decreased antibody production have also been noted.

Need

An acceptable intake (AI) has been developed for pantothenic acid and is given in Table 10.1. Age has an effect on pantothenic need.[57,58]



(B)

FIGURE 10.28 (continued)

BIOTIN

At the end of the 19th century it was discovered that yeast needed a factor for growth that was not any of the already discovered essential nutrients. This factor was called "bios." Later, scientists realized that bios was a mixture of inositol and biotin, and that bios could overcome "egg-white injury." At this point it was named vitamin H or factor H and was found to be needed for cellular

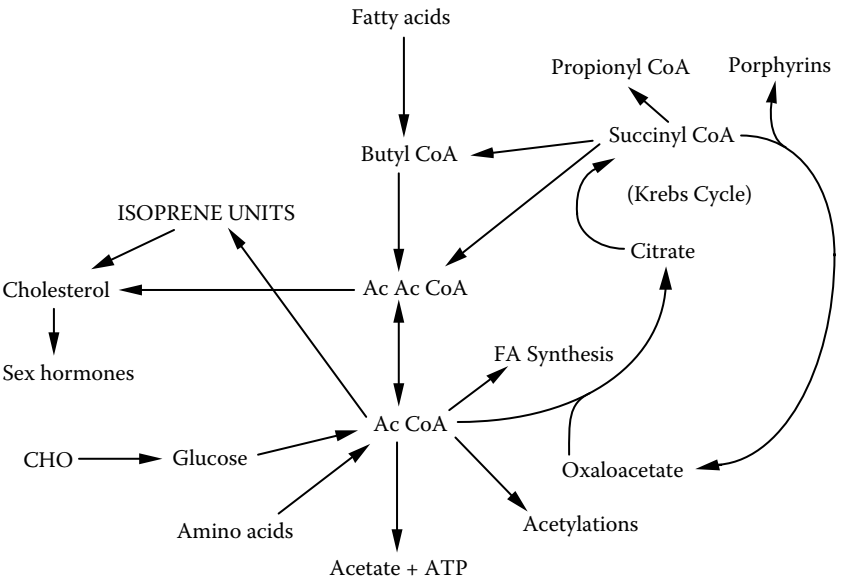


FIGURE 10.29 The central role of acetyl CoA in intermediary metabolism.

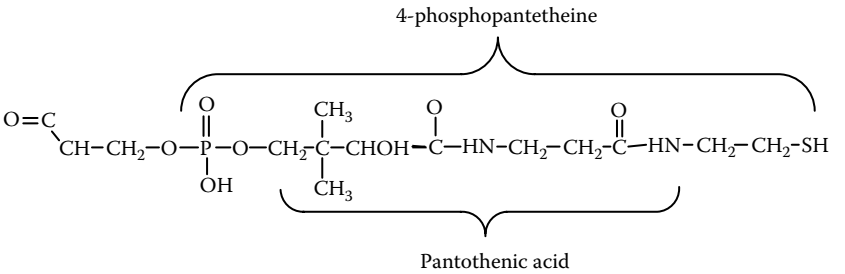


FIGURE 10.30 Pantothenic acid attached to the seryl residue of the acyl carrier protein.

respiration. Because of its essentiality for respiration it was named coenzyme R. In the late 1930s Gyorgy finally integrated all these bits and pieces, and together with Kšgl, du Vigneaud, and Harris realized the essential nature of a material they had isolated and synthesized. Biotin was chosen as its name.

Structure, Physical, and Chemical Properties

Biotin is the trivial name for the compound *cis*-hexahydro-2-oxo-1H-thieno (3,4-d) imidazole-4-pentanoic acid. Its structure is shown in Figure 10.31. In order to have vitamin activity, the structure must contain a conjoined ureido and tetrahydro-thiophene ring, and the ureido 3N is sterically hindered, preventing substitution. The ureido 1N is a poor nucleophile.

Biotin occurs in eight isomeric forms but only D-biotin has vitamin activity. Several biotin analogs have been synthesized or isolated from natural sources. Among these are oxybiotin or biotinol,

TABLE 10.7
Deficiency symptoms in rats, dogs, and pigs

Rat	Dog	Pig
Dermatitis	Appetite	Spastic gait
Achromolrichia (graying)	Hair loss	Hair loss
Adrenal necrosis	Runny nose	As above
Hemorrhage	Fatty liver	
Spectacle eye	Irritability	
Spastic gait	Hypoglycemia	
Anemia		
Leukopenia		
Infertility		
Impaired antibody formation		
Gonadal atrophy		

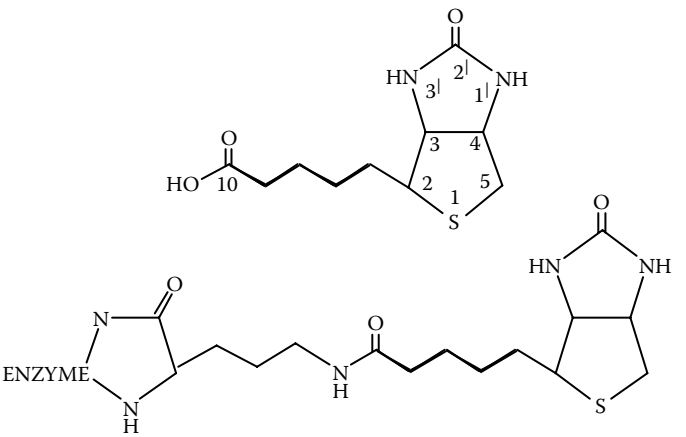


FIGURE 10.31 Structure of biotin and enzyme-bound biotin.

biocytin, dethiobiotin, and biotin sulfoxide. The latter two are inactive as vitamins whereas the first two have some vitamin activity.

Biotin is a white crystalline substance that in its dry form is stable to air, heat, and light. Its molecular weight is 244.3 and melting point is 167°C. It decomposes at 230–232°C. It has a limited solubility in water (22 mg/ml HOH) and is more soluble in ethanol. When in solution it is unstable to oxygen and strong acid or alkaline conditions, and will be gradually destroyed by ultraviolet light. The analytical method of choice is HPLC in combination with avidin-binding assays. Microbiological methods are also available. These methods use *Lactobacillus casei*, *Lactobacillus plantarum*, *Neurospira crassa*, *Ochromonas danica* or *Saccharomyces cerevisiae*. These microorganisms require biotin for growth and are sensitive to varying quantities of biotin in the growth media. Using avidin, a protein found in egg white that binds biotin at the ureido group, an isotope dilution assay has been developed and is sensitive in the range of 4–41 pmols. Colorimetric assays based on the

reaction of biotin with *p*-(dimethylamino) cinnamaldehyde or on the absorbance of iodine formed during the oxidation of biotin to its sulfone with potassium iodide have been developed. The colorimetric assays are not as sensitive as the avidin-binding assays.

Sources

There are numerous food sources for biotin. Biotin is found in every living cell in minute amounts where it exists either in its enzyme-bound form or as an amide. Rich sources include organ meats, egg yolk, brewer's yeast, and royal jelly. Soy flour or soybean, rice polishings, various ocean fish, and whole grains are good sources of the vitamin. Human milk contains biotin.[59,60]

Absorption and Metabolism

Biotin in food exists in the free and enzyme-bound form. The protein-bound form can be digested, which in turn yields biocytin, a combination of biotin and lysine. Biocytin is hydrolyzed via the action of biotinidase to its component parts. The resultant biotin is then available for absorption. Biotin is absorbed via facilitated diffusion. The jejunum is the major site for this absorption. Once absorbed, it circulates as free biotin. There may be some species differences in absorptive mechanism. In addition, there is synthesis of the biotin by the gut flora.

Biotinase is present in plasma as well, where it has a similar function, that is, to release biotin from the breakdown products of biotin dependent carboxylases (see below).[61] Biotinase, if mutated, results in an autosomal recessive disorder that results in a secondary biotin deficiency that can be overcome with biotin supplements. The clinical symptoms of this genetic disorder are the same as those of the biotin deficient state and relate to the function of biotin as a coenzyme in intermediary metabolism, especially the carboxylase reactions.

Biotinase has been cloned and sequenced, and its distribution throughout the body determined.[61] Although active in the intestinal tract, its activity is not sufficient to catalyze all of the bound biotin found in food. It has been estimated that less than 50% of the bound biotin found in foods of plant origin is hydrolyzed to provide the free form. The availability of biotin in food depends on the percent that is bound. In general, protein-bound biotin is more readily available from foods of animal origin compared to those of plant origin. Biotin can be rendered unavailable by avidin, a protein found in raw egg white. Once the egg is cooked the avidin is denatured and no longer binds the biotin. This binding is the explanation of the disorder "egg white injury." Other proteins, particularly membrane and transport proteins, bind biotin and are responsible for its entry into all cells that use the vitamin.

Function

Once absorbed, biotin travels to the liver where it is bound to a liver plasma membrane receptor.[62,63] Biotin has two major functions in metabolism. First, biotin serves as a mobile carboxyl carrier as it is attached to enzymes that catalyze carboxy group transfer.[64] The formation of this biotin-enzyme complex is shown in Figure 10.32. A number of enzymes require biotin as a prosthetic group for their function. These are listed in Table 10.8. Second, biotin is bound covalently to histones (DNA-binding proteins), as described in Chapter 5. Binding of biotin to both carboxylases and histones is mediated by holocarboxylase synthetase.[65]

Deficiency

In humans the symptoms of severe deficiency include dermatitis, skin rash, hair loss (alopecia), developmental delay, seizures, conjunctivitis, visual and auditory loss, metabolic ketolactic acidosis, hyperammonemia, and organic academia. Biotin deficiency results in reproductive failure and impairs the growth and development of the fetus.[66] Abnormal plasma fatty acid profiles are also

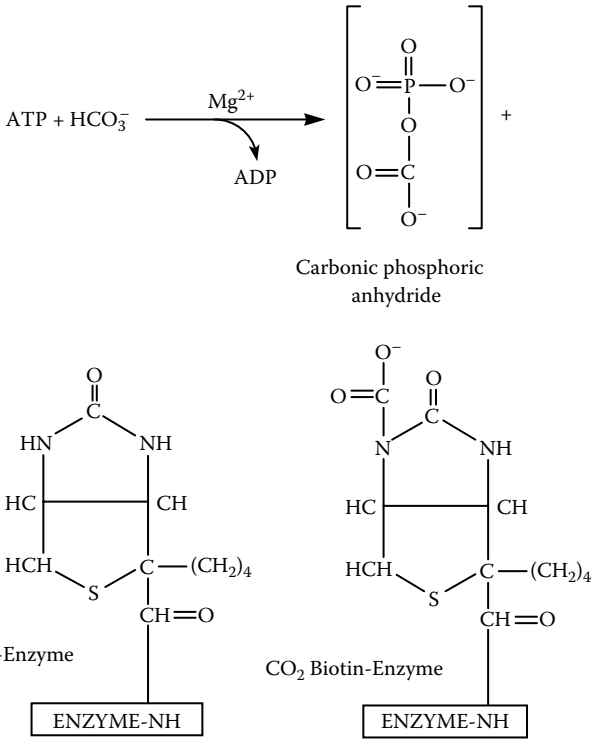


FIGURE 10.32 Formation of the CO_2-biotin enzyme complex.

TABLE 10.8
Biotin-dependent enzymes in animals

Enzyme	Role	Location
Pyruvate carboxylase	First reaction in pathway that converts 3-carbon precursors to glucose (gluconeogenesis).	Mitochondria (rate-limiting step in gluconeogenesis).
	Replenishes oxaloacetate for citric acid cycle.	
Acetyl-CoA cacarboxylase	Commits acetate units to fatty acid synthesis by forming malonyl-CoA.	Cytosol (rate limiting step in fatty acid synthesis).
Propionyl-CoA carboxylase	Converts propionate to succinate, which can be converted to succinyl CoA, and intermediate of the citric acid cycle.	Mitochondria
β-Methylcrotonyl-CoA carboxylase	Catabolism of leucine and certain isoprenoid compounds.	Mitochondria

observed in biotin deficiency.[67] These symptoms have been reported in persons lacking normal biotinidase activity through a genetic error.[68] In a genetically normal human population a true biotin deficiency is extremely rare; however, in persons that are suffering from protein-energy malnutrition, biotin deficiency may develop secondarily. Only a few instances have been reported. In one, the deficient state was caused by the chronic consumption of 30 raw eggs/day for several months. In this individual, the symptoms were primarily related to the skin.

Need

Because the vitamin is present in a wide variety of foods and because it can be synthesized by the intestinal flora, a fixed intake figure has been difficult to determine. However, the National Academy of Sciences Food and Nutrition Board has published an adequate dietary intake (AI) for this vitamin (Table 10.1).

FOLIC ACID

More than 50 years ago folate was discovered as a necessary constituent of every living cell of every organism whether plant or animal.[69] It took years of meticulous work to separate its function from that of vitamin B_{12} for both are involved in the one carbon transfer that is so important in the synthesis of the purines and pyrimidines that are constituents of DNA and RNA. Actually, until the genetic material and its function in the cell were worked out, there was no progress in understanding the role of the folates in nucleic acid synthesis. With the advent of our growing knowledge of how genes are made and how they work, we finally have come to understand the importance of folic acid in cellular function.

Structure, Chemical, and Physical Properties

Folic acid or folate or folacin is the generic term for pteroylmonoglutamic acid and its related biologically active compounds. A number of derivatives have vitamin activity. The basic structure of pteroylglutamic acid is shown in Figure 10.32. The derivatives (Table 10.9) include the addition of hydrogen at N5 and N10 and only one glutamate attached to para aminobenzoic acid. This derivative is called tetrahydrofolic acid (THF). Other derivatives can have a methyl group attached at N5, a methyl bridge between N5 and N10, or a methylene bridge at this position or an aldehyde group at either N5 or N10, or a HCNH group at N5. All of these derivatives have vitamin activity because vitamin activity is dependent on the presence of a pteridine (pterin) structure with variable hydrogenation or methyl addition at N5 or N10, and the presence of at least one glutamyl residues linked via peptide bonds to *p*-amino benzoic acid. Methotrexate (4-amino-N^{10}-methyl folic acid, an anti neoplastic agent) and aminopterin (4-amino folic acid, a rodenticide) are folate antagonists and as such are useful pharmaceutical agents against cell growth. The pteroyl monoglutamate form has a molecular weight of 441.1 and is moderately soluble in water (0.0016 mg/1 mL water). It has an absorption maxima at 256, 283, and 368 mM. It is an orange yellow crystal with a melting point of 250°C. It is unstable to ultraviolet light, heat, oxygen, acid conditions, and divalent metal ions such as iron and copper. As mentioned, it is present in all living cells in small amounts so until the advent of sensitive HPLC techniques,[70] its determination in food and animal tissues relied on microbiological techniques. In addition to the HPLC methodology there is also a radioimmunological technique

TABLE 10.9
Derivatives of folic acid

Derivative	N-5	N-10
Tetrahydrofolic acid	–H	–H
5-Methyfolic acid	$–CH_3$	–H
5,10-Methanylfolic acid	–CH=	$–CH_2=$
5,10-Methylenefolic acid	$–CH_2–$	$–CH_2=$
5-Formylfolic acid	–HCO	H
10-Formylfolic acid	H	–HCO
5-Formiminfolic acid	–HCN	H

that involves the binding of the vitamin to an isotopically labeled protein, followed by antibody precipitation and quantification.

Sources

Folate is found in a wide variety of foods of both animal and plant origin. However, because it is so unstable, food sources may be insufficient to meet need. Good sources include meats, fruits, vegetables (especially asparagus), dry beans, peas and nuts, and whole grain cereal products.

Absorption and Metabolism

Folate transport in the intestine is a carrier-mediated, pH-dependent process with maximum transport occurring after glutamation in the jejunum.[71–73] There are specific folate-binding proteins that function in the absorption process.[74] One is a low-affinity folate-binding protein found in the brush-border membrane of the absorptive cell. There is another high-affinity folate- binding protein that is localized to the jejunal brush-border cells. Affinity is optimized at pH 5.5–6.0. The high-affinity-binding protein is similar to one found in the kidney. A number of drugs inhibit active folate transport.[75] These include ethacrynic acid, sulfinpyrazone, phenylbutazone, sulfasalazine, and furosemide. All of these are amphipathic substances. That is, they are compounds with a polar–apolar character. Absorption is also inhibited by cyanide and 2,4-dinitrophenol, drugs that poison oxidative phosphorylation and thus reduce the ATP supply. ATP is necessary for the active transport process to work. Absorption can also occur by passive diffusion, but this is a secondary means for folate uptake. Very little folate appears in the feces.

After folate is absorbed, it circulates in the plasma as pteroylmonoglutamate. That which is not used by the cells is excreted in the urine as pteroylglutamic acid, 5-methyl-pteroylglumic acid, 10-formyl tetrahydrofolate, or acetamidobenzoylglumate. Uptake by cells is mediated by a highly specific folate-binding protein. This protein has been isolated from the membranes of a variety of cells and a cDNA probe has been prepared.[76] Folate appears to stimulate the transcription of the mRNA for this protein.

Function

Folate's main function is as a coenzyme in one carbon transfer.[77,78] However, before it can do this it must be activated. Activation consists of the reduction of folic acid to dihydrofolic acid and thence to tetrahydrofolic acid, as shown in Figure 10.33. Dihydrofolate reductase is a therapeutic target for a number of anticancer drugs that work through inhibiting cell cycle. A number of folate derivatives have vitamin activity, and these derivatives are interconvertible, as shown in Figure 10.34. Methyl group transfer also involves vitamin B_{12}, as illustrated in Figure 10.35.

The regulation of methyl group transfer is complex and involves a number of enzymes and substrates. Although serine is a good source for the methyl group, methyl groups arise from other substrates as well. The major source of single methyl groups involves a cycle of reactions catalyzed by serine, hydroxymethyltransferase, 5,10-methylene-FH_4 reductase, and methionine synthetase. The last of these reactions is rate-limiting for the cycle, whereas the second is inhibited by an *S*-adenosylmethionine (SAM) as well as 5-methyl FH4. As described in the next section on vitamin B_{12}, methionine synthesis depends on the transfer of labile methyl groups from 5-methyl folate to B_{12} which, as methyl B_{12}, donates this methyl group to homocysteine making methionine. One carbon transfer is important in purine and pyrimidine synthesis. The mechanism of this transfer that involves folate (and also B_{12}) is illustrated in Figure 10.36. The whole reaction sequence for purine and pyrimidine synthesis is presented in Chapter 5. In this sequence, methyl transfer occurs at several different points (listed in Table 10.10), and it is immediately apparent why folate and vitamin B_{12} are so important to gene expression.[80–82] Although nuclear DNA, once made, merely reproduces itself within the cell cycle, new messenger RNA is made every minute as new proteins are needed

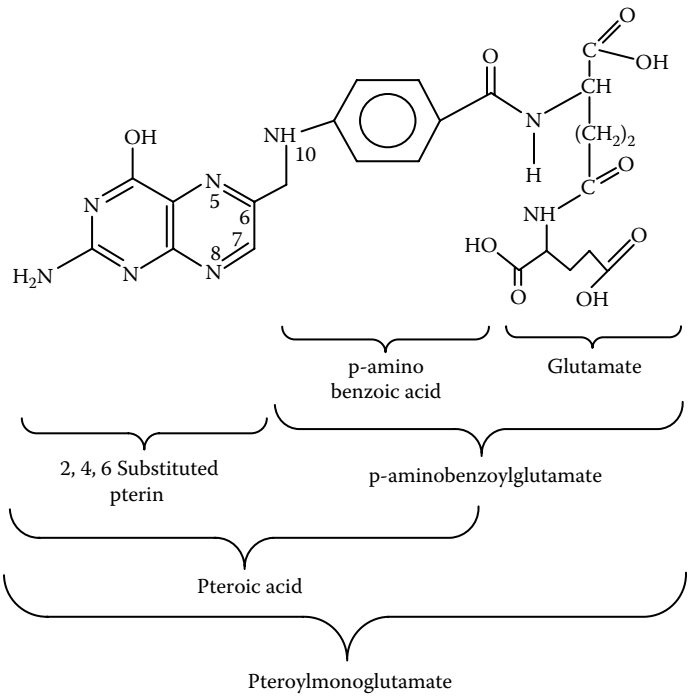


FIGURE 10.33 Structure of folic acid.

by the cell. Some of the purine and pyrimidine bases can be salvaged and reused, but this recycling is not 100% efficient. Messenger RNA has a very short half life (seconds to hours) compared to the other nucleic acid species in the cell. Thus, newly synthesized purines and pyrimidines must be available. If not available, de novo protein synthesis, and of course new cell formation will be adversely affected.

More recently, the roles of folate-dependent methylation of DNA and histones (DNA-binding proteins) have attracted considerable attention (see Chapter 5).[81] For example, methylation of cytosine residues in cytosine/guanosine-rich regions ("CpG islands") in gene promoters is associated with gene silencing. Methylation of cytosine residues is mediated by DNA methyltransferases DNMT1, DNMT3a, and DNMT3b; SAM serves as a methyl donor in these reactions. DNMT1 is considered a "maintenance" methyl transferase (reestablishing methylation patterns after cell division), whereas DNMT3a and DNMT3b are de novo methyl transferases (creating new methylation marks in DNA). Moreover, methylation of lysine residues in histones may cause transcriptional silencing or activation of genes, depending on the site where the methylation mark resides. For example, folate-dependent dimethylation (= binding of two methyl groups) to the ε-group of lysine-9 in histone H3 is associated with gene silencing, whereas trimethylation of lysine-4 is associated with gene activation. In these reactions, SAM serves as a donor of methyl groups, and the generation of SAM depends on folate.

Deficiency

Although anemia, dermatitis, and impaired growth are the chief symptoms of folate deficiency in the human, scientists are now beginning to recognize the importance of adequate folate intake in early embryonic development. Inadequate intake by the mother prior to and/or during the early stages of

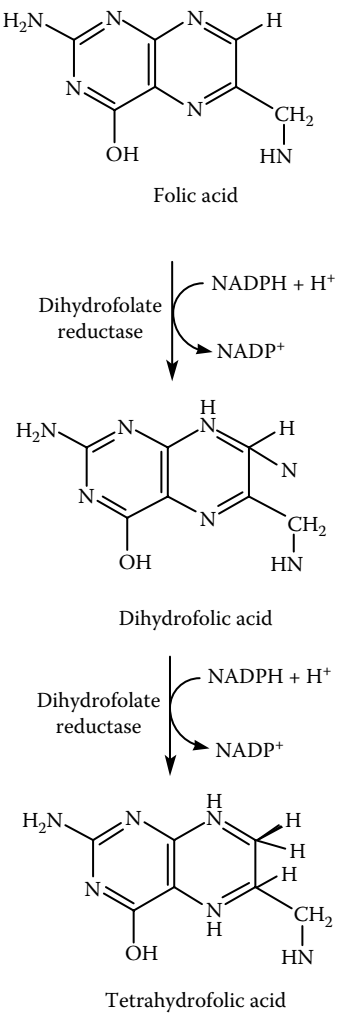


FIGURE 10.34 Activation of folic acid.

development can have teratogenic effects on the embryo. Embryonic development, particularly the neural tube, is impaired in folate deficiency.[83] As a result, infants with spina bifida and other neural tube defects are born. It is estimated that about 2,500 infants per year are born with these defects. Available evidence indicates that women contemplating pregnancy should consume 400 µg/d as a prophylactic measure. Low folate intake has been suggested as a factor in the development of colon cancer as well as in the bronchial squamous metaplasia (premalignant lesions) of smokers, and cervical dysplasia (another premalignant lesion) in women.[84] Folate antagonists serve important roles as antiinfective, antineoplastic, and antiinflammatory drugs.[79,85] These antagonists work by inhibiting dihydrofolate reductase, a key enzyme in the synthesis of thymidylate and therefore DNA.[86] Low zinc intake compromises folate status by negatively affecting intestinal folate uptake. Other symptoms of deficiency are leukopenia (low white cell count), general weakness, depression, and polyneuropathy. The latter sign is probably related to the folate-B_{12} interaction. Alcoholism drives up the need for folacin as well as that of vitamin B_{12}.[87]

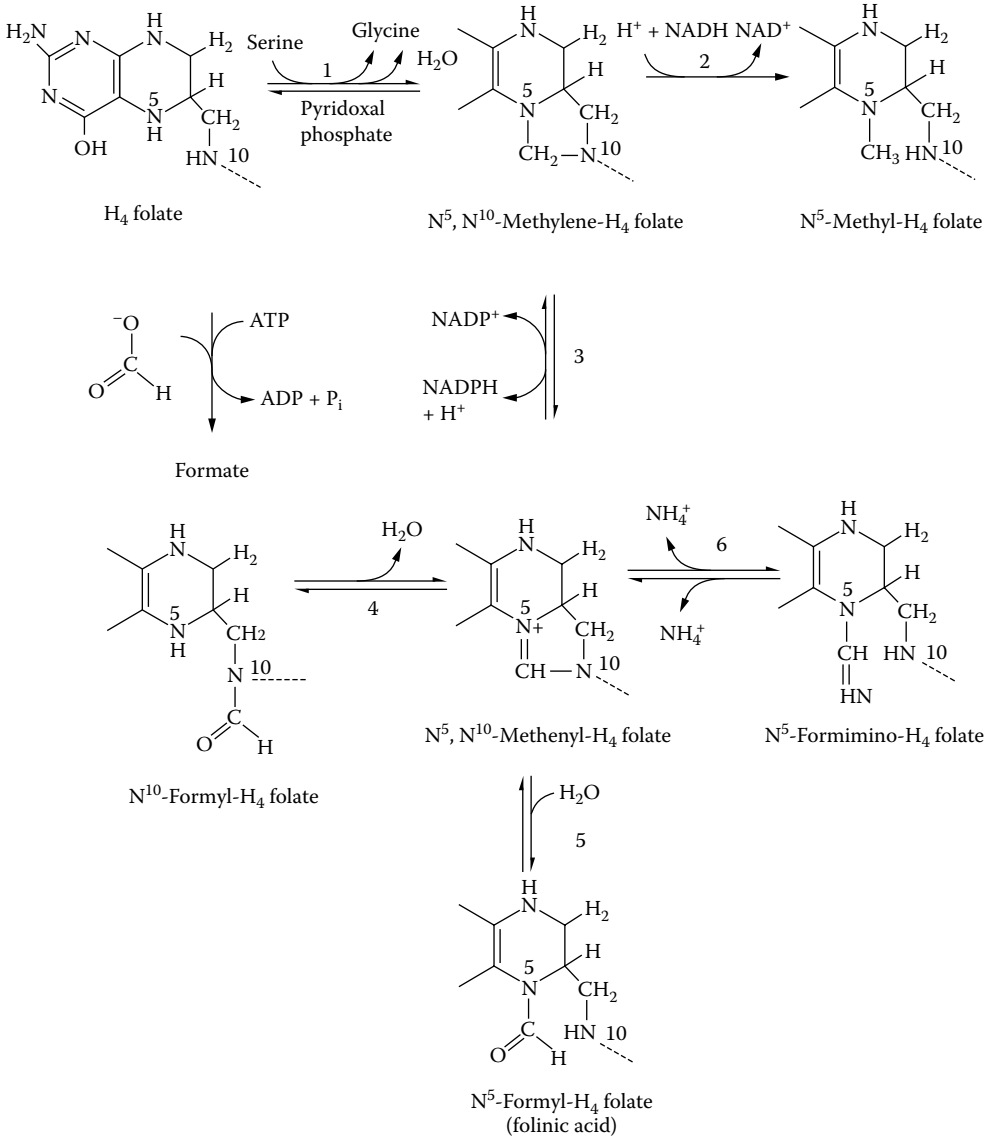


FIGURE 10.35 Interconversions of one carbon moieties attached to tetrahydrofolate.

In rats, folate deficiency has been shown to result in an increased rate of mitochondrial DNA deletion, decreased mitochondrial DNA content, and decreased mitogenesis.[88] These changes were accompanied by an increase in expression of nuclear encoded genes that regulate mitochondrial gene expression as well as biogenesis. Folate deprivation induced aberrant mitochondrial function via aberrations in mitochondrial DNA, and the cell tried to compensate for this by increasing the synthesis of nuclear factors that upregulate mitochondrial gene expression. Whether this decrease in expression ultimately impinges upon mitochondrial oxidative phosphorylation has not been determined. Studies have not been performed in humans to determine whether folate status affects mitochondrial DNA or mitochondrial function, yet there is every indication that folate may play an important role in mitochondrial health.

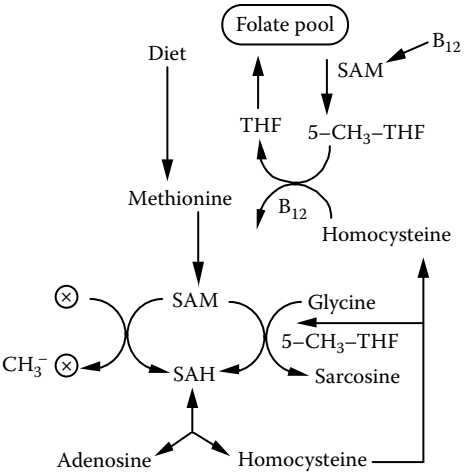


FIGURE 10.36 Involvement of B_{12} in methyl group transfer via *S*-adenylation (SAM).

TABLE 10.10
Choline and related metabolites

As methyl donor:
Choline
Methionine
Folacin
Betaine
As choline metabolite:
Choline, acetylcholine
Phosphorylcholine
Betaine
Phosphatidylcholine (lecithin)
Lysophosphatidylcholine (lysolecithin)
Sphingomyelin

Need

The latest DRIs are given in Table 10.1. However, as mentioned in the preceding section, women contemplating pregnancy should increase their intake twofold. There are racial and ethnic differences in the usual folic acid intake, and it has been suggested that despite food fortification every woman of child-bearing age should take a folic acid supplement. Because folate is not toxic, this is probably a good idea, with the caveat that B_{12} status is normal.[89,90] If this is not the case, excess folate could mask a B_{12} deficiency somewhat until the irreversible neurologic features of B_{12} deficiency appear. Excess folate intake leads to specific and significant downregulation of intestinal and renal folate uptake.[91]

VITAMIN B_{12}

Vitamin B_{12} is one of the most recently discovered vitamins yet it is the most potent.[92] Very little is required to prevent the symptoms of pernicious anemia and subsequent neurological change. It was isolated in 1948 and shortly thereafter shown to be the required substance needed to prevent pernicious anemia.

Structure, Physical, and Chemical Properties

Vitamin B_{12} is a very complex structure as shown in Figure 10.37. The term B_{12} is the generic descriptor for all corrinoids, those compounds having a corrin ring. Cyanocobalamin is the trivial designation for this compound. In order to have vitamin activity it must contain a cobalt centered corrin ring. Below the ring it may have a side chain heterocyclic nitrogen or a solvent or may have nothing attached here. Above the ring it may have a hydroxo, aqua, methyl, 5-deoxyadenosyl, CN^-, Cl^-, Br, nitro, sulfito or sulfato group. There are a number of structural analogs that have vitamin activity. Regardless of the substituents present above or below the cobalt centered corrin ring, unless the ring is present, there will be no vitamin function. The ring consists of four reduced pyrrole rings linked by three methylene bridges and one direct bond. The cobalt atom is in the 3^+ state and can form up to 6 coordinate bonds. It is tightly bound to the four pyrrole N atoms and can also bond a nucleotide and a small ligand below and above the ring, respectively. Commercially viable synthesis of this compound is very difficult although one such process has been developed using two moles of 5-aminolevulinate to form porphobelinogen, a pyrrole ring with an aminomethyl group on C-2, an acetate group on C-3 and a proprionate on C-4. Four of these are linked together to form hydroxymethylbelane, which in turn cyclizes and then the cobalt atom is added to form the vitamin. Vitamin B_{12} has a molecular weight of 1355.4 and is moderately soluble in water (12.5 mg/mL). It is insoluble

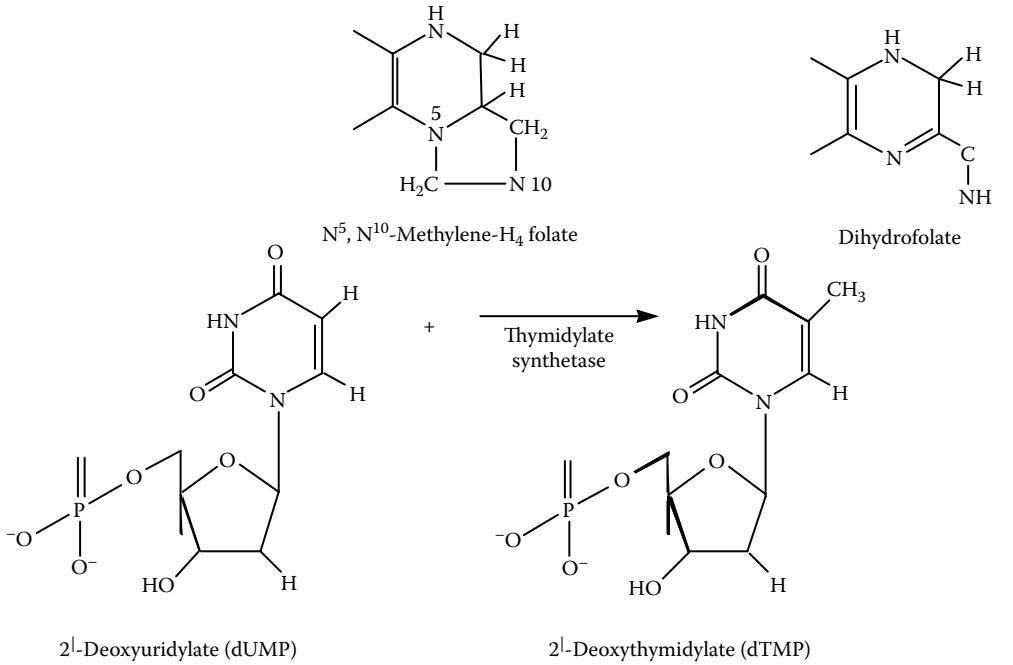


FIGURE 10.37 Addition of methyl groups to deoxyuridylate using the methyl group transfer function of folate.

in fat solvents. Its absorption maxima occurs at 278, 361, and 550 nm. It is a heat stable red crystal but will decompose at temperatures above 210°C. The crystal will melt at temperatures above 300°C. It is unstable to ultraviolet light, acid conditions, and the presence of metals such as iron and copper. Vitamin B_{12} is very difficult to assay. It is primarily the product of microbial synthesis and thus is not usually present in large amounts in most foods. Organ meats are good sources of B_{12}. It is synthesized in the gastrointestinal system by the resident flora. At this time, the best assay system is one which uses B_{12}-dependent microorganisms. The most responsive and specific of these is *Ochromonas malhamensis*. Although sensitive to small amounts of the vitamin these procedures are tedious. Spectrophotometric assays that can detect as little as 25 μg/mL are also available but not practical because their sensitivity is so limited.

Absorption and Metabolism

Most mammals depend on a very complex system to extract vitamin B_{12} and absorb it.[93-95] The process of absorption begins in the stomach where preformed B_{12} is bound to a carrier protein called intrinsic factor.[94] As B_{12} is made by the gut flora it too is bound to a carrier protein.[95] Whether this carrier is identical to that available in the stomach is not known. It probably is. Likely, future research will show that the synthesis of this carrier is directed by the vitamin in a manner analogous to that of retinol and the retinol receptor protein (see Chapter 9). Actually, there are four structurally distinct B_{12} carrier proteins. Intrinsic factor is one of these and another, called R binder, is found in the proximal part of the alimentary tract. R binder is degraded by the pancreatic peptidases and proteases, whereas the intrinsic factor-B_{12} complex proceeds intact to the distal portion of the ilium where in the presence of calcium and neutral pH, the complex binds to a receptor (called IF) on the surface of the luminal epithelial cell. Subsequent to binding of the complex to IF, the vitamin appears in the portal blood bound to a fourth protein called transcobalamin II or TCII. The blood contains an additional R-type protein called transcobalamin I (TCI) which assists in the transport of the vitamin to its target cells. Although absorption occurs mainly in the distal ilium, it also occurs in the large intestine.[95] This takes advantage of the fact that B_{12} is synthesized by the intestinal flora in this part of the intestinal tract.[96] The mechanism of absorption by the ileum likely is an active one mediated by the intrinsic factor; however, the details of this mechanism are unknown except for the need for the carriers as described above. Similarly, the transport of B_{12} through the enteral cell likely involves these carriers and probably involves divalent ions. Calcium in particular is needed for the attachment of the intrinsic factor-B_{12} complex to its cognate receptor on the enteral cell plasma membrane. Likely other factors are also involved; persons with a variety of diseases such as pancreatitis, tropical sprue, fluoroacetate poisoning, and pancreatic insufficiency do not absorb B_{12} efficiently and often show signs of pernicious anemia until provided with oral B_{12} supplements or injected with B_{12}. Absorption by the large intestine likely occurs via passive diffusion.

Once absorbed, B_{12} is transported in the blood bound to one of three transport proteins: transcobalamin I, II, or III. Small amounts are stored (as methylcobalamin) in the liver, kidney, heart, spleen, and brain. Thus, if an individual lacks intrinsic factor due perhaps to a genetic disease or surgical loss of the stomach (gastrectomy), or, as described above, has one of more diseases that affect B_{12} absorption, an injection of B_{12} can be given once a month (rather than daily) and this will correct the problem of inadequate supply.

Function

Vitamin B_{12} functions in one of two ways: (1) It participates as a coenzyme in reactions that utilize 5′-deoxyadenosine linked covalently to the cobalt atom (adenosylcobalamin). (2) It participates as a coenzyme in reactions that utilize the attachment of a methyl group to the central cobalt atom (methylcobalamin). The conversion of cobalamin to methylcobalamin is catalyzed by the enzyme, B_{12} coenzyme synthetase. It catalyzes the reduction of the molecule then catalyzes the reaction with

deoxyadenosyl mostly derived from ATP. In addition to ATP, this reaction needs a diol or a dithiol group, a reduced flavin or a reduced ferredoxin as the biological alkylating agent. The enzymes requiring B_{12} as a coenzyme are listed in Table 10.11. The first of these is required for thymidine synthesis. This reaction removes a methyl group from methyl folate and delivers it to homocysteine which, in turn, delivers it to deoxyuridylate converting it to thymidine. Thus, nucleic acid synthesis is arrested because it is dependent on adequate B_{12} intake. This process is also dependent on folacin. B_{12} deficient animals show decreased methylation of tetrahydrofolate and decreased cellular folate levels despite dietary folate sufficiency. One of the characteristics of the B_{12} deficient state is an anemia characterized by few mature red cells. Immature nucleated cells (megaloblasts) can be found but not the mature ones. Among the other enzymes listed in Table 10.11 is methylmalonyl CoA mutase, which participates in propionate metabolism. The overall reaction using B_{12} as a coenzyme in proprionate metabolism is shown in Figure 10.38.

Proprionate metabolism although a minor pathway in monogastric animals is of some importance in neural tissue.[97] Loss of this metabolic activity may explain the peripheral neural loss that characterizes long term B_{12} deficiency.

Methylmalonic aciduria characterizes the B_{12} deficient individual. Studies with rats made B_{12} deficient show an increase in odd numbered fatty acids in the neural and hepatic lipids and low hepatic methylmalonyl CoA mutase activity. Replacement of B_{12} in the diet corrects both these responses.[98] The effect of B_{12} on mutase activity is such that it is likely that the vitamin serves not only as the coenzyme in the reaction but also serves a role in the synthesis of that enzyme. Several reports of B_{12} activity vis à vis protein synthesis have appeared in the literature in addition to the one concerning the synthesis of the mutase. Whether this relates to the role of B_{12} in DNA and RNA synthesis, i.e., the synthesis of pyrimidines and purines, or whether a B_{12}-protein complex acts as a *cis*- or *trans*-acting factor in the pathway for the expression of the specific genes for these enzymes is unknown. Methionine synthetase is another of the cobalamin dependent enzymes.[99] Severe inhibition of this enzyme in humans results in megaloblastic anemia and eventually in subacute combined degeneration of the spinal cord. It also results in an abnormal distribution of folate and folate derivatives. Epidermal growth factor appears to serve as a local mediator of the neurotrophic action of cobalamin in the rat CNS and likely in the human.[100]

TABLE 10.11
Enzymes requiring B_{12} as a coenzyme

N^5-methyltetrahydrofolate homocysteine methyltransferase
Acetate synthetase
Glutamate mutase
Methylmalonyl-CoA mutase
α-Methyleneglutarate mutase
Dioldehydrase a
Dioldehydrase b
Glyceroldehydrase
Ethanolamine ammonia-lyase
L-β-lysine mutase
D-α-lysine mutase
Ornithine mutase
L-β-leucine aminomutase

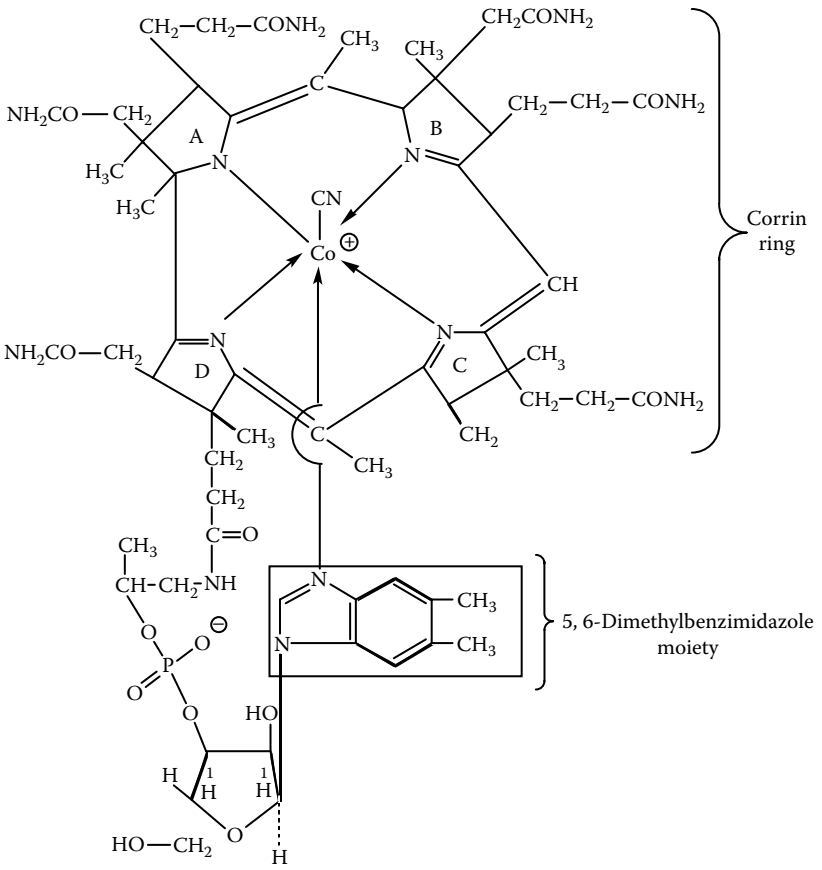


FIGURE 10.38 Structure of vitamin B_{12}.

Deficiency

As described in the preceding section, the deficiency state has as its main characteristic, pernicious anemia. Although inadequate B_{12} intake can result in this anemia, this is a rather unusual nutritional state because most foods of animal origin contain B_{12} and so little is needed. More common as a cause of pernicious anemia is a genetically determined deficiency of intrinsic factor. This trait is inherited as an autosomal dominant trait and occurs in about 1 in 1000. It can be treated with monthly B_{12} injections (~60–100 μg/dose). In the absence of this trait, the people most at risk for pernicious anemia are those who abstain from eating foods of animal origin. In addition to these are those who have had one of the illnesses described earlier that impair absorption. Humans who have had a gastrectomy or some disease of the gastric mucosa or some disease resulting in malabsorption are in this category.

Following the development of pernicious anemia (which is reversible) is the irreversible loss of peripheral sensation. This is due to the degenerative changes in these nerves including demyelination or loss of the lipid protective coat that surrounds the nerve tracts. Once the myelin is lost the nerve dies. Neural loss begins in the feet and hands and progresses upward to the major nerve trunks such that a progressive neuropathy can be followed. Because both folate and B_{12} are interactively involved in DNA and RNA synthesis it used to be difficult to segregate one deficiency anemia from

the other. However, given the presence of methylmalonicaciduria and differential analysis of the red cell, one can determine the cause of the anemia. In addition to folacin and B_{12} deficient intakes of iron, copper, and zinc can also explain anemia (see Chapter 12).

Need

Daily requirements for B_{12} are very small. The normal turnover rate is about 2.5 μg/day thus the recommendation for adults is close to this turnover rate or 2 μg/day. Table 10.1 gives the DRI for humans of different ages. The need for B_{12} is also related to the intake of ascorbic acid, thiamin, carnitine, and fermentable fiber.[101-104] Each of these nutrients affect the production of proprionate and in their absence or relative deficiency proprionate production is increased and this, in turn, drives up the need for B_{12}. As already mentioned, the needs for B_{12} and folate are related. Compromised cobalamin status during pregnancy may put both mother and child at risk for further deterioration of vitamin B_{12} status during lactation compromising the growth and development of the infant.[105]

CARNITINE

As is the case with choline and inositol, carnitine can be synthesized in the body in amounts usually sufficient to meet needs. Thus, carnitine is not considered an essential nutrient. However, just as there may be instances where choline or inositol must be provided from external sources, this is also true for carnitine. Interest in the essentiality of carnitine was stimulated by Broquist and colleagues who showed that carnitine was synthesized from lysine, an amino acid frequently in short supply in third world malnourished patients. Follow up work by Borum[106] showed that the premature infant could not synthesize sufficient carnitine to meet the needs for growth and normal metabolism. These reports thus stimulated the consideration of carnitine as an essential nutrient.

Structure, Physical, and Chemical Properties

Carnitine is a quaternary amine, β-hydroxy-γ-*N*-trimethyl aminobutyric acid. Its structure is shown in Figure 10.39. It is very hygroscopic with a molecular weight of 161.2. As mentioned, it is synthesized in the body (primarily the liver) from lysine. This pathway is shown in Figure 10.40. There are a number of essential nutrients involved: niacin as part of NAD, iron in the ferrous state, ascorbate to keep iron in its ferrous state and of course lysine together with methionine as a methyl donor. As long as the diet provides these nutrients carnitine will be synthesized to meet the needs of the normal individual. There are instances, however, where despite the provision of these essential nutrients carnitine synthesis does not take place to the extent that is needed.

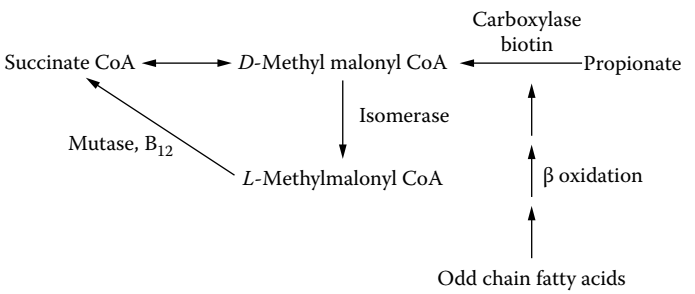


FIGURE 10.39 Overall reaction in the conversion of proprionate to succinate using biotin and vitamin B_{12} as coenzymes.

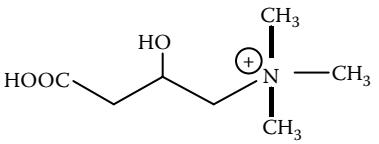


FIGURE 10.40 Structure of carnitine.

In this instance, carnitine becomes an essential nutrient. This occurs in the premature infant. Due to its prematurity its biochemical pathways, especially that for carnitine synthesis, are not well developed. In another instance the need for carnitine exceeds endogenous synthesis. This occurs in the severely traumatized individual. The whole-body response to trauma involves a catecholamine-glucocorticoid response that greatly increases lipolysis and fatty acid oxidation. This drives up the need for carnitine and in this situation endogenous synthesis is inadequate.

Sources

The techniques for food carnitine analysis are not well developed. However, despite the inadequacies of the methodology, it is safe to indicate that good sources of carnitine include red meats and some organ meats. Milk, whole grains and some vegetables (i.e. spinach, cauliflower, avocado and peanuts) contain modest amounts.

Absorption and Metabolism

Carnitine is absorbed via an active process involving sodium and a carrier. Carnitine in large amounts is also absorbed by passive diffusion. Concurrent with its absorption is its acetylation. It is transported in both the free and acetylated form to the muscles where 90% of the total body carnitine may be found. The turnover of carnitine is quite slow. Although needed for fatty acid oxidation particularly by the working muscle, it is continuously recycled rather than degraded and excreted. The kidney plays an important role in carnitine conservation in that 90% of the carnitine that arrives at the kidney is reabsorbed by the glomerulus and returned to the circulation. In instances of kidney failure this conservation is lost and again we have a situation where exogenous carnitine must be supplied. A small amount of acylcarnitine ester may be found in the urine of normal subjects. This probably represents less than 1% of the total body carnitine pool.

Function

Carnitine serves as part of the carnitine acyl transferase system located in the mitochondrial membrane. This system is shown in Figure 10.41. There are two transferases involved: carnitine acyl transferase I located on the outer side of the inner mitochondrial membrane and carnitine acyl transferase II located on the matrix side of the membrane. These enzymes catalyze the synthesis and hydrolysis of the fatty acyl carnitine esters as well as work with the transporter protein (acetyl translocase) that catalyze the movement of the fatty acids into the mitochondrial matrix. There is no other known function for carnitine.

Deficiency

Low levels of carnitine in tissues and blood typify the carnitine deficient individual in addition to hyperlipidemia, cardiomyopathy, and muscle spasm.

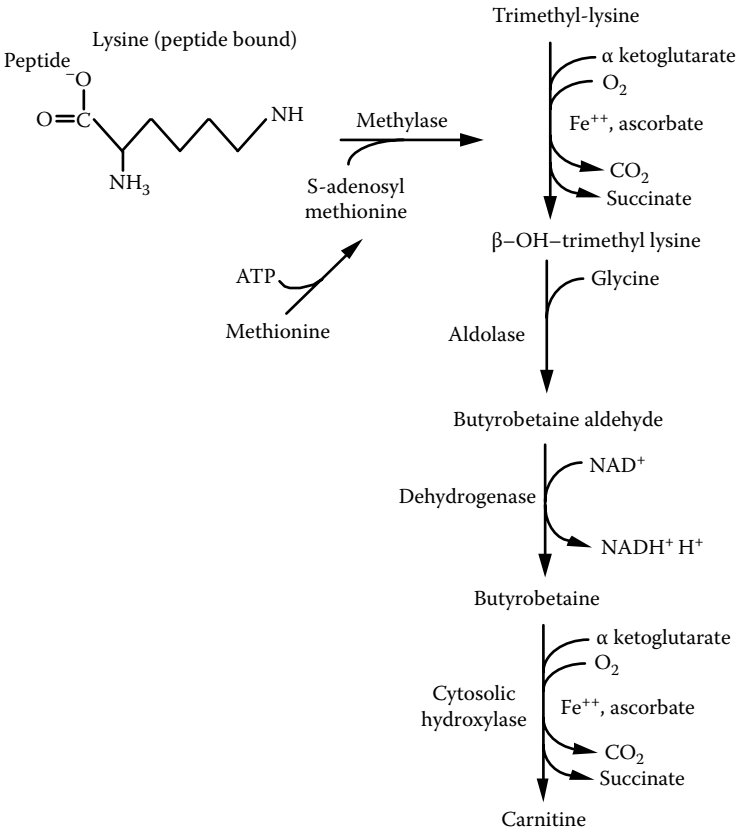


FIGURE 10.41 Synthesis of carnitine.

Because carnitine can be synthesized in the body no recommended intake levels have been set for normal children and adults.[107] Work continues on developing intake recommendations for preterm infants and others with special needs.

CHOLINE

Although many nutrition scientists have presumed that the list of required vitamins is complete, others would argue that there are certain circumstances where a dietary supply of a compound is essential for the support of normal metabolism. Choline is one of these compounds. In rats given a choline deficient diet, one can observe a fatty liver as well as certain CNS deficits. Human cells grown in culture also have an absolute requirement for choline and humans sustained by choline free parenteral solutions develop symptoms similar to those of the deficient rat. It is on this basis that the inclusion of choline in a list of essential micronutrients is argued.[108,109]

Structure, Physical, and Chemical Properties

Choline is the trivial name for 2-hydroxy-*N,N,N*-trimethyl-ethanaminium. The structure for this compound is shown in Figure 10.42. Choline is freely soluble in water and ethanol but insoluble in such organic solvents as ether or chloroform. It is extremely hygroscopic. It is a strong base and readily decomposes in alkaline solutions, resulting in the production of trimethylamine. Because of

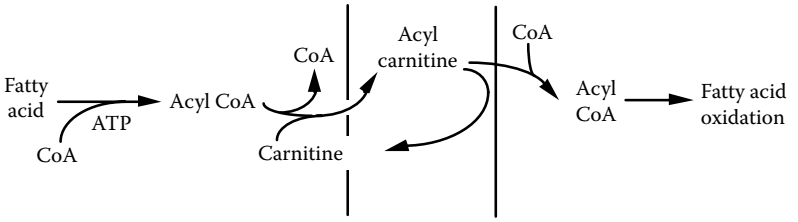


FIGURE 10.42 The carnitine acyl transferase system.

TABLE 10.12
Metabolic reactions in which folate plays a role as a coenzyme

Enzyme	Role
Thymidylate synthetase	Transfers formaldehyde to C-5 of dUMP to form a dTMP in pyrimidine synthesis.
Glycinamide ribonucleotide transformylase	Donates formate in purine synthesis.
5,5-amino-4-imidazolecarboxamide transformylase	Donates formate in purine synthesis.
Serine hydroxymethyl transferase	Accepts formaldehyde in serine catabolism.
10-formyl-FH_4 synthetase	Accepts formaldehyde from tryptophan catabolism.
10-formyl-FH_4 dehydrogenase	Transfers formate for oxidation to CO_2 in histidine catabolism.
Methionine synthase	Donates methyl group to homocysteine to form methionine.
Formiminotransferase	Accepts formimino group from histidine.

its unique structure, choline serves as a donor of methyl groups. It has a molecular weight of 121.2 and belongs to a class of compounds that function either as methyl donors or as membrane constituents. Related compounds are listed in Table 10.12. Because of its instability, the determination of choline in food and biological tissues is fraught with difficulty. Commonly used is the reineckate method, which involves the precipitation of choline as a reineckate salt and the development of a characteristic color. Unfortunately, this method lacks the sensitivity and specificity provided by newer chromatographic and isotopic methods that are combined with rapid inactivation, via microwave, of choline degradative enzymes. Work is ongoing for the development of sensitive and specific methods for choline assay.

Sources

Choline is widely distributed in foods and is consumed mainly in the form of lecithin (phosphatidylcholine). Lecithin is not only a naturally occurring common food ingredient but is also a common additive to processed foods. It serves as a good food stabilizer and emulsifying agent. Choline chloride and choline bitartrate are added to infant formula to assure equivalency to breast milk, which contains 7 mg/100 kcal (7 mg/420 kJ); 7 mg is about 50 μmol. Recent assessments of commercial infant formulas, however, showed less choline in the preparation than given on the label. Choline content in this report ranged from 100–647 μmol. In part this discrepancy may be due to the lability of choline once in a solution (the infant formula) that is mildly alkaline and, in part, due to the relative difficulty in assessing choline content accurately and sensitively.

Absorption and Metabolism

There is a good bit of difficulty in assessing choline absorption. Currently, it is believed to be absorbed via a sodium-dependent carrier-mediated mechanism. If large amounts of choline are consumed, uptake of the excess is by passive diffusion. One study using labeled choline showed that about 65% of the dose was found in the urine as trimethylamine within 12 hours of ingestion. When labeled choline was incorporated into lecithin and ingested, significantly less of the label was recovered in the urine as trimethylamine. About 50% of the ingested labeled lecithin entered the thoracic duct intact. The use of antibiotics to reduce the population of gut flora reduced the loss of trimethylamine in the urine. This showed that one of the major degradative steps in the loss of choline is through the action of the gut flora. The route of choline and lecithin metabolism and degradation is shown in Figure 10.43. In addition to the action of the gut flora, phosphatidylcholine is subject to enzymatic degradation. Phospholipase A_1, A_2, and B catalyze the ester bonds that link the fatty acids at the glycerol carbons 1 and 2 resulting in free fatty acids and glycerophosphocholine. Most of the lecithin that is ingested has only one of its fatty acids removed prior to absorption. Sphingomyelin, a related complex lipid containing choline, is not degraded at all in the intestinal lumen. All of the phospholipids are transported into the lymphatic circulation from the gut and appear in the plasma lipoproteins. All classes (high-density lipoproteins, low-density lipoproteins, very low-density lipoproteins and chylomicrons) contain phosphatidylcholine. The chylomicrons are the major carriers from the gut but once into the blood the phosphatidylcholine is redistributed among the lipoprotein classes. From the blood it is then taken up by all cell types following the lipase action and resynthesis pattern used by the transport and uptake of the triacylglycerides.

Most species can synthesize choline using methionine as the methyl donor. This synthesis is shown in Figure 10.44. Methionine is converted to SAM. The methyl groups are then joined to form choline. The de novo synthesis of phosphatidylcholine usually occurs by using phosphatidylethanolamine, another phospholipid, as the starting material. Aside from the diet, this conversion of phosphatidylethanolamine to phosphatidylcholine is the only source of de novo synthesized choline in the mammalian body. This synthesis is outlined in Figure 10.45. Also shown in Figure 10.45 is the interconversion, through base exchange, of other phospholipids to phosphatidylcholine.

Function

Choline serves as a precursor for the neurotransmitter, acetylcholine. The intake of choline can affect brain levels of acetylcholine, and this may be a benefit to patients showing acetylcholine deficits as in tardive dyskinesia. Some benefit is also achieved with people having short-term memory loss as in Alzheimer's disease. In addition, choline plays an important role in fetal neuronal development. Dietary choline supplementation of rat diets enhances memory performance and increases the hippocampal phospholipase D activity in the offspring.[110,111]

As important as the synthesis of acetylcholine, is the synthesis of the membrane phospholipid, phosphatidylcholine. This phospholipid is an important structural element of the membrane and, depending on the chain length and saturation of the fatty acids attached to carbons 1 and 2, contributes to the degree of fluidity of that membrane. The consideration of fluidity is important to the

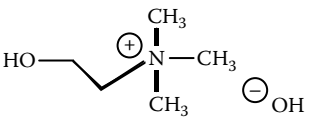


FIGURE 10.43 Structure of choline.

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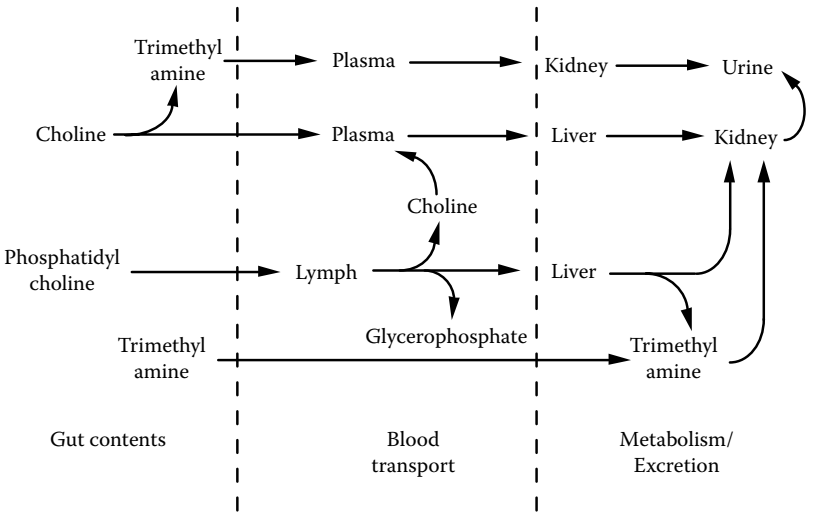


FIGURE 10.44 Absorption and metabolism of choline.

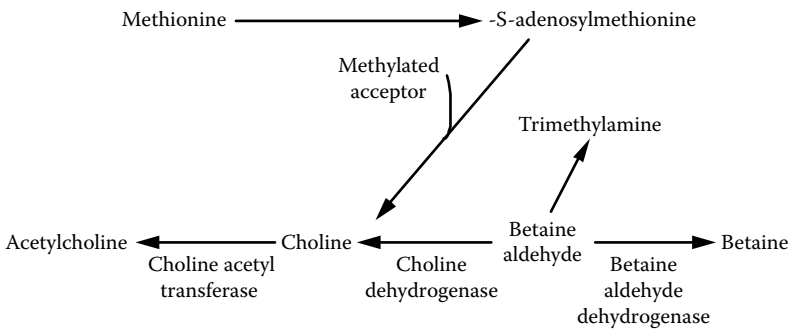


FIGURE 10.45 Use of methionine in the synthesis of choline and subsequent use of choline in betaine and acetylcholine synthesis.

function of the membrane-embedded proteins. Many of these proteins change shape as part of their action, and the fluidity of the surrounding lipid determines the ease with which they can do this.

Phosphatidylcholine functions in the transport of lipids not only as part of the lipoproteins but also in the transmembrane lipid transport system. In this role, phosphatidylcholine serves as a lipotrope. It also plays a role in cell signaling.[109]

Lastly, because choline serves as a precursor of betaine, it acts as a methyl donor in the 1-carbon metabolic pathways. These pathways include the formation of methionine from homocysteine and the formation of creatine from guanidoacetic acid.

Deficiency

Because choline can be synthesized in the body and because it is universally present in our food supply, a true deficiency in normal humans is rare indeed. Choline deficiency can occur in poultry

fed a low choline diet, an individual deficient in methionine, and/or methyl donors. Depressed growth, fatty liver, and hemorrhagic renal disease have been reported to occur in deficient animals of a number of species. Choline deficiency induces apoptosis in SV40-immortalized CWSV-1 rat hepatocytes in culture, and it is possible that choline might have this effect in vivo.[112] However, this has not been shown. Of interest is an early report of an effect of choline deficiency on body carnitine pools.[113] A 50% reduction has been reported, and due to the importance of carnitine in fatty acid oxidation, this relationship may explain the fatty liver of deficient animals.

Need

There are suggested intakes (AI) for choline (Table 10.1). Rebouche has outlined these needs.[107]

INOSITOL

Until fairly recently, little attention has been paid to the role of inositol in the diet. This has occurred despite the recognition that dietary inositol has been shown to prevent the development of a fatty liver in rats and to cure alopecia in rats and mice, and despite a report made over a 100 years ago that diabetic humans excreted large quantities of this substance in the urine. Inositol is an essential part of every cell.[114] It is a key ingredient for one of the membrane lipids, phosphatidylinositol, and it is critical to the inositol signaling system (the phosphatidyl inositol system) that plays a key role in the regulation of hormone release, and cellular response to a variety of signals.[115–118] It is not considered an essential nutrient for humans.

Structure, Physical, and Chemical Properties

Inositol is a 6-carbon sugar that is configuratively related to D-glucose. Its structure is shown in Figure 10.46. It occurs in nature in nine possible isomeric forms. However, only one, myoinositol, is biologically important as a nutrient. Myoinositol is a water-soluble, cyclic, 6- carbon compound (*cis*-1,2,3,5 *trans*-4,6-cyclohexane-hexanol). Inositol is widely distributed in foods of both plant and animal origin. In plants and animals it exists as part of the phosphatidylinositol (PI) of the cell membranes or as free inositol. Phytic acid, a component of many grain products, can be converted to myoinositol with the removal of the phosphate groups (Figure 10.47). Phytate or phytic acid can bind calcium, magnesium, and other divalent ions[119] within the intestinal compartment, making them unavailable for absorption by mucosal cells. Once the phytate is dephosphorylated through the action of phytase, the inositol residue remains. The divalent ions are released, and the free inositol is absorbed. Both free inositol and cell membrane phosphatidylinositol are found in foods of animal origin.

Absorption and Metabolism

Dietary phosphatidylinositol is acted on by the luminal enzyme, phospholipase, and converted to lysophosphatidylinositol. This compound can then be further hydrolyzed to produce glycerophosphorylinositol and then free inositol or acted upon by an acyltransferase in the intestinal cell, which converts it back to phosphatidylinositol. This is then transported out of the gut absorptive cell as a component of the lipoproteins.

Free inositol in the lumen is transported into the luminal cells via an active, energy-dependent, sodium-dependent transport process quite similar to that which transports glucose. Although similar it is not identical to it. Free inositol is then transported in the blood at a concentration of about 300 μM.

As mentioned, inositol can be synthesized from glucose by a variety of mammalian cells. Synthesis in the testes, brain, kidney, and liver has been reported. Humans can synthesize up to 4 grams/day in the kidneys alone. Synthesis from glucose proceeds from glucose to glucose-6-

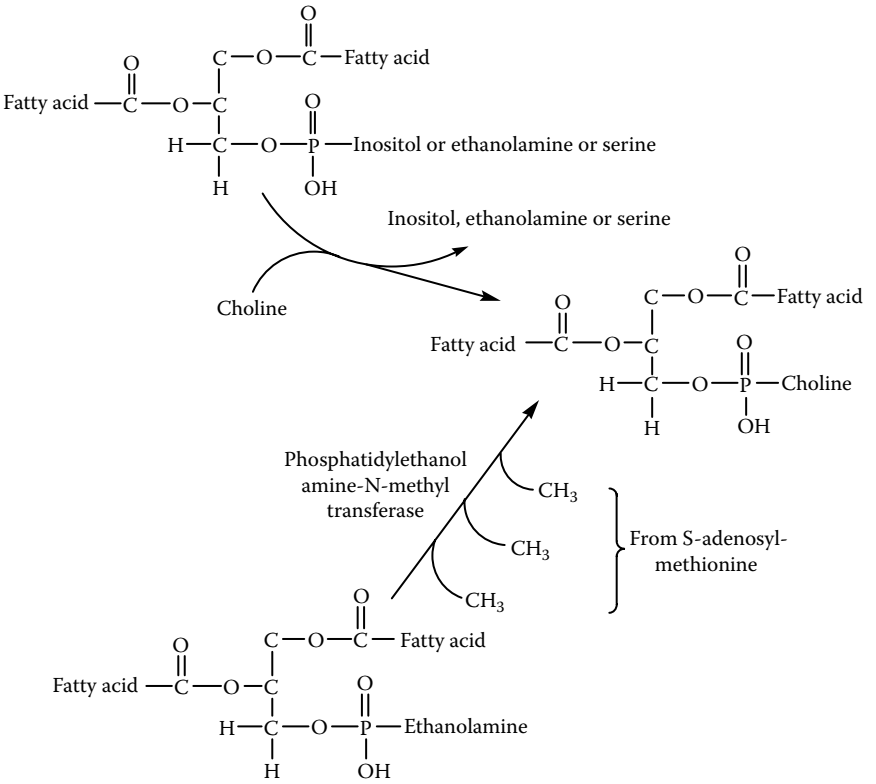


FIGURE 10.46 Biosynthesis of phosphatidylcholine.

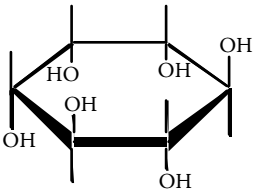


FIGURE 10.47 Myoinositol structure.

phosphate to inositol-1-phosphate to inositol. The enzymes are glucokinase or hexokinase, followed by inositol-1-synthetase and then NAD-inositol-dephosphorylase. Figure 10.47 illustrates the pathway for the production of inositol from glucose and also from phytate.

Function

Inositol functions as a constituent of the membrane phospholipid, phosphatidylinositol. Free inositol is added to diacylglycerol via a CDP reaction, producing phosphatidylinositol and CMP. The enzyme catalyzing this reaction is CDP diacylglycerol:inositol phosphatidyltransferase, sometimes called phosphatidylinositol synthetase. This synthesis takes place in the microsomes, and the

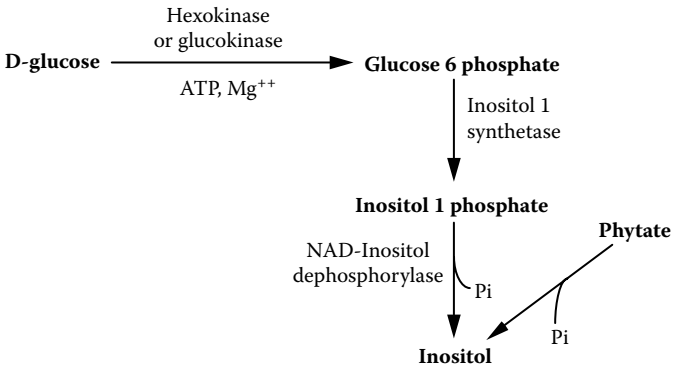


FIGURE 10.48 Synthesis of free inositol from either glucose or phytate. The conversion of glucose to inositol is an insulin-dependent pathway, whereas the dephosphorylation of phytate is not.

enzyme has a Km of 4.6 mM for inositol. This reaction is illustrated in Figure 10.48. Phosphatidylinositol can also be synthesized via an exchange reaction where free inositol can exchange for either choline or ethanolamine in either phosphatidylcholine or phosphatidylethanolamine. The Km for the Mn^{++}-dependent reaction is 0.024 mM. Once formed, the phosphatidylinositol migrates from the microsomes where it is formed, to any one of the membranes within and around the cell. It comprises approximately 10% of the phospholipids in the cellular membranes. Recently, its function as a part of a unique cellular second messenger system (different from the cyclic AMP system) has been explored. This system, called the PIP system or PIP cycle, has been reported to function in insulin release by the pancreatic insulin-producing β cells,[115–118] in the regulation of protein kinase C, in the mobilization of intracellular calcium, in the regulation of Na^+K^+ ATPase activity, and may have a role in blood clotting, blood pressure regulation, and renal function.

Once formed, phosphatidylinositol serves as a substrate for one of several enzymes. These reactions are shown in Figure 10.48. Phospholipase A_1 acts on phosphatidylinositol to produce lyso PI (2 acyl PI). Phospholipase A_2 acts to produce a 1 acyl PI. Both A_1 and A_2 act to remove one of the fatty acids from the phospholipids; A_1removes the fatty acid (usually stearic acid, 18:0) from the glycerol carbon 1, whereas A_2 removes the fatty acid (usually arachidonic acid, 20:4) from the glycerol carbon 2. When phospholipase A_2 is activated, arachidonic acid is released, and this fatty acid serves as the substrate for prostaglandin synthesis. Prostaglandins are another group of hormone-like substances that are important in the regulation of blood pressure and blood clotting. A third enzyme also has phosphatidylinositol as its substrate. This enzyme is ATP PI kinase (phospholipase C) and initiates the PIP cycle also illustrated in Figure 10.49. Phospholipase C action is mediated by the guanine-nucleotide-binding protein called the G protein. Phospholipase C cleaves the phosphorylated inositol from the glycerol backbone, producing diacylglycerol (DAG) and phosphatidylinositol 4,5 phosphate (PIP_2). DAG serves to activate protein kinase C, an important regulatory enzyme discovered in 1979. Neutral DAG remains within the membrane, whereas the liberated inositol 4,5 bisphosphate migrates into the cytoplasm and, in the process, is again phosphorylated to form inositol 1,4,5 phosphate (PIP_3). This compound causes a release of calcium ion from nonmitochondrial vesicular, intracellular stores. The triphosphate inositol binds to a receptor protein associated with these stores to effect this release. Ca^{++} release from the endoplasmic reticulum is elicited via an opening of a gated channel. Cyclic-AMP-dependent phosphorylation of the receptor protein seems to be involved. The magnesium ion is also involved. Inositol 1,4,5 phosphate can then either be dephosphorylated to release free inositol or be phosphorylated once again to form inositol

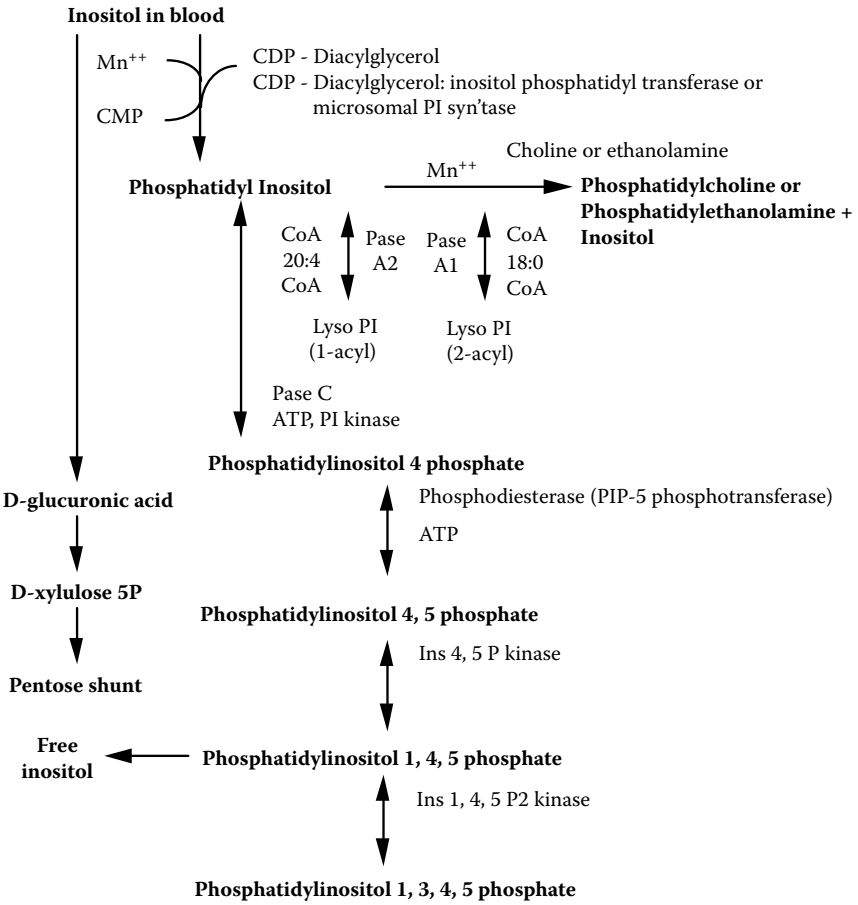


FIGURE 10.49 Inositol metabolism and the PI cycle.

1,3,4,5 phosphate (PIP_4) via the enzyme D-myo-inositol 1,4,5 triphosphate 3-kinase. This kinase is stimulated by a Ca^{++} in the presence of calmodulin and protein kinase C, and thus the level of the inositol 1,4,5 phosphate is carefully regulated. Inositol 1,3,4,5 phosphate also is an active metabolic regulator in that it modulates calcium ion through either the reuptake of Ca^{++} into the intracellular stores or through control of the Ca^{++} transfer process between inositol 1,4,5 phosphate sensitive and insensitive pools. The kinase enzyme might be the target for the enzyme protein tyrosine kinase. All of these phosphorylations of inositol are reversible, and the amounts of each of the phosphorylated intermediates depends on the hormonal status of the individual as well as on the availability of inositol for phosphatidylinositol synthesis. Insulin, various growth factors, and PGF2α (one of the prostaglandins) have all been shown to stimulate the phosphatidylinositol cycle. Of particular interest are the reports that inositol turnover is increased in acute diabetes. Other observations on the relationship of diabetes to inositol status include the following:

Antiinsulin antiserum treatment of the diabetic neutralizes the effect of the exogenous insulin on inositol turnover.

Insulin treatment of acute insulin deficiency reverses the effect of diabetes on inositol turnover.

Insulin increases the synthesis of phosphatidylinositol, DAG and protein kinase C activity in a variety of cells from normal animals.

All of these findings suggest that there may be circumstances where the synthesis of the inositol by the body might be inadequate to meet the body's need and that this substance must be provided in the diet. Under these circumstances inositol becomes an essential nutrient. One of these circumstances is the disease diabetes mellitus.

Diabetes, regardless of whether it is insulin dependent or noninsulin dependent, is characterized by a failure to appropriately regulate blood glucose levels. With high blood levels of glucose the sorbitol pathway is stimulated.[120] If this pathway is stimulated, endogenous synthesis of myoinositol is reduced.[114] Further, cellular uptake of glucose is impaired in the diabetic state. If cellular glucose uptake is impaired, less inositol is synthesized from glucose and less is available for phosphatidylinositol synthesis.[121] Hence, the diabetic excretes more inositol in the urine while having an intracellular deficiency because of inadequate endogenous synthesis. In turn, this means that more preformed inositol should be provided to the body via the diet. Thus, the diabetic may have a significantly greater requirement for inositol than the nondiabetic. Indeed, there may be as broad a range in inositol requirements as there is the range of severity of diabetes. Recently, there have been reports in the medical literature that have suggested that the secondary complications of diabetes (e.g., renal disease) could be ameliorated by dietary inositol supplementation. Some investigators have shown a reversal of the diabetes-induced increased glomerular filtration rate with a seven- to tenfold increase in dietary levels of myoinositol. At any rate, it would appear that diabetics have a larger than normal need for dietary inositol because: (1) they excrete more than do normal people; (2) when hyperglycemic, they synthesize less from glucose because less glucose is available inside the cell and because the first two steps in glucose metabolism are insulin dependent; (3) they absorb less from the diet, and (4) when hyperglycemic they have greater sorbitol production which, in turn, inhibits the pathway for inositol synthesis.

Deficiency

In normal humans, inositol needs are presumed to be met by endogenous synthesis, so detailed studies have not been performed using healthy volunteers. Indirect evidence of need has been reported sparsely in the medical literature but detailed controlled-feeding studies have not been conducted. Such studies have been performed in laboratory rats and mice. In these animals the most striking feature of the deficient state was the development of a fatty liver. This was reversed with dietary inositol supplements. Hair loss and poor growth were also reported for deficient animals. At the time these animal studies were conducted (1979–1980) the PIP cycle was not known. Researchers knew about the presence of phosphatidylinositol in the cell membrane, but they did not recognize its importance in the lipid signal transduction process. Studies on the PIP cycle in rats made inositol deficient have yet to be conducted.

Is inositol essential in the human diet? In normal people it probably is not, but in the diabetic, inositol may be an essential nutrient. As mentioned, the healthy human adult probably meets his or her daily need easily because of the ubiquitous presence of inositol in much of the food humans consume. Even in the event of inadequate intake, healthy humans can probably synthesize more than they need to maintain optimal health. However, just as there may be special circumstances that dictate an increased intake of other nutrients, so, too, can this occur with respect to inositol. This research arena is particularly active these days as we learn more about the PIP cycle and the nutritional implications of this signal system.

Need

Many nutrition scientists argue that inositol is not an essential nutrient because the body can synthesize it from glucose. However, as we become more aware of the genetic diversity of humans (as well as other species), we have come to realize that some individuals vary in their needs for the required nutrients. This is particularly true for those nutrient needs that are partially satisfied through endogenous synthesis. In this category are niacin, arginine, histidine, and choline. Individuals who can not synthesize these nutrients in the quantities they need must have a dietary supply. In addition, some individuals may excrete larger than normal amounts of the nutrient or catabolize it faster than usual. In all of these instances, the individual will require a far greater dietary supply of a given nutrient than other individuals of the same species. Thus, in normal adult populations, some individuals may have a far greater need for certain nutrients than others. It is for this reason that the DRIs are set up to accommodate the needs of a wide variety of people. A safety factor is built into the recommendation to allow for individual differences in need. There is no recommendation for inositol intake. As mentioned, there are several nutrients in the current list of required ones that can be synthesized in the body. Although these nutrients can be synthesized, they also must be provided in the diet because endogenous synthesis may be inadequate. Niacin, one of the B vitamins, is typical of this group of nutrients. It can be synthesized from tryptophane. Choline, carnitine, arginine, tyrosine, para amino benzoic acid, and vitamin D are others. Inositol can also be placed on this list. On top of these considerations is the greatly increased need for a nutrient to support growth. Growth can be so rapid that it outstrips endogenous synthesis. Such is the case with arginine. During growth it is an essential amino acid. At maturity it is not. Certain disease states can alter a person's need for and tolerance of nutrients. Hence, carnitine is an essential nutrient for the premature infant and the severely traumatized individual. Diseases such as diabetes can also affect nutrient needs. People with diabetes mellitus may have a need for inositol that exceeds their bodies' capacity to synthesize it. Research is needed to determine whether a nutrient recommendation should be developed for this compound.

OTHER COMPOUNDS THAT MAY OR MAY NOT BE VITAMINS

The bulk of the work identifying and describing compounds we now know as vitamins occurred in the first part of the 20th century. Recent work has suggested other compounds that may also be essential to the maintenance of normal metabolism. In this list are pyrroloquinoline quinone, ubiquinone, orotic acid, para amino benzoic acid, lipoic acid, and the bioflavinoids. Brief descriptions of these substances follow.

Pyrroloquinoline Quinone

Pyrroloquinoline quinone, sometimes called *ethoxatin*, serves as a cofactor in the reaction catalyzed by lysyl oxidase. Lysyl oxidase catalyzes the cross linking of collagen and elastin. It also requires copper as a cofactor.

Pyrroloquinoline quinone is a tricarboxylic acid with a fused heterocylic (*o*-quinone) ring system. Its C-5 carbonyl group is very reactive toward nucleophiles, and it is this action that allows this substance to function in collagen and elastin crosslinking. At present, information is scarce with respect to its food sources and essentiality. Likely it can be endogenously synthesized but, whether or not this compound meets the definition of a vitamin has yet to be established.

Ubiquinone

Ubiquinone is another substance whose vitamin status is unclear. It is an essential component of the mitochondrial respiratory chain, and it has been assumed to be synthesized endogenously. Actually,

ubiquinone is a group of related substances. They are a group of *tetra*-substituted 1,4-benzoquinone derivatives with isoprenoid side chains of various lengths. The biochemists have termed these substances as coenzyme Q. They function as reversible donors/acceptors of reducing equivalents from NAD, passing electrons from flavoproteins to the cytochromes via cytochrome b_5. Because the ubiquinones can be synthesized endogenously in large amounts even when diets lacking the ubiquinones are offered, these substances fail to meet the definition of the word "vitamin."

Orotic Acid

Orotic acid is an important metabolic intermediate in the synthesis of pyrimidines. It is synthesized endogenously from *N*-carbamylphosphate by dehydration and oxidation. It is another of those substances that fails to meet the definition of a vitamin. However, when used as a dietary supplement (0.1% of diet), it has resulted in a fatty, enlarged liver and increased the levels of hepatic uracil, presumably due to an influence on pyrimidine synthesis. Orotic-acid-induced fatty liver is accompanied by falling plasma cholesterol levels, and falling activity of HMG CoA reductase. This hepatic enzyme is greatly influenced by its product (cholesterol) and so if the liver does not export it, it feeds back to inhibit its synthesis.

Para Amino Benzoic Acid (PABA)

p-Amino benzoic acid is an essential growth factor for a number of bacteria that use it as a precursor of folacin. Animals, however, cannot synthesize folacin, so *p*-amino benzoic acid does not meet the definition of the word "vitamin." Its involvement in folacin use is discussed in the section on folacin.

Lipoic Acid

Lipoic acid is essential to oxidative decarboxylation of α-keto acids. It participates in pyruvate dehydrogenase complex. This is a multienzyme complex where lipoic acid is linked to the ε-amino group of a lysine residue from the enzyme dihydrolipoyl transacetylase. As lipoamide undergoes reversible acylation/deacylation, it transfers acyl groups to coenzyme A and results in reversible ring opening/closing in the oxidation of the α-keto acid. Lipoic acid can be synthesized endogenously in amounts sufficient to meet need. Therefore, it does not meet the definition of the word vitamin.

Bioflavinoids

Bioflavinoids are a group of compounds that augment the action of ascorbic acid in the prevention of scurvy. They are mixtures of phenolic derivatives of 2-phenyl-1,4-benzopyrone. The bioflavinoids are present in a large variety of foods and were first isolated by Szent–Gyorgy from lemon juice and red peppers. More than 800 different flavinoids have been found. They occur naturally as glycosides, which are hydrolyzed by the gut flora prior to absorption. No single unique deficiency syndrome has been found or reported in animals fed a bioflavinoid-free diet. Furthermore, there has not been a unique response to the addition of bioflavinoids to the diet. On this basis, despite its activity as a potentiator of ascorbic acid, the bioflavinoids cannot be considered vitamins.

PSEUDOVITAMINS

The term *vitamin* was coined many years ago to designate those organic compounds that are needed to sustain normal growth and metabolism, and which are needed in small amounts. This term developed by nutritional biochemists and physiologists has been used commercially as well as scientifically. Unfortunately, there have been (and continue to be) commercial uses of the term that are inappropriate. Hence we have compounds such as laetrile (an extract from fruit pits), pangamic

acid or vitamin B_{15}, and methylsulfonium salts of methionine called vitamin U and gerovital. Gerovital, also called vitamin H_3 or CH_3 is a buffered solution of procaine hydrochloride (Novacain), a local anesthetic. To be effective as an anesthetic it must be injected. Gerovital is advertised as an antiaging substance but claims of its effects have not been substantiated. The advertised use of methylsulfonic salts of methionine to prevent peptic ulcers likewise has not been substantiated. Pangamic acid, another of these pseudovitamins, is not a chemically defined substance. Rather it is a mixture of compounds. Of the materials labeled pangamic acid one of the compounds is *N,N*-diisopropylamine dichloroacetate. This is a drug and when administered to normal rats caused death preceded by respiratory failure, extreme hypotension, and hypothermia. There is no evidence of essentiality for pangamic acid.

Lastly, laetrile is included in this list of pseudovitamins. This compound has been the focus of a number of litigations due to the claim by its suppliers that it can serve as an anticancer drug. This claim was investigated and found wanting by the U.S. Food and Drug Administration (FDA). The term *laetrile* has several synonyms: amygdalin and vitamin B_{17}. Amygdalin is a β-cyanogenic glucoside and is a major constituent in preparations named laetrile. Amygdalin is a substance found in peach pits, apricot pits, and the kernels and seeds of many fruits. Neither the U.S. FDA or the Canadian equivalent of this regulatory agency recognize laetrile as a vitamin.

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11 Macrominerals

The term *macrominerals* refers to those elements needed by the body in milligram quantities on a daily basis. The category includes sodium, potassium, chloride, calcium, phosphorus, and magnesium. Although the body content of the first three is relatively small because of high turnover, the body content of calcium and phosphorous, by comparison, is relatively large. All of the macrominerals serve as electrolytes, and their critical use relates to this function. Most minerals are found in the bones and teeth and thus have a structural function, however, they also serve as metabolic regulators.

SODIUM

Sodium is the major extracellular electrolyte. It circulates as a fully dissociated ion due to its +1 charge. It is thus fully water soluble. It has been estimated that the adult body contains 52–60 mEq/kg (male) and 48–55 mEq/kg (female). One mEq/L equals one mmol/L. Thus, the average adult male would have about 83–97 g of sodium in his 70-kg body. Between 2/3 and 3/4 of this sodium is "fixed" in the mineral apatite of the bone. The remaining comprises a pool that undergoes considerable flux as it participates in a sodium–potassium exchange. The exchangeable sodium in the adult human body can be predicted using the following equation:

$$Na_e\ (mEq) = 163.2\ (\text{total body water}) - 69 - Ke\ (mEq)$$

where Ke is the exchangeable potassium.

Exchangeable potassium can be predicted as follows:

$$Ke(mEq) = 150\ (\text{intracellular body water}_L) + 4\ (\text{total body water} - \text{intracellular body water})$$

Total body water can be determined or estimated using one of several prediction equations. Sodium is primarily an extracellular ion and as such the serum will contain 136–145 mEq/L. Normal sodium intake varies from less than 2 grams to 10 grams per day. Most of this sodium comes from table salt, NaCl. Foods rich in added salt are usually the snack foods such as potato chips, salted nuts, pretzels, and so forth. Processed foods have more added salt than nonprocessed foods because salt not only improves the taste of the finished product but also serves as a preservative. Luncheon meats, cheese spreads, pickles, relishes, catsup, canned and frozen vegetables, crackers, breads, and frozen desserts are but a few of the foods that contain more sodium than their raw or nonprocessed components. Every living thing contains sodium and, except for pure fats and carbohydrates, no major food source lacks this element. There is a suggested acceptable intake (AI) figure for sodium (Table 11.1) These estimates presume that the individual has a moderately active lifestyle and lives in a temperate environment. Heavy work in a hot, dry environment and a variety of medical conditions will affect the need for sodium as well as for other electrolytes that are lost through the skin. Although sodium intake can be highly variable, serum sodium is not. As described, the normal range of serum sodium is quite small. Values in excess of 150 mEq/L (hypernatremia) are considered abnormal.

Values below 135 mEq/L (hyponatremia) are also considered abnormal and are of clinical concern. There are several reasons why hypernatremia or hyponatremia can develop. These are

TABLE 11.1
USDA Daily Dietary Reference Intakes (bold) and Acceptable Intakes (AI*) of various age/gender groups for sodium, potassium, chloride, calcium, phosphorus, and magnesium

Group	Sodium	Potassium	Chloride	Calcium	Phosphorus	Magnesium
	g	g	g	mg	mg	mg
Infants						
0–6 mo	0.12*	0.4*	0.18*	210*	100*	30*
7–12 mo	0.37*	0.7*	0.57*	270*	275*	75*
Children						
1–3 yr	1.0*	3.0*	1.5*	500*	**469**	**80**
4–8 yr	1.2*	3.8*	1.9*	800*	**500**	**130**
Males						
9–13 yr	1.5*	4.5*	2.3*	1,300*	**1,250**	**240**
14–18 yr	1.5*	4.7*	2.3*	1,300*	**1,250**	**410**
19–30 yr	1.5*	4.7*	2.3*	1,000*	**700**	**400**
31–50 yr	1.5*	4.7*	2.3*	1,000*	**700**	**420**
51–70 yr	1.3*	4.7*	2.0*	1,200*	**700**	**420**
70+	1.2*	4.7*	1.8*	1,200*	**700**	**420**
Females						
9–13 yr	1.5*	4.5*	2.3*	1,300*	**1,250**	**240**
14–18 yr	1.5*	4.7*	2.3*	1,300*	**1,250**	**360**
19–30 yr	1.5*	4.7*	2.3*	1,000*	**700**	**310**
31–50 yr	1.5*	4.7*	2.3*	1,000*	**700**	**320**
51–70 yr	1.5*	4.7*	2.0*	1,200*	**700**	**320**
70+ yr	1.5*	4.7*	1.8*	1,200*	**700**	**320**
Pregnancy						
14–18 yr	1.5*	4.7*	2.3*	1,300*	**1,250**	**400**
19–30 yr	1.5*	4.7*	2.3*	1,000*	**700**	**350**
31–50 yr	1.5*	4.7*	2.3*	1,000*	**700**	**360**
Lactation						
14–18 yr	1.5*	5.1*	2.3*	1,300*	**1,250**	**360**
19–30 yr	1.5*	5.1*	2.3*	1,000*	**700**	**310**
31–50 yr	1.5*	5.1*	2.3*	1,000*	**700**	**320**

Source: www.nap.edu

summarized in Table 11.2. In normal individuals the level of sodium in the serum is tightly controlled, and this control is intertwined with the control of potassium concentration, chloride concentration, and water balance.

Regulation of Serum Sodium

The system that regulates sodium levels in the blood also is involved in the regulation of water balance, pH, and osmotic pressure. Both hormones and physical/chemical factors are involved. Table 11.3 lists the hormones and their role in sodium balance as well as in water balance. The hormones listed are all involved indirectly or directly because of the need to regulate the osmotic

TABLE 11.2
Causes of hypernatremia and hyponatremia

Hypernatremia (serum Na > 150 mEq/L)	Hyponatremia (serum Na < 135 mEq/L)
Dehydration–excess sweating, deficient water intake	Cachexia (any wasting disease, such as cancer)
Excess solute loading	Anorexia nervosa
Diabetes insipidus (too little ADH)	Ulcerative colitis
Brain stem injury	Liver disease
	Congestive heart failure
	Ascites, edema
	Major trauma
	Severe infection
	Excess water intake
	Inappropriate ADH release
	Diarrhea
	Certain drugs (chlorothiazide, mercurial diuretics, etc.)
	Adrenalectomy

TABLE 11.3
Hormones involved in the regulation of serum sodium

Hormone	Function
Vasopressin (ADH)	Serves to stimulate the reabsorption of water by the glomerulus and renal convoluted tubule
Atrial natriuretic hormone	Counteracts Vasopressin and thus induces water loss, sodium loss, and potassium loss. It also decreases blood pressure and increases glomerular filtration rate. Suppresses renin release and aldosterone release. Antagonizes angiotensin II and norepinephrine
Renin	Catalyzes the conversion of inactive angiotensin I to active angiotensin III
Angiotensin II	At low serum sodium levels it conserves sodium by stimulating its reabsorption. At high serum sodium levels it has the reverse effect. Stimulates vasoconstriction which increases blood pressure. Reduces water loss, decreases glomerular filtration rate. Stimulates aldosterone release
Aldosterone	Conserves sodium by increasing sodium resorption by the kidney

pressure within and around the cells. Osmolality—that is, the concentration of solutes on each side of a semipermeable membrane—is maintained by the passage of water through that membrane. This osmolality is maintained at roughly 270–290 milliosmols. The proteins, many of which can not pass through the plasma membrane, are part of this solute load. In the well nourished, healthy individual, the intracellular and extracellular proteins are maintained at fairly constant levels. This leaves the small fully ionized solutes (Na^+, K^+, Cl^-) as major determinants of the osmotic pressure of the system. Osmotic pressure is the physical force required to keep the osmolality on both sides of the membrane approximately equal. If the solute load on one side exceeds that of the other, water and the small solutes (Na^+, K^+, Cl^-) will pass through the membrane to equalize the concentration of solutes.

Because the number of particles determines osmotic pressure, substances that ionize affect osmotic pressure according to the degree of dissociation. Thus, the fully dissociated NaCl produces the ions, Na^+ and Cl^- in such a fashion that at 0.154 molar there are 1.85 particles each of Na^+ and Cl^-, which exert a pressure of 286 milliosmols. There are several ways to measure osmolality in the laboratory using rather simple instruments called osmometers. For example, the osmolality of a

solution can be determined using the effect of a solute on freezing point. The addition of one osmole to 1 liter of water will depress the freezing point by 1.86°C.

Several of the hormones listed in Table 11.3 are released in response to changes in sodium concentration or to signals generated by osmoreceptors located in the anterolateral hypothalamus. The sodium ion, of all of the circulating ions, is the most potent of the solutes that activate the osmoreceptors, which in turn signal the release of hormones regulating osmolality. Vasopressin (also called antidiuretic hormone or ADH) is one of these; aldosterone is another. When the osmoreceptors sense a change (increase) in the solute load, ADH release is stimulated. This results in an increase in renal water resorption, and thus dilution of the solute load. Aldosterone release is stimulated by low serum sodium-high potassium levels in the blood. Aldosterone, released by the adrenal cortex, functions in sodium conservation by increasing renal reabsorption of this ion. Of interest is the action of these ions at the level of the expression of the aldosterone synthetase gene. At low sodium high potassium levels, the transcription of the aldosterone synthetase gene is stimulated. More messenger RNA is produced and more enzyme is synthesized.[1]

The pathway for the synthesis of aldosterone, as part of the steroid hormone synthetic network, is shown in Figure 11.1. Aldosterone synthesis from corticosterone via the enzyme 11β-hydroxylase (an enzyme that catalyzes 11-deoxycorticosterone conversion to corticosterone) is enhanced by angiotensin II. Angiotensin II release is stimulated by low sodium levels. Figure 11.2 illustrates the roles of sodium and potassium in sodium conservation. Angiotensin II action requires the calcium ion and high potassium levels to facilitate calcium ion movement from outside the cell to the inside of the cell. As aldosterone levels increase, the calcium ion facilitates its release by the cortex cell. Aldosterone then moves to the kidney where it stimulates electrolyte conservation. Thus, the circle is completed. Sodium is regulated by several hormones and in turn regulates the synthesis of these hormones through effects on gene transcription.[1] Sodium also stimulates the transcription of the genes for cholesterol SCC, a P-450 enzyme, the gene for adrenodoxin, and, in hypertensive animals, has been found to stimulate the transcription of the gene for endothelin 1.[2] Endothelin 1 is an important vasoactive peptide that acts as a diuretic and as a natriuretic. It stimulates vasoconstriction and

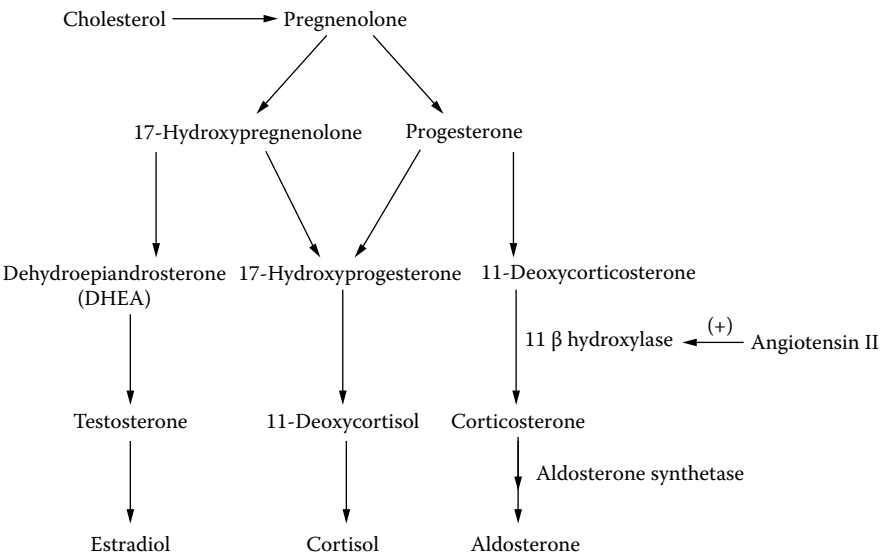


FIGURE 11.1 The steroid hormone synthetic pathway showing the synthesis of aldosterone as well as the other steroid hormones.

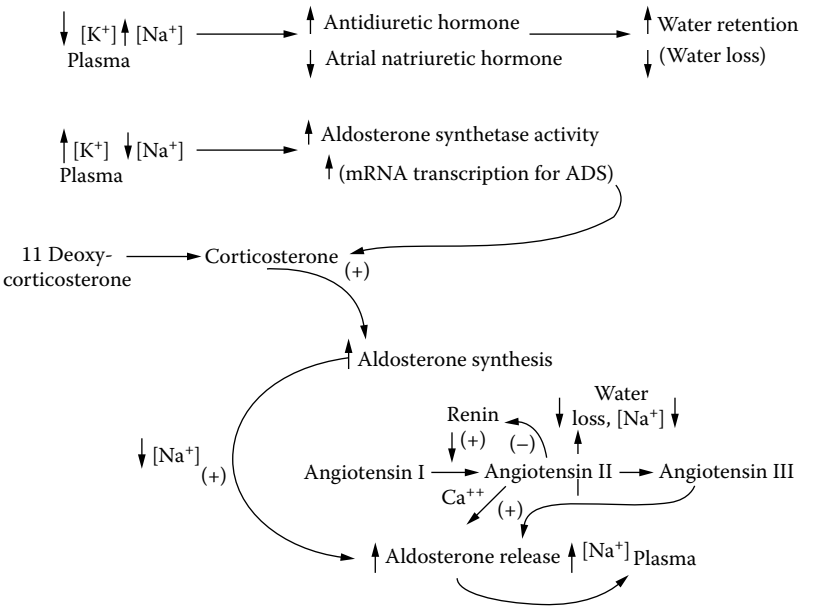


FIGURE 11.2 Schematic representation of how low-potassium, high-sodium levels affect sodium conservation. Two systems are used: One involves the posterior pituitary, which releases antidiuretic hormone (ADH, also called vasopressin). ADH stimulates water and sodium retention. The other system involves the adrenal cortex, which is stimulated to release aldosterone, the mineralcorticoid hormone responsible for sodium conservation.

in genetic hypertension it seems to reduce sodium loss. Sodium conservation is a part of the regulation of water balance. This regulation is shown in Figure 11.3.

The disorder *diabetes insipidus* develops in the absence of ADH. The disease is characterized by extreme urinary water loss (up to 30 L/d) and extreme thirst. In this disorder, the posterior pituitary may be diseased or have a tumor that interferes with the production of ADH. In this disease, the resorption of water by the renal tubules is not stimulated and the patient excretes a large volume of very dilute urine. Patients with diabetes insipidus can be successfully treated. Interestingly, their treatment consists of providing the hormone in an aerosol that allows the patient to inhale the hormone through the nose. An even rarer disease of water balance is a form of diabetes insipidus that does not involve the production and release of ADH by the posterior pituitary but is primarily of renal origin. In this disorder, the kidney is unresponsive to the hormone. The reasons for this unresponsiveness are not known nor has a suitable treatment been devised.

Function

Indirectly, the function of the sodium ion as a participant in the regulation of osmotic pressure has already been discussed when the regulation of serum sodium levels was discussed. In addition to its function in this system, it also functions in nerve conduction, active transport both by the enterocyte and by other cell types, and plays a role in the formation of the mineral apatite of the bone. The common thread to its role in nerve conduction, active transport, and water balance is its function in the sodium-potassium ATPase. This enzyme, embedded in the plasma membrane of most cells, is perhaps the most thoroughly studied enzyme of the active transport systems. The ATPase transmembrane protein was first isolated in 1957 and consists of two types of subunits; a 110-kD nonglycosylated α subunit that contains the enzyme's catalytic activity and ion-binding site, and a

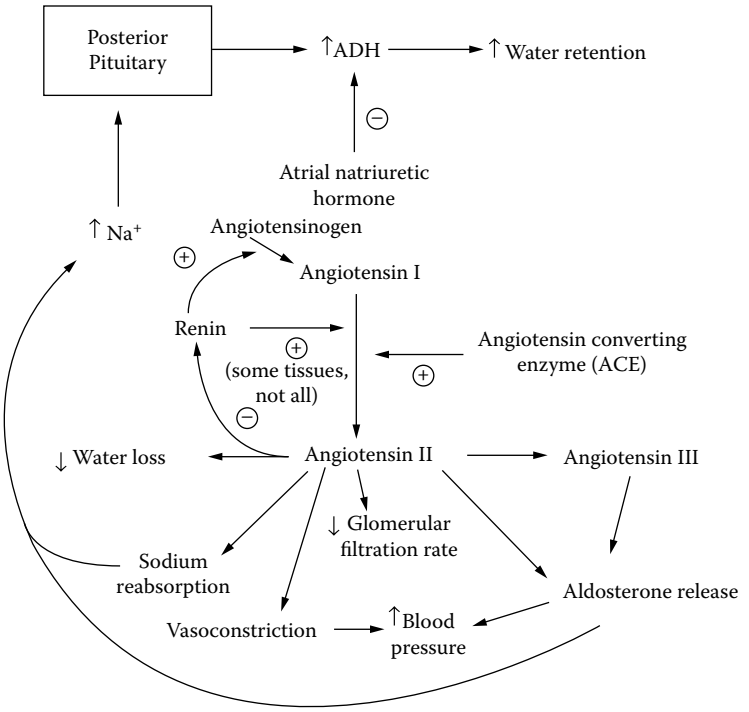


FIGURE 11.3 Water balance is controlled by a cascade of hormones. These hormones also have a role in the control of blood pressure. One of the drugs used for the control of blood pressure inhibits ACE. Others inhibit water loss by inhibiting angiotensin or rennin.

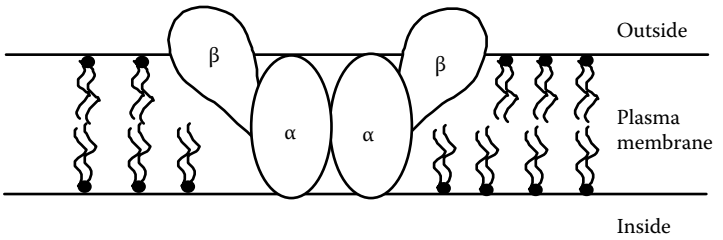


FIGURE 11.4 Structure of the membrane-bound Na^+K^+ATPase.

55-kD glycoprotein β subunit. The enzyme has two of each of these subunits. Figure 11.4 illustrates the structure of this enzyme. It is speculated that glycoprotein plays a role in the recognition of appropriate substrates, but this role is yet unsubstantied. The ATPase is frequently called the Na^+K^+ pump because it pumps sodium out as potassium returns to the cell; when this exchange takes place, there is a concomitant hydrolysis of ATP. The equation which describes this process is as follows:

$$3Na^+ \text{ (in)} + 2\ K^+ \text{ (out)} + ATP \leftrightarrow 3Na^+ \text{ (out)} + 2K^+ \text{ (in)} + ATP + Pi$$

The pump is an electrogenic system, illustrated in Figure 11.5, with the extrusion of three positively charged particles (the Na^+) in return for two negatively charged ones (the K^+). As an electrogenic system, the Na^+K^+ATPase generates an electrochemical potential gradient that is responsible for

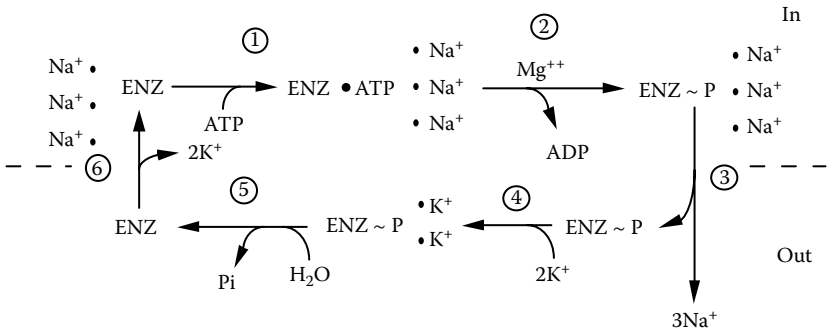


FIGURE 11.5 Mechanism of action of the Na^+K^+ATPase. The ion exchange occurs via a series of reactions utilizing the a subunits of the enzyme, which has an inward-facing high-affinity Na^+-binding site that reacts with ATP to form the activated ENZ~P only when Na^+ is bound to it. On the exterior aspect of the α subunits is a high-affinity K^+-binding site, which will hydrolyze, releasing inorganic phosphate only when K^+ is bound to it. The obligatory sequence begins with the sodium-ATP-binding and ends with the K^+ import into the cell. The sequence of these steps is numbered in this illustration.

nerve action. Signal transmission along a nerve path occurs via a depolarization/repolarization scheme whereby potassium leaves the neuron and sodium enters (depolarization); through ATPase activity the reverse occurs (repolarization). Much of the ATP that cells produce by the mitochondria is used by this ATPase. In fact, in nerve cells, up to 70% of their ATP production is consumed by the sodium pump as it functions in signal transmission. Other tissues as well use the ATPase to sustain the resting electric potential of the cells within them.[3]

Other cell types use the pump for other purposes. As mentioned, the active transport of needed nutrients into the enterocyte uses the pump. Should the pump malfunction, diarrhea will result. Food-borne illness as well as food allergies or genetic errors in the genes for needed digestion/absorption processes can cause diarrhea. Various organisms such as those from the Salmonella family or the *Vibrio cholerae* can cause massive diarrhea and fluid loss. In the case of the latter, the cholera-causing organism produces a toxin which binds to specific receptors on the intestinal cell wall. The toxin activates adenyl cyclase by causing ADP-ribosylation of the GαS protein involved in regulating the cyclase. This results in an elevation in cAMP levels that turns on electrolyte secretion and thereby inhibits the active transport processes necessary for the absorption of nutrients (and water) from the gut. Whereas the water can pass freely from the intestine, other materials cannot and, as such, create an osmotic pull on the water. In turn, the water and unabsorbed nutrients fill the intestine, stimulating peristalsis and evacuation of the colon. If the massive fluid and electrolyte losses are not replaced, the cholera victim does not survive and recover. Cholera victims can be given an oral solution rich in glucose (~110 mM), sodium (99 mM), chloride (74 mM), bicarbonate (39 mM), and potassium (4 mM). The WHO (World Health Organization) recommends a solution containing 20 g glucose, 3.5 g NaCl, 2.5 g $NaHCO_3$, and 1.5 g KCl per liter of clean drinking water. This solution takes advantage of the fact that the cholera toxin does not "poison" the sodium-dependent ATP-dependent glucose uptake system. By giving excess glucose, sodium is "pushed in" to the cell, and electrolyte balance is restored. If the victim can not swallow, an intravenous replenishment must be followed. If untreated, the victim becomes severely dehydrated and death ensues. In countries where fresh human excreta is used as fertilizer for the fields, epidemics of cholera occur frequently. Epidemics are particularly evident in very warm climates because the cholera organisms multiply very rapidly under these conditions. Food contamination by organisms other than *V. cholerae* is also common in areas where both animal and human excreta are used in the untreated state. If the

excreta is allowed to ferment, the heat generated by the fermentation process will kill most of the organisms likely to cause food-borne illness.

Muscle contraction/relaxation uses a Na^+K^+ ATPase or pump. In the muscle, there is an additional pump, the Na^+Ca^{++} antiport system. This system pumps sodium out and allows calcium into the cell. Calcium triggers muscle contraction, and so the two pump systems work together to regulate muscle action. When heart muscle degenerates the clinician has several drugs that can be used to stimulate muscle action by inhibiting the Na^+K^+ATPase. These drugs, called *cardiac glycosides* (digitalis, ouabain), inhibit the ATPase by binding to the α subunits (see Figure 11.4). This results in an increase in intracellular Na^+, which in turn stimulates the Na^+Ca^{++} antiport system. The cell extrudes Na^+, and the resultant influx of Ca^{++} triggers an increase in the force of the cardiac muscle contraction. The drug dose must be carefully monitored because too much Ca^{++} influx could be lethal unless counteracted. The regulation of intracellular calcium levels together with its role in metabolic regulation is the topic of intense interest by nutritionists, biochemists, and physiologists.

POTASSIUM

Potassium is the major intracellular electrolyte.[4] The healthy young adult male has between 42 to 48 mEq K^+/kg body weight or 2940–3360 mEq in the 70 kg man. Persons with above-average muscle mass (e.g., athletes) will have more body potassium than persons of average muscle mass. Virtually all the body potassium is exchangeable with the exception of small amounts that are irretrievably bound up in the bone mineral. Because potassium is primarily an intracellular ion the number of cells in the body can be estimated using an infusion of heavy isotope K^{40}. The infused and exchangeable potassium equilibrates and by determining the dilution of the isotope, one can determine cell number and mass while correcting for fat mass. The Na^+K^+ATPase actively works to ensure that K^+ stays within the cell and that very little (3.5–5.0 mEq/L) is present in extracellular fluids.[5,6]

Just as all living things serve as sources of sodium, so, too, are they sources of potassium. Only highly refined food ingredients (e.g., pure sugars, fats, and oils) lack this essential nutrient. Especially good sources are orange juice, avocados, fish, and bananas. Potassium passes freely from the gastrointestinal system into the enterocyte and thence into the body. Potassium is distributed in response to energy-dependent Na^+ redistribution. Almost all of the consumed potassium is excreted in the urine with a very small amount found in the feces in healthy, normal adults. In persons experiencing diarrhea, however, the loss of potassium can be quite large and debilitating.[6] If the diarrhea is of short duration (less than 12 hours) the body will compensate and the person will survive. However, should this condition persist, potassium supplementation will be needed. Such is the case with the disease cholera and for a number of the malabsorption syndromes. The plasma or serum level of potassium is not a reliable index of whole-body potassium status simply because potassium within the cell (not the serum) is what is needed. Causes for concern with too little potassium (hypokalemia) or too much (hyperkalemia) have to do with muscle contractility. If hypokalemia persists the person could die of cardiac arrest. This occurs because too much K^+ has left the contractile unit, and the heart muscle loses its ability to contract. Common causes of hypo and hyperkalemia are listed in Table 11.4. The regulation of potassium balance follows that of sodium balance, and its participation in the sodium potassium pump has already been discussed.

CHLORIDE

Chloride is the third leg upon which osmotic pressure and acid-base balance rests.[7] Normal chloride levels in plasma are 100–106 mEq/L and vary very little. The glomerular filtrate contains 108 mEq/L and urine contains 138 mEq/L. Sweat can contain as much as 40 mEq/L but usually contains only trace amounts. The intracellular fluid contains very little Cl^- (~4 mEq/L) whereas intestinal juice contains 69–127 mEq/L. In instances of secretory diarrhea, the chloride content of the excrement can be as high as 45 mEq/L. Most of the chloride in the intestinal tract does not appear in the

TABLE 11.4
Causes of hypokalemia and hyperkalemia

Hypokalemia (plasma levels < 3.5 mEq/L)	Hyperkalemia (plasma levels > 7 mEq/L)
Vomiting in excess (loss of chloride)	Chronic renal failure
Diuretics that enhance K^+ loss	Addison's disease (no aldosterone)
Cushing's disease (excess steroids)	Major trauma, infection
Rehydration therapy without K^+	Metabolic acidosis
Chronic renal disease	
Metabolic alkalosis	
Diarrhea	

TABLE 11.5
Causes of hypochloremia and hyperchloremia

Hypochloremia	Hyperchloremia
Increased extracellular water volume due to trauma and/or cachexia	Dehydration
Vomiting with large loss of gastric HCL	Diabetes insipidus
Overuse of diuretics	Brain stem injury
Overuse of adrenal steroids with retention of Na^+	Ureterointestinal anastomoses due to reabsorption of Cl^-
Chronic respiratory acidosis (high CO_2, low pH)	
Chronic renal disease, renal failure	

feces of normal individuals. Rather, this ion recirculates as sodium and potassium are carried into the body. The main excretory pathway is urine. This ion (a member of the halogen family of elements that includes fluoride, iodide, and bromide, in addition to chloride), although very reactive, is passively distributed throughout the body. As mentioned, it moves to replace anions lost to cells via other processes. It is the other half of table salt, NaCl, and as such, is found in abundance in most foods. Dietary intake is in excess of that of sodium yet the usual plasma Na^+:Cl^- ratio is about 3:2. This imbalance is due to the passive nature of chloride transfer between water compartments and to the active system which serves to retain Na^+.[8] Instances of below- and above-normal plasma levels of Cl^- are not diet related but are due to metabolic reasons usually associated with Na^+ and K^+ homeostasis. Cystic fibrosis, for example, is a disease where chloride transport is abnormal.[9–11] In these patients there are abnormalities in chloride transport in the epithelia of the respiratory tract, sweat glands, and intestinal tract. The disease is characterized by defective chloride channels.[12] Actually, cystic fibrosis is just one of several diseases in the group of diseases called *channelopathies*.[13] Not only can chloride channels be abnormal, so, too, can potassium channels. Altogether, problems in ion exchange can have marked effects on normal cell function. Listed in Table 11.5 are reasons why hypochloremia and hyperchloremia develop. Note the similarities between these causes and those listed for sodium in Table 11.2 and those for potassium in Table 11.4.

Function

As an electronegative element, Cl^- is a good oxidizing agent. In typical reactions it is reduced to its electronegative form. One of its main functions is as an essential ingredient of the gastric acid, hydrochloric acid. This is completely dissociated into a strong electron donor (H^+) and a strong electron acceptor (Cl^-). Gastric juice contains 120–160 mEq/L. The function of chloride aside from its passive participation in electrolyte balance has to do with hemoglobin and its function as a carrier for oxygen and carbon dioxide. The process is called "the chloride shift." The Cl^- is bound more

tightly to deoxyhemoglobin than to oxyhemoglobin. Hence the affinity of hemoglobin is directly proportional to the concentration of Cl^-. The carbonate ion, HCO_3^- freely permeates the erythrocyte membrane so that once formed it equilibrates with the plasma. The need for charge neutrality on both sides of the red cell membrane requires that Cl^- replace HCO_3^- as it leaves the erythrocyte. Consequently, because cations do not shift, the Cl^- ion in the venous blood erythrocyte is higher than in the arterial blood erythrocyte. Thus, the Cl^- modulates hemoglobin oxygen affinity.

CALCIUM

Calcium is the fifth most abundant element in the body exceeded only by carbon, hydrogen, oxygen, and nitrogen. It is the primary mineral in bones and teeth where it is present as hydroxyapatite ($3Ca_3(PO_4)_2 \bullet Ca(OH)_2$). On a dry weight basis, bone contains about 150 mg calcium/gram bone. By comparison, soft tissue such as liver, muscle, or brain contains less than 35 μg calcium/gram tissue. A normal 70 kg man will have about 22 g calcium/gram fat-free tissue or a total of 1.54 kg calcium. Whereas the calcium in the teeth is seldom mobilized, that which is in the skeletal muscle is mobilized and replaced at about 0.5 g/day. This daily turnover of calcium is essential to the maintenance of metabolic homeostasis because not only does calcium serve as a structural element, it also serves, in its ionized form, as an essential element in cell signaling systems. Of the total body calcium, 1% serves as an intracellular/intercellular messenger/regulator. Calcium mobilization and deposition changes with age, diet, hormonal status and physiological state. Bone calcium homeostasis is related to bone strength and if mobilization exceeds deposition, the bones will become porous (osteoporosis) and break easily.

Sources

The average daily calcium intake for adults in the United States ranges from 500 to 1200 mg. The range is quite broad because it depends on the percent of the diet that comes from dairy products. Milk, cheese, ice cream, yogurt, sour cream, buttermilk, and other fermented and nonfermented milk products can provide as much as 72% of the daily calcium intake. Nuts and whole grain products are also good sources of calcium whereas other foods are relatively poor sources of this mineral. Shown in Table 11.6 are a number of foods and their calcium content.

Some foods contain calcium-binding agents that reduce the availability of the calcium to the enterocyte. For example, some plant foods contain calcium in measurable quantities but these same foods also contain phytate, a six carbon anomer of glucose having six phosphate groups. Phytate will bind calcium, reducing its availability for absorption. Once the phytate is degraded by the enzyme phytase the bound calcium is then released and is once again available for absorption. Unfortunately, this release occurs in the lower third of the intestine and in the large intestine, areas which are less active in terms of calcium absorption. Phytate binds other divalent ions (Zn, Fe, Mg) as well, and has a similar effect on their absorption. In addition, oxalate and some tannins can have this effect.

Food Mixtures

Milk and milk products are excellent sources of calcium, as mentioned earlier. The reason why milk calcium absorption is so good is because of the type of protein found in these foods.

Casein, the main protein in milk, is a relatively small protein (mol. wt. 23,000) having numerous phosphorylated serine residues. These residues have a negative charge to them that enables the protein to bind the positively charged calcium ions. Lactalbumin, another milk protein, is also a calcium-binding protein. In addition to calcium, it will also bind zinc. This is true for a number of proteins; those that bind one divalent ion will also bind other divalent ions. Hence, there could be a competition for binding that would affect the availability of the minerals involved. As the proteins

TABLE 11.6
Food sources of calcium in 100-g portions

Source	mg	Weight of average serving (g)
Skim milk	123	245
Whole milk	119	244
Ice cream	129	66
Yogurt	120	227
Oysters	45	84
Cheddar cheese	728	28
Spinach	135	90
Mustard greens	74	70
Broccoli	48	44
White bread	125	24
Whole wheat bread	72	25
Carrots	26	72
Potatoes	10	202
Winter squash	14	102
Egg	50	50
Hamburger	10	100
Hot dog	19	57

Source: www.nal.usda.gov/fnic/foodcomp/Data/foods.82nutrients

are degraded by the digestive enzymes, these protein-bound minerals become available for uptake by the enterocyte. In mixtures of foods this availability can be enhanced or compromised depending, on the food mixture. As mentioned, cereal foods and green leafy vegetables contain oxalates or phytate that bind calcium. When these foods are mixed with dairy foods, one could anticipate a reduction in the availability of the calcium in the dairy food. In contrast, foods that are rich in vitamin C enhance calcium availability probably due to the redox nature of ascorbic acid. This vitamin readily changes from an oxidized to a reduced form and assists not only calcium absorption but also assists the absorption of those minerals that have more than one charged state (Fe^{++}, Fe^{+++}, Cu^{++}, Cu^{+++}, etc.).

Foods and food mixtures that provide calcium and phosphorus together in a ratio of 2:1 to 1:2 optimize calcium absorption. Both minerals are actively transported and yet do not share a single transport mechanism (see absorption, below). When the food mixtures are unbalanced with respect to this ratio, then calcium uptake will be impaired. Table 11.7 summarizes the influence of food components on calcium availability.

Bioavailability

In contrast to the macronutrients, the vitamins, and sodium, potassium, and chloride, not all of the calcium that is consumed is absorbed. The fraction that is absorbed can vary, depending on the food source, the mixture of foods consumed, and the physiological status of the individual. That which is actually involved in biological processes is the bioavailable fraction.[14] The determination of calcium bioavailability and absorption efficiency is a complicated process that involves careful food analysis to determine calcium content and careful measurements of the food consumed, followed by feces and urine analysis of calcium excretion, corrected for calcium recycling. The amount of calcium in

TABLE 11.7
Food components that affect calcium absorption

Component	Effect
Alcohol	↓
Ascorbic acid	↓↑
Cellulose	↓
Fat[a]	↓↑
Fiber	↓
Lactose	↑
Medium-chain triglycerides	↑
Oxalates	↓
Pectin	↓↑
Phytate	↓
Protein[b]	↓↑
Sodium alginate	↓
Uronic acid	↓

[a] In cases of steatorrhea, calcium absorption is reduced.
[b] Certain proteins, such as those in milk, enhance calcium availability, whereas others, such as those in plants, reduce it.

the urine reflects the calcium that is absorbed, but it does not reveal the amount of calcium that has been recycled. Likewise, the calcium in the feces reflects not only the calcium that was not absorbed from the food, but also the calcium that was secreted into the intestine and not reabsorbed. The use of the calcium balance technique likewise does not measure the amount of calcium deposited in the bones and teeth, nor does it provide an estimate of the amount of calcium that is mobilized from these depots. However, the balance technique can be combined with tracer techniques that provide reasonably good estimates of absorption and bioavailability. Bioavailability estimates can be obtained using isotopes of calcium as tracers.[15,16] The heavy isotope Ca (^{46}Ca, ^{48}Ca) or the radioisotope calcium (^{45}Ca, ^{47}Ca) can be incorporated into a food and its presence in blood, urine and feces monitored over time. If a plant food, the plant can be grown in an isotope-enriched growth medium, and the edible portions provided to the subject or animal. If an animal food, the animal either consumes an isotopically labeled feed or is infused with a solution of labeled $CaCl_2$. Knowing the total food calcium, and the percent that was isotopically labeled will then allow for calculations of intestinal uptake, recycling, excretion, storage, and use. The double isotope technique involves the consumption of a ^{45}Ca-enriched food, followed by an infusion of ^{47}CaCl in the vein.[47] Ca has a short half-life (~4.7 d), whereas ^{45}Ca has a much longer one (~163.5 d). The stable isotopes (^{48}Ca, ^{49}Ca) can be substituted for the radioisotopes. The infused ^{47}Ca will be diluted in the blood by the calcium mobilized from the depots as well as by the absorbed calcium which also dilutes the food ^{45}Ca. The fraction that is absorbed is calculated as follows:

$$J_{ms} = \frac{J_{max}[Ca_L^{++}] + A\,[Ca_L^{++}]}{K_T + [Ca_L^{++}]} \quad (11.1)$$

where J_{ms} = total calcium flux from lumen

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J_{max} = maximum saturable flux
Ca^{++} = lumen, $J_{max}/2$ is observed
and A = diffusion constant

In practice, absorption = fraction of oral ^{45}Ca in urine/fraction of ^{47}Ca in urine. The use of ^{47}Ca assumes that the infused calcium will behave as though it had been absorbed. In each instance the measurement is a single time point. Urine is collected for 24 hours after isotope administration. To obtain an estimate of recycling the investigator will collect blood, urine, and feces at intervals over a 2–4-d period, monitor the dilution of the consumed and infused labels and, using this information, calculate rate constants for each calcium pool. Absorption can also be calculated from the feces, using the loss of the consumed label to estimate the absorption. However, because there is considerable individual variation in gut passage time, which can influence calcium absorption, feces must be collected for periods of up to 12 d. Given the shorter half-life of ^{47}Ca that is infused to provide the estimate of recycling, this method for determining apparent absorption is not as popular.

Combining the use of both radioactive and stable isotopes also has some problems. The stable isotope ^{48}Ca degrades to ^{49}Ca with the emission of a γ ray. ^{49}Ca has a half-life of 8.8 minutes. ^{46}Ca converts to ^{47}Ca, which has radioactivity. The use of heavy isotopes requires the use of mass spectrometry to detect their presence, whereas liquid scintillation or gamma counters are needed for detecting the radioisotopes. Small animals such as the rat, mouse, and chicken with their smaller body sizes, faster rates of growth, and faster metabolic rates reduce the expense and increase the number of measurements as well as the validity of these measurements due to their genetic homogeneity.

Apparent Absorption

Apparent absorption is the fraction of the consumed calcium that disappears from the gastrointestinal tract. Thus, it is the net movement of calcium from the lumen into the animal. This movement can be tracked in vitro using an intestinal segment and a calcium isotope. The apparent absorption is calculated.

$$\text{Apparent Absorption} = \frac{V[^{40}Ca_i - (^{40}Ca_f)(PRR)]}{L \text{ or } W} \tag{11.2}$$

The subscripts i and f refer to initial and final concentrations of calcium in μmoles/ml. PRR is the ratio of phenol red in the initial and final samples. L is the length and W is the weight of the intestinal segment, and V is the volume perfused in milliliters. The lumen to plasma flux is unidirectional and can be calculated as follows:

$$\text{Flux} = \frac{V[^{45}Ca_i - (^{45}Ca_f)\,(PRR)]}{[(SA_i + SA_f)/2]\,W} \tag{11.3}$$

In this calculation, SA refers to the specific activity of the calcium isotope, and W is the dry weight of the intestinal segment. All the other definitions are the same as in Equation 11.2. These equations have been successfully used to calculate calcium absorption in normal rats as shown in Table 11.8.

Physiological Status

Absorption efficiency is greater in the young growing individual than in the mature adult, which in turn has a greater efficiency than in the aged individual.[17] As well, there are gender differences due in part to hormonal status. Postmenopausal females have a less efficient calcium absorption than premenopausal females.[18] Men, both young and mature, have a greater calcium absorption efficiency

TABLE 11.8
Apparent absorption of calcium in vitro by duodenal loops in normal rats

Volume perfused	240 ml
Duration of perfusion	2 h
Conc. Ca	0.8 mM
Conc. ^{45}Ca (sp act., 2000 μCi/mg)	12 μCi/L
Length of intestine	87 cm
Weight of intestine	5.4 g
Mucosal dry weight	7.2 mg/cm
Net absorption, μmol/h	
Per cm segment length	0.140
Per g dry weight mucosa	20.1
Lumen to plasma flux	
Per cm segment length	0.196
Per g dry weight mucosa	27.6

than females of the same age.[17] Testosterone has been shown to enhance calcium absorption.[19] Calcium absorption is impaired in vitamin D deficient individuals[20,21] as well as in persons with insulin-dependent diabetes mellitus.[22] Clearly, the endocrine status of the individual affects calcium uptake, and this in turn can influence calcium status in terms of bone structure and calcium content.

Mechanisms of Absorption

Only 20–50% of ingested calcium is absorbed, yet this mineral is so important to metabolism that two mechanisms exist for its absorption.[23,24] One of these is vitamin-D-dependent, whereas the other is not. The latter is one that uses passive diffusion. The former is an energy-dependent active transport system and is illustrated in Figure 11.6. This is a saturable system that operates actively when calcium is in short supply in the diet. Thus, when the diet is calcium poor, this system is more active than when a calcium-rich diet is consumed. Thus, a wide range of apparent calcium absorption exists. In the active system, calcium diffuses across the brush border down its thermodynamic gradient into the cell as free unbound Ca^{++}. This calcium is then bound by an intracellular protein called calbindin D_{9k}, which serves to maintain the level of calcium in the cell at a low, nontoxic level.[25] Calcium released from calbindin enters and leaves the subcell compartments such as the mitochondria[26,27] and endoplasmic reticulum.[28] This occurs in an orderly oscillatory manner.[29] The calcium ion leaves the enterocyte either in exchange for sodium or is extruded from the cell by a calcium-activated ATPase. This ATPase has been found in both the brush border and at the basal-lateral membrane. As calcium is extruded, ATP is cleaved to ADP and Pi. Hence the energy dependence of this system. Because the sodium that is exchanged for calcium must be actively removed from the intracellular compartment by the Na^+K^+ATPase, this process is also part of this energy-dependent system.

Absorption of calcium by the enterocyte is but one of the roles of vitamin D. Other roles include renal calcium conservation, intracellular calcium movement, and bone calcium deposition, as well as bone calcium mobilization. Calcium homeostasis is thus one of the main functions of vitamin D. Vitamin D or 1,25-dihydroxycholecalciferol, has these effects on calcium homeostasis due to its effects on the synthesis calcium-binding proteins (Table 11.9). In the intestine the protein of interest is calbindin D_{9k}. Vitamin D binds to its cognate receptor in the enterocyte, and this steroid-receptor complex migrates to the nucleus where it binds to a specific DNA sequence, which encodes

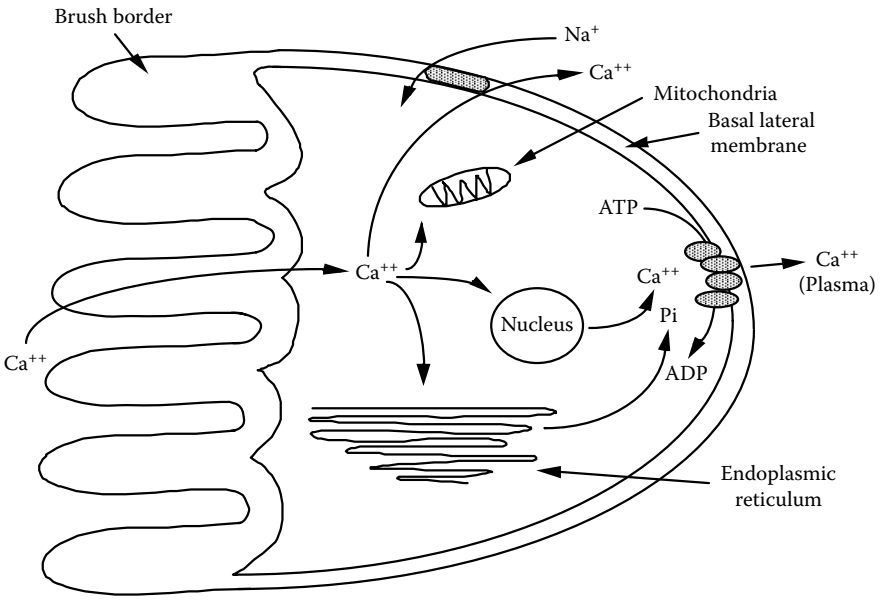


FIGURE 11.6 Calcium absorption by the enterocyte. Both the $Ca^{2+}Na^{+}$ exchange and the energy dependent systems are shown.

TABLE 11.9
Calcium-binding proteins

Protein	Function
α lactalbumin	Carries calcium in milk
Casein	Carries calcium in milk
Calmodulin	Serves as major intracellular calcium receptor; activates cyclic nucletoidephosphodiesterase
Calbindin—D_{9k} and D_{28k}	Facilitates intracellular Ca^{++} translocation
Osteocalcin	Essential for calcium deposition in bone
$Ca^{++}Mg^{++}$ ATPase	Essential to movement of calcium across membranes
Prothrombin	Essential to blood clot formation
Calcitonin	Inhibits osteoclast-mediated bone resorption. Regulates blood calcium levels by preventing hypercalciumemia
Parathyroid hormone	Stimulates calcitonin synthesis, bone Ca resorption, renal Ca conservation
Albumin	Carries calcium in the blood
Globulin	Carries calcium in the blood
Osteopontin	Essential for calcium mobilization from bone
Troponin C	Muscle contraction
Alkaline phosphatase	Mineralization of bone
Sialoprotein	Embryonic bone growth
GLA-rich clotting proteins	Binds calcium in the coagulation cascade (see vitamin K)
Villin, gelsolin	Cytoskeleton stabilization

the protein, calbindin D_{9k}. Studies of vitamin D deficient animals have shown a rapid induction of calbindin mRNA transcription when the missing vitamin was provided. This was followed by a restoration of normal calcium absorption in these repleted animals. In addition to the rapid increase in transcription was an effect of the vitamin on calbindin mRNA half-life. There was an increase in this half-life that indicated a vitamin effect on mRNA stability. Thus, the vitamin both stimulates transcription and has a posttranscriptional effect on calbindin synthesis.

Vitamin D is not the only hormone involved in calbindin synthesis. Testosterone, retinoic acid, growth hormone, progesterone, insulin-like growth factor 1, and estrogen augment, whereas glucocorticoids inhibit the synthesis of calbindin D_{9k}.[18,19,25,30,31] Calbindin D_{28k}, another vitamin-D-dependent calcium-binding protein in the enterocyte and renal cell, has its synthesis regulated by retinoic acid. In the brain this calcium-binding protein is not regulated by vitamin D. The multiplicity of hormonal controls of calbindin synthesis explains in part the gender and age differences in calcium absorption and, indeed, helps to elucidate the reasons why postmenopausal females are more at risk for calcium inadequacy than are young growing males and females. Those hormones that have a positive effect on calbindin D synthesis are the same hormones that are missing (or in short supply) in the postmenopausal female. Should calbindin D_{9k} synthesis be inadequate, calcium absorption will decline. As well, calbindin D_{28k}, an important calcium-binding protein in the renal cell responsible for renal calcium reabsorption, could also be synthesized at a reduced rate, and thus calcium conservation would be reduced. The two calbindins are subject to nearly the same hormonal influences. With decreased calcium absorption and conservation, bone calcium mobilization increases with the result of an age/gender-related loss in bone calcium: osteoporosis.

Calcium Transport, Blood Calcium Regulation

The concentration of calcium in the plasma is the result of three processes that are integrated so as to maintain a constancy of calcium in the circulation. Blood calcium is regulated at 100 mg/L (2.50 mmol/L) across the life span, although in late adulthood there may be a small (10%) decline due to an age-related decline in the total calcium-binding capacity of the serum proteins. Most (80%) of the blood calcium bound to protein is carried by albumin, with the remainder bound to a variety of globulins. About 60% of blood calcium circulates as the free ion or as an ion complex. Calcium levels in the blood are regulated mainly by three hormones: active vitamin D (1,25-dihydroxycholecalciferol), calcitonin, and parathyroid hormone (PTH), a hormone released by the parathyroid glands. These glands are embedded in the thyroid gland and are stimulated by falling calcium levels to release PTH. PTH acts on the bone and the kidneys. In the kidneys PTH reduces calcium loss by stimulating its reabsorption. In the bone PTH stimulates calcium release. It also inhibits collagen synthesis by osteoblasts. Osteoblasts, through the synthesis of collagen, provide the organic matrix on which the minerals of the bone are deposited. Osteoclasts, in contrast, are those bone cells responsible for bone resorption. PTH stimulates osteoclast activity indirectly because PTH stimulates renal inorganic phosphate loss. Because phosphate is counterion of Ca^{++} in the bone, phosphate loss causes $Ca_5(PO_4)_3OH$ to leach out of the bone and thus raise serum calcium levels. Finally, PTH has one other effect. It stimulates the synthesis of 1,25-dihydroxycholecalciferol, which in turn stimulates intestinal calcium uptake. This active form of vitamin D enhances the PTH effect on bone calcium mobilization. Thus, PTH has a major role in blood calcium regulation, and it has its effects at several different sites.

Counteracting PTH is calcitonin. Calcitonin, synthesized by C cells in the thyroid gland, inhibits osteoclast-mediated bone resorption. Calcitonin also inhibits the activation of vitamin D and inhibits renal calcium conservation. Altogether PTH-active vitamin D and calcitonin regulate blood calcium levels such that there is little variation in normal individuals.[32] Should blood levels fall below the 2.2–2.5 mmol/L level, calcium tetany will occur. In newly lactating females this is called "milk fever." It can quickly be reversed with an infusion of a calcium lactate or gluconate. Milk fever develops because of a great demand for calcium by the mammary gland. When the demand

exceeds the elasticity of the blood calcium homeostatic system, hypocalcemia results. As mentioned, this is usually temporary and reversible.

Hypocalcemia due to underactive parathyroid glands or to chronic renal failure, and vitamin D deficiency or hypomagnesemia is more difficult to manage because the underlying causes are more difficult. Hormone replacement, renal transplant, or correction of blood magnesium levels are the usual strategies followed. With respect to the low blood magnesium, this is one of the consequences of alcoholism.[33] Excess ethanol intake can interfere with the intake and use of magnesium, which in turn results in a loss of responsiveness of osteoclasts to PTH. This interrupts or interferes with the homeostatic mechanisms needed to control blood calcium levels. The pathways of calcium use are illustrated in Figure 11.7.

Function

Bone mineralization. Virtually 99% of the total body calcium is found in the bones and teeth. This calcium is part of a mineral complex that is deposited on an organic matrix comprised primarily of type I collagen. This collagen has a unique amino acid composition consisting of large amounts of glycine, proline and hydroxyproline.[34] A single molecule of type I collagen has a molecular mass of ~285 kD, a width of ~14 Å, and a length of ~300 Å. There are at least 17 different polypeptides found in collagen. The polypeptides used vary throughout the body, and each collagen uses at least three of them. Collagen is about 30–33% glycine with another 15–30% of the amino acid residues as proline and 3, 4, or 5 hydroxyproline. Collagen is a left-handed triple helix stabilized by hydrogen bonding. These bonds may involve bridging water molecules between the hydroxyprolines. The collagen fibrils are also held together by covalent cross linking. These cross links are between the side chains of lysine and histidine, and the linkage is catalyzed by the copper-dependent enzyme lysyl oxidase. Up to four side chains can be covalently bonded to each other. All in all, this collagen provides a network of fibers upon which the crystals of hydroxy apatite ($Ca_3(PO_4)_3OH$) are deposited. The hydroxy apatite is by no means pure calcium phosphate. Some ions (magnesium, iron, sodium and chloride) are adsorbed on to the surface of the hydroxyapatite crystallites, whereas other ions (strontium, fluoride, and carbonate) are incorporated into the mineral lattice. The presence of these other ions affects the chemical and physical properties of the calcified tissue. Solubility, for example, is decreased when strontium and fluoride ions are present. Hardness is enhanced by the presence of fluoride.

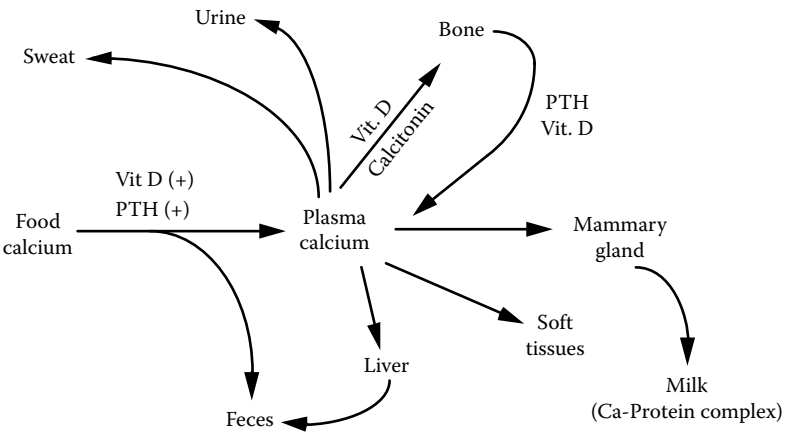


FIGURE 11.7 Pathways of calcium use.

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Bone formation begins in the embryo and continues throughout life. The nature of the process, and the cells involved, changes as the individual ages. Osteoprogenitor cells in early development synthesize the extracellular matrix described earlier and also regulate the flux of minerals into that matrix. As the calcified tissue begins to form, osteoclasts appear on the surface of this tissue and osteoblasts, connected to one another by long processes, are totally surrounded by mineralizing matrix. Each type of mineralized tissue has some unique properties but all share several histologic features in the early mineralization process. Hunziker (1984) has described this process in the epiphysial growth plate during the ossification of the endochondral cartilage as it becomes bone. Initial mineral deposition occurs at discrete sites on membrane-bound bodies (matrix vesicles) in the extracellular matrix. These initial deposits are diffuse and lack orientation. The mineral crystals proliferate and mineralization proceeds, filling the longitudinal but not the transverse septa. Changes in the activities of enzymes that catalyze the hydrolysis of phosphate esters and those that catalyze certain proteolytic reactions follow or accompany this mineralization. These reactions are prerequisite to vascular invasion. Following vascular invasion, lamellar bone is formed by osteoblasts directly on the surface of the preexisting mineralized cartilage. These osteoblasts secrete type I collagen with very little proteoglycan and few extracellular matrix vesicles. This process is repeated over and over until the bone has finished growing. At this point the growth plate closes and the mature length and shape of the bone is apparent. This bone, however, is not at all metabolically inert. It continues to lose and gain mineral matter; that is, it is continuously remodeled through the action of the osteoblasts, which synthesize the collagen matrix, and the osteoclasts, which are stimulated by PTH to reabsorb calcium (and other minerals) in times of need. Although the number of osteoblasts declines with age, the number of osteoclasts increase especially in postmenopausal females. This helps to explain some of the age-related loss in bone mineral that occurs in aging females. Osteoclasts act first during bone remodeling by producing cavities on either the cortical or cancellous (trabecullar) bone surfaces. When these cavities develop, osteoblasts are recruited for bone remineralization, thereby filling (or refilling) the cavities. The bone matrix reforms and remineralizes as described, resulting in new bone formation. The remodeling process is an ongoing one with rates of resorption equaling the rates of new bone formation as long as the hormones controlling each process are in balance and as long as the nutrients needed to support this ongoing process are provided. Figures 11.8 and 11.9 illustrate calcium turnover in bone and the influence of hormones on this process.

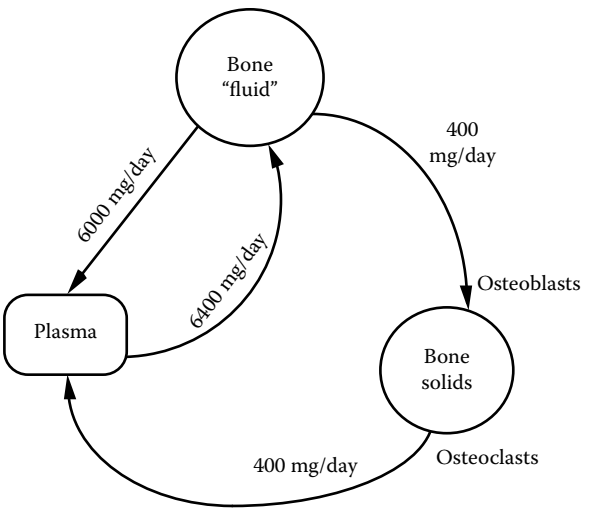


FIGURE 11.8 Calcium turnover in bone.

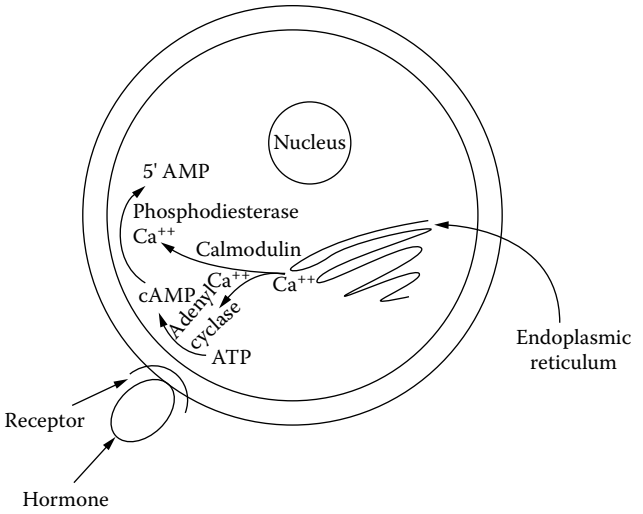


FIGURE 11.9 Calmodulin moves Ca^{++} from the endoplasmic reticulum to serve as a cofactor in reactions catalyzed by phosphodiesterase and adenyl cyclase in the cylic AMP second messenger system.

Bone mass can remain constant for many decades. However, once the hormone balance changes, this constancy changes. In females, bone mass declines by an estimated 1–2% per year after menopause. In senile men, bone mass loss also occurs. In fact, both senile males and females experience about a 1% loss per year. Not only is there a loss in bone mass but there is a loss in structural integrity. The bones lose their mineral apatite and become porous (osteoporosis) and as well lose their architecture upon which the mineral has rested.[35] The very compact cortical portions of the bone disappear, leaving a fragile, largely trabecular bone. The result of these changes is a fragile skeletal system subject to nontrauma-related fracture.

Although the importance of appropriate hormone balance (PTH, calcitonin, active vitamin D, and estrogen) can not be overemphasized, it should also be recognized that dietary calcium (as well as phosphorous and other nutrients) plays an important role in the maintenance of bone mass.[36] Intakes at or exceeding 800 mg/d have been shown to counteract the age-related loss in bone mass.

Cell Signaling

Metabolic regulation and the integration of a variety of metabolic pathways and cell systems depends largely on the communications the cells, and the organelles within cells have, within and between each other. Signaling systems exist that orchestrate this communication. An integral part of these signaling systems is the calcium ion. Although less than 1% of the total body calcium store serves this function, its importance can not be overestimated. The flux of calcium from one compartment to another plays a vital role in metabolic regulation. This flux is facilitated by an intracellular calcium-binding protein called *calmodulin* (Figure 11.10).

Calmodulin contains four Ca^{++}-binding sites with affinities in the micromolar range. It is ubiquitous in all eukaryotic cells and mediates many of calcium's effects. Among these is the activation of phosphodiesterase, a component of the cAMP second messenger system. Phosphodiesterase catalyzes the conversion of cAMP to 5′ AMP and requires calcium as a cofactor. Calcium is translocated from its storage site on the endoplasmic reticulum by calmodulin to the interior aspect of the plasma membrane, whereupon it is released to serve as a cofactor for phosphodiesterase. A similar

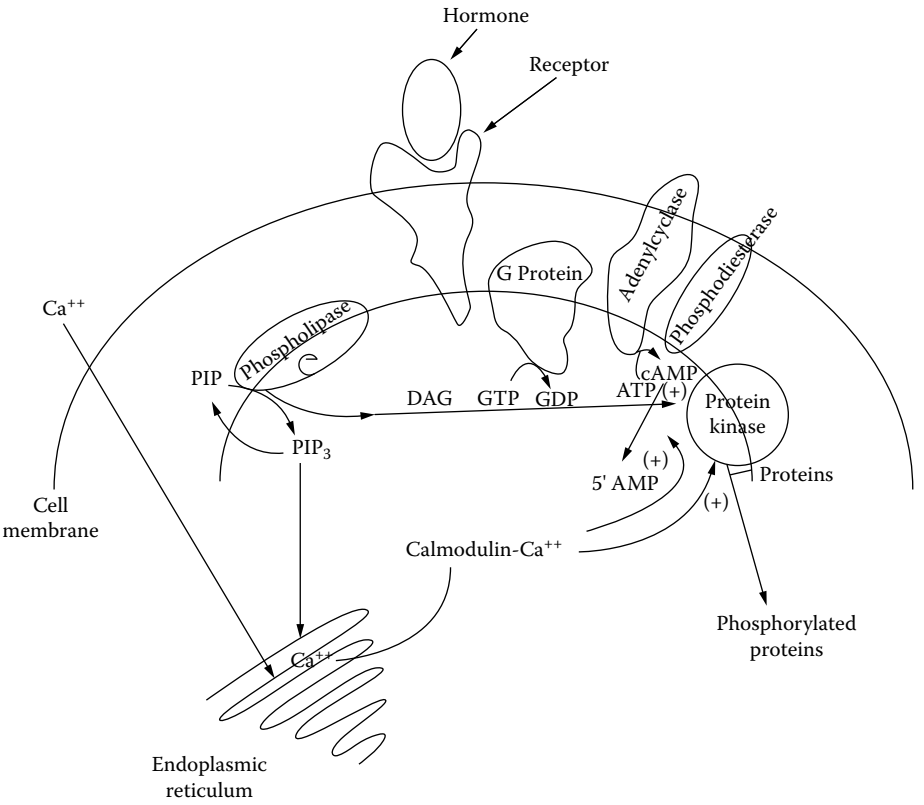


FIGURE 11.10 Integration of cAMP and PIP second messenger systems showing the role of calcium in both.

mechanism exists for the action of calcium in another cell second messenger system, the phosphatidyl inositol system (Figure 11.10). In this instance, phospholipase C, a membrane-bound protein, is activated by the binding of an external compound, a hormone, for example, to its cognate receptor. Phospholipase C catalyzes the release of inositol 1,4,5-phosphate from phosphatidylinositol, one of the plasma membrane's phospholipids. Diacylglycerol and inositol 1,4,5-phosphate then migrate forward into the cytoplasm. DAG binds to protein kinase C and activates it with the help of the calcium ion. Inositol 1,4,5-phosphate in the meantime migrates to the endoplasmic reticulum, stimulating the release of more calcium which in turn further stimulates protein kinase C. Protein kinase C catalyzes the phosphorylation of a variety of proteins. Some of these are enzymes that must be phosphorylated to increase or decrease their metabolic activity, whereas others are necessary for secretion processes (i.e., gastric acid release or hormone release) or for substrate uptake (i.e., glucose transport), or for any of a number of energy-driven processes.

Calcium and the cAMP signaling system, together with the phosphatidylinositol signaling system, have been shown to explain the action of many hormones and cell regulators. Figure 11.11 illustrates these two systems. Angiotensin, cholecystokinin, acetylcholine, insulin-like growth factors, insulin, and glucagon are but a few hormones whose mode of action involves the calcium ion in one or the other of these signaling systems. There is considerable cross talk between these systems as a result of their mutual need for Ca^{++}.[37,38]

In addition to its role in these second messenger systems, Ca^{++} flux between cytoplasm and mitochondria regulates mitochondrial activity. Ca^{++} uptake by mitochondria is energetically less

demanding than its export.[26] Uptake followed by active export is an oscillatory process. Ca^{++} flows in until a 200 μM concentration is reached, whereupon it is actively pumped out using the energy of ATP. This active system costs about 63% of the ATP hydrolysis energy. There are three separate Ca^{++} transport mechanisms at work (Figure 11.11). One is the calcium uniporter. The uniporter rapidly sequesters external Ca^{++} into the mitochondrial matrix and in a similar fashion also sequesters Fe^{++}, Sr^{++}, Mn^{++}, Ba^{++}, and Pb^{++}. (Mg^{++} uptake is much slower than Ca^{++} and probably is not mediated by this uniporter system.) The other two Ca^{++} transport mechanisms facilitate Ca^{++} efflux rather than influx. There is a sodium-independent and a sodium-dependent mechanism. The latter is more powerful of the two for facilitating Ca^{++} efflux. Its velocity is much higher than the sodium-independent mechanism. Altogether these three mechanisms function to control Ca^{++} flux and hence mitochondrial metabolism, particularly oxidative phosphorylation.

The mitochondria Ca^{++} cycle is designed to regulate intramitochondrial Ca^{++} levels and to relay changes in cytosolic Ca^{++} to the mitochondrial matrix. Surges in cytosolic Ca^{++} via the cell signaling systems activate a variety of ATP-requiring reactions. In turn, as ATP is used, its metabolic end product, ADP or AMP, is transported back into the mitochondria, whereupon it is rephosphorylated and exported (along with the Ca^{++}) to the cytosol. This multifaceted system provides flexibility and responsivity to changing cellular environments and has considerable control strength with respect to the balance of catabolic and anabolic metabolic pathways. In this instance, Ca^{++} is more than a mere signal; it is a key element in metabolic control. Shown in Table 11.10 are some of the many reactions or reaction sequences that are activated or suppressed by Ca^{++}.

Calcium and Cell Death

Just as Ca^{++} is important to the regulation of cell metabolism, it has another role: it can mediate the death of a cell or a group of cells.[39,40] When the integrity of the cell's membrane is breached either through injury, sepsis, chemical insults, or anoxia, the normal flux of Ca^{++} from storage depots to sites of use and return is interrupted. Just as the extracellular Ca^{++} is carefully regulated so as not to exceed that very narrow range of 2.2–2.5 mmol, so, too, is the intracellular Ca^{++} concentration. As described, Ca^{++} flows into the mitochondria but is actively exported. If the injury to the cell interrupts this active, energy-driven export, the Ca^{++} will continue to flow into the mitochondria and raise the ionic concentration of the mitochondrial matrix. This has negative effects on oxidative

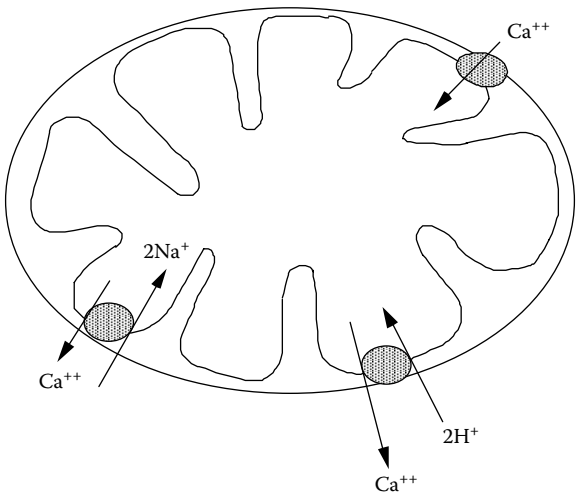


FIGURE 11.11 Ca^{++} flux in the mitochondrial compartment.

TABLE 11.10
Ca^{++} as a metabolic regulator: reactions or reaction sequences stimulated by Ca^{++}

Fatty acid oxidation
Amino acid transport into hepatocytes
Citric acid cycle (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase)
Pyruvate dehydrogenase
ATP-Mg/Pi carrier (mitochondrial carrier)
Glucose-stimulated insulin release
Phosphodiesterase
Stimulation of olfactory neurons
Trypsinogen conversion to trypsin
Pancreatic α-amylase activation
Pancreatic phospholipase A_2
Hydrolysis of troponin in muscle to tropomyosin
Phospholipase C
Blood clotting (binding of calcium to GLA rich proteins)

phosphorylation, and the stage is set for a downward spiral towards mitochondrial dysfunction. Ca^{++} influx into the cytosol as well as the nucleus also occurs because it is downward energetically under these conditions. When sufficient calcium accumulates, the cell dies. Although the loss of one cell is not devastating, the loss of many cells can be, and is. Of particular concern is the accumulation of Ca^{++} by interconnected muscle such as the heart. Muscle contraction is a Ca^{++}-mediated event. Ca^{++} flows into the myocyte mitochondria and is pumped out using ATP. If Ca^{++} accumulates in the myocyte and is not actively pumped out, the myocyte dies.

Counteracting this Ca^{++} movement in cells under duress is a class of pharmaceutical agents called *calcium blockers*. These drugs postpone cell death by interfering with Ca^{++} influx. Since their discovery, they have been very useful in the management of hypertension and heart disease, and have been found useful in several other settings. Calcium ionophores, agents that facilitate Ca^{++} flux, have also been discovered. The most widely used one is A23187. Two molecules of this compound surround Ca^{++} and because the agent is lipophillic, it is able to passively cross the plasma membrane and deliver the Ca^{++} to the cytosol.

Other functions of calcium, particularly those relating to clot formation and embryonic development, have been discussed in the sections on vitamin K and vitamin A, respectively. In these sections the synthesis of specific proteins that bind calcium and that perform specific roles in the processes of interest are described.

Muscle Contraction

The muscle cell has unique calcium storage sites called the sarcoplasmic reticulum. This reticulum is similar to that found in other cell types but is highly specialized. It contains ribosomes and many of the same enzymes found in the endoplasmic reticulum of other cell types but in addition also contains large quantities of calcium-activated ATPase. Approximately 75% of the sarcoplasmic reticulum is this ATPase. The ATPase also requires magnesium, so its name indicates this: $Ca^{++}Mg^{++}$ATPase. It serves as a calcium pump using the energy released by ATP to drive the calcium ion from the cytosol to the sarcoplasmic reticulum. There the calcium resides until needed for muscle contraction. Upon receipt of a signal to contract, a Ca^{++} channel is opened, and Ca^{++} flows

into the cytoplasm of the muscle cell where it binds to troponin, a contractile protein of skeletal and cardiac muscle. This protein is a long rod-like structure that extends over the length of the muscle fiber. When Ca^{++} binds to it, it changes its shape, becoming shorter. The muscle cell contains two other filaments, actin and myosin, which interact when troponin shortens due to Ca^{++} binding. When troponin is in the relaxed state these two filaments are too far apart to interact.

Muscles are signaled to contract by a wave of depolarization–repolarization flowing down the muscle fiber from its point of contact at the neuromuscular junction. During depolarization of skeletal muscle, extracellular Na^{+} flows into the cell and potentiates the Ca^{++} release from the sarcoplasmic reticulum. In the heart muscle, with its slightly different muscle fiber organization the signal for contraction is generated by the AV sinus node on the right side of the heart. This signal is regularly spaced and results in depolarization–repolarization, just as happens in skeletal muscle. However, with depolarization Ca^{++} flows into the cytosol from the extracellular fluid as well as from the sarcoplasmic reticulum. This has the result of increasing the strength of the contraction because more calcium is present. During repolarization Ca^{++} is then pumped, via the Ca^{++} Mg^{++} pump, back into the sarcoplasmic reticulum. In heart muscle there are many more mitochondria providing ATP than are found in skeletal muscle, hence the need for Ca^{++} by the heart muscle is greater than that of skeletal muscle.

In contrast to the heart and skeletal muscle, smooth muscle does not contract and relax strongly. These muscles can sustain contraction for a longer period of time and are far less dependent on calcium for contractile strength. Rather, the chief role of calcium in this muscle type is that of serving in the various hormone-mediated cell signaling systems.

Calcium Deficiency

Considering the vital role of vitamin D and other micronutrients in determining calcium status, it is truly difficult to produce a "pure" calcium deficient state.[41] Calcium deficiency does indeed occur but it is usually due to other factors: lack of vitamin D activation, loss of estrogen production, adrenal dysfunction, parathyroid gland dysfunction, and so forth. If any of these conditions develop, then signs of calcium deficiency occur. These signs include inadequate bone calcification and growth in children (rickets) and weak porous bones in adults (osteoporosis). Rickets characterized by malformed poorly calcified bones is more of a disease of inadequate vitamin intake than one of inadequate calcium intake. Should blood calcium levels fall acutely, calcium tetany will result and unless calcium is provided quickly by the intravenous route, death will ensue.

Need

Because calcium absorption is dependent on so many different factors, there has been vigorous discussion among experts as to what the recommended intake of calcium should be. Acceptable intakes have been devised (www.nap.edu, Table 11.1) and these figures should be used as guidance for intake targets. In addition there are a number of dietary factors (excess protein, calcium: phosphorus ratio, calcium:magnesium ratio, oxalates, phytates) that increase calcium loss from the system and these are hard to quantitate such that an age-appropriate recommendation can be made. Several diseases stem from or affect calcium use, and these must be considered. For example, hypertension has been linked to inadequate milk-product calcium intake.[42,43] Critical experiments that would irrefutably support such a linkage have yet to be conducted; nonetheless, there are suggestions arising from population studies that those who consume calcium by way of dairy foods have less hypertension than those who avoid dairy foods. Follow-up studies in hypertensive rats have shown that increasing dietary calcium reduces the hypertensive state.

As mentioned, vitamin D and the estrogen status affect calcium use. Increasing the calcium intake can compensate for a reduction in absorption efficiency, and thus the postmenopausal female could benefit from the increased calcium intake. More recently, it has been noted that

postmenopausal women given calcium and vitamin D supplements have a reduced risk of postmenopausal weight gain.[44] The relationship between obesity or weight gain and dietary calcium intake, as well as the relationship between calcium and insulin resistance, is an interesting consideration with respect to calcium need. Pittas et al.[45] reviewed the role of vitamin D and calcium in Type 2 diabetes. They used a meta analysis to systematically review a variety of studies of people with diabetes. They concluded that vitamin D and calcium insufficiency may have a negative impact on glucose homeostasis. Results of the CARDIA study[46] showed that dietary patterns characterized by increased dairy consumption (increased vitamin D and calcium with increased milk consumption) had a strong inverse association with insulin resistance in young adults who were overweight. This same dietary characteristic has also been reported to reduce obesity and to have a beneficial effect on weight loss by nondiabetic overweight and obese subjects[47-50] and genetically obese mice.[51] The mechanism of action of vitamin D and calcium in the problem of excess fat stores has yet to be elucidated; however, it is apparent that hypocaloric diets that are used to promote fat loss and that include calcium–vitamin-D-rich foods have positive effects on weight loss.

Note in Table 11.1 that older people have a larger DRI for calcium than young adults. In part this may be an indirect indication that increased calcium intakes could ameliorate the age-related increase in fat accumulation and its effect on Type 2 diabetes. It could also be a compensatory dietary recommendation for the older adult who often becomes more sedentary later in life than in the younger years. Physical activity influences calcium turnover; physical activity increases calcium retention. Bedridden individuals tend to lose calcium whereas the person who maintains a moderately active lifestyle optimizes his/her calcium retention.

PHOSPHORUS

Calcium and phosphorus are essential minerals that are usually considered together because the formation of bone and the uptake of calcium for this purpose is closely tied to an optimal ratio of calcium:phosphorus of 1:2 to 2:1.[36] However, phosphorus, as does calcium, has other functions in addition to bone formation. Because bone formation was discussed in the preceding section concerning calcium, this section will present information about other aspects of the need for phosphorus.

Phosphorus is a member of Group V in the 4th period of the periodic table. It has an atom number of 15 and an atomic weight of 31. There are no heavy isotopes but there are some useful radioisotopes. ^{32}P is the radioisotope most frequently used in biological systems. It has a short half-life (14.3 d).

Little free phosphorus is found in the living body.[52] Most phosphorus is in the form of phosphate PO_4^-. Phosphate is comprised of a central atom of phosphorus surrounded by four atoms of oxygen. At pH 7, hydrogen is joined to the phosphorus and oxygen to form HPO_4^-. At low pH it is phosphoric acid, H_3PO_4. Other phosphates include H_3PO_4, H_2PO_{4-}, and PO_4^-. Phosphate in the free form is called *inorganic phosphate* with the abbreviation Pi. Phosphates are widely distributed in nature and, thus, a deficiency due to inadequate intake is highly unlikely. Soft drinks, processed foods, and foods of animal origin are excellent sources of phosphate. The usual intake of humans consuming a mixed diet is about 1 g per day for females and 1.5 g per day for males. Even though a deficiency is unlikely, a daily DRI very close to that of calcium is available as shown in Table 11.1. Symptoms of deficiency have been observed in premature infants fed a low phosphorus milk.[53,54] These symptoms include anorexia, muscle weakness, rickets, impaired growth, and bone pain. Adults consuming large amounts of aluminum oxide antiacids and a low phosphate diet can also develop some of these same symptoms.[54] Rickets, of course, is only seen in the young. There is a genetic disease (Fanconi syndrome) related to phosphate deficiency carried on the X chromosome that phenotypes as phosphate deficiency. These symptoms can be ameliorated by phosphate supplements, and it is clear from the biochemical studies made using tissues from these patients that many of their symptoms are due to phosphate-related deficits in intermediary metabolism. The bone pain and poor skeletal growth and mineralization are due to lack of phosphate and hence hydroxyapatite for deposition in

the bone matrix. Phosphates are readily absorbed with little loss except that which is tightly bound in indigestible portions of food. Phytic acid, a hexose containing 6-phosphate groups, a component of some plant foods, can form insoluble salts with calcium, magnesium, and iron, rendering these minerals unavailable for absorption. However, there is a phytase lower in the intestinal tract that will dephosphorylate phytic acid, and when this happens, these minerals are released. Usually this happens too far away from their absorption sites to be of benefit.

Phosphorus as phosphate is readily absorbed via an active saturable sodium-dependent mechanism. Calcitriol (active vitamin D) facilitates the absorption as it facilitates calcium uptake. Of the total body pool of phosphorus (~750 g), ~600 g is found in the bones and teeth. One gram is found in the extracellular compartment whereas the remainder (~150 g) is found within the cells. Urinary excretion is the route for excretion although up to 25% of the food phosphorus can appear in the feces. Renal conservation of phosphorus is the main mechanism for phosphorus homeostasis. Vitamin D, glucocorticoids, and growth hormone enhance, whereas estrogen, thyroid hormones, parathyroid hormones, and elevated plasma Ca^{++} levels inhibit renal phosphate conservation.

Function

A principal use of phosphorus is as the anion in hydroxyapatite used in bone mineralization. However, more important, is the role of phosphate in intermediary metabolism. It is a crucial part of the genetic material DNA and RNA, a part of phospholipids, phosphoproteins, the adenine nucleotides (ATP, ADP, AMP), guanine nucleotides (GTP, GDP, GMP), and of the second messenger systems, and plays a critical role in all anabolic and catabolic pathways. In the genetic material the purine and pyrimidine bases are linked together by deoxyribose or ribose and phosphate groups (see Chapter 5 for structure).

In this structure the phosphate groups serve as the links between the bases and, because of the polarity of the phosphate group, serve to stabilize the structure. The DNA polymer is a very stable structure under nonenzymatic conditions. It owes its stability to the fact that the phosphate group can bind the bases yet still have sufficient polarity to retain a negative charge, repelling other negatively charged molecules such as peroxides. Of course, the phosphate group is not the only negatively charged group in the DNA molecule. Hydrogen bonding leaves some atoms vulnerable to attack but, overall, the phosphate group is the important group when nonenzymatic degradation is considered.

The ATP molecule (Figure 11.12) is another important use of phosphate. The bonds that hold the phosphate groups to the ribose and thence to the adenine are called *high-energy bonds* represented by the symbol ≈. When broken, these bonds release about twice the energy of a normal bond. As such, ATP and its related high-energy compounds GTP and CTP, are the keystones of energy transfer from one metabolite to another. A full discussion of ATP synthesis and degradation can be found in the section on energy in Chapter 1. The phosphorylation of intermediates in both catabolic and anabolic pathways are important uses of phosphate. Again because of its unique structure, it provides a necessary electronegative charge that in turn creates a vulnerable position in the molecule to which it is attached. For example, glucose could not be metabolized to pyruvate unless it was phosphorylated. The glucokinase (or hexokinase) using the high-energy phosphate group from ATP phosphorylates glucose at carbon #6. In so doing, the molecule becomes somewhat unstable, such that in the next few steps it can be rearranged to form fructose (a 5-member ring) 6-phosphate. This structure now has a vulnerable carbon at position 1, and the next step is to attach another phosphate group at position 6. With two strong electronegative centers, one at each end of the molecule, this 6- carbon structure can be split in half to two phosphorylated 3-carbon structures, and on it goes. The phosphate group is reused over and over, and in each use it provides a means for subsequent reactions in metabolism, only to be discarded by the resultant metabolic product. Its highly charged structure thus is its reason for use and reuse.

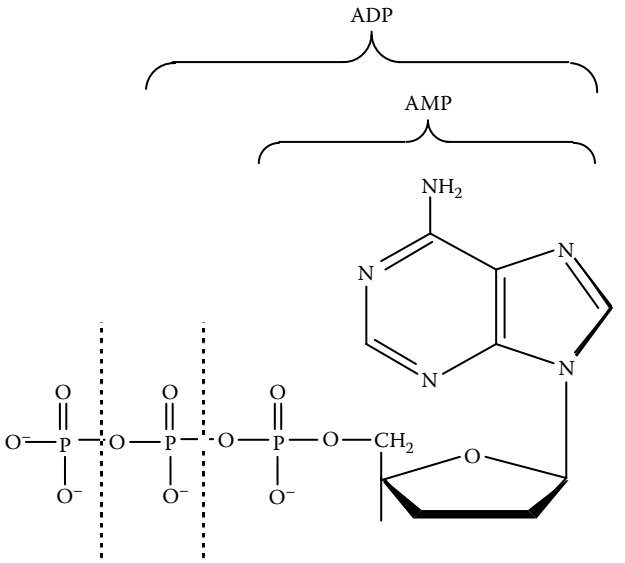


FIGURE 11.12 Structure of ATP. ADP has two phosphate groups, whereas AMP has only one.

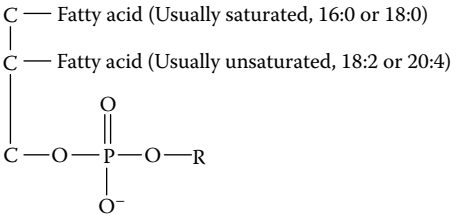


FIGURE 11.13 Structure of a phospholipid.

Use and reuse also applies to the role of phosphate in the structure and function of the membrane phospholipids. Phospholipids have both a lipophilic and a lipophobic portion in their structure. The lipophilic portion (see Figure 11.13) is the fatty acid portion, whereas the phosphatidylated compounds provide the lipophobic (or hydrophilic) portion. This structure is absolutely required for the functional attributes of the membranes. The membranes serve as geographical barriers around the cells and organelles. As such, some materials are refused entry into the cell or organelle, whereas other materials are either embedded in these phospholipids and held there or are allowed to pass into or out of the cell or organelle. Lipid soluble materials can diffuse through the lipophilic portion of the phospholipid, whereas lipophobic materials are excluded unless carried through by one of the embedded proteins. The charge contributed by the phosphate group in the phospholipid is what holds the proteins in place and which in turn functions to permit entry or exit of lipophobic metabolites and substrates. It should be noted, however, that highly charged metabolites such as the phosphorylated intermediates do not cross membranes. They are repelled by the phosphate group of the phospholipids.

From this discussion of the function of phosphorus in living systems, it is easy to understand why phosphorus is so widely distributed in the food supply. Every living thing must have phosphorus in its cells or it would not survive. It is as essential to life as oxygen, carbon, and nitrogen.

MAGNESIUM

Magnesium is an alkaline earth metal with an atomic number of 12 and an atomic weight of 24. It is in group II of the 3rd period of the periodic table. Magnesium has two naturally occurring isotopes, ^{25}Mg and ^{26}Mg and seven radioisotopes. ^{28}Mg is the most commonly used radioisotope with a half-life of 21 hours.

Magnesium is the most abundant divalent cation in living systems. As such its distribution in the food supply is broad. Both vegetables and meats are good sources of magnesium, whereas milk and milk products are relatively poor sources of this mineral. Magnesium stabilizes mammalian membranes, and in plants this mineral is ionically bound in the center of the chlorophyll molecule. In addition, magnesium is a cofactor in almost all phosphorylation reactions involving ATP. Because of the universality of its presence in the food supply, deficiency states are unlikely to develop in persons consuming a variety of foods.

Absorption, Metabolism, Excretion

Magnesium is absorbed by both passive diffusion and active transport. Although there are two systems for Mg^{++} uptake, neither is particularly efficient. Between 30 and 70% of that consumed in food is absorbed. When food is supplemented with Mg^{++}, the percent absorbed falls. Thus, a meal containing 40 mg in food will result in 28 mg actually entering the enterocyte and appearing in the blood, whereas in an enriched Mg^{++} containing meal (40 mg + a 920 mg magnesium salt) only 11–14% will be absorbed, or 105–134 mg. The usual ~300 mg Mg^{++} intake has an apparent absorption of about 100 mg, a 33% efficiency.

Magnesium is recirculated via bilary secretion into the intestinal contents. The recirculated mineral can be reabsorbed and, in times of need, this reabsorption can be very efficient. If not reabsorbed, this magnesium will be excreted via the feces. The usual excretory route for absorbed magnesium is via urine. In fact, the renal absorption mechanism is the main means for regulating magnesium status. About 100 mg of magnesium is lost via the urine per day by normal adults consuming about 300 mg/d in the food. In times of need, magnesium reabsorption by the renal tubules will occur, and urinary magnesium levels will fall. In the deficient state the level will fall to zero as the deficiency proceeds.

After magnesium is absorbed, it circulates throughout the body; about 30–35% of the circulating magnesium is protein-bound, whereas the remaining circulates as magnesium salts (13% as citrate or phosphate complexes) or as free magnesium (~55%). Magnesium is principally an intracellular ion occupying a central role in intermediary metabolism, as described in the next section.

Function

More than 300 metabolic reactions require magnesium as a cofactor. The role of magnesium is one of forming a labile association with a substrate, allowing the enzyme to complex with it; once a product is formed, the magnesium and the enzyme separate for further use. This is illustrated in Figure 11.14.

Mg^{++} reduces the high negative charge of the substrate (usually ATP) by chelate formation with two phosphate groups (the β and γ P). The adenine ring is not involved. Actually, this coupled reaction sequence occurs concurrent with the transfer of the liberated phosphate group to another substrate such as glucose. It is facilitated by the enzyme hexokinase or glucokinase. Any kinase, however, will use this same reaction sequence as will a number of other reactions involving a

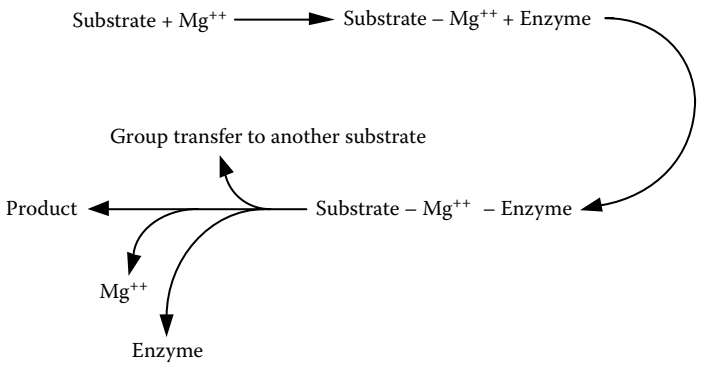


FIGURE 11.14 Use of magnesium as a cofactor in metabolic reactions.

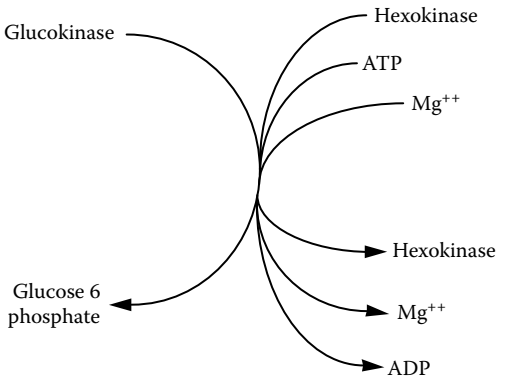


FIGURE 11.15 Role of magnesium as a cofactor in the phosphorylation of glucose.

cleavage of high-energy bond and the use of that energy to activate via attachment of a reactive electronegative group to a formerly inactive metabolic substrate or intermediate. Thus, in the instance of the phosphorylation or glucose, the coupled reaction is represented in Figure 11.15.

Some coupled reactions use more than one Mg^{++}. Enolase for example uses 4 Mg^{++}. The Mg^{++} ion binds to enolase, activating it by keeping it in its active conformation. Magnesium is probably binding to the sulfur group of sulfur containing amino acids in the enzyme structure, thus favoring the reaction by polarizing the P-O bonds. Conformational change likely explains the Mg^{++} in many of the enzymes in which it is a required cofactor for activation. In contrast, Mg^{++} can be an inhibitor; it can bind with tyrosyl residues. If these residues are part of the active site of an enzyme, then the enzyme will be less active. Actually, the binding of Mg^{++} to the tyrosyl residue of albumin accounts for its transport in the blood. Much (32%) of the magnesium in the blood is carried by albumin.

Phospholipids form complexes with both Mg^{++} and Ca^{++}. These phospholipid complexes are integral parts of the various membranes in the cell (plasma membrane, endoplasmic reticulum, mitochondrial membrane, and nuclear membrane). The degree to which these minerals are held by the membrane depends on the type of phospholipid in that membrane. The phospholipids are not uniformly distributed. The plasma membrane, for example, has very little cardiolipin, whereas the mitochondrial membrane has very little phosphatidyl serine (see lipids, Chapter 8). Highly negative

phospholipids will attract and bind more Ca^{++} and Mg^{++} than phospholipids having a lesser charge. In addition, Ca^{++}, due to its stronger 2^{++} charge, will be more attracted than will Mg^{++}. Nonetheless, the attraction of these minerals lends a stabilizing effect to an otherwise labile and mobile membrane constituent.

Bone accounts for 60–65% of the total magnesium in the body. It is deposited in the bone matrix along with calcium phosphate as part of the mineral apatite. Hormones which affect calcium deposition and mobilization also affect magnesium deposition and mobilization.

Deficiency

Deficiency states have been produced in animals under stringent magnesium exclusion conditions. As early as 1926, mice were shown to develop magnesium deficiency using a magnesium-deficient diet. Cattle, and later rats, were also made deficient. The first clinical evidence in humans appeared in 1934, and subsequent studies have shown that persons experiencing prolonged malabsorption, renal dysfunction or failure, alcoholism, and a number of endocrine disorders can become magnesium deficient.[33,55–59] Magnesium deficiency is characterized by very low blood levels of magnesium and neuromuscular symptoms such as muscle spasms, twitching, muscle fasciculations, tremor, personality changes, anorexia, nausea, and vomiting. Acutely deficient individuals may have convulsions and lapse into coma. In almost every instance of clinically evident magnesium deficiency there has been a clinically important predisposing condition as cited earlier. Marginally deficient states have also been suggested in patients with normal serum or blood levels but depleted tissue levels of magnesium. In these patients the symptoms are varied and it may be difficult to assign magnesium deficiency as their cause unless tissue samples (muscle) are obtained and assayed. A positive magnesium balance of 1 mEq/kg can be demonstrated in such individuals. Persons at risk are those with diabetes, renal disease, or malabsorption syndrome which, in turn, reduces magnesium conservation in the former and decreases magnesium absorption in the latter. Chronic ulcerative colitis and chronic granulomatous enteritis are the most common causes of the diarrhea of malabsorption syndrome. Other causes are gluten-induced enteropathy, celiac disease, and unrecognized lactose intolerance. Frequently, magnesium deficiency is accompanied by hypocalcemia. This appears to be due to a failure of bone to exchange calcium for magnesium. The osteoclast receptor for PTH loses its responsiveness to this hormone, with the resultant loss in active bone resorption. This means that there is a failure in the system used to maintain blood calcium levels and also that magnesium is a key feature in the PTH-calcium regulatory pathway. Once magnesium is restored, the sensitivity of the osteoclast receptor to PTH is restored, and the serum calcium returns to normal levels.

Magnesium deficiency also has effects on the vitamin-D-calcium relationship. Vitamin D (1,25-dihydroxycholecalciferol) is not as active in promoting intestinal calcium uptake in the absence of magnesium. Even though neither ion is absorbed very efficiently, this efficiency is further reduced when one or the other is absent or in short supply. Both are poorly absorbed in vitamin-D-deficient states because vitamin D mediates the uptake of both.

The use of mercurial or thiazide diuretics in the management of hypertension can result in excess magnesium loss. These diuretics interfere with renal magnesium conservation. They also enhance potassium loss as do some other drugs (e.g., furosemide, ethacrynic acid, goutamicin, cabenicillin, cisplatin, and amphotericin B). Many of these drugs are cytotoxic drugs used in cancer chemotherapy and, as such, are used over a short time span. Although minerals are lost through the use of these drugs, they can be replaced from the body store or through supplementation. Many physicians using these therapies may elect to supplement the patients' diet after the chemotherapeutic regimen is complete, anticipating that the cancer cell also needs magnesium. Chemotherapy consists of using highly toxic materials, anticipating that the fast-growing cancer cell will be more likely to take up the drug and die than will the normal cell. Part of the drug action is to interfere with cell replication which, in turn, requires magnesium. If the cancer cell becomes magnesium-deficient then the drug will be a more effective chemotoxic agent.

Magnesium deficiency in rats has been shown to result in elevated blood lipids as well as proliferation of smooth muscle cells.[55] These responses are key elements in the atherogenic process, and it has been proposed that a relative magnesium deficiency in humans could pave the way for atherosclerosis or other degenerative diseases. However, other studies of alcoholism, renal disease, and various endocrinopathies have shown that these conditions must be in place prior to the development of magnesium deficiency. Thus, atherosclerosis could occur as a secondary complication of alcoholism or renal disease or diabetes, etc., and this secondary complication might develop as a result of an induced magnesium deficiency.

Need

As mentioned, magnesium is widely distributed in the food supply, and thus a deficiency in an individual having access to a variety of foods is unlikely. Nonetheless, the Food and Nutrition Board of the National Academy of Sciences has recommended intakes based on age and gender of 40 to 400 mg/d as detailed in Table 11.1. As mentioned in the section on deficiency, certain predisposing diseases may affect either magnesium absorption or reabsorption by the kidney. In these circumstances, magnesium status may be negatively affected and an intake above that recommended as desirable may be needed. Assessment of that need, however, is imperative before an increased intake is undertaken. Excess intake can be toxic with symptoms similar to those of uremia (nausea, vomiting, hypotension). Other responses to excess intake include changes in heart action (bradycardia, vasodilation) and CNS function.

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12 Trace Minerals

In addition to the minerals already described as members of the macromineral class of nutrients, there are two groups of minerals needed in far smaller amounts.[1,2] These groups fall into the general class of microminerals. One of these is the trace mineral group that includes iron, copper, and zinc, whereas the other group, the ultra trace minerals, includes chromium, manganese, fluoride, iodide, cobalt, selenium, silicon, arsenic, boron, vanadium, nickel, cadmium, lithium, lead, and molybdenum. Dietary Reference Intakes (DRI) or suggested Adequate Intakes (AI) have been made for iron, zinc, iodide, chromium, fluoride, selenium, manganese, and molybdenum (http://www.nap.edu). With some of these minerals there are recommendations for intakes for some of the population groups but not for all. There are insufficient data for intake recommendations for silicone, sulfate, vanadium, potassium, arsenic, and chromium. Table 12.1 gives the recommended intakes (in bold type) and suggested adequate intakes (indicated by an asterisk*). For some of the minerals an excess intake could be toxic. For these minerals, an upper intake limit has been set that defines the upper intake at which no adverse effects would be expected. Thus, the National Academy of Sciences Food and Nutrition Board has developed a table of Tolerable Upper Limits (TUL or UL). These are shown in Table 12.2. Tolerable upper limits also exist for nonmineral nutrients as discussed in earlier chapters.

ESSENTIAL MICROMINERALS

The definition of essentiality for the trace and ultra trace minerals is different from that used for amino acids, vitamins, and essential fatty acids. That difference is due to the problem of not having clear, incontrovertible evidence of the essentiality of each of these nutrients.[2] A mineral is thought to be essential if its lack adversely affects an essential biological function that can be reversed by replacement of that mineral in the diet. A mineral is also considered essential if its lack interrupts an essential phase of the life cycle, for example, the completion of a normal pregnancy. The latter definition has been disputed, however, because not all trace minerals with a defined biochemical function interfere with the completion of a normal pregnancy. Lastly, some of the trace minerals become essential only when there is some other disturbance in the normal physiology of the individual. In these instances there might be genetic diseases that interfere with the absorption or transport of a given mineral, or there might be some disease that interferes with the use of that mineral. An example of the latter is seen in pernicious anemia. In this disease the individual lacks the intrinsic factor needed for the absorption of vitamin B_{12}. Cobalt is an essential component of this vitamin and constitutes 4% of its molecular weight. If the vitamin is not absorbed, then cobalt is not absorbed.

Almost all of the trace and ultra trace minerals can be found in the bones and teeth. These minerals are deposited in the organic matrix of these structures along with calcium phosphate. Bone mineralization (the formation of the bone apatite) involves many of the trace and ultra trace minerals known to be consumed by man and other animals. These minerals can be mobilized from the bones, but the extent of this mobilization is variable. To a large degree, the mobilization of the trace elements is very slow. This results in a long residence time and long half-life. When coupled with a low absorption efficiency,[3] this long residence time is part of a system designed to protect the body from excess mineral exposure and mineral toxicity. For many of the trace elements, their

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TABLE 12.1
Daily Dietary Reference Intakes (DRI) for iron, zinc, iodide, selenium, chromium, copper, fluoride, manganese, and molybdenum

Group	Age	DRI			
		Iron mg	Zinc mg	Iodide µg	Selenium µg
Infants	0–6 months	0.27*	2*	110*	15*
	7–12 months	**11**	**3**	130*	20*
Children	1–3 years	**7**	**3**	**90**	**20**
	4–8 years	**10**	**5**	**90**	**30**
Males	9–13 years	**8**	**8**	**120**	**40**
	14–18 years	**11**	**11**	**150**	**55**
	19–30 years	**8**	**11**	**150**	**55**
	31–50 years	**8**	**11**	**150**	**55**
	50–70 years	**8**	**11**	**150**	**55**
	70 + years	**8**	**11**	**150**	**55**
Females	9–13 years	**8**	**8**	**120**	**40**
	14–18 years	**15**	**9**	**150**	**55**
	19–30 years	**18**	**8**	**150**	**55**
	31–50 years	**18**	**8**	**150**	**55**
	51–70 years	**8**	**8**	**150**	**55**
	70+ years	**8**	**8**	**150**	**55**
Pregnancy	14–18 years	**27**	**12**	**220**	**60**
	19–30 years	**27**	**11**	**220**	**60**
	31–50 years	**27**	**11**	**220**	**60**
Lactation	14–18 years	**10**	**13**	**290**	**70**
	19–30 years	**9**	**12**	**290**	**70**
	31–50 years	**9**	**12**	**290**	**70**

	Chromium µg	Copper µg	Fluoride mg	Manganese mg	Molybdenum µg
Infants, 0–6 months	0.2*	200*	0.01*	0.003*	2*
Infants, 7–12 months	.5*	20*	.5*	.6*	3*
Children, 1–3 years	11*	**340**	0.7*	1.2*	**17**
4–8 years	15*	**440**	1.0*	1.5*	**22**
Males, 9–13 years	25*	**700**	2.0*	1.9*	**34**
14–18 years	35*	**890**	3.0*	2.2*	**43**
19–30 years	35*	**900**	4.0*	2.3*	**45**
31–50 years	30*	**900**	4.0*	2.3*	**45**
51–70 years	30*	**900**	4.0*	2.3*	**45**
70+ years	30*	**900**	4.0*	2.3*	**45**

TABLE 12.1 (continued)
Daily Dietary Reference Intakes (DRI) for iron, zinc, iodide, selenium, chromium, copper, fluoride, manganese, and molybdenum

	Chromium	Copper	Fluoride	Manganese	Molybdenum
	µg	µg	mg	mg	µg
Females, 9–13 years	21*	**700**	2.0*	1.6*	**34**
14–30 years	24*	**890**	3.0*	1.6*	**43**
31–50 years	25*	**900**	3.0*	1.8*	**45**
51–70 years	20*	**900**	3.0*	1.8*	**45**
70+ years	20*	**900**	3.0*	1.8*	**45**
Pregnancy					
14–18 years	29*	**1,000**	3.0*	2.0*	**50**
19–30 years	30*	**1,000**	3.0*	2.0*	**50**
31–50 years	30*	**1,000**	3.0*	2.0*	**50**
Lactation					
14–18 years	44*	**1,300**	3.0*	2.6*	**50**
19–30 years	45*	**1,300**	3.0*	2.6*	**50**
31–50 years	45*	**1,300**	3.0*	2.6*	**50**

Figures in bold type are the DRIs whereas the figures followed by an asterisk (*) are those of adequate intake. The latter are extrapolated figures and are based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate. These figures are used when the DRIs cannot be determined directly. For further information see: http://www.nap.edu

TABLE 12.2
Daily tolerable upper limits of copper, fluoride, iodide, iron, and magnesium

Group	Copper	Fluoride	Iodide	Iron	Magnesium
	µg	mg	µg	mg	mg
Infants					
0–6 mo	ND	0.7	ND	40	ND
7–12 mo	ND	0.9	ND	40	ND
Children					
1–3 years	1,000	1.3	200	40	65
4–8 years	3,000	2.2	300	40	110
Males and females					
9–13 years	5,000	10	600	40	350
14–18 years	8,000	10	900	45	350
19–70 years	10,000	10	1,100	45	350
70+ years	10,000	10	1,100	45	350
Pregnancy					
14–18 years	8,000	10	900	45	350
19–50 years	10,000	10	1,100	45	350
Lactation					
14–18 years	8,000	10	900	45	350
19–50 years	10,000	10	1,100	45	350

Source: www.nap.edu

discovery as essential nutrients was preceded by their recognition as toxic elements. A summary of the functions of the trace and ultra trace minerals is provided in Table 12.3.

TRACE MINERAL TOXICITY

Inadvertent exposure to a variety of minerals, whether it be via inhalation, absorption through the skin, or ingestion with food or drink, can elicit a toxic response. Some of the essential minerals can be toxic if subjects are exposed to high quantities. As indicated in Table 12.2 there are tolerable upper limits to these minerals that if exceeded might elicit an undesirable set of symptoms. There are other minerals whose essentiality for humans has yet to be proven. Some of these have been shown to be needed by other species. As with the essential minerals, excess exposure can elicit an adverse (toxic) response. Minerals in this category include arsenic, vanadium, nickel, silicone, tin, aluminum, boron, cadmium, lead, germanium, lithium, asbestos, mercury, and rubidium. Mercury, used in the silver amalgam of teeth fillings, has been identified as a toxic element.[4] It has been found in fish such as those at the top of the marine food chain (e.g., tuna, swordfish, and shark). When consumed or released from teeth amalgam fillings, it is sequestered in the bone, brain, and kidney. If the individual is pregnant, the mercury crosses the placental barrier and can be found in the fetal liver and kidney. Mercury is usually excreted through the kidneys, and urinary mercury can be used as an index of exposure.

With most minerals, the first line of defense against food-borne toxicity is that offered by the gastrointestinal system: vomiting and diarrhea. Through vomiting, contaminated food is expelled. Through diarrhea, malabsorption as well as excretion of recirculated (via bile) mineral is facilitated, reducing the intestinal exposure and subsequent uptake of the mineral. Failing to ablate the toxic state, the kidney tubules will attempt to reduce the body load, and some minerals can be excreted in the urine. However, some minerals (i.e., copper, iron, zinc) and lead are not as subject to renal filtration as are other minerals (i.e., selenium, magnesium, calcium, molybdenum and mercury).[5–14] Reduction of the body load that is in circulation then is accomplished by depositing the excess mineral in the bones. Bone mineral content has been used to document cases of suspected toxicity. Accidental or intentional poisoning can sometimes be masked by other nonspecific symptoms, but bone analysis can provide the documentation needed to support or deny a supposition of toxicity.

To a lesser extent, hair analysis together with blood analysis can reveal the mineral status of an individual. The difficulty in using these analyses is that the mineral content can be transient.[15] That is, blood levels of trace minerals represent the immediate mineral intake rather than long-term exposure, whereas hair mineral content can represent not only food or drink mineral but also airborne mineral. Hair mineral can be contaminated by shampoos and other hair treatments.

The adverse effects of trace minerals are as diverse as the minerals themselves. Each mineral has its preferred target in the body. For some, the target is DNA.[8,16–18] Certain minerals (copper, arsenic, nickel, and chromium) bind to DNA. The binding is a covalent one and produces either a nonfunctional DNA or a DNA that can not repair itself. Evidence of this crosslinking has been demonstrated in vitro using a variety of cell types. Chinese hamster cells have been used to show copper-induced, chromium-induced,[19] and nickel-induced DNA crosslinking, whereas human fibroblasts and epithelial cells have shown arsenic-induced crosslinking.[16,20]

The concept of chemically induced cross linkage of DNA as a factor in carcinogenesis has been proposed to explain the role of asbestos and the development of mesothelioma. Mesothelioma is a malignant growth of the pleural and peritoneal cavities. These tumors are stimulated to grow by the presence of asbestos fibers that act as artificial linkers of DNA, resulting in mutations within the pleural and peritoneal cells. Changes in the CDKN2 (p16) gene seem to be involved. This gene is either lost or mutated. Its gene product is a regulator of the phosphorylation of protein 105, a tumor suppresser. Unphosphorylated p105 can inhibit passage from G1 to S phase of the cell cycle, whereas phosphorylated 105 permits this passage. Passage inhibition is a common feature of cancer cell initiation. Thus, any substance that interferes with this passage could be regarded as a

TABLE 12.3
Trace elements and key features of their use

Micromineral	Function	Remarks
Trace minerals		
Copper	Essential cofactor for a variety of enzymes involved in iron use, collagen synthesis, and antioxidants	Wilson's disease results from excess intake
Iron	Essential for hemoglobin synthesis, cytochrome activity, urea cycle activity, lipogenesis, and cholesterogenesis	Hemochromatosis results from excess intake. Anemia is the major sign of inadequate intake
Zinc	Essential to the function of over 70 enzymes; important to activity of DNA and RNA polymerase	Poor growth in children with renal disease due to excess zinc loss during dialysis. Deficiency may occur with use of diuretics. Zinc loss is increased in traumatized patients
Ultra trace minerals		
Arsenic	Needed for growth and optimal iron use	Toxic; well-known metabolic poison
Chromium	Needed for optimal action of insulin at target tissue	Widely dispersed in a variety of foods. Brewers yeast and beer are good sources. Deficiency is unlikely
Cobalt	Important component of vitamin B_{12}; deficiency symptoms are those of B_{12} deficiency	Large excess intake can block iron absorption.
Fluorine	Increases the hardness of bones and teeth, activates adenylate cyclase	Fluorosis (mottling of teeth) results from excess intake
Iodine	Needed for thyroid hormone synthesis	Goiter and cretinism result from inadequate intake
Manganese	Cofactor in a wide variety of enzymes; essential to reactions using ATP or UTP	Widely distributed throughout the body. Deficiency is unlikely
Molybdenum	Activates adenylate cyclase; cofactor in sulfite oxidase and xanthine oxidase	Widely distributed in the food supply. Deficiency is unlikely
Nickel	The need for this element has not been shown for humans	Widely distributed in the food supply. Deficiency is unlikely
Selenium	Essential to glutathione peroxidase and thyroxine deiodinase	Toxicity and need are influenced by environmental factors
Silicon	The need for this element has not been shown for humans but has been shown for animals	Widely distributed in the food supply. Deficiency is unlikely
Vanadium	The need for this element has not been shown for humans but has been shown for animals	Widely distributed in the food supply. Deficiency is unlikely

carcinogen. Minerals in excess can have this effect, and excess intakes of some have been linked with certain forms of cancer. Iron, for example, has been linked to colon cancer. The mechanism whereby excess iron has its effect is far from clear. It may induce DNA cross linking as described above, but it may also act as an ion that stimulates free radical formation and attacks cells and their DNA, causing them to mutate. In either scenario, excess iron and colon cancer are associated.

ANTAGONISMS AND INTERACTIONS AMONG TRACE MINERALS

No general discussion of trace minerals would be complete without mention of mineral interactions. Numerous antagonisms and synergisms have been reported. This should be expected when it

is considered that many of the trace minerals have more than one charged state and that living cells have preferences with respect to these states. For example, the uptake of iron is much greater when the iron is in the ferrous (+2) state than when in the ferric (+3) state. Minerals that keep iron in the ferric state will interfere with its absorption and use. Minerals that do the reverse will enhance iron uptake. Such is the beneficial action of copper on iron. The cuprous ion keeps the ferrous ion from losing electrons, becoming the ferric ion. In addition, there is an interaction among nickel, copper, and iron,[21] and between nickel and copper.[22,23] These interactions make it difficult to assess the relative need for nickel. Nickel supplementation accentuates copper deficiency, and this effect was influenced by iron. In the ruminant, the potency of molybdenum as a copper antagonist depends on an adequate supply of sulfur.[18] Tin, copper, iron, zinc, and calcium also interact affecting the needs for each of these minerals.[24] Copper and zinc mutually antagonize the absorption of each.[25] The interactions of essential minerals are best illustrated in Figure 12.1. To the nutrition scientist seeking to design a purified diet providing all the known nutrients in needed amounts, these mineral interactions can be quite a problem. Sometimes the so-called purified ingredients contain (or fail to contain) minerals unknown to the producer or user. This is especially a problem in the protein portion of the diet. Proteins can bind minerals (as described in each of the sections on each of the minerals), and these minerals are found in the proteins. Soybean protein can contain phytate-bound phosphorus and magnesium; casein and lactalbumin depending on origin contain variable amounts of calcium, magnesium, and selenium. Unless the investigator determines the mineral content of the diet ingredients, there is the possibility that an unexpected mineral imbalance could occur, and this imbalance could affect the outcome of the experiment. The specifics of these imbalances and interactions will be detailed as each of the trace and ultra trace minerals is discussed.

IRON

Iron, element 26 in the periodic table, is the fourth most prevalent mineral in the Earth's crust. Neolithic man learned to mine iron and to forge tools from iron. The Romans used iron preparations as tonics, but the clinical recognition of iron as an essential nutrient was not accomplished until the 17th century.[26] Sydenham was the first to propose that chlorosis (a sickness in adolescent females characterized by a pale skin color) was due to iron deficiency anemia. He showed that iron salts were an effective treatment.

In 1713, Remmery and Jeffrey demonstrated the presence of iron in the mineral matter of blood, and in 1852 Funke showed that this mineral was contained by the red cell.[26] Thus, it was learned that iron and red cell number were related, and that the red cell function of carrying oxygen depended on its hemoglobin content.

Iron is present in a variety of inorganic salts in the environment. It exists mainly in a trivalent form as ferric oxide or hydroxide or its polymers. Absorption of these salts is very limited unless they can be solubilized and ionized by the intestinal contents. Both ferric and ferrous salts are

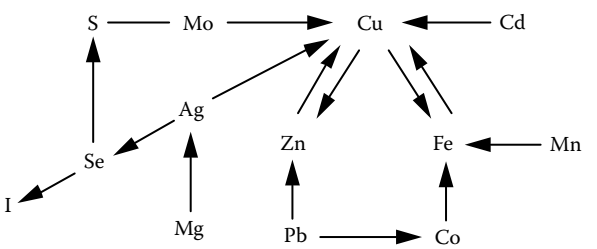


FIGURE 12.1 Trace mineral interactions.

present in the diet, but only the ferrous salts are absorbed from the gastrointestinal tract. Ferric compounds must be reduced in order to be absorbed. This is accomplished in the stomach by the low pH of the gastric juice.

The availability of iron from food depends on its source.[27–30] Soybean protein, for example, contains an inhibitor of iron uptake. Diets such as those in Asia contain numerous soybean products, and iron absorption is adversely affected by this soybean inhibitor. Tannins, phytates, certain fibers (not cellulose), carbonates, phosphates, and low-protein diets also adversely affect the apparent absorption of iron. In contrast, ascorbic acid, fructose, citric acid, high-protein foods, lysine, histidine, cysteine, methionine, and natural chelates (e.g., heme), all enhance the apparent absorption of iron. Zinc and manganese reduce iron uptake by about 30–50% and 10–40%, respectively. Excess iron reduces zinc uptakes by 13–22%. Stearic acid, one of the main fatty acids in meat, also enhances iron uptake.

In foods, as well as in animal tissues, iron is present in a variety of metalloproteins, which include hemoglobin, myoglobin, cytochromes, transferrin, ferritin, etc.[31] In the heme proteins, iron is coordinated within a tetraporphyrin moiety which, in turn, is bound to a polypeptide chain. Hemoglobin is a tetramer with a molecular weight of 64,500 that contains two α-subunits and two β-subunits. These give the protein allosteric properties in the uptake and release of oxygen. Each polypeptide subunit contains 1 atom of ferrous iron which amounts to 0.34% of the protein by weight.

Absorption, Excretion, Metabolism

Iron uptake by the gut, iron use and reuse, and iron loss comprises a system that for all intents and purposes is a closed system.[31] The gain through the gut is very inefficient, and there is virtually no mechanism aside from blood loss that rids the body of its iron excess. This system is shown in Figure 12.2. The total iron content of the body averages 4.0 g in men and 2.6 g in women. As shown in Table 12.4, there are two groups of iron-containing compounds that are considered essential to life. Essential iron compounds in the body include hemoglobin, myoglobin, and the cytochromes.

In addition, there are a number of enzymes whose active site has an iron-sulfur center. Hemoglobin is the most abundant and easily sampled of the heme proteins and accounts for greater than 65% of body iron. The second group of molecules are those involved in iron transport (transferrin) and storage (ferritin, hemosiderin).[32]

Transferrin is the iron-transport protein that carries ferric iron between the sites of its absorption, storage, and utilization. It is a β-glycoprotein, molecular weight 76,000, that binds 2 atoms of ferric iron per mole. Iron is transferred from the intestinal mucosa to transferrin and is carried through the blood to peripheral tissues containing receptor sites for transferrin. Transferrin is synthesized in the liver, brain, and testes, as well as other tissues. The regulation of the gene for transferrin varies from cell type to cell type, and each cell type has its own array of promoters and transcription factors that control the amount of transferrin synthesized. The amount of transferrin synthesized is inversely related to the iron supply. In times of low intake, more transferrin is produced so as to optimize iron availability.[33,34]

Once iron enters the cell, it is chelated to a protein that is then called ferritin. The enzyme that catalyzes this chelation is called ferrochelatase. This reaction then represents the ultimate destination for the majority of the iron that enters the cell. Chelation of iron to its storage protein occurs at the outer aspect of the mitochondrial membrane. Ferritin has a molecular weight of 450,000. It is composed of 24 subunits which form an outer shell within which there is a storage cavity for polynuclear hydrous ferric oxide phosphate. Its synthesis is highly regulated at the level of translation by the ferric atom[35–37] and, posttranscriptionally, by a cytoplasmic protein called the *iron regulatory protein*.[38] When iron is present, the mRNA is available for translation. In the absence of iron, the mRNA folds up on itself such that the start site for translation is covered up. Over 30% of the weight of ferritin may be iron. It is present in the liver, gut, reticulo-endothelial cells, and

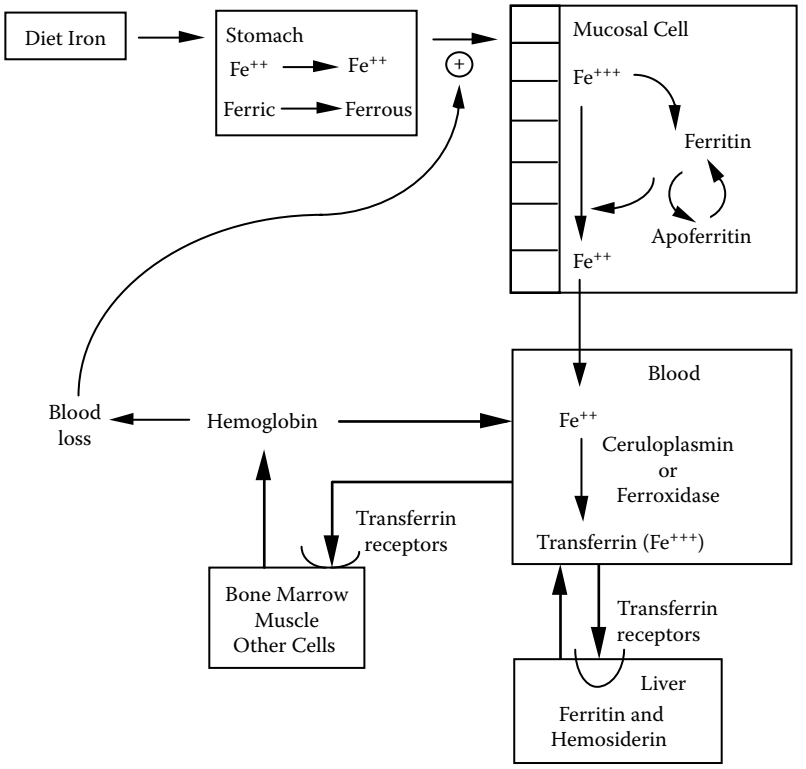


FIGURE 12.2 Overview of iron uptake and use. This is an apparently closed system with the recycling and conservation of iron absorbed from the gut.

TABLE 12.4
The body content of iron

Types of iron	Male 70 kg	Female 60 kg
Essential iron	3.100 g	2.100 g
Hemoglobin	2.700	1.800
Myoglobin, cytochromes and other enzymes	0.400	0.300
Storage and transport iron	0.900	0.500
Ferritin, hemosiderin	0.897	0.407
Transferrin	0.003	0.003
Total iron	4.000	2.600

bone marrow. Hemosiderin is a form of denatured ferritin that contains about one-third of the iron stores. The posttranscriptional regulation of ferritin synthesis is regarded as a protective mechanism when excess iron is present. In this situation the iron regulatory protein binds to the iron responsive element in the ferritin mRNA decreasing ferritin synthesis. When the iron-responsive protein dissociates from the iron-responsive element, ferritin synthesis resumes. Aconitase, an enzyme that catalyzes the dehydrogenation of citrate to α-ketoglutarate has been found to serve as an iron regulatory protein.[39] Ferritin also serves as a zinc detoxicant and a zinc ion donor.[40] This is important in instances where a zinc overload has occurred.

The hormone hepciden has emerged as key to the regulation of iron balance. When elevated it serves to increase iron in macrophages and decrease gastrointestinal iron uptake.[41,42] The synthesis of hepcidin is induced by infection, inflammation, and elevated iron intake. Lower levels of hepcidin are associated with anemia, hypoxia, iron deficiency, and hereditary hemochromatosis. Hepciden mRNA fall quickly after blood loss.[42] In response to inflammation, IL6 levels rise followed by rises in hepcidin.[41] Hepcidin inhibits iron release by the macrophages, and this inhibition results in a fall in serum iron. The export of iron from enterocytes and macrophages is mediated by ferroportin. Hepcidin acts by binding ferroportin on the plasma membranes, causing it to be internalized and degraded. This increases enterocyte iron that eventually passes this iron into the lumen of the gastrointestinal tract. Hepcidin deficiency seems to be the basis for hemochromatosis.

Iron-Containing Materials in the Body

The cytochromes are enzymes involved in electron transport system which is located principally in the mitochondria. Cytochrome P-450, a specialized cytochrome, is used to oxidize organic compounds. This cytochrome is located in the endoplasmic reticulum. Whereas the cytochrome P-450 enzymes are active in the detoxification of drugs and chemicals, these enzymes also activate carcinogens. The cytochrome P-450I has this function, whereas P-450IIE has a propensity to form oxygen radicals which are both cytotoxic and carcinogenic.[43] Other cytochromes generate oxygen radicals by futile cycling. In some respects, the ability to generate peroxides has a protective effect in that peroxides will kill invading pathogens. Peroxide formation is in fact the first line of defense against such an invasion. Other enzymes in which iron is not bound to heme, include iron sulfur proteins, metalloflavoproteins, and certain glycolytic enzymes.

Because the lifetime of a red cell is about 120 days in humans, the flow of iron through the plasma space amounts to about 25–30 mg/d in the adult (about 0.5 mg/kg body weight). This amount of iron corresponds to the degradation of about 1% of the circulating hemoglobin mass per day. Iron is conserved in the body in males and postmenopausal females to a great degree; only 10% being lost per year in normal men, or about 1 mg/d. This loss of 1 mg/day has to be made up by absorption of iron from the diet, which is only about 10% efficient, requiring about 10 mg of dietary iron/d. In menstruating females the loss is increased to 2 mg/d, which means that the intake and absorption of iron must be increased or these females will develop iron deficiency.

In contrast to the turnover of hemoglobin in the red cell, tissue iron compounds, which include the cytochrome enzymes and a variety of other nonheme enzymes, are heterogeneous in respect to life span. Furthermore, these compounds are subject to degradation at exponential rates similar to the rate of turnover of the subcellular organelle with which they are associated. For example, in rats, mitochondrial cytochrome C has a half-life of about 6 days, whereas hemoglobin has a half life of about 63 days.

The apparent absorption of iron (e.g., the amount absorbed from food) can vary from less than 1% to more than 50%. The percentage that is absorbed depends on the nature of the diet, on the type of iron compound in the diet, and on regulatory mechanisms in the intestinal mucosa that reflect the body's physiological need for iron.

Two types of iron are present in the food, namely heme iron, which is found principally in animal products, and nonheme iron, which is inorganic iron bound to various proteins in the plant. Most of the iron in the diet, usually greater than 85%, is present in the nonheme form. The absorption of nonheme iron is strongly influenced by its solubility in the upper part of the intestine. Absorption of nonheme iron depends on the composition of the meal and is subject to enhancers of absorption such as animal protein and by reducing agents such as vitamin C. On the other hand, heme iron is absorbed more efficiently. It is not subject to these enhancers. Although heme iron accounts for a smaller proportion of iron in the diet, it provides quantitatively more iron to the body than dietary nonheme iron.

The regulation of iron entry into the body takes place in the mucosal cells of the small intestine. Its iron gate is very sensitive to the iron stores, so if the iron stores are low, which is true for most women and children, the intestinal mucosa takes up iron and increases the proportion absorbed from the diet. On the other hand, if the body is replete with iron, as is typical of healthy men and postmenopausal women, then the percentage of iron absorbed is low. This mechanism offers some protection against iron overload. In infancy, lactoferrin, an iron-binding protein in human milk, promotes the absorption of iron through lactoferrin receptors on the surface of the intestinal mucosa of infants. This may explain why iron is well-absorbed from human milk. Milk is not usually considered a good source of iron but for the breast-fed infant, this lactoferrin-iron mechanism prevents deficiency from developing. As the infant matures, however, this mechanism becomes inadequate.

On the average, only about 10% of dietary iron is absorbed. In order to be absorbed, the iron must be in the ferrous state. Upon entry into the enterocyte, it is incorporated (in part) into ferritin in which the iron is in the ferric state. When the iron is transported from the mucosal to the serosal side of the enterocyte it is transported in the ferrous state, probably bound to cytoplasmic proteins. When the iron is pumped out of the enterocyte it must be oxidized to the ferric state in order to bind to transferrin. This is accomplished by ceruloplasmin (molecular weight 160,000), which contains eight copper ions in the divalent state. Ceruloplasmin copper is reduced by the iron, resulting in the formation of cuprous ions in ceruloplasmin and ferric iron in transferrin. As mentioned earlier, transferrin is recognized in the periphery by cells that have transferrin receptors. The transferrin receptors vary, depending on the tissue and the condition. Tissue such as erythroid precursors, placenta, and liver, which have a large number of transferrin receptors, have a proportionately high intake of iron. When these cells are in an iron-rich environment, the number of receptors decreases and, conversely, when they are in an iron-poor environment, the number of receptors increases. The up- and downregulation of transferrin receptors is accomplished at the level of transcription.[34,35] Measurements of messenger RNA for the transferrin receptors indicate that there can be as much as a 20-fold change in messenger RNA and, presumably, as much as a 20-fold difference in transcription of the transferrin receptor gene. The change in mRNA and its transcription is related to the concentration of iron. When the iron is delivered to an erythroid precursor cell in the bone marrow, the ferric iron has to be again reduced to the ferrous state in order to be incorporated into the heme prosthetic group. The reduction is accomplished by an NADH-dependent reductase, and the insertion of iron in the heme ring is accomplished by another enzyme known as *chelatase*. When the ferrous iron is inserted into heme associated with specific subunits in hemoglobin, the various subunits polymerize to form the tetramer of hemoglobin A.

The presence of oxygen, of course, tends to oxidize a certain small percent of the iron each day, and the formation of ferric iron in the hemoglobin molecule results in its conversion to methemoglobin, which has no capacity to take up and release oxygen. In order to minimize this effect of the oxidation of ferrous iron in hemoglobin by cellular oxygen concentrations, methemoglobin reductase, which is also an NADH-dependent enzyme, reduces the ferric iron in methemoglobin back to the ferrous state, which regenerates ordinary hemoglobin.

Iron Needs

A normal red cell lasts for about 120 days in the circulation of humans and is then taken up by the reticuloendothelial system and degraded into bile pigments and ferric iron, which enters the transferrin pool and is recirculated to the extent of about 25 mg/day. Thus, the turnover of iron within the body is 10–20 times the amount absorbed. A similar small amount, ~1 mg/d, is lost by the sloughing of G.I. cells and by skin cells. Fecal losses of iron are about 0.6 mg/d. Urinary losses are essentially nil.

In menstruating women, or in individuals with hemorrhage, the iron losses can be considerable and anemia can occur as a result of menstrual losses or bleeding. This is the basis for the chlorosis (chronic hypochromic microcytic anemia) observed in adolescent girls. This symptom is one of the

first characteristic associated with a nutrient deficiency and was first identified as iron deficiency in the 17th century. Females, during the childbearing years, must replace the iron lost in menstrual blood, which over a month, amounts to about 1.4 mg/d. During infancy and childhood, about 40 mg of iron are required for the production of essential iron compounds associated with the gain of 1 kg of new tissue. Obviously the iron needs are great in rapid growth phases of infancy and adolescence. The needs of pregnant women are also great because during pregnancy a total of about 1.0 gm of iron is needed to cover the growth need of the fetus and that of the mother as she prepares for delivery and lactation. It is difficult to obtain this amount of iron from the usual diet. It is estimated that about 27 mg/d of elemental iron is needed in the diet to provide sufficient iron to the pregnant female.

The elderly in good health have no apparent increase in their needs for iron[44]; however, their need might be increased should they develop chronic disease. Table 12.1 gives the DRIs for iron.

Iron Deficiency (Anemia)

Iron deficiency is probably the most common nutritional deficiency present in the world population. This is true because iron is poorly absorbed and because many diets, especially those consumed by third-world populations, are iron poor. Diets that contain whole grain cereals and legumes contain only nonheme iron, which is poorly absorbed. Furthermore, women and children are at constant risk for iron deficiency. Assessment of deficiency includes the determination of levels of tissue ferritin, transferrin, receptor activity, heme iron, red cell number, and hemoglobin levels.

The appearance of clinical iron deficiency anemia occurs in three stages: the first involves depletion of iron stores as measured by decrease in serum ferritin, which reflects the ferritin supply (iron stores) in the body, without loss of essential iron compounds and without any evidence of anemia. The second stage is characterized by biochemical changes that reflect the lack of iron sufficient for the normal production of hemoglobin and other iron compounds. This is indicated by a decrease in transferrin saturation levels and an increase in erythrocyte protophyrin—so-called iron-deficiency without anemia. In the final stage, the appearance of iron-deficiency anemia occurs with depressed hemoglobin production and a change in the mean corpuscular volume of the RBC to produce a microcytic hypochromic anemia. This is expressed clinically as pallor and weakness. There are also changes in the nails, which take on a spoon shape when the iron deficient state is severe. In rats made iron deficient several changes in intermediary metabolism have been reported. These include an increase in peripheral tissue sensitivity to insulin, an increase in hepatic glucose production (gluconeogenesis), a decrease in the conversion of thyroxine to triiodothyronine, impaired fatty acid oxidation, and ketogenesis, an increased need for carnitine, evidence of oxidative damage to the erythrocyte membrane, abnormal monoamine metabolism in the brain (increased dopamine synthesis and downregulation of dopamine receptors), increased serum triglycerides and cholesterol and slightly less pentose shunt activity. In addition to these metabolic changes, iron-deficient rats had an impaired immune response to a pathogen challenge. There was a decrease in antibody production and a decrease in the natural killer cell population. Iron deficiency in chicks results in a compromised development of the elastic fibers in the aorta and lungs.[45] Whether these same responses also exist in iron-deficient humans is not certain; however, it is likely that there are numerous similarities.

Pharmacological Treatment of Iron Deficiency

The treatment of iron-deficiency anemia is a pharmacologic activity and involves giving large doses of iron, usually equivalent to 60 mg of elemental iron or 300 mg of ferrous sulfate, once or twice a day. It is usually given between meals to minimize gastrointestinal side effects. Fortunately, the smaller the dose and the more severe the anemia, the greater will be the percentage of iron absorbed. This treatment is usually continued for 2–3 months to normalize hemoglobin levels and iron stores. These should be monitored until satisfactory values are obtained.

Toxicology

Iron toxicity is a result of excess iron intake. This can occur acutely in children who ingest iron pills or iron vitamin supplements, not realizing that they can be toxic. Severe iron poisoning is characterized by damage to the intestine with bloody diarrhea, vomiting, acidosis, and sometimes liver failure. Effective treatment includes induced emesis, food and electrolyte treatment to prevent shock, and the use of iron-chelating agents to bind the iron. This treatment has substantially decreased the mortality from about 50% in 1950 to less than a few percent in recent years. Table 12.2 provides information on the upper tolerable limit for iron intake, and when these intakes are exceeded, toxicity results.

Chronic overload of iron can result either from chronic excess intake or from a genetic disorder, hemochromatosis. Hemochromatosis is intermittent in nature. The genetic disorder is carried on chromosome 6. Two descendants of the British King George III have been diagnosed with the disease, and it has been suggested that the intermittent psychiatric state of King George III during the American Revolution might have been due to hemochromatosis. Hemochromatosis is characterized by increased iron absorption with damage to the pancreas, liver, brain, and heart. This damage has been associated with diabetes, liver failure, and heart failure. There is also an associated psychiatric abnormality that is likewise intermittent in character. Persons with hemochromatosis may also develop hepatocellular carcinoma and colon cancer. Two lines of evidence have been put forth that support this suggestion. The first line concerns the production of free radicals.[46] Iron, in excess, can catalyze the production of free radicals, which in turn can damage cell membranes as well as act as mutagens through damaging DNA. The DNA can repair itself but the free-radical damage could be so great that the repair is inadequate. The second line concerns the fact that cancer cells, like normal cells, require iron as an essential ingredient of metabolism. Having a surplus of iron in the system could increase the survival and proliferation of the cancer cell. Several population studies have provided support for this line of thought. In these studies a dose-response relationship—that is, a correlation of iron intake, ferritin levels, and the development of colon cancer—was found that was highly suggestive of a causal role for excess iron intake in colon cancer development.

Carcinogenesis can also be instigated by other minerals. Nickel subsulfide, for example, is a potent carcinogen having the renal tissue as a target. In the presence of high-to-moderate iron levels, the activity of the nickel compound is increased. In copper excess due to a genetic disorder involving the protein that transports copper, hepatic cancer develops, and this cancer is potentiated by high iron levels. It would appear in these last examples that the role of iron is that of a cancer promoter rather than that of an initiator as described for colon cancer.

Although high iron intakes can be harmful, it should be noted that optimal iron intakes can protect against lead toxicity. Lead competes with iron for uptake by the enterocyte. If the transporter is fully saturated by its preferred mineral, iron, then the lead will be poorly absorbed and excreted in the feces. Well-nourished individuals with respect to iron nutriture are at less risk for lead toxicity than are those whose iron intake is marginal or deficient.

Zinc

Zinc is the last transition element in the series of the fourth period of the periodic table. It has an atomic number of 30 and an atomic weight of 65.4. There are 15 isotopes of which ^{65}Zn is the most useful. This radioisotope has a half-life of 244 days.

Zinc is a good reducing agent and will form stable complexes with other ions as well as form a wide range of salts with members of the halogen family, as well as with carbonates, phosphates, sulfates, oxalates, and phytate.

Absorption, Metabolism, Excretion

Like iron, zinc absorption is relatively poor.[47] Of the approximately 4–14 mg/d consumed, only 10–40% is absorbed. Absorption is decreased by the presence of binding agents or chelating agents that render the mineral unabsorbable. Zinc binds to ligands that contain sulfur, nitrogen, or oxygen. Zinc will form complexes with phosphate groups (PO_4^-), chloride (Cl^-), and carbonate groups (HCO_3^-) as well as with cysteine and histidine. Buffers such as *N*-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid (HEPES) have little effect on zinc binding to these ligands. Clay, a mixed mineral soil fragment, for example, can render zinc unavailable. So, too can fiber, phosphate, and phytate (inositol hexaphosphate). Zinc bound in this fashion is excreted via the feces. People who are geophagic (Pica) and/or who consume large amounts of phytate-containing foods (mainly cereal products) are at risk for developing zinc deficiency. Oberleas[48] has calculated that diets having a phytate-to-zinc ratio greater than 10 will induce zinc deficiency regardless of the total zinc content in these diets. Unlike iron, zinc exists in only one valence state: Zn^{++}. The normal 70 kg human absorbs 1–2 mg/d (Figure 12.3) using both a nonsaturable and a saturable process. The former is passive diffusion whereas the latter involves zinc-binding metallothionein protein. Studies on the mechanisms of zinc absorption by the enterocyte have shown that fast zinc uptake is attributable to extracellular binding of zinc, followed by internalization of the zinc ligand mediated by an unknown molecular entity. After entry into the enterocyte the zinc is bound to a cysteine-rich intestinal protein (CRIP), which in turn transfers the zinc to either metallothionine or through the serosal side of the enterocyte to albumin, which carries it to its site of use. Figure 12.4 shows this proposed uptake system. Vitamin D enhances zinc uptake, probably due to an effect on the synthesis of metallothionine. From the enterocyte it is transferred to the serum where ~77% is loosely bound to albumin, ~20% is tightly bound to an α-2 macroglobulin and 2–8% (1–0.1 nm) is ultrafilterable.[49] This ultrafiltrate is excreted either in the urine (0.5–0.8 mg/d) or in the feces via bilary excretion. The liver appears to be a major site of Zn^{++} uptake after it has been absorbed. There is both rapid uptake ($t_{1/2}$ = 20 seconds) and a slower linear uptake.[50]

Function

Zinc has two important functions. One is that of serving as an essential cofactor for more than 70 enzymes. Table 12.5 provides a partial list of these enzymes. In this role, zinc binds to the histidine

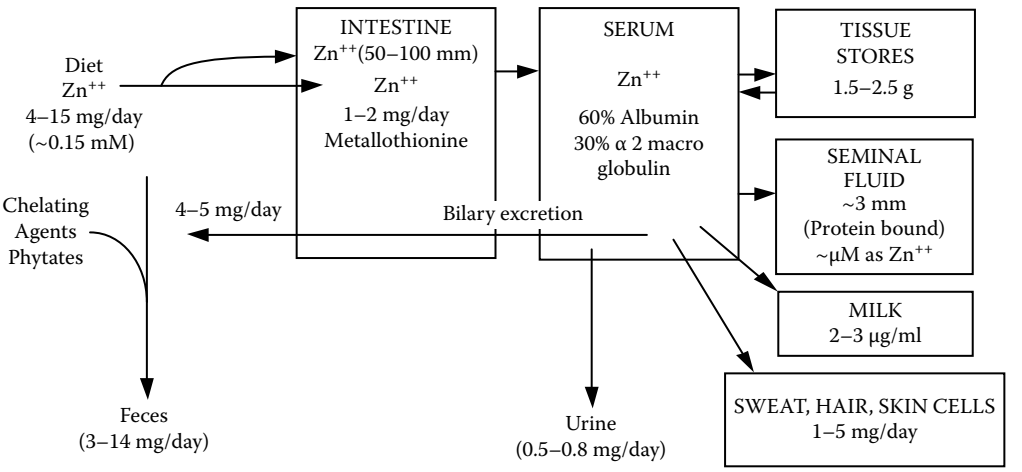


FIGURE 12.3 Zinc balance in normal adult humans.

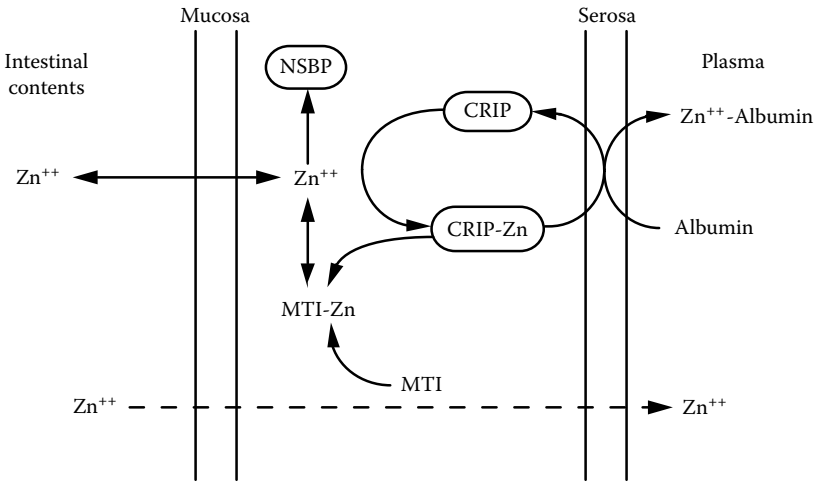


FIGURE 12.4 Intestinal zinc absorption. Passive diffusion is shown at the lower part of the diagram whereas mediated transport involving metallothionine (MTI), the cysteine-rich protein (CRIP) and the nonspecific binding proteins (NSBP) are shown in the upper part of the diagram.

TABLE 12.5
Enzymes requiring zinc as a cofactor*

Adenosine deaminase	Glyoxalase
Alcohol dehydrogenase	Lactate dehydrogenase
Alkaline phosphatase	Leukotriene hydrolase
Angiotensin converting enzyme	5′ Nucleotidase
Aspartate transcarbamylase	Phosphodiesterase
Carbonic anhydrase	Proteases and peptidases
Carboxypeptidase A, B, and DD	Pyruvate dehydrogenase
Cytoplasmic superoxide dismutase (also requires copper)	Reverse transcriptase
δ-amino levulinate dehydrase	Thymidine kinase
DNA and RNA polymerases	Thymulin
Elastase	Transcarboxylases
Fructose 1,6-bisphosphatase	Transcription factor Sp1

* The enzymes listed here are representative of the many requiring zinc for activity.

and cysteine residues of the enzyme proteins (Figure 12.5), and in so doing stabilizes and exposes the active sites of these enzymes such that catalysis of the reaction in question can take place. Even though these enzymes require zinc as a cofactor, these enzymes appear to function at near-normal levels in deficient animals. In part, this occurs because these enzymes are intracellular enzymes and tenaciously retain the zinc so as to continue to function. There obviously is a hierarchy of zinc need by the living body. Tissue stores and dispensable zinc uses are raided well before the intracellular zinc needed by these enzymes. Furthermore, deficient states are characterized by an increase in zinc absorption efficiency, further protecting the system from self destruction.

Equally important is the binding of zinc to specific DNA-binding proteins found in the nucleus. In this role, zinc binds again to histidine and cysteine residues of the linear portions of these binding

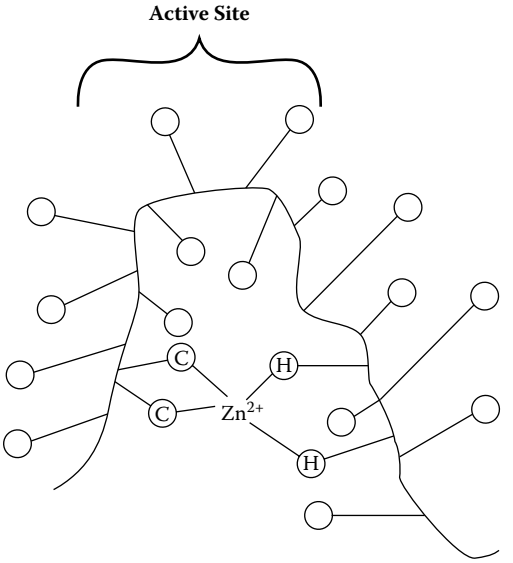


FIGURE 12.5 Zinc binds to cysteine (C) and histidine (H) residues of an enzyme protein stabilizing it so as to expose its active catalytic site. The rods and balls shown in this illustration are used to represent the various amino acid residues sticking out from the amino acid chain of the enzyme.

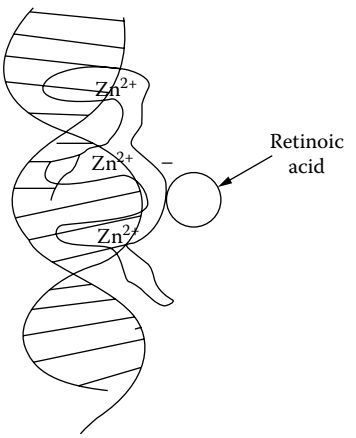


FIGURE 12.6 A zinc-DNA binding protein in action. In this instance retinoic acid is bound to its zinc-containing DNA-binding protein.

proteins, giving these proteins a sausage-like shape (Figure 12.6). These proteins with zinc attached are called *zinc finger proteins* or simply *zinc fingers*.[8] A number of nutrients (i.e., vitamin A, vitamin D, and hormones, i.e., steroids, insulin-like growth factor I, growth hormone, and others) have their effects on the expression of specific genes because they can bind to very specific zinc fingers, which in turn bind to very specific DNA regions.

If there is a mutation in the genes that encode these DNA-binding proteins such that they lack the requisite two residues each of histidine and cysteine in the linear part of their structure, then the functional attributes of these vitamins and hormones at the genetic level will be ablated. Instances of such mutations have been published, as well as instances where these zinc fingers have been

purposefully modified as a therapeutic approach to disease control. Zinc-containing transcription factor Zif268 has been modified with the result of a loss in sequence-specific recognition of DNA by viruses, thus ablating the viral invasion and takeover of their target cells. Although this modification was done in vitro and not tested in whole animals, this approach might have therapeutic application in the future as a means to avert the consequences of viral diseases such as AIDS.[51]

Zinc as part of a zinc finger or by itself stimulates the expression of its own transporter protein, metallothionine.[52–58] Metallothionine exists as two distinct yet related compounds termed MT-1 and MT-2. These proteins are hydrophilic, low-molecular-weight proteins (6–7 kDa) containing a high percentage of cysteine residues (23–22 mol%). The function of the cysteine is to bind heavy metals via clusters of thiolate bonds. The synthesis of metallothionines is regulated by zinc through its action on the expression of the genes for these proteins. The level of MT-1 is a very sensitive indicator of zinc deficiency.[48] The metallothionine gene was identified by Palmiter et al.[56] By combining the results of DNA sequence information with the results of deletion mapping studies, unique short sequences of DNA were found that mediated the role of zinc in metallothionine gene expression. The metal response units were sites for *trans*-acting transcription factors that bind and enhance the basal rate of transcription of the genes for metallothionine. In the absence of dietary zinc, gene transcription is impaired and metallothionine levels are low. In addition, in zinc-deficient animals, numerous breaks in single-strand DNA have been observed.[59] This can be reversed when dietary zinc is restored. Incidentally, transgenic mice that overexpress metallothionine I are very resistant to zinc deficiency.[53] The overexpression of metallothionine increases the absorption efficiency of these mice, thus compensating for inefficient absorption. The cytokine, interleukin 1, has been shown to direct and regulate zinc metabolism in the traumatized or septic individual as well as normal persons. Interleukin 1 increases the expression of the metallothionine gene, thereby increasing zinc uptake through the gut and its transport to and uptake by bone marrow and thymus with relatively less zinc taken up by other body components. Trauma and sepsis both require zinc for new protein synthesis for tissue repair, and both conditions are characterized by a rise in interleukin 1 levels in the blood. In addition to its function in metallothionine transcription, as a component of numerous enzymes and in the zinc fingers, zinc also is important for the stabilization of membranes and provides structural strength to bone as part of the bone mineral apatite.

Storage

Zinc is found in all cell types and tissues. However, it is notably stored in the β cells of the islets of Langerhans in the pancreas. There, zinc is incorporated into the hormone insulin. Insulin contains two to four atoms of zinc as part of its crystalline structure. Zinc may play a role in insulin release but the details of this role have not been completely elucidated. Pharmaceutical preparations of insulin needed by diabetics for hormone replacement therapy contain zinc. It should be noted that not all species incorporate zinc in the insulin structure.

Zinc Interactions

Zinc can sometimes be displaced on the zinc fingers by other divalent metals. Iron, for example, has been used to displace zinc on the DNA-binding protein that binds estrogen. This protein binds to the estrogen response element of the DNA in the promoter regions encoding estrogen-responsive gene products. When this occurs in the presence of H_2O_2 and ascorbic acid, damage to the proximate DNA, the estrogen response element, occurs. It has been suggested that in this circumstance of an iron-substituted zinc finger, free radicals are more readily generated with the consequence of genomic damage.[8] This suggestion has been offered as an explanation of how excess iron (iron toxicity) could instigate the cellular changes that occur in carcinogenesis.

In excess, cadmium can also substitute for zinc in the zinc fingers. In this substitution, the resultant zinc fingers are nonfunctional. Because of the importance of these fingers in cell survival and

renewal, a cadmium substitution is lethal. Cadmium toxicity is an acute illness with little lag time needed for the symptom of cell death to manifest itself.

The metallothionine proteins, in addition to binding zinc and copper, also bind other heavy metals such as mercury and cadmium. This occurs when the individual is acutely exposed to toxic levels of these metals. Excess zinc intake can adversely affect copper absorption and also affect iron absorption. Further, excess zinc can interfere with the function of iron as an antioxidant and can interfere with the action of cadmium and calcium as well. Ferritin, the iron storage protein, can also bind zinc. In zinc excess, zinc can replace iron on this protein. Other interactions include the copper–zinc interaction. Copper in excess can interfere with the uptake and binding of zinc by metallothionine in the enterocyte. In humans consuming copper-rich diets, the apparent absorption of zinc is markedly reduced. In part, this is due to a copper–zinc competition for enterocyte transport, and in part due to a copper effect on metallothionine gene expression. Metallothionine has a greater affinity for copper than for zinc, and thus zinc is left behind, whereas copper is transported to the serosal side of the enterocyte for export to the plasma whereupon the copper rather than the zinc is picked up by albumin and transported to the rest of the body. Fortunately, excess copper in the normal diet is not common. Zinc is usually present in far greater amounts, and this interaction is of little import in the overall scheme of zinc metabolism.

Deficiency

Until the early 1960s, zinc as an essential nutrient for humans had not been demonstrated. Prasad in 1961[60] and Halsted in 1958[47] and 1963[47] described conditions in humans later found to be due to inadequate zinc intake. Among the symptoms were growth failure, anemia, hypogonadism, enlarged liver and spleen, rough skin and mental lethargy. These features can be attributed both to the loss of zinc as a cofactor in many enzymatic reactions, and to the loss of zinc as an essential component of the DNA-binding zinc fingers. Detailed studies of populations having these symptoms among its members revealed the custom of clay eating as well as diets that were very low in animal protein and high in cereal products. Geophagia (clay eating) can affect the bioavailability of not only zinc but also iron and other minerals needed for optimal growth and development. In Iran, Prasad and Halsted found that the provision of iron and protein supplements corrected the anemia and enlarged spleen and liver. Pubic hair and the gonads also began to develop. It was difficult to explain all of the clinical features (and their reversal) solely on the basis of iron deficiency and/or protein deficiency because other investigators had not reported the features of hypogonadism as part of the iron or protein deficient state. However, studies in animals showed that this feature is characteristic of zinc deficiency. Later, Prasad in Egypt reported on growth retardation and testicular atrophy in young men. Geophagia was not a custom in this group nor were there signs of enlarged spleens and livers. The dietary patterns were similar to those found in Iran, in that the diets were high in cereals and low in animal protein. Zinc concentrations in hair, plasma, and red cells were lower than normal. Zinc turnover using ^{65}Zinc was increased above normal and the 24-hour exchangeable pool of zinc was smaller than expected. There were no signs of liver disease or any other chronic disease that would affect zinc status except that the Egyptian subjects were infected with shistosomiasis and hookworms. Studies of populations in Egypt where these infections were absent but where the diets were similar lead to the conclusion that the growth failure and hypogonadism were indeed signs of zinc deficiency. This was proven without a doubt when zinc supplements were provided and these signs were reversed. The zinc deficiency signs of growth failure and sexual immaturity are the result of an individual's inability to support cell division and hence tissue growth likely due to a decreased expression of insulin-like growth fctor and growth hormone.[61] The skin symptoms are the most obvious because skin cells turn over very rapidly (~7 days). These symptoms include a moist eczematoid dermatitis found in the nasolabial folds and around other body orifices. There is a failure in zinc-deficient individuals to replace these routinely desquamated cells. In infants and young children, inadequate zinc intake can result in abnormal CNS development[62] as well as impaired skeletal

development.[63] In the latter instance, zinc deficiency results in an impaired calcium uptake probably due to a decreased synthesis of intestinal calbindin. Impaired immune response and impaired taste sensitivity also characterize the deficient state. These features again relate to the role of zinc in cell turnover. Immunity requires antibody synthesis involving zinc fingers whereas the taste sensation involves short-lived epithelial cells on the surface of the tongue and oral cavity. The features of zinc deficiency have been reported in infants having an autosomal recessive mutation in one of the genes that encode the zinc-carrying metallothionine found in the enterocyte. This condition is treated with oral zinc supplements, which, through mass action, provides enough zinc to the enterocyte by passive diffusion. Because only one of the metallothionine genes has mutated, this strategy overcomes the inherent zinc-deficient state. Other zinc deficient states have been reported in severely traumatized individuals and in patients with end-stage renal disease with or without dialysis. In renal disease, more zinc is lost via the kidneys than normal.[64] In renal failure, excess zinc is lost through the dialysate when patients are maintained on regular dialysis treatment. Children who are anephric and on dialysis must be monitored with respect to their zinc status. Failure to do so will result in growth failure and lack of sexual maturation. Again, a zinc supplement can reverse these symptoms.

Status

Zinc status can be difficult to assess sensitively. Plasma and neutrophil zinc levels can give a static measure of status; however, these blood levels can only evaluate the amount of zinc in transport, not the functional state of the individual. Measurement of alkaline phosphatase activity is quite useful because it is a zinc requiring enzyme and is sensitive to zinc deprivation. The level of metallothionine I in blood is also a very sensitive indicator of zinc intake adequacy.[65] Hair zinc levels have been suggested as indicators of chronic zinc status; however, hair samples are frequently contaminated by zinc-containing shampoos or zinc-containing water. Hair growth rate could also influence hair zinc content.[15] Hence, assessing zinc status using hair samples probably is not very useful. Currently, the intake recommendation is set at 2–13 mg/d, depending on the life stage of the individual (see Table 12.1). Assuming that the individual consumes a wide variety of foods and that the intake recommendation for good-quality protein is met, the average individual should be well nourished with respect to zinc.

COPPER

Since the bronze age, copper alloyed with tin has been used by man to fabricate a vast array of useful items. However, it has only been a few decades since it was recognized as an essential nutrient for man and other animals. In the 1920s it was found that copper was needed, in addition to iron, for hemoglobin synthesis. This came about when anemic animals supplemented with iron did not improve unless copper was also provided. The pioneering work of Cartwright and his associates[66] showed the relationship between the two metals in heme biosynthesis. Other roles for copper have because been elucidated.[67]

Copper is a transition metal in the fourth period of the periodic table. It has a molecular weight of 63.4 and an atomic weight of 29 and two valence states, cuprous (Cu^{++}) and cupric (Cu^{+++}). There are two naturally occurring isotopes, Cu^{63} and Cu^{65} and two radioisotopes, ^{64}Cu and ^{67}Cu. The former has a half life of 12.7 hours, whereas the latter has a half-life of 62 hours.

Copper is present in nearly all foods in varying amounts. Dairy products are poor sources of copper, whereas legumes and nuts are rich in this mineral. Raisins, whole grains, beef liver, shellfish, and shrimp are excellent sources as well. Surveys of foods consumed by a variety of population groups in the United States indicate a range of intake from 0.7 to 7.5 mg/d. On low intakes, absorption is markedly more (56% of intake) than when intake is high (12% of intake). The form of copper (cuprous or cupric) is a key factor in determining the percentage of intake that is absorbed.[68,69] Zinc, tin, ascorbic acid, and iron adversely affect copper absorption.

Absorption, Metabolism, Excretion

Copper absorption takes place in the small intestine and to a limited extent in the stomach.[70–72] Copper status affects absorption; where need is great, uptake is high. The amount absorbed also depends on the food mixture consumed and on the presence of other divalent minerals that may compete for uptake. Absorption efficiency is low with an average uptake of 12%. Copper absorption is affected (reduced) by phytate[68] and by zinc.[25,73] That which is not absorbed is excreted in the feces. Also excreted in the feces is copper transported to the intestine from the liver via the bile. About 2 mg/d is excreted via the bilary route. Copper is also excreted in the urine and lost through the skin and hair. The percent lost via the urine, bilary excretion, skin and hair is between 12 and 43% of the intake. The daily urinary copper in the human is quite small, amounting to 10–50 μg/d. In the rat urinary loss accounted for 2–7% of the intake.[74]

Once copper is absorbed by the enterocyte, it passes to the blood where it is bound to either albumin or transcuprein.[75] The half-life of albumin-bound copper is on the order of 10 minutes. The copper is then delivered to the liver where it is incorporated into an α globulin transport protein called *ceruloplasmin*. Ceruloplasmin can carry six atoms of copper. Blood levels of copper are about 1 mg/L whereas ceruloplasmin is about 150–600 mg/L. Ceruloplasmin is not only useful in transporting copper to all parts of the body, it also has enzyme activity as a ferroxidase, an amide oxidase, and as a superoxide dismutase. As a ferroxidase it is an active participant in the release of iron from its liver storage sites to transferrin in the plasma. It is active in the conversion of iron from the ferrous to ferric state and in the linkage of the ferric iron to apotransferrin to form transferrin which, in turn, transports the iron to the reticulocyte for hemoglobin synthesis. Its role in iron metabolism relates to the cuprous–cupric interconversion. On average, the copper contained by the ceruloplasmin is half cuprous and half cupric.

Although ceruloplasmin may indeed be the most active of the copper transporters, other transporters also participate in the delivery of copper to the cells which use and/or store this mineral. Albumin, as mentioned, can serve in this function as can a 270-kDa protein, transcuprein, and certain of the amino acids, notably histidine.[72] The liver is the major user and repositor of copper. The copper levels in the liver remain relatively constant. Bilary excretion and ceruloplasmin release are the major mechanisms used to maintain copper levels in this tissue. Ceruloplasmin has a high copper content and accounts for 70–90% of the approximately 1 μg/mL copper in the plasma.

Transcuprein has been shown to compete with albumin at the intestine for copper, yet functions in the portal circulation as a donor of copper to albumin. The existence and function of transcuprein in the maintenance of copper status has not been fully explored.

Although the transport of copper in the blood has received considerable attention, its transport into the cell has not been as well studied. Copper passes through the plasma membrane via fixed membrane transporter proteins. These membrane proteins may either reversibly bind the copper or form channels through which the copper passes. The kinetics of copper transport have been studied. The Km values are uniformly in the low micromolar range, whereas the Vmax is highly variable, depending on cell type, incubation conditions, and media used.

Function

Copper, zinc, and iron are all involved in the regulation of gene expression. Already discussed are the specific roles for zinc and iron. That of copper vis-â-vis metallothionine has been described.[11] The specific metallothionine locus (CUP1) encodes metallothionine, a 6570 molecular weight protein that binds heavy metals. The CUP1 promoter does not respond directly to copper. Rather, this is a property of an upstream-activating sequence (UAS) present as a tandem sequence designated UASp and UASd located between −105 and −108 bp from the transcription start site. It would appear that transcription of the metallothionine is a function of both copper and zinc because this

metal binding protein can only be synthesized in the presence of both. This cascade-like process for the expression of the copper-responsive metallothionine is illustrated in Figure 12.7.

Using the messenger RNA differential display method, ten other genes have been shown to have a copper response element needed for expression (Wang et al., 1996).[76] Seven of these had substantial homology with: ferritin mRNA, fetuin mRNA, mitochondrial 12S, and 16S rRNA, and with mitochondrial tRNA for phenylalanine, valine, and leucine. These homologies suggest roles for copper in mitochondrial gene expression, which in turn relate to the observation of decreased oxidative phosphorylation in copper deficient rats.[77–79] Copper is an important component of one of the mitochondrial respiratory enzymes, cytochrome C, and serves as a cofactor for a variety of enzymes (Table 12.6). Of the remaining RNAs identified as having a copper-response element, no gene product has yet been found. It is possible that these products might be enzymes requiring copper as a cofactor or may be copper transport proteins. This would follow the paradigm of gene expression found with zinc and iron.

Deficiency

Copper deficiency is rare in humans consuming a variety of foods. One of the major characteristics of copper deficiency is anemia and poor wound healing similar to that observed in vitamin C deficiency. The anemia is not responsive to iron supplementation. Weakness, lassitude, joint ache, osteoporosis, small petechial hemorrhaging, and arterial aneurysms can all be attributed to the vital role of copper in the synthesis of connective tissue and, in particular, collagen synthesis. Heart rupture is a frequently reported feature of copper-deficient rats.[80] Central nervous system degeneration can be related to a decline in respiratory-chain activity; however, in the hierarchy of enzymes requiring copper for function, this enzyme is about the last to be affected in a copper-deficient animal. Reduced immune response has also been reported. Copper-deficient animals have been shown to have decreased T lymphocyte and neutrophil activities.[81] Copper and chromium depletion has been shown to affect lymphocyte proliferation. Both minerals are needed simultaneously to elicit this response to a mitogen challenge.[82]

Other signs of the deficient state include elevated levels of plasma cholesterol, neutropenia, achromatism, twisted, kinky hair, and hemacytic, hypochromic anemia.[83] Copper-deficiency causes elevated cholesterol levels in the blood, elevated HMG CoA reductase activity, and increased hepatic glutathione levels in rats.[84] If the rise in glutathione level is inhibited, then the hypercholesterolemia associated with copper deficiency is abolished. Heme biosynthesis is impaired in copper deficient swine.[85] Adrenal steroidogenesis is impaired, as is catecholamine synthesis in copper deficiency. The latter is due to a deficiency of the copper-containing enzyme dopamine beta hydroxylase. Chronic

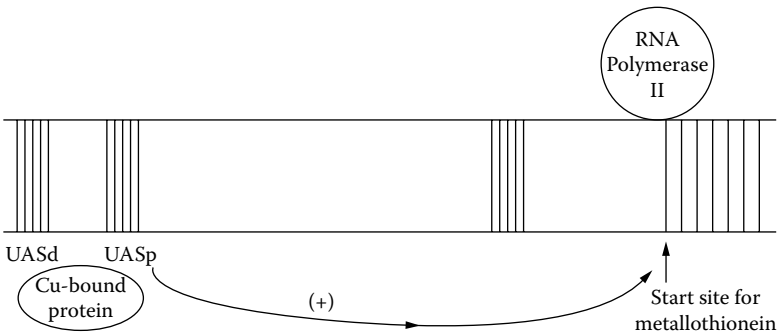


FIGURE 12.7 Copper plays a role in the expression of the gene for the cysteine-rich metallothionine by using a part of a DNA binding protein. This protein has several zinc fingers that attach to the USSd and UASp sites. When bound, it stimulates transcription.

TABLE 12.6
Enzymes requiring copper as a cofactor

α-amidating enzyme
Amine oxidase
Ascorbate oxidase
Cytochrome C oxidase
Cytoplasmic superoxide dismutase
Dopamine β hydroxylase
Ferroxidase II
Lysyl oxidase
Metallothionine
Monoamine oxidase
Phenylalanine 4 monooxygenase
Tyrosine oxidase

Note: The enzymes listed are representative of the many requiring zinc for activity.

diarrhea and malabsorption have been reported in infants fed copper-deficient formulas. In male rats fed purified diets, the use of pure sugars (mono and disaccharides) in a high carbohydrate diet accelerate the development of the copper-deficient state. Probably this has to do with the purity of this dietary ingredient rather than on any copper–sugar interaction at the cell or subcellular level.

Abnormal Copper Status

In normal humans, copper intake excess is rare. Although copper toxicity can develop if the exposure is high enough and long enough, the body can protect itself from occasional excess intake by lowering its absorption. The normal human should consume 0.2–1.3 mg/d of copper, depending on life stage to maintain an optimal nutritional status. The estimate of an adequate dietary intake (Table 12.1) is similar to the population studies that indicate the usual intake is between 1 and 1.5 mg/d. At intakes of 1–1.5 mg/d no signs of deficiency have been observed. Turnlund[71] has reported that young men consuming between 0.75–7.53 mg/d were able to attain positive copper balance regardless of intake. Likely, the figure given for optimal intake is a little high because of the paucity of data on copper status under controlled conditions.

There are two genetic disorders that have assisted scientists in understanding the function and metabolism of copper. In one, Menkes syndrome, copper absorption is faulty. Intestinal cells absorb the copper but can not release it into the circulation.[55,86] Parenteral copper corrects most of the condition that resembles copper deficiency but care must be exercised in its administration. Too much can be toxic. In addition, parenterally administered copper does not reach the brain and cannot prevent the cerebral degeneration and premature death characteristic of patients with Menke's disease.

Another genetic disorder in copper status is Wilson's disease.[87–90] This condition is also associated with premature death and is due to an impaired incorporation of copper into ceruloplasmin and decreased bilary excretion of copper. This results in an accumulation of copper in the liver and brain. Early signs of Wilson's disease include liver dysfunction, neurological disease, and deposits of copper in the cornea manifested as a ring that looks like a halo around the pupil. This lesion is called the Kayser–Fleischer ring. Renal stones, renal aciduria, neurological deficits, and osteoporosis also characterize Wilson's disease. Periodic bleeding, which removes some of the excess copper, can be helpful in managing Wilson's disease as can treatment with copper-chelating agents such as D-penicillamine, and increasing the intake of zinc, which interferes with copper absorption.

SELENIUM

Selenium is one of the "newer" minerals discovered during the 20th century as being both required and toxic with a relatively narrow range of intake between the two.[2] The existence of selenium as a metal was first reported by J.J. Berzelius in 1817 and can occur in nature in a variety of forms, referred to as *allotropic* forms. Metallic selenium is silver grey. If ground it appears black but if ground extremely fine it appears red. Vitreous selenium is black and shiny. Again, when powdered, it is red in color. When suspended in water it provides a dark red liquid. In nature, selenium is frequently found in combination with lead, copper, mercury, and silver. These combinations are called *selenides*. Its chemistry is similar to that of sulfur. Selenium is an allotropic metal in group 6 of the fourth period of the periodic table. Its molecular weight is 78.96, and its atomic weight is 34. Although selenium has 26 isotopic forms, only five of these are naturally occurring: Se^{76}, Se^{78}, Se^{77}, Se^{80}, and Se^{82}. Of the radioisotopes, ^{75}Se is the most useful, having a half-life of 118 days. Selenium has three valence states: Se^{++}, Se^{++++}, and Se^{++++++}. It can form selenides, as described above, selenate and, because it will react with sulfur and oxygen, will form selenomethionine, selenocystine, methylselenocysteine, and dimethyl selenide. The latter compound is quite volatile.

Although the presence of selenium in the Earth's mineral matter was known, its recognition as an essential nutrient did not occur until Schwarz and Foltz[91] showed that a form of liver necrosis in rats could be cured if either vitamin E or selenium were administered. This report and others showed that for some purposes these two nutrients were interchangeable. It was this interchangeability that interfered with the identification of selenium as an essential nutrient. In hindsight, we now realize that selenium and vitamin E both play important roles in the detoxification of peroxides and free radicals.

Absorption, Excretion, Metabolism

Absorption is very efficient, with most of the ingested selenium absorbed readily from a variety of foodstuffs. The source of the mineral can have effects on its absorption. Figure 12.8 provides an overview of the daily selenium flux in a normal human.

Selenium is transported from the gut on the low- and very-low-density lipoproteins. Red cells, liver, spleen, muscle, nails, hair, and tooth enamel all contain significant quantities of this mineral. Normal blood values for adults range from 55 to 72 μg/L. People with disorders that are characterized by increased oxidative stress to the red cell (i.e., β-thalassemia, diabetes, and/or smoking) tend to have slightly higher blood selenium levels, whereas pregnant and lactating women tend to have lower selenium levels. Excess (greater than 10 ppb in water or 0.05 ppm in food) intake is toxic, but unlike iron and copper, which have inefficient excretory pathways, selenium is actively excreted in the urine. The urinary system functions to maintain optimal selenium status. Figure 12.9 shows

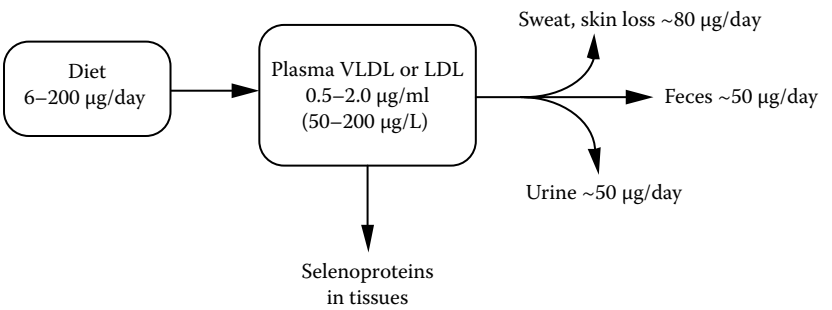


FIGURE 12.8 Daily selenium flux in a 70 kg man.

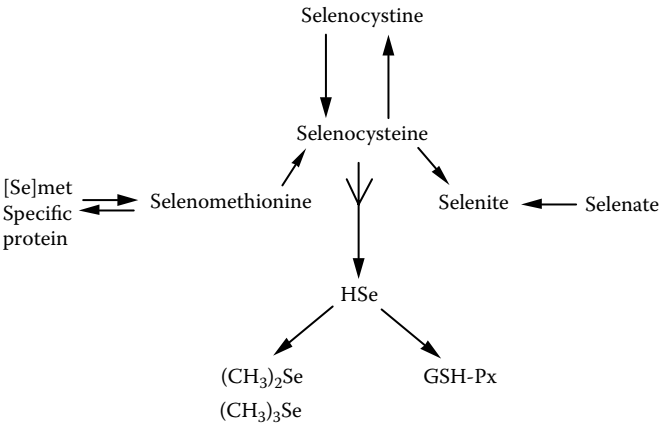


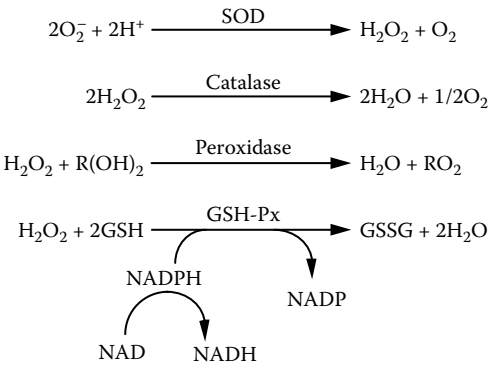
FIGURE 12.9 General scheme for selenium metabolism. Selenium is used by almost every cell type because of its vital role in glutathione peroxidase.

how selenium is utilized. The metabolic use of selenium follows that of sulfur. It involves the formation of a selenium–sulfur bond using the SH group of either cysteine or methionine. From there it is incorporated into one of a group of proteins called *selenoproteins*. Under normal intake conditions (50–200 μg Se/d), about 50 μg can be found in the feces, and a similar amount is excreted in the urine. Sweat and desquamated skin cells account for another 80 μg/d. These losses can vary depending on dietary conditions. When intakes are low, losses are low, and when intakes are high, there are corresponding rises in fecal and urine losses. Diets that include whole grain products, seafood, and organ meats will provide an optimal amount of selenium. In addition to these rich food sources, selenium in lesser amounts can be found in a wide variety of foods. In the United States, selenium deficiency is rare. This is not the case in China or in some parts of New Zealand and perhaps other parts of the world where the variety of foods available for consumption is severely constrained or reduced to those items produced locally. If the locality is a selenium-poor area, humans as well as domestic animals may become deficient. Where there is a high selenium area, humans as well as animals may suffer from a selenium overload. Low plasma selenium concentrations have been reported to associate with poor muscle strength in older adults.[92]

Function

Selenium is an essential element in a group of proteins called the *selenoproteins*.[93,94] The synthesis of these proteins involves four gene products, one of which is tRNA for serine.[95,96] It also involves sulfur-containing amino acids and selenium. These are joined via selenophosphate to form selenocysteine. The reaction is catalyzed by the enzyme selenophosphate synthetase. Vitamin B_6 serves as a coenzyme in this synthetic reaction. Selenocysteine in proteins is encoded by either a UGA opal codon or the TGA triplet which is normally a stop codon. In order to read the TGA codon as selenocysteine there must be a stem-loop structure in the 3′-untranslated region of the selenoprotein genes.[97] Although this stem-loop structure appears to be absolutely required for the incorporation of selenocysteine into a protein, documentation of this requirement is not fully complete.

Of the selenoproteins, glutathione peroxidase (GSH-PX or GPX, EC 1.11.1.9) has been the most widely studied. This enzyme catalyzes the reduction of peroxide (H_2O_2) and organic peroxides (fatty acid peroxides) in the presence of glutathione (GSH) to water and oxidized glutathione. This enzyme is an essential component of the series of reactions (shown in Figure 12.10) designed to



SOD	Superoxide dismutase (Requires Mg^{++} or Cu^{++} or Mn^{++} or Cu^{++} and Zinc)
GSH-Px	Glutathione peroxidase (Requires Se)
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H_2O_2	Hydrogen peroxide

FIGURE 12.10 The reduction of oxygen radicals in the red cell. Protection from free-radical attack preserves the function of the red cell membrane and hemoglobin.

maintain the redox state of the membrane and cell contents. Other enzymes include catalase, a peroxidase, and superoxide dismutase (SOD). Superoxide dismutase is found in both cytoplasm and mitochondria, and requires different divalent ions as cofactors. Manganese, zinc, copper, and magnesium function in this respect. Glutathione peroxidase is of major importance to the red blood cell, which lacks mitochondria, the organelles that also work to maintain an optimal redox state. The red cell, because it contains the oxygen-carrying hemoglobin, must regulate the redox state so that this hemoglobin can release oxygen in exchange for CO_2. In this exchange there are opportunities for peroxide formation. These must be suppressed and glutathione peroxidase does just this.

Glutathione peroxidase is less active in deficient animals than in normally nourished animals. In fact, a decline in enzyme activity is a sensitive indicator of selenium status.[98] At least four isozymes exist, and these have been isolated and characterized. The four enzymes are numbered 1 through 4 as GPX1, GPX2, GPX3, and GPX4. Two of these, GPX1 and GPX4, are expressed in most tissues. GPX1 is found in large quantities in red blood cells, liver, and kidney, where as GPX4 is found largely in testes. GPX2 is mainly in the gastrointestinal tract, and GPX3 is expressed mainly in kidney, lung, heart, breast, and placenta. There are species differences in the expression of these isozymes. In humans, GPX2 and 3 are expressed in the liver. In rodents this is not the case. Rodent liver expresses only GPX1, not GPX2 or 3.

As described, the active site of the selenium-dependent glutathione peroxidase contains a selenocysteine encoded by a UGA opal codon. However, not all of the isozymes are selenium dependent. There is an androgen-induced epididymal cell enzyme that shares sequence homology with GPX3 but is not selenium dependent nor does it have the UGA codon in its mRNA. The GPX2 maps to chromosome 14, whereas GPX3 and GPX4 map to chromosomes 5 and 19, respectively. GPX1 maps to human chromosome 3 and has sequences that are homologous to those found in

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chromosome 21 and the x chromosome. Glutathione peroxidase catalyzes the reduction of various organic peroxides as well as hydrogen peroxides. This reaction is shown in Figure 12.10.

Glutathione furnishes the reducing equivalents in this reaction. Because membranes contain readily oxidizable unsaturated fatty acids, the stability of these membranes (and hence their function) is dependent on the activity of the antioxidant system of which this selenium-containing enzyme is a part. If the diet is marginal in selenium but adequate in copper, iron, and vitamins A, E, and C, which also serve as antioxidants, then cell damage by free radicals will be minimized. These other antioxidants can and do suppress free radical formation. Whereas vitamin E serves an important role in suppressing free radical production, its site of action is separate from that of selenium in its role as an essential component of glutathione peroxidase. Studies of selenium-deficient rats given a vitamin E supplement showed that these rats had no change in enzyme activity. Similarly, vitamin E deficient rats show little improvement in red-cell fragility with selenium supplements despite the overlap in antioxidant function of the two nutrients. The antioxidant properties of these cell components, however, are of particular interest to the pharmacologist because many drugs are, in themselves, oxidizing agents that work by disrupting the membranes of invading pathogens. The aging process as well as carcinogenesis may well be related to the adequacy of the body's antioxidant system.[99,100] Because free radicals damage cells, these cells may not be replaced at the same rate as the rate of destruction or the damage that, in turn, may make the cell vulnerable to invasion by cancer-producing viruses or agents that initiate cancer growth. Anticancer drugs, as mentioned, may be strong oxidizing agents. Normal as well as infected cells of the host will be affected by drug treatment. Should the host's antioxidant system be compromised either by nutritional deficiency or because of some genetically determined deficiency in the maintenance of intracellular redox states, cell survival and normal function will be compromised.

Several drugs have been developed that have antiinflammatory properties and that also contain selenium. They work by catalyzing the degradation of peroxides much like glutathione peroxidase or by reducing the production of leukotriene B. Both actions serve to reduce inflammation.

Although approximately 36% of the total selenium in the body is associated with glutathione peroxidase, a number of other proteins in the body also contain this mineral. Table 12.7 provides a list of these proteins. Thirteen different selenoproteins, ranging in weight from 10 to 71 kDa have been identified. Several of these are glutathione peroxidase isozymes, and several have been isolated from a variety of cell lines. One, having thioredoxin reductase activity, has been isolated from human lung adenocarcinoma cells. Another, selenoprotein W, is thought to be responsible for white muscle disease when this protein is not made because of deficient selenium intake. Selenoprotein P, a protein that accumulates in plasma, may be a selenium transport protein, but its true function has yet to be elucidated. Selenium is an integral part of the enzyme type 1 iodothyronine deiodinase (DI, EC 3.8.1.4), which catalyzes the deiodination of the iodothyronines, notably the deiodination of

TABLE 12.7
Selenoproteins of biological importance

Cytosolic glutathione peroxidase
Extracellular glutathione peroxidase
Gastrointestinal glutathione peroxidase
Iodothyronine deiodinase
Phospholipid hydroperoxide glutathione peroxidase
Selenoprotein P
Selenoprotein W
Sperm capsule selenoprotein

thyroxine (T_4) to triiodothyronine (T_3), the most active of the thyroid hormones.[101,102] This deiodination is also catalyzed by type II and type III deiodinases, which are not selenoproteins (Figure 12.11). Whereas all the deiodinases catalyze the conversion of thyroxine to triiodothyronine, there are differences in the tissue distribution of these enzymes. The pituitary, brain, central nervous system, and brown adipose tissue contain types II and III, whereas type I is found in liver, kidney, and muscle. These two isozymes (II, III) contribute very little triiodothyronine to the circulation except under conditions (i.e., starvation) that enhance reverse triiodothyronine (rT_3) production. In selenium-deficient animals, type I synthesis is markedly impaired, and this impairment is reversed when selenium is restored to the diet. Under these same conditions the ratio of T_3 to T_4 is altered. There is more T_4 and less T_3 in the deficient animals, and the ratio of the two is reversed when selenium is restored. Because type II and III deiodinase also exist, these enzymes should increase in activity so as to compensate for the selenium-dependent loss of function. However, they do not do this because their activity is linked to that of the type I. When T_4 levels rise (as in selenium deficiency), this rise feeds back to the pituitary, which in turn alters (reduces) TSH release. The conversion of T_4 to T_3 in the pituitary is catalyzed by the type II deiodinase, yet TSH release falls (Figure 12.12). T_4 levels are high because the type I deiodinase is less active (Figure 12.13). Whereas the deficient animal might have a T_3/T_4 ratio of 0.01, the sufficient animal has a ratio of 0.02, a doubling of the conversion of T_4 to T_3. The effect of selenium supplementation on the synthesis and activity of the type I deiodinase probably explains the poor growth of deficient animals. Sunde and coworkers[103] have reported significant linear growth in deficient rats given a single selenium supplement, and this growth was directly related to the supplement-induced increase in type I deiodinase activity. In turn, the observations of changes in selenium status coincident with changes

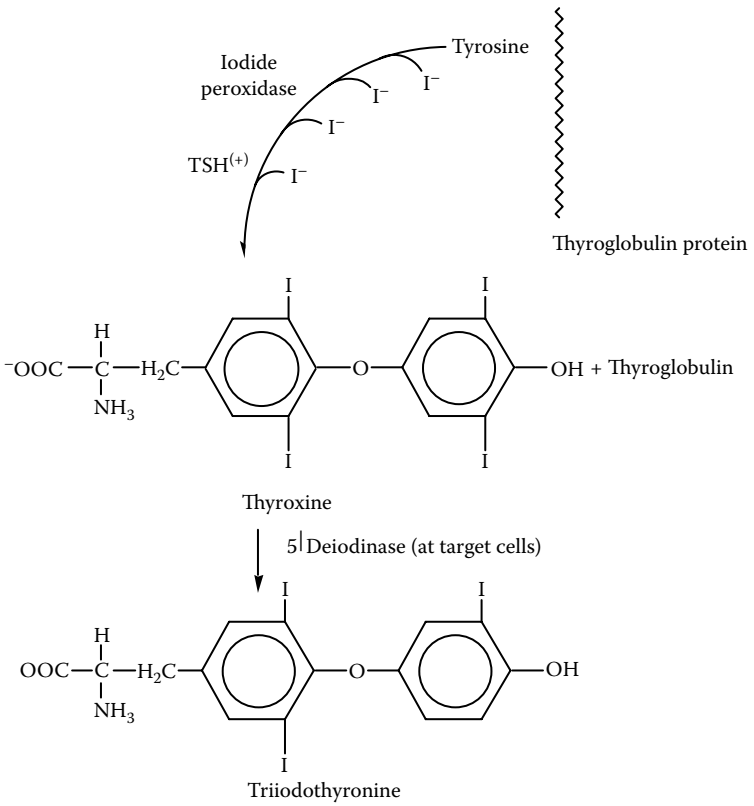


FIGURE 12.11 Schematic representation of thyroid hormone synthesis.

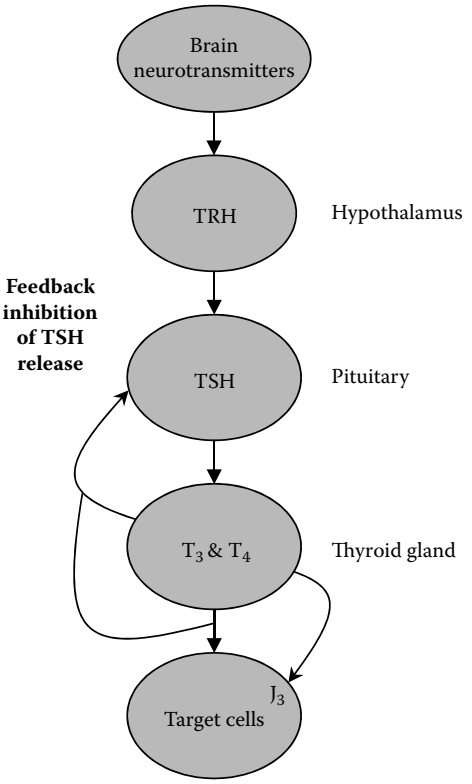


FIGURE 12.12 Feedback inhibition of TSH release.

in thyroid hormone status provided the necessary background for establishing the selenium–iodine interaction that today is taken for granted. The role of selenium in the synthesis of type I deiodinase clearly explains the lack of goiter (enlarged thyroid gland) in cretins who lack both iodine and selenium in their diet. Thus, low selenium intakes impair thyroid hormone activity, and there is a selenium-iodine interaction.

Selenium–Mineral Interactions

Other trace mineral interactions also exist.[11] Copper-deficient rats and mice have been shown to have reduced glutathione peroxidase activity. Copper deficiency increases oxidative stress yet oxidative stress affects all of the enzymes involved in free radical suppression. Even though glutathione peroxidase does not contain copper, the expression of the genes for this enzyme and for catalase is reduced in the copper-deficient animal. Note in Figure 12.10 that other trace minerals are involved as required components for SOD. Copper, zinc, magnesium, and manganese are part of the antioxidant system as is NADPH and NAD (niacin-containing coenzymes). The NAD, although not usually shown as part of the system, is involved because it can transfer reducing equivalents via the transhydrogenase cycle to NADP. Clearly, there are numerous nutrient interactions required for the maintenance of the optimal redox state in the cell.[104] This is important not only because it stabilizes the lipid portion of the membranes within and around the cells but also because it optimizes the functional performance of the many cellular proteins.

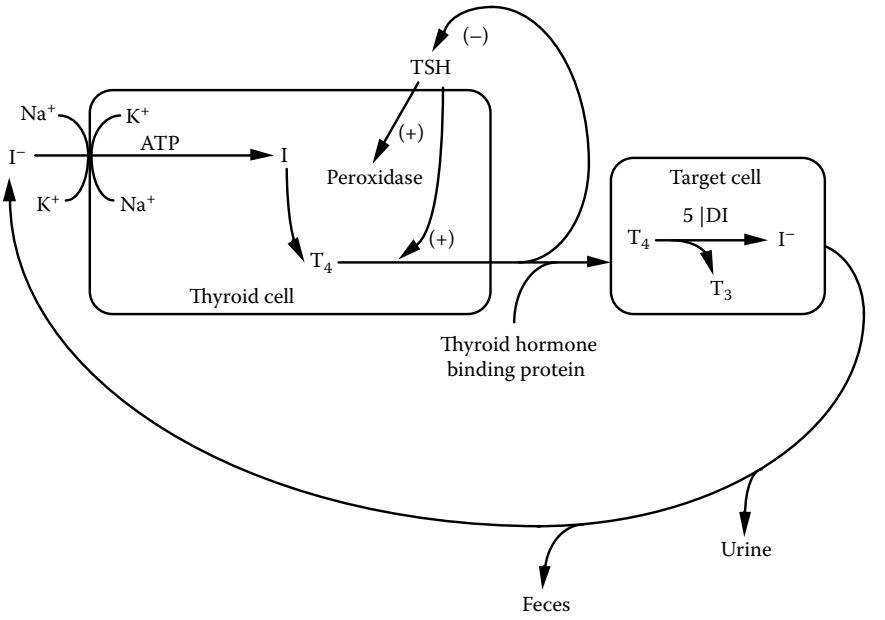


FIGURE 12.13 Overview of thyroid hormone production. Abbreviations used: TSH, thyroid-stimulating hormone from the pituitary; T_4, thyroxine; T_3, triiodothyronine; 5´DI, 5´-deiodinase. The thyroid gland is stimulated to produce and release thyroxine by TSH. Thyroxine is carried to the target cells by a binding hormone. The target cells then deiodinate the thyroxine producing T_3.

Deficiency

Selenium deficiency can develop in premature infants and in persons sustained for long periods of time by selenium-free enteral or parenteral solutions.[105] Symptoms characteristic of deficiency in humans include a decline in glutathione peroxidase activity in a variety of cell types,[106] fragile red blood cells, enlarged heart, cardiomyopathy, growth retardation, cataract formation, abnormal placenta retention, deficient spermatogenesis, and skeletal muscle degeneration. Some of these characteristics are shared with other species as shown in Table 12.8. In China, selenium deficiency is called Keshan disease.[12] It appears mainly in children and is marked by degenerative changes in the heart muscle (cardiomyopathy). This develops in children whose intakes are less than 17 µg/d. Selenite-enriched salt has been shown to reverse this deficiency, and the time needed for this reversal in all cells depends on the half-life of the affected cells. Those cells that turn over rapidly will quickly show signs of deficiency and just as quickly show signs of reversal. Should cells that are not normally replaced quickly be affected by the deficient state, that effect will not be quickly reversed by selenium supplementation. Blood levels of selenium are not especially informative when it comes to the assessment of selenium deficiency.[107]

Toxicity

Selenium intake in excess of 400 µg/d is the upper tolerable limit for this mineral.[88,108] An intake of 750 µg/d is toxic. Toxicity does not usually occur unless the individual is exposed not only to high diet levels but also to industrial conditions (smelters, selenium rich smoke, etc.) that increase entry of the mineral into the body. Selenium toxicity in farm animals has been observed when the food supply for these animals consists of selenium-rich pastures and grain. Selenium toxicity in these

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TABLE 12.8
Signs and symptoms of selenium deficiency in animals

Poultry	
Chickens, Turkeys, Ducks	Exudative diathesis, skeletal myopathy, encephalomalacia, pancreatic necrosis, reduced growth, reduced egg production, reduced fertility, reduced feather growth
Bovines	Reduced growth, skeletal and cardial myopathy, embryonic death, retained placenta
Equines	Skeletal myopathy, reduced performance, foals also have muscle steatitis
Ovines	Skeletal and cardial myopathy, poor growth, reduced fertility, embryonic death
Porcines	Poor growth, skeletal myopathy, "mulberry heart disease," gastric ulcers, hepatic dysfunction
Rodents	
Rabbits, Mice, Rats	Skeletal myopathy, erythrocyte hemolysis, testicular degeneration, fetal death, and resorption, increased hepatic malic enzyme and glutathione reductase activity

animals is characterized by hoof loss and a neuromuscular condition known as "blind staggers." Damage to the liver and muscle is observed as well. Excess selenium intake interferes with zinc absorption and use, reduces tissue iron stores, and increases copper level in heart, liver, and kidney. Clearly, selenium excess upsets the normal trace element balance in the body.

IODINE (IODIDE)

The essentiality of iodine has been recognized since the late 1800s. Its relationship to the production of thyroxine was not fully realized but even the ancient medical literature recommended the consumption of seaweed or burnt sponges (both of which are rich in iodine) for the treatment of goiter. Iodine deficiency used to be endemic in all but the coastal regions of the world. Presently, it is frequently observed in third-world nations whose access to iodine is limited. With the advent and use of iodized salt and the development of means to process and distribute frozen seafoods, this once common nutritional disorder has all but disappeared.[109]

Iodine is a member of the halogen family which includes fluorine, chlorine, and bromine. These appear as group VII in the fifth period of the periodic table. Iodine has an atomic number of 53 and an atomic weight of 127. There are no naturally occurring isotopes, but two radioisotopes are useful in biological systems. These are ^{131}I with a half-life of 8 days and ^{125}I with a half-life of 60 days. Iodine is very labile but forms stable salts, the most common being Na I. The iodide ion has a negative one valence, I^-.

Absorption, Metabolism, Excretion

The average human in the United States consumes between 170–250 μg/d of iodine. It is converted to the iodide ion (I^-) and is easily absorbed. Once absorbed, it circulates in the blood to all the tissues of the body. Salivary glands, the gastric mucosa, choroid plexus, and the lactating mammary gland as well as the thyroid gland can concentrate iodine. Of the iodine consumed, 80% is trapped by the thyroid gland, which uses it for the synthesis of thyroxine.

As shown in Figure 12.11 and Figure 12.13, thyroxine is synthesized through the stepwise iodination of tyrosine. The gland produces and releases thyroxine when stimulated by the pituitary thyroid- stimulating hormone (TSH). TSH acts on the tyrosine-rich thyroglobulin serving to

unravel this protein to make tyrosine available for iodination via the enzyme, iodide peroxidase. First, monoiodothyronine is produced, then diiodothyronine, triiodothyronine, and then thyroxine. TSH stimulates the thyroid to release this thyroxine, which is transported to its target tissues (all the cells of the body) by way of a transport protein called *thyroid-binding protein*. Upon delivery to the target cell, the thyroxine is carried into the cell and deiodinated to triiodothyronine. The enzyme catalyzing this reaction is 5′ deiodinase, a selenium-containing enzyme. Triiodothyronine is the active form of the hormone having at least 10 times the activity of thyroxine. The iodine released by this deiodination is conserved and sent back into the bloodstream for further use by the thyroid gland. Iodide not used or sequestered is either excreted as organic iodine in the feces or as free iodine in the urine. The urinary loss is 40 times greater than the fecal loss. Figure 12.13 illustrates the endocrine pathway for thyroid hormone production, function, and excretion.

Deficiency

The enlargement of the thyroid gland (goiter) is characteristic of iodine deficiency and is marked by a hypertrophic, hyperplasic change in the gland's follicular epithelium. These epithelial cells synthesize thyroxine, and their growth is stimulated by the pituitary hormone, TSH. The cells become enlarged when iodine is not available to complete the thyroxine synthetic process. A goiter is not only unsightly but may also cause obstruction of the airways and damage the laryngeal nerves. The synthesis of thyroxine is the only known function of iodine. Thyroid hormone production can be influenced not only by iodine deficiency but also by selenium deficiency. In the latter instance, the active thyroid hormone, triiodothyronine, is not produced because the deiodination of thyroxine is dependent on the selenoprotein, 5′ deiodinase.

A diet deficient in iodine can cause a number of illnesses depending on the age of exposure.[110] The developing embryo or fetus of an iodine-deficient woman will suffer a neurological deficit that is quite profound. Infants and children consuming a deficient diet fail to grow normally and develop intellectually. Pregnant women may lose their pregnancies as the fetus fails to develop and dies in utero.

The deficient state can develop in instances where the population subsists on a marginal iodine intake and consume a diet rich in vegetables of the *Brassica* genus (*Cruciferae* family). Although cabbage and related vegetables have been shown to contain goitrogens (compounds that inhibit thyroxine release by the thyroid gland or that inhibit iodine uptake by the gland) other vegetables contain these substances as well.

Need

Because iodine is conserved very efficiently and is not toxic even at 10–20 times the required intake, iodine is considered to be a benign (but essential) trace element. An intake of 1–2 μg/kg should be adequate for most adult humans. Pregnant females need slightly (10–20%) more. Thus, the Food and Nutrition Board of the National Academy of Sciences has recommended an intake of 150 μg/d for adults, 6 μg/kg/d for infants, 5 μg/kg/d for young children, 4 μg/kg/d for older children, and 175 μg/d for pregnant and lactating females. Table 12.1 provides the details of these DRIs.

MOLYBDENUM

Molybdenum as an essential nutrient for humans and animals was first recognized in 1953. Its importance in relation to copper and iron was appreciated in that an excess of molybdenum interfered with copper and iron absorption. Molybdenum also interferes with the binding of copper by ceruloplasmin. In ruminants, molybdenum excess when combined with a high sulfur intake results in a copper-deficient state. The three elements (Mo, Cu, S) combine to form cupric thiomolybdate complexes. In humans, excess molybdenum intakes occur rarely. This is because most human foods that contain molybdenum also contain copper in amounts that exceed that of molybdenum.

Molybdenum is a transition metal in the fifth period of the periodic table. It has an atomic weight of 95.94 and an atomic number of 42. There are several naturally occurring isotopes ranging in weight from 92 to 100; however, Mo^{96} is the most useful in biological research. There are eight radioisotopes with weights ranging from 88 to 93. Molybdenum has four charged states: +3, +4, +5, and +6. The most common is the +6 state. Where this mineral serves as a cofactor in enzymatic reactions, it vacillates between +4 and +6.

Absorption and Excretion Function

Molybdenum is readily absorbed from most foods. Between 40 and 100% of the ingested mineral will be absorbed by the epithelial cells of the gastrointestinal system and will be found in the blood in a protein bound complex. A plasma concentration range of 0.5–15 μg/dL has been reported, and molybdenum may be found in very small amounts (0.1–1.0 μg/g) in most cell types. Some accumulation occurs in the liver, kidneys, bones, and skin. Molybdenum is readily excreted in the urine with small amounts excreted via the bilary route. The average intake of 300 μg/d is one-third to one-fifth that of copper.

Molybdenum's function is as a cofactor for the iron and flavin enzymes, xanthine oxidase, aldehyde oxidase, and sulfite oxidase. The molybdenum cofactor common to these enzymes is a pterin structure shown in Figure 12.14. This pterin is a monophosphate ester susceptible to cleavage by alkaline phosphatase.[111] One molecule of pterin phosphatase is associated with each molybdenum atom in sulfite oxidase and xanthine dehydrogenase. In situ oxidation of the pterin leads to an inactivation of sulfite oxidase. Molybdenum also serves to activate adenylate cyclase in brain, cardiac, and renal tissue and erythrocytes.[112] It has no effect on testicular adenylate cyclase, nor does it have an effect on adenylate cyclase previously stimulated with fluoride or GTP.

Food Sources and Recommended Intake

Molybdenum is found in very small amounts in most foods. The germ of grain is a good source of this mineral; however, much of this germ is lost when the grain is milled. Table 12.1 shows the recommendations for intakes by the different age/life stage groups.

MANGANESE

Although manganese (Mn) was recognized as an essential nutrient in 1931, its ubiquitous role as a cofactor in a variety of enzymatic reactions has only recently been appreciated. Structural abnormalities in growing birds and animals are the chief symptoms of deficiency. Depression of mucopolysaccharide synthesis and decreased mitochondrial manganese superoxide dismutase activity accompany these skeletal abnormalities as does congenital ataxia due to abnormal inner ear development and abnormal brain function.[113] Biochemical abnormalities were hard to document because many of the requirements for manganese as a cofactor in a number of enzymatic reactions could be

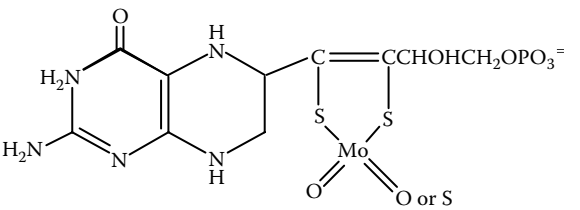


FIGURE 12.14 Pterin structure with molybdenum as part of its molecule.

met by magnesium. Nonetheless, bone and connective tissue growth are abnormal in manganese deficient animals. Few cases of human manganese deficiency have been reported.

Manganese, like magnesium, is a transition element. It can exist in 11 oxidation states. However, in mammalian systems, it usually exists in either the +3 or +2 state. In the +2 state it is less easily chelated than in the +3 state. The +3 state is one which interacts with the ferric ion (Fe^{+++}); it is also the state needed for service as a cofactor in the Mn superoxide dismutase enzyme.

Absorption and Excretion Function

Manganese is poorly absorbed through the gut. Between 2 and 15% of that ingested in the food appears in the blood. This has been traced using radioactive ^{54}Mn. As with many minerals, the route of excretion is via resecretion into the small intestine. That which is unabsorbed appears in the feces as does that which is excreted from the body via the bile. The mechanism whereby manganese is transported from the site of absorption to the site of use is not fully known. It may share transport with iron using transferrin or another protein, notably α-2-macroglobulin. Manganese circulates throughout the body at a level of 10–20 μg/L (~2 μmol/L). Widely varying blood levels of manganese have been reported. Smith et al.[114] contend that this wide variation has to do with contamination introduced by both sampling technique and analytical technique. Contamination can double the amount of manganese in the food, blood, or tissue sample. It is for this reason that balance studies to determine apparent absorption are not useful. Actually, this problem is common to many of the trace minerals. They are present in such small amounts in biological samples that extreme precautions must be taken to control contamination. Once transported from the gut to the liver, almost all of the manganese is cleared from the blood. In the liver it enters one of several pools. It can be found in the bile canaliculi from which it can be exported via the bile back into the small intestine, or it can enter the hepatocytes, where it is used by its various organelles (lysosomes, mitochondria, and nucleus), as a cofactor for several enzymes, or it can remain in the cytoplasm.

Manganese is present in the bone as part of the mineral apatite, and in the lactating gland, and liver to a greater extent than in other tissues.[114] However, no tissue is free of this mineral. The turnover of manganese in the body varies depending on location. It is very short (~3-min half-life) in hepatic mitochondria and, in bone, its half life is about an hour. The pool size likewise varies from 2 mg/kg in liver to 3.5 mg/kg in bone. As mentioned, it is an essential cofactor in a wide variety of enzymatic reactions[115–117] and is essential for normal fetal and neonatal development.[118] Listed in Table 12.9 are some of the many enzymes shown to require manganese as a cofactor. Manganese and calcium share a uniport mechanism for mitochondrial transport. It can accumulate in this organelle because it is cleared very slowly. Mitochondrial manganese efflux is not sodium

TABLE 12.9
Enzymes requiring manganese

Acetyl CoA carboxylase
Arginase
Galactose transferase
Glucokinase
Hydroxymethyl transferrase
Isocitrate dehydrogenase
Mitochondrial superoxide dismutase
Pyruvate carboxylase
Superoxide dismutase

dependent and in fact appears to inhibit both the sodium dependent and independent calcium efflux. This manganese effect on calcium is not reciprocal; calcium has no effect on manganese efflux.[116]

Evidence has been reported that manganese deficiency in experimental animals causes the downregulation of the mitochondrial manganese-containing superoxide dismutase (SOD) at the level of the activation of transcription of the gene that encodes this protein.[115,119] Superoxide dismutases are a class of metalloproteins that catalyze the dismutation of the superoxide radical (O^{-}_{2}) to oxygen (O_2) and hydrogen peroxide (H_2O_2). These enzymes play a critical role in protecting cells against oxidative stress, particularly that produced by drugs (see Figure 12.7). Most mammalian cells contain two forms of this enzyme: one in the cytosol requiring iron or zinc and copper, and the other requiring manganese. It is generally thought that the latter, present in the mitochondria, protects that organelle from potential damage by the superoxide radical that could possibly be produced through the activity of the respiratory chain. The important role of Mn SOD has been demonstrated using cell cultures. Those cultures in which the gene for this enzyme was altered, or in which the enzyme was inhibited, died. Apoptic cell death has been reported in cultured spinal neurons and in PC12 neuronal cells. Maintenance of mitochondrial manganese dependent SOD is especially important if a drug is used that disrupts the control or function of the respiratory chain. Redox active drugs (e.g., antibiotics, tetracycline, or pesticides, e.g., paraquat, have this effect.) When these compounds are given to animals or cells in culture, the activity of the manganese-superoxide dismutase increases. Interleukin-1 and tumor necrosis factor also increase the activity of this enzyme.[117] Some tumor cells respond to chemotherapeutic agents by increasing the activity of this enzyme as well. As a result, these tumors are resistant to certain cytotoxic agents. This is particularly true for melanoma cells and may explain the resistance of this cancer to many of the therapies found successful for other tumor cell types.

Food Sources, Recommended Intake

Rich food sources include nuts, whole grains, and leafy vegetables. Meats, milk, seafood, and other animal products are poor sources. The germ of grains can contain up to 14 ppm. The average diet consumed in the United States contains about 3–4 mg/d. The range of intakes suggested by the National Academy of Sciences is from 2–11 mg/d, depending on the age and life stage of the consumers (see Table 12.1).

Toxicity

Manganese toxicity with symptoms resembling Parkinson's disease has been reported to occur in up to 25% of miners and metal mill workers in Russia, North Africa, Chile, and the former Yugoslavia. The inhalation of mineral dust in mines and mills is the main route of excess exposure.

COBALT

The discovery of cobalt as an essential nutrient for mammals was first made in Australia by animal scientists seeking to understand the pathophysiology of a wasting disease in sheep and cattle. At first it was thought to be an iron deficiency disorder because iron supplements seemed to cure the condition. The iron supplements, however, were rather impure substances. In the mid 1930s, Underwood and associates discovered that it was not the iron per se but an impurity in the supplement that cured the condition. That impurity was cobalt.

Cobalt as an essential nutrient for humans was assumed based on the results of these animal studies. Many human foods, especially green leafy vegetables, contain cobalt. Meats, including the organ meats, provide cobalt as a component of vitamin B_{12}. Of the free cobalt found in these foods, very little is used although absorbed by the enterocyte. The results of tracer studies have shown that almost 100% of ingested cobalt appears in the urine. Very little appears in the feces and very little

is retained in the tissues. As discussed in the section on vitamin B_{12} in the chapter on water-soluble vitamins, the absorption of this vitamin (and its cobalt component) is dependent on the presence of intrinsic factor in the stomach. Although in ruminants the consumption of cobalt cures wasting disease (pernicious anemia), it does this not because the ruminant can absorb and then use this metal, but because the rumen flora can synthesize vitamin B_{12}, which in turn is absorbed. This, then, is the basis for understanding why vegetarians consuming large amounts of cobalt-rich green leafy vegetables are at risk for developing pernicious anemia. Vitamin B_{12} is found in foods of animal origin. Humans do not synthesize this vitamin as do the flora found in the ruminant. Whereas vegetarians obtain sufficient cobalt, they may be vitamin B_{12} deficient. Cobalt has no other known function aside from its central action in vitamin B_{12} function.

Toxicity, Mineral Interactions

Although cobalt is readily absorbed by the enterocyte, it is just as readily excreted by the kidney. Thus, toxicity in the usual sense is not a significant problem with respect to environmental exposures. Excess cobalt (1000 times normal) can be tolerated by a variety of species with little ill effect. However, cobalt does interfere with the absorption of iron and in fact can completely block iron uptake. Symptoms of iron deficiency anemia as well as symptoms of disturbed thyroid function (enlarged hyperplasic thyroid gland, myxedemia) and congestive heart failure have been observed when excess cobalt was consumed accidentally as a contaminant of beer. Cobalt salts were once used in the production of beer as a foaming agent. Whereas the occasional beer would not provide toxic amounts of cobalt, regular large intakes could and have done so. Once this was recognized, this production practice was discontinued. Actually, it was not the cobalt content of the beer alone that was responsible for the toxic response. The alcohol of the beer plus the likelihood of inadequate protein, iron, and B vitamin intake were also part of the problem. Cobalt together with manganese and iodine are involved in the synthesis of thyroxine. Whether they are interacting or merely essential cofactors in thyroxine synthesis has not been satisfactorily resolved.

OTHER MINERALS

Shown in Table 12.3 are a variety of minerals which have been shown to be essential to one or more species, not necessarily man.[2] Included in this list is fluorine (fluoride), an element known to provide hardness to teeth and bones, and which also inhibits tooth decay. Arsenic, a known poison, has been shown to be essential to chickens, rats, pigs, and goats, but its biological function is not known. Animals with arsenic deficiency exhibit depressed growth, myocardial degeneration, and premature death. Boron, likewise, is an essential nutrient for rats, but its function is not known. Deficient rats have a reduced stress response, which probably relates to a change in brain electrical activity. Boron-copper and boron–calcium-phosphate interactions have been reported. These interactions in boron-deprived animals have to do with increased brain copper concentrations with the former, and increased calcium and phosphate concentrations with the latter.

Chromium as an essential mineral is controversial.[2,120] Early in its history it was reported to be essential for optimal peripheral insulin action with respect to glucose uptake.[121] Studies of elderly humans with noninsulin-dependent diabetes mellitus showed an improvement in glucose tolerance in about half of the subjects following a period of chromium supplementation.[122] However, chromium supplementation of subjects with established diabetes had no effect on glucose tolerance in other studies.[123,124] There is thus a question as to whether chromium has an effect on insulin-mediated glucose metabolism. In part, some of the confusion may be related to the analytical methods used for chromium analysis. These have been found inadequate.[2] Chromium has been found in bone[114] and in breast milk.[125] Whether excess chromium is toxic is also questionable because of its low availability. Chromium picolinate in a dose response study has been shown to cause chromosome damage in vitro to hamster ovary cells.[19]

Nickel is another mineral whose function has not been defined, yet animals fed nickel-deficient diets fail to thrive. Nickel is a component of the urease enzyme family and although not found in mammalian urease has been found in ureases isolated from lower life forms. Similarly, nickel has been found associated with hydrogenases in lower life forms, and in these species has a role in oxidation-reduction reactions as well as in methane formation.

Silicon has been found essential to chickens and rats, and appears to be involved in bone formation.[2] Skeletal abnormalities typify silicon deficiency in these species.

Vanadium is needed by chickens for appropriate pigmentation;[2] however, whether this need is a true need or one induced by the particular diet ingredients used to compose the deficient diet is subject to discussion.

Aluminum, boron, tin, cadmium, lead, germanium, lithium, and rubidium have all been examined with respect to their essentiality. Data are lacking that document man's need for these elements. As mentioned above, these minerals can be toxic if exposure is great.

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Glossary

Throughout this text the reader may find terms or expressions that are unfamiliar. Scientists in this hybrid field of nutrition–biochemistry–genetics–physiology sometimes "speak in code." They adopt a text shorthand that is known to them but seldom explained to the uninitiated. It is for this reason that a list of definitions for these abbreviations and terms are provided. This glossary can also serve as a review for the reader whose background preparation for a course in advanced nutrition may be a bit "rusty." Some of the terms are medical ones, some are abbreviations common to biochemistry and/or biotechnology, and still others are relevant to the epidemiology of nutrition related diseases.

Acarbose A drug that inhibits α-glucosidase, an enzyme important to the digestion of starch

ACAT acyl coenzyme A:cholesterol acyl transferase. An enzyme that catalyzes the formation of an ester linkage between a fatty acid and cholesterol

Acetaldehyde An aldehyde formed from the oxidation of ethanol; can be converted to acetate and then to acetyl CoA

Acetaldehyde dehydrogenase An enzyme that catalyzes the conversion of acetaldehyde to acetate

Acetoacetate A 4-carbon compound found in large amounts in the uncontrolled insulin-dependent diabetic. It is a normal end product of fatty acid oxidation and can be further metabolized and used as fuel by normal tissues. Its further metabolism yields carbon dioxide and water

Acetone A 3-carbon ketone found in normal tissues and blood. It can accumulate if the citric acid cycle is not working properly. Levels of acetone are high in the uncontrolled insulin-dependent diabetic and can be toxic

Acetonemia Higher-than-normal levels of acetone in the blood. Normal levels can be between 3 and 20 mg/mL. In the uncontrolled insulin-dependent diabetic, individual acetone levels can significantly exceed 20 mg/ml

Acetonuria Higher-than-normal (1 mg/L/24 h) levels of acetone in the urine

Acetyl CoA A 2-carbon metabolic intermediate that is the starting substrate for fatty acid synthesis. It is also the end product of fatty acid degradation (fatty acid oxidation). When joined to oxalacetate it enters the citric acid cycle as citrate, and the CoA portion of the molecule is removed for use in other reactions

Acetylcholine A neurotransmitter released by a calcium-dependent process in response to the arrival of an action potential. Acetylcholine initiates changes in ion permeability that occur at the neuromuscular junction. Acetylcholine is hydrolyzed by acetylcholine esterase. The choline is then reused

Achlorhydria Absence of hydrochloric acid in the stomach

Acid-base balance Minute-to-minute regulation of the hydrogen ion concentration (pH) in the body; accomplished through the action of the bicarbonate buffering system, the phosphate buffering system, and the action of body proteins as buffers

Acidic amino acids Amino acids having two carboxyl groups in their structures. These amino acids are aspartic and glutamic acids

Acidosis When the blood pH falls below 7.4, acidosis develops

ACP Acyl carrier protein. A pantothenic acid-protein-thio-ethanolamine structure upon which fatty acid biosynthesis occurs

ACTH (Adrenocorticotropin hormone) Hormone released by the anterior pituitary that stimulates the release of the adrenal cortical steroid hormones (cortisol, corticosteroid, hydrocortisone, dehydroepiandrosterone, aldosterone)

Active site That part of an enzyme or hormone or carrier or other substance to which a substrate binds

Activity increment The energy needed to sustain body activities

Acylation The addition of an acyl group (carbon chain) to a substrate

ADH Antidiuretic hormone, also called vasopressin, produced by the posterior pituitary; plays a role in water conservation by the body. It stimulates water reabsorption by the renal tubules

Adenine A purine base that is an essential component of the genetic material, DNA

Adenyl cyclase The enzyme responsible for the conversion of ATP to cyclic AMP, an important second messenger for hormones that stimulate catabolic processes

Adipocytes Cells that store fat

Adiponectin Hormone secreted by adipocytes; acts as an insulin-sensitizing hormone

Adipsia Lack of thirst sensation

ADP Adenosine diphosphate. A metabolite of ATP. Energy is released when ATP releases a phosphate group (Pi) and becomes ADP

Aerobic metabolism Metabolic reactions that require oxygen

Albumin A small molecular weight protein found in blood and sometimes in urine

Alcohol A hydrocarbon containing a hydroxyl group (–OH) attached to one of the carbons in the carbon chain

Aldehyde A carbon compound containing a –C =O group

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Aldosterone A steroid hormone produced by the adrenal cortex that acts on the cells bordering the distal tubules of the kidney, stimulating them to reabsorb sodium

Allele Any one of a series of different genes that may occupy the same location (locus) on a specific chromosome in the nuclear DNA

Aliphatic A chain of carbon atoms with associated hydrogen and/or hydroxyl groups

Aliphatic amino acids Amino acids which consist of a chain of carbon atoms. These include alanine, cysteine, glycine, isoleucine, leucine, methionine, serine, threonine, and valine

Alkalosis A condition where the blood pH rises above 7.4

Allergens Substances capable of eliciting an immunologic response

Allosterism The binding of an inhibitor or activator to an enzyme at a site removed from the active site of that enzyme

Alopecia Loss of hair

Alveoli Cells in the lungs responsible for O_2/CO_2 exchange

Amide bond (Peptide bond) A bond involving an amino ($–NH_3$) group

Amino acids Simple organic nitrogenous compounds that are the building blocks of proteins

Aminoacidopathy Inborn errors (mutations in specific base sequences for specific gene products) resulting in inadequate utilization of one or more amino acids

Aminotransferases (transaminases) Enzymes that catalyze the transfer of an amino group ($–NH_2–$ or $–NH_3$) from one carbon chain to another

AMP Adenosine monophosphate. Metabolite of ADP. Energy is released when ADP releases a phosphate group (Pi) to become AMP

Amphipathic The characteristic of a compound containing both polar and nonpolar groups. This allows the compound to be soluble in both polar and nonpolar solvents. Water is a polar solvent; chloroform is a nonpolar solvent

Amphoteric The characteristic of a compound that allows it to have either an acidic or a basic function, depending on the pH of the solution

Amylase An enzyme that catalyzes the cleavage of glucose molecules from amylose (starch)

Amylin a 37 amino acid peptide cosecreted with insulin; it serves as a satiety signal

Amylopectin A branched-chain polymer of glucose found in plants

Amylose A straight-chain polymer of glucose also found in plants
Anabolism The totality of reactions that account for the synthesis of the body's macromolecules
Androgens Male sex hormones
Android obesity A form of obesity in which fat stores accumulate over the shoulders and abdominal area. Sometimes referred to as the "apple" shape
Anemia Below normal amounts of hemoglobin and/or red blood cells
Aneurism A bulge in the vascular tree (usually arterial)
Angina pectoris Chest pain due to lack of oxygen supplied to the heart muscle
Angiotensin The natural substrate for renin
Angiotensin converting enzyme (ACE) The enzyme responsible for converting angiotensin I to angiotensin II in the kidney
ANH (Atrial Natriuretic Hormone) The hormone that counteracts ADH
Anosmia Loss of the sense of smell
Anoxia Lack of oxygen in blood or tissues
Antecedents Events that precede or are causally linked to an event
Anthropometry Measurement of body features (i.e., weight, height, etc.)
Antibiotics Drugs that inhibit growth of (or kill) pathogens
Antibody Proteins produced by the B cells and T cells. (Parts of the body's immune system) that in turn react with antigens. Each antigen has a specific antibody that senses and reacts to it. In autoimmune disease, there is a loss in antigen specificity and antibodies react to groups of similar proteins some of which may be the body's own protein
Anticoagulants Compounds that interfere with blood clotting
Anticodon A sequence of three bases in the transfer RNA which is complimentary to that in the messenger RNA
Anticoding strand A strand of DNA that is used as a template to direct the synthesis of RNA that is complementary to it
Antigen A compound external (usually) to the body that elicits the synthesis of antibodies specific to its structure
Antioxidants Agents that prevent or inhibit oxidation reactions
Anus Opening at the posterior end of the digestive tract
Apoenzyme The inactive part or "backbone" of an enzyme protein
Apoprotein Carrier proteins that carry specific compounds such as lipid or vitamins or some other material requiring a carrier
Apparent digestibility Nutrients may not be completely available from foods that are consumed. To determine availability the amount of the nutrient in the feces is subtracted from the amount of the nutrient in the ingested food
Apparent digested energy (DE) Energy in the food consumed less the energy in the feces: DE=IE–FE
Apoptosis Programmed cell death
Arachidonic acid Long-chain fatty acid having four double bonds and 20 carbons
Archimedes' principle An object's volume which, when submerged in water, equals the volume of the water it displaces. If the mass and volume are known, the density can be calculated
Aromatic amino acids Amino acids that have a ring structure. Included are phenylalanine, tyrosine, and tryptophan
ARS Autonomously replicating sequence; the origin of replication in yeast
Arteriography A method of examining the major arteries using X-rays and an infusion of an opaque dye solution
Aseptic Sterile; absence of pathogens
Atherogenic Producing or inducing atherosclerosis
Atherosclerosis, Arteriosclerosis A progressive degenerative condition occurring within the vascular tree and resulting in occlusions and loss of elasticity of the vessel

ATP Adenosine triphosphate, the energy-rich compound that is produced by oxidative phosphorylation in the mitochondria. ATP can also be produced by substrate phosphorylation

ATPase A group of enzymes that catalyze the removal or addition of a single phosphate group to the high energy compound containing adenine

Atrophy Reduced size of an organ, tissue, or cell

Attenuated Weakened or lessened activity of a given biologically active compound

Autocrine system A variation of the paracrine system where the hormone secreting cell and the target of the hormone are located in the same tissue

Autoimmunity The destruction of self through the development of antibodies to the body's own proteins

Autonomic nervous system The system that includes portions of the brain, spinal cord, and adrenals and that serve to regulate the synthesis and release of epinephrine, enkephalins, and norepinephrine

Autosomal recessive trait A genetic trait that will express itself only if the individual inherits two identical copies of the same gene (one from each parent)

Autoxidation Reaction of an organic compound with elemental oxygen under mild conditions

B cells Lymphocytic cells produced in the bone marrow that are not processed further by the thymus gland

ß cells Cells in the islets of Langerhans in the endocrine pancreas that release insulin upon a glucose signal

Bacteriophage Bacteria eater

Bariatrics A medical term referring to the management of people who are overfat or obese

Baroreceptors Sensory nerve endings in the walls of the auricles of the heart, carotid artery sinus, vena cava, and aortic arch that respond to changes in pressure

Basal Energy Expenditure (BEE) Estimated energy used by the body at rest in a fasting state to maintain essential body functions. The BEE is then adjusted for activity, injury or disease, and thermic effect of food to predict the total daily energy expenditure. This is then used to estimate the energy intake requirement

Basal Metabolism (Basal Metabolic Rate, BMR) The minimum amount of energy needed to maintain the activity of metabolic processes occurring in the body. When rate is used an element of time is added

Basic amino acids Amino acids whose side chains have positively charged amino groups. These are histidine, lysine, and arginine

BCAA Branched chain amino acids. These are valine, leucine, and isoleucine

ß Alanine A naturally occurring form of alanine that is not incorporated into protein but which is an essential component of coenzyme A, pantothenic acid, and carnosine

ß-Carotene Precursor of active vitamin A

ß Hydroxybutyrate, ß hydroxybutyric acid Included in the group of compounds called ketone bodies

ß Oxidation Process in the mitochondria for the oxidation of fatty acids

Betaine Precursor of choline

Bicarbonate Any salt containing HCO_3; bicarbonate in the blood is indicative of the alkali reserve

Bile acids Cholic and chenodeoxycholic acid synthesized by the liver from cholesterol

Bile pigments Breakdown products of the heme portion of hemoglobin, myoglobin, and the cytochromes (bilirubin, biliverdin, and heme) excreted by way of bile

Bile salts Anionic forms of bile acids

Bioavailability Quantity of nutrient available to the body after absorption

Biogenic amines Compounds formed when amino acids are decarboxylated

BMI Body mass index = body weight (kg)/height $(cm)^2$

Bombesin A 14 amino acid peptide hormone found in the central nervous system, the thyroid gland, lung, adrenal, and skin and which has a broad spectrum of action

Brown Adipose Tissue (BAT) Highly vascularized adipose tissue with mitochondria-rich adipocytes

Buffer A compound that resists a change in hydrogen ion concentration in solution

Bulimia Habitual self-induced vomiting. Frequently accompanies anorexia nervosa

Cachexia Tissue wasting as in disease or overwhelming trauma or starvation

Calculus Synonym for gallstones or kidney stones; complex mineral precipitants

cAMP (Cyclic AMP) Cyclic 3′5′ adenosine monophosphate. A second messenger for catabolic hormone effects. Produced when adenyl cyclase acts on ATP

Cancer A group of diseases characterized by abnormal growth of cells which, because it is uncontrolled, subsume the normal functions of vital organs and tissues

Candidate gene A gene, if mutated, that is suspected to play a causal role in a clinical syndrome

Cap The structure at the 5′ end of eukaryotic mRNA, introduced after transcription by linking the terminal phosphate of the 5′ GTP to the terminal base of the mRNA. The added G and sometimes other bases are methylated

Carbohydrate Polyhydroxy aldehydes or ketones and their derivatives

Carbonyl group An aldehyde (–CH=O) group or ketone (–C=O) group; a group that characterizes aldehydes and ketones

Carboxylase An enzyme that catalyzes the addition of a carboxyl group to a carbon chain. This enzyme class usually requires thiamin as thiamin pyrophosphate (TPP) as a coenzyme.

Carboxylation The addition of a carboxyl (-COOH) group to a carbon chain

Carcinogenesis The process of developing cancer

Cardiomyopathy Structural or functional disease of the heart muscle (the myocardium)

Cardiovascular disease A group of diseases characterized by a diminution of heart action

Cariogenesis The process of tooth decay

Carnosine A dipeptide synthesized from ß alanine and histidine. Found in muscle where it activates myosin ATPase. Carnosine also serves to buffer the acidic state that develops in working muscle

Carotenes, carotinoids A group of yellow-orange pigments, some of which can be converted to active vitamin A. α, β, and γ carotene, are considered provitamins because they can be converted to active vitamin A. The carotenes also have an antioxidant function

Carrier A substance, usually a protein, that binds to a substrate and transports it from its point of origin to its point of use

Catabolism The totality of those reactions that reduce macromolecules to usable metabolites, carbon dioxide, and water

Catalase An enzyme which catalyzes the conversion of peroxide to water and oxygen; this is a key enzyme in the cytoplasmic free radical suppression system

Catalyst A substance, usually a protein, in living (or nonliving) systems that enhances or promotes a chemical reaction without being a reactant. Enzymes are catalysts

Catecholamines Neurotransmitters (epinephrine, dopamine, and norepinephrine) that are produced in the adrenal medulla from tyrosine. These hormones are instrumental in orchestrating the "fight or flight" response to stress conditions

Cation A positively charged ion

CDC Centers for Disease Control; an agency of the U.S. Department of Health and Human Services

cDNA A single-stranded DNA molecule that is complimentary to an mRNA and is synthesized from it by the action of reverse transcription

CDP Cytidine diphosphate. A nucleotide consisting of cytosine (a pyrimidine), ribose, and two-high energy phosphate groups

Cecum A blind appendage of the intestinal tract located at the juncture of the small and large intestine. Also called the appendix

Cellulose A structural polysaccharide found primarily in plants and composed of glucose units linked together with ß 1,4 bonds

Cephalin A phospholipid, similar to lecithin, present in the brain

Cerebroside A complex lipid in nerve and other tissues. A cerebroside is a sphingolipid

Cerebrovascular accident Also referred to as a stroke; sudden circulatory impairment in one or more blood vessels that supply the brain

Cerebrovascular disease Similar to cardiovascular disease in that the vascular tree of the brain has developed atherosclerotic lesions that restrict or occlude the blood supply to this organ

Ceruloplasmin A copper-containing globulin in blood plasma with a molecular weight of 150,000 and 8 copper atoms. It catalyzes the oxidation of amines, phenols, and ascorbic acid

Chelate, chelation A combination of metal ions chemically held within a ring of heterocyclic structures by bonds from each of these structures to the metal. Iron is held by heme in this fashion as part of the hemoglobin structure

Chimeric molecule A molecule of DNA or RNA or protein containing sequences from two different species

Chloride shift Part of the system which maintains the blood acid-base balance. Chloride and bicarbonate ions exchange across the erythrocyte plasma membrane and is called "the shift"

Cholecystitis Inflammation of the gallbladder

Cholecystokinin (CCK) A hormone released from duodenal cells and which stimulates the gall bladder to contract and release bile into the duodenum

Cholecholithiasis Obstruction of the common bile duct; presence of a stone in the duct

Cholelithiasis Presence or formation of gallstones

Cholestasis Arrest of bile excretion

Cholesterol A four-ringed structure in the nonsaponifiable lipid class that is an important substrate for steroid hormone synthesis

Chondrocytes Cartilage precursor cells or cartilage cells

Chorionic gonadotropin A heterodimeric glycoprotein hormone of 57 kDa consisting of a noncovalent bound α (92 amino acid residues) and a distinctive ß (134 amino acid residues) subunit found in the blood of pregnant females. The major function of this hormone is to stimulate the production of progesterone by the corpus luteum

Chromaffin cells Cells in the adrenal medulla and also the kidney, ovary, testis, heart and gastrointestinal tract. Similar cell types are found in the carotid and aortic bodies. In the adrenal medulla these cells produce epinephrine

Chromosomes When DNA is extracted from the cell nucleus, it is not one continuous strand. Rather, it breaks up into fairly predictable arrangements called chromosomes

Chromosome walking The sequential isolation of clones carrying overlapping sequences of DNA, allowing large regions of the chromosomes to be spanned. (Walking is often performed in order to reach a locus of interest.)

Chyle A turbid white or yellow fluid, taken up by the lacteals in the process of lipid digestion and absorption

Chylomicron Fat-protein complex formed to carry absorbed dietary fats from the intestine to other tissues. Not normally found in the blood of a fasting individual

Chyme The semifluid mass of partly digested food extruded from the stomach into the duodenum of the small intestine

Cilia, Cilium Hair-like processes extending out from the surface of epithelial cells

Citrate An intermediate in the citric acid cycle; the essential starting metabolite for fatty acid synthesis in the cytosol

Citric Acid Cycle (Kreb's Cycle) A cycle found in mitochondria that produces reducing equivalents for use by the respiratory chain and which also produces carbon dioxide

Clone A large number of cells or molecules that are identical to a single parental cell or molecule

CMP Cytidine monophosphate. A nucleotide consisting of cytosine (a pyrimidine), ribose, and one high-energy phosphate group

CoA, Coenzyme A A coenzyme containing the vitamin, pantothenic acid. CoA forms a thio ester bond with acetyl and acyl groups and with amino acids and facilitates their metabolism

CoQ, Coenzyme Q A coenzyme also called *ubiquinone*. It serves as an essential component of the respiratory chain and transfers electrons between the flavoproteins and the cytochromes

Coding strand A strand of DNA that serves as the template for mRNA

Codon A sequence of three bases (triplet) that specifies a particular amino acid in the sequence of reactions comprising protein synthesis. Amino acids can have more than one codon

Cofactor An essential ingredient for an enzyme catalyzed reaction. Cofactors are usually minerals

Competitive inhibition Inhibition of an enzymatic reaction that occurs when a substance, closely related to the substrate, binds to the active site of the enzyme yet is not catalyzed by it

Complementary base pairs The pairing of purine and pyrmidine bases of the DNA. Adenine is paired with thymine and cytosine with guanine. These pairs are linked together via hydrogen bonds that form the cross links between the two strands of DNA for the double helix typical of the nuclear genetic material. In RNA, adenine pairs with uracil

Concordance The chance that an identical disease will occur in related family members

Consensus sequence An idealized sequence in which each position represents the base most often found when many actual sequences are compared

Constitutive genes Genes that are expressed as a function of the interaction of RNA polymerase with the promoter, without additional regulation; sometimes called "housekeeping genes" in the context of describing functions expressed in all cells at a low level

Corepressor A small molecule that triggers repression of transcription by binding to a regulator protein

Cori cycle A cycle that occurs between the red blood cell, kidneys, muscle, platelets, and the liver and which helps maintain a normal blood glucose level in the face of changes in lactate production

Cosmid A plasmid into which the DNA sequences from bacteriophage lambda that are necessary for the packaging of DNA (cos sites) have been inserted; this permits the plasmid DNA to be packaged in vitro. Coupled reaction: two reactions that have a common intermediate that transfers electrons or reducing equivalents or some other element from one set of reactants to another

C-peptide When proinsulin is cleaved to form active insulin, the fragment removed is the c-peptide

Creatine A metabolite in muscle that is synthesized from glycine and arginine and which can be phosphorylated to form creatine phosphate

Creatine phosphate A high-energy compound in muscle that has a higher phosphate group transfer potential than ATP. It acts as a reservoir for phosphate so that the muscle can maintain a steady level of ATP to support muscle contraction. It also serves this function in nerve so that a steady supply of ATP for nerve conduction is provided

Creatine phosphokinase (creatine kinase) Enzyme in skeletal muscle, cardiac muscle, and in brain tissue that catalyzes the transfer of high energy phosphate from phosphocreatine to ADP to make ATP

Creatinine The urinary excretion product of creatine breakdown

Crenation Shriveling of the cell

Crude fiber The residue of plant food left after extraction by dilute acid and alkali

CTP Cytidine triphosphate. A nucleotide consisting of cytosine (a pyrimidine), ribose, and three high energy phosphate groups

Cyclooxygenase Two forms exist: COX I and COX II. Enzyme responsible for the formation of eicosanoids particularly those that act as inflamatories. COX I is a constitutive enzyme present in most cell types and works under noninflammatory conditions. COX II is

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inducible in cells as part of an inflammatory response to injury or irritation. COX II is the target of several prescription antiinflammatory drugs useful in the treatment of arthritis

Cytidine A nucleotide consisting of cytosine (a pyrimidine) and ribose

Cytochromes Electron carriers in the respiratory chain and elsewhere. All cytochromes have a heme prosthetic group that contains iron. The iron fluctuates between the +2 and +3 states. The cytochrome P-450 enzymes are important to the detoxification processes in the body

Cell sap (Cytosol) The medium in which the organelles of the cell are suspended

Cytosine A pyrimidine base found in the genetic material, DNA and RNA

Cytotoxic agents Chemicals that destroy specific cells; streptozotocin is an example. This agent destroys the insulin-producing ß cells of the pancreas

Cytokines A family of peptide hormones produced mainly by macrophages and lymphocytes. Some cytokines are produced by the brain, adipose tissue and other cell types

Daily Reference Intake (DRI) Revised guidelines for the intakes of nutrients including fats, carbohydrates, proteins, energy, and micronutrients. These guidelines are designed to prevent chronic disease and encompasses several types of recommendations including the Recommended Dietary Allowance (RDA) by the Food and Nutrition Board (National Academy of Sciences) that provide guidance for the prevention of deficiency diseases

DE Digestive energy. The energy of food after the costs (and losses) of digestion are subtracted

Deaminase An enzyme which catalyzes the removal of an amino group from a carbon chain

Deamination The process of amino group removal

Decarboxylase An enzyme that catalyzes the removal of a carboxy group from a carbon chain

Decarboxylation The reaction in which a carboxyl group is removed

Degrade To reduce large molecules to smaller ones

Dehydratases Enzymes which catalyze the removal of a molecule of water from a carbon chain. These enzymes frequently require pyridoxine as a coenzyme

Dehydration The process of water removal

Dehydrogenases Enzymes that catalyze the removal of reducing equivalents (hydrogen ions) from carbon chains

Dehydrogenation Removal of hydrogen ions; catalyzed by a group of enzymes called dehydrogenases

Delta (Δ) Change. The Greek symbol is Δ

ΔG° Free energy that is available to do work

ΔG°′ Standard free energy of biochemical reactions

Densitometry Measurement of body density

Deoxyadenosine A nucleoside containing adenine (a purine) and deoxyribose

Deoxycytidine A nucleoside containing cytosine (a pyrimidine) and deoxyribose

Deoxyguanine A nucleoside containing guanine (a purine) and deoxyribose

Deoxyribonucleic acid (DNA) The genetic material in the nucleus and mitochondria

Dermatitis Inflammation of the skin

Detoxification Removal of toxic properties of a substance; metabolic conversion of pharmacologically active compounds to nonactive compounds

Deuterium A hydrogen isotope having twice the mass of the common hydrogen atom

Deuterium oxide Heavy water that contains two molecules of deuterium and one of oxygen

DEXA Dual energy x-ray absorptiometry. It is a method used to estimate bone density and soft tissue composition

Dextran A glucose polymer

Dextrose Synonym of glucose

DHHS United States Department of Health and Human Services

Diacylglycerol (Diglyceride, DAG) A lipid having two fatty acids esterified to glycerol; also called diglyceride

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Diet-induced thermogenesis (DIT) A rise in heat production associated with the consumption of food. This energy loss represents the energy cost of the processing of food for metabolic use

Diffusion A uniform distribution of solutes on both sides of a permeable membrane

Dipeptide Two amino acids linked together by a peptide bond

Direct calorimetry The measurement of heat produced by a body through the use of a calorimeter

Disaccharide A carbohydrate containing two sugar (saccharide) units. Sucrose, mannose, and lactose are common disaccharides in the human diet

Distal Away from the center of the body

Distention The condition of being expanded or extended beyond normal

Disulfide bridge A bond containing two sulfur molecules

Diuresis Urine excretion in excess of normal

Diuretics Pharmaceutic agents which increase urinary water loss

Diurnal variation Cyclical changes in one or more features of the body over a 24-hour period

Duodenum The first 12 inches of the small intestine of the human

Edema Excess accumulation of water in the body, particularly in the periphery

Eicosanoids A group of hormone-like compounds synthesized from arachidonic acid

Electrolyte An electrically charged particle

Electron donor In an electron acceptor/donor pair, the electron donor gives or donates electrons to the acceptor. An electron donor is a reducing agent

Elongation factors Proteins that associate with ribosomes cyclically during the addition of each amino acid to the amino acid chain

Embolism Obstruction of a blood vessel by blood clot or foreign body

Embryo The initial stage of development of the fertilized egg; usually the first 12 weeks in human pregnancy

Emulsion A mixture of fat and water held together by an agent that has a lipophilic and a hydrophilic portion to its structure. Bile salts serve as emulsifying agents facilitating lipid digestion and absorption

Endocytosis The process of forming a vesicle within the secreting cell of material the cell will release upon appropriate signaling

Endonuclease An enzyme that cleaves internal bonds in DNA or RNA

Endorphin A peptide produced in the brain that has sedative properties; ß endorphin stimulates prolactin release

Endothelin A 21-amino-acid peptide that is a potent vasoconstrictor

Energetic efficiency The percentage of the intake energy that the body traps energy in the high energy bond and subsequently stores that energy or uses it to synthesize body components

Enhancer element A cis-acting base sequence that binds one or more promoters. It can function in either orientation and in any location (upstream or downstream) relative to the promoter region

Enterocyte Absorptive cells lining the gastrointestinal tract

Enteral nutrition The provision of nutrients in a solution infused via a nasogastric tube

Epithelium The layer of cells that provide the surface covering of the body; the nonvascular cell layer of skin, gastrointestinal tract, and respiratory tract

ER Endoplasmic reticulum

Ester A compound containing an oxygen linkage (-O-); the product of the condensation of an acid and alcohol with the loss of a molecule of water

Etiology The study of the causes of disease

Excinuclease An enzyme that is involved in nucleotide exchange/repair of DNA

Exocrine cells Cells that produce secretions into the surrounding environment

Exon The sequence of bases used to transcribe mRNA

Exonuclease An enzyme that cleaves nucleotides from either the 3′ or 5′ ends of DNA or RNA

Extracellular Outside of the cell, as in serum that surrounds the blood cells

Extravascular Outside the vascular system, as the fluids which are between the cells in the tissues or between the capillaries and the cells they supply

Facilitated transport Transport against a concentration gradient using a carrier but which is not energy- or sodium-dependent

FAD, FMN Flavin adenine dinucleotide, flavin mononucleotide. Coenzymes that carry reducing equivalents (H^+) in reactions of intermediary metabolism. Riboflavin is an essential component of these nucleotides

Fatty acids Carbon chains having a carboxyl group at one end and a methyl group at the other end

FDA Food and Drug Administration. A subunit of the U.S. Department of Health and Human Services

Fecal energy (FE) The gross energy of the feces

Feces Excrement discharged from the bowel via the anus; contains undigested food, intestinal secretions, intestinal flora, and desquamated intestinal cells

Fetus An unborn child; stage of development of a human from embryo to birth or from about 12 weeks postfertilization to full gestational age (approximately 40 weeks)

Fibroblasts Progenitor cells that can differentiate into other more specific cells

Fibrous plaque Lipids that collect on a fibrous network within or on the arterial walls during the atherogenic process, creating a projection into the lumen of the vessel and impeding flow

Fingerprinting the use of restriction fragment LPs or repeat sequences of DNA to establish a unique pattern of DNA fragments for an individual

Flatulence Release from the anus of gases (methane, sulfur dioxide, etc.) produced in the large intestine through the action of intestinal flora on food residue. If the gas is not released, intestinal distention and discomfort result

Footprinting A technique for identifying the site on DNA bound by proteins that protect certain bonds from attack by nucleases

Free energy Energy not trapped or used further; usually dissipated as heat

Free radicals Very reactive products of the autooxidation of unsaturated fatty acids and some amino acids

FSH Follicle-stimulating hormone

GABA Gamma amino butyric acid. A neurotransmitter formed through the decarboxylation of glutamic acid. It is an inhibitory neurotransmitter that quiets excited neurons

Galanin A 30-amino-acid peptide found in the neurons of the gastrointestinal submucosa. It can inhibit the release of somatostatin, insulin, pancreatic polypeptide, and neurotensin

Gaseous products of digestion (GPE) Combustible gases that are products of digestion/fermentation in the gastrointestinal tract. Especially important in the calculation of energy balance of ruminant animals

Gastrin A small peptide hormone produced by gastric cells. Gastrin stimulates the parietal cells to release hydrochloric acid

GDP Guanosine diphosphate. A dephosphorylated form of GTP important in the activation of substances participating in the biosynthesis of proteins

Gene Carrier of the genetic codes for specific protein components of an organism

Genotype The inherited characteristic of an organism

Geophagia The eating of dirt; an abnormal appetite

Ghrelin Hormone secreted by the stomach that stimulates food intake

GIP Gastric-inhibitory peptide. A peptide hormone inhibiting gastric motility and acid secretion

GMP Guanosine monophosphate. A nucleotide containing guanine (a purine), ribose, and a high-energy phosphate group

GRP Gastrin releasing peptide. A neuroactive peptide originating in nerves of the gut and stimulating the release of gastrin from gastric cells

GSH Glutathione, reduced

GSSG Glutathionine, oxidized. A tripeptide containing glutamic acid, cysteine, and glycine. Its sulfhydryl group can undergo reversible oxidation and reduction allowing the peptide to serve as a buffer. Its chief function is to serve as a reductant of toxic peroxides

GTP Guanosine triphosphate. A high-energy, phosphate-containing compound needed for protein synthesis

Gynoid obesity Excess body fat deposited mainly on the hips and thighs; sometimes referred to as the "pear" form of obesity

Hairpin A double-helical stretch formed by base pairing between neighboring complementary strands of a single strand of DNA or RNA

Half-life The time required for half of the amount of a given substance to disappear or be degraded

HANES (NHANES I, II, III, OR HHANES) Health and Nutrition Examination Survey sponsored by the U.S. Centers for Disease Control, a unit of the U.S. Public Health Service

Heat shock protein A 90-kDa protein involved in gene transcription

Hematocrit Volume of erythrocytes packed by centrifugation in a given volume of blood

Hematopoiesis The production of blood cells

Heme The protein portion of hemoglobin, which holds iron and is responsible for the carriage and release of oxygen by red blood cells

Heme iron Iron held by heme

Hemoglobin (Hb) The iron-containing protein in red blood cells that carries oxygen to the periphery and carbon dioxide back to the lungs for excretion

Heterozygote An individual carrying two different copies of a gene for a given characteristic

Heterodimerization The polymerization of two unlike compounds to DNA

Histones Conserved DNA-binding proteins that protect the DNA from damage

HLA genes Genes that encode elements of the immune system

HMG CoA 3-Hydroxy-3-methylglutaryl coenzyme A. A metabolic intermediate in cholesterol synthesis

HNIS Human Nutrition Information Service of the U.S. Department of Agriculture

Homodimerization The binding of two identical compounds to DNA

Homozygote An individual carrying two identical copies of a gene for a particular gene product

Hybridization The specific reassociation of complementary strands of nucleic acids

Hydroxylation The addition of a hydroxyl (–OH) group to a compound

Hyperlipidemia Abnormally high levels of lipids in the blood

Hypermetabolism Higher than normal rates of metabolism

Hypertension High blood pressure; systolic/diastolic reading higher by 20% of normal (120/80)

Hypertrophy Increased cell size

IBW Ideal body weight

IE Intake of food energy

Ileum The last third of the small intestine

Incidence The number of new events or cases of a disease in a population within a specified time period

Indirect calorimetry Estimation of energy need based on oxygen consumption

Infarct Death of local tissue where its blood supply is obstructed

Initiation factors Proteins that associate with the small subunit of ribosomal RNA that play a role in the initiation of the assemblage of amino acids into an amino acid chain

Insert An additional length of base pairs introduced artificially

Insulitis Inflammation of the pancreatic islets

Intron The sequence of bases in a gene that are transcribed but removed by mRNA editing before the mRNA leaves the nucleus

Inverted repeats Two copies of the same sequence of DNA repeated in opposite orientation on the same molecule; adjacent inverted repeats constitute a palindrome

Intercellular compartment The water that surrounds cells and is contained in the intracellular space. Plasma is part of this compartment and surrounds the red blood cells in the blood

Interstitial fluid Fluid located between cells and in some body cavities such as joints, pleura, and the gastrointestinal tract

Interstitial spaces Space between tissues. Intracellular compartment: body water compartment consisting of fluids within the cells

Intravascular Within the vascular tree. An intravascular (I.V.) injection is one where the substance is injected into a vein

Ion An element with either a positive or negative charge

IRS-1 A 131-kDa intracellular protein essential for most of insulin's intracellular actions

Ischemia Impaired blood flow causing oxygen and nutrient deprivation resulting in pain and, if severe, death of some or all parts of the tissue

Islets of Langerhans Endocrine clusters of cells embedded in the exocrine pancreas; responsible for the secretion of glucagon, somatostatin, amylin, insulin, and probably some other small hormones

Isoprenoids Members of the steroid class of compounds. Isoprenoids include the carotenes and ubiquinone

IU (International unit) An amount defined by the International Conference for Unification of Formulae

Jejunum The middle third of the small intestine

Joule A unit of energy in the metric system

Kinetic energy Mechanical energy

Km Michaelis constant. The substrate concentration that produces half maximal velocity of the reaction. The affinity of the enzyme for its substrate determines Km. If the affinity is high, the reaction will proceed very quickly. If two enzymes work on the same substrate, the one with the greater affinity will be more active and have a higher Km

Labile Easily degraded; unstable

Lean body mass LBM; that fraction of the body exclusive of stored fat. This fraction is considered to be the active metabolic fraction

Leptin A cytokine produced by the adipocyte that signals satiety

LH Leutinizing hormone: released by the pituitary, serves to stimulate estrogen and progesterone production in females and testosterone in males

LHA Lateral hypothalamus

LNAA Large neutral amino acids; branched-chain and aromatic amino acids

Lumen Interior aspect of a vessel

M (μ) Greek letter prefix that indicates 10^{-6} fraction of a liter or gram

Macrophages Large mononuclear cells that phagocytosize foreign compounds and neutralize them

Magnetic resonance imaging (MRI) A technology allowing the imaging of a body without radiation hazard

MAO Monoamine oxidase. A copper-containing enzyme that catalyzes the removal of oxygen from epinephrine, tyramine, and serotonin, inactivating them and reducing neuronal activity. Inhibitors of MAO are valuable drugs for treating depression

MCV Mean corpuscular volume. The ratio of the volume of packed cells to the volume of the blood sample; an indirect measure of the number of red blood cells in the blood sample

Mean Average value for a group of values

Median Value where half the values fall below and half fall above this value

Messenger RNA (mRNA) A single strand of purine and pyrimidine bases synthesized in the nucleus so that its base sequence complements DNA

Metabolic water Water formed as a result of metabolic reactions

Metabolizable energy (ME) The energy in the food less that is lost in feces, urine, and combustible gases

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Microsatellite marker A dispersed group of 2–5 base sequences that are repeated up to 50 times. May occur at 50–100 thousand locations in the genome. Used in linkage analysis

Microsatellite polymorphism Heterozygosity of a certain microsatellite marker in an individual

Milliequivalent A measurement of the concentration of electrolytes in solution. It is determined by multiplying the milligrams per liter by the valence of the chemical and dividing by the molecular weight of the substance: mEq/L = (mg/L) · Valence/molecular weight. A measurement of the osmotic activity of a solution that is determined by dividing the milliequivalent value by its valence

Morbidity Illness leading to death

Mortality Cause of death

Motilin A gastrointestinal hormone that plays a role in controlling the motility of the small intestine during periods without food

Na^+K^+ Pump An energy dependent pump that operates to keep Na^+ on the outside of cells and K^+ on the inside of cells

NAD, NADH, NADP, NADPH Niacin-containing coenzymes which function as carriers for hydrogen ions in dehydrogenase catalyzed reactions

National Center for Health Statistics (NCHS) (U.S.) An agency that collects data on the causes of death and disease in the United States as well as in other nations

National Research Council (NRC) A division of the U.S. National Academy of Sciences established in 1916 to promote the effective utilization of scientific and technical resources

National Science Foundation (NSF) (U.S.) A U.S. government funding organization for the basic sciences

Nationwide Food Consumption Survey (NFCS) (U.S.) Data collected by scientists at the U.S. Department of Agriculture to document the kinds and amounts of food consumed in the United States

Neuropeptide Y A small peptide hormone released by the GI tract and by the central nervous system, having a possible role in food intake regulation

NIH (National Institutes of Health—U.S.) Located primarily in Bethesda, Maryland. One of the largest U.S. government-funded health and disease research centers

Nucleosome The basic structural subunit of chromatin, consisting of ~200 bp of DNA and an octomer of histones

Null mutation A mutation that completely eliminates the function of the gene, usually because it has been eliminated

Nutrient density The nutrient composition of food expressed in terms of nutrient quantity per 100 kcal

Obesity Excess accumulation of body fat (more than 20% of the body as fat)

Odds ratio (OR) A good approximation of the relative risk

Olfaction The sense of smell

Oligonucleotide A short, defined sequence of nucleotides joined together in the typical phosphodiester linkage

Oliguria Decreased urine production

Oncogene A gene related to cancer

Oncotic pressure The pressure exerted by the plasma proteins on the walls of the vascular system

Oocyte An egg produced by the ovary

Oogenesis The production of oocytes (eggs) by the ovary of the female

Open reading frame A series of triplets coding for amino acids without any termination codons

Ophthalmia Inflammation of the conjunctiva (membrane that lines the eyelid) of the eye

Opioid receptors Structures on the surface of neuronal cells that mediate the function of opiates

ORI The origin of replication in prokaryotes

Orthostatic hypotension Hypotension that results from standing and often associated with dehydration

Osmolality Quantity of solutes per liter of solution that contributes to the pressure of that solution on a membrane

Osmoreceptors Structures in the hypothalamus that detect changes (elevations) in blood tonicity. When tonicity is increased, a signal is generated that inhibits water loss and water uptake into the blood

Osmosis The passage of solvents across a membrane so as to equalize the concentrations of solutes on each side of the membrane

Osmotic effect The effects of solutes on water passage

Osmotic pressure The pressure that must be applied to prevent the passage of a solvent

Ossification The process of bone formation

Osteoarthritis Degenerative bone and joint disease due to wear and tear; age-associated

Osteoblasts Bone-forming cells

Osteocalcin A protein whose synthesis is dependent on Vitamin K and which acts to promote mineral deposition in bone

Osteoclasts Cells responsible for bone remodeling. These cells mobilize bone mineral

Osteomalacia A condition characterized by a weakening and softening of the bone and in which the bending of long bones can develop. Associated with Vitamin D deficiency

Osteopenia Decreased bone mass

Osteoporosis Disease in which the bone loses its mineral content and becomes porous; associated with aging, particularly in females lacking estrogen

Oxidase An enzyme group responsible for oxygen removal; catalyzes oxidation/reduction reactions using oxygen as the electron acceptor

Oxidation The removal of electrons using oxygen as the electron acceptor

Oxidation/reduction Reactions in which electrons are lost by one reactant and gained by another

Oxidative deamination The removal of an amino group together with the loss of electrons that are accepted by oxygen

Oxidative decarboxylation The removal of a carboxyl group together with the loss of electrons accepted by oxygen.

Oxidative phosphorylation (OXPHOS) Two processes whereby water and ATP are synthesized simultaneously. One, respiration, joins hydrogen and oxygen ions to make water, and the other, ATP synthesis, uses some of the energy so released to synthesize ATP. These coupled processes occur in the mitochondria of the cell

Parenteral nutrition Nutritional support furnished through the vascular system

Passive diffusion A process for the passage of solutes across a membrane that does not involve a carrier or energy

PEP Phosphoenopyruvic acid, a key metabolite in gluconeogenesis

PEPCK Phosphoenolpyruvate carboxykinase; the rate-limiting enzyme in gluconeogenesis

PGI, PGE Prostaglandins in the I or E series

pH The numerical representation of the hydrogen ion concentration in a solution. A low pH represents an acid solution while a high pH represents a basic or alkaline solution. Physiological pH is 7.4

Phenotype A category or group to which an individual is assigned based on one or more inherited characteristics; the overt expression of the genotype

Phosphatase An enzyme which catalyzes the removal of a phosphoryl group from a substrate

Physiological fuel value The energy provided by food with a correction for the energy lost through digestion and absorption

PLP Pyridoxal phosphate

Polyunsaturated Fatty Acid (PUFA) A fatty acid having more than one double bond

PP-Fold Polypeptide fold hormones: polypeptide Y (PYY), pancreatic polypeptide (PP) and neuropeptide Y (NPY). NPY serves as a neurotransmitter. All three have a role (inhibitory) in food intake as well as other functions

Polyadenylation The addition of a sequence of polyadenylate to the 5′ end of a eukaryotic RNA after its transcription
Polymerase chain reaction (PCR) An enzymatic method for repeated copying of DNA
Postprandial After a meal
Precision No random errors in the measurements. Also referred to as reproducibility
Prevalence The number of existing cases of X in a given population at a given time
Probe A molecule used to detect the presence of a specific fragment of DNA or RNA
Prokaryotes Single cell organisms
Promoter A region of DNA involved in binding RNA polymerase and various regulatory factors to initiate the transcription of RNA
Prosthetic group A nonprotein component of an enzyme (or other body protein) that is necessary to the activity of that enzyme or protein
Proton A particle in the nucleus of an atom that has a positive charge
Proximal Toward the center of the body
P/S ratio The ratio of polyunsaturated fatty acids to saturated fatty acids
Pseudogene An inactive segment of DNA arising from the mutation of parental genes; an artifact of gene isolation
PTH Parathyroid hormone
Purine A nitrogenous base that is an essential structural unit of DNA and RNA. Includes adenine and guanine
PVN Paraventricular nucleus in the hypothalamus
Pyrimidines A group of nitrogenous bases needed for DNA and RNA structures. Includes cytosine, thymine, and uracil
RBC Red blood cell. Erythrocyte
RBP Retinol-binding protein
Reading frame One of three possible ways of reading a nucleotide sequence as a series of triplets
Receptor A general term applied to any protein in any part of the body that binds to a specific compound and allows that compound to do its job
Recombinant DNA DNA isolated and/or synthesized in the laboratory
Regression Equation A statistical method for calculating the causal relationship of an independent variable to a dependent variable
Replication Duplication of the genetic code
Reporter gene A coding unit whose product is easily detected; may be connected to any promoter of interest so that the expression of that gene can be monitored
RER Rough endoplasmic reticulum
Respiratory quotient (RQ) The ratio of carbon dioxide exhaled to oxygen consumed
Restriction enzyme An endonuclease enzyme that causes cleavage of both strands of DNA at highly specific sites dictated by base sequence
Restriction fragment length polymorphism RFLP; inherited differences in sites for binding restriction enzymes
Reverse transcription RNA-directed synthesis of DNA catalyzed by reverse transcriptase
RIA Radioimmunoassay
Ribonuclease An enzyme that catalyzes the destruction of RNA
Ribonucleic acid (RNA) A single strand of nucleosides having ribose instead of deoxyribose, and uracil in place of thymine
Ribosome The organelle of the cell where protein synthesis occurs
RBP Retinol-binding protein
RDA Recommended daily dietary allowance; a term that has been replaced by DRI
SAM *S*-adenosylmethionine. A principal methyl donor
SER Smooth endoplasmic reticulum

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Sex-linked trait A genetic characteristic carried on either the X or the Y chromosome
SGOT Serum glutamate-oxaloacetate transaminase. A vitamin B_6-dependent enzyme whose level in the serum rises after muscle damage, particularly after a heart attack
SGPT Serum glutamate-pyruvate transaminase. Another Vitamin B_6-dependent enzyme
SI units Standard units for expressing biological values
Signal transduction The process by which a receptor interacts with a ligand at the surface of the cell and then transmits a signal to trigger a series of reactions within that cell
Skinfold thickness A double fold of skin and underlying tissue that can be used to estimate body fat stores
SOD Superoxide dismutase
Southern blotting A method for transferring DNA from an agarose gel to a nitrocellulose filter on which DNA can be detected by a suitable probe
Specific dynamic action (SDA) Heat production resulting from the metabolism of food
Splicing The removal of introns from RNA accompanied by the joining of the exons
Splicisome The macromolecule complex responsible for precursor mRNA slicing. The splicisome consists of at least five small nuclear RNAs and many proteins
Stable isotope An isotope that is nonradioactive. K^{40} is a stable isotope
Sticky-ended DNA Complementary single strands of DNA that protrude from opposite ends of a DNA duplex or from the ends of different duplex molecules
Stool Feces
Stroke Blockage or rupture of blood vessels supplying the brain with resulting loss of consciousness, paralysis, and other symptoms. Apoplexy is a common term for stroke
Supine Lying on one's back
Symptoms Signs or indications of disease
T_3 Triiodothyronine. The most active of the thyroid hormones
T_4 Thyroxine. The form of thyroid hormone released by the thyroid gland to the blood
T cells Cells of the immune system that originated from the thymus gland
Tandem Describes multiple copies of the same sequence that lie adjacent to each other in a gene
Targeted mutation An animal ("knockout") that has a specific gene altered or deleted at a specific location
TBF Total body fat
TBG Thyroid-hormone-binding globulin; protein that carries the thyroxine from the thyroid gland to its target tissue
TBW Total body water
TDP, TPP Thiamin-containing coenzyme required for decarboxylation reactions
Thymidine phosphate (TMP, TDP, TTP) A nucleotide consisting of thymine (a pyrimidine base) deoxyribose and 1, 2, or 3 phosphate groups
Thymine A pyrimidine base in DNA
TPN Total parenteral nutrition. A method of providing all nutrient needs through a solution infused into a large blood vessel
TSH Thyroid-stimulating hormone. A hormone released by the pituitary that stimulates the thyroid gland to make and release thyroxine
tRNA Transfer ribonucleic acid. Form of nucleic acid responsible for transferring specific amino acids to specific sites on the mRNA in the process of protein synthesis
Translation The synthesis of protein using the message for amino acid sequence carried by messenger RNA. Occurs on the ribosomes
Translocases Proteins that move smaller molecules through a membrane
Tritium ($^3H^+$) Radioactive hydrogen
TXA_2 Thromboxane A_2. An eicosanoid involved in stimulating platelet aggregation
Ubiquinone Coenzyme Q, an electron carrier that is part of the respiratory chain in the mitochondria

UMP, UDP, UTP Uridine mono-, di- or triphosphate. A high energy compound essential to glycogen synthesis

Uracil Pyrimidine base used in DNA

Uridine Uracil with a ribose group attached

VIP Vasoactive peptide. A neuropeptide originating in the neurons of the gastrointestinal system

VLDL Very-low-density lipoprotein. A lipid–protein complex involved in the transport of lipids from the liver and gut to storage sites

Western blot A method for transferring protein to a nitrocellulose filter on which the protein can be detected by an antibody

Xeropthalmia Vitamin A deficiency

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