FATTY ACID CATABOLISM

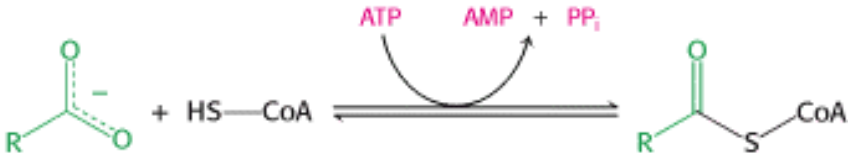
Beta-Oxidation of fatty acids:

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.

The enzymes of fatty acid oxidation in animal cells are located in the mitochondrial matrix, as demonstrated in 1948 by Eugene P. Kennedy and Albert Lehninger. The fatty acids are activated before they enter the mitochondrial matrix. The fatty acids with chain lengths of 12 or fewer carbons enter mitochondria without the help of membrane transporters. Those with 14 or more carbons, which constitute the majority of the FFA obtained in the diet or released from adipose tissue, cannot pass directly through the mitochondrial membranes—they must first undergo the three enzymatic reactions of the **carnitine shuttle.** The first reaction is catalyzed by a family of isozymes present in the outer mitochondrial membrane, the **acyl-CoA synthetases,** which promote the general reaction

Fatty acid + CoA + ATP ⟶ fatty acyl–CoA + AMP + PPi

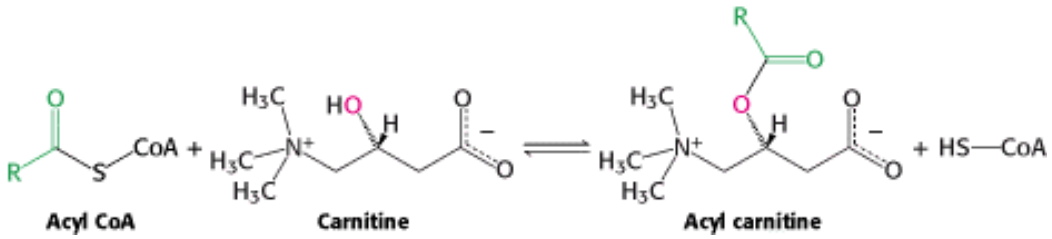
Thus, acyl-CoA synthetases catalyze the formation of a thioester linkage between the fatty acid carboxyl group and the thiol group of coenzyme A to yield a **fatty acyl–CoA,** coupled to the cleavage of ATP to AMP and PPi.



The 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.

Fatty acids are activated on the outer mitochondrial membrane, whereas they are oxidized in the mitochondrial matrix. 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 trans esterification is catalyzed by **carnitine acyltransferase I,** in outer mitochondrial membrane.



The fatty acyl–carnitine ester then enters the matrix by facilitated diffusion through the **acyl-carnitine/carnitine transporter** of the inner mitochondrial membrane. In the third and final step of the carnitine shuttle, the fatty acyl group is enzymatically transferred from carnitine to intra mitochondrial coenzyme A by **carnitine acyltransferase II.** This isozyme, located on the inner face of the inner mitochondrial membrane, regenerates fatty acyl–CoA and releases it, along with free carnitine, into the matrix. Carnitine reenters the intermembrane space via the acyl-carnitine/carnitine transporter.

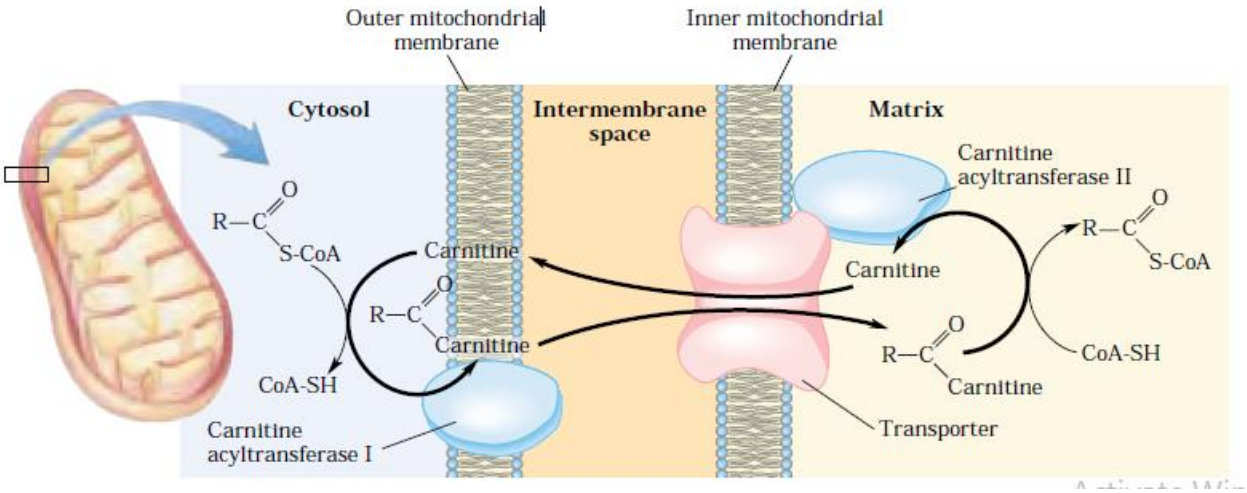


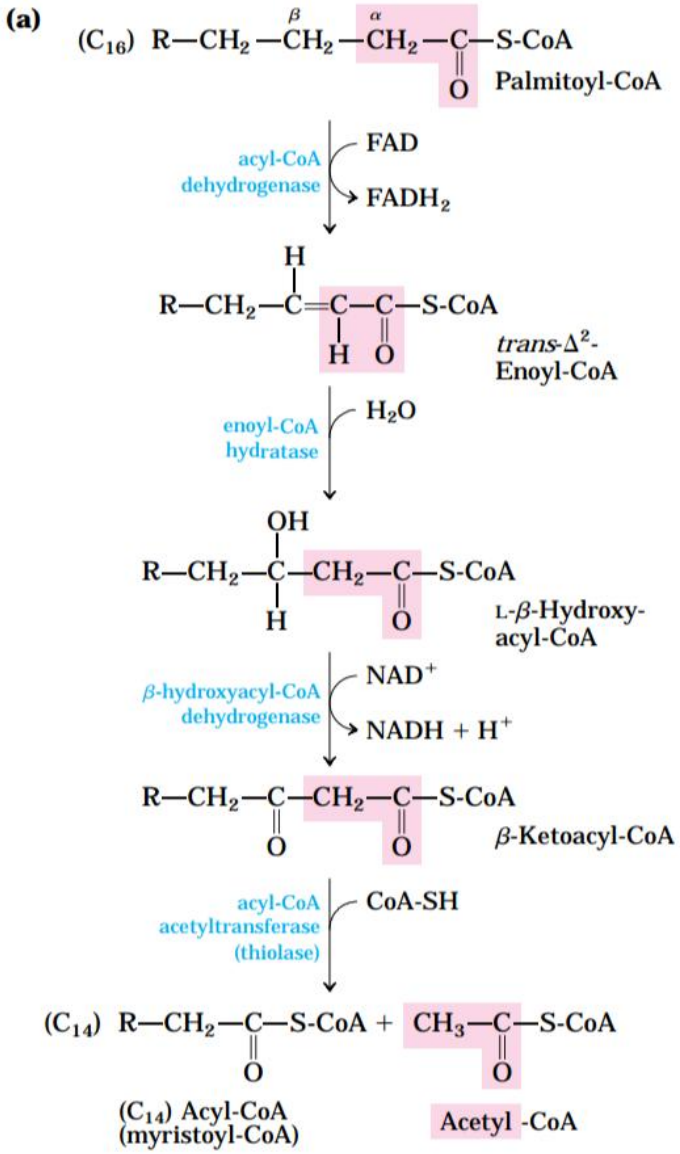
Figure: Fatty-acyl carnitine/carnitine shuttle

Beta-oxidation of saturated fatty acids:

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.

- The first reaction in each round of degradation is the *oxidation* of acyl CoA by an ***acyl CoA dehydrogenase*** to give a trans- Δ^2enoyl CoA with a trans double bond between C-2 and C-3. There are three isozymes of acyl-CoA dehygrogenase all of which are flavoproteins with FAD. The electrons removed from the fatty acyl–CoA are transferred to FAD, and the reduced form of the dehydrogenase immediately donates its electrons and become oxidized.
- The next step is the *hydration* of the double bond between C-2 and C-3of enoyl CoA by ***enoyl CoA hydratase*** to form the L stereoisomer of **β-hydroxyacyl-CoA (3-hydroxyacyl-CoA).**
- In the third step, L-*β* hydroxyacyl-CoA is dehydrogenated to form *β*-**ketoacyl-CoA,** by the action of β-**hydroxyacyl-CoA dehydrogenase;** NAD^+ is the electron acceptor. The NADH formed in the reaction donates its electrons to **NADH dehydrogenase,** an electron carrier of the respiratory chain.

- The fourth and last step of the *β*-oxidation cycle is catalyzed by **acyl-CoA acetyl transferase,** more commonly called **thiolase,** which promotes reaction of- β ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now shortened by two carbon atoms.



In one pass through the β-oxidation sequence, one molecule of acetyl-CoA, two pairs of electrons, and four protons (H^+) are removed from the long-chain fatty acyl–CoA, shortening it by two carbon atoms (14-C myristoyl-CoA). The myristoyl-CoA can now go through another set

of four *β*-oxidation reactions, exactly analogous to the first, to yield a second molecule of acetyl-CoA and lauroyl-CoA, the coenzyme A thioester of the 12-carbon laurate. Altogether, seven passes through the β-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight molecules of acetyl-CoA.

Palmitoyl-CoA + 7CoA + 7FAD + 7NAD^+ + 7H2O → 8 acetyl-CoA + 7FADH2 + 7NADH + 7H^+

Energetics:

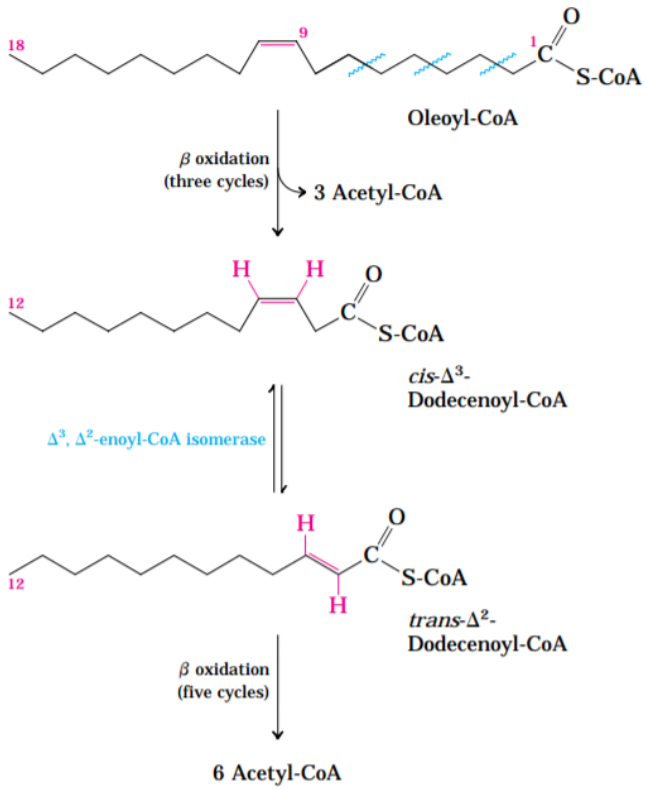
In each reaction cycle, an acyl CoA is shortened by two carbon atoms, and one molecule each of FADH2, NADH, and acetyl CoA is formed. The degradation of palmitoyl CoA (C16-acyl CoA) requires seven reaction cycles. In the seventh cycle, the C4-ketoacyl CoA is thiolyzed to two molecules of acetyl CoA. Hence, the stoichiometry of oxidation of palmitoyl CoA is

$$\text{Palmitoyl CoA} + 7\,\text{FAD} + 7\,\text{NAD}^+ + 7\,\text{CoA} + 7\,\text{H}_2\text{O} \longrightarrow 8\,\text{acetyl CoA} + 7\,\text{FADH}_2 + 7\,\text{NADH} + 7\,\text{H}^+$$

Approximately 2.5 molecules of ATP are generated when the respiratory chain oxidizes each of the 7 molecules of NADH, whereas 1.5 molecules of ATP are formed for each of the 7 molecules of FADH2 because their electrons enter the chain at the level of ubiquinol. Recall that the oxidation of acetyl CoA by the citric acid cycle yields 10 molecules of ATP. Hence, the number of ATP molecules formed in the oxidation of palmitoyl CoA is 10.5 from the 7 molecules of FADH2, 17.5 from the 7 molecules of NADH, and 80 from the 8 molecules of acetyl CoA, which gives a total of 108. The equivalent of 2 molecules of ATP is consumed in the activation of palmitate, in which ATP is split into AMP and 2 molecules of Pi. Thus, *the complete oxidation of a molecule of palmitate yields 106 molecules of ATP*.

For monounsaturated fatty acids:

Oleate is an abundant 18-carbon monounsaturated fatty acid with a cis double bond between C-9 and C-10. Oleoyl-CoA then undergoes three passes through the fatty acid oxidation cycle to yield three molecules of acetyl-CoA and the coenzyme A ester of a Δ^3, 12-carbon unsaturated fatty acid, *cis*-Δ3-dodecenoyl- CoA. This product cannot serve as a substrate for enoyl-CoA hydratase, which acts only on trans double bonds. The auxiliary enzyme **Δ^3, Δ^2-enoyl-CoA isomerase** isomerizes the *cis*-Δ^3-enoyl-CoA to the *trans*-Δ^2-enoyl CoA, which is converted by enoyl-CoA hydratase into the corresponding L-*β*-hydroxyacyl-CoA (*trans*-Δ^2-dodecenoyl-CoA). This intermediate is now acted upon by the remaining enzymes of *β*-oxidation to yield acetyl CoA and the coenzyme A ester of a 10- carbon saturated fatty acid, decanoyl-CoA. The latter undergoes four more passes through the pathway to yield five more molecules of acetyl-CoA. Altogether, nine acetyl-CoAs are produced from one molecule of the 18-carbon oleate.



For Polyunsaturated Fatty Acids:

Linoleate is a C18 polyunsaturated fatty acid with cis-Δ^9 and cis-Δ^{12} double bonds. The cis-Δ^3 double bond formed after three rounds of β-oxidation is converted into a trans-Δ^2 double bond by the aforementioned isomerase. The acyl CoA produced by another round of β-oxidation contains a cis-^{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 β -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*-Δ^3-enoyl CoA. *cis*- Δ^3-Enoyl CoA isomerase then converts *trans*-Δ^3-enoyl CoA into the trans-Δ^2 form, a customary intermediate in the β -oxidation pathway.