## 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-bystep fashion into acetyl CoA, which is then processed in the citric acid cycle.
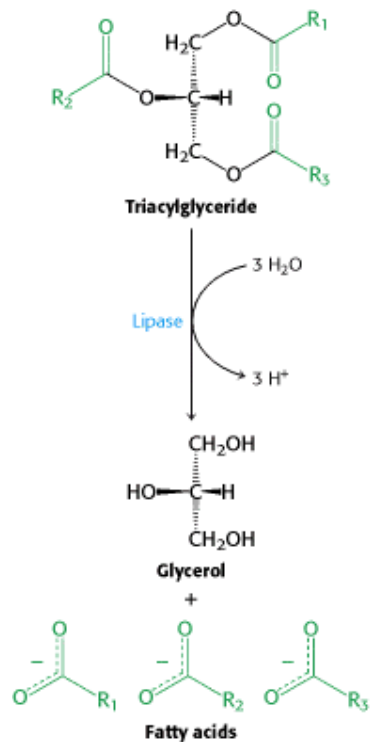
Triacylglycerols Are Hydrolyzed by Cyclic AMP-Regulated Lipases

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 (Section 15.1.3). 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.
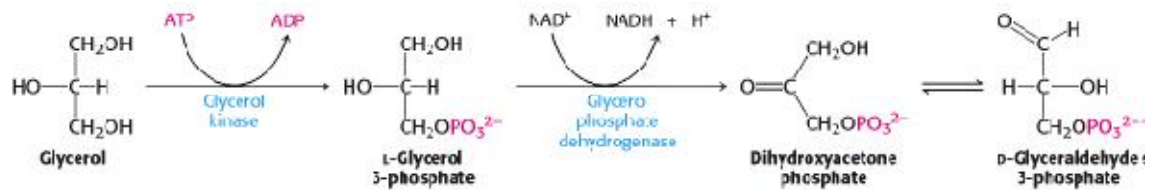
## Fatty Acids Are Linked to Coenzyme A Before They Are Oxidized

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*).
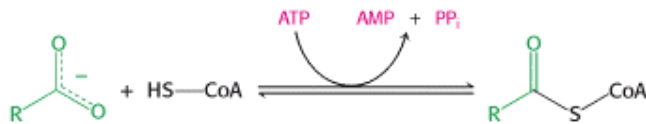
Activation of a fatty acid is accomplished in two steps. First, the fatty acid reacts with ATP to
form an *acyl adenylate*. In this mixed anhydride, the carboxyl group of a fatty acid is bonded to the phosphoryl group of
AMP. The other two phosphoryl groups of the ATP substrate are released as pyrophosphate. 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 PPi and AMP) and one high-transfer-potential compound is
formed (the thioester acyl CoA).

Fatty acid + ATP ⇌ **Acyl adenylate** + PP_i (1)

Acyl CoA + AMP   (2)


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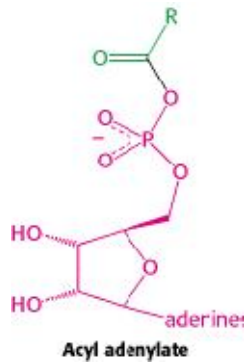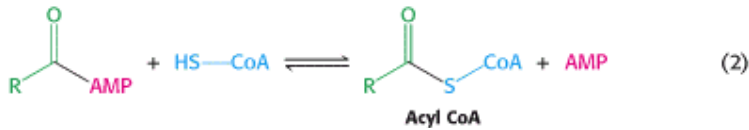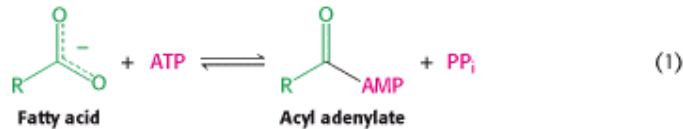
How is the overall reaction driven forward? The answer is that pyrophosphate is rapidly hydrolyzed by a pyrophosphatase, and so the complete reaction is

$$RCOO^- + CoA + ATP + H_2O \longrightarrow RCO{-}CoA - AMP + P_i + 2\,H^+$$

This reaction is quite favorable because the equivalent of two molecules of ATP is hydrolyzed, whereas only one hightransfer-
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 CoA   Carnitine   Acyl carnitine
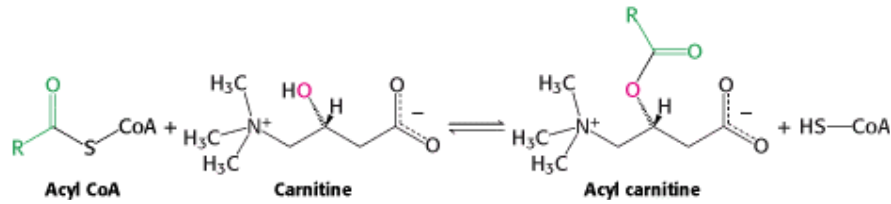
Acyl carnitine is then shuttled across the inner mitochondrial membrane by a translocase (Figure 22.7). The acyl group is
transferred back to CoA on the matrix side of the membrane. This reaction, which is catalyzed by *carnitine*
acyltransferase II (carnitine palmitoyl transferase II), is simply the 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 carnatine 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 prolonged exercise is an important
characteristic of a deficiency of carnitine acyl transferases because muscle relies on fatty acids as a long-term source of
energy. Medium-chain (C8-C10) fatty acids, which do not require carnitine to enter the mitochondria, are oxidized
normally in these patients. These diseases illustrate that the impaired flow of a metabolite from one compartment of a
cell to another can lead to a pathological condition.

Acetyl CoA, NADH, and FADH2 Are Generated in Each Round of Fatty Acid
Oxidation

A saturated acyl CoA is degraded by a recurring sequence of four reactions: oxidation by flavin adenine dinucleotide
(FAD), hydration, oxidation by NAD+, and thiolysis by CoA (Figure 22.8). The fatty acyl chain is shortened by two
carbon atoms as a result of these reactions, and FADH2, NADH, and acetyl CoA are generated. Because oxidation is on
the β carbon, this series of reactions is called the β-*oxidation pathway*.
The first reaction in each round of degradation is the *oxidation* of acyl CoA by an *acyl CoA dehydrogenase* to give an
enoyl CoA with a trans double bond between C-2 and C-3.

$$\text{Acyl CoA} + \text{E-FAD} \longrightarrow \textit{trans-}\Delta^2\text{-enoyl CoA} + \text{E-FADH}_2$$
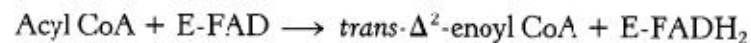
As in the dehydrogenation of succinate in the citric acid cycle, FAD rather than NAD+ is the electron acceptor because
the value of ΔG for this reaction is insufficient to drive the reduction of NAD+. Electrons from the FADH2 prosthetic
group of the reduced acyl CoA dehydrogenase are transferred to a second flavoprotein called *electron-transferring*
flavoprotein (ETF). In turn, ETF donates electrons to *ETF:ubiquinone reductase*, an iron-sulfur protein. Ubiquinone is
thereby reduced to ubiquinol, which delivers its high-potential electrons to the second proton-pumping site of the
respiratory chain. Consequently, 1.5 molecules of ATP are generated per molecule of FADH2 formed in
this dehydrogenation step, as in the oxidation of succinate to fumarate.

The next step is the *hydration* of the double bond between C-2 and C-3 by *enoyl CoA hydratase*.

$$\textit{trans-}\Delta^2\text{-Enoyl CoA} + H_2O \rightleftharpoons \text{L-3-hydroxyacyl CoA}$$
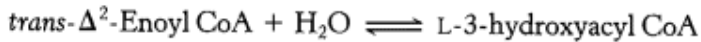
The hydration of enoyl CoA is stereospecific. Only the L isomer of 3-hydroxyacyl CoA is formed when the trans-$\Delta 2$
double bond is hydrated. The enzyme also hydrates a cis-$\Delta 2$ double bond, but the product then is the D isomer. We shall
return to this point shortly in considering how unsaturated fatty acids are oxidized.
The hydration of enoyl CoA is a prelude to the second *oxidation* reaction, which converts the hydroxyl group at C-3 into
a keto group and generates NADH. This oxidation is catalyzed by *L-3-hydroxyacyl CoA dehydrogenase*, which is specific
for the L isomer of the hydroxyacyl substrate.

$$\text{L-3-Hydroxyacyl CoA} + NAD^+ \rightleftharpoons \text{3-ketoacyl CoA} + NADH + H^+$$

The preceding reactions have oxidized the methylene group at C-3 to a keto group. The final step is the cleavage of 3- ketoacyl CoA by the thiol group of a second molecule of CoA, which yields acetyl CoA and an acyl CoA shortened by two carbon atoms. This thiolytic cleavage is catalyzed by β-*ketothiolase*.

$$\begin{array}{c} \text{3-Ketoacyl CoA} + \text{HS—CoA} \rightleftharpoons \text{acetyl CoA} + \quad \text{acyl CoA} \\ (n \text{ carbons}) \hspace{13em} (n - 2 \text{ carbons}) \end{array}$$

The table below summarizes the reactions in fatty acid oxidation.
The shortened acyl CoA then undergoes another cycle of oxidation, starting with the reaction catalyzed by acyl CoA
dehydrogenase (Figure 22.9). Fatty acyl chains containing from 12 to 18 carbon atoms are oxidized by the long-chain
acyl CoA dehydrogenase. The medium-chain acyl CoA dehydrogenase oxidizes fatty acyl chains having from 14 to 4
carbons, whereas the short-chain acyl CoA dehydrogenase acts only on 4- and 6- carbon acyl chains. In contrast, β-
ketothiolase, hydroxyacyl dehydrogenase, and enoyl CoA hydratase have broad specificity with respect to the length of
the acyl group.