BMLT-2nd SEM

Biochemistry & Biophysics: Paper-V, Unit-9

Topic: LIPID METABOLISM

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I. OVERVIEW

Fatty acids exist "free" in the body (that is, they are unesterified), and are also found as fatty acyl esters in more complex molecules, such as triacylglycerols. Low levels of free fatty acids occur in all tissues, but substantial amounts can sometimes be found in the plasma, particularly during fasting. Plasma free fatty acids (transported on serum albumin) are in route from their point of origin (triacylglycerol of adipose tissue or circulating lipoproteins) to their site of consumption (most tissues). Free fatty acids can be oxidized by many tissues — particularly liver and muscle—to provide energy. Fatty acids are also structural components of membrane lipids, such as phospholipids and glycolipids. Fatty acids are attached to certain intra cellular proteins to enhance the ability of those proteins to associate with membranes. Fatty acids are also precursors of the hormone-like prostaglandins. Esterified fatty acids, in the form of triacylglycerols stored in adipose cells, serve as the major energy reserve of the body. Figure 16.1 illustrates the metabolic pathways of fatty acid synthesis and degradation, and their relationship to carbohydrate metabolism.

II. STRUCTURE OF FATTY ACIDS

A fatty acid consists of a hydrophobic hydrocarbon chain with a terminal carboxyl group that has a pKa of about 4.8 (Figure 16.2). At physiologic pH, the terminal carboxyl group (–COOH) ionizes, becoming –COO–. This anionic group has an affinity for water, giving the fatty acid its amphipathic nature (having both a hydrophilic and a hydro- phobic region). However, for long-chain fatty acids (LCFAs), the hydrophobic portion is predominant. These molecules are highly waterinsoluble, and must be transported in the circulation in association with protein. More than 90% of the fatty acids found in plasma are in the form of fatty acid esters (primarily triacylglycerol, cholesteryl esters, and phospholipids) contained in circulating lipoprotein particles. Unesterified (free) fatty acids are transported in the circulation in association with albumin.

Figure 16.2
Structure of a fatty acid.

A. Saturation of fatty acids

Fatty acid chains may contain no double bonds—that is, be saturated—or contain one or more double bonds—that is, be mono- or polyunsaturated. When double bonds are present, they are nearly always in the cis rather than in the trans configuration. The introduction of a cis double bond causes the fatty acid to bend or "kink" at that position (Figure 16.3). If the fatty acid has two or more double bonds, they are always spaced at three-carbon intervals.

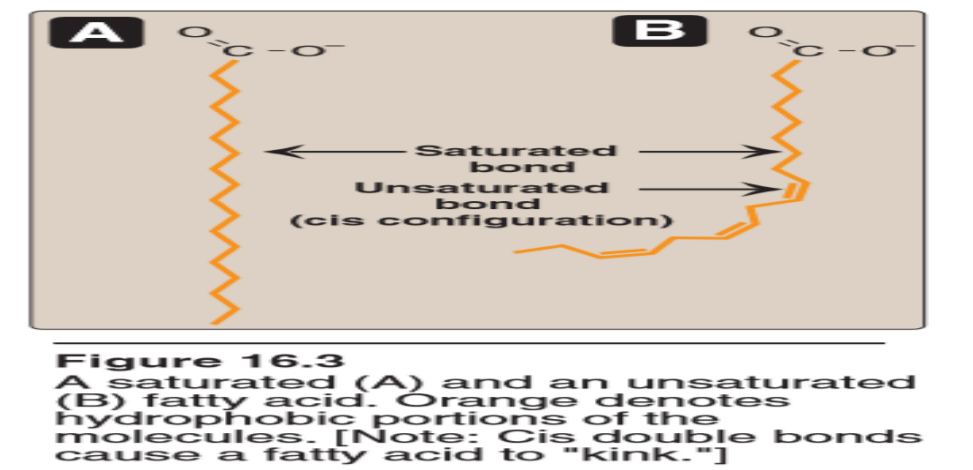

Figure 16.3
A saturated (A) and an unsaturated (B) fatty acid. Orange denotes hydrophobic portions of the molecules. [Note: Cis double bonds cause a fatty acid to "kink."]

B. Chain lengths of fatty acids

The common names and structures of some fatty acids of physiologic importance are listed in Figure 16.4. The carbon atoms are numbered, beginning with the carboxyl carbon as carbon 1. The number before the colon indicates the number of carbons in the chain, and those after the colon indicate the numbers and positions (relative to the carboxyl end) of double bonds. For example, as shown in Figure 16.5A, arachidonic acid, 20:4(5,8,11,14), is 20 carbons long and has 4 d ouble bonds (between carbons 5–6, 8–9, 11–12, and 14–15). [Note: Carbon 2, the carbon to which the carboxyl group is attached, is also called the α-carbon, carbon 3 is the β-carbon, and carbon 4 is the γ-carbon. The carbon of the terminal methyl group is called the ω-carbon regardless of the chain length.] The double bonds in a fatty acid can also be denoted relative to the ω (or methyl-terminal) end of the chain. Arachidonic acid is referred to as an ω-6 fatty acid acid (also an n-6, Figure 16.5A) because the terminal double bond is six bonds in from the ω end (Figure 16.5B). Another ω-6 fatty acid is the essential linoleic acid, 18:2(9,12). In contrast, α-linol enic acid, 18:3(9,12,15), is an essential ω-3 fatty acid.

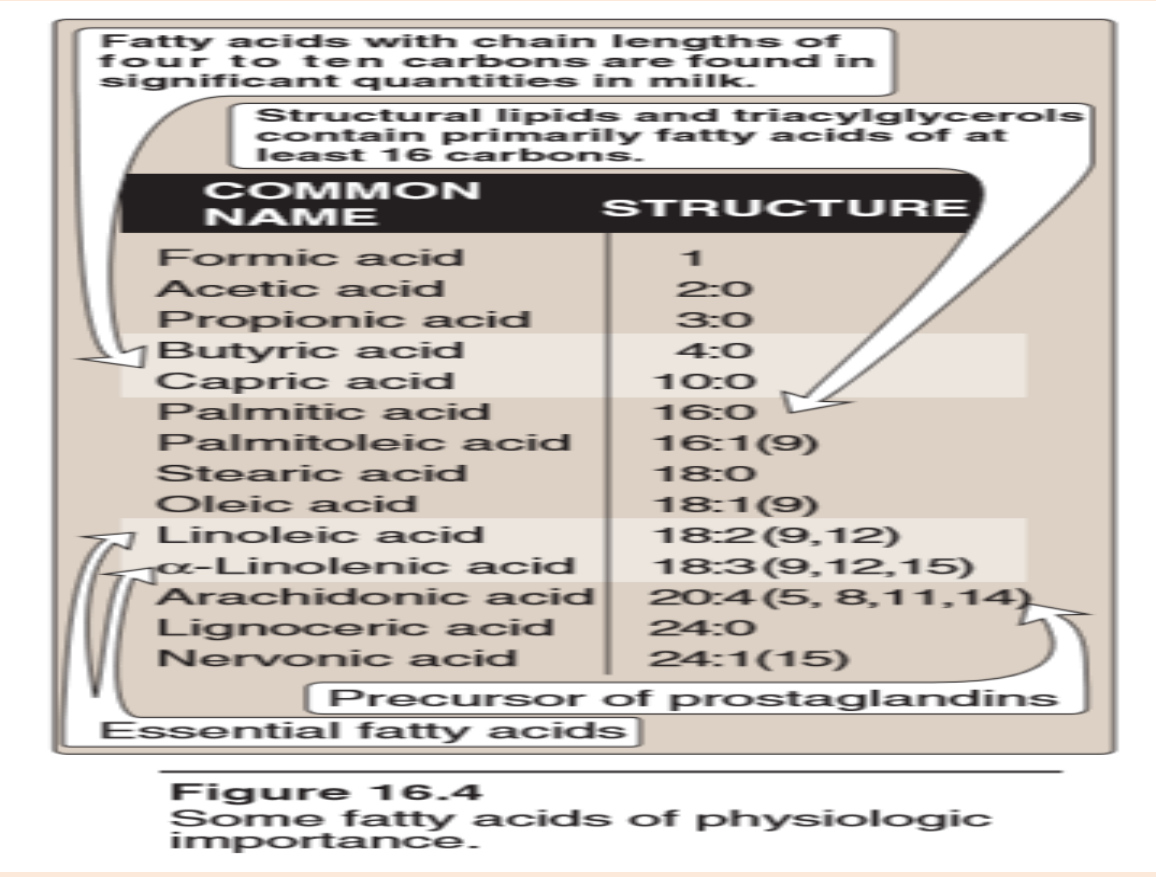

Figure 16.4
Some fatty acids of physiologic importance.

C. Essential fatty acids

Two fatty acids are dietary essentials in humans because of our inability to synthesize them: linoleic acid, which is the precursor of ω-6 arachidonic acid, the substrate for prostaglandin synthesis, and α-linolenic acid, the precursor of other ω-3 fatty acids important for growth and development. Plants provide us with the essential fatty acids.

III. MOBILIZATION OF STORED FATS AND OXIDATION OF FATTY ACIDS

Fatty acids stored in adipose tissue, in the form of neutral triacylglycerol(TAG), serve as the body's major fuel storage reserve. TAGs provide concentrated stores of metabolic energy because they are highly reduced and largely anhydrous. The yield from the complete oxidation of fatty acids to CO2 and H2O is 9 kcal/g fat (as compared to 4 kcal/g protein or carbohydrate).

A. Release of fatty acids from TAG

The mobilization of stored fat requires the hydrolytic release of fatty acids and glycerol from their TAG form. This process is initiated by hormone-sensitive lipase , which removes a fatty acid from carbon 1 and/or carbon 3 of the TAG. Additional lipases specific for diacylglycerol or monoacylglycerol remove the remaining fatty acid(s).

1. **Activation of hormone-sensitive lipase (HSL):** This enzyme is activated when phosphorylated by a 3 ',5 ' -cyclic AMP(cAMP)– dependent protein kinase . 3',5'-Cyclic AMP is produced in the adipocyte when one of several hormones (such as epinephrine or glucagon) binds to receptors on the cell membrane, and activates adenylyl cyclase (Figure 16.15). The process is similar to that of the activation of glycogen phosphorylase (see Figure 11.10). [Note: Because acetyl CoA carboxylase is inhibited by hormone-directed phosphorylation when the cAMP-mediated cascade is activated (see Figure 16.8), fatty acid synthesis is turned off when TAG degradation is turned on.] In the presence of high plasma levels of insulin and glucose, HSL is dephosphorylated, and becomes inactive.

2. Fate of glycerol: The glycerol released during TAG degradation cannot be metabolized by adipocytes because they apparently lack glycerol kinase . Rather, glycerol is transported through the blood to the liver, where it can be phosphorylated. The resulting glycerol phosphate can be used to form TAG in the liver, or can be converted to DHAP by reversal of the glycerol phosphate dehydrogenase reaction illustrated in Figure 16.13. DHAP can participate in glycolysis or gluconeogenesis.

3**. Fate of fatty acids:** The free (unesterified) fatty acids move through the cell membrane of the adipocyte, and bind to plasma albumin. They are transported to the tissues, enter cells, get activated to their CoA derivatives, and are oxidized for energy. Regardless of their levels, plasma free fatty acids (FFA) cannot be used for fuel by erythrocytes, which have no mitochondria. Brain, too, does not use fatty acids for energy, but the reasons are less clear.

B. *β-Oxidation of fatty acids*

The major pathway for catabolism of fatty acids is a mitochondrial pathway called β-oxidation, in which two-carbon fragments are successively removed from the carboxyl end of the fatty acyl CoA, producing acetyl CoA, NADH, and FADH2.

1. **Transport of long-chain fatty acids (LCFA) into the mitochondria:** After a LCFA enters a cell, it is converted in the cytosol to its CoA derivative by long-chain fatty acyl CoA synthetase (thiokinase), an enzyme of the outer mitochondrial membrane. Because β-oxida tion occurs in the mitochondrial matrix, the fatty acid must be transported across the inner mitochondrial membrane that is impermeable to CoA. Therefore, a specialized carrier transports the long-chain acyl group from the cytosol into the mitochondrial matrix. This carrier is carnitine, and this rate-limiting transport process is called the carnitine shuttle (Figure 16.16).

a. ***Steps in LCFA translocation:*** First, the acyl group is transferred from CoA to carnitine by carnitine palmitoyltransferase I (CPT-I)—an enzyme of the outer mitochondrial membrane. [Note: CPT-I is also known as CAT-I for carnitine acyltransferase I .] This reaction forms acyl carnitine, and regenerates free CoA. Second, the acylcarnitine is transported into the mitochondrial matrix in exchange for free carnitine by carnitine–acylcarnitine translocase . Carnitine palmitoyltransferase II (CPT-II , or CAT-II)—an enzyme of the inner mitochondrial membrane—catalyzes the transfer of the acyl group from carnitine to CoA in the mitochondrial matrix, thus regenerating free carnitine.

b. ***Inhibitor of the carnitine shuttle:*** Malonyl CoA inhibits CPT-I , thus preventing the entry of long-chain acyl groups into the mitochondrial matrix. Therefore, when fatty acid synthesis is occurring in the cytosol (as indicated by the presence of malonyl CoA), the newly made palmitate cannot be transferred into the mitochondria and degraded. [Note: Muscle, though it does not synthesize fatty acids, contains the mitochondrial isoform of acetyl CoA carboxylase (ACC2), allowing muscle to regulate β-oxidation.] Fatty acid oxidation is also regulated by the acetyl CoA to CoA ratio: as the ratio increases, the Co-A requiring thiolase reaction decreases (Figure 16.17).

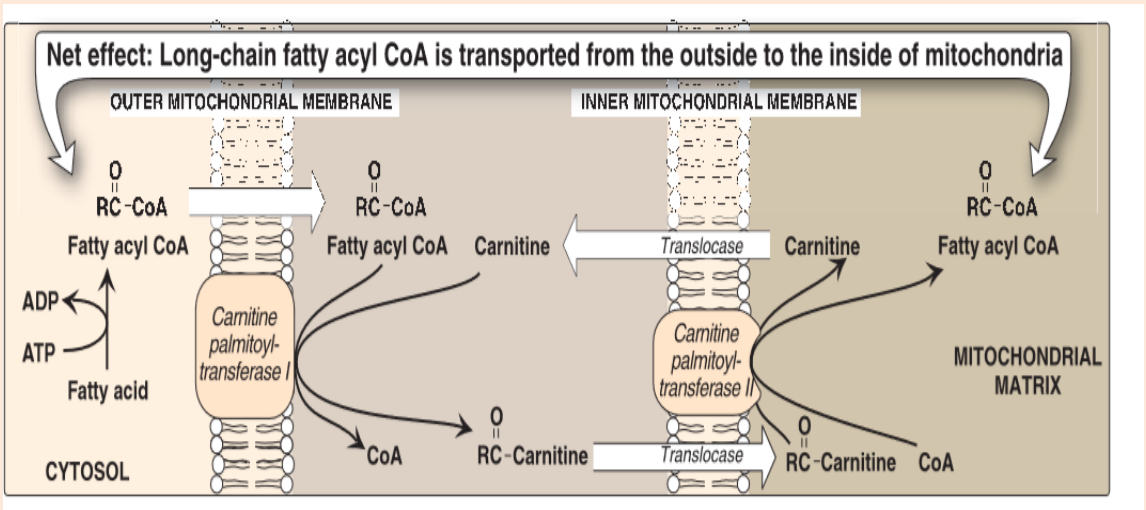

Figure 16.16
Carnitine shuttle. [Note: *Long-chain fatty acyl CoA synthetase* is in the outer mitochondrial membrane; active site faces the cytosol.]

c. ***Sources of carnitine:*** Carnitine can be obtained from the diet, where it is found primarily in meat products. Carnitine can also be synthesized from the amino acids lysine and methionine by an enzymatic pathway found in the liver and kidney but not in skeletal or heart muscle. Therefore, these latter tissues are totally dependent on uptake of carnitine provided by endogenous synthesis or the diet, and distributed by the blood.

d. ***Carnitine deficiencies***: Such deficiencies result in a decreased ability of tissues to use LCFA as a metabolic fuel. Secondary carnitine deficiency occurs in many situations, including: 1) in patients with liver disease causing decreased synthesis of carnitine; 2) in individuals suffering from malnutrition or those on strictly vegetarian diets; 3) in those with an increased requirement for carnitine as a result of, for example, pregnancy, severe infections, burns, or trauma; or 4) in those undergoing hemodialysis, which removes carnitine from the blood. Congenital deficiencies in one of the components of the carnitine palmitoyltransferase system, in renal tubular reabsorption of carnitine, or in carnitine uptake by cells cause primary carnitine deficiency.

2. Entry of short- and medium-chain fatty acids into the mitochondria:

Fatty acids shorter than 12 carbons can cross the inner mitochondrial membrane without the aid of carnitine or the CPT system. Once inside the mitochondria, they are activated to their CoA derivatives by matrix enzymes, and are oxidized.

3. Reactions of β-oxidation:

The first cycle of β-oxidation is shown in Figure 16.17. It consists of a sequence of four reactions involving the β-carbon (carbon 3) that results in shortening the fatty acid chain by two carbons. The steps include an oxidation that produces FADH2, a hydration step, a second oxidation that produces NADH, and a thiolytic cleavage that releases a molecule of acetyl CoA. Each step is catalyzed by enzymes with chain-length specificity. These four steps are repeated for saturated fatty acids of even-numbered carbon chains (n/2) – 1 times (where n is the number of carbons), each cycle producing an acetyl group plus one NADH and one FADH2. The final thiolytic cleavage produces two acetyl groups.

4. Energy yield from fatty acid oxidation:

The energy yield from the β-oxidation pathway is high. For example, the oxidation of a molecule of palmitoyl CoA to CO2 and H2O produces 8 acetyl CoA, 7 NADH, and 7 FADH2, from which 131 ATP can be generated; however, activation of the fatty acid requires 2 ATP. Thus, the net yield from palmitate is 129 ATP (Figure 16.18). A comparison of the processes of synthesis and degradation of long-chain saturated fatty acids with an even number of carbon atoms is provided in Figure 16.19.

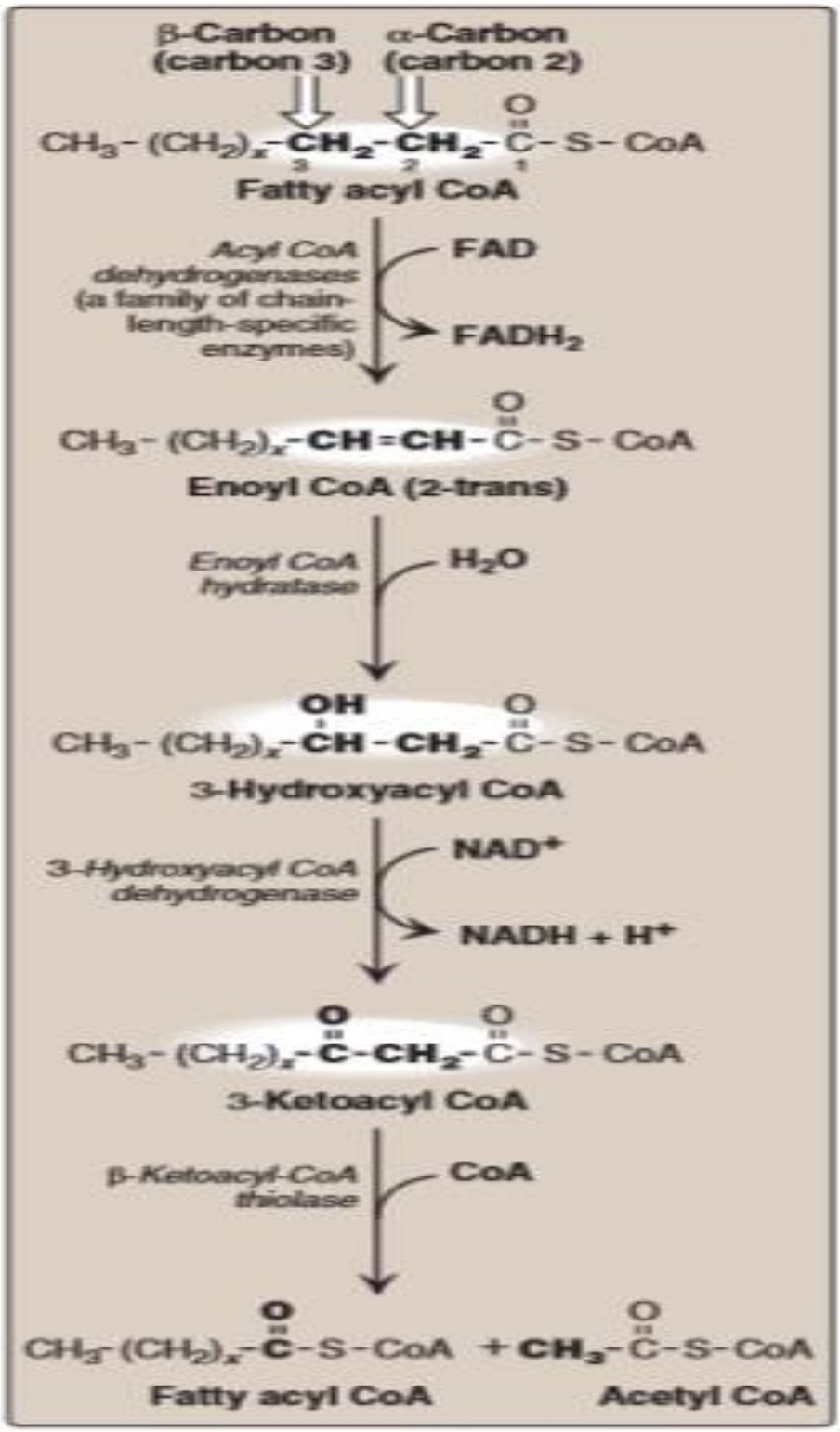

Figure 16.17
Enzymes involved in the β-oxidation of fatty acyl CoA. [Note: *Enoyl CoA hydratase* requires a trans double bond between carbon 2 and carbon 3.]

5. **Medium-chain fatty acyl CoA dehydrogenase (MCAD) deficiency:**

In mitochondria, there are four fatty acyl CoA dehydrogenase species, each with a specificity for either short-, medium-, long-, or very-long-chain fatty acids. MCAD deficiency, an autosomal recessive disorder, is one of the most common inborn errors of metabolism, and the most common inborn error of fatty acid oxidation, being found in 1:14,000 births worldwide, with a higher incidence in Northern Europeans. It results in decreased ability to oxidize fatty acids with six to ten carbons (these accumulate and can be measured in urine), and severe hypoglycemia (because the tissues must increase their reliance on glucose). Treatment includes avoidance of fasting. MCAD deficiency has been identified as the cause of some cases originally reported as sudden infant death syndrome (SIDS) or Reye syndrome.

6. Oxidation of fatty acids with an odd number of carbons:

The β-oxidation of a saturated fatty acid with an odd number of carbon atoms proceeds by the same reaction steps as that of fatty acids with an even number, until the final three carbons are reached. This compound, propionyl CoA, is metabolized by a three-step pathway (Figure 16.20).

a. ***Synthesis of D-methylmalonyl CoA*:** First, propionyl CoA is carboxylated, forming D-methylmalonyl CoA. The enzyme propionyl CoA carboxylase has an absolute requirement for the coenzyme biotin, as do most other carboxylases.

b. ***Formation of L-methylmalonyl CoA*:** Next, the D-isomer is converted to the L-form by the enzyme, methylmalonyl CoA racemase .

c***. Synthesis of succinyl CoA:*** Finally, the carbons of L-methylmalonyl CoA are rearranged, forming succinyl CoA, which can enter the tricarboxylic acid (TCA) cycle.The enzyme, methylmalonyl CoA mutase , requires a coenzyme form of vitamin B12 (deoxy-adenosylcobalamin) for its action. The mutase reaction is one of only two reactions in the body that require vitamin B12.

7. Oxidation of unsaturated fatty acids:

The oxidation of unsaturated fatty acids provides less energy than that of saturated fatty acids because unsaturated fatty acids are less highly reduced and, therefore, fewer reducing equivalents can be produced from these structures. Oxidation of monounsaturated fatty acids, such as 18:1(9) (oleic acid) requires one additional enzyme, 3,2-enoyl CoA isomerase , which converts the 3-trans derivative obtained after three rounds of β-oxidation to the 2-trans derivative required as a substrate by the enoyl CoA hydratase . Oxidation of polyunsaturated fatty acids, such as 18:2(9,12) (linoleic acid), requires an NADPH-dependent 2,4-dienoyl CoA reductase in addition to the isomerase .

8. β-Oxidation in the peroxisome:

Very-long-chain fatty acids (VLCFA), or those 22 carbons long or longer, undergo a preliminary β-oxidation in peroxisomes. The shortened fatty acid (linked to carnitine)

diffuses to a mitochondrion for further oxidation. In contrast to mitochondrial β-oxidation, the initial dehydrogenation in peroxisomes is catalyzed by an FAD-containing acyl CoA oxidase . The FADH2 produced is oxidized by molecular oxygen, which is reduced to H2O2; thus, no ATP is generated by this step. The H2O2 is reduced to H2O by catalase (see p. 148).

C. α-Oxidation of fatty acids Branched-chain, 20 carbon fatty acid, phytanic acid:

This is not a substrate for acyl CoA dehydrogenase because of the methyl group on its β carbon (Figure 16.21). Instead, it is hydroxylated at the α-carbon by phytanoyl CoA α hydroxylase (PhyH), carbon 1 is released as CO2, and the product, 19 carbon pristanic acid, is activated to its CoA derivative and undergoes β-oxidation. Refsum disease is a rare, autosomal recessive disorder caused by a deficiency of peroxisomal PhyH . This results in the accumulation of phytanic acid in the plasma and tissues. The symptoms are primarily neurologic, and the treatment involves dietary restriction to halt disease progression.

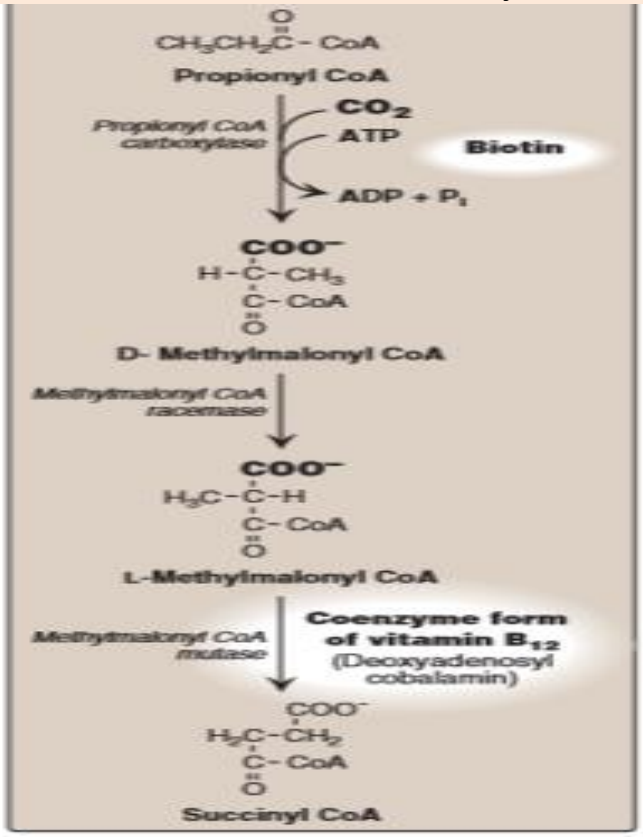

Figure 16.20
Metabolism of propionyl CoA.

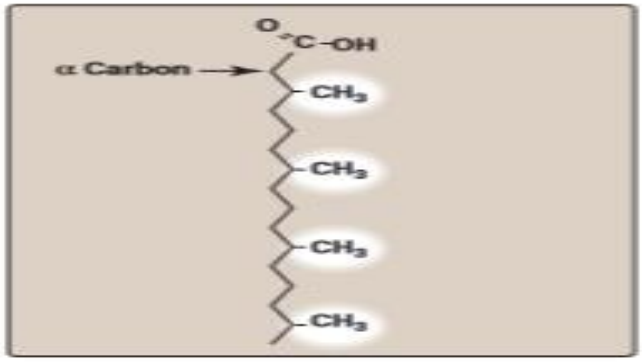

Figure 16.21
Phytanic acid—a branched-chain fatty acid.

KETONE BODIES: AN ALTERNATE FUEL FOR CELLS

Liver mitochondria have the capacity to convert acetyl CoA derived from fatty acid oxidation into ketone bodies. The compounds categorized as ketone bodies are acetoacetate, 3-hydroxybutyrate (also called β-hydroxybutyrate), and acetone (a non-metabolized side product, Figure 16.22).

Acetoacetate and 3-hydroxybutyrate are transported in the blood to the peripheral tissues. There they can be reconverted to acetyl CoA, which can be oxidized by the TCA cycle. Ketone bodies are important sources of energy for the peripheral tissues because

1) they are soluble in aqueous solution and, therefore, do not need to be incorporated into lipoproteins or carried by albumin as do the other lipids;

2) they are produced in the liver during periods when the amount of acetyl CoA present exceeds the oxidative capacity of the liver; and

3) they are used in proportion to their concentration in the blood by extrahepatic tissues, such as the skeletal and cardiac muscle and renal cortex. Even the brain can use ketone bodies to help meet its energy needs if the blood levels rise sufficiently; thus, ketone bodies spare glucose.This is particularly important during prolonged periods of fasting .

A. Synthesis of ketone bodies by the liver: ketogenesis

During a fast, the liver is flooded with fatty acids mobilized from adipose tissue. The resulting elevated hepatic acetyl CoA produced primarily by fatty acid degradation inhibits pyruvate dehydrogenase, and activates pyruvate carboxylase. The OAA thus produced is used by the liver for gluconeogenesis rather than for the TCA cycle. Therefore, acetyl CoA is channeled into ketone body synthesis. [Note: Fatty acid oxidation decreases the NAD+ to NADH ratio, and the rise in NADH shifts OAA to malate.This pushes acetyl CoA away from gluconeogenesis and into ketogenesis (Figure 16.24).]

1**. Synthesis of 3-hydroxy-3-methylglutaryl (HMG) CoA:** The first synthetic step, formation of acetoacetyl CoA, occurs by reversal of the thiolase reaction of fatty acid oxidation. Mitochondrial HMG CoA synthase combines a third molecule of acetyl CoA with acetoacetyl CoA to produce HMG CoA. HMG CoA synthase is the rate-limiting step in the synthesis of ketone bodies, and is present in significant quantities only in the liver.

2. Synthesis of the ketone bodies: HMG CoA is cleaved to produce acetoacetate and acetyl CoA, as shown in Figure 16.22. Acetoacetate can be reduced to form 3-hydroxybutyrate with NADH as the hydrogen donor. Acetoacetate can also spontaneously decarboxylate in the blood to form acetone—a volatile, biologically nonmetabolized compound that can be released in the breath. The equilibrium between acetoacetate and 3-hydroxybutyrate is determined by the NAD+/NADH ratio. Because this ratio is low during fatty acid oxidation, 3-hydroxyb utyrate synthesis is favored.

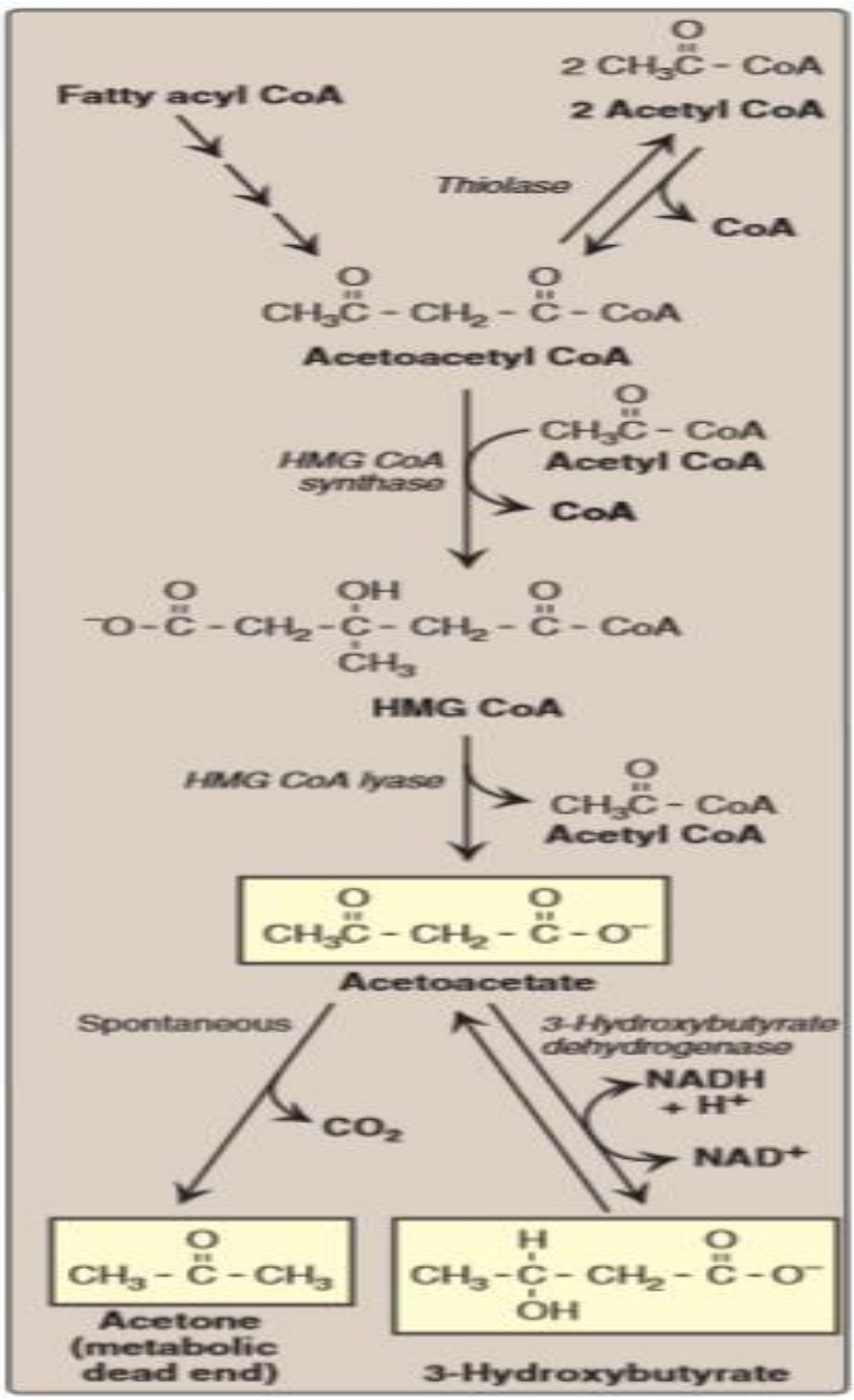

Figure 16.22
Synthesis of ketone bodies. HMG – hydroxymethylglutaryl CoA.

B. Use of ketone bodies by the peripheral tissues: **ketolysis**

Although the liver constantly synthesizes low levels of ketone bodies, their production becomes much more significant during fastingwhen ketone bodies are needed to provide energy to the peripheral tissues. 3-Hydroxybutyrate is oxidized to acetoacetate by 3-hydroxy butyrate dehydrogenase , producing NADH (Figure 16.23). Acetoa cetate is then provided with a CoA molecule taken from succinyl CoA by succinyl CoA:acetoacetate CoA transferase (thiophorase). This reaction is reversible, but the product, acetoacetyl CoA, is

actively removed by its conversion to two acetyl CoA. Extrahepatic tissues, including the brain but excluding cells lacking mitochondria (for example, red blood cells), efficiently oxidize acetoacetate and 3-hydroxybutyrate in this manner. In contrast, although the liver actively produces ketone bodies, it lacks thiophorase and, therefore, is unable to use ketone bodies as fuel.

C. Excessive production of ketone bodies in diabetes mellitus

When the rate of formation of ketone bodies is greater than the rate of their use, their levels begin to rise in the blood (ketonemia) and, eventually, in the urine (ketonuria). This is seen most often in cases of uncontrolled, type 1 diabetes mellitus. In diabetic individuals with severe ketosis, urinary excretion of the ketone bodies may be as high as 5,000 mg/24 hr, and the blood concentration may reach 90 mg/dl (versus less than 3 mg/dl in normal individuals). A frequent symptom of diabetic ketoacidosis is a fruity odor on the breath, which results from increased production of acetone. An elevation of the ketone body concentration in the blood results in acidemia.

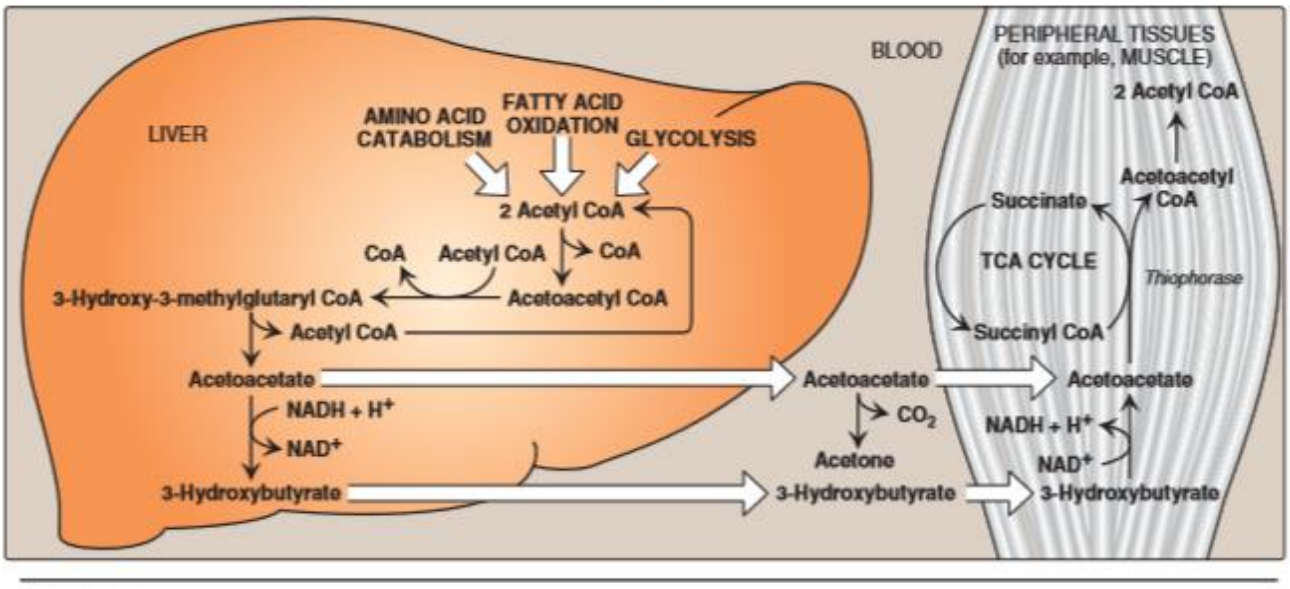

Figure 16.23
Ketone body synthesis in the liver and use in peripheral tissues. [Note: *Thiophorase* is also known as *succinyl CoA: acetoacetate CoA transferase.*]

SYNTHESIS OF CHOLESTEROL

Cholesterol is synthesized by virtually all tissues in humans, although liver, intestine, adrenal cortex, and reproductive tissues, including ovaries, testes, and placenta, make the largest contributions to the body's cholesterol pool. As with fatty acids, all the carbon atoms in cholesterol are provided by acetate, and NADPH provides the reducing equivalents. The pathway is endergonic, being driven by hydrolysis of the high-energy thioester bond of acetyl coenzyme A (CoA) and the terminal phosphate bond of adenosine triphosphate (ATP).

Synthesis requires enzymes in both the cytosol and the membrane of the smooth endoplasmic reticulum (ER). The pathway is responsive to changes in cholesterol concentration, and regulatory mechanisms exist to balance the rate of cholesterol synthesis within the body against the rate of cholesterol excretion. An imbalance in this regulation can lead to an elevation in circulating levels of plasma cholesterol, with the potential for vascular disease.

A. *Synthesis of 3-hydroxy-3-methylglutaryl (HMG) CoA*

The first two reactions in the cholesterol synthetic pathway are similar to those in the pathway that produces ketone bodies (see Figure 16.22, p. 196). They result in the production of HMG CoA (Figure 18.3). First, two acetyl CoA molecules condense to form acetoacetyl CoA. Next, a third molecule of acetyl CoA is added, producing HMG CoA, a six-carbon compound.

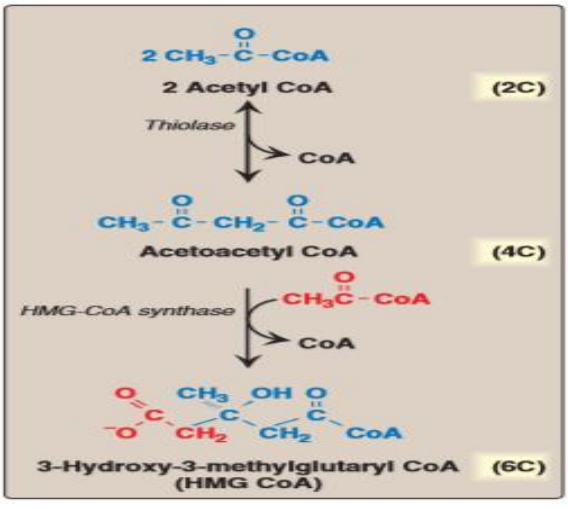

Figure 18.3
Synthesis of HMG CoA.

B. Synthesis of mevalonate

The next step, the reduction of HMG CoA to mevalonate, is catalyzed by HMG CoA reductase , and is the rate-limiting and key regulated step in cholesterol synthesis. It occurs in the cytosol, uses two molecules of NADPH as the reducing agent, and releases CoA, making the reaction irreversible (Figure 18.4).

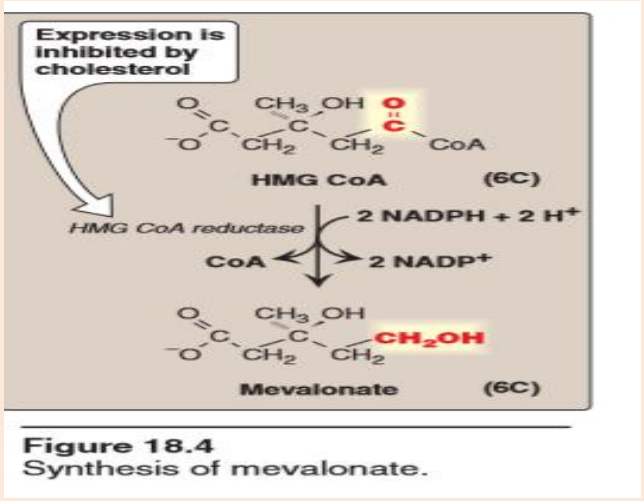

Figure 18.4
Synthesis of mevalonate.

C. Synthesis of cholesterol

The reactions and enzymes involved in the synthesis of cholesterol from mevalonate are illustrated in Figure 18.5. [Note: The numbers shown in brackets below correspond to numbered reactions shown in this figure.]

[1] Mevalonate is converted to 5-pyrophosphomevalonate in two steps, each of which transfers a phosphate group from ATP.

[2] A five-carbon isoprene unit—isopentenyl pyrophosphate (IPP)— is formed by the decarboxylation of 5-pyrophosphomevalonate. The reaction requires ATP.

[3] IPP is isomerized to 3,3-dimethylallyl pyrophosphate (DPP).

[4] IPP and DPP condense to form ten-carbon geranyl pyro phosphate (GPP).

[5] A second molecule of IPP then condenses with GPP to form 15carbon farnesyl pyrophosphate (FPP). [Note: Covalent attachment of farnesyl to proteins, a process known as "prenylation," is one mechanism for anchoring proteins to plasma membranes.]

[6] Two molecules of FPP combine, releasing pyrophosphate, and are reduced, forming the 30-carbon compound squalene.

[7] Squalene is converted to the sterol lanosterol by a sequence of reactions catalyzed by ER-associated enzymes that use molecular oxygen and NADPH. The hydroxylation of squalene triggers the cyclization of the structure to lanosterol.

[8] The conversion of lanosterol to cholesterol is a multistep process, resulting in the shortening of the carbon chain from 30 to 27 carbons, removal of the two methyl groups at carbon 4, migration of the double bond from carbon 8 to carbon 5, and reduction of the double bond between carbon 24 and carbon 25.

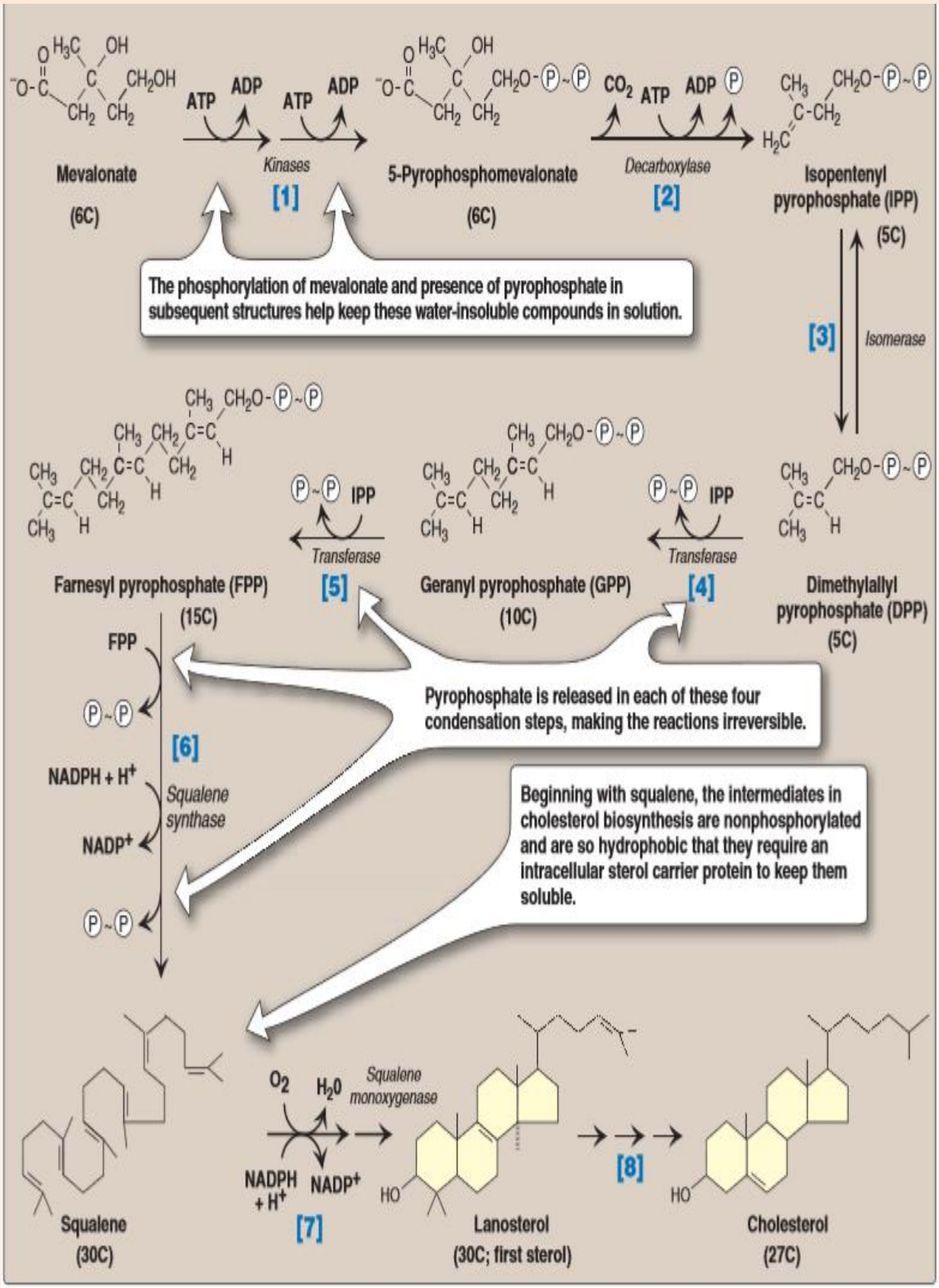

Figure 18.5
Synthesis of cholesterol from mevalonate.

Assisssgnment Questions:

1. Explain the structure of fatty acids.
2. Differentiate between saturated and unsaturated fatty acids. Give examples for each.
3. What is essential fatty acids. Give examples.
4. Write about β-Oxidation of fatty acids with diagram..
5. What do you mean by carnitine shuttle with diagram..
6. Explain about β-Oxidation of fatty acids with an odd number of carbons with diagram..
7. Explain the process of ketone bodies synthesis with diagram..
8. Define ketolysis.
9. Explain the overall process of cholesterol synthesis with diagram.
10. Write about the Synthesis of 3-hydroxy-3-methylglutaryl (HMG) CoA with diagram.