6.4.5.1 Nucleotide Kinases. The terminal high-energy bond of ATP can be transferred to various nucleoside diphosphates (e.g., UDP, CDP, GDP) by nucleotide kinases. For example, UDP generated from UTP in the pathway that incorporates glucose 1-phosphate into glycogen is regenerated using ATP as the high-energy phosphate donor:

$$\text{UDP} + \text{ATP} \rightleftharpoons \text{UTP} + \text{ADP}$$

6.4.5.2 Myokinase. Myokinase or adenylate kinase is a nucleotide kinase that generates ATP from two molecules of ADP:

$$\text{ADP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{AMP}$$

High levels of myokinase are found in muscle cells, where it serves to regenerate ATP during periods of high energy demand. The AMP generated in the myokinase reaction is an allosteric activator of key regulatory enzymes of glycolysis, thus stimulating ATP synthesis.

6.4.6 Storage of High-Energy Phosphate Bonds

Creatine phosphate is used by muscle and other cells to store energy from ATP in a readily available form. *Creatine kinase* (CK), also called *creatine phosphokinase* (CPK), is an energy-transfer enzyme that catalyzes the synthesis of creatine phosphate from creatine and ATP (Fig. 6-6):

$$\text{creatine} + \text{ATP} \rightleftharpoons \text{creatine phosphate} + \text{ADP}$$

CK is present in many different types of cells (brain, colon), but the highest CK level in the body is found in the cytosol and mitochondria of skeletal and heart muscle. Mitochondrial CK is located on the outer surface of the inner mitochondrial membrane, where it uses the ATP generated by oxidative phosphorylation to catalyze the production of creatine phosphate. The high-energy creatine phosphate molecule diffuses into the cytosol, where it is stored until needed for muscle work. Cytosolic CK is closely associated with myofibrils and catalyzes the formation of ATP from creatine phosphate and ADP, thus providing ATP for muscle contraction.

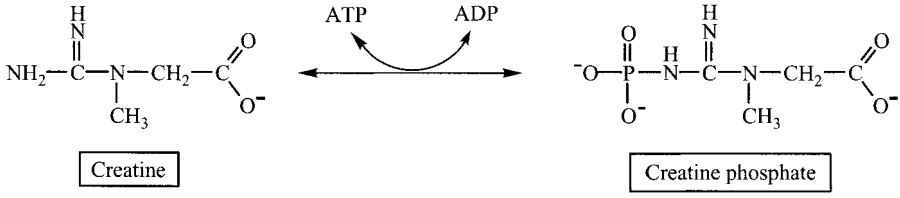

FIGURE 6-6 Synthesis of creatine phosphate.

6.4.6.1 Creatine Kinase as a Marker of Heart and Skeletal Muscle Injury. For decades measurement of serum levels of CK served as a marker for heart or skeletal muscle injury. CK is a dimeric protein made up of two distinct polypeptide subunits, designated M (muscle) and B (brain). Three CK isoenzymes were found in human tissues: CK-MM in skeletal muscle, CK-BB in brain, and the heterodimer CK-MB, considered the myocardial isozyme. More recent studies have demonstrated, however, that cardiac muscle normally contains CK-MM, and that the CK-MB isozyme is present in cardiac muscle only after the muscle has sustained prior injury. The diagnostic value of plasma CK-MB measurements thus reflects the fact that many people who have myocardial infarctions have a prior history of subclinical cardiac damage. CK-MB is also present in skeletal muscle that has experienced prior injury, including muscle of highly trained long-distance runners. Although an increased plasma level of CK-MB is often still suggestive of myocardial injury, quantification of plasma levels of cardiac isoforms of the structural proteins called *troponins* has superseded CK as the definitive marker of myocardial injury.

6.4.7 Oxidative Phosphorylation as a Source of Reactive Oxygen Species

Mitochondria are the major source of oxygen free radicals in tissues. In the process of consuming large amounts of molecular oxygen while producing ATP coupled to the oxidation of respiratory substrates, some of the oxygen is only partially reduced, generating superoxide ($O_2^{\bullet}$) and other reactive oxygen species (ROS). Superoxide anion is produced in the mitochondrial matrix by complex I (NADH–ubiquinone oxidoreductase) and complex III (ubiquinone–cytochrome *c* oxidoreductase). In contrast, cytochrome *c* oxidase (complex IV) has such a high oxidative capacity that it reduces oxygen completely to H_2O and does not produce ROS.

Fortunately, there are many cellular defenses against ROS. For example, superoxide anion is converted to hydrogen peroxide (H_2O_2) by mitochondrial and cytosolic superoxide dismutases:

$$2O_2^{\bullet} + 2H^+ \rightarrow O_2 + H_2O_2$$

H_2O_2 can, in turn, be neutralized by several cellular scavenger systems, including glutathione peroxidase, catalase, thioredoxin, and glutaredoxin.

6.5 REGULATION OF OXIDATIVE PHOSPHORYLATION

Electron transport and oxidative phosphorylation are normally tightly coupled; that is, electron transport depends on concurrent ATP synthesis. The process by which electron transport pumps protons out of the mitochondrion ceases unless there is a mechanism for returning protons to the matrix of the mitochondrion. Under normal conditions, this return of protons from the intermembrane space into the

mitochondrion is accomplished through the action of ATP synthase. Thus, both electron transport and oxidative phosphorylation occur only when ADP is available as a substrate for oxidative phosphorylation. Both pathways are less active when the energy needs of the cell are low and the ATP/ADP ratio is high. Inhibition of electron transport, in turn, increases the intramitochondrial NADH/NAD^+ ratio, which results in inhibition of both the TCA cycle and fatty acid oxidation.

6.5.1 Brown Fat

One tissue in which electron transport is normally dissociated from oxidative phosphorylation is brown fat, which derives its color and name from the fact that it has a much greater mitochondrial content than that of white adipose tissue and more extensive vascularization. Although virtually no brown fat is present in adults, brown fat may account for as much as 5% of the body weight of neonates. The main function of brown fat is thermogenesis, which provides heat when the infant is exposed to a cold environment. The molecular basis for this nonshivering thermogenesis is the presence of thermogenin or mitochondrial uncoupling protein 1. Thermogenin is a transmembrane protein in the inner mitochondrial membrane that contains a proton channel which allows protons to reenter the mitochondrial matrix independent of the activity of ATP synthase. Thermogenin thus acts to uncouple electron transport from oxidative phosphorylation, which results in the dissipation of the energy of the proton gradient as heat.

6.6 ABNORMAL FUNCTIONING OF ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

6.6.1 Genetic Diseases

Many degenerative diseases result from mutations in genes that code for the components of electron transport and the oxidative phosphorylation apparatus. Whereas some of these mutations alter nuclear DNA and follow classical Mendelian inheritance patterns, others result from mutations in mitochondrial DNA. Mitochondrial DNA (mtDNA) encodes 13 of the approximately 1000 proteins that comprise the mitochondrion, including seven subunits of respiratory complex I and one or more of the subunits of respiratory complexes III and IV and ATP synthase. In addition, mtDNA encodes ribosomal and transfer RNA molecules required for the intramitochondrial synthesis of these proteins. Point mutations in or deletions of mitochondrial genes can impair electron transport and oxidative phosphorylation.

The syndromes resulting from disorders of mtDNA-encoding protein components of electron transport and oxidative phosphorylation include LHON (Leber hereditary optic neuropathy) and some forms of Leigh disease. Disease resulting from point mutations in tRNA include MERRF (myoclonic epilepsy associated with ragged-red fibers) and MELAS (mitochondrial myopathy, encephalomyopathy, lactic acidosis, and strokelike episodes).

Mitochondrial disorders resulting from mutations in mtDNA are usually sporadic or follow matrilineal inheritance. Since most cells contain numerous mitochondria, each with multiple DNA molecules, individual cells are usually heteroplasmic in that they have a mixture of mitochondrial genomes. Furthermore, cellular replication may divide the mitochondria unevenly between the daughter cells, resulting in cells with an increased mutant load and correspondingly increased impairment of oxidative phosphorylation. The earliest manifestations of mitochondrial mutations are neurological and muscular abnormalities and are a consequence of the greater dependence of brain, heart, and skeletal muscle on mitochondrial ATP synthesis to maintain cellular functions.

6.6.2 Inhibitors of Electron Transport

Substances that inhibit electron transport and thus ATP generation are highly toxic. Among these are carbon monoxide, azide (N_3^-), and cyanide, all of which inhibit the cytochrome *c* oxidase activity of respiratory complex IV. Other inhibitors of electron transport include the rat poison rotenone, which inhibits respiratory complex I, and antimycin A, which inhibits respiratory complex III. Electron transport is also inhibited by the antifungal agent oligomycin, which inhibits ATP synthase.

6.6.3 Uncoupling Agents

Lipophilic weak acids readily penetrate the inner mitochondrial membrane and can transport protons back into the mitochondrial matrix. These acids thereby uncouple electron transport from oxidative phosphorylation, thus dissipating the energy derived from electron transport as heat. As such, they are the toxicologic equivalent of thermogenin and prevent ATP synthesis. One well-known uncoupling agent is 2,4-dinitrophenol, which was used briefly in the 1930s as a weight-loss pharmaceutical. Since 2,4-dinitrophenol causes cataracts and fatal fevers, the drug was quickly removed from the market.

6.6.4 Thyroid Disease

One of the major functions of thyroid hormones (e.g., thyroxine) is to maintain energy homeostasis. Thyroxine stimulates the synthesis of many of the proteins of the electron transport system and oxidative phosphorylation, including cytochrome oxidase, ATP synthase, and the adenine nucleotide transporter, resulting in an increased capacity for mitochondrial ATP synthesis. At the same time, hyperthyroidism results in a decreased ratio of ATP generated to O_2 utilized, with an increased dissipation of the energy of electron transport as heat. By contrast, hypothyroid individuals have both an increase in the efficiency of ATP generation and a decrease in the maximal rate of ATP synthesis.

CHAPTER 7

THE PENTOSE PHOSPHATE PATHWAY

7.1 FUNCTIONS OF THE PENTOSE PHOSPHATE PATHWAY

We learned in Chapter 4 that the main function of glycolysis is to oxidize glucose. The *pentose phosphate pathway* is a second pathway for the oxidation of glucose that synthesizes NADPH and pentose phosphates, which are five-carbon sugar phosphates. Because the pathway for the synthesis of the pentose phosphate ribulose 5-phosphate branches from glycolysis after the formation of glucose 6-phosphate, this alternate pathway is sometimes referred to as the *hexose monophosphate shunt*. All the enzymes involved in the pentose phosphate pathway are localized to the cytosol.

Glycolysis is a catabolic pathway. Under aerobic conditions, its products are pyruvate, ATP, and reducing equivalents in the form of NADH. By contrast, the pentose phosphate pathway serves two distinct anabolic roles. First, as its name implies, the pathway generates pentose 5-phosphates, which are substrates for nucleic acid synthesis. Second, the pathway generates reducing equivalents in the form of NADPH. In contrast to glycolysis, the pentose phosphate pathway does not generate ATP.

7.1.1 Functions of NADPH

NADPH is formed from $NADP^+$ (or nicotinamide-adenine dinucleotide phosphate) by the addition of two electrons and a hydrogen ion (proton). The $NADP^+$/NADPH couple is thus very similar to the NAD^+/NADH couple. However, whereas NADH is

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primarily an electron carrier involved in fuel oxidation and ATP generation, NADPH serves to provide reducing equivalents for biosynthetic reactions such as fatty acid synthesis and cholesterol synthesis. Other roles for NADPH include recycling of oxidized glutathione to its reduced form and providing the reductant in the cytochrome P450–catalyzed hydroxylation of sterols.

NADH and NADPH provide the cell with two distinct pools of reducing equivalents, which can coexist at different redox levels. Since the electrons of cytosolic NADH are usually shuttled efficiently into the mitochondria, the cytosolic $NADH/NAD^+$ ratio is normally relatively low (usually < 0.01). By contrast, a high ratio of $NADPH/NADP^+$ is required for biosynthetic pathways. Since electrons are not transferred directly from cytosolic NADPH to NAD^+ or from NADH to $NADP^+$, the reducing equivalents associated with NADH and NADPH, respectively, can be effectively targeted for different needs of the cell.

7.2 TISSUES IN WHICH THE PENTOSE PHOSPHATE PATHWAY IS ACTIVE

Although the enzymes of the pentose phosphate pathway are present in all cells, their level of expression varies greatly from tissue to tissue. Relatively high levels of the pentose phosphate pathway enzymes are found in the liver where large quantities of fatty acids and cholesterol are synthesized, and in endocrine glands such as the ovaries, testes, and adrenal cortex, which synthesize cholesterol and steroid hormones. High levels of the pentose phosphate pathway enzymes are also found in cells of the early embryo and in other rapidly dividing cells, such as enterocytes, all of which require substantial amounts of ribose 5-phosphate for nucleic acid synthesis. By contrast, only low levels of hexose monophosphate shunt enzymes are present in skeletal muscle.

7.2.1 Phagocytic Cells

The highest levels of glucose 6-phosphate dehydrogenase, the first enzyme in the pentose phosphate pathway, are found in neutrophils and macrophages. In these phagocytic cells, NADPH is used to generate superoxide radicals from molecular oxygen in a reaction catalyzed by NADPH oxidase:

$$2O_2 + NADPH \rightarrow 2O_2^{\bullet} + NADP^+ + H^+$$

Superoxide anion, in turn, can serve to generate other reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), hydrochlorous acid (HOCl), and hydroxyl radical ($OH\cdot$), that kill phagocytized microorganisms (Fig. 7-1). Superoxide anion can also react with nitric oxide (NO) to generate peroxynitrite ($ONOO^-$), which in turn can lead to the formation of other nitrogen-containing radicals. The marked increase in

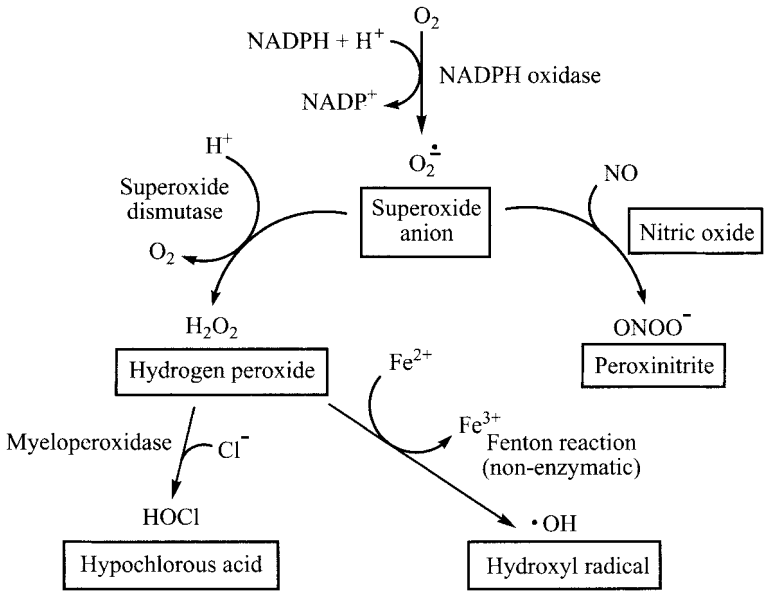

FIGURE 7-1 Role of NADPH oxidase in the generation of reactive oxygen species.

the rate of O_2 consumption by phagocytic cells following exposure to bacteria and other stimuli is often referred to as the *oxygen burst*.

7.2.2 Erythrocytes

Reactive oxygen species can attack proteins, membrane lipids, and DNA. Red blood cells rely on NADPH for protection against hemolysis caused by exposure to ROS such as hydrogen peroxide and lipid peroxides. The major intracellular antioxidant in red blood cells, as in most other cells, is glutathione, a tripeptide (γ-glutamylcysteinylglycine), which in the reduced state contains a sulfhydryl group, hence the abbreviation GSH. Vitamin E (Fig. 7-2) also plays a role in eliminating free

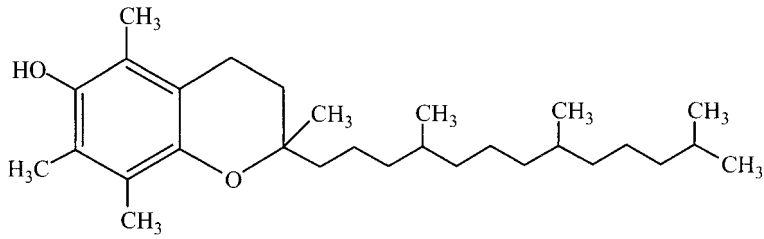

FIGURE 7-2 α-Tocopherol (vitamin E).

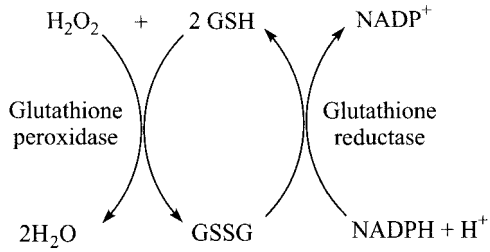

FIGURE 7-3 Role of NADPH in the generation of reduced glutathione. GSH, reduced glutathione; GSSG, oxidized glutathione.

radicals. Since it is fat soluble, vitamin E tends to partition into cellular membranes, which is where most of its antioxidant function is exerted.

The destruction of hydrogen peroxide is catalyzed by glutathione peroxidase, a ubiquitous, cytosolic, selenium-containing enzyme. The enzymatic destruction of hydrogen peroxide generates oxidized glutathione dimer (GSSG) (Fig. 7-3):

$$H_2O_2 + 2GSH \rightarrow 2H_2O + GSSG$$

NADPH is then used by glutathione reductase to restore oxidized glutathione to its original, reduced state:

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$$

Not surprisingly, glucose flux through the hexose monophosphate shunt increases when there is infection or exposure to certain drugs (e.g., primaquine, paraquat, naphthalene) that increase oxidant stress. In the absence of sufficient NADPH, the glutathione defense system is compromised and the risk of hemolysis is increased.

7.3 PHYSIOLOGICAL STATES AND CONDITIONS DURING WHICH THE PENTOSE PHOSPHATE PATHWAY IS PARTICULARLY ACTIVE

The pentose phosphate pathway is most active under conditions that require increased production of NADPH. Its activity is enhanced in granulocytes (e.g., neutrophils) during phagocytosis and in erythrocytes in response to oxidant stress.

NADPH provides reducing equivalents for biosynthetic processes such as fatty acid and cholesterol synthesis. In the liver, the pentose phosphate pathway is active in the fed state, when excess dietary carbohydrates are being converted into fatty acids and then into triacylglycerols. Indeed, the two NADPH-generating enzymes of the pentose phosphate pathway, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, are both induced by high levels of blood glucose and a high insulin/glucagon ratio (as is the case after a meal).

7.4 REACTIONS OF THE PENTOSE PHOSPHATE PATHWAY

Conceptually, the pentose phosphate pathway can be viewed as consisting of two phases. The first phase, which starts with glucose 6-phosphate, is the oxidative component of the pathway and the one that generates NADPH. In the process, 6-carbon glucose 6-phosphate is converted to five-carbon ribulose 5-phosphate and CO_2. The oxidative phase of the pentose phosphate pathway is irreversible. The second phase of the pentose phosphate pathway allows for the nonoxidative interconversion of sugar phosphates and functions to recycle excess pentose phosphates back into intermediates of the glycolytic pathway at the level of fructose 6-phosphate and glyceraldehyde 3-phosphate. Alternatively, the second phase of the pentose phosphate pathway can be utilized to generate pentose phosphates for nucleic acid synthesis when the synthesis of NADPH is not required and glucose flux through the oxidative phase does not occur.

7.4.1 Oxidative Phase

The three reactions that comprise the oxidative phase of the pentose phosphate pathway are shown in Fig. 7-4. Glucose 6-phosphate dehydrogenase catalyzes the initial oxidation of glucose 6-phosphate to 6-phosphoglucono-δ-lactone. This is the first of the two steps that generate NADPH and, as discussed below, is the regulated step of the pathway:

$$\text{glucose 6-phosphate} + NADP^+ \rightarrow \text{6-phosphoglucono-δ-lactone} + NADPH + H^+$$

Gluconolactonase then catalyzes the hydrolysis of the lactone bond of the cyclical 6-phosphoglucono-δ-lactone to produce a linear structure:

$$\text{6-phosphoglucono-δ-lactone} + H_2O \rightarrow \text{6-phosphogluconate} + H^+$$

6-Phosphogluconate dehydrogenase catalyzes the $NADP^+$-dependent oxidative decarboxylation of 6-phosphoglucononate to produce ribulose 5-phosphate and CO_2, thereby generating the second molecule of NADPH in the process:

$$\text{6-phosphogluconate} + NADP^+ \rightarrow \text{ribulose 5-phosphate} + CO_2 + NADPH + H^+$$

7.4.2 Nonoxidative Phase

The nonoxidative phase of the pentose phosphate pathway (Fig. 7-5) involves reshuffling the carbon skeletons of various sugar phosphates to reversibly interconvert pentose phosphates and hexose phosphates. It is best visualized as having three rearrangement steps:

$$C_5 + C_5 \rightleftharpoons C_3 + C_7 \qquad \text{(transketolase)}$$

$$C_3 + C_7 \rightleftharpoons C_6 + C_4 \qquad \text{(transketolase)}$$

$$C_4 + C_5 \rightleftharpoons C_6 + C_3 \qquad \text{(transketolase)}$$

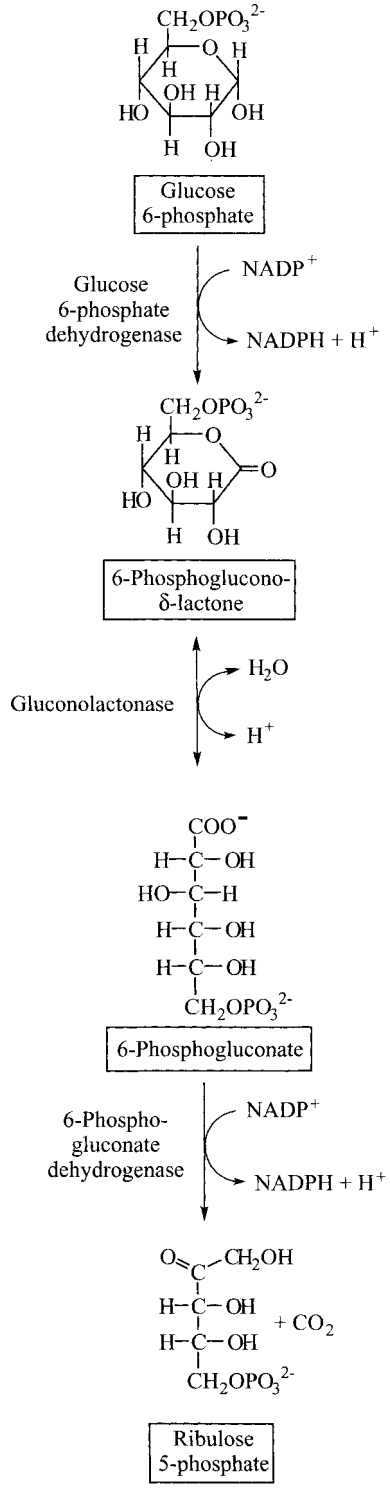

FIGURE 7-4 Oxidative phase of the pentose phosphate pathway.

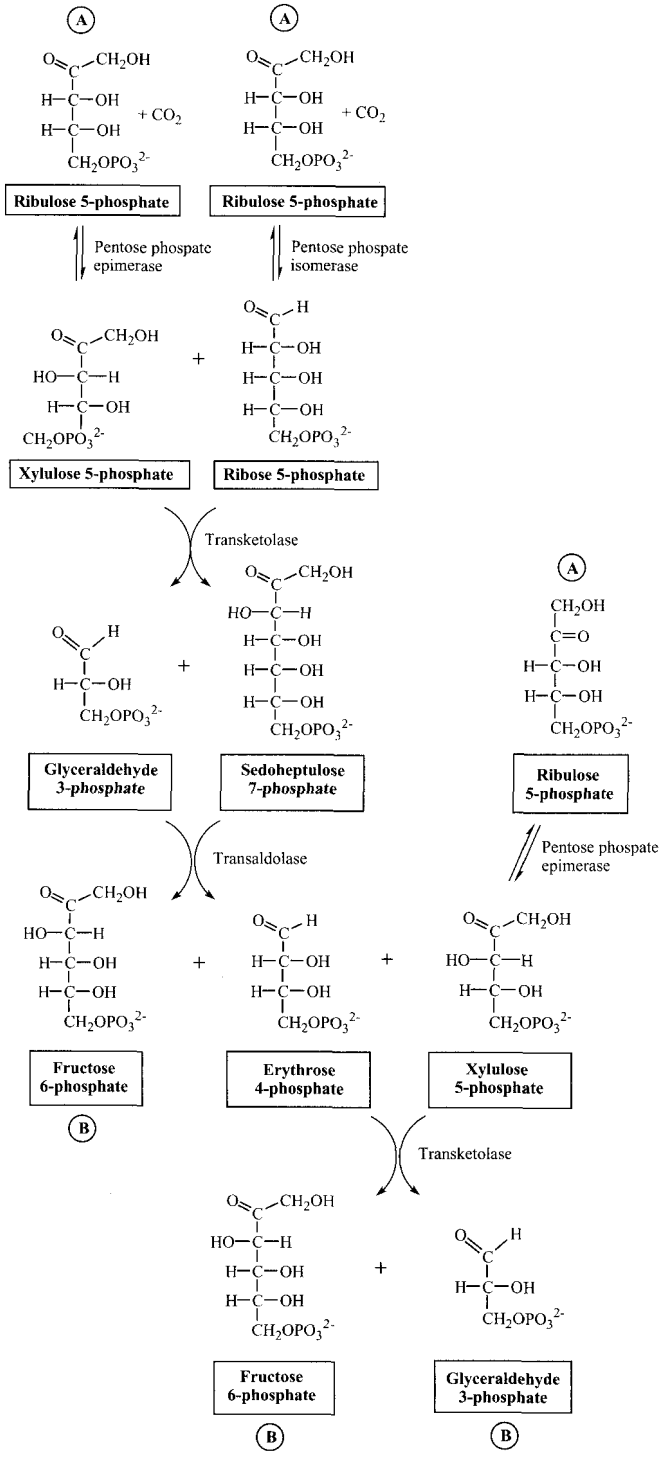

FIGURE 7-5 Nonoxidative phase of the pentose phosphate pathway. Ⓐ, substrates; Ⓑ, products.

The net effect of these reactions is

$$3C_5 \rightleftharpoons 2C_6 + C_3$$

where C_6 in fructose 6-phosphate and C_3 in glyceraldehyde 3-phosphate.

The nonoxidative phase of the pentose phosphate pathway starts with two pentose phosphates, ribose 5-phosphate and xylulose 5-phosphate, both of which are synthesized from ribulose 5-phosphate, which is the product of the oxidative phase of the pathway (Fig. 7-4). Ribose 5-phosphate is generated from ribulose 5-phosphate by phosphopentose isomerase, a step that is also required to generate ribose 5-phosphate for nucleotide synthesis. The reversible isomerization of the aldose sugar phosphate and the ketosugar phosphate is analogous to the conversion of glucose 6-phosphate to fructose 6-phosphate during glycolysis. Phosphopentose epimerase converts ribulose 5-phosphate to a second ketosugar phosphate, xylulose 5-phosphate.

The nonoxidative phase of the pentose phosphate pathway consists of three successive transfers of two- or three-carbon fragments between sugar-phosphates. First, transketolase transfers a two-carbon unit from xylulose 5-phosphate to ribose 5-phosphate, producing glyceraldehyde 3-phosphate plus the seven-carbon sedoheptulose 7-phosphate. Transketolase requires thiamine pyrophosphate as a cofactor. In the second step, transaldolase transfers a three-carbon unit from sedoheptulose 7-phosphate to glyceraldehyde 3-phosphate, forming four-carbon erythrose-4-phosphate plus fructose 6-phosphate. Transketolase then catalyzes a second two-carbon transfer reaction from the donor molecule, xylulose 5-phosphate. This time the acceptor molecule is erythrose 4-phosphate, thereby forming glyceraldehyde 3-phosphate plus a second molecule of fructose 6-phosphate.

7.5 REGULATION OF THE PENTOSE PHOSPHATE PATHWAY

The rate-limiting step of the oxidative phase is the first enzyme, glucose 6-phosphate dehydrogenase. The activity of this enzyme is regulated by the availability of the electron acceptor, $NADP^+$. Competition by the product, NADPH, with $NADP^+$ for binding to glucose 6-phosphate dehydrogenase acts to inhibit the enzyme. Under normal physiological conditions, most of the $NADP^+$ is in the reduced form (NADPH), and the activity of glucose 6-phosphate dehydrogenase is low. Activation of biosynthetic pathways such as fatty acid synthesis and cholesterogenesis results in consumption of NADPH and increases the flux of glucose 6-phosphate through glucose 6-phosphate dehydrogenase.

The oxidative phase is also regulated at the level of gene expression. In the liver, both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are induced upon refeeding after starvation when the availability of exogenous glucose promotes fatty acid synthesis and increases the need for NADPH.

As noted above, the oxidative and nonoxidative phases of the pentose phosphate pathway can function independently of each other. Flux through the nonoxidative

phase of the pathway is regulated by the supply and demand for ribose 5-phosphate. The nonoxidative phase is inactive when all the pentose phosphates produced by the oxidative phase are converted into ribose 5-phosphate for RNA and DNA synthesis. On the other hand, when the oxidative phase is producing pentose phosphates in excess of cellular requirements, the nonoxidative phase becomes active and converts the excess pentose phosphates into fructose 6-phosphate plus glyceraldehyde 3-phosphate. Since the pentose phosphate pathway usually functions during the fed state, the fructose 6-phosphate and glyceraldehyde 3-phosphate generated by the nonoxidative phase are utilized primarily for glycolysis, producing ATP for biosynthetic processes.

When the demand for ribose 5-phosphate exceeds that of the NADPH which is concurrently produced by the oxidative phase of the pathway, the nonoxidative phase operates in the reverse direction; that is, it converts fructose 6-phosphate plus glyceraldehyde 3-phosphate into ribose-5-phosphate. Since all of the reactions of the nonoxidative phase are reversible, a decline in concentrations of ribose 5-phosphate will stimulate pentose phosphate synthesis without a concomitant increase in the flux of glucose through the oxidative phase of the pathway.

7.6 ABNORMAL FUNCTIONING OF THE PENTOSE PHOSPHATE PATHWAY

7.6.1 Glucose 6-Phosphate Dehydrogenase Deficiency

Globally, deficiency of glucose 6-phosphate dehydrogenase (G6PD) is the most common inborn error of metabolism. The major clinical manifestation of G6PD deficiency is acute hemolytic anemia following exposure to oxidative stress. Glucose 6-phosphate dehydrogenase deficiency may also present as neonatal jaundice during the first few days of life. The G6PD gene is X-linked and affects hemizygous males and homozygous females; heterozygous females are usually relatively unaffected unless X chromosome inactivation is skewed unfavorably. Many persons with G6PD deficiency are normally asymptomatic. However, an episode of hemolytic anemia usually occurs within hours of exposure to an oxidative stress which markedly increases the demand for NADPH to restore oxidized glutathione to its reduced form. Viral and bacterial infections are the most common triggers of acute hemolytic episodes. Hemolysis may also result from the ingestion of specific drugs, such as antimalarials or sulfonamide antibiotics, or certain foods, such as unripe fava beans, that contain the pyrimidine β-glycosides, vicine, and convicine that react nonenzymatically with O_2 to produce reactive oxygen species. The anemic crisis is usually self-limiting and ends when older erythrocytes, those most deficient in G6PD, have been hemolyzed.

7.6.1.1 Why Are Erythrocytes Particularly Vulnerable to Deficiencies of Glucose 6-Phosphate Dehydrogenase? Red blood cells rely on NADPH to maintain glutathione in its reduced form, thereby protecting the cells from oxidative

stress. Other cells, such as neutrophils and hepatocytes, also require high levels of NADPH; however, they have alternative pathways for generating NADPH. The major alternative source of NADPH, particularly for fatty acid and cholesterol synthesis, is the malic enzyme, which catalyzes the reaction

$$\text{malate} + \text{NADP}^+ \rightarrow \text{pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+$$

The malate utilized in this reaction is a component of the metabolic pathway that shuttles acetyl-CoA out of the mitochondrion in the form of citrate (see Chapter 11). Since erythrocytes do not contain mitochondria, they lack this alternative source of NADPH.

A second problem for red blood cells is that the most common genetically based deficiency in glucose 6-phosphate dehydrogenase, G6PD A^-, results in a relatively unstable G6PD enzyme that has a shortened half-life. Whereas cells that have a nucleus can synthesize new molecules of the G6PD enzyme, erythrocytes lack this capacity.

7.6.1.2 What Happens with More Severe G6PD Deficiencies? The rare patient who has an exceeding low level of G6PD activity in his or her cells is likely to have chronic hemolytic anemia (even without added oxidative stress) and an increased susceptibility to infection, because the NADPH supply of neutrophils is inadequate to support sufficient generation of H_2O_2 during phagocytosis.

7.6.1.3 Why Is There Such a High Gene Frequency for Defective Alleles of G6PD? G6PD deficiency has a prevalence of 5 to 25% in areas where malaria is endemic. Male hemizygotes and female heterozygotes both have significant protection against severe malaria. Erythrocytes of persons with G6PD deficiency are more sensitive than normal to hydrogen peroxide generated by the malarial parasite. Free-radical damage to erythrocyte membrane lipids causes hemolysis and death of the intracellular parasite before the parasite can reach maturity.

7.6.2 Chronic Granulatomous Disease

Chronic granulatomous disease (CGD) is a relatively rare genetic disorder affecting phagocytic cells normally involved in killing pathogenic microorganisms. Mutations in any one of the proteins that comprise the phagocyte NADPH oxidase system can prevent the enzyme from generating superoxide anion and downstream reactive oxygen species (e.g., H_2O_2, hydroxyl free radical, hypochlorite) involved in killing infectious organisms. People with CGD are at increased risk for pneumonia, osteomyelitis, and abscesses.

CHAPTER 8

GLYCOGEN

8.1 FUNCTION OF GLYCOGEN

Glycogen is a highly branched homopolymer of glucose that serves as the main carbohydrate-based energy store in the body. Although crucial to both homeostasis of blood glucose and to muscle work, glycogen actually represents less than 1% of the body's caloric stores, with triacylglycerol and mobilizable proteins accounting for the rest.

Glycogen granules are located in the cytosol. They consist of linear chains of glucose in α-1,4 glycosidic linkage, with α-1,6 glycosidic linkages forming branches after approximately every 8 to 10 glucose residues (Fig. 8-1). Starch, the glucose homopolymer in plants, consists of two types of molecules: amylose, which is a linear structure with glucose units in α-1,4 glycosidic linkages, and amylopectin, which contains α-1,6 glycosidic branches off the linear α-1,4 glycosidic chain. Glycogen is thus similar to, but more highly branched than, amylopectin. Because the anomeric carbons of the outermost glucose moieties of glycogen are all in glycosidic linkages with adjacent glucose moieties and thus not free to open up into the aldehyde form, the outer ends of the glycogen branches are all nonreducing. The glucose moiety in the core of glycogen is also nonreactive because it is covalently bound to glycogenin, the protein which, as described below, serves as the primer that initiates glycogen synthesis.

Glycogenolysis (glycogen breakdown) provides a readily available source of glucose when it is needed. Glycogen synthesis functions to replenish the glycogen stores in liver and muscle when dietary carbohydrates are available.

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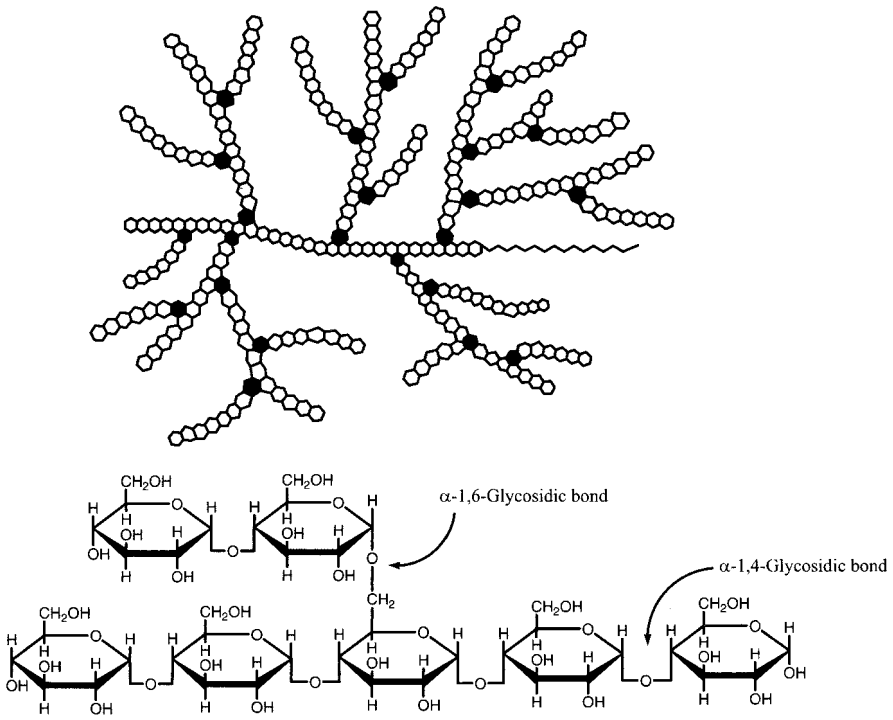

FIGURE 8-1 Structure of glycogen. The solid hexagons represent glucose units at α1,6 branch points. The zig-zag line represents glycogenin.

8.2 TISSUES THAT STORE GLYCOGEN

Although nearly all cells of the body contain trace amounts of glycogen, the major glycogen stores are found in liver and skeletal muscle. The liver contains the greatest concentration of glycogen of any organ, accounting for as much as 10% of tissue wet weight. On a mass basis, however, the largest portion of the body's total glycogen stores is in muscle. In a healthy adult in the fed state, the liver contains about 400 g of glycogen, whereas muscle contains approximately 1200 g of glycogen. Liver and muscle glycogen stores serve two distinctly different purposes. When mobilized, the glucose derived from liver glycogen is secreted by hepatocytes and used to maintain the concentration of glucose in the blood. By contrast, glucose arising from glycogen breakdown in skeletal and heart muscle remains in the muscle cells and is used to provide energy for muscle work.

The brain also contains a small but significant amount of glycogen, which is localized primarily in astrocytes. Brain glycogen accumulates during sleep and is mobilized upon waking, suggesting a functional role for brain glycogen in the conscious brain. The glycogen reserves in the central nervous system also provide at least a moderate degree of protection against hypoglycemia.

Another organ with a specialized role for glycogen stores is the fetal lung. Type II pulmonary cells begin to accumulate glycogen at about 26 weeks of gestation. Late in gestation, these cells shift their metabolism toward the synthesis of pulmonary surfactant, with the intracellular glycogen serving as a major substrate for the synthesis of surfactant lipids, of which dipalmitoylphosphatidylcholine is the major component.

8.3 PHYSIOLOGICAL STATES AND CONDITIONS DURING WHICH GLYCOGEN METABOLISM IS ESPECIALLY ACTIVE

Glycogenolysis in liver occurs in the fasted state and is stimulated primarily by the hormone glucagon. Resynthesis of glycogen occurs during the fed state and is stimulated both by insulin and the increased availability of glucose.

Glycogenolysis in muscle occurs during muscle contraction and affects only those muscles that are actually engaged in physical activity. Resynthesis of glycogen in muscle following exercise is more rapid when blood glucose levels are high and insulin is available to stimulate the activity of the GLUT4 transporters that facilitate the entry of glucose into muscle cells.

8.4 REACTIONS OF GLYCOGEN SYNTHESIS AND GLYCOGENOLYSIS

8.4.1 Glycogenolysis

Glycogenolysis (glycogen mobilization) is the process by which glucose units are removed, one at a time, from the numerous nonreducing ends of glycogen. In contrast to the cleavage of the glycosidic bonds of starch by α-amylase in the intestinal tract, cleavage of α-1,4-glycosidic bonds during glycogenolysis in muscle and liver is a phosphorolytic rather than a hydrolytic process and utilizes orthophosphate (P_i) rather than water.

8.4.1.1 Glycogen Phosphorylase. The first step in glycogenolysis is catalyzed by glycogen phosphorylase, commonly called *phosphorylase*. This enzyme cleaves the α-1,4 glycosidic bonds of glycogen (Fig. 8-2):

$$\text{glycogen}_{(n \text{ glucose residues})} + HPO_4^{2-} \rightarrow \text{glucose 1-phosphate} + \text{glycogen}_{(n-1 \text{ glucose residues})}$$

The reaction catalyzed by glycogen phosphorylase is physiologically irreversible and is the regulated step in glycogen mobilization. Different isozymes of glycogen phosphorylase are present in muscle and liver, permitting organ-specific regulation of glycogenolysis.

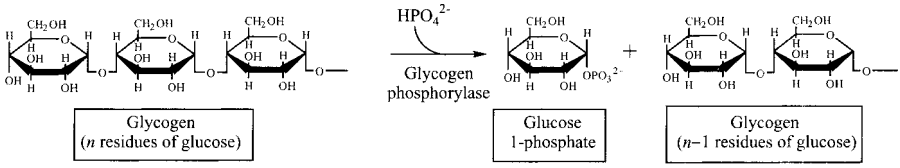

FIGURE 8-2 Glycogen phosphorylase catalyzes glycogenolysis.

8.4.1.2 Debranching Enzyme. Glycogen phosphorylase catalyzes the cleavage of α-1,4 glycosidic bonds but not of α-1,6 glycosidic bonds. Furthermore, glycogen phosphorylase is incapable of removing glucose residues that lie within four residues of a branch point. The debranching enzyme that solves this problem has two distinct catalytic activities (Fig. 8-3). First, the oligo-α-1,4 → α-1,4-glucanotransferase catalytic site transfers three glycosyl residues en bloc from the α-1,6 branch to an adjacent α-1,4 chain, leaving behind a single glucose unit in α-1,6 glucosidic linkage. The α-1,6-glucosidase catalytic site of the debranching enzyme then hydrolyzes the α-1,6 glycosidic bond of the remaining glucose unit, releasing free glucose and producing a long, unbranched α-1,4 glycosidic chain that is a suitable substrate for continued glycogenolysis by glycogen phosphorylase.

8.4.1.3 Significance of the Branched Structure of Glycogen. Given the complications involved in removing the branch-point glucose units from glycogen during glycogenolysis, it is reasonable to ask why these branches exist in the first place. There are two advantages to the highly branched structure of glycogen. First, the branched glycogen molecule provides numerous nonreducing termini that can serve as substrate for attack by multiple molecules of glycogen phosphorylase. This permits rapid mobilization of glycogen when the resulting glucose 1-phosphate is urgently needed either for muscle work or blood glucose homeostasis. The only alternative for providing multiple nonreducing ends of glycogen as available substrate for rapid mobilization by glycogen phosphorylase would be to have many smaller glucose polymers (oligosaccharides) instead of the larger glycogen granules. Given the substantial amounts of glycogen that can be stored in hepatocytes and muscle cells, having a comparable number of nonreducing ends on multiple linear (amylose) chains would substantially increase intracellular osmotic pressure and could cause cellular damage.

Second, the highly branched structure of glycogen permits formation of a compact glycogen granule. By contrast, long chains of relatively unbranched glucose polymers would also cause cellular damage. Evidence for this comes from the liver pathology associated with Anderson disease, a rare genetic disorder in which the ability to form branches in glycogen is impaired due to a deficiency of the branching enzyme. The presence of intracellular glycogen with very long outer branches leads to progressive cirrhosis of the liver and fatal liver failure.

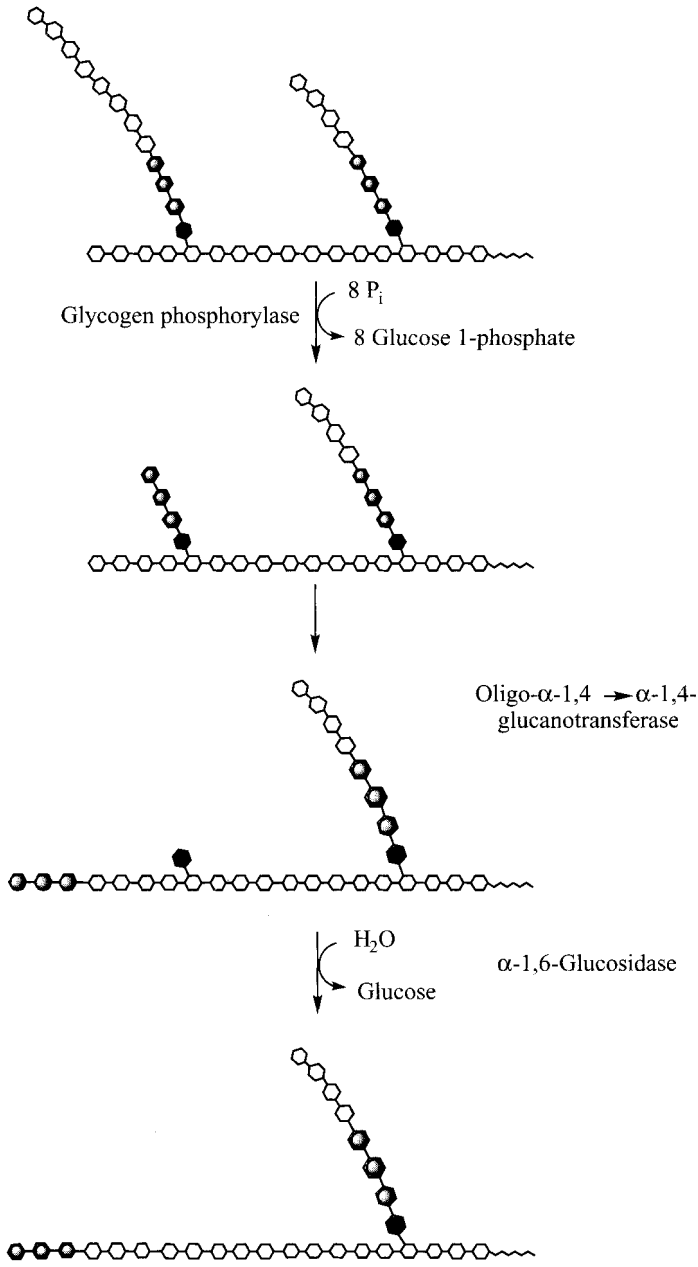

FIGURE 8-3 Role of the debranching enzyme in glycogenolysis.

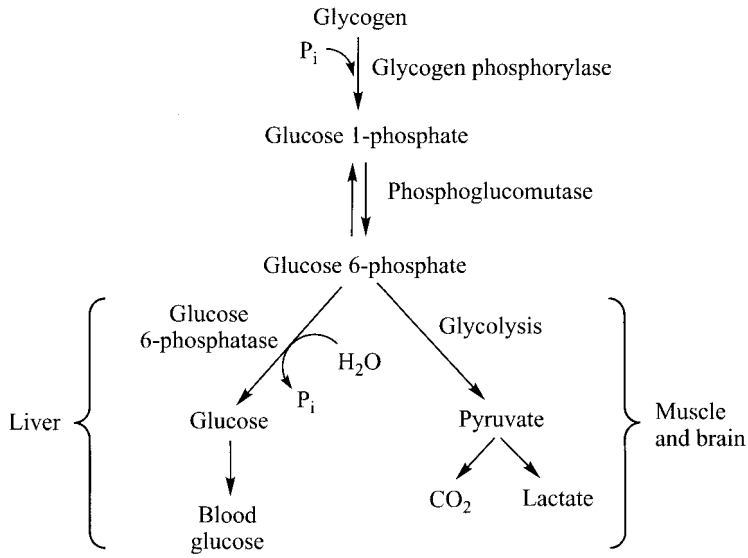

FIGURE 8-4 Alternate fates of glycogen-derived glucose 6-phosphate in muscle and liver.

8.4.1.4 What Is the Fate of Glucose 1-Phosphate in Skeletal Muscle?

One additional metabolic step is required before the glucose 1-phosphate generated in the glycogen phosphorylase reaction can be utilized as an energy source by muscle cells, and that is the transfer of the phosphate group from carbon-1 to carbon-6. Phosphoglucomutase catalyzes the following reaction:

$$\text{glucose 1-phosphate} \rightleftharpoons \text{glucose 6-phosphate}$$

This reaction is freely reversible, with the direction being determined by the relative concentrations of substrate and product. In muscle cells, the resulting glucose 6-phosphate molecule then enters the glycolytic pathway and is catabolized to provide energy for muscle contraction (Fig. 8-4).

8.4.1.5 What Is the Fate of Glucose 1-Phosphate in Hepatocytes?

Hepatocytes also contain phosphoglucomutase; however, the fate of glucose 6-phosphate is different in liver than in muscle (Fig. 8-4). The glucose 6-phosphate generated by glycogenolysis in liver is not catabolized within the hepatocyte but is, instead, released from the cell as free glucose. This irreversible dephosphorylation step is catalyzed by glucose 6-phosphatase:

$$\text{glucose 6-phosphate} + H_2O \rightarrow \text{glucose} + P_i$$

Glucose 6-phosphatase is an intrinsic enzyme embedded in the membrane of the endoplasmic reticulum (ER) (Fig. 8-5). Hydrolysis of glucose 6-phosphate occurs not

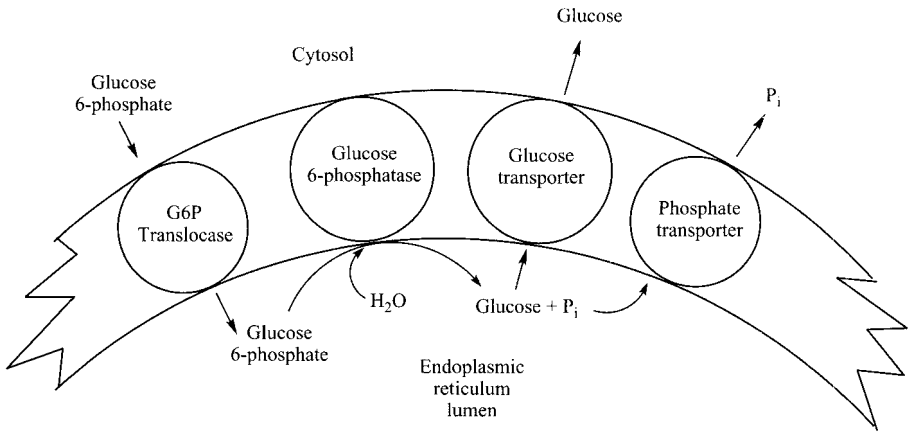

FIGURE 8-5 Role of transporters in the functioning of glucose 6-phosphatase.

in the cytosol but in the lumen of the endoplasmic reticulum. A specific transporter, glucose 6-phosphate translocase, is required to transport glucose 6-phosphate from the cytosol into the lumen of the ER; an anion transporter returns the resulting inorganic phosphate to the cytosol. Although the hepatic ER also contains a glucose transporter, most of the resultant glucose is not transported back into the cytosol of the hepatocyte; instead, the glucose is secreted to maintain blood-glucose homeostasis. Glucose 6-phosphatase is expressed in liver and the renal cortex, but not in muscle, and plays an important role in providing blood glucose from gluconeogenesis as well as glycogenolysis.

8.4.1.6 What Is the Advantage of Intracellular Phosphorolysis Versus Hydrolysis of Glycogen? Phosphorolysis is particularly advantageous for skeletal muscle and heart, which utilize the glucose units of glycogen for energy within the cell. As discussed in Chapter 4, phosphorylation of sugars serves both to activate them and to trap them within the cell. Mobilization of glycogen by phosphorolysis thus ensures that the exercising muscle can utilize the glycogen-derived glucose for its energy needs directly rather than allowing that glucose to be released into the bloodstream.

Another advantage of phosphorolytic degradation of glycogen as opposed to hydrolysis of glycogen is that generation of glucose phosphate instead of free glucose permits the glycogen-derived glucose to bypass the initial activation step of glycolysis: the hexokinase or glucokinase reaction. As also discussed in Chapter 4, each glucose molecule entering the glycolytic pathway requires 2 ATP to produce the activated glycolytic intermediate, fructose 1,6-bisphosphate. Subsequent glycolysis to lactate generates 4 ATP, for a net yield of 2 ATP/glucose. Phosphorolysis of glycogen, in effect, conserves some of the activation energy required for glycogen synthesis and only requires the input of one ATP to generate fructose 1,6-bisphosphate. This

means that the net yield of ATP per glucose obtained from glycogen is 3 rather than 2; the 50% increase is clearly beneficial to strenuously exercising muscle.

It should be noted that not all of the glucose molecules of glycogen are mobilized as glucose 1-phosphate. As discussed above, during glycogenolysis the α-1,6-glycosidase component of the debranching enzyme releases a molecule of free glucose from each of the branch points of the glycogen molecule.

8.4.2 Glycogen Synthesis

As in the case of other anabolic pathways, the process of glycogen synthesis requires more energy input than is obtained during glycogen breakdown. The expenditure of an additional high-energy phosphate bond for each glucose molecule incorporated into glycogen during glycogen synthesis is the price the cell must pay for being able to store glucose in glycogen when fuel is plentiful and have that endogenous glucose available when exogenous fuels are scarce. The activated form of glucose that is used for glycogen synthesis is UDP-1-glucose.

8.4.2.1 Pathway for Glycogen Synthesis from Free Glucose. Following a meal, glucose is taken up by cells and phosphorylated by hexokinase (in muscle) or glucokinase (in liver) to generate glucose 6-phosphate:

$$\text{glucose} + \text{ATP} \rightarrow \text{glucose 6-phosphate} + \text{ADP}$$

When liver and muscle are poised to synthesize glycogen, phosphoglucomutase then shifts the phosphate group from C_6 to C_1:

$$\text{glucose 6-phosphate} \rightleftharpoons \text{glucose 1-phosphate}$$

This reversible reaction is catalyzed by the same enzyme that catalyzes the conversion of glucose 1-phosphate to glucose 6-phosphate during glycogenolysis.

8.4.2.2 UDP-Glucose Pyrophosphorylase. UDP-glucose pyrophosphorylase catalyzes the synthesis of UDP-1-glucose from glucose 1-phosphate (Fig. 8-6):

$$\text{glucose 1-phosphate} + \text{UTP} \rightleftharpoons \text{UDP-1-glucose} + \text{PP}_i$$

Although the enzyme is named for the reverse reaction (glucose 1-phosphate production), the reaction is driven to the right by the rapid hydrolysis of inorganic pyrophosphate to inorganic phosphate by pyrophosphatase. Note that the pathway of glycogen synthesis has thus far consumed two high-energy bonds (1 ATP and 1 UTP) per molecule of glucose activated to the level of UDP-1-glucose.

8.4.2.3 Glycogen Synthase. Glycogen synthase then transfers the activated glucose moiety of UDP-1-glucose to the 4-hydroxyl group of a glucose residue

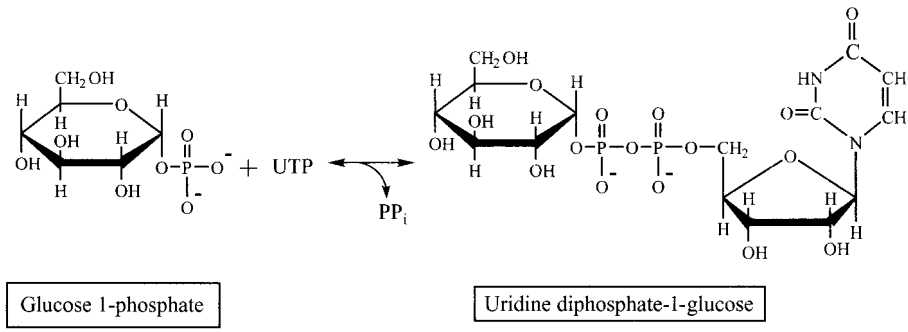

FIGURE 8-6 Synthesis of uridine diphosphate-1-glucose (UDP-1-glucose).

at one of the numerous nonreducing ends of the branched glycogen molecule, thereby forming a new α-1,4 linkage and extending the carbohydrate chain by one glucose unit.

8.4.2.4 Formation of Branches in the Growing Glycogen Molecule. The formal name of the branching enzyme is amylo-(α-1,4 → α-1,6) transglycosylase. The enzyme transfers en bloc a six- to seven-residue oligosaccharide from the nonreducing end of a newly elongated α-1,4 glucose polymer to form a new α-1,6 glycosidic linkage (Fig. 8-7).

8.4.2.5 How Is the Synthesis of a New Molecule of Glycogen Initiated? Glycogen synthase adds glucose moieties to an already existing α-1,4 glucose polymer. It is reasonable to ask about the origins of the initial glucose polymer. The answer is that a specialized protein called *glycogenin* serves as a primer for glycogen synthesis. Glycogenin is self-glucosylating and catalyzes both the covalent attachment of the initial glucose moiety to the protein and the subsequent addition of glucose units to form an α-1,4 glucose oligomer which can serve as substrate for glycogen synthase. The glycogenin molecule remains in the core of the resulting glycogen polymer, where it is attached to the reducing end of the initial glucose unit (Fig. 8-1).

8.5 REGULATION OF GLYCOGEN METABOLISM

As indicated above, glycogen synthesis occurs in the fed state, whereas glycogenolysis occurs both in the fasted state and in response to strenuous exercise. The two pathways—glycogen synthesis and glycogenolysis—are reciprocally regulated so that both processes are not active at the same time, thus preventing the operation of a futile, energy-wasting cycle. Glycogen metabolism is regulated by both allosteric and hormonal mechanisms. We first discuss the reciprocal hormonal regulation of the two pathways and then the specific allosteric mechanisms that apply to each of the key regulatory enzymes.

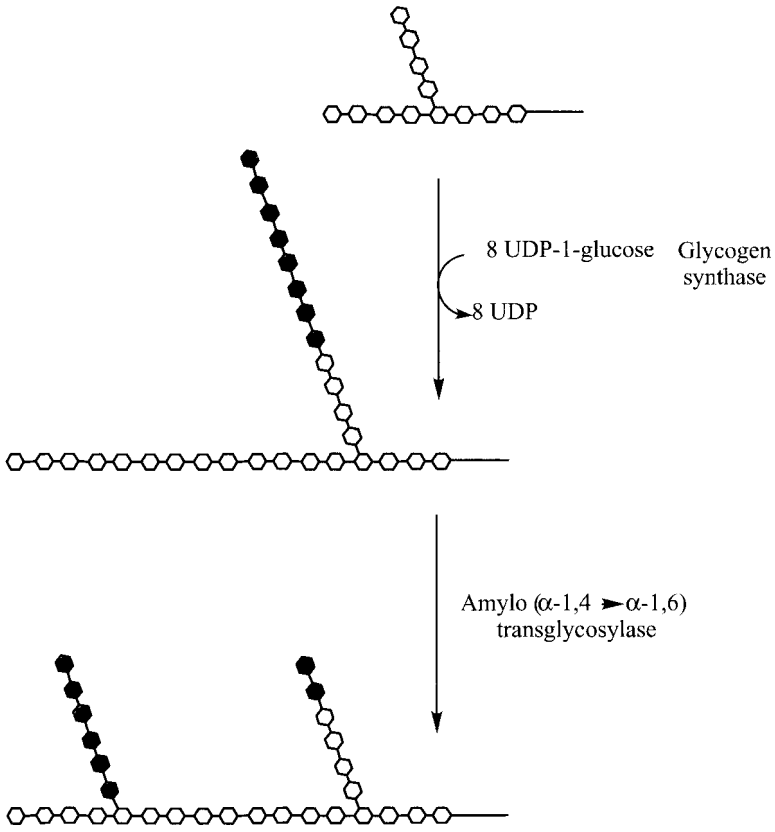

FIGURE 8-7 Glycogen synthesis and the role of amylo (α-1,4 → α-1,6)transglycosylase (the branching enzyme).

8.5.1 Hormonal Regulation

The major regulated steps of glycogenolysis and glycogen synthesis are catalyzed by glycogen phosphorylase and glycogen synthase, respectively. As discussed in Chapter 2, the signal transduction cascade initiated by glucagon or epinephrine acts through protein kinase A (PKA) to phosphorylate specific serine residues on certain key regulatory enzymes, including glycogen synthase and a highly specific protein kinase, phosphorylase kinase, which is dedicated to phosphorylating glycogen phosphorylase (Fig. 8-8). Protein phosphorylation activates glycogen phosphorylase and inactivates glycogen synthase, thereby stimulating glycogenolysis while concurrently inhibiting glycogen synthesis.

Binding of insulin to its receptor on hepatocytes and muscle cells leads to many downstream intracellular effects, one of which is activation of protein phosphatase 1. Protein phosphatase 1, in turn, reverses the effects of protein kinase A by dephosphorylating the enzymes glycogen synthase, glycogen phosphorylase, and phosphorylase

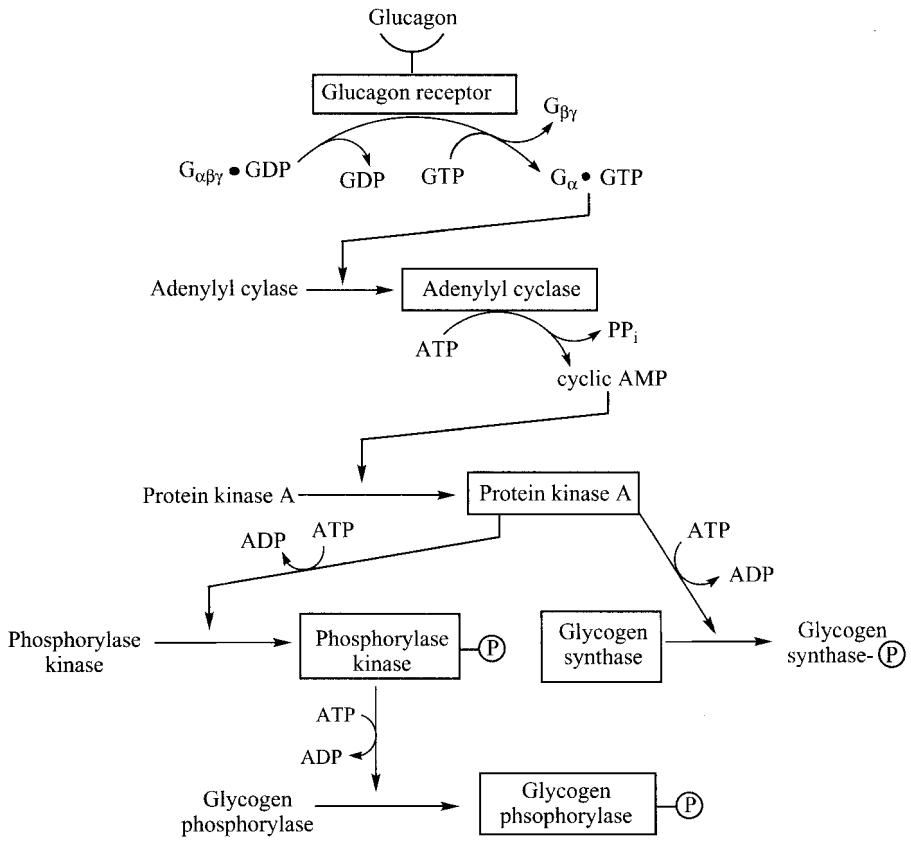

FIGURE 8-8 Role of glucagon in the coordinated regulation of glycogenolysis and glycogen synthesis. The active forms of the receptor and the enzymes in the signaling cascade are indicated in boxes.

kinase. Insulin thus acts to reverse the effects of glucagon and/or epinephrine on these enzymes, effectively activating glycogen synthesis while inactivating glycogen mobilization.

8.5.1.1 Regulation of Glycogen Phosphorylase. Glycogen phosphorylase is a dimeric enzyme that can assume one of two states, R (relaxed) and T (tense or taut), that are in equilibrium with each other. In the R state, the active site of glycogen phosphorylase is accessible to the substrate (glycogen) and the enzyme is active; conversely, in the T state, the active site is inaccessible and the enzyme is inactive. The nonphosphorylated form of glycogen phosphorylase (referred to as *phosphorylase b*) exists primarily in the inactive T form. Phosphorylation of phosphorylase *b* (producing phosphorylase *a*) shifts the equilibrium in favor of the R form, thus increasing glycogen phosphorylase activity.

The equilibrium between the R and T forms of glycogen phosphorylase is also affected by allosteric regulators. Muscle and liver contain different isozymes

of glycogen phosphorylase, thus permitting tissue-specific modulation of enzyme activity.

Muscle Phosphorylase. Phosphorylase *b* (the nonphosphorylated form of the enzyme) is inactive under most physiological conditions. Both ATP and glucose 6-phosphate are allosteric modulators that act to keep the glycogen phosphorylase primarily in the inactive T state. Thus, when fuel and energy are plentiful, glycogen phosphorylase is inactive. Exercise changes this situation by depleting supplies of both ATP and glucose 6-phosphate. The decreased energy charge of exercising muscle results in increased concentrations of AMP, which shift the equilibrium toward the R form, allosterically activating the otherwise inactive phosphorylase *b*.

Liver Phosphorylase. Glucose is an allosteric inhibitor of liver glycogen phosphorylase which acts to inhibit the otherwise active phosphorylase *a*. This mechanism serves to restrict glycogenolysis when it is not needed to maintain blood glucose.

8.5.1.2 Regulation of Phosphorylase Kinase. Phosphorylase kinase is a serine–threonine protein kinase that phosphorylates and activates glycogen phosphorylase. Like glycogen phosphorylase, there are muscle and liver isozymes of phosphorylase kinase. Muscle phosphorylase kinase is only partially activated by phosphorylation. Phosphorylase kinase is also partially activated by an increase in the intracellular concentration of calcium ions that bind to calmodulin, a ubiquitous calcium-binding regulatory protein. Each molecule of phosphorylase kinase contains one molecule of calmodulin as its δ-subunit. Nerve impulses and muscle contraction increase cytosolic [Ca^{2+}], thereby enhancing hormone-stimulated activation of phosphorylase kinase and subsequent mobilization of intramuscular glycogen. Full activation of phosphorylase kinase requires both PKA-catalyzed phosphorylation and binding of Ca^{2+} to the calmodulin subunit.

8.5.1.3 Regulation of Glycogen Synthase. As described above, PKA-stimulated phosphorylation of glycogen synthase inactivates the enzyme. Several other protein kinases, including glycogen synthase kinase-3 (GSK3), can also phosphorylate specific serine residues of glycogen synthase and thus contribute to inactivation of the enzyme. By contrast, high concentrations of glucose 6-phosphate increase the activity of the otherwise inactive phosphorylated glycogen synthase in both muscle and liver. This allosteric regulation explains how blood glucose concentration modulates glycogen synthesis by the liver.

8.6 ABNORMALITIES OF GLYCOGEN METABOLISM

8.6.1 Glycogen Storage Diseases

There are a number of inborn errors of metabolism which result in excessive accumulation of intracellular glycogen; these are called *glycogen storage diseases*. Deficiencies in the activity of the liver isozymes of glycogen phosphorylase or phosphorylase

kinase result in liver enlargement (hepatomegaly) and fasting hypoglycemia. The symptoms of a deficiency of glycogen phosphorylase or phosphorylase kinase are not as severe as those associated with a deficiency of glucose 6-phosphatase (von Gierke disease) where neither glycogenolysis nor gluconeogenesis is available to support blood glucose homeostasis because glucose 6-phosphate cannot be dephosphorylated and released from the hepatocytes.

By contrast, a genetic deficiency of the muscle isozyme of glycogen phosphorylase (McArdle disease) is accompanied by muscle-specific accumulation of glycogen and a limited ability to exercise strenuously. In the absence of glycogenolysis to support exercise, affected persons rapidly develop a depleted energy charge within the muscle that causes severe muscle pain and weakness.

8.6.1.1 Pompe Disease. During cellular recycling and tissue remodeling, most cells of healthy persons accumulate some glycogen within lysosomes. Catabolism of glycogen within lysosomes utilizes an alternative pathway in which lysosomal acid maltase hydrolyzes both α-1,4- and α-1,6-glycosidic bonds. Unlike the other glycogen storage diseases, deficiency of acid maltase results in accumulation of glycogen in lysosomes rather than as granules in the cytosol. Whereas many organs are affected in Pompe disease, the most serious problem is cardiac damage, which ultimately results in death due to cardiorespiratory failure.

8.6.2 Neonatal Hypoglycemia

At delivery, the maternal supply of glucose is cut off abruptly, resulting in a transient hypoglycemic state in the first 1 to 2 hours. In response to hypoglycemia, increased concentrations of epinephrine and glucagon in the blood stimulate the liver to initiate glycogenolysis, which is crucial to neonatal survival. However, some newborns lack adequate hepatic glycogen reserves to support blood glucose homeostasis in the first few hours after birth. During the third trimester, the fetal liver normally accumulates 10 to 12 g of glycogen, which provides the neonate with about a 12-hour supply of glucose. It is common for premature infants to be born before they have had time to accumulate a sufficient quantity of this crucial glucose reserve. Inadequate neonatal glycogen reserves can also be the result of in utero malnutrition. In either case, the result can be life threatening if intravenous glucose is not supplied in a timely manner.

A related problem can arise in an infant born to a mother who has any form of poorly controlled diabetes mellitus, including *gestational diabetes*, which is also called *glucose intolerance of pregnancy*. Although fetal exposure to a constant oversupply of glucose results in higher-than-normal glycogen stores in what are often large-for-gestational-age-infants, it also results in increased plasma insulin levels in the fetus. The persistence of an elevated insulin level in the infant after birth and the concomitant lower-than-normal concentration of glucagon act to suppress mobilization of glycogen from the neonatal liver. Thus, although these infants have ample glycogen reserves, hyperinsulinemia may prevent them from maintaining adequate circulating levels of blood glucose and put them at risk for developing life-threatening hypoglycemia.

8.6.3 Glycogen Loading

Although the preferred fuel of resting muscle is fatty acids, exercising muscle uses a mixture of glucose and fatty acids. With an increased rate of work, the percentage contribution of glucose to energy metabolism is increased. Sustained vigorous exercise normally depletes glycogen stores within 1 to 3 hours; since muscle glycogen is available only for the intracellular use of the muscle, glycogen stores are depleted only in those muscles that are actively exercising. Following exercise, repletion of the intramuscular glycogen stores usually occurs within 1 to 2 days, particularly if the diet provides at least 50 to 60% of total calories as carbohydrates. Interestingly, such repletion may increase glycogen stores to levels that are twice their original level. An athlete who trains vigorously two or three days before a competitive event and then consumes a high-carbohydrate diet can therefore enter the competition with higher-than-normal glycogen reserves and an increased capacity for sustained physical exertion. It should be noted that with training, the muscles also become less dependent on glucose, further increasing one's ability to go further on their glycogen reserves.

CHAPTER 9

GLUCONEOGENESIS

9.1 FUNCTION OF GLUCONEOGENESIS

In Chapter 3 we learned that some tissues, particularly the brain, red blood cells, and the renal medulla, depend heavily on glycolysis to satisfy their ATP needs. Yet, as discussed in Chapter 8, the glycogen stores of the body are limited and provide only about an 8- to10-hour supply of glucose to maintain the plasma glucose concentration within normal limits.

Gluconeogenesis is the process by which the body synthesizes glucose from endogenous noncarbohydrate precursors, primarily lactate and glycerol, and the carbon skeletons of the amino acids alanine and glutamine. This pathway is essential for maintaining the concentration of blood glucose in the fasted state. The term *gluconeogenesis* is used to indicate that this process is explicitly distinct from the interconversion of hexose sugars, through which glucose is generated from dietary fructose or galactose.

Unlike other fuel sources such as amino acids, lactate, and glycerol, the carbon skeletons of most fatty acids cannot be utilized for gluconeogenesis. Virtually all physiological fatty acids contain an even numbers of carbons (usually, C16 or C18) and their catabolism involves cleavage of the fatty acid chain into two-carbon acetyl-CoA units. Humans and other animals lack a pathway for converting acetyl-CoA to glucose. On the other hand, the oxidation of the relatively rare odd-chain fatty acids and branched methyl fatty acids that are present in human diets do generate small amounts of propionic acid that can be converted into glucose.

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9.2 TISSUES IN WHICH GLUCONEOGENESIS IS ACTIVE

The liver has long been considered the major gluconeogenic organ. However, recent studies indicate that contributions of the renal cortex to gluconeogenesis have been underappreciated. Current estimates indicate that, on a wet weight basis, the renal cortex produces more glucose than the liver, much of it for use by the renal medulla. Indeed, renal gluconeogenesis protects the body from severe hypoglycemia under conditions of liver failure. It should be noted, however, that the kidney lacks significant glycogen stores and, unlike the liver, can contribute to glucose homeostasis via gluconeogenesis but not by way of glycogenolysis.

9.3 PHYSIOLOGICAL CONDITIONS IN WHICH GLUCONEOGENESIS IS ESPECIALLY ACTIVE

Gluconeogenesis is active primarily when a person is in the fasted state, when dietary carbohydrates have been utilized or stored as glycogen, and the plasma concentration of glucose has declined. The liver starts synthesizing glucose in response to the decreased insulin/glucagon ratio which occurs after postprandial processing of absorbed nutrients and increases the rate of gluconeogenesis as glycogen stores become depleted during a subsequent fasting period.

Gluconeogenesis also increases during prolonged physical exercise and serves to provide glucose for heart and active skeletal muscle. After exercise, the rate of gluconeogenesis remains elevated and contributes to modest replenishment of muscle glycogen stores prior to the availability of dietary glucose. During both fasting and recovery from prolonged exercise, the substantial energy cost of gluconeogenesis is met primarily by concurrent β-oxidation of fatty acids to acetyl-CoA in the liver.

Fasting gluconeogenesis is particularly important in the neonate. In the first few hours after delivery, the newborn experiences a period of transient hypoglycemia resulting from loss of the continuous glucose infusion that had been provided through the umbilical cord from the maternal circulation. Since glycogen stores in the newborn are insufficient to meet the resulting need for blood sugar, the healthy neonate responds by increasing the rate of gluconeogenesis.

9.4 REACTIONS OF GLUCONEOGENESIS

9.4.1 Enzymes Unique to Gluconeogenesis

The pathway for gluconeogenesis utilizes many, but not all, of the enzymes of glycolysis (Fig. 9-1). The reactions that are common to glycolysis and gluconeogenesis are the reversible reactions. This is best illustrated when one considers lactate as the starting point for gluconeogenesis. As discussed in Chapter 3, lactate is the endpoint of glycolysis when pyruvate cannot be metabolized through pyruvate dehydrogenase and the TCA cycle. Anaerobic glycolysis occurs in red blood cells, in the renal medulla, and

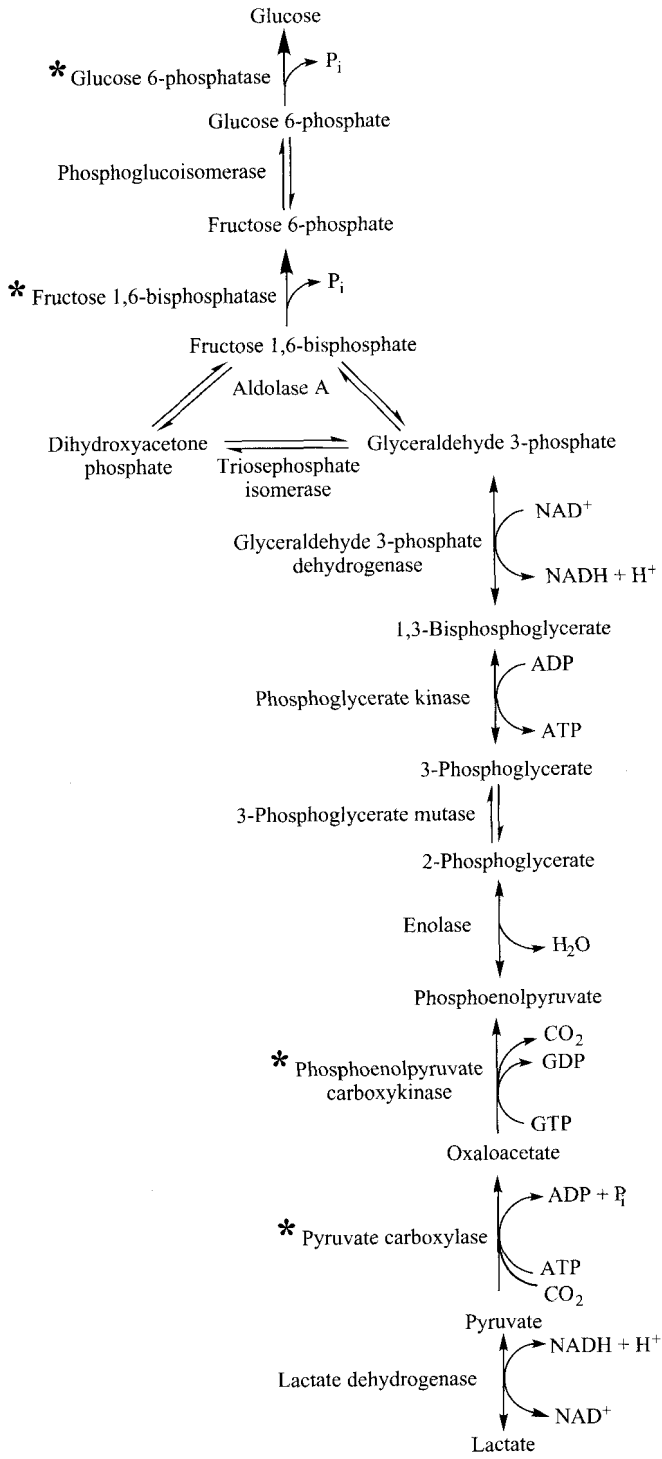

FIGURE 9-1 Pathway for gluconeogenesis from lactate. Asterisks indicate irreversible steps.

in skeletal muscle during strenuous exercise. Gluconeogenesis serves to regenerate glucose from lactate, thereby ensuring a constant supply of glucose for those cells and tissues that are highly dependent on glycolysis for their energy needs.

There are three physiologically irreversible steps in the glycolytic pathway that must be bypassed by gluconeogenesis-specific enzymes. Two of these irreversible steps are the two ATP-requiring activation reactions of glycolysis catalyzed by glucokinase and phosphofructokinase-1; they are bypassed by glucose 6-phosphatase and fructose 1,6-bisphosphatase, respectively. The third irreversible step of glycolysis is the second ATP-generating reaction, which is catalyzed by pyruvate kinase. (The other substrate-level phosphorylation reaction that generates ATP is catalyzed by phosphoglycerate kinase; it is reversible and is utilized in gluconeogenesis as well as glycolysis.) The gluconeogenesis pathway utilizes the reactions catalyzed by pyruvate carboxylase and phosphoenolpyruvate carboxykinase to bypass the irreversible pyruvate kinase reaction of glycolysis (Fig. 9-2).

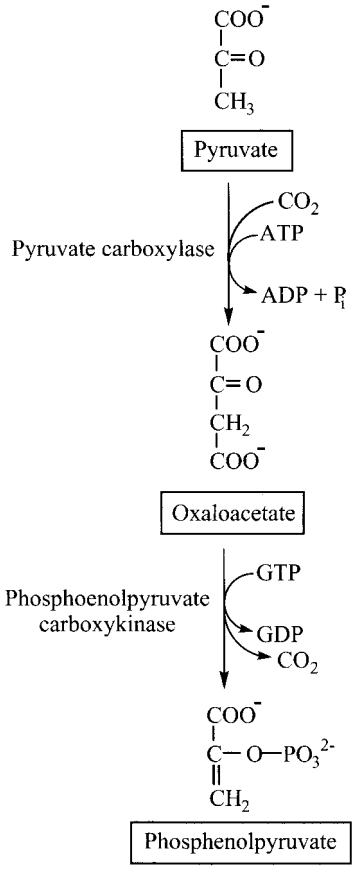

FIGURE 9-2 The two initial steps of gluconeogenesis.

Thus, the pathway for gluconeogenesis from lactate shown in Figure 9-1 has many reactions in common with the glycolytic pathway shown in Figure 3-3. The reactions catalyzed by enzymes such as phosphoglucoisomerase, aldolase A, glyceraldehyde 3-phosphate dehydrogenase, and phosphoglycerate kinase are reversible and are at near-equilibrium under the normal intracellular conditions of pH and concentrations of substrates and products. These reversible reactions can therefore be used for either glycolysis or gluconeogenesis, depending on the activity of each of the enzymes that catalyze the irreversible steps that regulate the availability of substrates for the two pathways.

Let us consider those steps that are unique to gluconeogenesis.

9.4.1.1 Pyruvate Carboxylase. Carboxylation of pyruvate by pyruvate carboxylase generates oxaloacetate and requires input of energy in the form of ATP:

$$\text{pyruvate} + CO_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP}$$

The mitochondrial synthesis of oxaloacetate was discussed briefly in Chapter 4 in the context of anaplerotic reactions that serve to replenish the supply of citric acid cycle intermediates. Similar to other carboxylases, the catalytic domain of pyruvate carboxylase contains a molecule of biotin that is covalently attached to the enzyme by means of an amide bond between the carboxyl group of the biotin side chain and the ε-amino group of a lysine residue of the enzyme (Fig. 9-3). Biotin serves as a carrier of the activated CO_2 that is transferred to pyruvate to form oxaloacetate. This same CO_2 molecule is released during the next step in gluconeogenesis and does not appear in the resulting glucose molecule. Pyruvate carboxylase is activated by

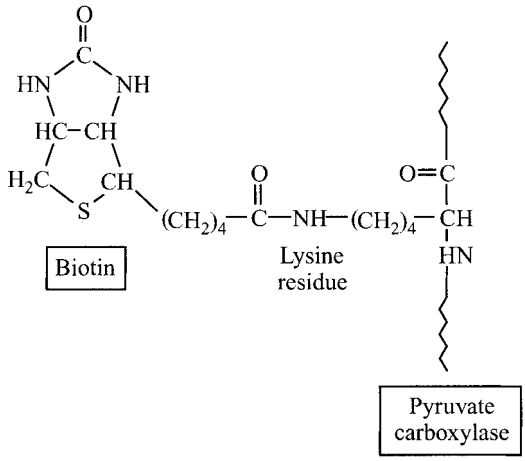

FIGURE 9-3 Covalent linkage of biotin to pyruvate-CoA carboxylase.

acetyl-CoA, ensuring that gluconeogenesis occurs only when there is sufficient fatty acid oxidation to provide the energy needed for glucose synthesis.

9.4.1.2 Phosphoenolpyruvate Carboxykinase. The next step in gluconeogenesis involves the simultaneous decarboxylation and phosphorylation of oxaloacetate. GTP provides the high-energy phosphate group that ends up in the product, phosphoenolpyruvate (PEP):

$$\text{oxaloacetate} + \text{GTP} \rightarrow \text{phosphoenolpyruvate} + \text{CO}_2 + \text{GDP}$$

By virtue of the phosphate group it contains, PEP is trapped in the cytosol and thus remains available for the next step in gluconeogenesis.

The sequence of reactions catalyzed by pyruvate carboxylase and pyruvate carboxykinase serves to bypass the irreversible pyruvate kinase reaction of glycolysis. The utilization of these two reactions is not, however, without an extra energy cost. Whereas one ATP is generated during glycolysis by the conversion of PEP to pyruvate in the pyruvate kinase reaction, it takes two high-energy phosphate groups (one from ATP and one from GTP) to generate PEP from pyruvate during gluconeogenesis.

9.4.1.3 Fructose 1,6-Bisphosphatase. Hydrolysis of fructose 1,6-bisphosphate is the next irreversible step of gluconeogenesis:

$$\text{fructose 1,6-bisphosphate} + \text{H}_2\text{O} \rightarrow \text{fructose 6-phosphate} + \text{P}_\text{i}$$

This reaction reverses the step in glycolysis that is catalyzed by phosphofructokinase-1, but it does not regenerate the ATP utilized in that reaction. Like phosphofructokinase-1, which is the main step that is regulated in glycolysis, fructose 1,6-bisphosphatase is a critical allosteric enzyme that is involved in regulation of gluconeogenesis.

9.4.1.4 Glucose 6-Phosphatase. This enzyme, which is as integral protein of the endoplasmic reticulum, catalyzes the intraluminal hydrolysis of glucose 6-phosphate to generate free glucose:

$$\text{glucose 6-phosphate} + \text{H}_2\text{O} \rightarrow \text{glucose} + \text{P}_\text{i}$$

Glucose 6-phosphatase-catalyzed dephosphorylation of glucose 6-phosphate is essential for the release of glucose from the cell. Expression of glucose 6-phosphatase in liver and renal cortex permits these tissues to utilize gluconeogenesis to maintain blood glucose. As discussed in Chapter 8, hepatic glucose 6-phosphatase is also necessary for the release of glucose from the liver during glycogenolysis. In the absence of glucose 6-phosphatase, glucose 6-phosphate is trapped within the cell and either utilized for glycolysis or incorporated into glycogen.

9.4.2 Precursors for Gluconeogenesis

9.4.2.1 Lactate. The gluconeogenesis pathway described above started with lactate as the substrate and essentially reversed glycolysis to produce glucose. Gluconeogenesis thus provides a mechanism by which the liver and renal cortex can synthesize glucose from the lactate produced by skeletal muscle during strenuous exercise and continuously by red blood cells. The metabolic interchange between lactate-generating cells and gluconeogenic cells, called the *Cori cycle*, is illustrated in Figure 4-5.

The Cori Cycle Requires Net Energy Input. More energy is required to generate glucose from lactate in the liver than is obtained by oxidizing glucose in red blood cells. Glycolysis of glucose to lactate produces a net of two molecules of ATP per molecule of glucose oxidized. By comparison, gluconeogenesis from lactate requires 6 ATP equivalents (4 ATP, 2 GTP) to produce one molecule of glucose. The two-step sequence catalyzed by pyruvate carboxylase and pyruvate carboxykinase requires one ATP plus one GTP per molecule of pyruvate. An additional ATP is required for the phosphoglycerate kinase-catalyzed conversion of 3-phosphglycerate to 1,3-bisphosphoglycerate. Since two molecules of lactate are utilized for the synthesis of each molecule of glucose, the total energy cost is 2×3 or 6, high-energy bonds.

Is the Cori Cycle Just a Waste of Energy? It may appear that continued breakdown and resynthesis of glucose is wasteful. It is, however, the small energy cost paid by the liver and renal cortex to permit effective functioning of other cells. Since erythrocytes lack both mitochondria and a nucleus, they are smaller than most cells; their relatively small cell size permits easy passage of red cells through tiny capillaries but renders red blood cells completely dependent on glycolysis to lactate for their ATP. The conversion of lactate to glucose occurs in the liver, where ample ATP can be generated from the β-oxidation of long-chain fatty acids.

Glycolysis to lactate is also advantageous during strenuous exercise. Although the yield of ATP per glucose molecule metabolized is much lower than when glucose is oxidized all the way to CO_2 and water, the rate at which ATP can be generated by glycolysis is greater than the rate at which ATP can be produced by oxidative phosphorylation. Most of the lactate generated by muscle is secreted into the circulation and returned to the liver, where it is converted back into glucose by the process of gluconeogenesis. The energy required to convert lactate into glucose is derived from the mitochondrial β-oxidation of fatty acids and transfer of the reducing equivalents from NADH and $FADH_2$ into the ATP-generating oxidative phosphorylation system of mitochondria.

9.4.2.2 Alanine. Alanine is the major gluconeogenic amino acid substrate of the liver. In the fasted state, proteolysis of muscle proteins provides substrates for maintaining blood glucose homeostasis. However, not all amino acid carbon skeletons can be converted into glucose. In particular, muscle protein contains a significant percentage (approximately 20%) of branched-chain amino acids that are ketogenic or

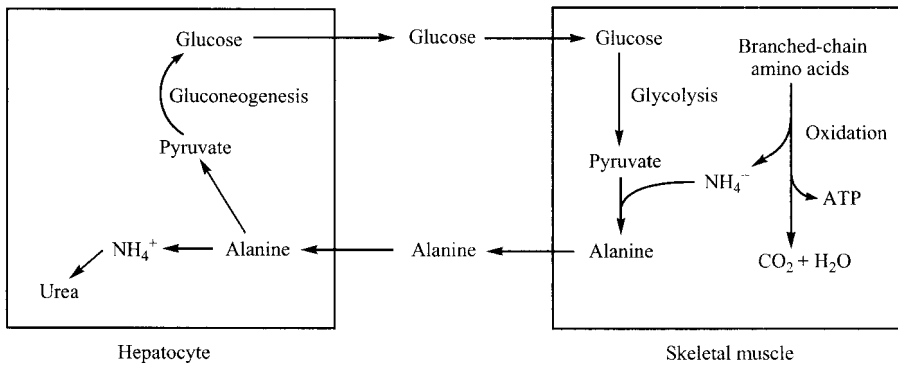

FIGURE 9-4 Alanine cycle.

mixed ketogenic and glucogenic. Oxidation of the carbon chains of branched-chain amino acids occurs primarily within muscle and serves as a significant energy source for muscle during fasting. Before branched-chain amino acids can be oxidized, the α-amino groups must be removed by transamination and exported from the muscle primarily as alanine and glutamine.

In the synthesis of alanine in muscle, pyruvate serves as the acceptor molecule for the α-amino groups transferred, with the pyruvate being derived from glycolysis. This means that muscle cells need a constant supply of glucose to sustain the net export of gluconeogenic precursors. That glucose supply is provided mainly by hepatic gluconeogenesis from alanine. The interorgan cycle of glucose catabolism in the muscle to generate alanine and the recycling of the carbon skeletons of alanine to glucose in the liver is called the *alanine cycle* (Fig. 9-4). Like the Cori cycle discussed above, the alanine cycle has a net energy cost. Nevertheless, the alanine cycle has significant advantages to the organism as a whole since it permits efficient catabolism of muscle proteins that provide substrates for gluconeogenesis.

The pathway for hepatic gluconeogenesis from alanine is similar to that from lactate in that both lactate and alanine are readily converted to pyruvate. In the case of alanine, the reaction involves transamination in which the α-amino group of an amino acid is transferred to α-ketoglutarate and subsequently excreted as urea:

$$\text{alanine} + \alpha\text{-ketoglutarate} \rightarrow \text{pyruvate} + \text{glutamate}$$

9.4.2.3 Glutamine. In the renal cortex, glutamine is the preferred substrate for gluconeogenesis. Like alanine, glutamine is synthesized by skeletal muscle in the fasted state as a means of exporting the amino groups of amino acids. In the kidney, the two amino groups of glutamine are removed by glutaminase and glutamate dehydrogenase, producing free ammonium ions and α-ketoglutarate. The ammonium ions serve to buffer acids excreted in the urine, while the α-ketoglutarate provides substrate for gluconeogenesis. As a result of the linkage between the generation of free ammonium ions and α-ketoglutarate, gluconeogenesis in the kidney increases

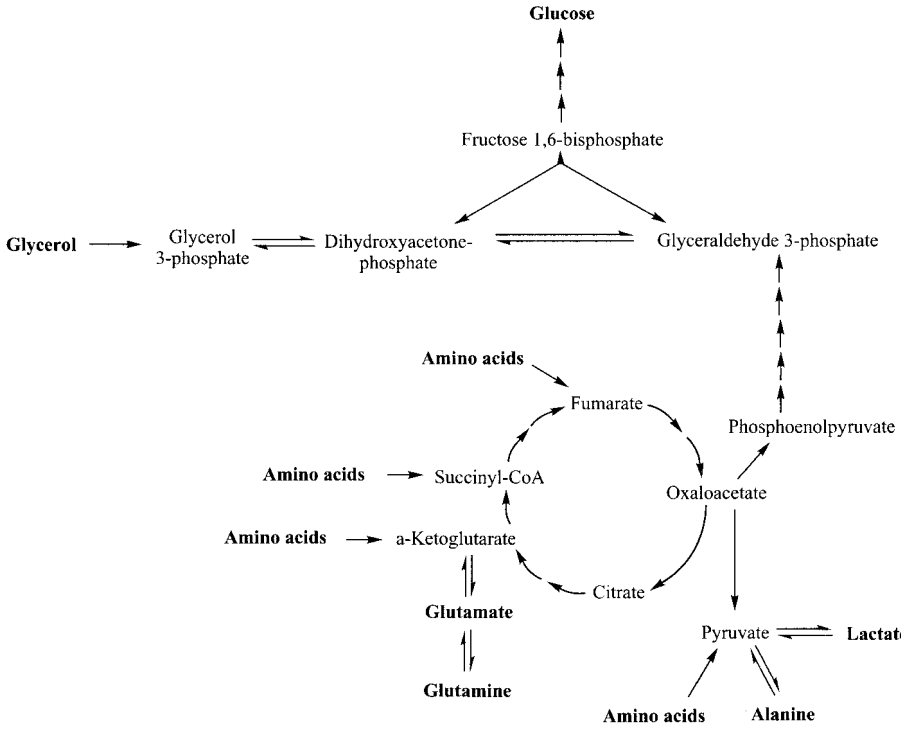

FIGURE 9-5 Pathway for gluconeogenesis from substrates other than lactate. Amino acids that generate pyruvate: alanine, cysteine, glycine, methionine, serine, threonine, and tryptophan. Amino acids that generate α-ketoglutarate: arginine, glutamate, glutamine, histidine, and proline. Amino acids that generate succinyl-CoA: isoleucine, threonine, and valine. Amino acids that generate fumarate: phenylalanine, tyrosine, and aspartate (via the urea cycle). Amino acids that generate oxaloacetate: asparagine and aspartate.

significantly during conditions of acidosis as well as fasting. Oxidation of α-ketoglutarate via the TCA cycle produces oxaloacetate (Fig. 9-5), which then enters the same pathway as that used to synthesize glucose from lactate (Fig. 9-1).

9.4.2.4 Many Other Amino Acids, Including Histidine, Proline, and Asparagine, Are Glucogenic. A number of the other amino acids can contribute all or a part of their carbon skeletons to gluconeogenesis. In each instance, the carbon skeletons of these glucogenic amino acids are metabolized either to pyruvate or to one of the TCA-cycle intermediates, such as oxaloacetate, succinyl-CoA, or α-ketoglutarate (Fig. 9-5).

Not all amino acid carbon skeletons can be utilized for gluconeogenesis, because the catabolism of certain amino acids generates acetyl-CoA, and humans cannot convert acetyl-CoA into glucose. The pyruvate dehydrogenase reaction is irreversible, and animal cells lack an alternative pathway for utilizing acetyl-CoA for the net

synthesis of TCA-cycle intermediates. Amino acids such as leucine which are catabolized to acetyl-CoA do not provide carbon skeletons that are suitable for glucose synthesis in humans. Still other amino acids are both glucogenic and ketogenic. For example, catabolism of tryptophan produces both pyruvate and acetyl-CoA: The pyruvate can be utilized for gluconeogenesis, but the acetyl-CoA cannot.

9.4.2.5 Glycerol. In the fasted state, mobilization of adipose triacylglycerols provides free fatty acids and glycerol. Although even-chain fatty acids are catabolized to acetyl-CoA and, like ketogenic amino acids, are not substrates for gluconeogenesis, the glycerol that is released during lipolysis can be a significant source of substrate for glucose synthesis.

Glycerol released from adipocytes is taken up by the liver and phosphorylated by glycerol kinase:

$$\text{glycerol} + \text{ATP} \rightarrow \text{glycerol 3-phosphate} + \text{ADP}$$

NAD^+-dependent glycerol 3-phosphate dehydrogenase then oxidizes the glycerol 3-phosphate to dihydroxyacetone phosphate, which enters the gluconeogenic pathway at the level of the aldolase A reaction (Fig. 9-5):

$$\text{glycerol 3-phosphate} + NAD^+ \rightarrow \text{dihydroxyacetone phosphate} + \text{NADH} + H^+$$

9.4.3 Localization of Gluconeogenesis

All of the enzymes of the glycolytic pathway are located in the cytosol. Although most of the enzymes of gluconeogenesis are also found in the cytosol, there are two exceptions: pyruvate carboxylase and glucose 6-phosphatase.

9.4.3.1 Pyruvate Carboxylase. Pyruvate carboxylase is a mitochondrial enzyme that provides oxaloacetate for the TCA cycle as well as for gluconeogenesis. Before oxaloacetate can be converted into glucose, it must be shuttled out of the mitochondrion. This is accomplished by first reducing oxaloacetate to malate inside the mitochondrion; following its transport into the cytosol, malate is then reoxidized to oxaloacetate. Oxaloacetate derived from α-ketoglutarate or other TCA-cycle intermediates is also transported out of the mitochondrion as malate. Since both the mitochondrial and cytosolic malate dehydrogenase isozymes are NAD^+-linked, the net effect of this process is the conversion of NADH to NAD^+ in the mitochondrion and the generation of NADH from NAD^+ in the cytosol (Fig. 9-6). Cytosolic NADH is required for the subsequent conversion of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate (Fig. 9-1). It should be noted that the gluconeogenic pathway from lactate generates NADH in the initial lactate dehydrogenase reaction:

$$\text{lactate} + NAD^+ \rightarrow \text{pyruvate} + \text{NADH} + H^+$$

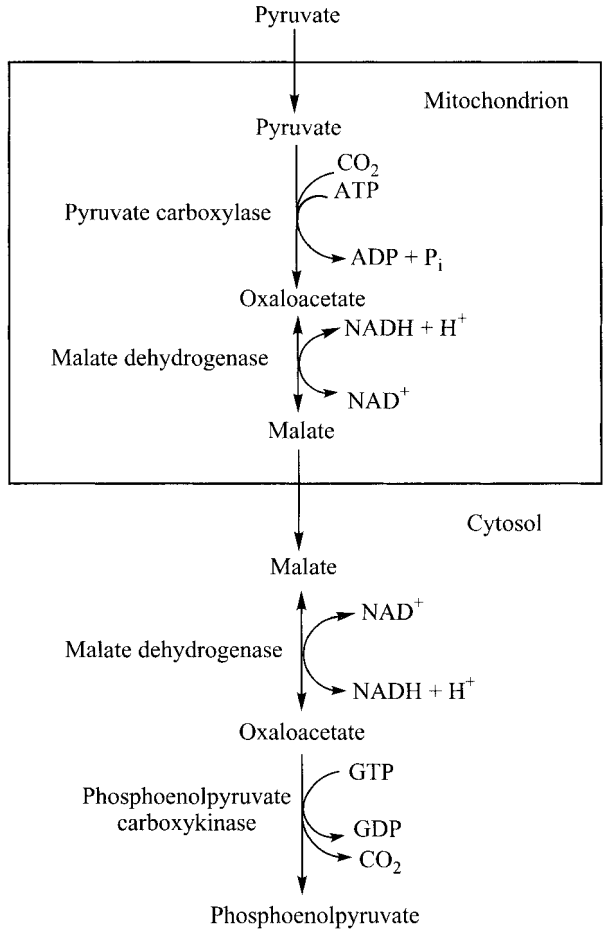

FIGURE 9-6 Intracellular localization of the initial steps of gluconeogenesis.

whereas gluconeogenesis from alanine does not:

$$\text{alanine} + \alpha\text{-ketoglutarate} \rightarrow \text{pyruvate} + \text{glutamate}$$

9.4.3.2 Glucose 6-Phosphatase. The hydrolysis of glucose 6-phosphate to glucose is required for the release of glucose from the cell and as such is the last step in both gluconeogenesis and glycogenolysis in the liver. As discussed in Chapter 8, the hydrolysis of glucose 6-phosphate to free glucose occurs not in the cytosol but in the lumen of the endoplasmic reticulum (Fig. 8-5). Although some glucose may be transported back into the cytosol, most of it remains extracellular, where it serves to maintain blood glucose homeostasis.

9.5 REGULATION OF GLUCONEOGENESIS

Glycolysis and gluconeogenesis in hepatocytes are reciprocally regulated in that physiological conditions that activate one pathway concurrently inactivate the other. As described in Chapter 3, the major regulated steps of glycolysis are those catalyzed by phosphofructokinase 1 (PFK-1) and pyruvate kinase. The major regulated steps of gluconeogenesis are the reactions that bypass these two irreversible reactions of glycolysis: those catalyzed by fructose 1,6-bisphosphatase, pyruvate carboxylase, and phosphoenolpyruvate carboxykinase (Fig. 9-7). Regulation of gluconeogenesis occurs on several levels, as described below.

9.5.1 Allosteric Regulation

9.5.1.1 Pyruvate Carboxylase and Phosphoenolpyruvate Carboxykinase. Both of these enzymes are inhibited when the energy charge of the cell

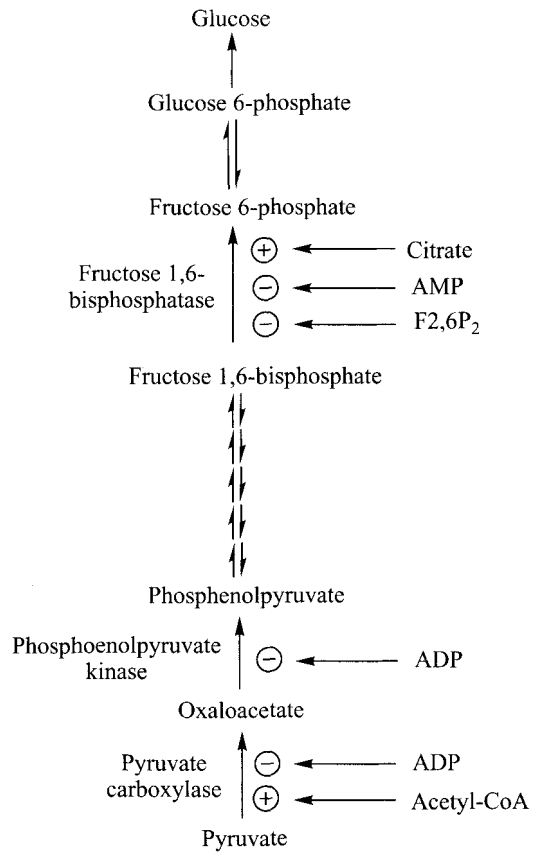

FIGURE 9-7 Regulation of gluconeogenesis. $F2,6P_2$, fructose 2,6-bisphosphate.

is low; in both cases the allosteric inhibitor is ADP rather than AMP. In addition, pyruvate carboxylase is stimulated by high concentrations of mitochondrial acetyl-CoA.

9.5.1.2 Fructose 1,6-Bisphosphatase. This enzyme is activated by citrate and inhibited by AMP. Both of these regulatory mechanisms serve to ensure that gluconeogenesis occurs only when sufficient energy is available for the synthesis of glucose. Citrate is exported from the mitochondrion during the process of shuttling the acetyl moiety of acetyl-CoA into the cytosol for the synthesis of cholesterol and fatty acids. Thus, the cytosolic citrate concentration increases when mitochondrial acetyl-CoA is present at a concentration which exceeds that required for ATP generation via the combined actions of the TCA cycle and the electron transport chain. Conversely, the high levels of AMP and low cytosolic citrate concentrations that occur when the energy charge of the cell is low act to inhibit gluconeogenesis. Since phosphofructokinase-1 is inhibited by citrate and stimulated by AMP, the low-energy charge of the cell also increases the rate of glycolysis.

9.5.2 Hormonal Regulation of Enzymatic Activity

Synthesis of glucose by means of gluconeogenesis is essential to blood glucose homeostasis in the fasted state. Glucagon and epinephrine stimulate gluconeogenesis in part by increasing substrate availability (e.g., glycerol and amino acids) and also through their effects on the activity of fructose 1,6-bisphosphatase. These two hormones also act to inhibit glycolysis in liver by inhibiting phosphofructokinase-1 (PFK-1). By contrast, insulin acts to inhibit fructose 1,6-bisphosphatase and stimulate PFK-1.

The mechanism of hormonal effects on both gluconeogenesis and glycolysis involves regulation of the concentration of fructose 2,6-bisphosphate, which is an allosteric inhibitor of the gluconeogenic enzyme fructose 1,6-bisphosphatase as well as an activator of the glycolytic enzyme phosphofructokinase-1. As described in Chapter 3 and illustrated in Figure 3-9, synthesis and degradation of fructose 2,6-bisphosphate are catalyzed by two enzyme activities, phosphofructokinase-2 and fructose 2,6-bisphosphatase, respectively, which are both contained on one bifunctional protein. Phosphorylation of the liver isozyme by protein kinase A inhibits phosphofructokinase-2 and activates fructose 2,6-bisphosphatase, thereby decreasing the intracellular concentration of fructose 2,6-bisphosphate and stimulating gluconeogenesis. By contrast, insulin initiates a signal transduction cascade, which results in the dephosphorylation of the bifunctional protein, increasing the intracellular concentration of fructose 2,6-bisphosphate and thus inhibiting gluconeogenesis while increasing the rate of glycolysis.

9.5.3 Transcriptional Regulation of Gene Expression

Expression of three of the four gluconeogenic enzymes—phosphoenolpyruvate carboxykinase, fructose 1,6-bisphosphatase, and glucose 6-phosphatase—is increased

in response to a high glucagon/insulin ratio in the fasted state, with phosphoenolpyruvate carboxykinase being the major regulated enzyme. Although the transcriptional response is slower than the hormonal regulation involving enzyme phosphorylation, increases in enzyme activities occur within 40 minutes of the return to fasting blood glucose levels. Expression of gluconeogenic enzymes is also stimulated by other hormones, including hydrocortisone. Indeed, enhancement of glucose synthesis is one of the major physiological roles of the glucocorticoids.

Unlike the other enzymes required for gluconeogenesis but not glycolysis, pyruvate carboxylase is expressed constitutively. This is consistent with the dual role of pyruvate carboxylase: It is a gluconeogenic enzyme in the fasted state and an important anaplerotic enzyme in the fed state.

9.6 ABNORMAL FUNCTION OF THE GLUCONEOGENIC PATHWAY

9.6.1 Genetic Disease

Type I glycogen storage disease or *von Gierke disease* is actually a condition of impaired gluconeogenesis as well as glycogen metabolism. The reason for this is that the defective enzyme in von Gierke disease, glucose 6-phosphatase, is needed for the export of glucose derived from gluconeogenesis as well as that derived from hepatic mobilization of glycogen stores. Insufficient glucose 6-phosphatase activity results in accumulation of excess glucose 6-phosphate and consequent excessive glycogen storage in both liver and kidneys. Von Gierke disease can also be a result of defects in the transporter systems that transport glucose 6-phosphate from the cytosol to the lumen of the endoplasmic reticulum and return P_i and glucose to the cytosol (Fig. 8-5). Since neither gluconeogenesis nor glycogenolysis can provide glucose to the blood in the absence of glucose 6-phosphatase, the fasting hypoglycemia of von Gierke disease is more severe than that due to glycogen phosphorylase deficiency.

9.6.2 Poorly Controlled Diabetes Mellitus Type 1

Diabetes mellitus derives its name from the fact that the urine of patients with this disease has a characteristic sweetness caused by urinary excretion of glucose. The underlying pathology of type I diabetes mellitus is inadequate insulin production secondary to autoimmune damage to the β-cells of the pancreas. The result is a hormonal milieu in which the body perceives that it is starving even in the postprandial state. The liver responds to the elevated glucagon/insulin ratio by increasing gluconeogenesis, thus resulting in hyperglycemia and glycosuria.

9.6.3 Excess Ethanol Consumption

As discussed to a greater extent in Chapter 13, the major pathway that metabolizes ethanol involves two successive oxidation steps, both of which require NAD^+ and generate NADH. The resulting elevated $NADH/NAD^+$ ratio depletes gluconeogenic

substrates by driving all of the following reactions to the right:

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \rightarrow \text{lactate} + \text{NAD}^+$$

$$\text{dihydroxyacetone phosphate} + \text{NADH} + \text{H}^+ \rightarrow \text{glycerol 3-phosphate} + \text{NAD}^+$$

$$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{malate} + \text{NAD}^+$$

Acute ethanol intoxication is thus often associated with severe, even life-threatening hypoglycemia, especially if the person consuming the ethanol is malnourished and has limited glycogen stores. A similar problem can occur when ethanol consumption follows strenuous, glycogen-depleting exercise. The remedy in either case is to provide the person with oral or, if necessary, intravenous glucose.

CHAPTER 10

FATTY ACID OXIDATION AND KETONES

10.1 FUNCTIONS OF FATTY ACID OXIDATION

10.1.1 Fatty Acid Oxidation Provides Energy for Cellular and Metabolic Work

Triacylglycerols (TAG) are the major energy store of the body and the major endogenous fuel in the fasted state. Triacylglycerols are not only a more concentrated energy source than glucose (or glycogen), generating 9 kcal/g compared to 4 kcal/g from glucose, but they can also be stored in a more compact, nonhydrated form. Current American diets typically contain 35 to 50% of calories as fat. In addition, after a meal, dietary carbohydrates in excess of immediate caloric needs are converted to fat and stored for future use.

Most of the fatty acids oxidized by the β-oxidation pathway are linear, unbranched molecules comprised of 16 or 18 carbon atoms. These long-chain fatty acids (LCFA) include the saturated fatty acids palmitic acid (16:0) and stearic acid (18:0), where the notation in parentheses indicates the number of carbons and, after the colon, the number of double bonds (Fig 10-1). The most common monounsaturated fatty acid is oleic acid (9c–18:1) in which the *cis* carbon–carbon double bond starts on carbon atom 9 from the carboxyl end of the molecule. The most common polyunsaturated fatty acid is linoleic acid, which is essential in the diet. Linoleic acid is an 18-carbon, diunsaturated fatty acid with carbon–carbon double bonds starting on carbon atoms 9 and 12 from the carboxyl end of the molecule (9c,12c–18:2). Linoleic acid may also be written as 18:2ω6 (18:2*n*-6) to indicate that that the first carbon–carbon double

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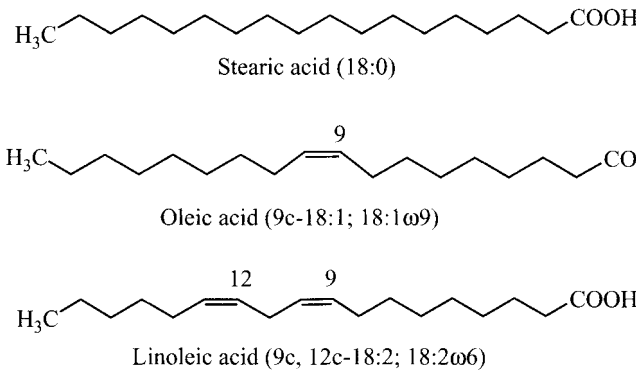


FIGURE 10-1 Structures of some common fatty acids.

bond starts on the sixth carbon from the methyl or omega (ω) end of the molecule. Using the latter nomenclature, oleic acid would be written 18:1ω9.

β-Oxidation is the pathway by which the long-chain fatty acids from both dietary fat and adipose tissue TAG are oxidized to acetyl-CoA. The reducing equivalents released during fatty acid oxidation are captured in the form of $FADH_2$ and NADH, which are used to support oxidative phosphorylation. In most circumstances, the acetyl-CoA units generated by β-oxidation will subsequently be oxidized through the tricarboxylic acid (TCA) cycle, generating additional $FADH_2$ and NADH and ultimately, additional ATP. Particularly in the fasted state, many cells and tissues depend on β-oxidation of fatty acids to provide the ATP needed to maintain ion gradients and to support biosynthetic processes such as gluconeogenesis.

10.1.2 Fatty Acid Oxidation Provides Fuel to the Brain During Starvation

Since the brain does not utilize long-chain fatty acids as an energy source, it is normally dependent on the oxidation of glucose to meet its energy needs. However, during prolonged fasting or starvation the brain meets its energy needs by oxidizing ketones (ketone bodies) as well as glucose. The ketones of physiological significance are four-carbon anions (β-hydroxybutyrate and acetoacetate) produced from acetyl-CoA generated by the β-oxidation of long-chain fatty acids in the liver (Fig. 10-2). Oxidation of ketones by the brain reduces the brain's dependence on glucose and thus decreases the body's need to catabolize muscle proteins to provide amino acid carbon skeletons for gluconeogenesis.

10.1.3 Fatty Acid Oxidation Generates Heat

Brown fat is a specialized tissue that has a high metabolic rate although it does not produce very much ATP from the NADH and $FADH_2$ generated during the oxidation of fatty acids. As discussed in Chapter 6, the presence of thermogenin or "uncoupling protein" in the inner membrane of the mitochondria of brown fat results in the

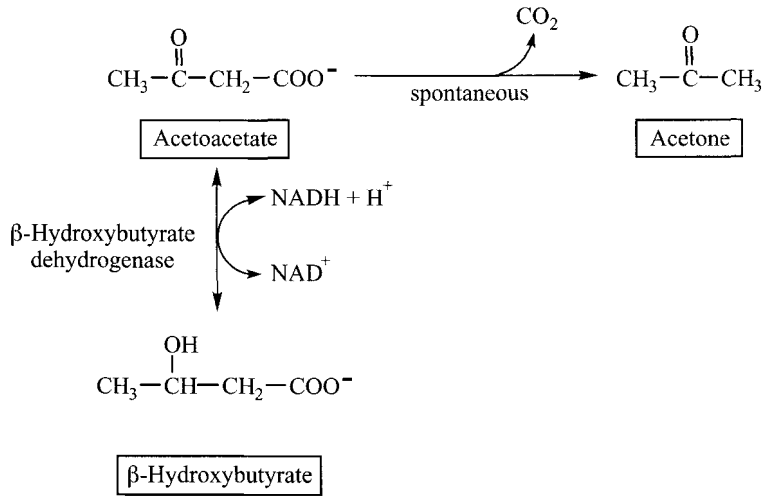


FIGURE 10-2 Ketone bodies; acetoacetate is the precursor of both β-hydroxybutyrate and acetone.

generation of heat rather than ATP from electron flow through the electron-transport chain. This process contributes to nonshivering thermogenesis, which is particularly important for maintaining body temperature in newborns.

10.1.4 Fatty Acid Oxidation Provides a Pathway for Catabolism of Diverse Dietary Fatty Acids

The major fatty acids of both dietary TAG and adipose stores in the body contain 16 to 18 carbons and are oxidized through mitochondrial β-oxidation. Essentially the same pathway is utilized for short- (C4–C6) and medium-chain (C8–C12) fatty acids. Related peroxisomal pathways, which are discussed later in the chapter, oxidize the less common branched-chain and very long-chain (≥C22) fatty acids.

10.2 TISSUES IN WHICH FATTY ACID OXIDATION IS ACTIVE

All cells and tissues except red blood cells and the brain oxidize fatty acids to generate ATP. The β-oxidation pathway is absent in red blood cells because they lack mitochondria. Although neuronal cells in the brain do contain mitochondria, there is only limited transport of fatty acids across the blood–brain barrier. This explains why fatty acids per se are not a significant fuel source for the brain.

Fatty acid oxidation is most active in tissues that are highly active metabolically. Thus, skeletal and heart muscle in particular have a large capacity for oxidizing fatty acids. Normally, 60 to 90% of the energy required for contraction of the heart is derived from the oxidation of fatty acids.

10.3 PHYSIOLOGICAL CONDITIONS IN WHICH FATTY ACID OXIDATION IS MOST ACTIVE

10.3.1 Fatty Acid Oxidation Increases in the Fasted State

In the fasted state, the plasma glucose concentration is depressed and tissues such as muscle rely primarily on oxidation of fatty acids to generate ATP. In addition, during a fast the liver has an increased requirement for ATP to provide the energy required for gluconeogenesis. The liver meets this requirement by β-oxidizing long-chain fatty acids to acetyl-CoA. The process of gluconeogenesis markedly reduces the intramitochondrial supply of oxaloacetate, thereby limiting subsequent oxidation of acetyl-CoA via the TCA cycle. Under these conditions, the two-carbon units of acetyl-CoA are utilized to synthesize four-carbon ketone bodies—acetoacetate and β-hydroxybutyrate—which leave hepatocytes and enter the blood. Oxidation of the ketone bodies to CO_2 and water occurs in tissues such as muscle and brain that do not carry out gluconeogenesis.

10.3.2 Fatty Acid Oxidation Increases During Exercise

Increased rates of fatty acid oxidation are required during exercise and provide a substantial fraction of the ATP required for muscle work. As discussed more fully in Chapter 25, the precise nature of the mix of glucose and fatty acids utilized by muscle depends on exercise intensity and duration and on a person's prior conditioning. It should be noted that, since substrate-level phosphorylation does not occur in the pathway of fatty acid oxidation, generation of ATP from β-oxidation is dependent on the availability of oxygen for oxidative phosphorylation. For this reason, strenuous exercise that depletes oxygen renders muscle more dependent on glycolysis to lactate than is the case when the work rate of muscle is lower.

10.4 PATHWAYS OF FATTY ACID OXIDATION

10.4.1 Transport of Fatty Acids

Since long-chain fatty acids are poorly soluble in aqueous media, they must be transported in the plasma complexed with albumin. When a fatty acid dissociates from albumin it is transferred from the capillary lumen through the capillary endothelium and interstitial space to the cells below. Long-chain (C16–C20) fatty acids enter cells both by simple diffusion and by carrier-mediated transport. The diffusion mechanism involves initial penetration of the outer leaflet of the plasma membrane by the hydrophobic tail of the fatty acid, followed by a "flip-flop" within the membrane. Thus, when the fatty acid emerges on the cytosolic side of the membrane, the carboxyl group of the fatty acid enters the cytosol ahead of the hydrocarbon tail.

There are three major fatty acid transporter proteins in the plasma membranes of human cells: fatty acid transporter (FAT/CD36), plasma membrane fatty acid

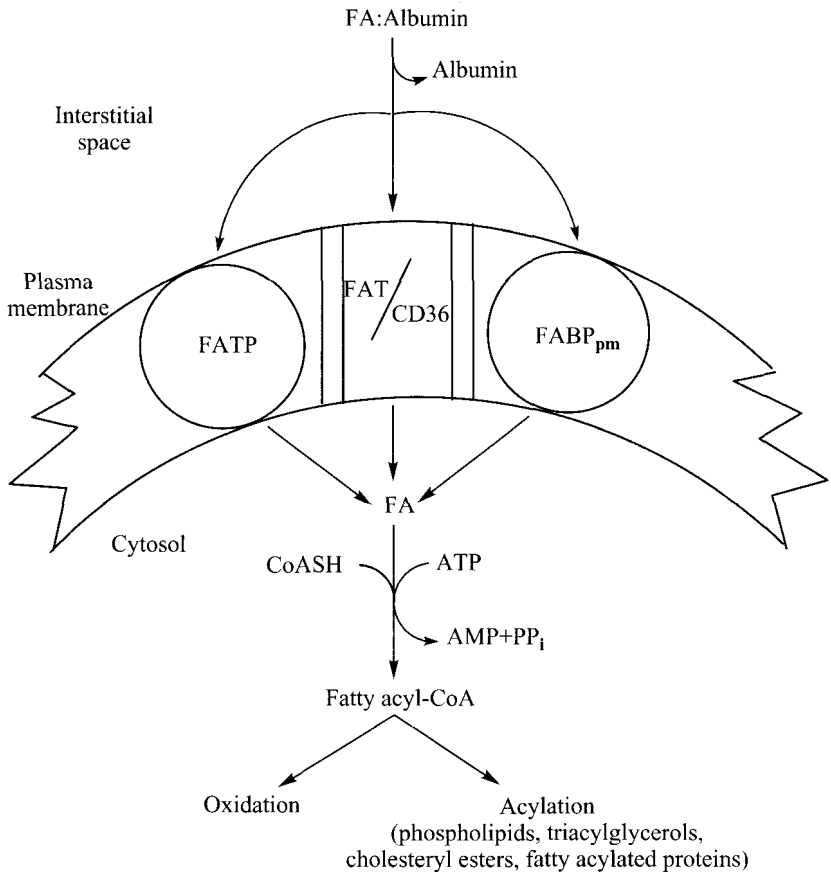


FIGURE 10-3 Fatty acid transporters in the plasma membrane. FATP, fatty acid transport protein; $FABP_{pm}$, fatty acid–binding protein of the plasma membrane.

transport protein (FABPpm), and fatty acid transport protein (FATP) (Fig. 10-3). Of these, FAT/CD36 is the major fatty acid transporter in heart muscle, skeletal muscle, adipocytes, and intestine. Intracellularly, fatty acids are bound to cytosolic fatty acid binding proteins (FABP), which deliver the fatty acids to the sites where they are metabolized.

10.4.2 Activation of Free Fatty Acids

Once inside cells, fatty acids must be activated before they can be metabolized. In contrast to glucose, which is activated and trapped within cells as glucose 6-phosphate, fatty acids are converted not to acyl phosphates but to thioesters of coenzyme A

(CoASH) by acyl-CoA synthetase:

$$\text{CoASH} + \text{ATP} + \text{R}{-}\text{COOH} \rightleftharpoons \text{R}{-}\overset{\overset{\text{O}}{\|}}{\text{C}}{-}\text{SCoA} + \text{AMP} + \text{PP}_\text{i}$$

This reversible reaction is pulled in the direction of acyl-CoA synthesis by pyrophosphatase, which hydrolyzes PP_i to $2P_i$.

Acyl-CoA synthetases are localized to three different sites in cells: the cytosolic face of the endoplasmic reticulum, the outer mitochondrial membrane, and the peroxisomal membrane. There are at least five genetically distinct acyl-CoA synthetase (ACS) isoforms, each having its own specificity with regard to the fatty acid substrate. For example, ACS4 prefers polyunsaturated fatty acids such as arachidonic acid (20:4ω6) and docosahexaenoic acid (DHA, 22:6ω3).

10.4.3 Mitochondrial β-Oxidation

10.4.3.1 The Carnitine–Fatty Acid Transport System. Long-chain fatty acids destined for β-oxidation are activated to their CoA forms primarily on the surface of the outer mitochondrial membrane. The inner mitochondrial membrane is, however, impermeable to long-chain fatty acyl-CoA molecules. Transport of fatty acids containing 16 to 20 carbon atoms across the inner mitochondrial membrane is facilitated by a fatty acid transport mechanism called the *carnitine translocase system.*

Carnitine is a quaternary amine that has a hydroxyl group to which a fatty acid can be attached (Fig. 10-4). The fatty acid is linked to carnitine by means of an oxygen ester bond that is unusual because it is energy-rich and highly reactive (unlike the more stable oxygen ester linkage between fatty acids and glycerol in TAG). Since carnitine can be synthesized in the liver and kidney from trimethyllysine, it is not usually considered an essential dietary nutrient. Synthesis of trimethyllysine occurs as a posttranslational modification of muscle proteins, with *S*-adenosylmethionine

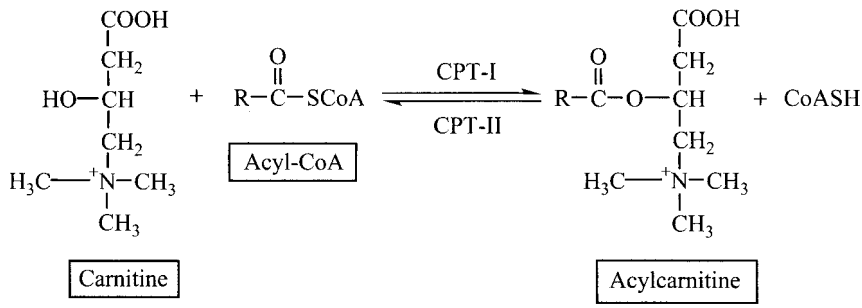


FIGURE 10-4 Transfer of acyl groups catalyzed by carnitine palmitoyltransferase I (CPT-I) and carnitine palmitoyltransferase II (CPT-II).

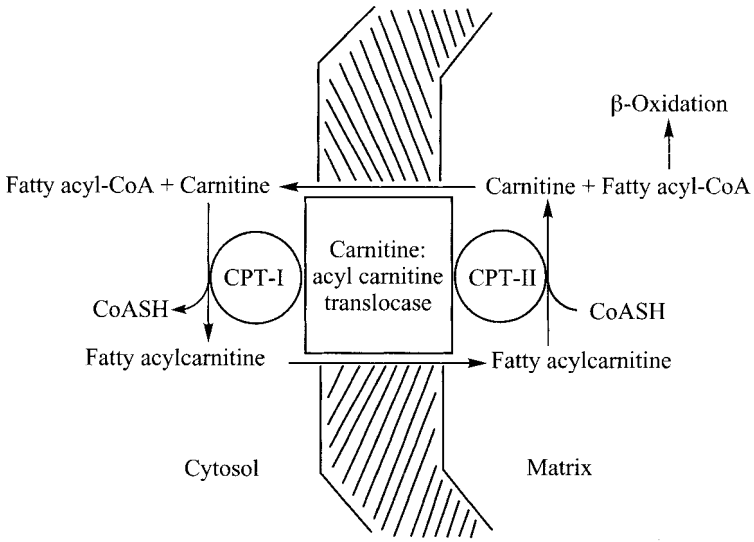


FIGURE 10-5 Transport of long-chain fatty acids into mitochondria by means of the carnitine–acylcarnitine translocase system. CPT-I, carnitine palmitoyltransferase I; CPT-II, carnitine palmitoyltransferase II.

(the activated form of methionine) serving as the methyl donor; when proteins turn over, trimethyllysine is released and made available for carnitine synthesis.

The crux of the system for transport of long-chain fatty acids into the mitochondrion is a carnitine translocase, which is embedded in the inner mitochondrial membrane (Fig. 10-5). This translocase transports fatty acylcarnitine into the mitochondria in exchange for free carnitine, which is concurrently exported from the mitochondrial matrix into the cytosol.

The activity of the carnitine translocase system is dependent on two enzymes, carnitine palmitoyltransferase I (CPT-I) and carnitine palmitoyltransferase II (CPT-II), both of which catalyze the reversible transfer of long-chain fatty acids between coenzyme A and carnitine:

$$\text{carnitine} + \text{R}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{SCoA} \rightleftharpoons \text{R}-\overset{\overset{\text{O}}{\|}}{\text{C}}-\text{O}-\text{carnitine} + \text{CoASH}$$

CPT-I is localized to the mitochondrial outer membrane and acts to generate acylcarnitine. CPT-II, which is localized to the matrix face of the inner mitochondrial membrane, forms intramitochondrial acyl-CoA from CoASH and acylcarnitine. As shown in Figure 10-5, the net effect is the transfer of a long-chain fatty acid from an extramitochondrial CoASH molecule to an intramitochondrial CoASH molecule.

10.4.3.2 Mitochondrial β-Oxidation Pathway. The term β-*oxidation* is derived from the fact that the critical chemistry of the four core reactions that comprise the pathway takes place on the third carbon from the carboxyl end: that is, the β-carbon atom.

The first of the four core reactions of the β-oxidation pathway is irreversible and is catalyzed by acyl-CoA dehydrogenase (Fig. 10-6). Two hydrogen atoms are removed—one each from the α and β carbons—generating a carbon–carbon double bond between the α and β carbons of the fatty acyl-CoA chain. These hydrogen atoms are transferred to FAD to form $FADH_2$. Mitochondria contain a family of FAD-linked acyl-CoA dehydrogenases: a long-chain acyl-CoA dehydrogenase, which is specific for fatty acids containing 14 to 20 carbon atoms; a medium-chain acyl-CoA dehydrogenase, which oxidizes intermediate chain-length fatty acids (C8–C12); and a short-chain acyl-CoA dehydrogenase, which oxidizes C4 and C6 fatty acids.

The second step in β-oxidation involves hydration of the carbon–carbon double bond between the α- and β-carbons by enoyl-CoA hydratase (Fig. 10-6). The hydroxyl group is introduced onto the β-carbon. A second dehydrogenase, NAD^+-linked β-hydroxyacyl-CoA dehydrogenase, then oxidizes the hydroxyacyl-CoA molecule to generate a β-ketoacyl-CoA and a molecule of NADH + H^+. The fourth and final step in β-oxidation involves cleavage of the fatty acid chain with attachment of a second molecule of CoASH to the β-carbon and generation of one molecule of acetyl-CoA. The enzyme that catalyzes this reaction is called β-ketoacyl-CoA thiolase, reflecting the fact that the cleavage of the carbon–carbon bond involves a sulfhydryl group.

The net effect of the four steps in β-oxidation is the production of one molecule of acetyl-CoA and one fatty acyl-CoA molecule whose carbon chain is two carbons shorter than the original substrate. The four steps are then repeated, with successive chain shortening by two carbon atoms. The final thiolytic cleavage reaction converts the 4-carbon β-ketobutyryl-CoA (acetoacetyl-CoA) into two molecules of acetyl-CoA.

10.4.3.3 Energy Yield from β-Oxidation. Calculation of the amount of ATP that can be derived from the oxidation of one long-chain fatty acid such as palmitate (16:0) is a useful exercise for demonstrating the energy-dense nature of fatty acids. The two dehydrogenase reactions that are part of each round of mitochondrial β-oxidation produce one molecule of $FADH_2$ and one molecule of NADH. As discussed in Chapter 6, passage of the reducing equivalents from these reduced cofactors into the electron transport chain yields 2.5 ATP/NADH and 1.5 ATP/$FADH_2$. The seven cycles of β-oxidation that are required to oxidize a 16-carbon palmitic acid molecule to 8 molecules of acetyl-CoA thus generate 7 × 4, or 28, ATP. Subsequent oxidation of these acetyl-CoA molecules via the TCA cycle generates 10 ATP/acetyl-CoA or 80 ATP/8 acetyl CoA, for a total yield of 108 ATP per palmitoyl-CoA. Subtracting the two high-energy bonds expended in the fatty acid synthetase and pyrophosphatase reactions that converts palmitic acid into palmitoyl-CoA, each molecule of palmitic acid can thus generate 106 molecules of ATP.

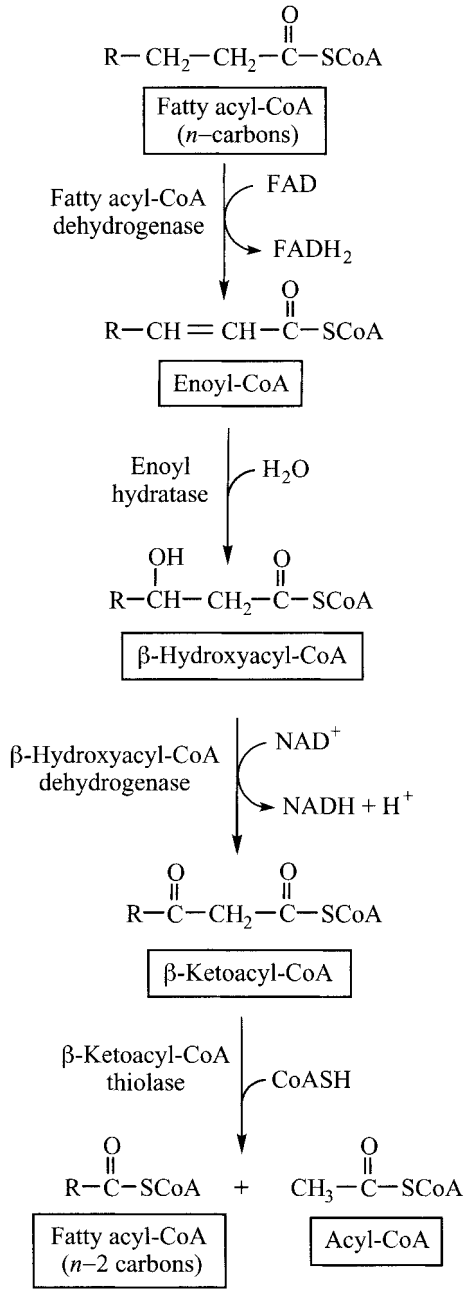


FIGURE 10-6 Mitochondrial β-oxidation pathway.

10.4.3.4 Ancillary Reactions to the Pathway of β-Oxidation. β-Oxidation of saturated fatty acids generates an unsaturated intermediate with a $\Delta^{2,3}$-*trans* double bond, which is then hydrated by enoyl-CoA hydratase. The metabolism of unsaturated and polyunsaturated fatty acids requires additional enzymes to act on preexisting *cis* double bonds. As illustrated in Figure 10-7, the oxidation of linoleic acid (c9,c12–18:2) proceeds in essentially the same manner as the β-oxidation of saturated fatty acids, with successive cleavage of two-carbon units as acetyl-CoA. However, after three cycles of β-oxidation, the chain-shortening process produces an acyl-CoA molecule that has a *cis*-3,4 double bond. At this point, an ancillary enzyme, Δ^3,Δ^2-enoyl-CoA isomerase, converts the $\Delta^{3,4}$-*cis* double bond to a $\Delta^{2,3}$-*trans* double bond, thus providing a suitable substrate for enoyl-CoA hydratase.

A slightly different situation arises when the chain-shortening process produces an acyl-CoA molecule that has a *cis*-4,5 double bond. Under these conditions, the acyl-CoA dehydrogenase step generates a $\Delta^{2,3}$-*trans*/$\Delta^{4,5}$-*cis* conjugated diunsaturated fatty acyl-CoA intermediate (Fig. 10-7). At this point a second ancillary enzyme, NADPH-dependent 2,4-dienoyl-CoA reductase, transfers hydrogen atoms from NADPH to carbons 4 and 5, generating a $\Delta^{3,4}$-*trans*-enoyl-CoA. Δ^3,Δ^2-Enoyl isomerase then converts $\Delta^{3,4}$-*trans*-enoyl-CoA to $\Delta^{2,3}$-*trans*-enoyl-CoA.

Note that by virtue of the carbon–carbon double bonds they contain, unsaturated fatty acids generate slightly less ATP than do saturated fatty acids. For example, β-oxidation of oleic acid bypasses one acyl-CoA dehydrogenase step and thereby generates one less $FADH_2$ molecule (and 1.5 fewer ATP) than its saturated counterpart, stearic acid.

10.4.3.5 Oxidation of Medium-Chain Fatty Acids. Breast milk contains relatively large amounts of medium-chain (C8–C12) fatty acids that provide nursing infants with substantial amounts of energy. These shorter fatty acids are more soluble than are their more common C16–C20 counterparts and can enter the mitochondrion directly from the cytosol without need for the carnitine transporter system. The C8–C12 fatty acids are activated to their corresponding acyl-CoA derivatives within the mitochondrion and then undergo β-oxidation. The initial oxidation reaction is catalyzed by medium-chain acyl-CoA dehydrogenase (MCAD). Because medium-chain fatty acids bypass both the carnitine transporter system and the β-oxidation enzymes specific to long-chain acyl-CoAs, TAG comprised of C8–C12 fatty acids are sometimes used as nutrient supplements to treat persons who cannot oxidize long-chain fatty acids due to genetic deficiencies of proteins involved in either the transport or oxidation of long-chain fatty acids.

10.4.3.6 Oxidation of Fatty Acids Containing an Odd Number Of Carbon Atoms. Dietary lipids often contain a small amount of odd-carbon fatty acids such as 17:0. Odd-chain fatty acids are oxidized by the normal mitochondrial β-oxidation process. However, the last thiolytic cleavage step produces one molecule of acetyl-CoA and one molecule of propionyl-CoA from the methyl end of the fatty acid. As indicated in Chapter 9, propionyl-CoA is gluconeogenic in a fasting person because it can be metabolized to glucose in the liver. The incorporation of propionyl-CoA

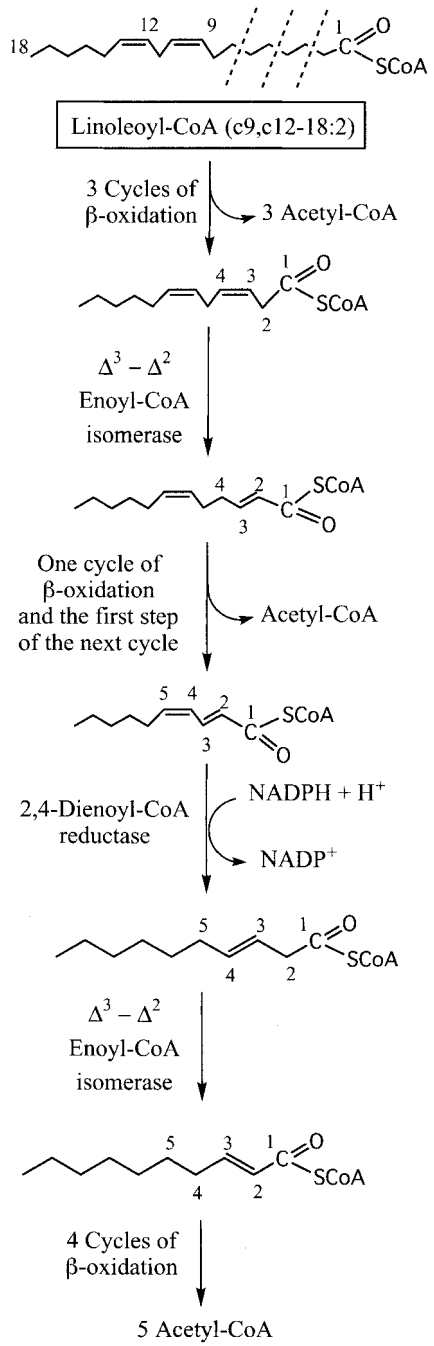


FIGURE 10-7 Role of ancillary enzymes in the oxidation of linoleic acid.

into mainstream metabolism involves carboxylation by propionyl-CoA carboxylase, followed by a vitamin B_{12}-requiring reaction that generates the TCA-cycle intermediate succinyl-CoA. It should be noted that only three of the carbons of an odd-chain fatty acid such as 17:0 are gluconeogenic; the other carbons, like those of even-chain fatty acids, generate only acetyl-CoA and cannot be utilized to synthesize glucose.

10.4.4 Ketone Bodies

The association of ketones (a.k.a., *ketone bodies*) with the ketoacidosis of diabetes has given these substances an undeservedly sinister connotation. However, ketones are normal metabolites that serve as circulating fuels, especially during periods of moderate (12 to 24 hours) or severe (>5 days) fasting. The two physiologically significant ketones are acetoacetate (β-ketobutyrate) and β-hydroxybutyrate (Fig. 10-2). Acetone is the product of the nonenzymatic decarboxylation of acetoacetate. Unlike hydrophobic long-chain fatty acids that require albumin for their transport in the plasma, ketone bodies are water-soluble and do not require a carrier protein for transport.

10.4.4.1 Ketone Synthesis. Ketones are synthesized mainly in the liver, with a smaller contribution from the renal cortex. In both tissues, the substrate for ketogenesis is mitochondrial acetyl-CoA, which is derived from the β-oxidation of long-chain fatty acids and, to a lesser extent, from the oxidation of ketogenic amino acids (e.g., leucine). In the fasted state, much of the acetyl-CoA generated by β-oxidation cannot enter the TCA cycle because of a relative shortage of oxaloacetate which has been diverted to gluconeogenesis. The pathway of ketone body synthesis (Fig. 10-8) converts two acetyl-CoA molecules into one four-carbon acetoacetate molecule while releasing two free CoASH molecules, which are required for continued β-oxidation. Continued β-oxidation, in turn, provides $FADH_2$ and NADH substrate for oxidative phosphorylation.

The first step in acetoacetate synthesis is catalyzed by β-ketothiolase, which also catalyzes the last step in β-oxidation:

$$2\,\text{acetyl-CoA} \rightleftharpoons \text{acetoacetyl-CoA} + \text{CoASH}$$

This reversible reaction is driven to the right by a high concentration of acetyl-CoA arising from β-oxidation.

The acetoacetyl-CoA from the β-ketothiolase reaction is then combined with a third molecule of acetyl-CoA to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA) in the reaction is catalyzed by HMG-CoA synthase:

$$\text{acetoacetyl-CoA} + \text{acetyl-CoA} \rightarrow \text{β-hydroxy-β-methyl-glutaryl-CoA} + \text{CoASH}$$

Most cells contain a second HMG-CoA synthase that is localized to the cytosol, where it is involved in the pathway of cholesterol synthesis rather than ketogenesis.

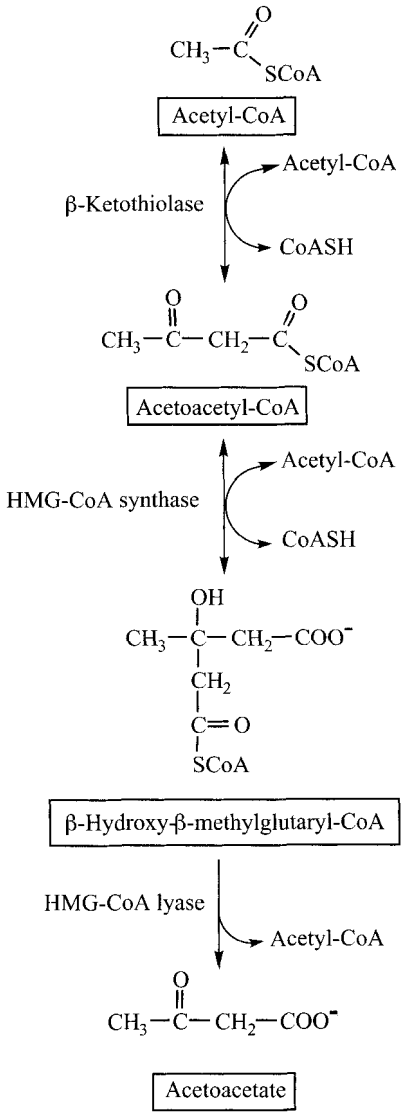


FIGURE 10-8 Ketogenesis pathway.

Mitochondrial HMG-CoA is then hydrolyzed by HMG-CoA lyase to produce acetoacetate plus acetyl-CoA:

$$\beta\text{-hydroxy-}\beta\text{-methylglutaryl-CoA} + H_2O \rightarrow \text{acetoacetate} + \text{acetyl-CoA}$$

While about one-third of the acetoacetate produced by HMG-CoA lyase is secreted by the liver into the circulation, the other two-thirds is first reduced by mitochondrial

β-hydroxybutyrate dehydrogenase and then secreted:

$$\text{acetoacetate} + \text{NADH} + \text{H}^+ \rightleftharpoons \beta\text{-hydroxybutyrate} + \text{NAD}^+$$

This reaction is driven in the direction of β-hydroxybutyrate synthesis by the relatively high mitochondrial ratio of NADH/NAD^+ generated by active β-oxidation of fatty acids. β-Hydroxybutyrate is more reduced and more energy-rich than acetoacetate.

10.4.4.2 Utilization of Ketones. Although the liver and red blood cells do not oxidize ketones, heart and skeletal muscle are capable of efficiently oxidizing ketones. Furthermore, after several days of fasting, the brain is also capable of utilizing ketones as an energy source. Ketone utilization is initiated by mitochondrial β-hydroxybutyrate dehydrogenase, which converts β-hydroxybutyrate back into acetoacetate:

$$\beta\text{-hydroxybutyrate} + \text{NAD}^+ \rightleftharpoons \text{acetoacetate} + \text{NADH} + \text{H}^+$$

Acetoacetate is then activated (and trapped within the cell) by one of two mitochondrial enzymatic reactions: The first trapping reaction is reversible and catalyzed by succinyl-CoA:β-ketoacid CoA-transferase:

$$\text{acetoacetate} + \text{succinyl-CoA} \rightleftharpoons \text{acetoacetyl-CoA} + \text{succinate}$$

The other trapping reaction is catalyzed by acetoacetyl-CoA synthetase:

$$\text{acetoacetate} + \text{ATP} + \text{CoASH} \rightarrow \text{acetoacetyl-CoA} + \text{AMP} + \text{PP}_\text{i}$$

β-Ketoacid CoA-transferase and acetoacetyl-CoA synthetase are both absent from hepatocytes, which accounts for the inability of liver to oxidize ketones.

As discussed above, acetoacetyl-CoA is the penultimate intermediate in the pathway of β-oxidation, and is cleaved by β-ketothiolase into two molecules of acetyl-CoA:

$$\text{acetoacetyl-CoA} + \text{CoASH} \rightarrow 2\ \text{acetyl-CoA}$$

Since tissues such as muscle that oxidize ketone bodies do not perform gluconeogenesis and thus do not deplete their supply of oxaloacetate in the fasted state, the acetyl-CoA molecules generated from acetoacetate and β-hydroxybutyrate are readily oxidized in the TCA cycle.

Overall, ketogenesis and ketone utilization constitute a multiorgan process that allows for the complete oxidation of long-chain fatty acids to CO_2 and water. Oxidation of acetyl-CoA derived from ketones can be a considerable source of ATP and is particularly significant in the brain during a period of prolonged fasting.

10.4.5 Peroxisomal Oxidation of Fatty Acids

10.4.5.1 Oxidation of Very Long-Chain Fatty Acids. The initial oxidation of very long-chain fatty acids (VLCFA) comprised of 22 carbon atoms or more is accomplished by a modified β-oxidation pathway that operates in peroxisomes (Fig. 10-9). One major difference between the mitochondrial and peroxisomal pathways is that, since peroxisomes lack an electron transport system, the reduced cofactors

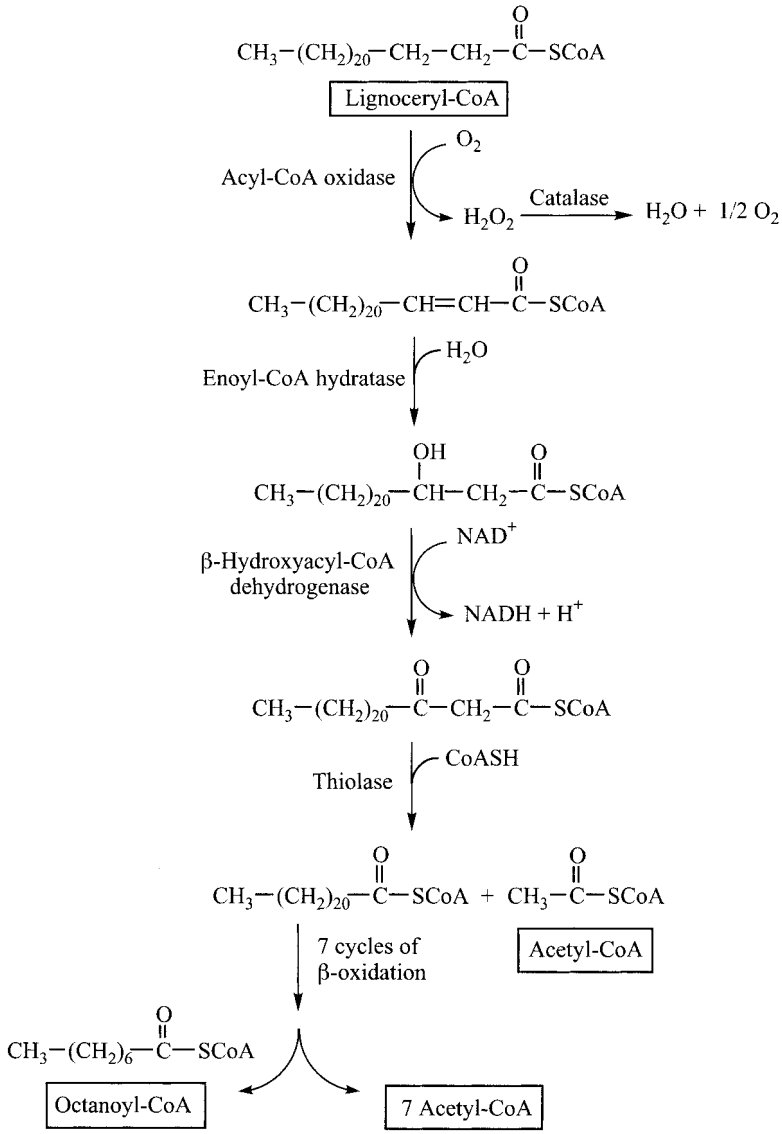


FIGURE 10-9 Pathway for oxidizing very long-chain fatty acids (≥C22) in peroxisomes.

generated during peroxisomal β-oxidation are not channeled directly into oxidative phosphorylation.

The VLCFA are first activated to acyl-CoAs by a distinct acyl-CoA synthase called *lignoceroyl* (24:0) *ligase*:

$$\text{lignoceric acid} + \text{ATP} + \text{CoASH} \rightarrow \text{lignoceroyl-CoA} + \text{AMP} + \text{PP}_i$$

The first FAD-linked dehydrogenase step in the peroxisomal β-oxidation pathway is different from the corresponding step in standard mitochondrial β-oxidation. The peroxisomal FAD-linked dehydrogenase (called *acyl-CoA oxidase*) that removes two hydrogen atoms from the fatty acid chain transfers those hydrogens to molecular oxygen, thus producing H_2O_2.

$$\text{R–CH}_2\text{–CH}_2\text{–C(=O)–SCoA} + \text{FAD} \rightarrow \text{R–CH=CH–C(=O)–SCoA} + \text{FADH}_2$$

$$\text{FADH}_2 + \text{O}_2 \rightarrow \text{FAD} + \text{H}_2\text{O}_2$$

Catalase within the peroxisome then breaks down the hydrogen peroxide:

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

The subsequent steps of the β-oxidation pathway in peroxisomes are similar to those that operate in mitochondrial β-oxidation. The reducing equivalents from the NADH generated by hydroxyacyl-CoA dehydrogenase are utilized for synthetic reactions within peroxisomes or shuttled out of the peroxisomes and eventually into mitochondria.

Once the peroxisomal β-oxidation pathway has reduced the very long-chain fatty acid chain to the level of an 8- or 10-carbon acyl-CoA molecule, the shortened fatty acid chain is transferred to mitochondria and further catabolized via the mitochondrial β-oxidation pathway. The peroxisomal acetyl-CoA units are probably hydrolyzed to acetate, which is subsequently oxidized in mitochondria.

10.4.5.2 α-Oxidation of Fatty Acids Containing a Branched Methyl Group. The chemistry of the β-oxidation pathway entails removal of both hydrogen atoms from the β-carbon atom. Therefore, fatty acids that have a methyl group on C3 (the β-carbon) cannot be oxidized by regular β-oxidation and require a specialized pathway, which is called *α-oxidation*. One such branched-chain fatty acid is phytanic acid, which is derived from the phytanol side chain of chlorophyll. Phytanic acid has methyl groups on carbon atoms 3, 7, 11, and 15 (Fig. 10-10). Humans do not derive phytanic acid directly from dietary chlorophyll, but do obtain it from dietary dairy products, beef, and fatty fish.

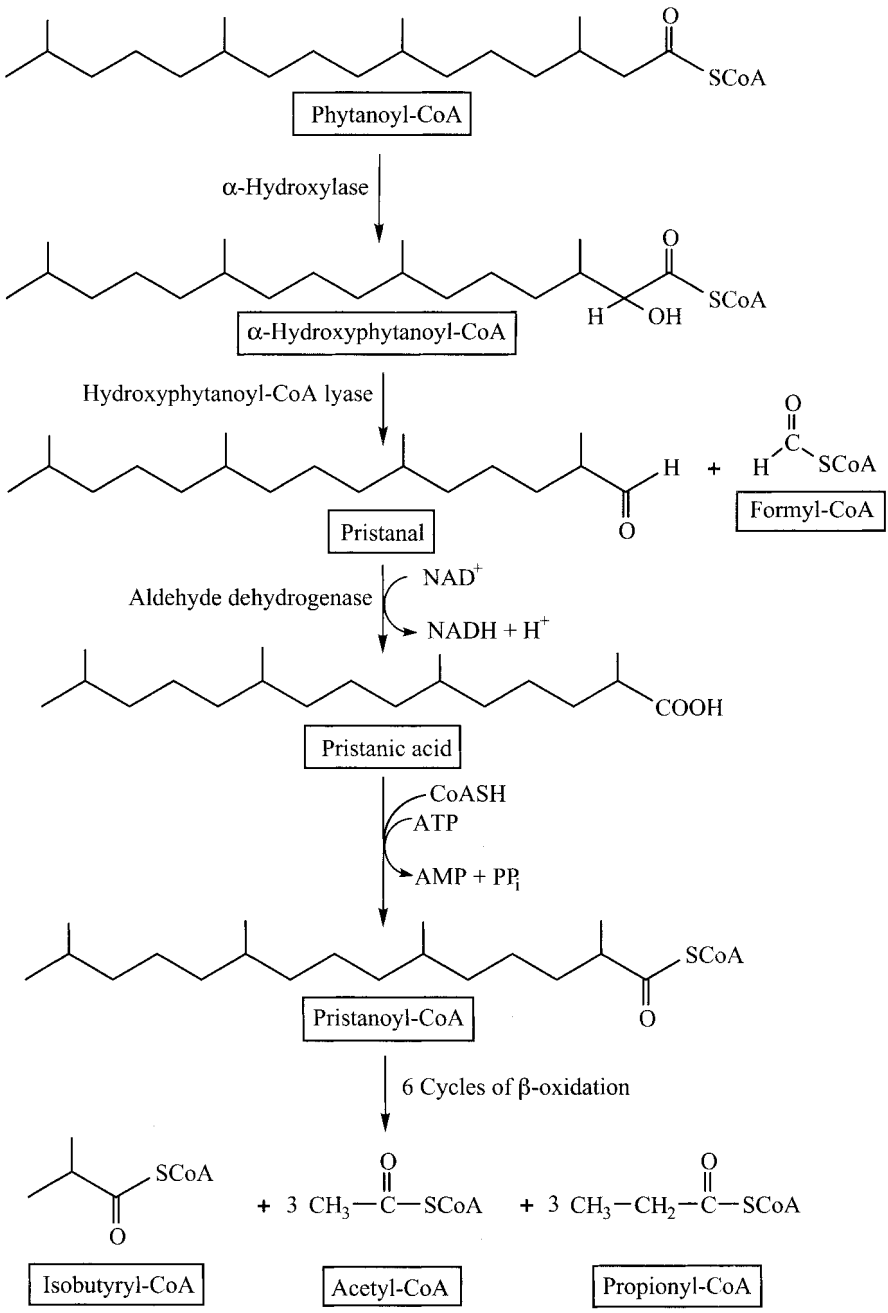


FIGURE 10-10 Peroxisomal α-oxidation of phytanic acid.

Peroxisomal α-oxidation of phytanoyl-CoA removes C1 of phytanic acid, thereby creating a shorter molecule in which the methyl group nearest the carboxyl is now on the α-carbon instead of the β-carbon; therefore, the product, pristanoic acid, is a suitable substrate for β-oxidation. Furthermore, the remaining methyl groups of the fatty acyl-CoA chain are now positioned on even-numbered carbon atoms and therefore do not present a problem for the enzymes of the standard β-oxidation pathway. Wherever a methyl group is attached to the α-carbon, cleavage of the carbon chain by β-ketothiolase will generate propionyl-CoA rather than acetyl-CoA.

10.5 REGULATION OF MITOCHONDRIAL FATTY ACID OXIDATION

10.5.1 Regulation by Energy Charge

The major site of regulation of the mitochondrial β-oxidation pathway is carnitine palmitoyltransferase-I (CPT-I), which controls the entry of long-chain (C16–C20) fatty acids into the mitochondrion. The activity of CPT-I is inhibited by malonyl-CoA, the product of the key regulatory enzyme of fatty acid synthesis: acetyl-CoA carboxylase. In the fed state, inhibition of CPT-I by malonyl-CoA prevents fatty acid oxidation when glucose is plentiful and when acetyl-CoA is being directed toward fatty acid synthesis. When a cell is actively synthesizing fatty acids de novo, the malonyl-CoA concentration in the cytosol increases. Subsequent inhibition of CPT-1 by malonyl-CoA decreases import of long-chain fatty acids into mitochondria, thereby preventing a futile cycle of simultaneous fatty acid synthesis and β-oxidation.

Conversely, when the energy charge of the cell is low, the increased concentration of AMP activates AMP-activated protein kinase (AMPK), which phosphorylates acetyl-CoA carboxylase, thereby inhibiting the enzyme so that it no longer produces malonyl-CoA. Thus, the effect of AMP activation of AMPK is to permit transport of fatty acids into the mitochondrion and ultimately increase the rate of β-oxidation.

β-Oxidation of fatty acids within the mitochondrion is also regulated by the energy charge of the cell. A high ATP/ADP ratio inhibits entry of reducing equivalents from NADH and $FADH_2$ into the electron-transport chain. The resulting increased concentrations of these reduced cofactors in turn prevent the two dehydrogenases of β-oxidation from acting when further generation of ATP is not required.

10.5.2 Regulation by Gene Transcription

Peroxisome proliferation-activator receptor-α (PPAR-α) is a ligand-activated transcription factor that stimulates fatty acid oxidation in liver and muscle. Ligands for PPAR-α include certain prostaglandins as well as some nonsteroidal anti-inflammatory drugs (e.g., indomethacin, ibuprofen). Ligand-activated PPAR-α induces the synthesis of many different genes, including members of the family of enzymes and proteins involved in β-oxidation.

10.6 DISEASES RELATED TO FATTY ACID OXIDATION

10.6.1 Medium-Chain Acyl-CoA Dehydrogenase Deficiency (MCADD)

The most common genetic defect in fatty acid oxidation is the one that affects the medium-chain acyl-CoA dehydrogenase. A deficiency in medium-chain acyl-CoA dehydrogenase activity is associated with high concentrations of both C8–C12 fatty acids and C8–C12 acylcarnitines in the plasma and urine of affected persons. Partial oxidation of these intermediate-chain-length fatty acids also generates unusual dicarboxylic fatty acids whose presence in body fluids is diagnostic of MCADD. These medium-chain dicarboxylic acids arise by a process called ω-*oxidation*, which takes place in the endoplasmic reticulum and involves oxidation of a fatty acid from its methyl end.

MCADD causes fasting hypoglycemia and muscle weakness. Limited utilization of fatty acids as fuels results in an increased dependence on glucose for muscle work. At the same time, gluconeogenesis is impaired because of the limited production of both ATP and NADH substrates needed to drive hepatic gluconeogenesis. Treatment of persons with MCADD involves avoiding periods of fasting that would tend to produce hypoglycemia. Patients with MCADD are advised to take frequent small meals that are relatively high in carbohydrates. They also benefit from consuming uncooked starch, which is digested and absorbed more slowly than cooked starch, thereby reducing the tendency toward hypoglycemia.

10.6.2 Genetic Defects in Long-Chain Fatty Acid Utilization

Genetic defects in many of the other proteins required for mitochondrial β-oxidation have also been documented. They include deficiencies in the genes encoding CPT-I, CPT-II, carnitine translocase, acyl-CoA dehydrogenase, and β-hydroxyacyl-CoA dehydrogenase. In all of these cases, the clinical manifestations include muscle weakness and fasting hypoglycemia, similar to those observed in patients with MCADD. Unlike the situation with MCADD, people with deficiencies in enzymes that metabolize long-chain fatty acids do benefit from diets that contain TAG composed primarily of medium-chain fatty acids. The utilization of these medium-chain fatty acids is not dependent on the palmitoylcarnitine translocase system or the β-oxidation enzymes that are specific for long-chain acyl-CoAs.

10.6.3 Systemic Carnitine Deficiency

Since mitochondrial oxidation of long-chain fatty acids depends on carnitine, anything that depletes the body of carnitine or which impairs intracellular carnitine availability will compromise a cell's capacity to carry out β-oxidation. Kidneys contain a carnitine transporter (the sodium-dependent organic cation transporter-2 or OCTN-2), which recovers 95% of filtered carnitine. The presence of OCTN-2 is also required for uptake of carnitine into peripheral tissues such as heart and skeletal

muscle. Thus, genetic loss of the function of this renal carnitine transporter results in both carnitine wastage and impaired β-oxidation of long-chain fatty acids.

Carnitine deficiency can also occur in newborns who have a limited capacity for carnitine synthesis. It is not uncommon for underweight or premature newborns to be born with relatively low stores of carnitine. Since human milk contains relatively low concentrations of carnitine, premature infants may benefit from carnitine supplementation.

10.6.4 Hypoglycin

There are also environmental factors that can reduce a person's ability to oxidize fatty acids. One such factor is hypoglycin, a substance that is present in the unripe fruit of the tropical akee tree. Ingested hypoglycin inhibits the mitochondrial acyl-CoA dehydrogenase responsible for oxidizing short- and medium-chain acyl-CoAs, thus causing severe, life-threatening hypoglycemia.

10.6.5 Impaired β-Oxidation of VLCFA

X-linked adrenoleukodystrophy (ALD) is a relatively common (1/20,000) genetic disease characterized by elevated levels of C26:0 and an elevated C26:0/C22:0 ratio in plasma. Pathology results from the accumulation of cholesteryl esters of VLCFA, particularly in the central nervous system, the adrenal glands, and the testes, with adverse effects on membrane structure and steroidogenesis. The genetic defect lies not in any of the enzymes of activation or β-oxidation of VLCFA, but rather in a gene (ALDP for adrenoleukodystrophy protein) that is a member of the adenosine triphosphate–binding cassette (ABC) family of transporters, and which appears to be involved in the activation of VLCFA-CoA synthetase.

Impaired β-oxidation of VLCFA is also observed in patients with peroxisomal biogenesis disorders such as Zellweger syndrome and neonatal ALD. These persons have a defect in one or more of the *PEX proteins* that are required to import enzymes into the peroxisome. Cells of people with peroxisomal biogenesis disorders are essentially devoid of peroxisomes and exhibit deficits in multiple peroxisomal metabolic pathways, including synthesis of unsaturated ether lipids, α-oxidation of phytanic acid, and processing of bile acid intermediates as well as β-oxidation of VLCFA.

10.6.6 Refsum Disease

This genetic disease is caused by a lack of the α-hydroxylase required for α-oxidation of fatty acids, such as phytanic acid, that have a methyl group on an odd-numbered carbon. Accumulation of large quantities of phytanic acid in the nervous tissue and liver results in chronic polyneuropathy and cerebellar disfunction.

10.6.7 Ketosis

The condition in which the blood and urine concentrations of ketones are markedly elevated is called *ketosis* or *ketoacidosis*. Ketosis occurs when hepatic gluconeogenesis is especially active and ketone production exceeds oxidation of ketones by muscle and other tissues. Children are more susceptible to ketosis than adults because of their higher metabolic rate, lower glycogen stores, and higher brain weight/liver weight ratio. Children may develop ketosis as a result of infections that induce anorexia and vomiting. Ketosis is also commonly seen in patients with untreated type 1 diabetes, where insulin insufficiency results in increased fat mobilization, gluconeogenesis, and ketone synthesis.

Since both acetoacetate and β-hydroxybutyrate are organic acids, ketosis is a form of metabolic acidosis. In order to be excreted in the urine, the anionic metabolic acids in the urine must be counterbalanced by equivalent numbers of cations. Therefore, ketosis may result in depletion of body stores of sodium and potassium and in some instances even in the loss of divalent cations such as calcium and magnesium.

CHAPTER 11

FATTY ACID SYNTHESIS

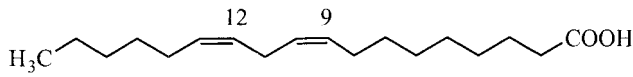
11.1 FUNCTIONS OF FATTY ACID SYNTHESIS

Fatty acid synthesis serves two main functions: One is to convert dietary carbohydrates and the carbon skeletons of excess amino acids into triacylglycerols (TAG) that can be stored until needed during periods of fasting. The other function is to produce a variety of fatty acids, which are components of the complex lipids of biological membranes and the precursors of the eicosanoid lipid hormones.

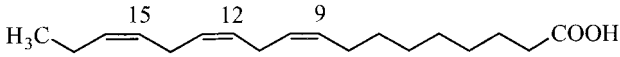
The major pathway of fatty acid synthesis converts acetyl-CoA molecules derived from dietary carbohydrates and amino acids into the long-chain fatty acid palmitic acid (16:0). Additional enzymes elongate and desaturate both endogenous palmitate and dietary fatty acids to produce a number of other fatty acids, of which the most common are stearic acid (18:0) and oleic acid (c9–18:1) (see Fig. 10.1).

Two fatty acids, linoleic acid (c9,c12–18:2ω6) and α-linolenic acid (c9,12, 15–18:3ω3), are essential fatty acids in the sense that they cannot be synthesized by humans, and as such must be obtained from the diet (Fig. 11–1). Although neither linoleic acid nor α-linolenic acid can be synthesized by humans, these dietary fatty acids can be elongated and further desaturated to produce 20- and 22-carbon polyunsaturated fatty acids such as arachidonic acid (20:4ω6) and docosahexaenoic acid (22:6ω3).

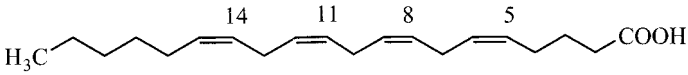
Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew



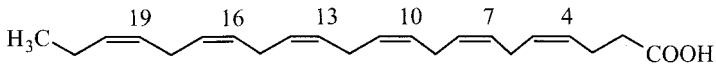
Linoleic acid (18:2ω6)



a-Linolenic acid (18:3ω3)



Arachidonic acid (20:4ω6)



Docosahexaenoic acid (22:6ω3)

FIGURE 11-1 Structures of some common polyunsaturated fatty acids.

11.2 LOCALIZATION OF FATTY ACID SYNTHESIS

Fatty acid synthesis takes place in the cytosol of most cells and tissues; however, hepatocytes and adipocytes are endowed with an especially high capacity for de novo fatty acid synthesis. In the case of fat cells, the fatty acids are esterified to glycerol and stored in the form of TAG. In the fasted state, the TAG in adipocytes are hydrolyzed sequentially by the triacylglycerol lipase desnutrin, hormone-sensitive lipase, and monoacylglycerol lipase, and the free fatty acids are released from adipocytes and transported through the blood bound to albumin.

Although the liver is the primary site of fatty acid synthesis in humans, hepatocytes do not normally accumulate TAG. Instead, the TAG are packaged into very low density lipoproteins (VLDL) and secreted into the circulation. In fact, the accumulation of extensive amounts of triacylglycerol in the liver is pathologic and can ultimately result in cirrhosis.

11.3 CONDITIONS WHEN FATTY ACID SYNTHESIS OCCURS

Fatty acid synthesis is most active following a meal. In the first few hours after foods containing carbohydrates such as starch and sucrose have been digested and absorbed, the body experiences a period of transient hyperglycemia, which triggers insulin secretion from the β-cells of the pancreas and suppresses the secretion of glucagon.

The resulting high insulin/glucagon ratio signals hepatocytes and adipocytes to take up glucose from the circulation and convert it into fatty acids, and ultimately into TAG. Fatty acid synthesis is thus greater when a person is consuming a high-carbohydrate diet than a diet that is relatively low in carbohydrates.

The rate of de novo fatty acid synthesis is very high during embryogenesis and in fetal lungs when there is a need for palmitic acid to support the synthesis of dipalmitoylphosphatidylcholine-rich pulmonary surfactant. Fatty acid synthesis is also greatly increased in cancer cells. Whereas normal cells obtain most of the fatty acids they need for membrane phospholipid synthesis from extracellular sources (e.g., plasma fatty acids and lipoprotein-associated triglycerides), cancer cells derive most of their fatty acids by means of de novo fatty acid synthesis.

11.4 REACTIONS THAT SYNTHESIZE AND MODIFY FATTY ACIDS

The pathway for de novo fatty acid synthesis generates palmitate (16:0) by the sequential addition of two-carbon units derived from acetyl-CoA to the growing fatty acid chain. These acetyl-CoA units are first activated by addition of CO_2 (HCO_3^{-1}) to form malonyl-CoA (see below). Synthesis of fatty acids also requires reducing equivalents that are provided by NADPH. The overall process of fatty acid synthesis can be summarized as follows:

$$8\,\text{acetyl-CoA} + 7\text{ATP} + 14\text{NADPH} + \text{H}^+ + \text{H}_2\text{O} \rightarrow$$
$$\text{palmitate} + 7\text{ADP} + 7\text{P}_\text{i} + 14\text{NADP}^+ + 8\text{CoASH}$$

Palmitate (as palmitoyl-CoA) can be elongated to form stearoyl-CoA, and both palmitoyl-CoA and stearoyl-CoA can be desaturated to generate palmitoleoyl-CoA (c9–16:1) and oleoyl-CoA (c9–18:1), respectively.

11.4.1 Sources of Cytosolic Acetyl-CoA

The major source of acetyl-CoA for fatty acid synthesis is glucose. Acetyl-CoA is also generated from oxidation of the carbon skeletons of excess dietary amino acids and from ethanol. In all cases, acetyl-CoA that is not needed for the immediate generation of ATP via the TCA cycle and electron-transport system is routed to the synthesis of fatty acids.

Generation of acetyl-CoA from glucose involves metabolism of glucose via glycolysis to pyruvate in the cytosol, and formation of acetyl-CoA from pyruvate by means of the pyruvate dehydrogenase reaction that occurs in mitochondria. Since the inner mitochondrial membrane is impermeable to acetyl-CoA, fatty acid–synthesizing cells transport acetyl-CoA equivalents from the mitochondrial matrix into the cytosol in the form of citrate. This process is accomplished as follows. First, acetyl-CoA condenses with oxaloacetic acid in the citrate synthase reaction that normally functions

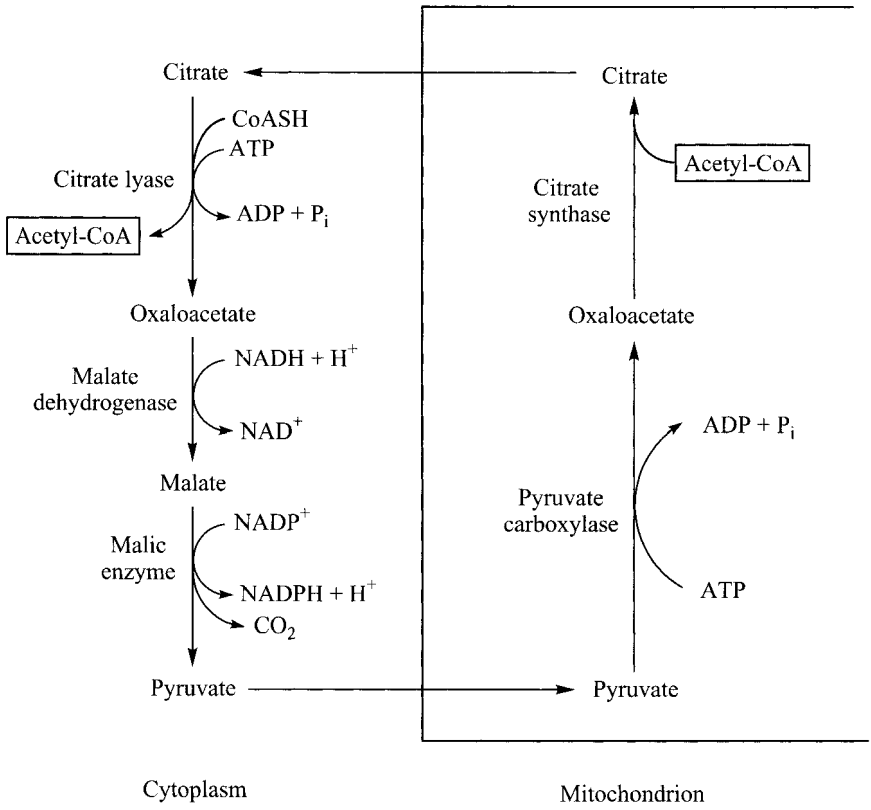


FIGURE 11-2 Pathway by which mitochondrial acetyl-CoA is transported into the cytosol and NADPH is concurrently generated from the reducing equivalents of NADH.

to introduce acetyl-CoA into the TCA cycle (Fig. 11-2):

$$\text{oxaloacetate} + \text{acetyl-CoA} \rightarrow \text{citrate} + \text{CoASH}$$

After being transported into the cytosol, citric acid is cleaved into acetyl-CoA and oxaloacetic acid by citrate lyase:

$$\text{citrate} + \text{CoASH} + \text{ATP} \rightarrow \text{acetyl-CoA} + \text{oxaloacetate} + \text{ADP} + P_i$$

This reaction serves two purposes: First, it provides acetyl-CoA substrate for fatty acid synthesis; second, it provides oxaloacetate for the NADPH-generating trans-hydrogenation pathway described below.

11.4.2 Sources of NADPH

11.4.2.1 Pentose Phosphate Pathway. Most of the NADPH that supplies the reducing equivalents for fatty acid synthesis is derived from the pentose phosphate pathway. The oxidative branch of this pathway utilizes two successive dehydrogenase reactions (catalyzed by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) to generate two molecules of NADPH for each molecule of glucose 6-phosphate oxidized to ribulose 5-phosphate.

11.4.2.2 Transhydrogenation Pathway. The net effect of the transhydrogenation pathway is the ATP-driven transfer of reducing equivalents from NADH to $NADP^+$ to produce NADPH. The pathway also serves to replenish the mitochondrial oxaloacetate pool needed to transport acetyl-CoA out of the mitochondrion in the form of citrate. The transhydrogenation pathway consists of three reactions (Fig. 11-2):

(1) $$\text{oxaloacetate} + \text{NADH} + \text{H}^+ \rightarrow \text{malate} + \text{NAD}^+$$

(2) $$\text{malate} + \text{NADP}^+ \rightarrow \text{pyruvate} + \text{CO}_2 + \text{NADPH} + \text{H}^+$$

(3) $$\underline{\text{pyruvate} + \text{ATP} + \text{CO}_2 \rightarrow \text{oxaloacetate} + \text{ADP} + \text{P}_i}$$

net reaction : $$\text{NADH} + \text{NADP}^+ + \text{ATP} \rightarrow \text{NAD}^+ + \text{NADPH} + \text{ADP} + \text{P}_i$$

Reaction (1) is catalyzed by cytosolic malate dehydrogenase. The oxaloacetate in this reaction comes from the citrate lyase reaction described above. The malic enzyme, which catalyzes reaction (2), converts malate to pyruvate by means of $NADP^+$-dependent, oxidative decarboxylation. The pyruvate produced by the malic enzyme enters the mitochondrion, where it is carboxylated in reaction (3) by pyruvate carboxylase.

The NADH substrate for reaction (1) is derived from the glyceraldehyde 3-phosphate dehydrogenase step in glycolysis. Since glycolysis and fatty acid synthesis usually operate at the same time, metabolism of glucose thus provides reducing equivalents as well as acetyl-CoA for fatty acid synthesis.

11.4.3 Generation of Malonyl-CoA

Acetyl-CoA is the immediate donor of the two carbons at the methyl end of a newly synthesized fatty acid. Malonyl-CoA serves as the high-energy, highly reactive donor of the additional acetyl units used during the process of fatty acid synthesis. Acetyl-CoA carboxylase, the cytosolic, biotin-containing enzyme that catalyzes the synthesis of malonyl-CoA, is the rate-limiting step of fatty acid synthesis (Fig. 11-3):

$$\text{acetyl-CoA} + \text{ATP} + \text{HCO}_3^- \rightarrow \text{malonyl-CoA} + \text{ADP} + \text{P}_i$$

As previously described for pyruvate carboxylase (Fig. 9-3), biotin is covalently attached to a lysine residue of acetyl-CoA carboxylase.

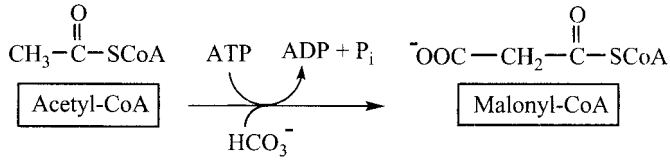


FIGURE 11-3 The acetyl-CoA carboxylase reaction.

Malonyl-CoA is an energy-rich compound that has a considerable fraction of the energy of the ATP molecule incorporated into its structure. During the process of fatty acid synthesis, release of the ionized carboxyl group of malonyl-CoA as CO_2 drives the formation of carbon–carbon bonds.

11.4.4 Fatty Acid Synthase Complex

Fatty acid synthase (FAS) is a multienzyme complex comprised of seven enzymes and one nonenzyme protein called *acyl carrier protein* (ACP). In humans, all seven enzymes and ACP occur as elements of a single large polypeptide. During fatty acid synthesis, all of the metabolic intermediates remain attached to the multienzyme fatty acid synthase complex.

The ACP domain of the FAS complex is similar to coenzyme A in that it contains a phosphopantothene group, which is composed of thioethanol amine in amide linkage to the vitamin pantothenic acid. However, unlike coenzyme A, the phosphopantothene group is esterified to the hydroxyl group of a serine residue of ACP rather than to adenosine 3′,5′-bisphosphate (Fig. 11-4). As its name indicates, the function of ACP is to carry the growing fatty acid chain during the process of fatty acid synthesis.

11.4.4.1 Initial Charging of the FAS Complex. The first two steps in fatty acid synthesis charge the FAS complex with acetyl and malonyl moieties from acetyl-CoA and malonyl-CoA, respectively. The first of the two reactions catalyzed by the malonyl/acetyl transferase component of the FAS affects the transfer of the acetyl unit of acetyl-CoA to the sulfhydryl group of ACP (Fig. 11-5):

$$\text{acetyl-CoA} + \text{ACP-SH} \rightarrow \text{acetyl-S-ACP} + \text{CoASH}$$

As soon as the acetyl unit has become attached to ACP, it is transferred to the catalytically active sulfhydryl group of the condensing enzyme (CE-SH):

$$\text{acetyl-S-ACP} + \text{CE-SH} \rightarrow \text{acetyl-S-CE} + \text{ACP-SH}$$

Shifting the acetyl group from ACP to the condensing enzyme frees up the sulfhydryl group of ACP so that it can accept a malonyl unit from malonyl-CoA. The transfer

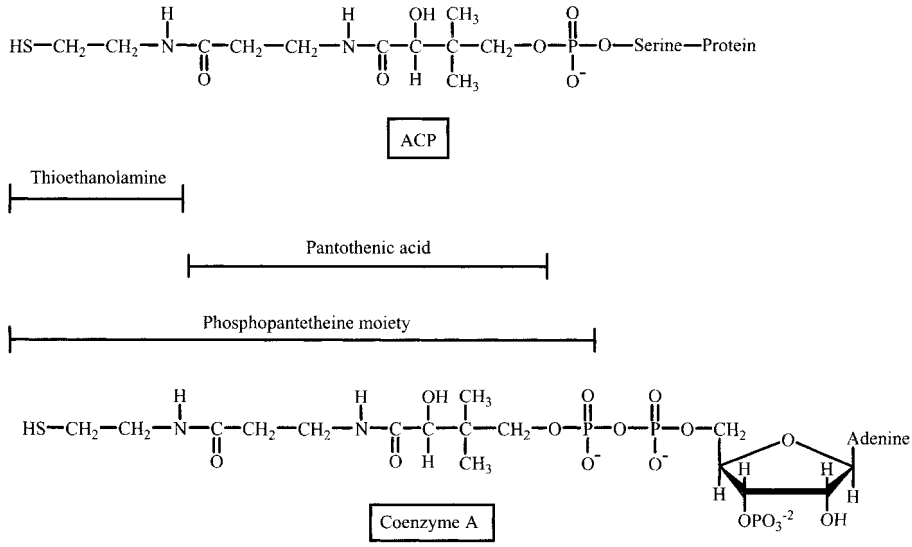


FIGURE 11-4 Comparison of the structures of acyl carrier protein (ACP) and coenzyme A (CoA).

of the malonyl moiety of malonyl-CoA onto ACP is accomplished by malonyl/acetyl transferase:

$$\text{malonyl-CoA} + \text{ACP-SH} \rightarrow \text{malonyl-S-ACP} + \text{CoASH}$$

With one acetyl and one malonyl moiety attached to the FAS complex, the stage is now set for the condensation step of the pathway.

11.4.4.2 Condensation Reaction. This bond-forming reaction is catalyzed by the condensing enzyme, β-ketoacyl-ACP synthase:

$$\text{acetyl-S-CE} + \text{malonyl-ACP} \rightarrow \beta\text{-ketobutyryl-ACP} + \text{CE-SH} + CO_2$$

Release of the high-energy carboxyl group of the malonyl moiety of malonyl-*S*-ACP as CO_2 pulls the reaction to the right.

11.4.4.3 Reduction Sequence. Next, there is a sequence of three reactions: two reduction reactions, both of which utilize NADPH as a source of reducing equivalents, and an intervening dehydration reaction (Fig. 11-6). The chemistry of the reduction reactions catalyzed by enzymes of the FAS complex resembles that of β-oxidation except that the fatty acid synthesis pathway operates in the opposite direction. Furthermore, both reduction reactions of fatty acid synthesis utilize NADPH, whereas the two oxidation reactions of β-oxidation generate $FADH_2$ and NADH.

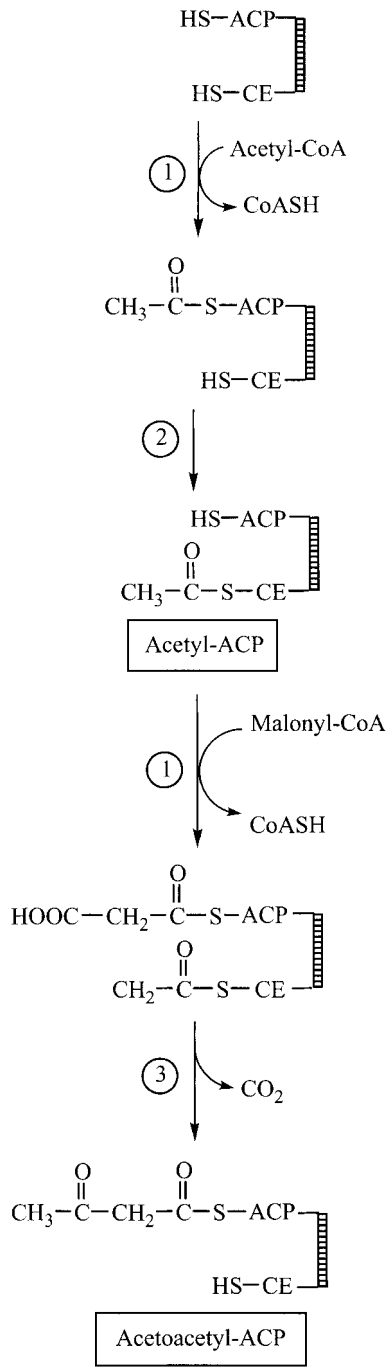


FIGURE 11-5 Reactions that load the fatty acid synthase complex and condense the two initial substrates. ①, ACP-malonyl/acetyl transferase; ②, acyl-group transfer within the fatty acid synthase complex; ③, β-ketoacyl synthase.

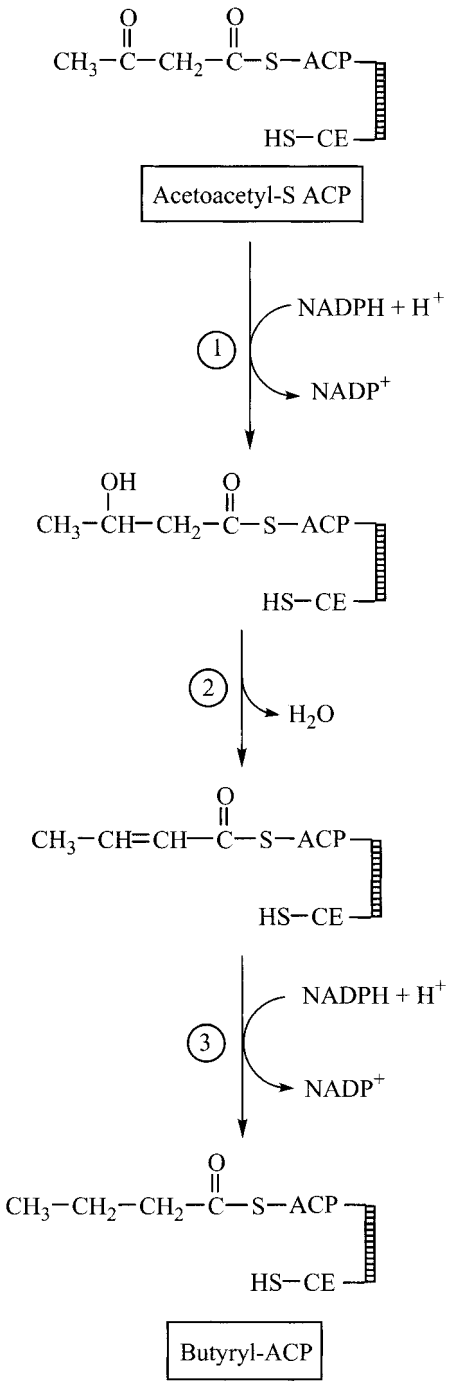


FIGURE 11-6 Reduction sequence of fatty acid synthesis: ①, β-ketoacyl-ACP reductase; ②, hydroxyacyl-ACP dehydratase; ③, enoyl-ACP reductase.

The first reduction reaction of fatty acid synthesis is catalyzed by β-ketoacyl-ACP reductase:

$$\beta\text{-ketoacyl-ACP} + \text{NADPH} + \text{H}^+ \rightarrow \beta\text{-hydroxyacyl-ACP} + \text{NADP}^+$$

The next step involves dehydration and is catalyzed by β-hydroxyacyl-ACP dehydratase:

$$\beta\text{-hydroxyacyl-ACP} \rightarrow \textit{trans}\text{-enoyl-ACP} + \text{H}_2\text{O}$$

A second NADPH-dependent reductase, enoyl-ACP reductase, reduces the carbon–carbon double bond of enoyl-ACP:

$$\text{enoyl-ACP} + \text{NADPH} + \text{H}^+ \rightarrow \text{butyryl-ACP} + \text{NADP}^+$$

11.4.4.4 Further Rounds of Fatty Acid Synthesis. Synthesis of butyryl-*S*-ACP completes one round of fatty acid synthesis. However, before another round of two-carbon addition to the growing fatty acid chain can occur, the butyryl moiety attached to ACP must first be shifted onto the sulfhydryl group of the condensing enzyme, which is the same –SH group that accepted the initial acetyl unit:

$$\text{butyryl-ACP} + \text{CE-SH} \rightarrow \text{butyryl-CE} + \text{ACP-SH}$$

This frees up the sulfhydryl group of ACP to accept a second malonyl unit and sets the stage for another round of reduction, dehydration, and reduction reactions. Additional rounds or cycles of the FAS complex take place, all with the growing acyl chain attached to ACP.

11.4.4.5 Chain Termination. Once the acyl chain has reached 16 carbons, the palmitoyl chain is released from the complex by the thioesterase component of the FAS complex:

$$\text{palmitoyl-ACP} + \text{H}_2\text{O} \rightarrow \text{palmitate} + \text{ACP-SH}$$

The one exception to the generation of palmitate as the product of fatty acid synthesis in human cells occurs during the synthesis of milk fat in the mammary gland. During lactation, some of the fatty acids that comprise the TAG of breast milk are derived from de novo fatty acid synthesis in mammary epithelial cells. De novo fatty acid synthesis in lactating mammary glands is especially active in women whose diets are based largely on cereal staples such as maize, rice, or millet. Under these circumstances, the mammary gland synthesizes mainly medium-chain-length (C8–C12) fatty acids. This occurs because the mammary epithelium expresses a specialized thioesterase called decanoyl-ACP thioesterase, which terminates fatty acid synthesis when the FAS complex has generated acyl chains comprised of 8 to 12 carbon atoms.

11.4.5 Modification Reactions

Most cells have the ability to increase the chain length and degree of unsaturation of long-chain fatty acids. Modification of both dietary-derived fatty acids and the palmitate synthesized de novo in the body accounts for the great diversity of fatty acids in membrane lipids and those involved in signaling (e.g., eicosanoids).

11.4.5.1 Fatty Acid Chain Elongation. Elongation of fatty acids occurs primarily in the endoplasmic reticulum and utilizes malonyl-CoA to add two-carbon units to long-chain fatty acyl-CoAs. There is a minor, secondary chain elongation system (elongase) in mitochondria that utilizes acetyl-CoA as the two-carbon donor and it appears to be involved primarily in the synthesis of lipoic acid, a cofactor for pyruvate dehydrogenase and α-ketoglutarate dehydrogenase.

The elongation system is comprised of a condensing enzyme that adds two carbons to a molecule of fatty acyl-CoA, and three additional enzyme activities, β-ketoacyl-CoA reductase, β-hydroxyacyl-CoA dehydratase, and enoyl-CoA reductase, whose activities are similar to the enzymes of FAS that catalyze the reduction sequence (Fig. 11-6).

The overall elongation reaction of palmitoyl-CoA that occurs in the endoplasmic reticulum is thus

$$\text{palmitoyl-CoA} + 2\text{NADPH} + \text{H}^+ + \text{malonyl-CoA} \rightarrow$$
$$\text{stearoyl-CoA} + 2\text{NADP}^+ + \text{CO}_2 + \text{CoASH}$$

There may be multiple elongation systems in the endoplasmic reticulum, with different specificities for the chain length and degree of unsaturation of the acyl chain substrate.

11.4.5.2 Δ^9-Desaturation of Endogenously Synthesized Fatty Acids. The major desaturase in human cells is stearoyl-CoA desaturase (Δ^9 desaturase), which introduces a double bond at carbon 9 from the carboxyl end of the fatty acid chain of fatty acyl-CoA. The enzyme desaturates both stearic acid (18:0) to oleic acid (*cis*9–18:1, *n*-9) and palmitic acid (16:0) to palmitoleic acid (*cis*9–16:1, *n*-7). Stearoyl-CoA desaturase is a mixed-function oxidase that utilizes molecular oxygen to oxidize both the long-chain fatty acyl-CoA and NADH:

$$\text{stearoyl-CoA} + \text{NADH} + \text{H}^+ + \text{O}_2 \rightarrow \text{oleoyl-CoA} + \text{NAD}^+ + 2\text{H}_2\text{O}$$

The desaturation complex includes the actual desaturase enzyme, cytochrome b_5 which serves as an electron acceptor, and NADH-cytochrome b_5 reductase, which contains FAD as a prosthetic group (Fig. 11-7).

11.4.5.3 Modification of Essential Fatty Acids. Human cells cannot introduce double bonds beyond carbon 9 from the carboxyl end of long-chain fatty acids. For this reason, linoleate (c9,c12–18:2; 18:2ω6) and α-linolenate (c9,c12,c15–18:3;

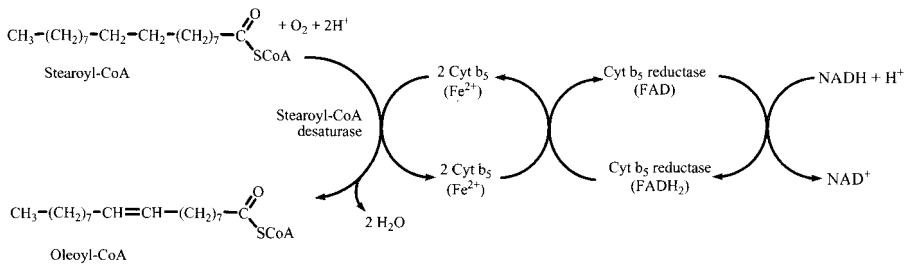


FIGURE 11-7 Desaturation of stearoyl-CoA.

18:3ω3) are essential dietary components that are obtained largely from vegetable oils. Both linoleate and α-linolenate can be modified by alternating desaturation and elongation steps to generate longer-chain polyunsaturated fatty acids. This process utilizes the fatty acid elongation system and fatty acid desaturases, which are specific for the Δ^5 and Δ^6 carbons of fatty acids. There does not appear to be a Δ^4 desaturase enzyme per se in humans; instead, Δ^4 desaturation is accomplished by a more complex, multistep process involving elongation and a subsequent β-oxidation atep.

The two parallel pathways by which the omega-6(ω6) fatty acid linoleic acid is converted into arachidonic acid (c5,c8,c11,c14–20:4) and the ω3 fatty acid α-linolenic acid is converted into docosahexaenoic acid (DHA, c4,c7,c10,c13,c16,c19–22:6) are shown in Figure 11-8. Note that although both ω6 and ω3 fatty acids such as linoleic acid and α-linolenic acid can be elongated and desaturated to generate longer-chain, more unsaturated ω6 and ω3 fatty acids, respectively, an ω3 fatty acid cannot be converted to an ω6 fatty acid, or vice versa.

11.5 REGULATION OF FATTY ACID SYNTHESIS

Fatty acid synthesis is regulated over the long term by insulin and in the short term by several different intracellular effectors: most important, citrate and long-chain fatty acyl-CoAs.

11.5.1 Regulation of the Activity of Acetyl-CoA Carboxylase

Acetyl-CoA carboxylase, the enzyme that catalyzes the rate-limiting step in fatty acid synthesis, is regulated both by allosteric modulators and by phosphorylation/dephosphorylation. The main allosteric activator of acetyl-CoA carboxylase is citrate. By contrast, palmitoyl-CoA and other long-chain fatty acyl-CoAs inhibit acetyl-CoA carboxylase; this phenomenon is an example of feedback inhibition.

Citrate activates acetyl-CoA carboxylase by inducing formation of an active, filamentous polymer from relatively inactive enzyme dimers. Activation of acetyl-CoA carboxylase by citrate reflects the energy status of the cell. In the fed state, when tissues are energy-replete, the high mitochondrial concentration of ATP inhibits

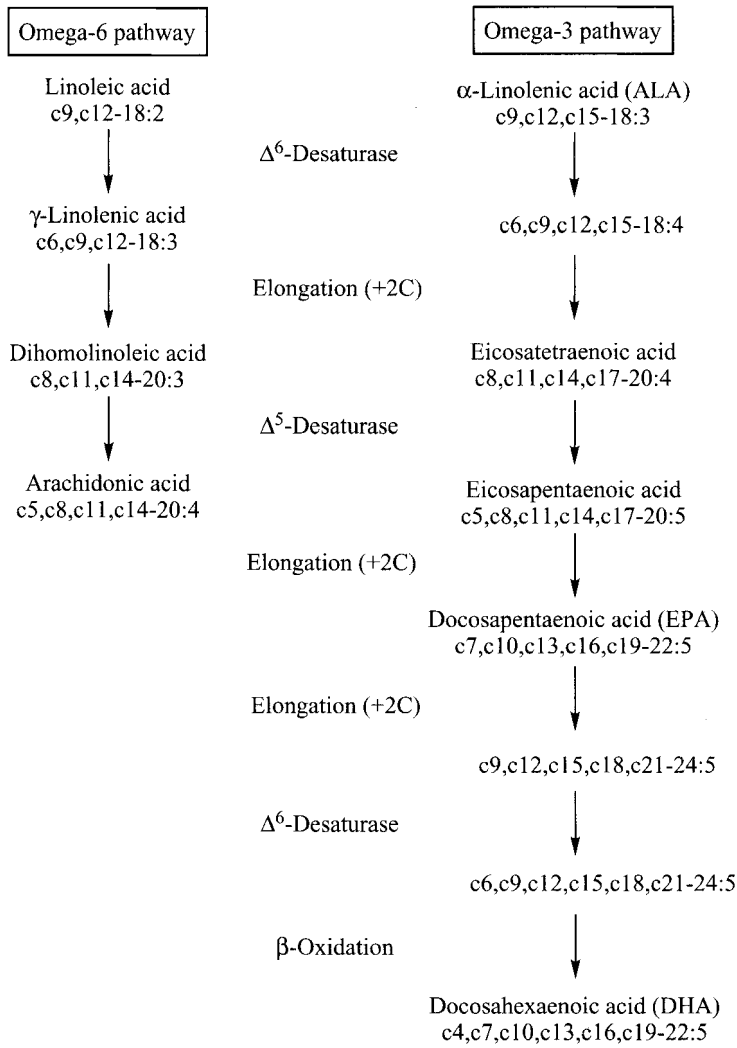


FIGURE 11-8 Elongation and desaturation of polyunsaturated fatty acids.

the TCA cycle by inhibiting isocitrate dehydrogenase. Since the citrate/isocitrate equilibrium favors citrate, it is citrate rather than isocitrate that accumulates within the mitochondrial matrix when the ATP concentration is high. As the mitochondrial concentration of citrate rises, citrate is transported into the cytosol, where it serves as a signal for the cell to synthesize fatty acids from excess acetyl-CoA.

Acetyl-CoA carboxylase activity is also regulated by protein phosphorylation/dephosphorylation. In the fasted state when the insulin/glucagon ratio is low, the intracellular concentration of cAMP increases, and cAMP-activated protein kinase A (PKA) phosphorylates and inhibits acetyl-CoA carboxylase. Acetyl-CoA

carboxylase is also inhibited by the action of a second protein kinase, AMP-activated protein kinase (AMPK), whose activity reflects depletion of intracellular ATP. AMPK is an energy sensor that responds to decreases in the cellular energy level as reflected by a high AMP/ATP ratio. AMPK phosphorylates and inactivates acetyl-CoA carboxylase, thereby decreasing the concentration of malonyl-CoA and diminishing fatty acid synthesis. As discussed in Chapter 10, a decrease in the concentration of malonyl-CoA also results in enhanced β-oxidation of long-chain fatty acids. In the fed state, insulin activates the protein phosphatase, which dephosphorylates acetyl-CoA carboxylase, thus increasing the activity of the carboxylase.

11.5.2 Regulation of Enzyme Synthesis

Insulin is a powerful anabolic signal, particularly in hepatocytes and adipocytes, where it induces synthesis of the lipogenic family of enzymes, which includes acetyl-CoA carboxylase, citrate lyase, the malic enzyme, glucose 6-phosphate dehydrogenase, pyruvate kinase, and the FAS complex. The mechanism underlying this action by insulin involves activation of the sterol regulatory element–binding protein-1 (SREBP-1), a membrane-bound transcription factor that enhances transcription of the genes encoding proteins required for fatty acid synthesis. Glucagon, on the other hand, represses de novo synthesis of these enzymes in adipocytes and liver, and stimulates degradation of the lipogenic family of enzyme proteins. AMP-activated protein kinase also suppresses expression of fatty acid synthase, acetyl-CoA carboxylase, and citrate lyase.

11.6 ABNORMAL FUNCTION OF FATTY ACID SYNTHESIS

There are no known diseases resulting from deficiencies of enzymes in the fatty acid synthesis pathway. However, an increased rate of fatty acid synthesis sufficient to damage hepatocytes may occur in the liver of a person who chronically consumes large amounts of ethanol. This pathological condition is known as *alcoholic liver disease*.

11.6.1 Essential Fatty Acid Deficiency

Synthesis of 20- and 22-carbon polyunsaturated fatty acids requires adequate dietary intake of both ω6 and ω3 fatty acids. Essential fatty acid (EFA) deficiency is primarily a deficiency of ω6 fatty acids, which are required in substantially larger quantities than are the ω3 fatty acids. EFA deficiencies are rare now that lipid emulsions are utilized clinically as a component of parenteral nutrition solutions when it is necessary to bypass the gut. The major clinical symptoms of EFA deficiency are skin rash and alopecia. Biochemical evidence of EFA deficiency appears before clinical symptoms and involves the elongation of oleate (18:1 ω9) to produce the abnormal polyunsaturated fatty acid c5,c8,c11–20:3 by the enzymes that normally elongate and desaturate linoleate and α-linolenate.

11.6.2 Deficiency of Omega-3 Fatty Acids

Synthesis of phospholipids containing the highly polyunsaturated fatty acid docosahexaenoic acid (22:6 ω3 or DHA) is critical for normal brain development and retinal function. This requirement is specific for ω3 fatty acids and cannot be met by members of the ω6 polyunsaturated fatty acid family. DHA is the most abundant polyunsaturated fatty acid in the central nervous systems and has been shown to modulate phosphatidylserine biosynthesis and neuronal signaling. Indeed, excess consumption of ω6 fatty acids such as linoleate actually exacerbates the deficiency of DHA by competing with α-linolenic acid as a substrate for the desaturation/elongation pathway. Premature infants who must sustain rapid brain growth and are born with limited fat stores are particularly at risk of DHA deficiency. Since prematurity is often associated with immature liver function and inadequate ability to elongate and desaturate polyunsaturated fatty acids, infant formulas are now supplemented with DHA and with arachidonic acid rather than with α-linolenate and linoleate.

CHAPTER 12

TRIACYLGLYCEROL TRANSPORT AND METABOLISM

12.1 FUNCTIONS OF TRIACYLGLYCEROLS

Both the triacylglycerols (TAG; also designated *triglycerides* or TG) stored in the body and most of the dietary triacylglycerols are comprised of three long-chain fatty acids (usually C16–C20) esterified to a molecule of glycerol (Fig. 12-1). The three hydroxyls are designated *sn* (stereospecific numbering)-1, *sn*-2, and *sn*-3. Fatty acids in the *sn*-1 and *sn*-3 positions tend to be long-chain saturated fatty acids (e.g., palmitic acid, stearic acid) or monounsaturated fatty acids (e.g., oleic acid), whereas those in the *sn*-2 position tend to be polyunsaturated fatty acids (e.g., linoleic acid, α-linoleic acid, arachidonic acid). The three most abundant fatty acids in the TAG of adipose tissue and plasma lipoproteins are palmitic acid, oleic acid, and linoleic acid.

12.1.1 Dietary Source of Energy

Populations in most Western countries consume 50 to 100 g of dietary fat per day, which amounts to 35 to 50% of their total daily energy intake. Dietary fat also contributes to the palatability of the diet.

12.1.2 Energy Storage

The main function of TAG in the body is to provide a compact and relatively unlimited means for storing energy. At 9 kcal/g, the energy content of TAG is more than

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

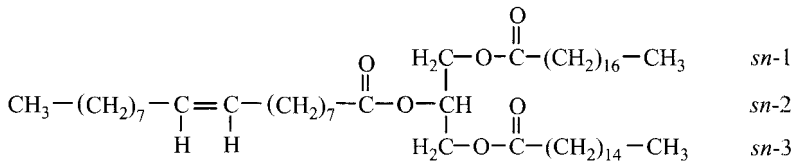


FIGURE 12-1 Structure of a typical triacylglycerol molecule illustrating the stereospecific numbering (*sn*) system of glycerol: 1, stearoyl; 2, oleoyl; 3, palmitoyl.

twice that of the other major form of energy that humans store, namely glycogen (4.1 kcal/g). The fatty acids that comprise TAG are highly reduced. Except for the carboxyl group, most of the carbon atoms of a fatty acid have two hydrogen atoms attached to them and are bonded to another carbon atom. It is the energy that is released during the oxidation of these C–H and C–C bonds that ultimately supports the synthesis of ATP by the oxidative phosphorylation apparatus of mitochondria. Furthermore, whereas glycogen binds more than twice its weight in water, TAG are hydrophobic, such that only about 15% of the mass of adipose tissue is water. This contrast in water-binding capacity between fat and glycogen means that on a weight basis, it is more economical to store energy in the form of fat than in the form of glycogen.

12.1.3 Physical Functions of Triacylglycerols

Fat acts as a cushion, protecting certain organs (e.g., the kidneys) from physical injury. Adipose layers in the skin provide thermal insulation.

12.1.4 Nonshivering Thermogenesis

Because of their larger skin surface area relative to their small body mass, newborn infants are especially prone to heat loss, which can result in hypothermia. About 5% of the body mass of newborns is attributable to brown fat, which is located in the neck, midline of the upper back, mediastinum, and other organs. Since newborns do not shiver, the main function of brown fat is to generate heat to maintain body temperature. Brown fat is a metabolically active tissue rather than primarily a fat store; its characteristic color comes from the pigmented cytochromes in the abundant mitochondria contained therein. The presence of a specialized uncoupling protein called *thermogenin* prevents the potential energy that is released during fatty acid oxidation from being captured as ATP. Instead, as described in Chapter 6, the energy provided by electron flow through the electron-transport chain in the mitochondria of brown fat is released as heat.

12.1.5 TAG Carry Fat-Soluble Vitamins

Dietary fats not only provide energy and essential fatty acids; they also serve as a carrier for the intestinal absorption of the fat-soluble vitamins A, D, E, and K and carotenoids.

12.2 SITES OF TAG STORAGE

The adipocyte depot is the major site of triacylglycerol storage. Small amounts of TAG are also found in other tissues, including muscle, pancreas, and liver. TAG are also found in the blood in the form of lipoproteins.

12.3 CONDITIONS UNDER WHICH TRIACYLGLYCEROL METABOLISM IS ACTIVE

12.3.1 TAG Metabolism in the Fed State

Dietary TAG are partially hydrolyzed during digestion, reassembled into TAG in the enterocyte, and transported from the small intestine in lymph in the form of lipoprotein complexes called *chylomicrons*. The lymph empties into the bloodstream, where it carries TAG to other organs. Excess dietary carbohydrates and proteins are catabolized and converted to fat, mainly in the liver; however, some conversion of glucose to fat also occurs in adipose tissue. Under normal circumstances, TAG synthesized in the liver are not stored there but are released into the blood as a component of VLDL (very low density lipoprotein). The TAG in circulating lipoproteins (both diet-derived and endogenously synthesized) are hydrolyzed in the capillaries of skeletal muscle, adipose, and other tissues by lipoprotein lipase, and the resulting free fatty acids are taken up by adipocytes and muscle cells.

Intravenous fat emulsions are a common component of parenteral nutrition formulas, where they provide a concentrated source of calories plus essential fatty acids. They provide a substrate for lipoprotein lipase in the capillaries, thereby making free fatty acids available for cellular metabolism.

12.3.2 TAG Metabolism in the Fasted State

Between meals or during a fast when glycogen stores have been depleted, TAG in adipocytes are hydrolyzed to free fatty acids and glycerol. These fatty acids circulate bound to albumin rather than as components of triacylglycerol-containing lipoproteins. Formation of noncovalent complexes between fatty acids and albumin solubilizes the long-chain fatty acids. Transport on albumin also minimizes the potential damage to membranes by the detergent activity of free fatty acid ions.

12.3.3 TAG Synthesis During Lactation

During lactation, mammary glands synthesize TAG for secretion into breast milk. The fatty acids can be synthesized de novo within the mammary gland or derived from fatty acids released from VLDL triacylglycerols by lipoprotein lipase.

12.4 PATHWAYS OF TRIACYLGLYCEROL METABOLISM

12.4.1 Synthesis of TAG

The pathway for triacylglycerol synthesis in most tissues, including liver and adipocytes, utilizes glycerol 3-phosphate and fatty acyl-CoA (Fig. 12-2). The activated fatty acids (i.e., fatty acids attached to coenzyme A) are derived either from endogenous, de novo fatty acid synthesis or from dietary fats. By contrast, triacylglycerol synthesis in the small intestine begins with 2-monoacylglycerol.

12.4.1.1 Generation of Glycerol 3-Phosphate. There are two sources of glycerol 3-phosphate: the glycerol kinase and glycerol 3-phosphate dehydrogenase reactions. Glycerol kinase is expressed primarily in liver and catalyzes the following reaction:

$$\text{glycerol} + \text{ATP} \rightarrow \text{glycerol 3-phosphate} + \text{ADP}$$

In the fed state, the major source of glycerol substrate for the glycerol kinase reaction is glycerol released from circulating lipoprotein TAG by lipoprotein lipase. Small amounts of glycerol can also be obtained from the digestion of dietary glycerolipids (e.g., TAG, phospholipids).

Nonhepatic tissues obtain glycerol 3-phosphate from dihydroxyacetone phosphate (DHAP), an intermediate in both glycolysis and partial gluconeogenesis (a.k.a., glyceroneogenesis). The reaction is catalyzed by glycerol 3-phosphate dehydrogenase:

$$\text{dihydroxyacetone phosphate} + \text{NADH} + \text{H}^+ \rightleftharpoons \text{glycerol 3-phosphate} + \text{NAD}^+$$

12.4.1.2 Synthesis of Phosphatidic Acid. The first step in triacylglycerol synthesis is the trapping and activation of fatty acid inside the lipid-synthesizing cell by fatty acid synthetase:

$$\text{fatty acid} + \text{CoASH} + \text{ATP} \rightarrow \text{fatty acyl-CoA} + \text{AMP} + \text{PP}_\text{i}$$

Phosphatidic acid is then generated by the transfer of two molecules of fatty acid from their coenzyme A derivatives to glycerol 3-phosphate. The two reactions are catalyzed by glycerol 3-phosphate acyltransferase and 1-acylglycerol 3-phosphate

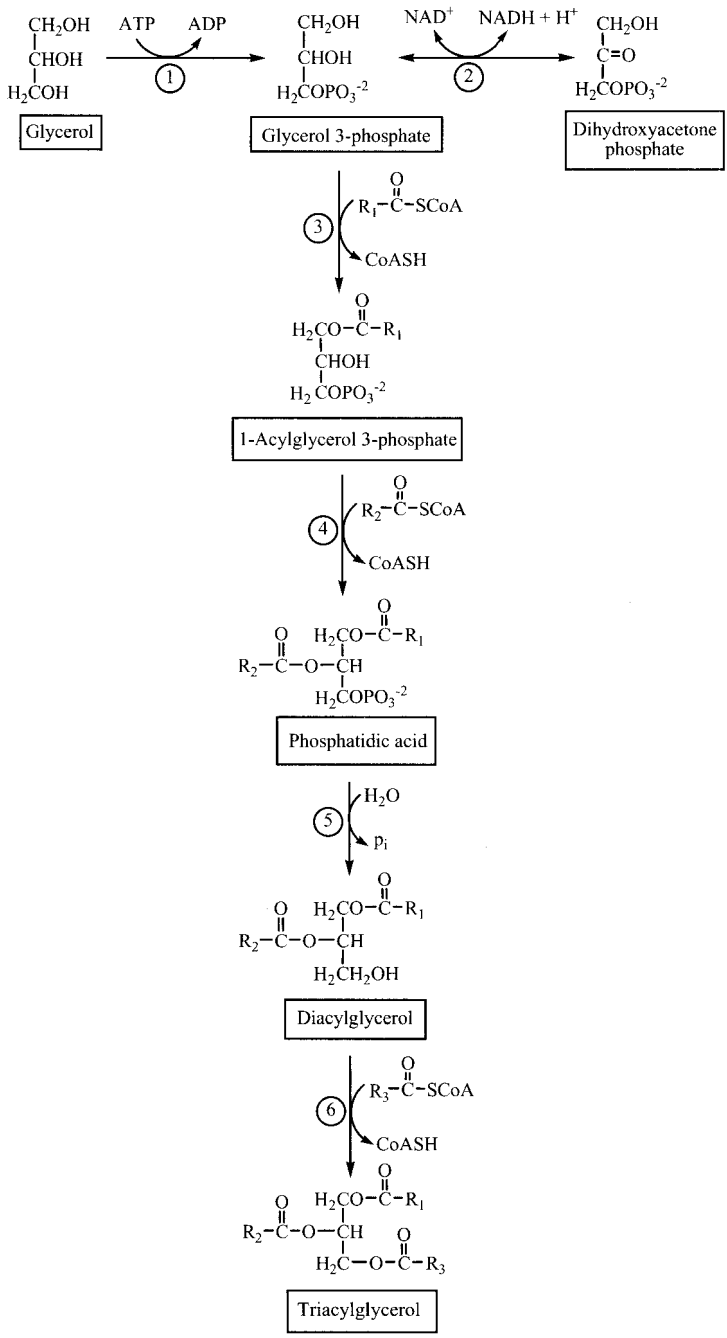


FIGURE 12-2 Biosynthesis of triacylglycerol: ①, glycerol kinase; ②, glycerol 3- phosphate dehydrogenase; ③, glycerol 3-phosphate acyltransferase; ④, 1-acylglycerol 3-phosphate acyltransferase; ⑤, phosphatidic acid phosphatase; ⑥, diacylglycerol acyltransferase.

acyltransferase, respectively (Fig. 12-2):

$$\text{glycerol 3-phosphate} + \text{fatty acyl-CoA} \rightarrow \text{1-acylglycerol 3-phosphate} + \text{CoASH}$$

$$\text{1-acylglycerol 3-phosphate} + \text{fatty acyl-CoA} \rightarrow \text{phosphatidic acid} + \text{CoASH}$$

The metabolic intermediate in this reaction sequence, 1-acyl glycerol 3-phosphate, is commonly called *lysophosphatidic acid* since it can also be generated by hydrolysis of the *sn*-2 fatty acid of phosphatidic acid.

12.4.1.3 Generation of TAG from Phosphatidic Acid. Phosphatidic acid is hydrolyzed by phosphatidic acid phosphatase to generate 1,2-diacylglycerol. Pathways for phospholipid synthesis also utilize phosphatidic acid phosphatase to generate 1,2-diacylglycerol:

$$\text{phosphatidic acid} + H_2O \rightarrow \text{diacylglycerol} + P_i$$

Addition of a third fatty acid group to the *sn*-3 hydroxyl by diacylglycerol acyltransferase yields triacylglycerol:

$$\text{diacylglycerol} + \text{acyl-CoA} \rightarrow \text{triacylglycerol} + \text{CoASH}$$

12.4.1.4 Intestinal TAG Synthesis. As discussed in Chapter 3, the products of the intestinal digestion of dietary fat, free fatty acids and 2-monoacylglycerol, are taken up by enterocytes. Intracellularly, the fatty acids are activated to their respective acyl-CoA form by fatty acid synthetases. Reassembly of fatty acids and 2-monoacylglycerol into triacylglycerol involves successive addition of two acyl groups to 2-monoacylglycerol in reactions catalyzed by acyl transferases located in the endoplasmic reticulum.

12.4.2 Lipoproteins Transport TAG in the Blood

Plasma contains a class of macromolecular aggregates called *lipoproteins* that disperse and transport otherwise highly water-insoluble lipids—cholesteryl esters and TAG in particular—in the circulation. Lipoproteins also play a key role in the metabolism of these lipids and facilitate the two-way exchange of TAG between tissues and the blood. In addition, cholesteryl esters and TAG undergo rapid exchange between the various lipoproteins.

Figure 12-3 illustrates the key structural features of a plasma lipoprotein. The surface of a lipoprotein particle is coated with proteins, phospholipids, and free (nonesterified) cholesterol. The polar ends of the amphipathic lipids face the surface of the lipoprotein, whereas the hydrophobic portions are oriented toward the center of the particle. The core of the lipoproteins is composed of highly nonpolar lipids such as TAG and cholesteryl esters.

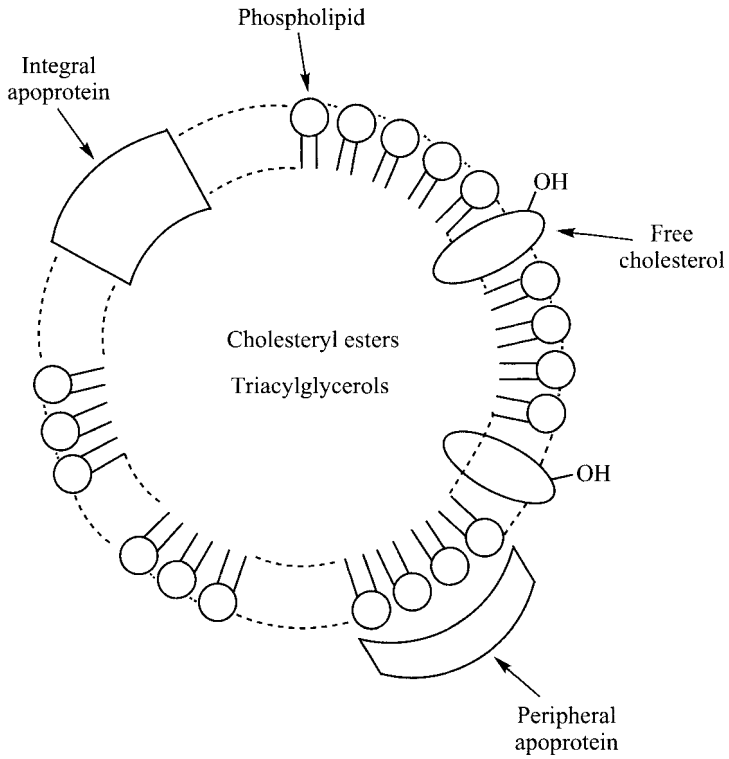


FIGURE 12-3 Generic structure of a plasma lipoprotein.

The proteins (or *apoproteins* as they are called when separated from the lipids) serve a number of functions, including stabilization of the lipoprotein's structure, recognition sites for lipoprotein receptors on cell membranes, and catalysis. For example, apo B100 is recognized by the LDL (low-density lipoprotein) receptor, whereas apo C2 activates the triacylglycerol-hydrolyzing enzyme, lipoprotein lipase.

For historical reasons, the lipoproteins are differentiated on the basis of density (Table 12-1). The most dense lipoprotein class is called high-density lipoprotein (HDL) (1.063 to 1.210 g/dL), followed by low-density lipoprotein (LDL) (1.016 to 1.063 g/dL), very low-density lipoprotein (VLDL) (0.95 to 1.006 g/dL), and chylomicrons (<0.95 g/dL). The major triacylglycerol-transporting lipoproteins are VLDL and chylomicrons. LDL and HDL transport primarily cholesteryl esters. HDL also contains some triacylglycerol. (See Chapter 17 for further discussion of lipoprotein metabolism.)

12.4.2.1 VLDL Transports Endogenous TAG. VLDL is synthesized and secreted mainly by the liver, with a small contribution by the small intestine following a meal. Its main function is to transport TAG that are synthesized in the liver. Functionally, the most prominent apoprotein component of VLDL is apo B100.

TABLE 12-1 Characteristics of the Major Lipoproteins

Lipoprotein	Density (g/mL)	Diameter (nm)	Major Apoprotein	Major Lipid
Chylomicrons	<0.95	75–1200	apo B48	Exogenous TAG
VLDL	0.95–1.006	30–80	apo B100	Exogenous TAG
LDL	1.019–1.063	12–25	apo B100	Cholesteryl ester
HDL2	1.063–1.12	10–20	apo A1	Cholesteryl ester
HDL3	1.12–1.21	7.5–10	apo A1	Cholesteryl ester

HDL, high-density lipoprotein, LDL, low-density lipoprotein, VLDL, very low-density lipoprotein, TAG, triacylglycerols.

While in the circulation, VLDL-triacylglycerols are hydrolyzed by lipoprotein lipase (see below), making free fatty acids available to adipose tissue for storage and to other peripheral tissues for energy production or biosynthetic purposes (e.g., phospholipid synthesis). As the TAG are hydrolyzed, the lipoprotein particles becomes smaller and less buoyant, passing through an intermediate-density lipoprotein (IDL) stage on their way to becoming low-density lipoproteins (LDL).

12.4.2.2 Chylomicrons Transport Exogenous TAG. Chylomicrons are transient in the sense that they are present in the circulation for only several hours following consumption of a fat-containing meal. The major apoprotein component of chylomicrons is apo B48, which contains only 48% of the full amino acid sequence of apo B100; its synthesis by enterocytes is the result of an RNA editing process in which a specific cytidine residue is deaminated to uridine, thus generating a stop codon within the RNA sequence.

Chylomicrons are synthesized by brush-border cells of the small intestine and secreted into the lymphatic system. Once in the blood, the TAG in chylomicrons are hydrolyzed by lipoprotein lipase. As they lose TAG, the chylomicrons shrink and become chylomicron remnants, which are eventually taken up by the liver.

12.4.3 Extracellular Hydrolysis of TAG

The enzymes that hydrolyze the ester bonds linking fatty acids to glycerol are classified as esterases and called *lipases*. The human body contains a number of lipases with distinctly different functions. The role of pancreatic lipase in the digestion of dietary TAG was discussed in Chapter 3. Lipases are also required to hydrolyze lipoprotein-associated TAG so that the constituent fatty acids can be taken up by cells. The lipases that act on TAG in lipoproteins include the three described below.

12.4.3.1 Lipoprotein Lipase (LpL). This glycoprotein is the main enzyme that hydrolyzes TAG in the blood. LpL is synthesized mainly in adipocytes, cardiac muscle, and lactating mammary glands. The enzyme is transported to the endothelial lining of capillaries, where it attaches to endothelial cells through noncovalent bonds to heparan sulfate proteoglycan. LpL catalyzes the hydrolysis of ester bonds in the

1- and 3-positions of the TAG in chylomicrons, VLDL and IDL:

$$\text{triacylglycerol} + 2H_2O \rightarrow 2 \text{ fatty acids} + \text{2-monoacylglycerol}$$

Some of the monoacylglycerol generated in this reaction is internalized by vascular cells. The remainder isomerizes spontaneously to 1-monoacylglycerol, which is then hydrolyzed by either LpL or a monoacylglycerol lipase in plasma:

$$\text{1-monoacylglycerol} + H_2O \rightarrow \text{fatty acid} + \text{glycerol}$$

Activation of lipoprotein lipase requires a cofactor or activator protein, apo C2, which is synthesized by the liver. Newly synthesized chylomicrons and VLDL contain apo B48 and apo B100, respectively, but do not contain apo C2; instead, as they circulate, they acquire apo C2 from HDL. As the chylomicrons and VLDL gradually become delipidated of TAG, apo C2 returns to HDL and is thus recycled.

12.4.3.2 Hepatic Lipase (HL). Hepatic lipase is synthesized by hepatocytes and tethered to the surface of liver capillaries by heparan sulfate proteoglycans. It hydrolyzes phospholipids as well as TAG contained in high-density lipoprotein (HDL). Hepatic lipase also plays a role in internalizing lipoprotein-associated lipids by hepatocytes.

12.4.3.3 Endothelial Lipase. Endothelial lipase is synthesized by various cell types, including vascular endothelial cells. It also cleaves fatty acids from TAG associated with HDL. Like lipoprotein lipase and hepatic lipase, endothelial lipase has a high affinity for cell-surface heparan sulfate proteoglycan.

12.4.4 Intracellular Lipases

12.4.4.1 Adipocyte Lipases. Most of the body's fat is stored in adipocytes. During a fast or when there is a rapid and critical need for energy, adipocytes hydrolyze some of their triacylglycerol stores and release free fatty acids and glycerol into the circulation.

Hormone-sensitive lipase is a key enzyme involved in the hydrolysis of adipocyte triacylglycerol; its name reflects the fact that the enzyme is activated by the signal transduction cascade involving cAMP and protein kinase A (PKA). In adipocytes, activation of PKA is initiated primarily by epinephrine and to a lesser extent by glucagon and growth hormone. Hormone-sensitive lipase catalyzes the hydrolysis of the fatty acids at positions 1 and 3 of triacylglycerol molecules to produce 2-monoacylglycerol (2-MAG).

For decades, hormone-sensitive lipase was considered to be the key regulatory enzyme in the lipolysis pathway of adipose tissue. However, it is now believed that a recently discovered lipase called *desnutrin* or *adipose triglyceride lipase* (ATGL)

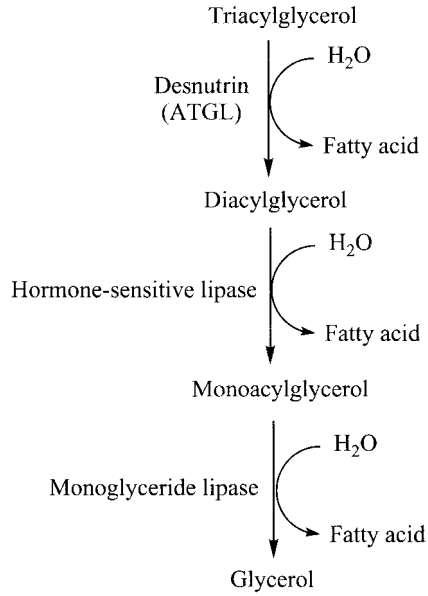


FIGURE 12-4 Pathway of triacylglycerol mobilization in adipocytes.

catalyzes the first step in triacylglycerol hydrolysis. Desnutrin catalyzes the hydrolysis of TAG to diacylglycerols (DAG) and is the rate-limiting step in triacylglycerol hydrolysis. Hormone-sensitive lipase is more active against DAG than against TAG. Thus, the current hypothesis regarding the lipolytic cascade in adipocytes involves three esterases acting sequentially: desnutrin hydrolyzes the first ester bond in TAG generating DAG; then DAG are hydrolyzed by hormone-sensitive lipase to produce 2-monoacylglycerols; and finally, monoacylglycerol lipase removes the third fatty acid to produce glycerol (Fig. 12-4).

12.4.4.2 Lipases in Muscle. Desnutrin and hormone-sensitive lipase are also present in those muscle fibers that have high aerobic oxidative capacity. These two lipases act to hydrolyze the normally modest intramuscular stores of TAG to provide energy during muscle work. Lipolysis in muscle is activated by muscular contraction as well as by epinephrine-induced signaling.

12.4.4.3 Lysosomal Acid Lipase. This lipase, which has a pH optimum near 5, catalyzes the hydrolysis of both LDL-derived triacylglycerols and cholesteryl esters that have been transported into lysosomes via the LDL receptor/endocytic pathway (see Chapter 17). The TAG are hydrolyzed to free fatty acids and monoacylglycerols which exit the lysosomes, whereupon these products can be used for cellular energy production or for phospholipid synthesis.

12.5 REGULATION OF TAG METABOLISM

Insulin is the most important regulator of triacylglycerol metabolism. Insulin enhances the rate of hydrolysis of lipoprotein-associated TAG by stimulating the synthesis and secretion of lipoprotein lipase by adipocytes and myocytes. Insulin also promotes TAG storage in adipocytes and TAG synthesis and VLDL export from hepatocytes. Simultaneously, insulin inhibits the breakdown of TAG, in fat cells. By contrast, hydrocortisone, epinephrine, and growth hormone oppose the action of insulin, inhibiting the synthesis of fatty acids in both hepatocytes and adipocytes, and promoting lipolysis in adipocytes in times of energy need, such as fasting and exercise.

12.5.1 Regulation of TAG Synthesis

Insulin stimulates dephosphorylation and activation of acetyl-CoA carboxylase, the enzyme that catalyzes the rate-limiting step in de novo fatty acid synthesis. Insulin also promotes fatty acid synthesis by inducing enzymes of the fatty acid synthesis family. In addition, insulin stimulates the catabolism of excess dietary carbohydrates, thereby increasing the supply of acetyl-CoA substrate for fatty acid and thus triacylglycerol synthesis.

12.5.2 Regulation of Adipocyte TAG Mobilization

Lipolysis in adipocytes is under tight hormonal control. As described above, desnutrin/ATGL is the enzyme that catalyzes the initial hydrolysis step involved in adipocyte triacylglycerol mobilization. The synthesis of desnutrin is induced by hydrocortisone and inhibited by insulin. Hormone-sensitive lipase is activated by epinephrine via a mechanism involving cAMP-dependent phosphorylation. By contrast, insulin acts to dephosphorylate hormone-sensitive lipase, thereby inhibiting lipolysis.

Adipocytes store TAG in the form of lipid droplets surrounded by a protein called *perilipin*. Like hormone-sensitive lipase, perilipin is phosphorylated by cAMP-dependent protein kinase A. In its unphosphorylated state, perilipin acts as a barrier that limits access of lipases to their substrates, thus maintaining a low rate of basal triacylglycerol hydrolysis. Phosphorylation of perilipin causes the lipid droplets to fragment and disperse, permitting efficient hydrolysis of adipocyte TAG.

12.5.3 Regulation of Lipoprotein Lipase Activity

Lipoprotein lipase (LpL) is active in both the fasted and fed states. In the fasted state, it plays an important role in making fatty acids from VLDL triacylglycerols available to cardiac and skeletal muscles, where the fatty acids serve as fuel. In contrast, in the fed state, LpL directs fatty acids from both chylomicrons and VLDL to adipocytes for storage in the form of TAG. Adipocyte LpL expression is reduced during fasting,

while its expression in muscle is up-regulated. Conversely, adipocyte LpL expression is up-regulated in the fed state.

In addition, muscle- and adipocyte-specific forms of LpL have different kinetic properties, with the muscle enzyme having a lower K_m for triacylglycerol than the adipocyte enzyme. Thus, the active site of the LpL enzyme, which is localized to the surface of muscle capillaries, is saturated even during the fasted state, when circulating triacylglycerol-containing lipoprotein levels are low. By contrast, the activity of LpL associated with adipose tissue capillaries increases in the fed state, when the levels of triacylglycerol-rich lipoproteins are relatively high.

12.6 DISEASES INVOLVING ABNORMALITIES IN TRIACYLGLYCEROL METABOLISM

12.6.1 Obesity

Obesity is defined as excess adiposity, which refers to the storage of excess TAG in fat depots. A common practice for estimating obesity utilizes the *body mass index* (BMI), which is calculated as weight (kg)/height $(m)^2$. Obesity is defined as a body mass index > 30 kg/m^2, or, for example, a weight of 175 pounds or more for a woman who is 5 ft 4 in. tall. Obesity is epidemic in the United States and in many other countries as well. The primary metabolic factors that cause obesity involve overconsumption of food (carbohydrates and protein as well as fats) and insufficient physical activity.

12.6.2 Hypertriglyceridemia

Hypertriglyceridemia is defined as an abnormally high triglyceride concentration in the blood. A normal fasting plasma triglyceride concentration is considered below 150 mg/dL, borderline high at 150 to 199 mg/dL, high at 200 to 499 mg/dL, and very high at 500 mg/dL or above. A high plasma triglyceride level is associated with increased risk for cardiovascular disease, especially myocardial infarction. Hypertriglyceridemia may be caused by a genetic defect or secondarily by acquired factors, such as obesity, physical inactivity, ethanol consumption, diabetes mellitus, hypothyroidism, and drugs that either stimulate triacylglycerol synthesis or retard triacylglycerol catabolism.

12.6.2.1 Type II Diabetes. People with type II are insulin resistant. One effect of this condition is increased lipolysis in adipocytes, which increases the supply of free fatty acids to the liver. Since free fatty acid uptake by hepatocytes is directly proportional to the plasma free fatty acid concentration, the increased free fatty acid flux stimulates triacylglycerol synthesis in the liver. Increased hepatic triacylglycerol synthesis, in turn, leads to increased synthesis and secretion of VLDL, resulting in an elevated plasma triglyceride concentration.

12.6.3 Fatty Liver

The term *nonalcoholic fatty liver disease* refers to a disease spectrum ranging from asymptomatic triacylglycerol accumulation in hepatocytes to nonalcoholic steatohepatosis (NASH), to cirrhosis (irreversible scarring of the liver) in persons who do not necessarily consume excessive amounts of ethanol. There is a strong association between insulin resistance and hepatic steatosis in such people.

As described above, insulin resistance results in increased uptake of fatty acids and triacylglycerol synthesis in the liver. Incorporation of TAG into VLDL depends on the synthesis of protein (primarily apo B100) and phospholipids (mainly phosphatidylcholine). Apolipoprotein B100 production is stimulated by insulin and elevated free fatty acid levels. Phosphatidylcholine synthesis depends on an adequate supply of choline and methionine. Conditions such as type II diabetes may result in a supply of free fatty acids for triacylglycerol synthesis that overwhelms the ability of the liver to assemble and secrete VLDL. As a result, there is abnormal accumulation of TAG in the liver, which eventually results in liver disease.

12.6.4 Triacylglycerol Accumulation in Other Organs

Small amounts of TAG are normally found in many tissues, including cardiac muscle, skeletal muscle, and the pancreas. Increased accumulation of TAG in skeletal muscle occurs in obesity and is associated with insulin resistance and type II diabetes. It is not yet clear whether the impaired insulin signalling in such persons is the result of accumulation of TAG per se or of elevated levels of intermediates in the triacylglycerol synthesis pathway.

Paradoxically, TAG also accumulate in the skeletal muscle of trained athletes, but not because of insulin resistance. Instead, exercise training appears to increase the ability of skeletal muscle to mobilize intramuscular TAG and oxidize the resulting fatty acids. Exercise also increases the expression of enzymes of triacylglycerol synthesis (e.g., diacylglycerol acyltransferase) in muscle.

12.6.5 Single Gene Defects That Impair Triacylglycerol Metabolism

12.6.5.1 Chylomicronemia. People with this rare disorder are characterized by extremely high fasting plasma triglyceride concentrations (> 1000 mg/dL). The main causes of chylomicronemia are genetic deficiencies in lipoprotein lipase activity or apo C2, which is a cofactor of this lipase that normally increases the rate of lipolysis. Patients with chylomicronemia usually present in childhood with recurrent attacks of pancreatitis. It is postulated that the chylomicrons impair circulation in pancreatic capillaries thus leading to inflammation. Cell damage then leads to release and activation of proteolytic enzymes in the pancreas rather than in the intestine, resulting in autodigestion of the pancreas. Treatment and prevention of chylomicronemia require restricting dietary fat. Patients deficient in apo C2 often benefit from plasma infusions, which provide an exogenous source of this apoprotein.

12.6.5.2 Abetalipoproteinemia. Abetalipoproteinemia is a rare genetic disease characterized by the absence of all apo B–containing lipoproteins (chylomicrons, VLDL, LDL). The underlying defect is in the gene for the microsomal triglyceride transfer protein (MTTP), which facilitates the uptake of TAG by both apo B100 and apo B48. In the absence of MTTP, lipoprotein assembly is impaired in both hepatocytes and intestinal cells. Lack of chylomicron formation results in malabsorption of dietary fat and steatorrhea, and deficiencies of fat-soluble vitamins. At the same time, lack of hepatic VLDL synthesis results in fatty liver. Malabsorption of vitamin E, a fat-soluble vitamin, and the marked impairment of the interorgan transport of vitamin E result in demyelination and serious neurological impairment.

12.6.5.3 Congenital Generalized Lipodystrophy. One form of this rare disorder results from loss of function of AGPAT2, the adipocyte isozyme of 1-acylglycerol-3-phosphate acyltransferase, which is required for the biosynthesis of both TAG and phospholipids. Affected persons present at birth or in early infancy with a marked deficit of adipose tissue and compensatory accumulation of TAG in other organs, such as liver and skeletal muscle. They also exhibit extreme insulin resistance, hypertriglyceridemia, hepatic steatosis, and early onset of diabetes.

CHAPTER 13

ETHANOL

13.1 FUNCTION OF ETHANOL METABOLISM

It is believed that the original physiological function of alcohol dehydrogenase was to remove ethanol formed by microorganisms in the intestinal tract. Ethanol is also a common component of wine, beer, and distilled spirits. Since there is no significant renal or pulmonary excretion of ethanol and no storage of ethanol in the body, whatever ethanol is consumed must be disposed of through metabolism.

Ethanol can be a significant source of energy for people who consume large quantities of alcoholic beverages. The caloric content of ethanol is approximately 7 kcal/g, which is intermediate between those of glucose (4 kcal/g) and fat (9 kcal/g).

13.2 LOCATION OF ETHANOL METABOLISM

The major site of alcohol metabolism is the liver, which contains both alcohol dehydrogenase and the microsomal ethanol-oxidizing system (MEOS), the two enzymes most responsible for ethanol metabolism. However, alcohol dehydrogenase activity is also present in the gastric mucosa (more so in men than women), and to a lesser extent in other organs, including the kidneys, lungs, and small intestine.

Medical Biochemistry: Human Metabolism in Health and Disease By Miriam D. Rosenthal and Robert H. Glew

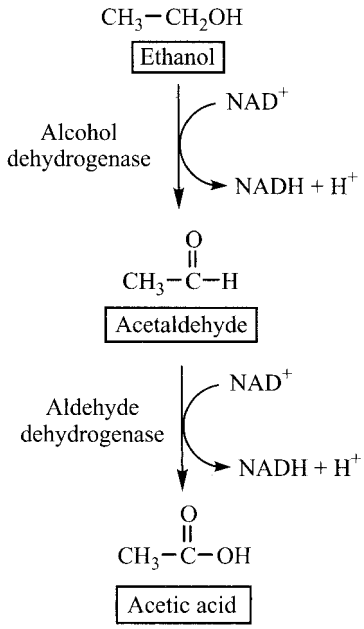


FIGURE 13-1 Major pathway from ethanol to acetyl-CoA.

13.3 PATHWAYS OF ETHANOL METABOLISM

The overall pathway for metabolizing ethanol involves oxidation of the alcohol to acetaldehyde, which is then oxidized to acetate (Fig. 13-1). The acetate derived from ethanol oxidation is activated to acetyl-CoA by acetate thiokinase (see below). The resulting acetyl-CoA can be metabolized through the TCA cycle or utilized for fatty acid synthesis. There are three enzymes or enzyme systems that convert ethanol to acetaldehyde: alcohol dehydrogenase, MEOS, and catalase.

13.3.1 Alcohol Dehydrogenase

In liver and stomach, ethanol metabolism is initiated mainly by NAD^+-dependent alcohol dehydrogenase (ADH), which is a cytosolic enzyme:

$$\text{ethanol} + NAD^+ \rightarrow \text{acetaldehyde} + NADH + H^+$$

There are several alcohol dehydrogenase isozymes that can oxidize ethanol. The gastric isozyme of ADH has a much higher K_m for ethanol than do the three ADH isozymes in liver. The NADH produced by alcohol dehydrogenase can be shuttled into the mitochondrion and utilized for ATP synthesis.

13.3.2 Microsomal Ethanol-Oxidizing System

The liver has a second pathway for oxidizing ethanol, which even though it can oxidize a variety of compounds in addition to ethanol, is designated the microsomal ethanol-oxidizing system (MEOS). Other substrates for MEOS include fatty acids, steroids, and barbiturates. MEOS oxidizes ethanol to acetaldehyde:

$$\text{ethanol} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{acetaldehyde} + \text{NADP}^+ + 2\text{H}_2\text{O}$$

MEOS is a mixed-function oxidase that oxidizes both ethanol and NADPH. In many ways MEOS is similar to stearoyl-CoA desaturase (see Chapter 11) in its use of a microsomal electron transport chain involving flavin nucleotides. In addition to its alcohol dehydrogenase activity per se, MEOS also contains cytochrome P450 and NADPH-dependent cytochrome P450 reductase. Since the major ethanol dehydrogenase component of MEOS, CYP2E1, has a much higher K_m for ethanol (11 mM) than the low-K_m forms of alcohol dehydrogenase (0.05 to 4 mM), MEOS functions only at relatively high concentrations of ethanol. To the extent that MEOS consumes NADPH, this reaction is a more wasteful way to metabolize ethanol than alcohol dehydrogenase, which generates NADH rather than consuming NADPH.

13.3.3 Catalase

Catalase, a ubiquitous enzyme, is also capable of oxidizing ethanol; however, its contribution to ethanol metabolism is minimal. Oxidation of ethanol by catalase utilizes hydrogen peroxide:

$$\text{ethanol} + \text{H}_2\text{O}_2 \rightarrow \text{acetaldehyde} + 2\text{H}_2\text{O}$$

The H_2O_2 required for catalase-catalyzed ethanol oxidation is derived mainly from the xanthine oxidase reaction of purine catabolism:

$$\text{hypoxanthine} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{xanthine} + \text{H}_2\text{O}_2$$

and from the sequential actions of NADPH oxidase (1) and superoxide dismutase (2):

$$(1)\ 2\text{O}_2 + \text{NADPH} + \rightarrow 2\text{O}_2^{\bullet} + \text{NADP}^+ + \text{H}^+$$

$$(2)\ 2\text{O}_2^{\bullet} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 + \text{O}_2$$

13.3.4 Metabolism of Acetaldehyde

Acetaldehyde generated by alcohol dehydrogenase, MEOS, or catalase is oxidized to acetate by NAD^+-dependent aldehyde dehydrogenase. Although the major isozyme

of aldehyde dehydrogenase is located in the mitochondria, there is also a cytosolic aldehyde dehydrogenase isozyme. Both isozymes of aldehyde dehydrogenase catalyze the reaction

$$\text{acetaldehyde} + \text{NAD}^+ \rightarrow \text{acetate} + \text{NADH} + \text{H}^+$$

13.3.5 Metabolic Fate of the Acetate Derived from Ethanol

The acetate produced by the oxidation of ethanol is activated by acetyl-CoA synthetase (a.k.a., acetate thiokinase):

$$\text{acetate} + \text{CoASH} + \text{ATP} \rightarrow \text{acetyl-CoA} + \text{AMP} + \text{PP}_\text{i}$$

The major liver isozyme of acetate thiokinase is cytosolic, and the acetyl-CoA it generates is used for fatty acid and cholesterol synthesis. However, when these two pathways are inactive (due primarily to a high ratio of glucagon to insulin), acetate will diffuse out of the hepatocytes and be taken up and oxidized by heart and skeletal muscle which have high concentrations of mitochondrial acetyl-CoA synthetase. Thus, if ethanol is consumed along with significant amounts of carbohydrate, the acetate generated from ethanol will be used mainly as a substrate for hepatic fatty acid synthesis. If, however, ethanol is consumed in the absence of carbohydrate, the acetate derived from the oxidation of ethanol will be used mostly as fuel and oxidized to CO_2 and water.

13.4 REGULATION OF ETHANOL METABOLISM

Chronic consumption of ethanol can increase hepatic levels of CYP2E1 many fold. Ethanol also increases the expression of other cytochrome P450 genes. When induction of MEOS increases the rate of metabolism of ethanol, the increased production of acetaldehyde may exceed the ability of the acetaldehyde dehydrogenases to further oxidize acetaldehyde. The resultant accumulation of acetaldehyde increases the risk of liver damage.

Gender differences and genetic variants in the enzymes responsible for metabolizing ethanol may account for some of the individual variation in tolerance to ethanol. As noted earlier, premenopausal women normally have lower levels of gastric alcohol dehydrogenase than men. The lower level of gastric ADH activity in women, as well as gender-based differences in body size and total body-water space, are believed to account for the lower tolerance to ethanol in women relative to men.

A number of genetic polymorphisms in ethanol-metabolizing enzymes have been characterized. The inducibility of CYP2E1 can vary as much as 10-fold between persons with polymorphisms in the 5′-flanking region of the gene. Similarly, many persons of East Asian descent have an inactive or less active form of ALDH2, the hepatic mitochondrial isozyme of acetaldehyde dehydrogenase. When people with a

mutation in ALDH2 consume ethanol, they are more susceptible to flushing, headache, and nausea, apparently because of acetaldehyde accumulation. The drug Antabuse, which is used to discourage alcoholics from drinking, acts by inhibiting acetaldehyde dehydrogenase; people who consume ethanol while taking Antabuse develop symptoms similar to those of people who have a genetic lack of ALDH2 activity.

13.5 METABOLIC ABNORMALITIES ASSOCIATED WITH ETHANOL METABOLISM

13.5.1 Alcoholic Hypoglycemia, Acidosis, and Ketoacidosis

Collectively, the successive reactions catalyzed by alcohol dehydrogenase and acetaldehyde dehydrogenase generate 2 mol of NADH per mole of ethanol oxidized. Metabolism of large or even moderate quantities of ethanol thus causes the $NADH/NAD^+$ ratio in the liver to increase markedly. Lack of NAD^+ inhibits lactate dehydrogenase and the entry of lactate into gluconeogenesis; the resulting increase in the plasma lactate concentration results in metabolic acidosis. A lack of NAD^+ also slows the action of other key enzymes required for gluconeogenesis, including glycerol 3-phosphate dehydrogenase, malate dehydrogenase, and glutamate dehydrogenase which is important in the removal of amino groups and subsequent entry of the carbon skeletons of amino acids into the gluconeogenesis pathway.

Metabolism of large quantities of ethanol can also lead to ketoacidosis, particularly when the plasma insulin concentration is depressed. Although much of the acetate derived from ethanol metabolism escapes the liver and is metabolized by other tissues, some of the acetate is activated by acetyl-CoA synthetase in hepatocytes. The high $NADH/NAD^+$ ratio slows entry of acetyl-CoA into the TCA cycle by decreasing the activity of NAD^+-linked malate dehydrogenase, thereby limiting the availability of oxaloacetate. The acetyl-CoA from ethanol metabolism is, instead, shunted to the synthesis of acetoacetate, which in turn is reduced to β-hydroxybutyrate, further exacerbating the metabolic acidosis. Alcoholic ketoacidosis is accompanied by dehydration, which results from a combination of vomiting, restricted fluid intake, and inhibition of antidiuretic hormone secretion by ethanol. Dehydration, in turn, impairs renal excretion of ketones.

13.5.2 Alcohol-Induced Fatty Liver

Chronic ethanol use perturbs normal hepatic metabolism, resulting in the accumulation of intracellular triacylglycerols. First, the high $NADH/NAD^+$ ratio decreases the activity of β-hydroxyacyl-CoA dehydrogenase, thus inhibiting β-oxidation of fatty acids. Second, metabolism of ethanol enhances fatty acid synthesis. Suppression of TCA-cycle activity leads to the accumulation of citrate in the cytosol, which stimulates acetyl-CoA carboxylase, the first enzyme in the pathway of fatty acid synthesis. At the same time, high concentrations of NADH increase the rate of

production of NADPH (the reductant required for fatty acid synthesis) by means of the NADH/NADPH transhydrogenation pathway (Chapter 10). Accumulation of fatty acids in the liver is further enhanced by hormonal factors, particularly glucagon and hydrocortisone, which stimulate lipolysis in adipocytes, and provides the liver both with additional free fatty acids and with the glycerol needed for their esterification into triacylglycerols.

13.5.3 Acetaldehyde Toxicity

Accumulation of acetaldehyde, produced both by alcohol dehydrogenase and MEOS, is believed to be responsible for most of the alcohol-induced liver damage known as *cirrhosis*. By virtue of its aldehyde group, acetaldehyde is a highly reactive molecule

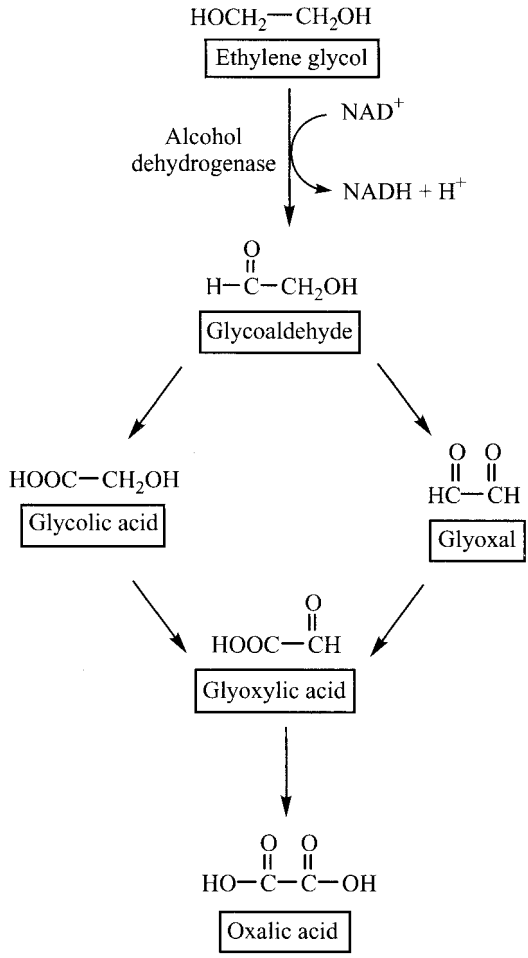


FIGURE 13-2 Metabolism of ethylene glycol.

that can form adducts with many different intracellular proteins. In particular, reaction of acetaldehyde with tubulin impairs secretion of serum proteins from hepatocytes, damaging these cells further. Increased oxidative stress, resulting from production of free radicals by CYP2E1, also contributes to liver damage in chronic alcoholics.

Alcohol dehydrogenase also plays a role in methanol and ethylene glycol toxicity. Methanol is oxidized by ADH to toxic formaldehyde, which in turn is oxidized to formic acid. ADH is also a key enzyme in the pathway that oxidizes ethylene glycol to three organic acids—glycolic acid, glyoxylic acid, and oxalic acid (Fig. 13-2)—which can cause life-threatening metabolic acidosis. Methanol and ethylene glycol poisoning can be treated by administration of the drug fomepizole (4-methylpyrazole) which inhibits ADH activity, thereby allowing these alcohols to be eliminated by the kidney and preventing formation of their more toxic metabolites.

Ethanol has also been utilized to treat ethylene glycol poisoning. Binding of ethanol to the catalytic site of alcohol dehydrogenase displaces ethylene glycol which can then be excreted by the kidneys instead of being oxidized. Since ethanol is a potent inhibitor of gluconeogenesis, concurrent provision of glucose is recommended to reduce the risk of inducing hypoglycemia, which could lead to brain damage.

13.5.4 Wernicke–Korsakoff Syndrome

People who chronically consume excessive amounts of ethanol are at risk for developing acute encephalopathy, peripheral nerve disfunction, and chronic impairment of short-term memory. This condition is known as *Wernicke–Korsakoff syndrome*. The

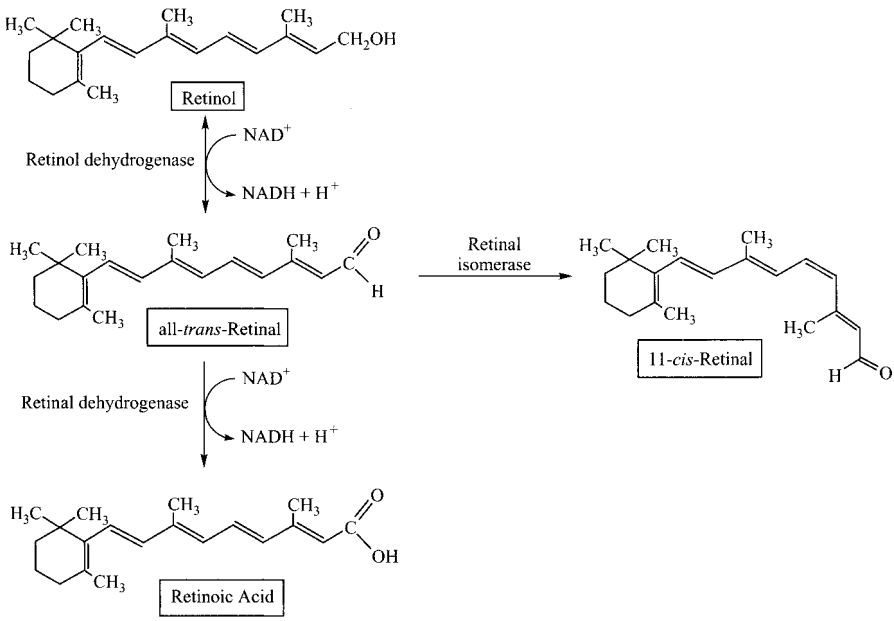


FIGURE 13-3 Metabolism of retinol.

underlying biochemical problem is a deficiency of thiamine (vitamin B_1), and early cases can be treated successfully with high doses of the vitamin. Thiamine pyrophosphate is the required cofactor for transketolase, which catalyzes two steps of the nonoxidative phase of the pentose phosphate pathway as well as for both pyruvate dehydrogenase and α-ketoglutarate dehydrogenase in the TCA cycle (Chapter 5), and for the α-keto acid dehydrogenase, which is involved in the catabolism of the carbon skeletons of branched-chain amino acids (Chapter 20). Dietary deficiency of thiamine is rare in Western societies except in people with alcoholism, who typically have poor diets. In addition, consumption of ethanol decreases thiamine absorption in the gut. Alcoholic cirrhosis impairs formation of the active cofactor thiamine pyrophosphate and contributes to excess excretion of thiamine.

13.5.5 Vitamin A Deficiency

Ethanol interferes with normal metabolism of vitamin A in two ways. First, ethanol is a competitive inhibitor of retinol dehydrogenase, which converts retinol to *all-trans*-retinal. Synthesis of retinal is essential for the formation of the visual pigment rhodopsin, which contains 11-*cis*-retinal. *All-trans*-retinal is also the precursor of retinoic acid, the active hormonal form of the vitamin (Fig. 13-3). Second, ethanol also induces the MEOS system, which enhances catabolism of retinol.

CHAPTER 14

PHOSPHOLIPIDS AND SPHINGOLIPIDS

14.1 FUNCTIONS OF PHOSPHOLIPIDS

Phospholipids are a heterogeneous class of molecules that are amphipathic; that is, they have both hydrophobic and hydrophilic domains. Most of the phospholipids are glycerophospholipids, in which the hydrophobic domain is 1,2-diacylglycerol. Phosphatidylcholine is an example of diacylglycerol phospholipid (Fig. 14-1). Other glycerophospholipids are ether lipids, in which the long-chain hydrocarbon in the 1-position is in ether linkage rather than ester linkage to glycerol. By contrast, sphingomyelin is a phospholipid that contains ceramide (*N*-acylsphingosine) in place of diacylglycerol (Fig. 14-1). The hydrophilic portion of all glycerophospholipids consists of a phosphodiester bridge that links the hydrophobic domain to the hydroxyl group of a small molecule such as choline, ethanolamine, serine, or inositol.

14.1.1 Membrane Structure

Most of the phospholipids in humans occur as structural elements of membranes where they separate the cytosol from the extracellular space or provide for intracellular compartmentalization (i.e., mitochondrial membranes). Phosphatidylcholine is the most abundant glycerophospholipid in membranes, and sphingomyelin is the most abundant ceramide-based lipid. The plasma membrane is asymmetric with respect to lipid composition in that phosphatidylcholine and sphingomyelin tend to be concentrated in the outer leaflet of the membrane bilayer, whereas

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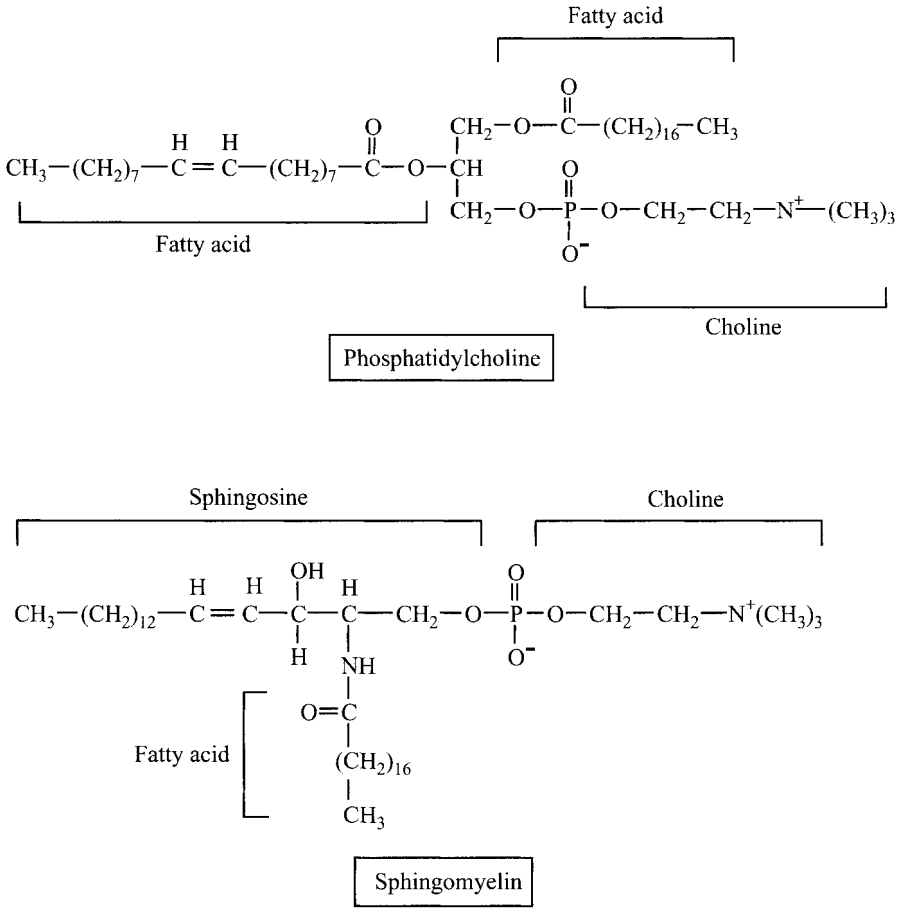

FIGURE 14-1 Structures of two common phospholipids: phosphatidylcholine and sphingomyelin.

phosphatidylethanolamine, phosphatidylserine, and phosphatidylinositol tend to be concentrated in the membrane's inner leaflet, facing the cytosol. Membranes of both myelin and gray matter of nervous tissue are particularly enriched in sphingomyelin.

Although phospholipids do move freely in the plane of the membrane, movement from one membrane leaflet to the other is limited because the polar head groups do not pass readily through the hydrophobic center of the membrane. Transfer of newly synthesized phospholipids from the cytosolic to the external face of the membrane and maintenance of appropriate membrane asymmetry requires the ATP-dependent action of transfer proteins known as *flippases*.

14.1.2 Phospholipids Are Emulsifiers

Phospholipids are amphipathic molecules that can disperse otherwise insoluble mixtures of hydrophobic molecules (e.g., triacylglycerols, cholesteryl esters) during

digestion and during transport of lipids in the circulation. Phospholipids in bile serve at least two functions. First, in the intestine, phospholipids aid in the dispersion of dietary triacylglycerols and cholesteryl esters, thereby promoting their digestion and absorption. Second, phospholipids solubilize cholesterol in bile (most of which is unesterified), thereby minimizing precipitation of cholesterol and gallstone formation in the biliary tract. Phospholipids are also critical components of plasma lipoproteins that coat the surface of lipoprotein particles (e.g., chylomicrons, VLDL, LDL, HDL), which transport triacylglycerols and cholesteryl esters in the circulation.

14.1.3 Surfactant

Pulmonary surfactant is the layer of lipid (90%) and protein (10%) that coats the alveolar surface of the lung. Surfactant, which is produced by type II pneumocytes, lowers the surface tension across the air–water interface of alveoli, thereby preventing collapse of terminal respiratory chambers and conducting airways. About 75% of the surfactant lipid is phosphatidylcholine; of this about half is a specialized phosphatidylcholine, dipalmitoylphosphatidylcholine, which contains two molecules of palmitic acid, a 16-carbon saturated fatty acid. Two acidic phospholipids, phosphatidylinositol and phosphatidylglycerol, together account for about 15% of the total phospholipid content of surfactant.

14.1.4 Protein Anchors

Phosphatidylinositol (PI) functions as a tethering mechanism that anchors certain proteins to the external leaflet of the plasma membrane of many different types of cells. Glycosylphosphatidylinositol, commonly called the *GPI anchor*, consists of amphipathic PI linked through an ethanolamine-containing oligosaccharide to the carboxyl group of the C-terminal amino acid of a mature glycoprotein (Fig. 14-2). Acetylcholinesterase, alkaline phosphatase, and 5′-nucleotidase are examples of proteins that are PI-anchored to membranes.

14.1.5 Activators of Enzymes

There are many instances where phospholipids function to promote enzyme activity. For example, glucocerebrosidase, a membrane-bound lysosomal enzyme that hydrolyzes glucosylceramide to glucose and ceramide, is activated by phosphatidylserine. Similarly, the activity of β-hydroxybutyrate dehydrogenase, which is involved in ketone body synthesis in the liver and β-hydroxybutyrate utilization in peripheral tissues (e.g., muscle, brain), is dependent on phosphatidylcholine. The activities of several of the proteases involved in blood coagulation require phosphatidylserine as well as calcium.

14.1.6 Precursors of Signaling Molecules

Hydrolysis of membrane phospholipids generates a variety of molecules that are involved in intracellular and cell–cell signaling. Diacylglycerol, ceramide,

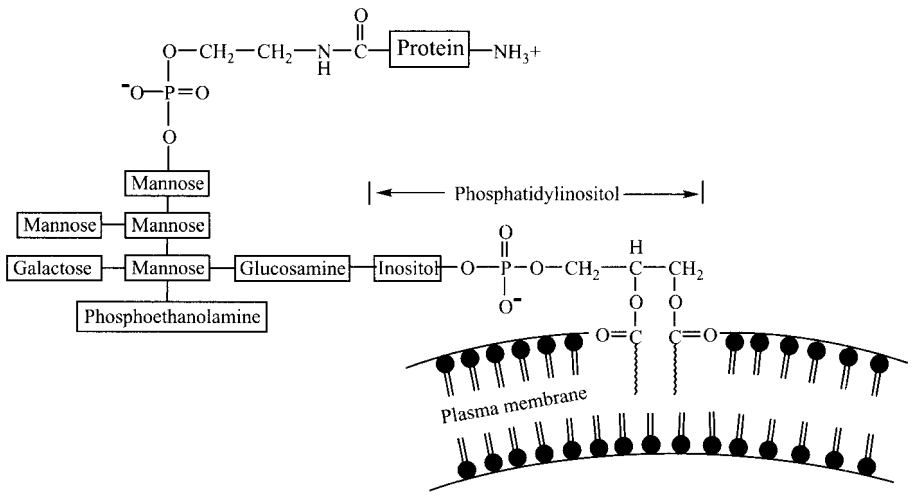

FIGURE 14-2 Generalized structure of a glycosylphosphatidylinositol-anchored membrane protein.

lysophosphatidic acid, and phosphatidic acid all activate various protein kinases, while sphingosine 1-phosphate and lysophosphatidic acid are extracellular signaling molecules whose respective roles include promoting angiogenesis and mitogenesis. Alkyl-linked choline phospholipids are the precursor for platelet-activating factor (PAF), which is an active hypotensive and inflammatory agent. Membrane phospholipids also provide a store of arachidonic acid, which is the precursor of prostaglandins and other autocoid eicosanoids.

14.1.7 Phospholipids as Scavengers of Free Radicals

Plasmalogens, a subclass of ether-linked glycerophospholipids that contain a vinyl ether double bond ($-CH_2-O-CH=CH-$) at the 1-position of the glycerol backbone, are present in high concentrations in the heart. By scavenging free radicals, plasmalogens may protect other membrane lipids from oxidative damage.

14.2 TISSUES IN WHICH PHOSPHOLIPIDS ARE SYNTHESIZED AND MODIFIED

All cells, with the possible exception of mature red blood cells, are capable of synthesizing one or more glycerophospholipids. Most of the reactions involved in phospholipid synthesis occur on the cytosolic face of the endoplasmic reticulum and Golgi complex.

The liver is a major site of phospholipid synthesis. In addition to making phospholipids for its own cellular needs, the liver generates phospholipids for secretion

into bile and for coating plasma lipoproteins (e.g., VLDL). Two other tissues with a high capacity for phospholipid synthesis are intestinal enterocytes, which reesterify lysophospholipids produced from biliary and dietary phospholipids during digestion, and type II cells of the lung, which synthesize pulmonary surfactant.

14.3 METABOLIC PATHWAYS OF PHOSPHOLIPID METABOLISM

14.3.1 Biosynthesis of the Lipid Backbone

14.3.1.1 Synthesis of Diacylglycerol. The pathway for synthesis of glycerophospholipids, like that for triacylglycerols, starts with glycerol 3-phosphate. Phosphatidic acid is then generated by two successive acyltransferase-catalyzed transfers of fatty acid from their CoA derivatives to glycerol 3-phosphate (see Fig. 12-2). The specificity of these two acyltransferases is not very strict: The first one, the one that attaches a fatty acid to the 1-position of glycerol, is selective for the 16-carbon saturated fatty acyl-CoA, palmitoyl-CoA, and other saturated long-chain fatty acids; the second acyltransferase is selective for monounsaturated and polyunsaturated fatty acyl-CoAs, particularly linoleoyl-CoA. Pathways that generate phospholipids involve either phosphatidic acid or diacylglycerol as intermediates. Generation of diacylglycerol is catalyzed by phosphatidic acid phosphatase:

$$\text{phosphatidic acid} + H_2O \rightarrow \text{diacylglycerol} + P_i$$

14.3.1.2 Synthesis of Ether Lipids. The pathway for synthesis of ether lipids starts with acylation of the glycolytic intermediate dihydroxyacetone phosphate by dihydroxyacetone phosphate acyltransferase (Fig. 14-3):

$$\text{dihydroxyacetone phosphate} + \text{acyl-CoA} \rightarrow \text{1-acyl dihydroxyacetone phosphate} + \text{CoASH}$$

1-Alkyl dihydroxyacetone phosphate synthase then catalyzes the addition of a long-chain fatty alcohol group (ROH) in exchange for the fatty acid:

$$\text{1-acyl dihydroxyacetone phosphate} + \text{ROH} \rightarrow$$
$$\text{1-alkyl dihydroxyacetone phosphate} + \text{RCOOH}$$

This is followed by NADPH-dependent reduction of the C2 keto group by acyl/alkyl-DHAP reductase and acylation of the resulting hydroxyl group to generate the alkyl analog of phosphatidic acid, which then serves as a substrate for ether phospholipid synthesis.

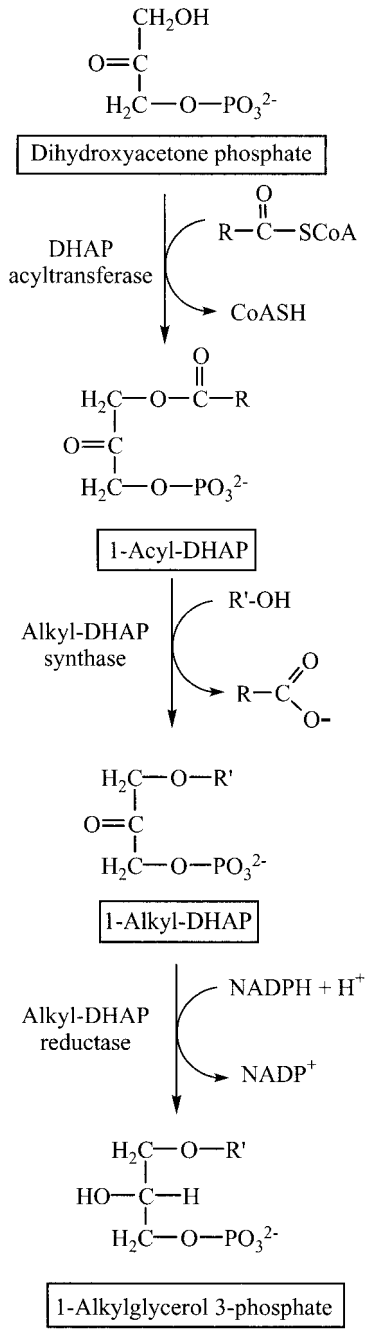

FIGURE 14-3 Synthesis of the 1-alkylglycerol 3-phosphate backbone of ether lipids. DHAP, dihydroxyacetone 3-phosphate; R′-OH, long-chain fatty alcohol.

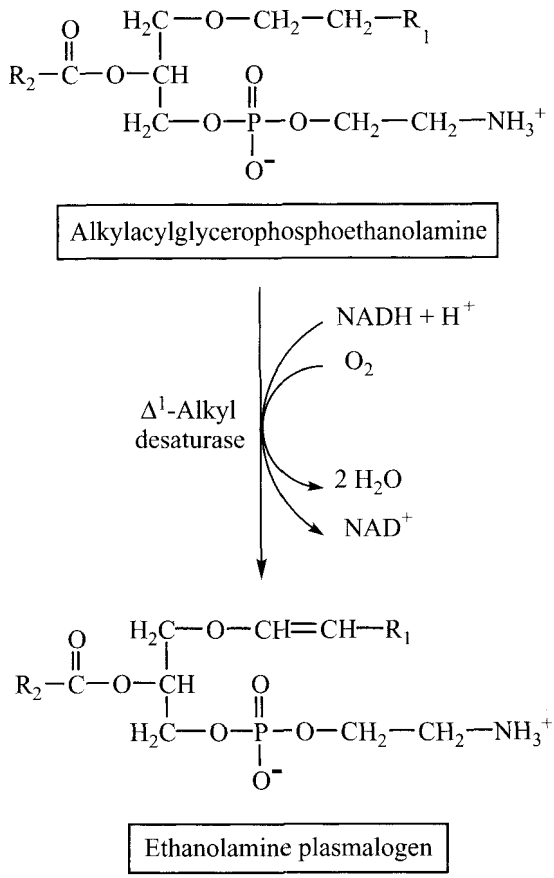

FIGURE 14-4 Synthesis of ethanolamine plasmalogen from the 1-alkyl ether lipid.

Plasmalogen Synthesis. Plasmalogens are ether lipids with a double bond between C1 and C2 of the alkyl chain ($-CH_2-O-CH=CH-$). Generation of the double bond occurs after completion of 1-alkyl-2-acylphospholipid synthesis and is catalyzed by Δ^1-alkyl desaturase, which is a peroxisomal NADH-dependent mixed function oxidase (Fig. 14-4).

14.3.1.3 Ceramide Synthesis. The sphingosine backbone is synthesized from palmitoyl-CoA and serine. In the initial reaction, catalyzed by pyridoxal phosphate-dependent serine-palmitoyl transferase, serine is decarboxylated, and the resulting amine-containing two-carbon fragment condenses with palmitoyl-CoA (Fig. 14-5):

$$\text{palmitoyl-CoA} + \text{serine} \rightarrow \text{3-ketosphinganine} + CO_2 + \text{CoASH}$$

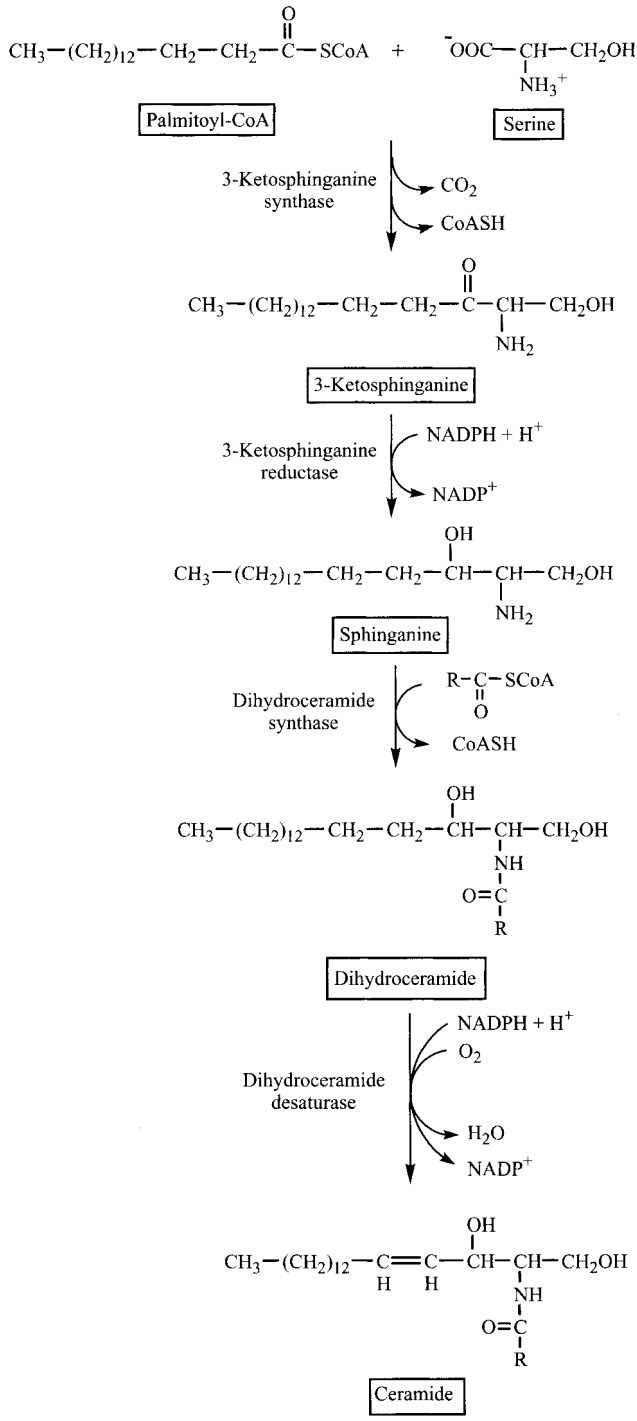

FIGURE 14-5 Synthesis of ceramide.

Next, 3-ketosphinganine reductase uses NADPH to reduce the keto group of 3-ketosphinganine, thereby generating *sphinganine* (also called *dihydrosphingosine*):

$$3\text{-ketosphinganine} + \text{NADPH} + \text{H}^+ \rightarrow \text{sphinganine} + \text{NADP}^+$$

Dihydroceramide synthase then attaches a long-chain fatty acid to the amino group of sphinganine to form dihydroceramide (*N*-acylsphinganine), which is then reduced to ceramide by *N*-acylsphinganine dehydrogenase.

14.3.2 Attachment of Polar Head Groups

14.3.2.1 Synthesis of Phosphatidylcholine and Phosphatidylethanolamine. In all human cells except hepatocytes, the major pathway of phosphatidylcholine (PC) synthesis starts with the activation of free choline. The initial step, the trapping of choline inside cells, is accomplished by the enzyme choline kinase (Fig. 14-6):

$$\text{choline} + \text{ATP} \rightarrow \text{phosphocholine} + \text{ADP}$$

Phosphocholine in turn is activated by CTP : phosphocholine cytidylyltransferase:

$$\text{phosphocholine} + \text{CTP} \rightarrow \text{CDP-choline} + \text{PP}_\text{i}$$

CDP-choline is then condensed with diacylglycerol in a reaction catalyzed by CDP-choline:diacylglycerol phosphocholine transferase:

$$\text{CDP-choline} + \text{diacylglycerol} \rightarrow \text{phosphatidylcholine} + \text{CMP}$$

An analogous pathway using CDP-ethanolamine is used to synthesize phosphatidylethanolamine (PE): namely, ethanolamine → phosphoethanolamine → CDP-ethanolamine → PE.

14.3.2.2 Phosphatidylinositol Synthesis. In contrast to the pathways of PC and PE synthesis, where their respective polar head groups, choline and ethanolamine, are activated at the expense of CTP, synthesis of PI involves CTP-dependent activation of the hydrophobic diacylglycerol moiety of phosphatidic acid, followed by condensation of CDP-diacylglycerol with inositol:

$$\text{phosphatidic acid} + \text{CTP} \rightarrow \text{CDP-diacylglycerol} + \text{PP}_\text{i}$$

$$\text{CDP-diacylglycerol} + \text{inositol} \rightarrow \text{phosphatidylinositol} + \text{CMP}$$

The inositol required for phosphatidylinositol synthesis is either obtained in the diet or synthesized by cyclization of glucose 6-phosphate. Major sites of inositol synthesis