

<b>COURSE CODE:</b>	<b>BCH 303</b>
<b>COURSE TITLE:</b>	<b>Metabolism of lipids</b>
<b>NUMBER OF UNITS:</b>	<b>2 Units</b>
<b>COURSE DURATION:</b>	<b>Two hours per week</b>

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### **COURSE DETAILS:**

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### **COURSE CONTENT:**

A survey of structure, functions, biosynthesis and catabolism of different classes of lipids. The regulatory aspects of biosynthesis and catabolism of the lipids, especially in relation to homeostasis in the organism will be stressed. The lipids include: fatty acids, glycerides, phosphoglycerides, waxes, prostaglandins, terpenoids and steroids such as bile acids, steroid hormones, carotenoids and vitamin A, vitamin D and terpenoid quinines. Biological membranes.

### **COURSE REQUIREMENTS:**

This is a compulsory course for all 300L Biochemistry students. In view of this, the students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

### **READING LIST:**

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- 1 Bohinski, R.C. 1983. Modern concepts in Biochemistry. 4<sup>th</sup> Edition. Allyn and Bacon.
- 2 Murray, R.K., Granner, D.K., Mayes, P.A. and Rodwell, V.W. 2003. Harper's Illustrated Biochemistry. 26<sup>th</sup> Edition. McGraw-Hill.

## LECTURE NOTES

### INTRODUCTION

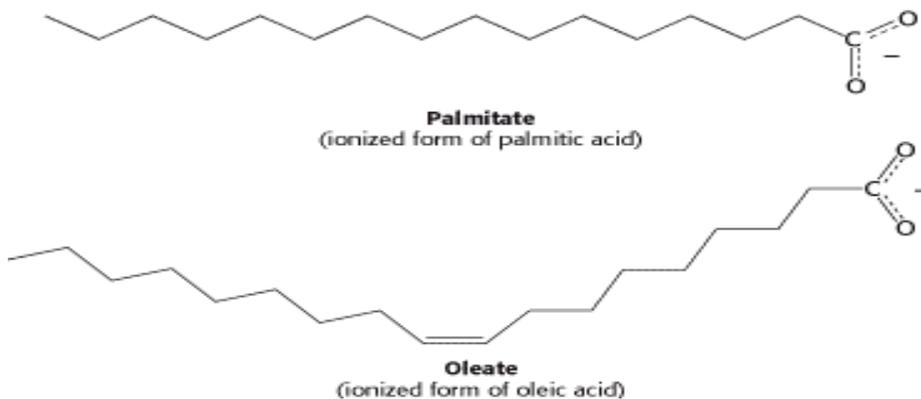
**Lipids** are a class of biological molecules defined by low solubility in water and high solubility in nonpolar solvents. As molecules that are largely hydrocarbon in nature, lipids represent highly reduced forms of carbon and, upon oxidation in metabolism, yield large amounts of energy. Lipids are thus the molecules of choice for metabolic energy storage.

The lipids found in biological systems are either **hydrophobic** (containing only nonpolar groups) or **amphipathic**, which means they possess both polar and nonpolar groups. The hydrophobic nature of lipid molecules allows membranes to act as effective barriers to more polar molecules.

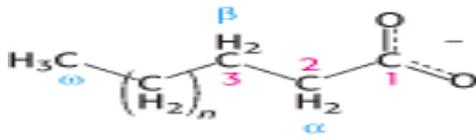
### Fatty Acids

A **fatty acid** is composed of a long hydrocarbon chain (“tail”) and a terminal carboxyl group (or “head”). The carboxyl group is normally ionized under physiological conditions. Fatty acids occur in large amounts in biological systems, but rarely in the free, uncomplexed state. They typically are esterified to glycerol or other backbone structures. Most of the fatty acids found in nature have an even number of carbon atoms (usually 14 to 24). Certain marine organisms, however, contain substantial amounts of fatty acids with odd numbers of carbon atoms. Fatty acids are either **saturated** (all carbon–carbon bonds are single bonds) or **unsaturated** (with one or more double bonds in the hydrocarbon chain). If a fatty acid has a single double bond, it is said to be **monounsaturated**, and if it has more than one, **polyunsaturated**. Fatty acids can be named or described in at least three ways, as listed in the Table below.

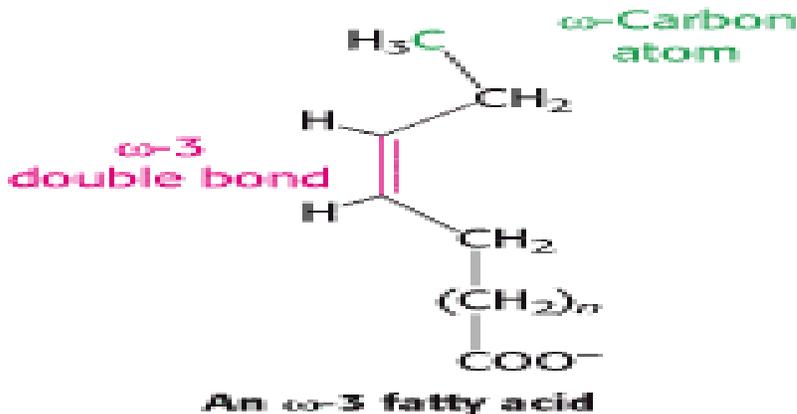
The systematic name for a fatty acid is derived from the name of its parent hydrocarbon by the substitution of *oic* for the final *e*. For example, the C18 saturated fatty acid is called *octadecanoic acid* because the parent hydrocarbon is octadecane. A C18 fatty acid with one double bond is called *octadecenoic acid*; with two double bonds, *octadecadienoic acid*; and with three double bonds, *octadecatrienoic acid*. The notation 18:0 denotes a C18 fatty acid with no double bonds, whereas 18:2 signifies that there are two double bonds. The structures of the ionized forms of two common fatty acids palmitic acid (C16, saturated) and oleic acid (C18, monounsaturated) are shown in Figure below.



*They are* numbered starting at the carboxyl terminus, as shown in the margin. Carbon atoms 2 and 3 are often referred to as  $\alpha$  and  $\beta$ , respectively. The methyl carbon atom at the distal end of the chain is called the  $\omega$ -carbon atom.



Alternatively, the position of a double bond can be denoted by counting from the distal end, with the  $\omega$ -carbon atom (the methyl carbon) as number 1. An  $\omega$ -3 fatty acid, for example, has the structure shown in the margin.



**Table showing some Common Biological Fatty Acids**

Number of Carbons	Common Name	Systematic Name	Symbol	Structure
<b>Saturated fatty acids</b>				
12	Lauric acid	Dodecanoic acid	12:0	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$
14	Myristic acid	Tetradecanoic acid	14:0	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$
16	Palmitic acid	Hexadecanoic acid	16:0	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
18	Stearic acid	Octadecanoic acid	18:0	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
20	Arachidic acid	Eicosanoic acid	20:0	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$
22	Behenic acid	Docosanoic acid	22:0	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$
24	Lignoceric acid	Tetracosanoic acid	24:0	$\text{CH}_3(\text{CH}_2)_{22}\text{COOH}$
<b>Unsaturated fatty acids (all double bonds are <i>cis</i>)</b>				
16	Palmitoleic acid	9-Hexadecenoic acid	16:1	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
18	Oleic acid	9-Octadecenoic acid	18:1	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$
18	Linoleic acid	9,12-Octadecadienoic acid	18:2	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_2(\text{CH}_2)_6\text{COOH}$
18	$\alpha$ -Linolenic acid	9,12,15-Octadecatrienoic acid	18:3	$\text{CH}_3\text{CH}_2(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_6\text{COOH}$
18	$\gamma$ -Linolenic acid	6,9,12-Octadecatrienoic acid	18:3	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_3(\text{CH}_2)_3\text{COOH}$
20	Arachidonic acid	5,8,11,14-Eicosatetraenoic acid	20:4	$\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_4(\text{CH}_2)_2\text{COOH}$
24	Nervonic acid	15-Tetracosenoic acid	24:1	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_{13}\text{COOH}$

Unsaturated fatty acids are slightly more abundant in nature than saturated fatty acids, especially in higher plants. The most common unsaturated fatty acid is **oleic acid**, or 18:1(9), with the number in parentheses indicating that the double bond is between carbons 9 and 10. The number of double bonds in an unsaturated fatty acid varies typically from one to four, but, in the fatty acids found in most bacteria, this number rarely exceeds one.

The double bonds found in fatty acids are nearly always in the *cis* configuration. As shown in Figure below, this causes a bend or “kink” in the fatty acid chain. This bend has very important consequences for the structure of biological membranes. Saturated fatty acid chains can pack closely together to

form ordered, rigid arrays under certain conditions, but unsaturated fatty acids prevent such close packing and produce flexible, fluid aggregates.

Some fatty acids are not synthesized by mammals and yet are necessary for normal growth and life.

These *essential fatty acids* include **linoleic** and  **$\alpha$ -linolenic acids**. These must be obtained by mammals in their diet (specifically from plant sources). **Arachidonic acid**, which is not found in plants, can only be synthesized by mammals from linoleic acid. At least one function of the essential fatty acids is to serve as a precursor for the synthesis of **eicosanoids**, such as *prostaglandins*, a class of compounds that exert hormone-like effects in many physiological processes.

In addition to unsaturated fatty acids, several other modified fatty acids are found in nature. Microorganisms, for example, often contain branched-chain fatty acids, such as **tuberculostearic acid**. When these fatty acids are incorporated in membranes, the methyl group constitutes a local structural perturbation in a manner similar to the double bonds in unsaturated fatty acids. Some bacteria also synthesize fatty acids containing cyclic structures such as cyclopropane, cyclopropene, and even cyclopentane rings.

The properties of fatty acids and of lipids derived from them are markedly dependent on chain length and degree of saturation. Unsaturated fatty acids have lower melting points than saturated fatty acids of the same length. For example, the melting point of stearic acid is 69.6°C, whereas that of oleic acid (which contains one *cis* double bond) is 13.4°C. The melting points of polyunsaturated fatty acids of the C18 series are even lower. Chain length also affects the melting point, as illustrated by the fact that the melting temperature of palmitic acid (C16) is 6.5 degrees lower than that of stearic acid (C18). Thus, *short chain length and unsaturation enhance the fluidity of fatty acids and of their derivatives*.

### ***Fatty Acids in Food: Saturated Versus Unsaturated***

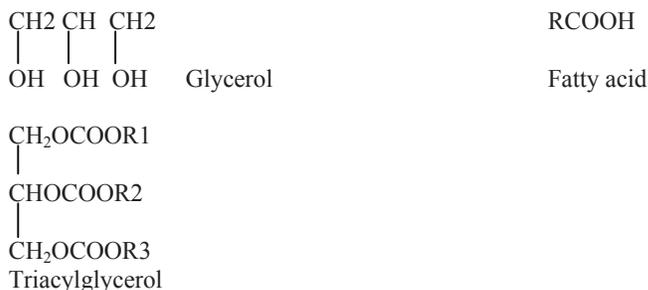
Fats consumed in the modern human diet vary widely in their fatty acid compositions. The table below provides a brief summary. The incidence of cardiovascular disease is correlated with diets high in saturated fatty acids. By contrast, a diet that is relatively higher in unsaturated fatty acids (especially polyunsaturated fatty acids) may reduce the risk of heart attacks and strokes. Corn oil, abundant in the United States and high in (polyunsaturated) linoleic acid, is an attractive dietary choice. *Margarine* made from corn, safflower, or sunflower oils is much lower in saturated fatty acids than is butter, which is made from milk fat. However, margarine may present its own health risks. Its fatty acids contain *trans*-double bonds (introduced by the hydrogenation process), which may also contribute to cardiovascular disease. Although vegetable oils usually contain a higher proportion of unsaturated fatty acids than do animal oils and fats, several plant oils are actually high in saturated fats. Palm oil is low in polyunsaturated fatty acids and particularly high in (saturated) palmitic acid (whence the name *palmitic*). Coconut oil is particularly high in lauric and myristic acids (both saturated) and contains very few unsaturated fatty acids.

Some of the fatty acids found in the diets of developed nations (often 1 to 10 g of daily fatty acid intake) are *trans* fatty acids—fatty acids with one or more double bonds in the *trans* configuration. Some of these derive from dairy fat and ruminant meats, but the bulk are provided by partially hydrogenated vegetable or fish oils. Substantial evidence now exists to indicate that *trans* fatty acids may have deleterious health consequences. Numerous studies have shown that *trans* fatty acids raise plasma LDL cholesterol levels when exchanged for *cis*-unsaturated fatty acids in the diet and may also lower HDL cholesterol levels and raise triglyceride levels. The effects of *trans* fatty acids on LDL, HDL, and cholesterol levels are similar to those of saturated fatty acids, and diets aimed at reducing the risk of coronary heart disease should be low in both *trans* and saturated fatty acids.

### **Triacylglycerols**

A significant number of the fatty acids in plants and animals exist in the form of **triacylglycerols** (also called **triglycerides**). Triacylglycerols are a major energy reserve and the principal neutral

derivatives of glycerol found in animals. These molecules consist of a glycerol esterified with three fatty acids.

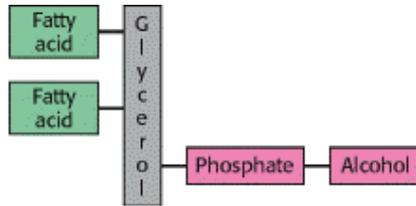


If all three fatty acid groups are the same, the molecule is called a simple triacylglycerol. Examples include **tristearoylglycerol** (common name *tristearin*) and **trioleoylglycerol** (*triolein*). Mixed triacylglycerols contain two or three different fatty acids. Triacylglycerols in animals are found primarily in the adipose tissue (body fat), which serves as a depot or storage site for lipids. Monoacylglycerols and diacylglycerols also exist, but are far less common than the triacylglycerols. Most natural plant and animal fat is composed of mixtures of simple and mixed triacylglycerols. Acylglycerols can be hydrolyzed by heating with acid or base or by treatment with lipases. Hydrolysis with alkali is called **saponification** and yields salts of free fatty acids and glycerol. This is how **soap** (a metal salt of an acid derived from fat) was made by our ancestors. One method used potassium hydroxide (*potash*) leached from wood ashes to hydrolyze animal fat (mostly triacylglycerols). (The tendency of such soaps to be precipitated by Mg and Ca ions in hard water makes them less useful than modern detergents.) When the fatty acids esterified at the first and third carbons of glycerol are different, the second carbon is asymmetric.

The various acylglycerols are normally soluble in benzene, chloroform, ether, and hot ethanol. Although triacylglycerols are insoluble in water, mono- and diacylglycerols readily form organized structures in water (discussed later), owing to the polarity of their free hydroxyl groups. Triacylglycerols are rich in highly reduced carbons and thus yield large amounts of energy in the oxidative reactions of metabolism. Complete oxidation of 1 g of triacylglycerols yields about 38 kJ of energy, whereas proteins and carbohydrates yield only about 17 kJ/g. Also, their hydrophobic nature allows them to aggregate in highly anhydrous forms, whereas polysaccharides and proteins are highly hydrated. For these reasons, triacylglycerols are the molecules of choice for energy storage in animals. Body fat (mainly triacylglycerols) also provides good insulation.

### Glycerophospholipids

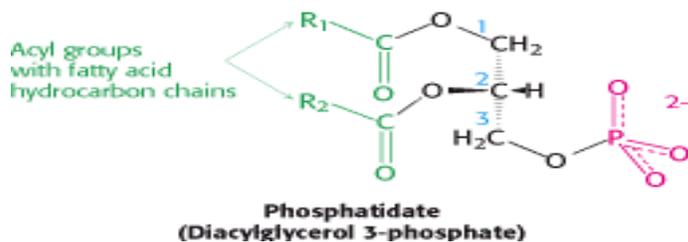
A 1,2-diacylglycerol that has a phosphate group esterified at carbon atom 3 of the glycerol backbone is a **glycerophospholipid**, also known as a *phosphoglyceride* or a *glycerol phosphatide*. These lipids form one of the largest classes of natural lipids and one of the most important. They are essential components of cell membranes and are found in small concentrations in other parts of the cell. It should be noted that all glycerophospholipids are members of the broader class of lipids known as **phospholipids**. The numbering and nomenclature of glycerophospholipids present a dilemma in that the number 2 carbon of the glycerol backbone of a phospholipid is asymmetric. It is possible to name these molecules either as D- or L-isomers. Thus, glycerol phosphate itself can be referred to either as D-glycerol-1-phosphate or as L-glycerol-3-phosphate. Instead of naming the glycerol phosphatides in this way, biochemists have adopted the *stereospecific numbering* or *sn-* system. In this system, the *pro-S* position of a prochiral atom is denoted as the *1-position*, the prochiral atom as the *2-position*, and so on. When this scheme is used, the prefix *sn-* precedes the molecule name (glycerol phosphate in this case) and distinguishes this nomenclature from other approaches. In this way, the glycerol phosphate in natural phosphoglycerides is named *sn-glycerol-3-phosphate*.

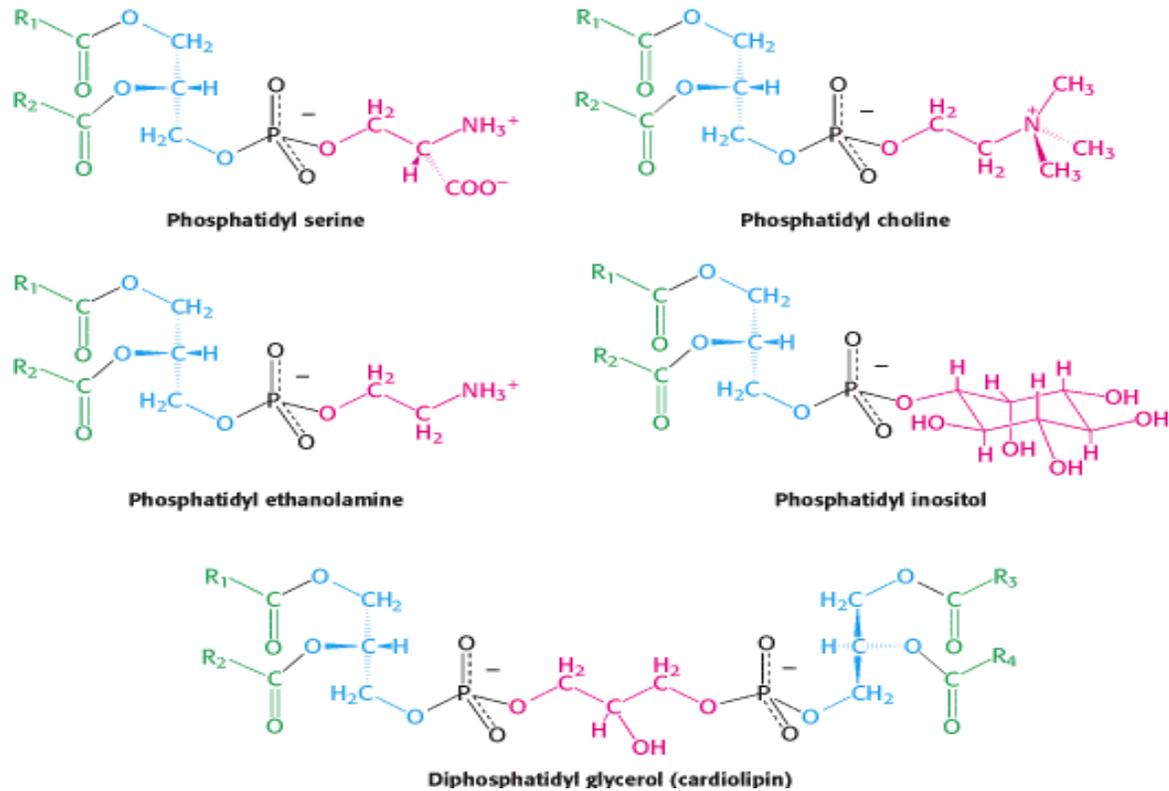


**Schematic Structure of a Phospholipid.**

### The Most Common Phospholipids

**Phosphatidic acid**, the parent compound for the glycerol-based phospholipids consists of *sn*-glycerol-3-phosphate, with fatty acids esterified at the 1- and 2-positions. Phosphatidic acid is found in small amounts in most natural systems and is an important intermediate in the biosynthesis of the more common glycerophospholipids. In these compounds, a variety of polar groups are esterified to the phosphoric acid moiety of the molecule. The phosphate, together with such esterified entities, is referred to as a “head” group. Phosphatides with choline or ethanolamine are referred to as **phosphatidylcholine** (known commonly as **lecithin**) or **phosphatidylethanolamine**, respectively. These phosphatides are two of the most common constituents of biological membranes. Other common *head groups* found in phosphatides include glycerol, serine, and inositol. Another kind of glycerol phosphatide found in many tissues is **diphosphatidylglycerol**. First observed in heart tissue, it is also called **cardiolipin**. In cardiolipin, a phosphatidylglycerol is esterified through the C-1 hydroxyl group of the glycerol moiety of the head group to the phosphoryl group of another phosphatidic acid molecule.

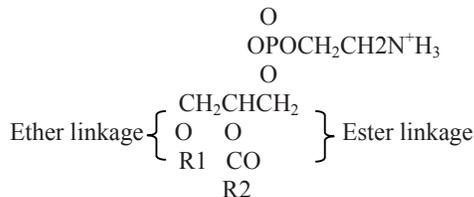




Phosphatides exist in many different varieties, depending on the fatty acids esterified to the glycerol group. As we shall see, the nature of the fatty acids can greatly affect the chemical and physical properties of the phosphatides and the membranes that contain them. In most cases, glycerol phosphatides have a saturated fatty acid at position 1 and an unsaturated fatty acid at position 2 of the glycerol. Thus, **1-stearoyl-2-oleoyl-phosphatidylcholine** is a common constituent in natural membranes, but **1-linoleoyl-2 palmitoylphosphatidylcholine** is not.

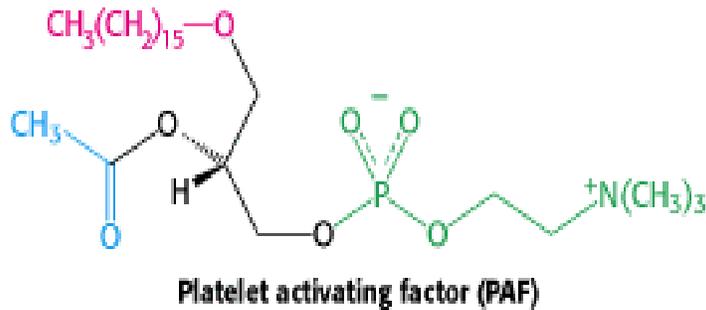
### Ether Glycerophospholipids

**Ether glycerophospholipids** possess an ether linkage instead of an acyl group at the C-1 position of glycerol.

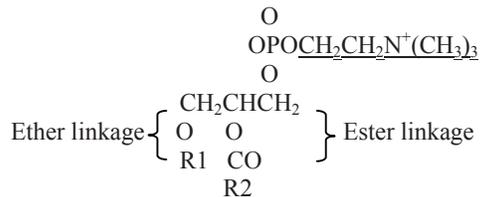


1-alkyl-2-acyl phosphatidylethanolamine, an ether glycerophospholipid.

One of the most versatile biochemical signal molecules found in mammals is **platelet activating factor**, or **PAF**, a unique ether glycerophospholipid called 1-alkyl 2-acetyl-phosphatidylcholine. The alkyl group at C-1 of PAF is typically a 16-carbon chain, but the acyl group at C-2 is a 2-carbon acetate unit. By virtue of this acetate group, PAF is much more water-soluble than other lipids, allowing PAF to function as a soluble messenger in signal transduction.



**Plasmalogens** are ether glycerophospholipids in which the alkyl moiety is *cis*- $\alpha,\beta$ -unsaturated. Common plasmalogen head groups include choline, ethanolamine, and serine. These lipids are referred to as phosphatidal choline, phosphatidal ethanolamine, and phosphatidal serine.



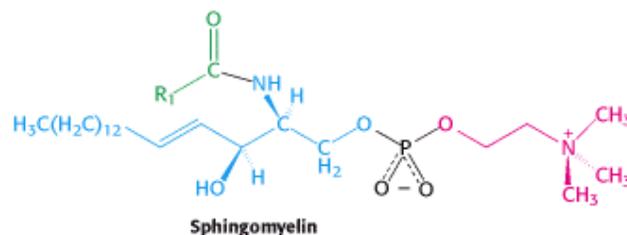
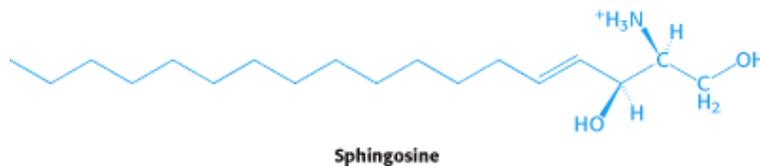
For phosphatidal choline,

R1 = -CH=CH(CH<sub>2</sub>)<sub>13</sub>CH<sub>3</sub>

R2 = -(CH<sub>2</sub>)<sub>16</sub>CH<sub>3</sub>

For phosphatidal ethanolamine, ethanolamine is in place of choline above.

**Sphingolipids** represent another class of lipids found frequently in biological membranes. An 18-carbon amino alcohol, **sphingosine**, forms the backbone of these lipids rather than glycerol. Typically, a fatty acid is joined to a sphingosine via an amide linkage to form a **ceramide**. **Sphingomyelins** represent a phosphorus-containing subclass of sphingolipids and are especially important in the nervous tissue of higher animals. A **sphingomyelin** is formed by the esterification of a phosphorylcholine or a phosphorylethanolamine to the 1-hydroxy group of a ceramide.



There is another class of ceramide-based lipids which, like the sphingomyelins, are important components of muscle and nerve membranes in animals. These are the **glycosphingolipids**, and they consist of a ceramide with one or more sugar residues in  $\alpha$ -glycosidic linkage at the 1-hydroxyl moiety.

The neutral glycosphingolipids contain only neutral (uncharged) sugar residues. When a single glucose or galactose is bound in this manner, the molecule is a **cerebroside** (Figure 8.13). Another class of lipids is formed when a sulfate is esterified at the 3-position of the galactose to make a **sulfatide**.

**Gangliosides** (Figure 8.14) are more complex glycosphingolipids that consist of a ceramide backbone with three or more sugars esterified, one of these being a **sialic acid** such as ***N*-acetylneuraminic acid**. These latter compounds are referred to as *acidic glycosphingolipids*, and they have a net negative charge at neutral pH.

The glycosphingolipids have a number of important cellular functions, despite the fact that they are present only in small amounts in most membranes.

Glycosphingolipids at cell surfaces appear to determine, at least in part, certain elements of tissue and organ specificity. Cell-cell recognition and tissue immunity appear to depend upon specific glycosphingolipids.

Gangliosides are present in nerve endings and appear to be important in nerve impulse transmission. A number of genetically transmitted diseases involve the accumulation of specific glycosphingolipids due to an absence of the enzymes needed for their degradation. Such is the case for ganglioside GM2 in the brains of *Tay-Sachs disease* victims, a rare but fatal disease characterized by a red spot on the retina, gradual blindness, and loss of weight, especially in infants and children.

## Waxes

**Waxes** are esters of long-chain alcohols with long-chain fatty acids. The resulting molecule can be viewed (in analogy to the glycerolipids) as having a weakly polar head group (the ester moiety itself) and a long, nonpolar tail (the hydrocarbon chains). Fatty acids found in waxes are usually saturated. The alcohols found in waxes may be saturated or unsaturated and may include sterols, such as cholesterol (see later section). Waxes are water-insoluble due

to the weakly polar nature of the ester group. As a result, this class of molecules confers water-repellant character to animal skin, to the leaves of certain plants, and to bird feathers. The glossy surface of a polished apple results from a wax coating.

**Lanolin**, a component of wool wax, is used as a base for pharmaceutical and cosmetic products because it is rapidly assimilated by human skin.

## Terpenes

The **terpenes** are a class of lipids formed from combinations of two or more molecules of 2-methyl-1,3-butadiene, better known as **isoprene** (a five-carbon unit that is abbreviated C5). A **monoterpene** (C10) consists of two isoprene units, a **sesquiterpene** (C15) consists of three isoprene units, a **diterpene** (C20) has four isoprene units, and so on. Isoprene units can be linked in terpenes to form straight chain or cyclic molecules, and the usual method of linking isoprene units is head to tail (Figure 8.16). Monoterpenes occur in all higher plants, while sesquiterpenes and diterpenes are less widely known. The **triterpenes** are C30 terpenes and include **squalene** and **lanosterol**, two of the precursors of cholesterol and other steroids (discussed later). **Tetraterpenes** (C40) are less common but include the carotenoids, a class of colorful photosynthetic pigments.

$\beta$ -Carotene is the precursor of vitamin A, while lycopene, similar to  $\beta$ -carotene but lacking the cyclopentene rings, is a pigment found in tomatoes.

Long-chain polyisoprenoid molecules with a terminal alcohol moiety are called **polyprenols**. The **dolichols**, one class of polyprenols (Figure 8.18), consist of 16 to 22 isoprene units and, in the form of

dolichyl phosphates, function to carry carbohydrate units in the biosynthesis of glycoproteins in animals. Polyprenyl groups serve to *anchor* certain proteins to biological membranes.

## Steroids

### Cholesterol

A large and important class of terpene-based lipids is the **steroids**. This molecular family, whose members effect an amazing array of cellular functions, is based on a common structural motif of three six-membered rings and one five-membered ring all fused together. **Cholesterol** is the most common steroid in animals and the precursor for all other animal steroids. The numbering system for cholesterol applies to all such molecules. Many steroids contain methyl groups at positions 10 and 13 and an 8- to 10-carbon alkyl side chain at position 17. The polyprenyl nature of this compound is particularly evident in the side chain. Many steroids contain oxygen at C-3, either a hydroxyl group in sterols or a carbonyl group in other steroids. Note also that the carbons at positions 10 and 13 and the alkyl group at position 17 are nearly always oriented on the same side of the steroid nucleus, the  $\alpha$ -orientation. Alkyl groups that extend from the other side of the steroid backbone are in an  $\alpha$ -orientation. Cholesterol is a principal component of animal cell plasma membranes, and much smaller amounts of cholesterol are found in the membranes of intracellular organelles. The relatively rigid fused ring system of cholesterol and the weakly polar alcohol group at the C-3 position have important consequences for the properties of plasma membranes. Cholesterol is also a component of *lipoprotein complexes* in the blood, and it is one of the constituents of *plaques* that form on arterial walls in *atherosclerosis*.

### Steroid Hormones

**Steroids** derived from cholesterol in animals include five families of hormones (the androgens, estrogens, progestins, glucocorticoids and mineralocorticoids) and bile acids. **Androgens** such as **testosterone** and **estrogens** such as **estradiol** mediate the development of sexual characteristics and sexual function in animals. The **progestins** such as **progesterone** participate in control of the menstrual cycle and pregnancy. **Glucocorticoids** (**cortisol**, for example) participate in the control of carbohydrate, protein, and lipid metabolism, whereas the **mineralocorticoids** regulate salt ( $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ) balances in tissues. The **bile acids** (including **cholic** and **deoxycholic acid**) are detergent molecules secreted in bile from the gallbladder that assist in the absorption of dietary lipids in the intestine.

## PROBLEMS

1. Draw the structures of (a) all the possible triacylglycerols that can be formed from glycerol with stearic and arachidonic acid, and (b) all the phosphatidylserine isomers that can be formed from palmitic and linolenic acids.
2. Describe in your own words the structural features of
  - a. a ceramide, and how it differs from a cerebroside.
  - b. a phosphatidylethanolamine, and how it differs from a phosphatidylcholine.
  - c. an ether glycerophospholipid, and how it differs from a plasmalogen.
  - d. a ganglioside, and how it differs from a cerebroside.
  - e. testosterone, and how it differs from estradiol.
3. From your memory of the structures, name
  - a. the glycerophospholipids that carry a net positive charge.
  - b. the glycerophospholipids that carry a net negative charge.
  - c. the glycerophospholipids that have zero net charge.
4. Compare and contrast two individuals, one of whose diet consists largely of meats containing high levels of cholesterol, and the other of whose diet is rich in plant sterols. Are their risks of cardiovascular disease likely to be similar or different? Explain your reasoning.

## **Fatty Acid Metabolism**

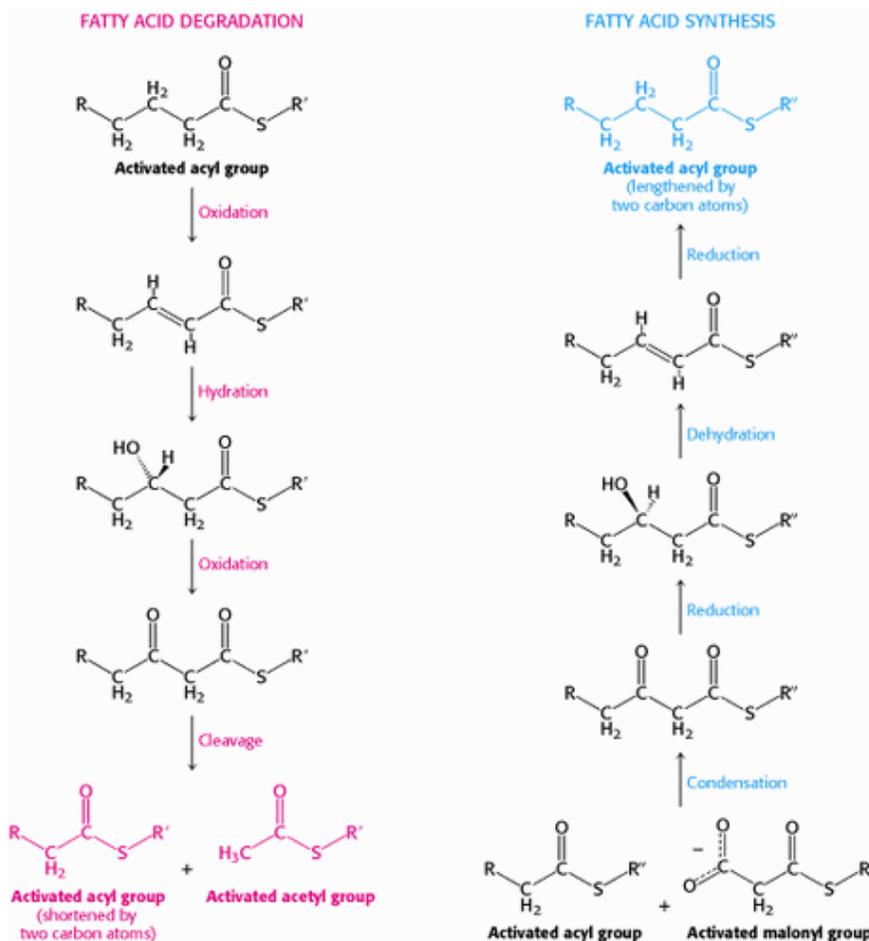
A fatty acid contains a long hydrocarbon chain and a terminal carboxylate group. Fatty acids have four major physiological roles. First, *fatty acids are building blocks of phospholipids and glycolipids*. These amphipathic molecules are the components of biological membranes.

Second, many proteins are modified by the *covalent attachment of fatty acids, which targets them to membrane locations*. Third, *fatty acids are fuel molecules*. They are stored as *triacylglycerols* (also called *neutral fats* or *triglycerides*), which are uncharged esters of fatty acids with glycerol. Fatty acids mobilized from triacylglycerols are oxidized to meet the energy needs of a cell or organism. Fourth, *fatty acid derivatives serve as hormones and intracellular messengers*. In this chapter, we will focus on the oxidation and synthesis of fatty acids, processes that are reciprocally regulated in response to hormones.

## An Overview of Fatty Acid Metabolism

Fatty acid degradation and synthesis are relatively simple processes that are essentially the reverse of each other. The process of degradation converts an aliphatic compound into a set of activated acetyl units (acetyl CoA) that can be processed by the citric acid cycle. An activated fatty acid is oxidized to introduce a double bond; the double bond is hydrated to introduce an oxygen; the alcohol is oxidized to a ketone; and, finally, the four carbon fragment is cleaved by coenzyme A to yield acetyl CoA and a fatty acid chain two carbons shorter. If the fatty acid has an even number of carbon atoms and is saturated, the process is simply repeated until the fatty acid is completely converted into acetyl CoA units.

Fatty acid synthesis is essentially the reverse of this process. Because the result is a polymer, the process starts with monomers in this case with activated acyl group (most simply, an acetyl unit) and malonyl units. The malonyl unit is condensed with the acetyl unit to form a four-carbon fragment. To produce the required hydrocarbon chain, the carbonyl must be reduced. The fragment is reduced, dehydrated, and reduced again, exactly the opposite of degradation, to bring the carbonyl group to the level of a methylene group with the formation of butyryl CoA. Another activated malonyl group condenses with the butyryl unit and the process is repeated until a C16 fatty acid is synthesized.



## Dietary Lipids Are Digested by Pancreatic Lipases

Most lipids are ingested in the form of triacylglycerols but must be degraded to fatty acids for absorption across the intestinal epithelium. Recall that lipids are not easily solubilized, yet they must be in order to be degraded.

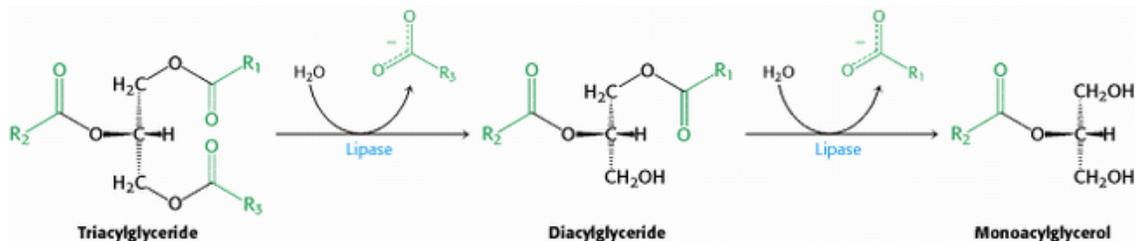
Triacylglycerols in the intestinal lumen are incorporated into micelles formed with the aid of *bile salt*, amphipathic molecules synthesized from cholesterol in the liver and secreted from the gall bladder. Incorporation of lipids into micelles orients the ester bonds of the lipid toward the surface of the micelle, rendering the bonds more susceptible to digestion by pancreatic lipases that are in aqueous solution. If the production of bile salts is inadequate owing to liver disease, large amounts of fats (as much as 30 g day<sup>-1</sup>) are excreted in the feces. This condition is referred to as steatorrhea, from the Greek *steato*, "fat."

The lipases digest the triacylglycerols into free fatty acids and monoacylglycerol. These digestion products are carried in micelles to the intestinal epithelium where they are absorbed across the plasma membrane.

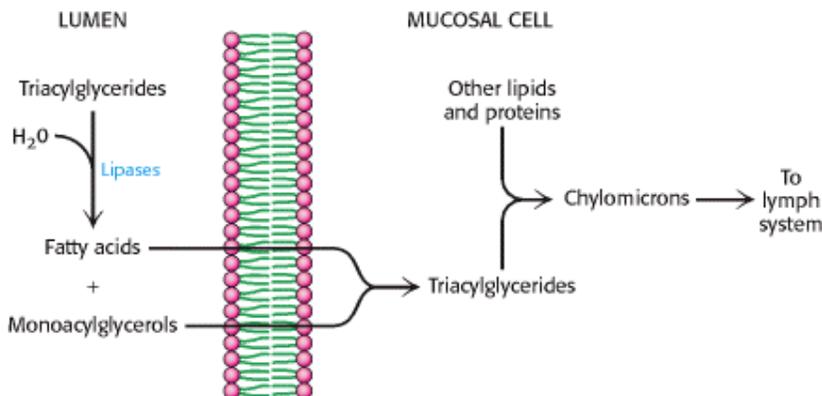
### Dietary Lipids Are Transported in Chylomicrons

In the intestinal mucosal cells, the triacylglycerols are resynthesized from fatty acids and monoacylglycerols and then packaged into lipoprotein transport particles called *chylomicrons*, stable particles ranging from approximately 180 to 500 nm in diameter. These particles are composed mainly of triacylglycerols, with apoprotein B-48 as the main protein component. Protein constituents of lipoprotein particles are called *apolipoproteins*. Chylomicrons also function in the transport of fat-soluble vitamins and cholesterol.

The chylomicrons are released into the lymph system and then into the blood. These particles bind to membrane-bound lipoprotein lipases, primarily at adipose tissue and muscle, where the triacylglycerols are once again degraded into free fatty acids and monoacylglycerol for transport into the tissue. The triacylglycerols are then resynthesized inside the cell and stored.



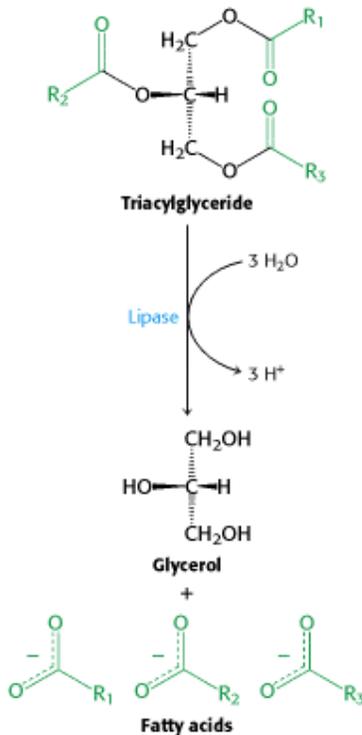
**Action of Pancreatic Lipases.** Lipases secreted by the pancreas convert triacylglycerols into fatty acids and monoacylglycerol for absorption into the intestine.



Free fatty acids and monoacylglycerols are absorbed by intestinal epithelial cells. Triacylglycerols are resynthesized and packaged with other lipids and apoprotein B-48 to form chylomicrons, which are then released into the lymph system.

## The Utilization of Fatty Acids as Fuel Requires Three Stages of Processing.

Peripheral tissues gain access to the lipid energy reserves stored in adipose tissue through three stages of processing. First, the lipids must be mobilized. In this process, triacylglycerols are degraded to fatty acids and glycerol, which are released from the adipose tissue and transported to the energy-requiring tissues. Second, at these tissues, the fatty acids must be activated and transported into mitochondria for degradation. Third, the fatty acids are broken down in a step-by-step fashion into acetyl CoA, which is then processed in the citric acid cycle.

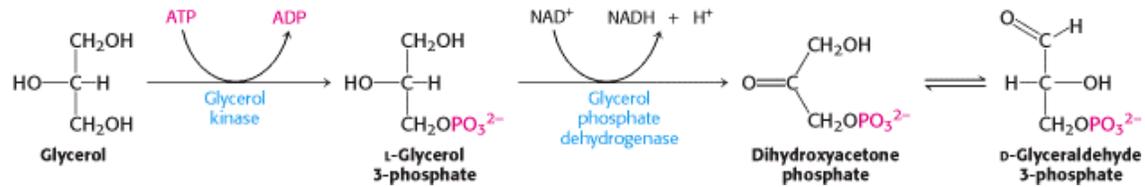


### Triacylglycerols Are Hydrolyzed by Cyclic AMP-Regulated Lipases

The initial event in the utilization of fat as an energy source is the hydrolysis of triacylglycerols by lipases, an event referred to as *lipolysis*. The lipase of adipose tissue are activated on treatment of these cells with the hormones epinephrine, norepinephrine, glucagon, and adrenocorticotropic hormone. In adipose cells, these hormones trigger 7TM receptors that activate adenylate cyclase. The increased level of cyclic AMP then stimulates protein kinase A, which activates the lipases by phosphorylating them. Thus, *epinephrine, norepinephrine, glucagon, and adrenocorticotropic hormone induce lipolysis* (Figure 22.6). In contrast, *insulin inhibits lipolysis*. The released fatty acids are not soluble in blood plasma, and so, on release, serum albumin binds the fatty acids and serves as a carrier. By these means, free fatty acids are made accessible as a fuel in other tissues.

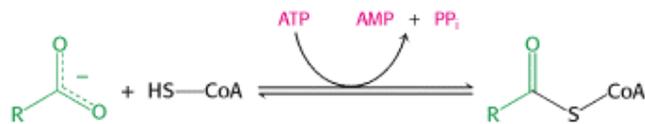
Glycerol formed by lipolysis is absorbed by the liver and phosphorylated, oxidized to dihydroxyacetone phosphate, and then isomerized to glyceraldehyde 3-phosphate. This molecule is an intermediate in both the glycolytic and the gluconeogenic pathways.

Hence, glycerol can be converted into pyruvate or glucose in the liver, which contains the appropriate enzymes. The reverse process can take place by the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate. Hydrolysis by a phosphatase then gives glycerol. Thus, glycerol and glycolytic intermediates are readily interconvertible.



## Fatty Acids Are Linked to Coenzyme A Before They Are Oxidized

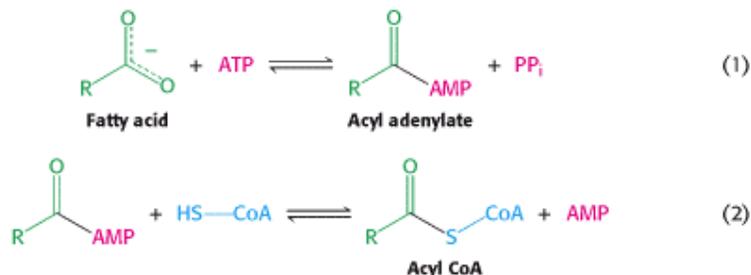
Fatty acids are oxidized in mitochondria. Subsequent work demonstrated that they are activated before they enter the mitochondrial matrix. Adenosine triphosphate (ATP) drives the formation of a thioester linkage between the carboxyl group of a fatty acid and the sulfhydryl group of CoA. This activation reaction takes place on the outer mitochondrial membrane, where it is catalyzed by *acyl CoA synthetase* (also called *fatty acid thiokinase*).

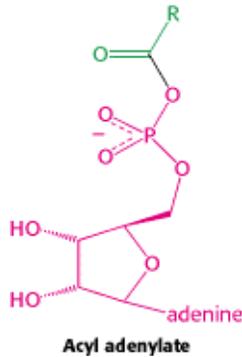


Activation of a fatty acid is accomplished in two steps. First, the fatty acid reacts with ATP to form an *acyl adenylate*. In this mixed anhydride, the carboxyl group of a fatty acid is bonded to the phosphoryl group of AMP. The other two phosphoryl groups of the ATP substrate are released as pyrophosphate. The sulfhydryl group of CoA then attacks the acyl adenylate, which is tightly bound to the enzyme, to form acyl CoA and AMP.

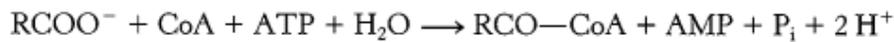
These partial reactions are freely reversible. In fact, the equilibrium constant for the sum of these reactions is close to 1.

One high-transfer-potential compound is cleaved (between PP<sub>i</sub> and AMP) and one high-transfer-potential compound is formed (the thioester acyl CoA).





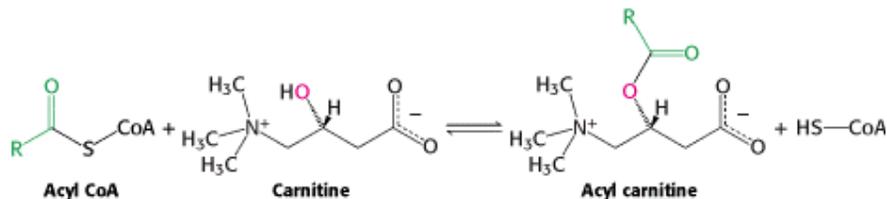
How is the overall reaction driven forward? The answer is that pyrophosphate is rapidly hydrolyzed by a pyrophosphatase, and so the complete reaction is



This reaction is quite favorable because the equivalent of two molecules of ATP is hydrolyzed, whereas only one high-transfer-potential compound is formed. We see here another example of a recurring theme in biochemistry: *many biosynthetic reactions are made irreversible by the hydrolysis of inorganic pyrophosphate.*

### **Carnitine Carries Long-Chain Activated Fatty Acids into the Mitochondrial Matrix**

Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix. A special transport mechanism is needed to carry long-chain acyl CoA molecules across the inner mitochondrial membrane. Activated long-chain fatty acids are transported across the membrane by conjugating them to *carnitine*, a zwitterionic alcohol. The acyl group is transferred from the sulfur atom of CoA to the hydroxyl group of carnitine to form *acyl carnitine*. This reaction is catalyzed by *carnitine acyltransferase I* (also called *carnitine palmitoyl transferase I*), which is bound to the outer mitochondrial membrane.



Acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase. The acyl group is transferred back to CoA on the matrix side of the membrane. This reaction, which is catalyzed by *carnitine acyltransferase II* (*carnitine palmitoyl transferase II*), is simply the reverse of the reaction that takes place in the cytosol.

Normally, the transfer of an acyl group from an alcohol to a sulfhydryl group is thermodynamically unfavorable. However, the equilibrium constant for this reaction for carnitine is near 1, apparently because carnitine and its esters are solvated differently from most other alcohols and their esters because of the zwitterionic nature of carnitine. As a result, the *O*-acyl link in carnitine has a high group-transfer potential. Finally, the translocase returns carnitine to the cytosolic side in exchange for an incoming acyl carnitine.

A number of diseases have been traced to a deficiency of carnitine, the transferase or the translocase. The symptoms of carnitine deficiency range from mild muscle cramping to severe weakness and even death. The muscle, kidney, and heart are the tissues primarily affected. Muscle weakness during

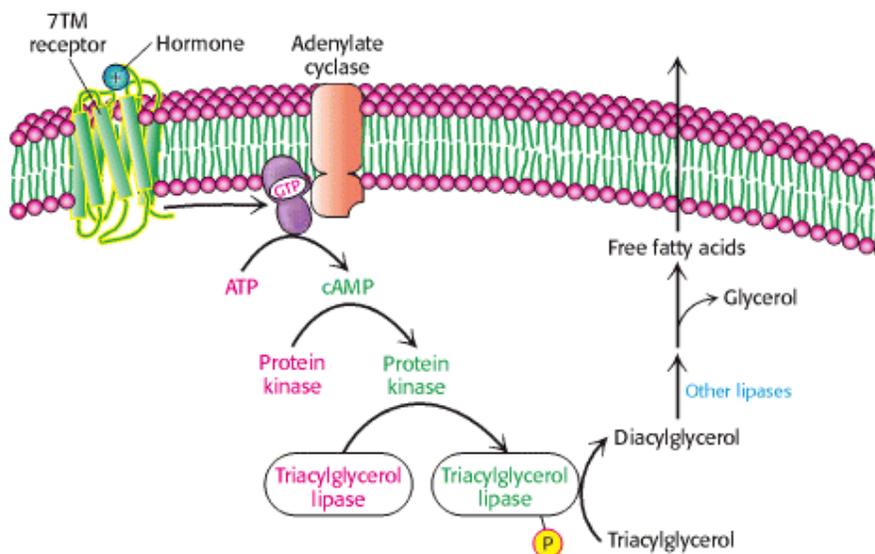
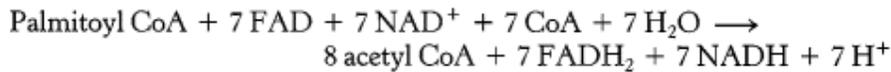


hydroxyacyl dehydrogenase, and enoyl CoA hydratase have broad specificity with respect to the length of the acyl group.

### The Complete Oxidation of Palmitate Yields 106 Molecules of ATP

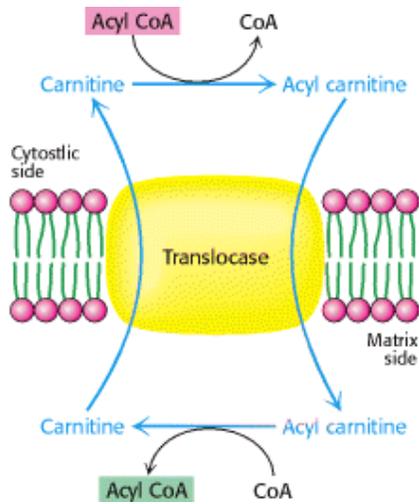
We can now calculate the energy yield derived from the oxidation of a fatty acid. In each reaction cycle, an acyl CoA is shortened by two carbon atoms, and one molecule each of FADH<sub>2</sub>, NADH, and acetyl CoA is formed.

The degradation of palmitoyl CoA (C16-acyl CoA) requires seven reaction cycles. In the seventh cycle, the C4-ketoacyl CoA is thiolized to two molecules of acetyl CoA. Hence, the stoichiometry of oxidation of palmitoyl CoA is



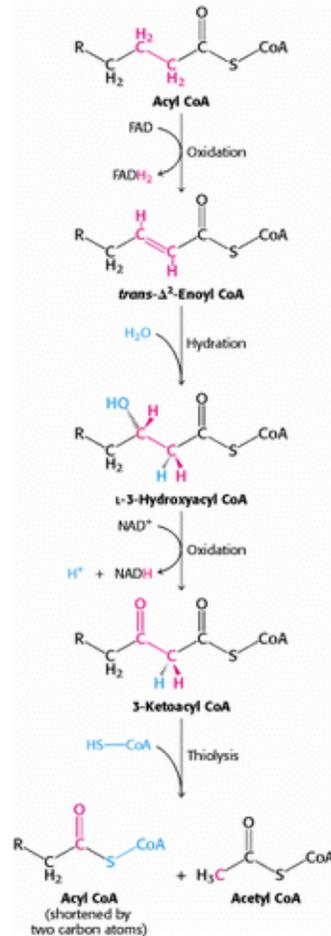
### Mobilization of Triacylglycerols.

Triacylglycerols in adipose tissue are converted into free fatty acids and glycerol for release into the bloodstream in response to hormonal signals. A hormone-sensitive lipase initiates the process.



**Acyl Carnitine Translocase.** The entry of acyl carnitine into the mitochondrial matrix is mediated by a translocase. Carnitine returns to the cytosolic side of the inner mitochondrial membrane in exchange for acyl carnitine.

**The figure below shows the Reaction Sequence for the Degradation of Fatty Acids.** Fatty acids are degraded by the repetition of a four-reaction sequence consisting of oxidation, hydration, oxidation, and thiolysis.



### Principal reactions in fatty acid oxidation

Step	Reaction	Enzyme
1	Fatty acid + CoA + ATP ↔ acyl CoA + AMP + PPi	Acyl CoA synthetase
2	Carnitine + acyl CoA ↔ acyl carnitine + CoA	Carnitine acyltransferase
3	Acyl CoA + E-FAD ↔ <i>trans</i> -Δ <sup>2</sup> -enoyl CoA + EFADH <sub>2</sub>	Acyl CoA dehydrogenases
4	<i>trans</i> -Δ <sup>2</sup> -Enoyl CoA + H <sub>2</sub> O ↔ 1-3-hydroxyacyl CoA	Enoyl CoA hydratase
5	1-3-Hydroxyacyl CoA + NAD <sup>+</sup> ↔ 3-ketoacyl CoA + NADH + H <sup>+</sup>	1-3-Hydroxyacyl CoA dehydrogenase
6	3-ketoacyl CoA + CoA ↔ acetyl CoA + acyl CoA (shortened by C <sub>2</sub> )	β-Ketothiolase (also called thiolase)

### Certain Fatty Acids Require Additional Steps for Degradation

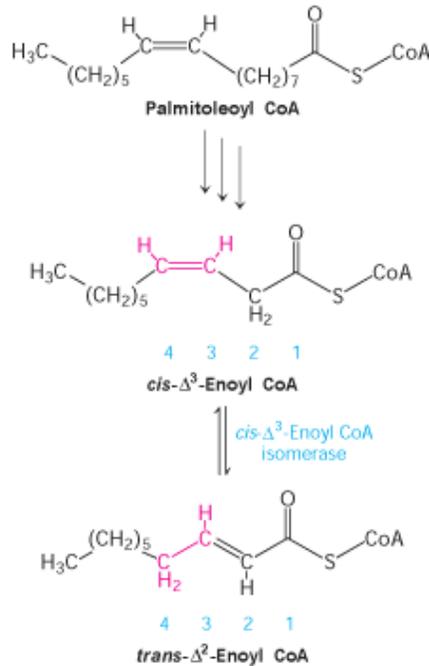
The β-oxidation pathway accomplishes the complete degradation of saturated fatty acids having an even number of carbon atoms. Most fatty acids have such structures because of their mode of synthesis. However, not all fatty acids are so simple. The oxidation of fatty acids containing double bonds requires additional steps. Likewise, fatty acids containing an odd number of carbon atoms yield a propionyl CoA at the final thiolysis step that must be converted into an easily usable form by additional enzyme reactions.

### An Isomerase and a Reductase Are Required for the Oxidation of Unsaturated Fatty Acids

The oxidation of unsaturated fatty acids presents some difficulties. Most of the reactions are the same as those for saturated fatty acids. In fact, only two additional enzymes an isomerase and a reductase are needed to degrade a wide range of unsaturated fatty acids.

Consider the oxidation of palmitoleate. This C<sub>16</sub> unsaturated fatty acid, which has one double bond between C-9 and C-10, is activated and transported across the inner mitochondrial membrane in the same way as saturated fatty acids.

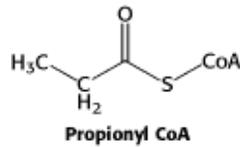
Palmitoleoyl CoA then undergoes three cycles of degradation, which are carried out by the same enzymes as in the oxidation of saturated fatty acids. However, the *cis*- $\Delta^3$ -enoyl CoA formed in the third round is not a substrate for acyl CoA dehydrogenase. The presence of a double bond between C-3 and C-4 prevents the formation of another double bond between C-2 and C-3. This impasse is resolved by a new reaction that shifts the position and configuration of the *cis*- $\Delta^3$  double bond. An *isomerase converts this double bond into a trans- $\Delta^2$  double bond*. The subsequent reactions are those of the saturated fatty acid oxidation pathway, in which the *trans- $\Delta^2$ -enoyl CoA* is a regular substrate.



Another problem arises with the oxidation of polyunsaturated fatty acids. Consider linoleate, a C<sub>18</sub> polyunsaturated fatty acid with *cis*- $\Delta^9$  and *cis*- $\Delta^{12}$  double bonds. The *cis*- $\Delta^3$  double bond formed after three rounds of  $\beta$  oxidation is converted into a *trans*- $\Delta^2$  double bond by the aforementioned isomerase. The acyl CoA produced by another round of  $\beta$  oxidation contains a *cis*- $\Delta^4$  double bond. Dehydrogenation of this species by acyl CoA dehydrogenase yields a *2,4-dienoyl intermediate*, which is not a substrate for the next enzyme in the  $\beta$ -oxidation pathway. This impasse is circumvented by *2,4-dienoyl CoA reductase*, an enzyme that uses NADPH to reduce the 2,4-dienoyl intermediate to *trans*- $\Delta^3$ -enoyl CoA. *cis*- $\Delta^3$ -Enoyl CoA isomerase then converts *trans*- $\Delta^3$ -enoyl CoA into the *trans*- $\Delta^2$  form, a customary intermediate in the  $\beta$ -oxidation pathway. These catalytic strategies are elegant and economical. Only two extra enzymes are needed for the oxidation of *any* polyunsaturated fatty acid. *Odd-numbered double bonds are handled by the isomerase, and even-numbered ones by the reductase and the isomerase.*

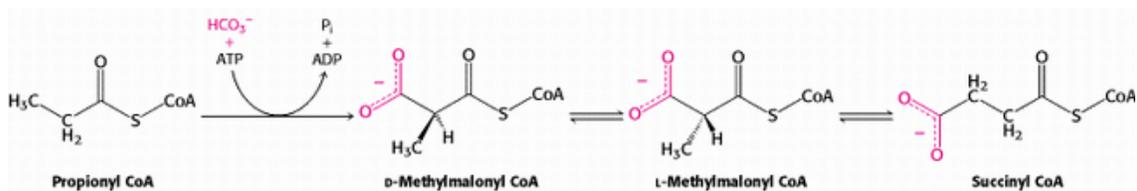
### **Odd-Chain Fatty Acids Yield Propionyl Coenzyme A in the Final Thiolysis Step**

Fatty acids having an odd number of carbon atoms are minor species. They are oxidized in the same way as fatty acids having an even number, except that propionyl CoA and acetyl CoA, rather than two molecules of acetyl CoA, are produced in the final round of degradation. The activated three-carbon unit in propionyl CoA enters the citric acid cycle after it has been converted into succinyl CoA.



## Propionyl CoA Is Converted into Succinyl CoA in a Reaction That Requires Vitamin B12

The pathway from propionyl CoA to succinyl CoA is especially interesting because it entails a rearrangement that requires *vitamin B12* (also known as *cobalamin*). Propionyl CoA is carboxylated at the expense of the hydrolysis of an ATP to yield the d isomer of methylmalonyl CoA. This carboxylation reaction is catalyzed by *propionyl CoA carboxylase*, a biotin enzyme that is homologous to and has a catalytic mechanism like that of pyruvate carboxylase. The d isomer of methylmalonyl CoA is racemized to the l isomer, the substrate for a mutase that converts it into *succinyl CoA* by an *intramolecular rearrangement*. The -CO-S-CoA group migrates from C-2 to C-3 in exchange for a hydrogen atom. This very unusual isomerization is catalyzed by *methylmalonyl CoA mutase*, which contains a derivative of vitamin B12, cobalamin, as its coenzyme.

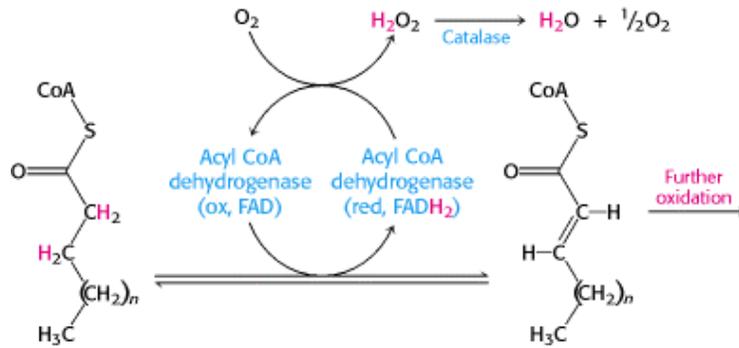


### Conversion of Propionyl CoA Into Succinyl CoA.

Propionyl CoA, generated from fatty acids with an odd number of carbons as well as some amino acids, is converted into the citric acid cycle intermediate succinyl CoA.

## Fatty Acids Are Also Oxidized in Peroxisomes

Although most fatty acid oxidation takes place in mitochondria, some oxidation takes place in cellular organelles called *peroxisomes*. These organelles are characterized by high concentrations of the enzyme catalase, which catalyzes the dismutation of hydrogen peroxide into water and molecular oxygen. Fatty acid oxidation in these organelles, which halts at octanoyl CoA, may serve to shorten long chains to make them better substrates of  $\beta$  oxidation in mitochondria. Peroxisomal oxidation differs from  $\beta$  oxidation in the initial dehydrogenation reaction. In peroxisomes, a flavoprotein dehydrogenase transfers electrons to O<sub>2</sub> to yield H<sub>2</sub>O<sub>2</sub> instead of capturing the high-energy electrons as FADH<sub>2</sub>, as occurs in mitochondrial  $\beta$  oxidation. Catalase is needed to convert the hydrogen peroxide produced in the initial reaction into water and oxygen. Subsequent steps are identical with their mitochondrial counterparts, although they are carried out by different isoforms of the enzymes. Zellweger syndrome, which results from the absence of functional peroxisomes, is characterized by liver, kidney, and muscle abnormalities and usually results in death by age six. The syndrome is caused by a defect in the import of enzymes into the peroxisomes. Here we see a pathological condition resulting from an inappropriate cellular distribution of enzymes.

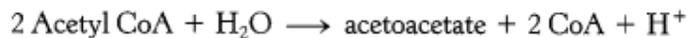


**Initiation of Peroxisomal Fatty Acid Degradation.** The first dehydration in the degradation of fatty acids in peroxisomes requires a flavoprotein dehydrogenase that transfers electrons to  $O_2$  to yield  $H_2O_2$ .

## Ketone Bodies Are Formed from Acetyl Coenzyme A When Fat Breakdown Predominates

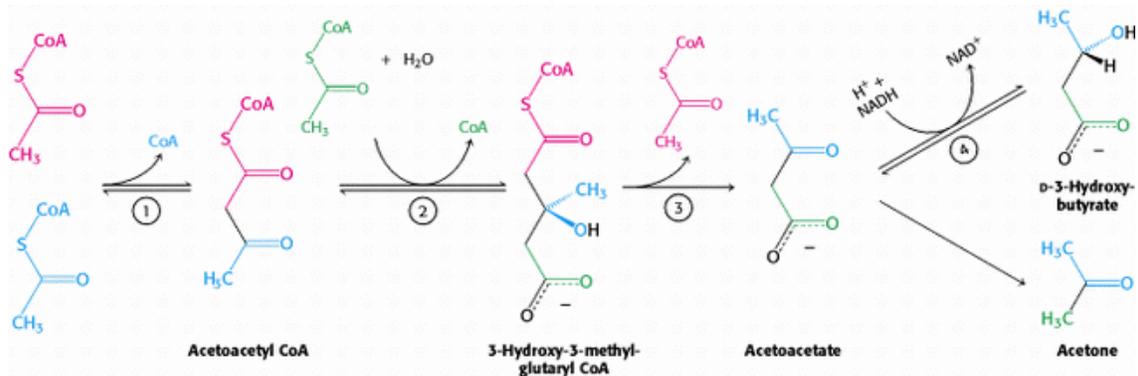
The acetyl CoA formed in fatty acid oxidation enters the citric acid cycle only if fat and carbohydrate degradation are appropriately balanced. The reason is that the entry of acetyl CoA into the citric acid cycle depends on the availability of oxaloacetate for the formation of citrate, but the concentration of oxaloacetate is lowered if carbohydrate is unavailable or improperly utilized. Recall that oxaloacetate is normally formed from pyruvate, the product of glycolysis, by pyruvate carboxylase. This is the molecular basis of the adage that *fats burn in the flame of carbohydrates*. In fasting or diabetes, oxaloacetate is consumed to form glucose by the gluconeogenic pathway and hence is unavailable for condensation with acetyl CoA. Under these conditions, acetyl CoA is diverted to the formation of acetoacetate and d-3-hydroxybutyrate. Acetoacetate, d-3-hydroxybutyrate, and acetone are often referred to as *ketone bodies*. Abnormally high levels of ketone bodies are present in the blood of untreated diabetics.

Acetoacetate is formed from acetyl CoA in three steps. Two molecules of acetyl CoA condense to form acetoacetyl CoA. This reaction, which is catalyzed by thiolase, is the reverse of the thiolysis step in the oxidation of fatty acids. Acetoacetyl CoA then reacts with acetyl CoA and water to give 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) and CoA. This reaction, which has a favorable equilibrium owing to the hydrolysis of a thioester linkage, compensates for the unfavorable equilibrium in the formation of acetoacetyl CoA. 3-Hydroxy-3-methylglutaryl CoA is then cleaved to acetyl CoA and acetoacetate. The sum of these reactions is



d-3-Hydroxybutyrate is formed by the reduction of acetoacetate in the mitochondrial matrix by d-3-hydroxybutyrate dehydrogenase. The ratio of hydroxybutyrate to acetoacetate depends on the NADH/NAD<sup>+</sup> ratio inside mitochondria.

Because it is a  $\beta$ -ketoacid, acetoacetate also undergoes a slow, spontaneous decarboxylation to acetone. The odor of acetone may be detected in the breath of a person who has a high level of acetoacetate in the blood.

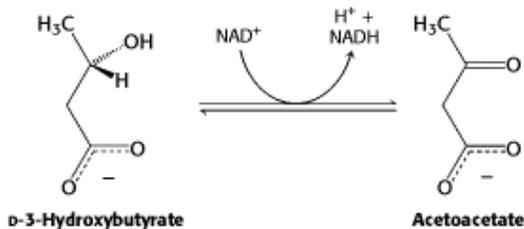


### Formation of Ketone Bodies.

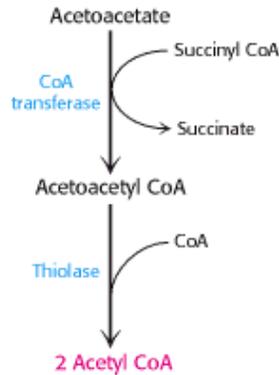
The Ketone bodies-acetoacetate, d-3-hydroxybutyrate, and acetone from acetyl CoA are formed primarily in the liver. Enzymes catalyzing these reactions are (1) 3-ketothiolase, (2) hydroxymethylglutaryl CoA synthase, (3) hydroxymethylglutaryl CoA cleavage enzyme, and (4) d-3-hydroxybutyrate dehydrogenase. Acetoacetate spontaneously decarboxylates to form acetone.

### Ketone Bodies Are a Major Fuel in Some Tissues

The major site of production of acetoacetate and 3-hydroxybutyrate is the liver. These substances diffuse from the liver mitochondria into the blood and are transported to peripheral tissues. These ketone bodies were initially regarded as degradation products of little physiological value. However, the results of studies by George Cahill and others revealed that these derivatives of acetyl CoA are important molecules in energy metabolism. *Acetoacetate and 3-hydroxybutyrate are normal fuels of respiration and are quantitatively important as sources of energy.* Indeed, heart muscle and the renal cortex use acetoacetate in preference to glucose. In contrast, glucose is the major fuel for the brain and red blood cells in well-nourished people on a balanced diet. However, the brain adapts to the utilization of acetoacetate during starvation and diabetes. In prolonged starvation, 75% of the fuel needs of the brain are met by ketone bodies. 3-Hydroxybutyrate is oxidized to produce acetoacetate as well as NADH for use in oxidative phosphorylation.



Acetoacetate can be activated by the transfer of CoA from succinyl CoA in a reaction catalyzed by a specific CoA transferase. Acetoacetyl CoA is then cleaved by thiolase to yield two molecules of acetyl CoA, which can then enter the citric acid cycle.



**Utilization of Acetoacetate as a Fuel.** Acetoacetate can be converted into two molecules of acetyl CoA, which then enter the citric acid cycle.

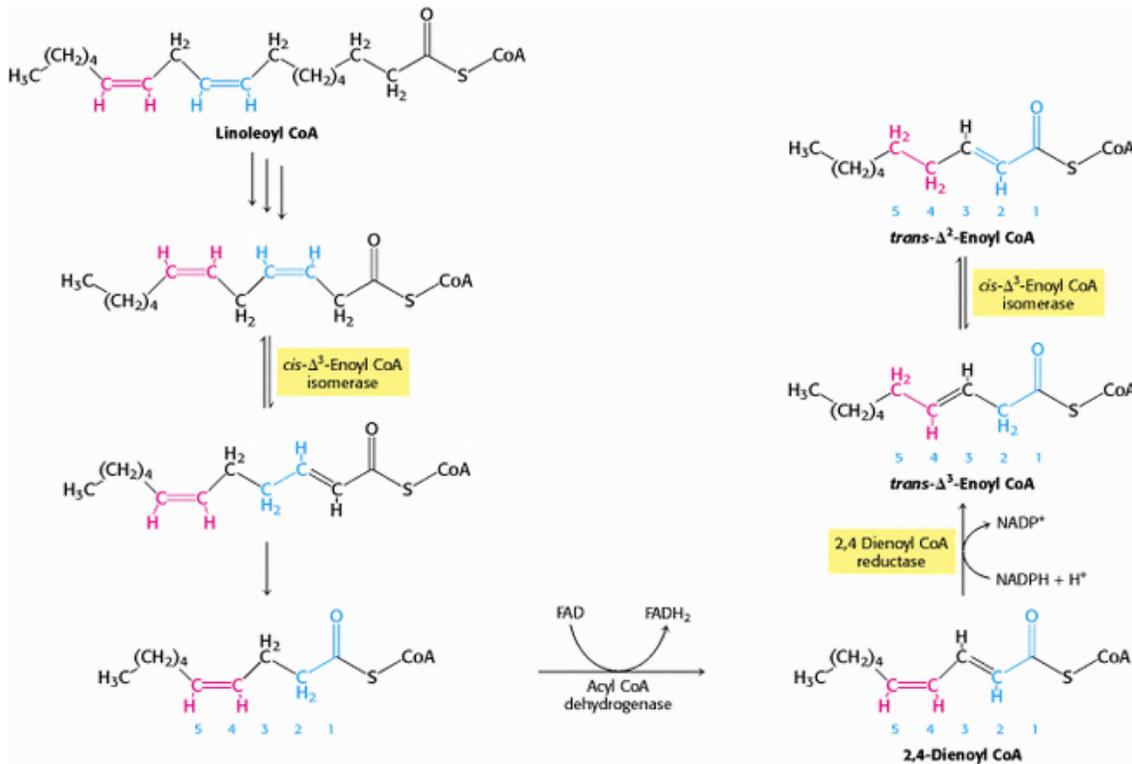
The liver has acetoacetate available to supply to other organs because it lacks this particular CoA transferase.

*Ketone bodies can be regarded as a water-soluble, transportable form of acetyl units.* Fatty acids are released by adipose tissue and converted into acetyl units by the liver, which then exports them as acetoacetate. As might be expected, acetoacetate also has a regulatory role. *High levels of acetoacetate in the blood signify an abundance of acetyl units and lead to a decrease in the rate of lipolysis in adipose tissue.*

Certain pathological conditions can lead to a life-threatening rise in the blood levels of the ketone bodies. Most common of these conditions is diabetic ketosis in patients with insulin-dependent diabetes mellitus. The absence of insulin has two major biochemical consequences. First, the liver cannot absorb glucose and consequently cannot provide oxaloacetate to process fatty acid-derived acetyl CoA. Second, insulin normally curtails fatty acid mobilization by adipose tissue. The liver thus produces large amounts of ketone bodies, which are moderately strong acids. The result is severe acidosis. The decrease in pH impairs tissue function, most importantly in the central nervous system.

### **Animals Cannot Convert Fatty Acids into Glucose**

It is important to note that *animals are unable to effect the net synthesis of glucose from fatty acids.* Specifically, acetyl CoA cannot be converted into pyruvate or oxaloacetate in animals. The two carbon atoms of the acetyl group of acetyl CoA enter the citric acid cycle, but two carbon atoms leave the cycle in the decarboxylations catalyzed by isocitrate dehydrogenase and  $\alpha$ -ketoglutarate dehydrogenase. Consequently, oxaloacetate is regenerated, but it is not formed de novo when the acetyl unit of acetyl CoA is oxidized by the citric acid cycle. In contrast, plants have two additional enzymes enabling them to convert the carbon atoms of acetyl CoA into oxaloacetate.



**Oxidation of Linoleoyl CoA.** The complete oxidation of the diunsaturated fatty acid linoleate is facilitated by the activity of enoyl CoA isomerase and 2,4-dienoyl CoA reductase.

## Fatty Acids Are Synthesized and Degraded by Different Pathways

Fatty acid synthesis is not simply a reversal of the degradative pathway. Rather, it consists of a new set of reactions, again exemplifying the principle that *synthetic and degradative pathways are almost always distinct*. Some important differences between the pathways are:

1. Synthesis takes place in the *cytosol*, in contrast with degradation, which takes place primarily in the mitochondrial matrix.
2. Intermediates in fatty acid synthesis are covalently linked to the sulfhydryl groups of an *acyl carrier protein* (ACP), whereas intermediates in fatty acid breakdown are covalently attached to the sulfhydryl group of coenzyme A.
3. The enzymes of fatty acid synthesis in higher organisms are joined in a *single polypeptide chain* called *fatty acid synthase*. In contrast, the degradative enzymes do not seem to be associated.
4. The growing fatty acid chain is elongated by the *sequential addition of two-carbon units* derived from acetyl CoA.

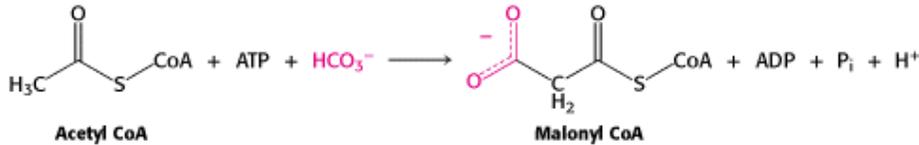
The activated donor of two carbon units in the elongation step is *malonyl ACP*. The elongation reaction is driven by the release of CO<sub>2</sub>.

5. The reductant in fatty acid synthesis is *NADPH*, whereas the oxidants in fatty acid degradation are *NAD<sup>+</sup>* and *FAD*.

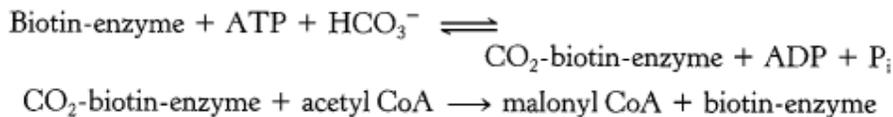
6. Elongation by the fatty acid synthase complex stops on formation of *palmitate* (C<sub>16</sub>). Further elongation and the insertion of double bonds are carried out by other enzyme systems.

## The Formation of Malonyl Coenzyme A Is the Committed Step in Fatty Acid Synthesis

Fatty acid synthesis starts with the carboxylation of acetyl CoA to *malonyl CoA*. This irreversible reaction is the committed step in fatty acid synthesis.



The synthesis of malonyl CoA is catalyzed by *acetyl CoA carboxylase*, which contains a biotin prosthetic group. The carboxyl group of biotin is covalently attached to the ε amino group of a lysine residue, as in pyruvate carboxylase and propionyl CoA carboxylase. As with these other enzymes, a carboxybiotin intermediate is formed at the expense of the hydrolysis a molecule of ATP. The activated CO<sub>2</sub> group in this intermediate is then transferred to acetyl CoA to form malonyl CoA.



This enzyme is also the essential regulatory enzyme for fatty acid metabolism.

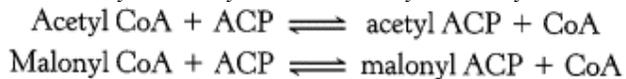
## Intermediates in Fatty Acid Synthesis Are Attached to an Acyl Carrier Protein

The intermediates in fatty acid synthesis are linked to an acyl carrier protein. Specifically, they are linked to the sulfhydryl terminus of a phosphopantetheine group, which is, in turn, attached to a serine residue of the acyl carrier protein. Recall that, in the degradation of fatty acids, a phosphopantetheine group is present as part of CoA instead. ACP, a single polypeptide chain of 77 residues, can be regarded as a giant prosthetic group, a "macro CoA."

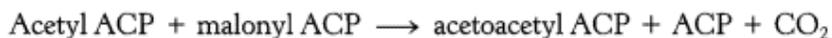
## The Elongation Cycle in Fatty Acid Synthesis

The enzyme system that catalyzes the synthesis of saturated long-chain fatty acids from acetyl CoA, malonyl CoA, and NADPH is called the *fatty acid synthase*.

The elongation phase of fatty acid synthesis starts with the formation of acetyl ACP and malonyl ACP. *Acetyl transacylase* and *malonyl transacylase* catalyze these reactions.



Malonyl transacylase is highly specific, whereas acetyl transacylase can transfer acyl groups other than the acetyl unit, though at a much slower rate. Fatty acids with an odd number of carbon atoms are synthesized starting with propionyl ACP, which is formed from propionyl CoA by acetyl transacylase. Acetyl ACP and malonyl ACP react to form acetoacetyl ACP. The *acyl-malonyl ACP condensing enzyme* catalyzes this condensation reaction.



In the condensation reaction, a four-carbon unit is formed from a two carbon unit and a three-carbon unit, and CO<sub>2</sub> is released. Why is the four-carbon unit not formed from 2 two-carbon units? In other words, why are the reactants acetyl ACP and malonyl ACP rather than two molecules of acetyl ACP?

The answer is that the equilibrium for the synthesis of acetoacetyl ACP from two molecules of acetyl ACP is highly unfavorable. In contrast, *the equilibrium is favorable if malonyl ACP is a reactant because its decarboxylation contributes a substantial decrease in free energy*. In effect, ATP drives the condensation reaction, though ATP does not directly participate in the condensation reaction. Rather, ATP is used to carboxylate acetyl CoA to malonyl CoA. The free energy thus stored in malonyl CoA is released in the decarboxylation accompanying the formation of acetoacetyl ACP. Although  $\text{HCO}_3^-$  is required for fatty acid synthesis, its carbon atom does not appear in the product. Rather, *all the carbon atoms of fatty acids containing an even number of carbon atoms are derived from acetyl CoA*.

The next three steps in fatty acid synthesis reduce the keto group at C-3 to a methylene group .

First, acetoacetyl ACP is reduced to d-3-hydroxybutyryl ACP. This reaction differs from the corresponding one in fatty acid degradation in two respects:

- (1) the d rather than the l isomer is formed; and
- (2) NADPH is the reducing agent, whereas  $\text{NAD}^+$  is the oxidizing agent in  $\beta$  oxidation.

This difference exemplifies the general principle that *NADPH is consumed in biosynthetic reactions, whereas NADH is generated in energy-yielding reactions*.

Then d-3-hydroxybutyryl ACP is *dehydrated* to form crotonyl ACP, which is a *trans*- $\Delta^2$ -enoyl ACP. The final step in the cycle *reduces* crotonyl ACP to butyryl ACP. NADPH is again the reductant, whereas FAD is the oxidant in the corresponding reaction in  $\beta$ -oxidation.

The enzyme that catalyzes this step, *enoyl ACP reductase*, is inhibited by triclosan, a broad-spectrum antibacterial agent. Triclosan is used in a variety of products such as toothpaste, soaps, and skin creams. These last three reactions - a reduction, a dehydration, and a second reduction convert acetoacetyl ACP into butyryl ACP, which completes the first elongation cycle.

In the second round of fatty acid synthesis, butyryl ACP condenses with malonyl ACP to form a C6- $\beta$ -ketoacyl ACP. This reaction is like the one in the first round, in which acetyl ACP condenses with malonyl ACP to form a C4- $\beta$ -ketoacyl ACP. Reduction, dehydration, and a second reduction convert the C6- $\beta$ -ketoacyl ACP into a C6-acyl ACP, which is ready for a third round of elongation.

The elongation cycles continue until C16-acyl ACP is formed. This intermediate is a good substrate for a thioesterase that hydrolyzes C16-acyl ACP to yield palmitate and ACP. *The thioesterase acts as a ruler to determine fatty acid chain length*.

## **Fatty Acids Are Synthesized by a Multifunctional Enzyme Complex in Eukaryotes**

Although the basic biochemical reactions in fatty acid synthesis are very similar in *E. coli* and eukaryotes, the structure of the synthase varies considerably. The fatty acid synthases of eukaryotes, in contrast with those of *E. coli*, have the component enzymes linked in a large polypeptide chain. Mammalian fatty acid synthase is a dimer of identical 260-kd subunits. Each chain is folded into three domains joined by flexible regions.

*Domain 1, the substrate entry and condensation unit*, contains acetyl transferase, malonyl transferase, and  $\beta$ -ketoacyl synthase (condensing enzyme).

*Domain 2, the reduction unit*, contains the acyl carrier protein,  $\beta$ -ketoacyl reductase, dehydratase, and enoyl reductase.

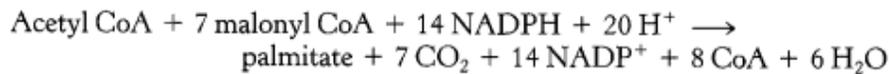
*Domain 3, the palmitate release unit*, contains the thioesterase.

Thus, *seven different catalytic sites are present on a single polypeptide chain*. It is multifunctional proteins in which different enzymes are linked covalently.

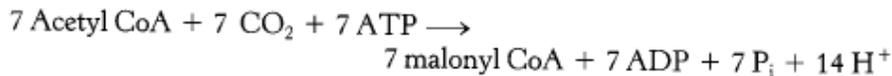
An advantage of this arrangement is that the synthetic activity of different enzymes is coordinated. In addition, a multienzyme complex consisting of covalently joined enzymes is more stable than one formed by non covalent attractions. Furthermore, intermediates can be efficiently handed from one active site to another without leaving the assembly.

## The Stoichiometry of Fatty Acid Synthesis

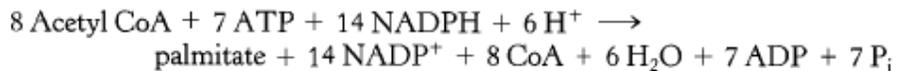
The stoichiometry of the synthesis of palmitate is



The equation for the synthesis of the malonyl CoA used in the preceding reaction is

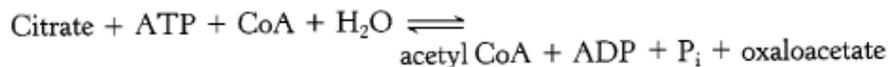


Hence, the overall stoichiometry for the synthesis of palmitate is



## Citrate Carries Acetyl Groups from Mitochondria to the Cytosol for Fatty Acid Synthesis

The synthesis of palmitate requires the input of 8 molecules of acetyl CoA, 14 molecules of NADPH, and 7 molecules of ATP. Fatty acids are synthesized in the cytosol, whereas acetyl CoA is formed from pyruvate in mitochondria. Hence, acetyl CoA must be transferred from mitochondria to the cytosol. Mitochondria, however, are not readily permeable to acetyl CoA. Recall that carnitine carries only long-chain fatty acids. *The barrier to acetyl CoA is bypassed by citrate, which carries acetyl groups across the inner mitochondrial membrane*. Citrate is formed in the mitochondrial matrix by the condensation of acetyl CoA with oxaloacetate. When present at high levels, citrate is transported to the cytosol, where it is cleaved by *ATP-citrate lyase*.



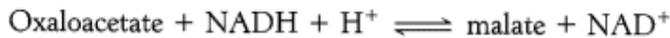
Thus, acetyl CoA and oxaloacetate are transferred from mitochondria to the cytosol at the expense of the hydrolysis of a molecule of ATP.

**Lyases-** These are nzymes catalyzing the cleavage of C-C, C-O, or C-N bonds by elimination. A double bond is formed in these reactions.

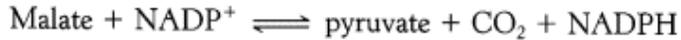
## Sources of NADPH for Fatty Acid Synthesis

Oxaloacetate formed in the transfer of acetyl groups to the cytosol must now be returned to the mitochondria. The inner mitochondrial membrane is impermeable to oxaloacetate. Hence, a series of bypass reactions are needed. Most important, these reactions generate much of the NADPH needed

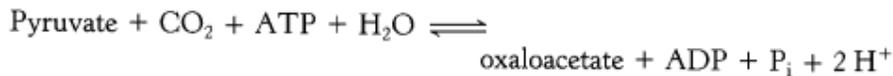
for fatty acid synthesis. First, oxaloacetate is reduced to malate by NADH. This reaction is catalyzed by a *malate dehydrogenase* in the cytosol.



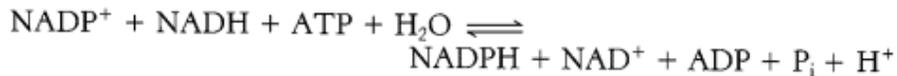
Second, malate is oxidatively decarboxylated by an *NADP*<sup>+</sup>-linked *malate enzyme* (also called *malic enzyme*).



The pyruvate formed in this reaction readily enters mitochondria, where it is carboxylated to oxaloacetate by pyruvate carboxylase.



The sum of these three reactions is

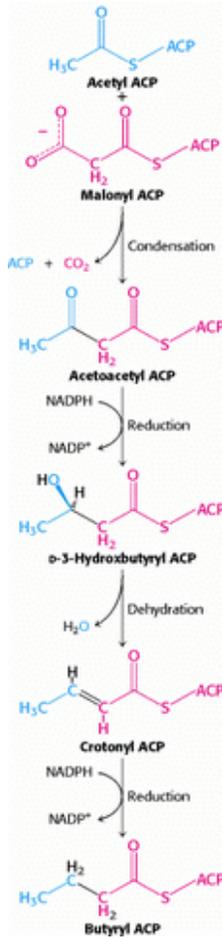


Thus, *one molecule of NADPH is generated for each molecule of acetyl CoA that is transferred from mitochondria to the cytosol*. Hence, eight molecules of NADPH are formed when eight molecules of acetyl CoA are transferred to the cytosol for the synthesis of palmitate. *The additional six molecules of NADPH required for this process come from the pentose phosphate pathway.*

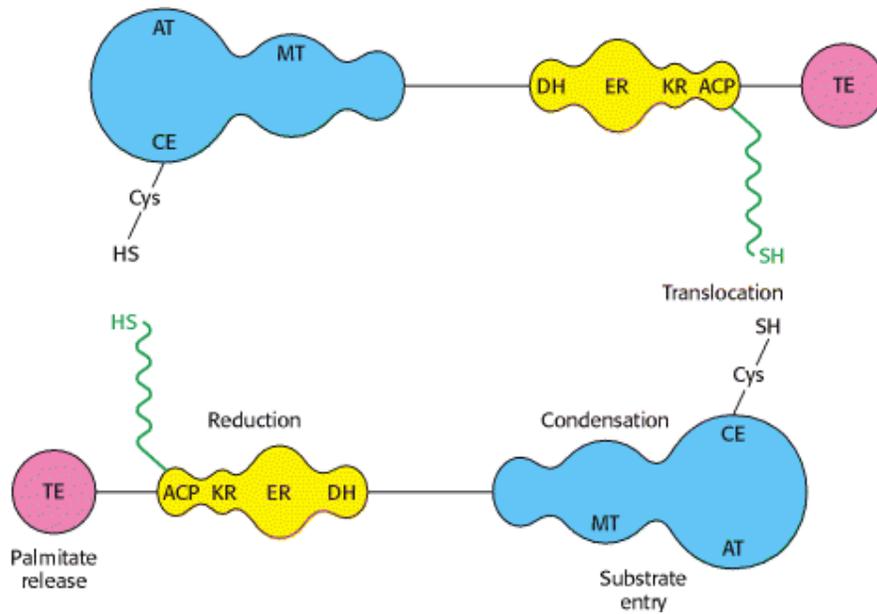
The accumulation of the precursors for fatty acid synthesis is a wonderful example of the coordinated use of multiple processes to fulfill a biochemical need. The citric acid cycle, subcellular compartmentalization, and the pentose phosphate pathway provide the carbon atoms and reducing power, whereas glycolysis and oxidative phosphorylation provide the ATP to meet the needs for fatty acid synthesis.

**Table. Principal reactions in fatty acid synthesis in bacteria**

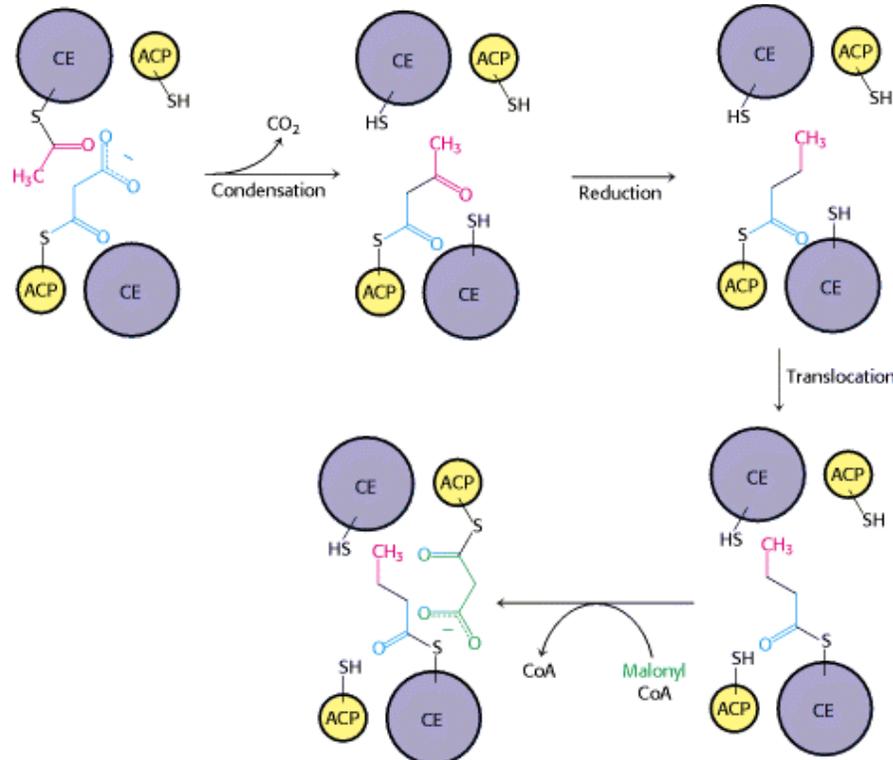
Step	Reaction	Enzyme
1	Acetyl CoA + HCO <sub>3</sub> <sup>-</sup> + ATP----- malonyl CoA + ADP + Pi + H <sup>+</sup>	Acetyl CoA carboxylase
2	Acetyl CoA + ACP===== acetyl ACP + CoA	Acetyl transacylase
3	Malonyl CoA + ACP===== malonyl ACP + CoA	Malonyl transacylase
4	Acetyl ACP + malonyl ACP----- acetoacetyl ACP + ACP + CO <sub>2</sub>	Acyl-malonyl ACP condensing enzyme
5	Acetoacetyl ACP + NADPH + H <sup>+</sup> =====d-3-hydroxybutyryl ACP + NADP <sup>+</sup>	β-Ketoacyl ACP reductase
6	d-3-Hydroxybutyryl ACP===== crotonyl ACP + H <sub>2</sub> O	3-Hydroxyacyl ACP dehydratase
7	Crotonyl ACP + NADPH + H <sup>+</sup> ----- butyryl ACP + NADP <sup>+</sup>	Enoyl ACP reductase



**Fatty Acid Synthesis.** Fatty acids are synthesized by the repetition of the following reaction sequence: condensation, reduction, dehydration, and reduction. The intermediates shown here are produced in the first round of synthesis.

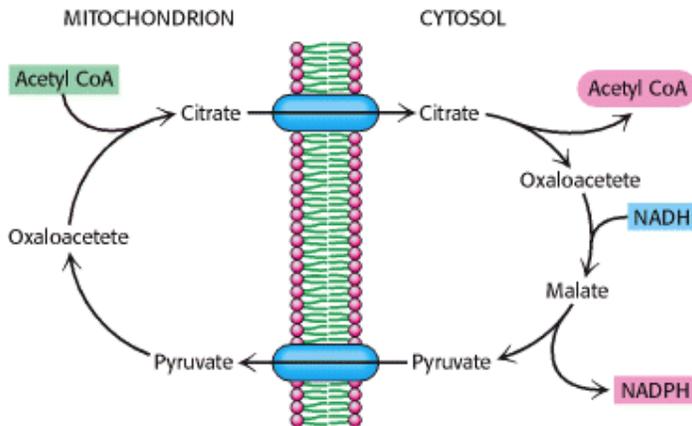


**Schematic Representation of Animal Fatty Acid Synthase.** Each of the identical chains in the dimer contains three domains. Domain 1 (blue) contains acetyl transferase (AT), malonyl transferase (MT), and condensing enzyme (CE). Domain 2 (yellow) contains acyl carrier protein (ACP), β-ketoacyl reductase (KR), dehydratase (DH), and enoyl reductase (ER). Domain 3 (red) contains thioesterase (TE). The flexible phosphopantetheinyl group (green) carries the fatty acyl chain from one catalytic site on a chain to another, as well as between chains in the dimer.



**Reactions of Fatty Acid Synthase.** Translocations of the elongating fatty acyl chain between the cysteine sulfhydryl group of the condensing enzyme (CE, blue) and the phosphopantetheine

sulfhydryl group of the acyl carrier protein (ACP, yellow) lead to the growth of the fatty acid chain. The reactions are repeated until the palmitoyl product is synthesized.



**Transfer of Acetyl CoA to the Cytosol.** Acetyl CoA is transferred from mitochondria to the cytosol, and the reducing potential NADH is concomitantly converted into that of NADPH by this series of reactions.

## Acetyl Coenzyme A Carboxylase Plays a Key Role in Controlling Fatty Acid Metabolism

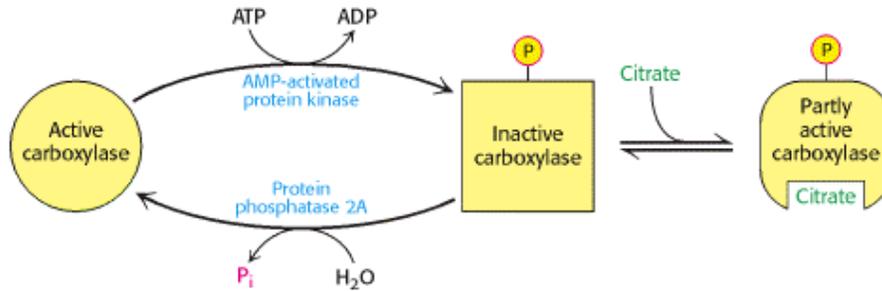
Fatty acid metabolism is stringently controlled so that synthesis and degradation are highly responsive to physiological needs. Fatty acid synthesis is maximal when carbohydrate and energy are plentiful and when fatty acids are scarce.

*Acetyl CoA carboxylase plays an essential role in regulating fatty acid synthesis and degradation.* Recall that this enzyme catalyzes the committed step in fatty acid synthesis: the production of malonyl CoA (the activated two-carbon donor). The carboxylase is controlled by three global signals glucagon, epinephrine, and insulin that correspond to the overall energy status of the organism. *Insulin stimulates fatty acid synthesis by activating the carboxylase, whereas glucagon and epinephrine have the reverse effect.* The levels of citrate, palmitoyl CoA, and AMP within a cell also exert control. *Citrate*, a signal that building blocks and energy are abundant, activates the carboxylase. Palmitoyl CoA and AMP, in contrast, lead to the inhibition of the carboxylase. Thus, this important enzyme is subject to both global and local regulation. We will examine each of these levels of regulation in turn.

### **Response to Diet.**

Fatty acid synthesis and degradation are reciprocally regulated so that both are not simultaneously active. *In starvation, the level of free fatty acids rises because hormones such as epinephrine and glucagon stimulate adipose-cell lipase. Insulin, in contrast, inhibits lipolysis.* Acetyl CoA carboxylase also plays a role in the regulation of fatty acid degradation. Malonyl CoA, the product of the carboxylase reaction, is present at a high level when fuel molecules are abundant. *Malonyl CoA inhibits carnitine acyltransferase I, preventing access of fatty acyl CoAs to the mitochondrial matrix in times of plenty.* Malonyl CoA is an especially effective inhibitor of carnitine acyltransferase I in heart and muscle, tissues that have little fatty acid synthesis capacity of their own. In these tissues, acetyl CoA carboxylase may be a purely regulatory enzyme. Finally, two enzymes in the  $\beta$ -oxidation pathway are markedly inhibited when the energy charge is high. NADH inhibits 3-hydroxyacyl CoA dehydrogenase, and acetyl CoA inhibits thiolase.

*Long-term control is mediated by changes in the rates of synthesis and degradation of the enzymes participating in fatty acid synthesis.* Animals that have fasted and are then fed high-carbohydrate, low-fat diets show marked increases in their amounts of acetyl CoA carboxylase and fatty acid synthase within a few days. This type of regulation is known as *adaptive control*.



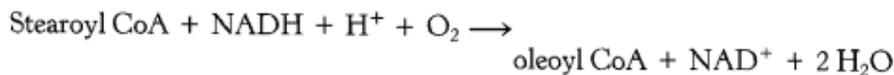
**Control of Acetyl CoA Carboxylase.** Acetyl CoA carboxylase is inhibited by phosphorylation and activated by the binding of citrate.

## Elongation and Unsaturation of Fatty Acids Are Accomplished by Accessory Enzyme Systems

The major product of the fatty acid synthase is palmitate. In eukaryotes, longer fatty acids are formed by elongation reactions catalyzed by enzymes on the cytosolic face of the *endoplasmic reticulum membrane*. These reactions add two carbon units sequentially to the carboxyl ends of both saturated and unsaturated fatty acyl CoA substrates. Malonyl CoA is the two-carbon donor in the elongation of fatty acyl CoAs. Again, condensation is driven by the decarboxylation of malonyl CoA.

## Membrane-Bound Enzymes Generate Unsaturated Fatty Acids

Endoplasmic reticulum systems also introduce double bonds into long-chain acyl CoAs. For example, in the conversion of stearoyl CoA into oleoyl CoA, a *cis*- $\Delta$  9 double bond is inserted by an oxidase that employs *molecular oxygen* and *NADH* (or *NADPH*).



This reaction is catalyzed by a complex of three membrane-bound enzymes: *NADH-cytochrome b5 reductase*, *cytochrome b5*, and a *desaturase*. First, electrons are transferred from NADH to the FAD moiety of NADH-cytochrome *b 5* reductase.

The heme iron atom of cytochrome *b 5* is then reduced to the Fe<sup>2+</sup> state. The nonheme iron atom of the desaturase is subsequently converted into the Fe<sup>2+</sup> state, which enables it to interact with O<sub>2</sub> and the saturated fatty acyl CoA substrate. A double bond is formed and two molecules of H<sub>2</sub>O are released. Two electrons come from NADH and two from the single bond of the fatty acyl substrate.

A variety of unsaturated fatty acids can be formed from oleate by a combination of elongation and desaturation reactions.

For example, oleate can be elongated to a 20:1 *cis*- $\Delta$  11 fatty acid. Alternatively, a second double bond can be inserted to yield an 18:2 *cis*- $\Delta$  6, $\Delta$  9 fatty acid. Similarly, palmitate (16:0) can be oxidized to palmitoleate (16:1 *cis*- $\Delta$  9), which can then be elongated to *cis*-vaccenate (18:1 *cis*- $\Delta$  11).

Unsaturated fatty acids in mammals are derived from either palmitoleate (16:1), oleate (18:1), linoleate (18:2), or linolenate (18:3). The number of carbon atoms from the  $\omega$  end of a derived unsaturated fatty acid to the nearest double bond identifies its precursor.

*Mammals lack the enzymes to introduce double bonds at carbon atoms beyond C-9 in the fatty acid chain.* Hence, mammals cannot synthesize linoleate (18:2 *cis*- $\Delta$  9,  $\Delta$  12) and linolenate (18:3 *cis*- $\Delta$  9,  $\Delta$  12,  $\Delta$  15). *Linoleate and linolenate are the two essential fatty acids.* The term *essential* means that they must be supplied in the diet because they are required by an organism and cannot be endogenously synthesized. Linoleate and linolenate furnished by the diet are the starting points for the synthesis of a variety of other unsaturated fatty acids.

## Eicosanoid Hormones Are Derived from Polyunsaturated Fatty Acids

*Arachidonate*, a 20:4 fatty acid derived from linoleate, is the major precursor of several classes of signal molecules: prostaglandins, prostacyclins, thromboxanes, and leukotrienes.

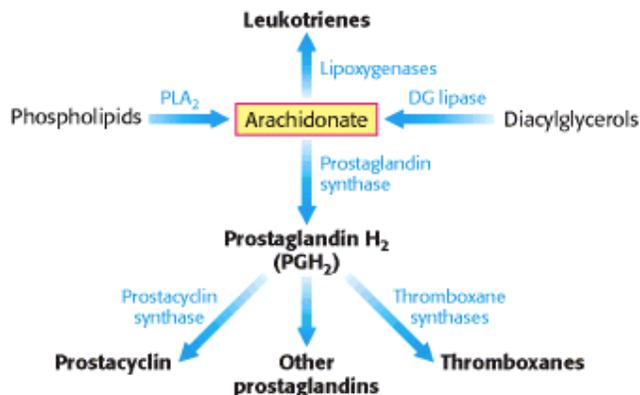
A *prostaglandin* is a 20-carbon fatty acid containing a 5-carbon ring. A series of prostaglandins is fashioned by reductases and isomerases. The major classes are designated PGA through PGI; a subscript denotes the number of carbon-carbon double bonds outside the ring. Prostaglandins with two double bonds, such as PGE<sub>2</sub>, are derived from arachidonate; the other two double bonds of this precursor are lost in forming a five-membered ring.

*Prostacyclin* and *thromboxanes* are related compounds that arise from a nascent prostaglandin. They are generated by *prostacyclin synthase* and *thromboxane synthase* respectively.

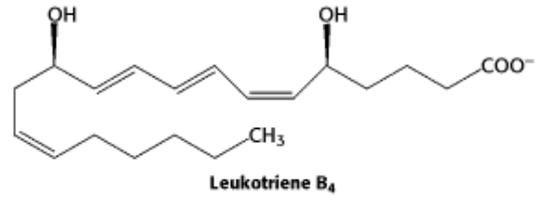
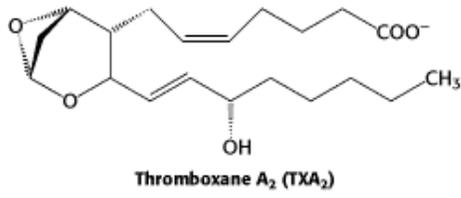
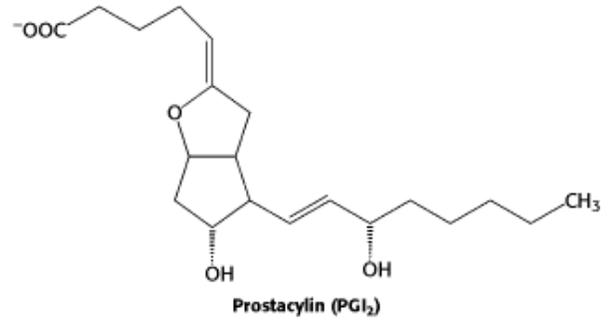
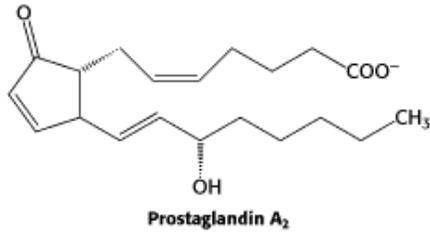
Alternatively, arachidonate can be converted into *leukotrienes* by the action of *lipoxygenase*. These compounds, first found in leukocytes, contain three conjugated double bonds hence, the name. Prostaglandins, prostacyclin, thromboxanes, and leukotrienes are called *eicosanoids* because they contain 20 carbon atoms.

Prostaglandins and other eicosanoids are *local hormones* because they are short-lived. They alter the activities both of the cells in which they are synthesized and of adjoining cells by binding to 7TM receptors. The nature of these effects may vary from one type of cell to another, in contrast with the more uniform actions of global hormones such as insulin and glucagon. Prostaglandins stimulate inflammation, regulate blood flow to particular organs, control ion transport across membranes, modulate synaptic transmission, and induce sleep.

Recall that aspirin blocks access to the active site of the enzyme that converts arachidonate into prostaglandin H<sub>2</sub>. Because arachidonate is the precursor of other prostaglandins, prostacyclin, and thromboxanes, blocking this step affects many signaling pathways. It accounts for the wide-ranging effects that aspirin and related compounds have on inflammation, fever, pain, and blood clotting.



**Arachidonate Is the Major Precursor of Eicosanoid Hormones.** Prostaglandin synthase catalyzes the first step in a pathway leading to prostaglandins, prostacyclins, and thromboxanes. Lipoxygenase catalyzes the initial step in a pathway leading to leukotrienes.



Structures of Several Eicosanoids.