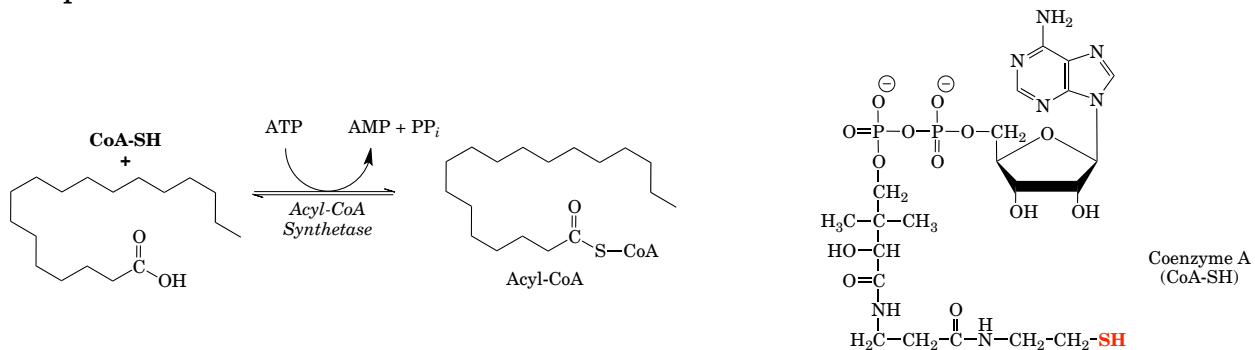


Fatty Acid Breakdown

The reactions involved in the actual breakdown of free fatty acids occur in the mitochondria. While short chain fatty acids (10 to 12 carbons or shorter) can enter the mitochondria by diffusion, long chain fatty acids require activation and translocation.

Activation of fatty acids

The enzyme *acyl-CoA synthetase* catalyzes the formation of a thioester bond between a fatty acid and coenzyme A. Thioester links are high-energy bonds; acyl-CoA synthetase uses the energy from ATP to drive the formation of the thioester. As drawn below, the reaction is reversible, but as with most similar reactions, the pyrophosphate released is converted to two molecules of inorganic phosphate by pyrophosphatase. Because AMP (rather than ADP) is the product from the reaction, acyl-CoA synthetase uses the equivalent of two ATP molecules to supply energy for the process.³



In the drawing above, the coenzyme A structure is given explicitly. This is relatively rarely done; coenzyme A participation in the reaction is limited to the altered chemistry it introduces in the thioester and to the improved ability for enzymes to bind the more complex coenzyme A structure rather than the simple carboxylic acid function.

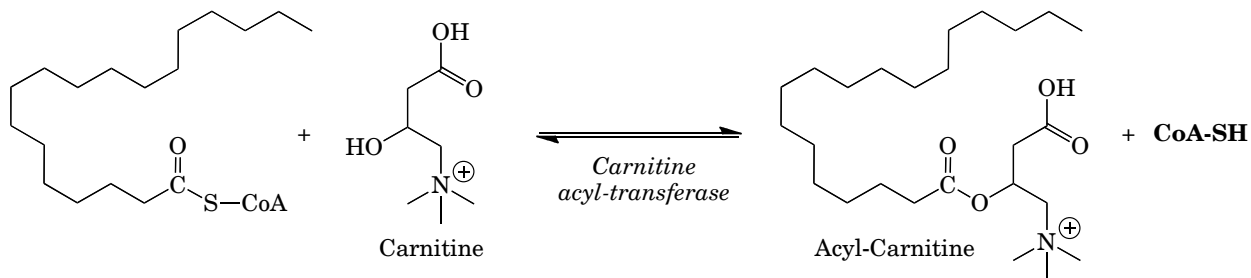
Translocation

Once activated by conjugation to coenzyme A, the acyl-CoA must be transported into the mitochondria. Entry of the activated fatty acid into the mitochondria is a multistep process. In order to maintain separate cytoplasmic and mitochondria pools of coenzyme A, the transport process uses a separate small molecule, carnitine.

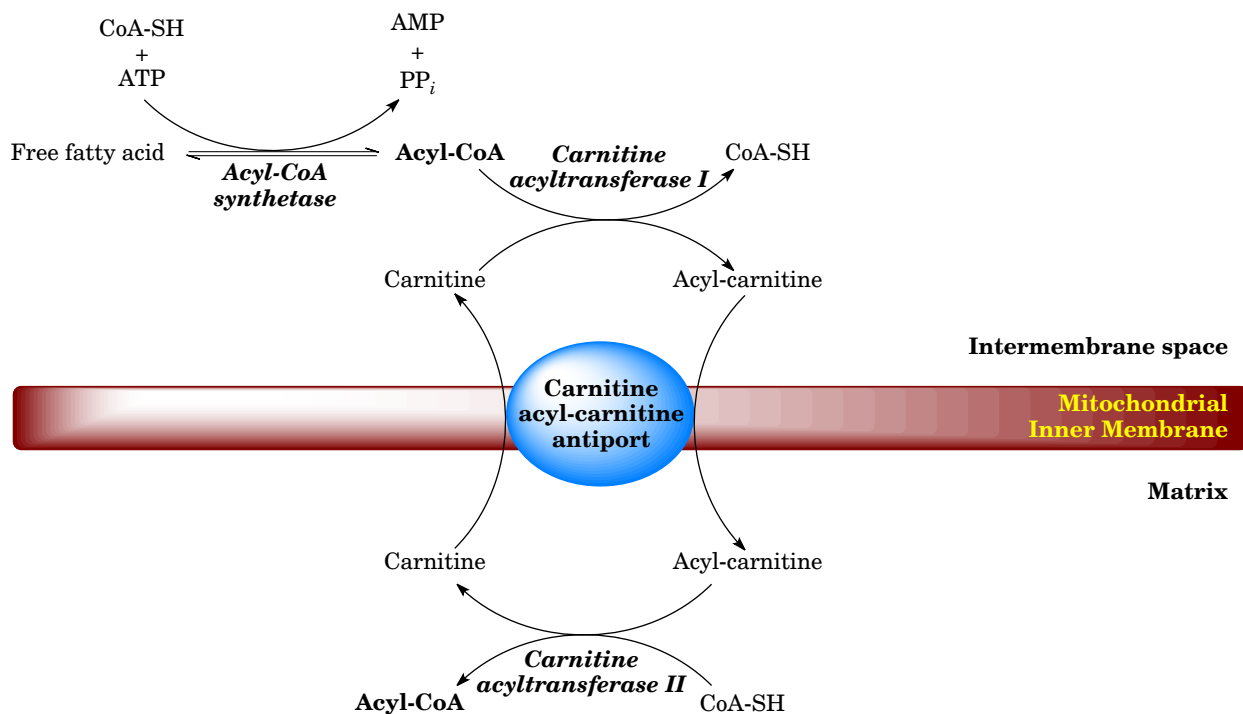
The cytosolic enzyme *carnitine acyltransferase I* reversibly exchanges the thioester bond to coenzyme A in the acyl-CoA for an ester bond to carnitine. Carnitine acyltransferase I is inhibited by malonyl-CoA, the substrate for fatty acid biosynthesis. Because entry into the mitochondria is required for breakdown of fatty

³The regeneration of ATP from AMP must occur in two steps. The first is the reversible reaction catalyzed by adenylate kinase that uses an ATP to phosphorylate the AMP, producing two ADP molecules. These must then both be converted back to ATP.

acids, and because acyl-carnitine (unlike acyl-CoA) can enter the mitochondria, carnitine acyltransferase I acts as a major control point for fatty acid breakdown.

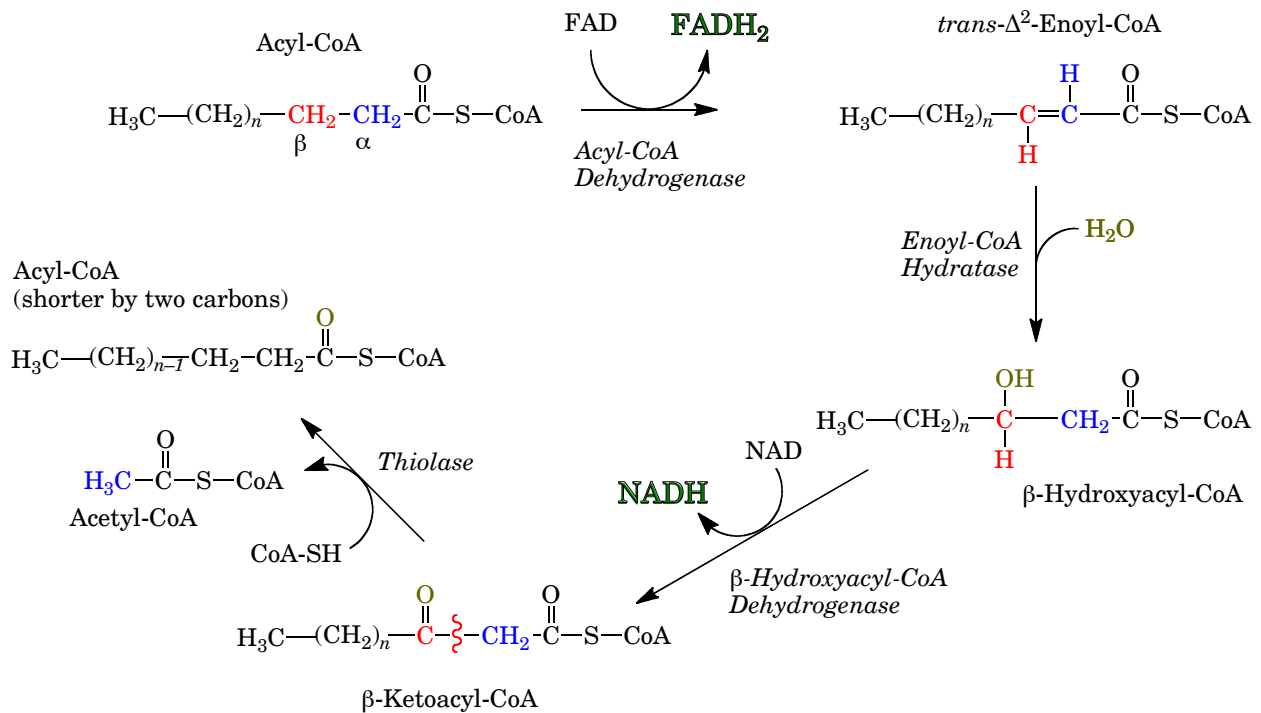


Acyl-carnitine is a ligand for a specific transporter, the carnitine/acyl-carnitine antiport. Once inside the mitochondrion, carnitine acyltransferase II reforms the Acyl-CoA. (Note that both of the carnitine acyltransferase reactions are readily reversible; no energy is added or lost during the transport process.) This multistep process of acyl-CoA entry into the mitochondria is summarized in the diagram below.

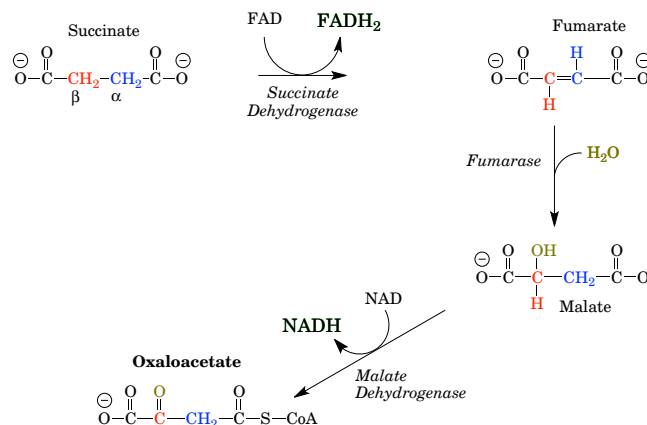


Fatty acid β -oxidation reactions

The β -oxidation pathway is called " β -oxidation" due to the fact that most of the chemistry involves the β -carbon of the acyl-CoA substrate. The initial acyl-CoA undergoes a series of four reactions, ending with the release of the two-carbon acetyl-CoA, and an acyl-CoA molecule two carbons shorter than the original. This shorter acyl-CoA then re-enters the pathway; the fatty acid β -oxidation pathway thus consists of a spiral, with the substrate decreasing in size until the final set of reactions releases two acetyl-CoA molecules.



In order to release a two-carbon unit from a fatty acid, an enzyme must break the bond between the α and β carbons (the blue and red carbons, respectively, in the pathway drawing). Direct cleavage of an unsubstituted carbon-carbon bond is an extremely difficult process to accomplish in a controlled fashion. In order to allow the process to occur, a three-enzyme pathway must first activate the β -carbon, followed by cleavage of the bond between the methylene α -carbon and the ketone on the oxidized β -carbon. The three enzymes involved in the activation events of the β -oxidation pathway are similar in many respects to the succinate dehydrogenase, fumarase and malate dehydrogenase enzymes of the TCA cycle.



Acyl-CoA dehydrogenase oxidizes the α - β bond single bond to a *trans* double bond while reducing FAD. The reaction is generally similar to that catalyzed by succinate dehydrogenase. Like succinate dehydrogenase, acyl-CoA dehydrogenase

transfers electrons to the electron transport chain. Unlike, succinate dehydrogenase, however, acyl-CoA dehydrogenase is not located in the mitochondrial inner membrane, but instead uses a short chain of soluble electron carriers to donate electrons from its FADH₂ cofactor to coenzyme Q.

Most organisms contain multiple acyl-CoA dehydrogenase enzymes. Although each isozyme catalyzes essentially identical reactions, the isozymes differ somewhat in acyl chain-length specificity. The effect of having different isozymes is most apparent in that genetic deficiencies of specific isozymes have somewhat different physiological consequences. As an example, deficiency in the medium chain acyl-CoA dehydrogenase (MCAD) has been linked to about 10% of “sudden infant death syndrome” (SIDS) cases.

Enoyl-CoA hydratase catalyzes a hydration reaction that adds a water molecule across the double bond formed by acyl-CoA dehydrogenase. This reaction is similar to the fumarase reaction of the TCA cycle. Enoyl-CoA hydratase results in the formation of a hydroxyl group on the β -carbon of the acyl chain.

β -Hydroxyacyl-CoA dehydrogenase uses NAD as a cofactor for the oxidation of the β -hydroxyl to a ketone, a reaction similar to that catalyzed by malate dehydrogenase. The result is the formation of β -ketoacyl-CoA, which contains a ketone on the carbon β to the thioester carbon.

Thiolase (also called Acyl-CoA:acetyltransferase) cleaves the β -ketoacyl-CoA, releasing an acyl-CoA two carbons shorter, and acetyl-CoA. The thiolase reaction forms a thioester bond between the β -ketone carbon and an additional coenzyme A, while breaking the bond between the α and β carbons of the original acyl-CoA. As we will see later, the thiolase reaction is potentially reversible. The thiolase cleavage reaction is inhibited by acetyl-CoA (largely because thiolase is capable of condensing two acetyl-CoA molecules in a reverse reaction).

The purpose of the first three reactions is to take an unsubstituted carbon and activate it by introducing a ketone in the β -position. The carbonyl destabilizes the carbon-carbon bond between the α and β carbons, and therefore allows the facile cleavage reaction catalyzed by thiolase to take place.

The acetyl-CoA produced usually enters the TCA cycle, although, especially in the liver, the acetyl-CoA can be used for lipid biosynthetic reactions.

The β -oxidation spiral is repeated until the fatty acid is completely degraded. If the original fatty acid contained an even number of carbons, the final spiral releases two molecules of acetyl-CoA. It is worth noting that the β -oxidation process is a spiral, not a cycle; each turn of the spiral results in a shorter substrate for the next turn. This contrasts with cyclic processes such as the TCA cycle, which begin and end with the same compound.

Energetics of fatty acid oxidation

It is useful to compare the energetics for glucose and fatty acid metabolism, because both glucose and fatty acids can ultimately be converted into acetyl-CoA, and then

oxidized to carbon dioxide. As we have seen previously, performing the TCA cycle results in production of one GTP (which is the equivalent of an ATP), three NADH, and one FADH₂ from each acetyl-CoA.

As mentioned in the section on oxidative phosphorylation, the yield of ATP from the reduced cofactors is a matter of some controversy; for the purpose of these comparisons, we will assume that three ATP are produced for every NADH, and two ATP for every FADH₂. These values apply to optimum conditions, with lower values being observed in actual physiology. In addition, we will assume that the cell is using the malate-aspartate shuttle for transport of NADH into the mitochondria. This shuttle results in entry of reducing equivalents as NADH rather than as FADH₂, and therefore in the maximal ATP yield from glucose. (Because fatty acid oxidation occurs entirely within the mitochondria, the shuttling of reducing equivalents is not relevant to fatty acid breakdown.)

The conversion of glucose to acetyl-CoA results in the net production of two ATP and four NADH (if you do not recall why this is true, please review the pathways for glucose breakdown). The two acetyl-CoA then result in the formation of an additional six NADH, two FADH₂, and two ATP. As is shown in the table, totaling these values reveals an overall yield of 38 ATP from glucose.

Comparison of Energetics of Metabolism for Glucose and Stearic Acid				
Energetic molecule	Glucose	Stearate ↓ Acetyl-CoA	9 Acetyl-CoA ↓ CO₂	Stearate (total)
Products				
ATP	4 → 4 ATP	-2	9	7 → 7 ATP
NADH	10 → 30 ATP	8	27	35 → 105 ATP
FADH ₂	2 → 4 ATP	8	9	17 → 34 ATP
Total	38 ATP			146 ATP

Breakdown of a fatty acid requires activation to the acyl-CoA, a process that costs two ATP equivalents. For the 18-carbon fatty acid stearic acid, eight spirals of the β-oxidation pathway, resulting in nine acetyl-CoA, eight NADH, and eight FADH₂, follow the activation step. The nine cycles of the TCA cycle required to consume the acetyl-CoA produced result in formation of nine ATP, 27 NADH, and nine FADH₂. Thus, the complete breakdown of stearic acids results in a net production of seven ATP + 35 NADH + 17 FADH₂. (Note that in the nine TCA cycles, nine ATP are produced; however, the activation reaction requires the equivalent of two ATP.) If we use the same values for ATP production from the reduced cofactors, this results in a total of 146 ATP.

Is this comparison fair? Perhaps not: glucose contains only six carbons, while stearic acid contains 18 carbons. A fairer comparison involves consideration of the amount

of ATP produced per carbon. Dividing the ATP production by the number of carbons in the compound reveals that glucose yields 6.3 ATP per carbon, while stearic acid yields 8.1 ATP per carbon. Thus the fatty acid results in slightly more ATP than does glucose.

An even more useful comparison, however, takes molecular weight into account. Glucose has a molecular weight of 180 g/mol, while stearic acid has molecular weight of 284 g/mol. Dividing ATP produced by the molecular weight of the compound reveals yields of 0.2 ATP/gram (dry weight) of glucose compared to 0.5 ATP/gram (dry weight) of stearic acid. Thus, on a dry weight basis, fatty acids have a higher energy density than do carbohydrates.

The energy density difference of fatty acids and glucose is even more striking when the hydration of the compound *in vivo* is taken into account; in aqueous solution, glucose is associated with roughly three times its weight in water, while fatty acids are stored as hydrophobic (and therefore nearly totally dehydrated) triacylglycerols. This means that, physiologically, fatty acids contain roughly eight-times more energy than carbohydrates per unit mass.

Special cases

The β -oxidation pathway discussed above applies to nearly all fatty acids and their derivatives. However, some fatty acids contain odd-numbers of carbons or sites of unsaturation. These compounds require additional reactions to complete their breakdown.

Odd-numbered fatty acids

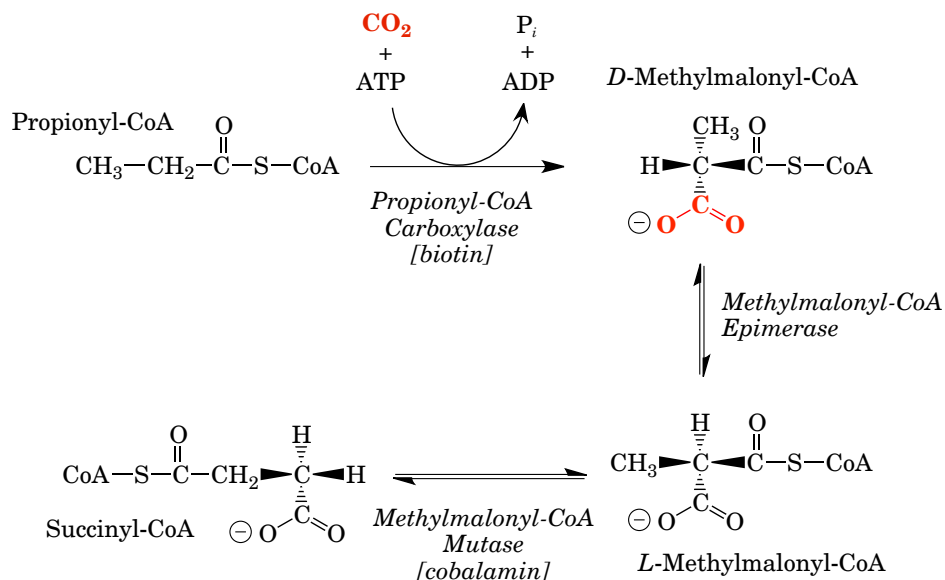
Fatty acids with odd numbers of carbons are found in some marine animals, in many herbivores, in microorganisms, and in plants. These fatty acids are subjected to β -oxidation in the same way as fatty acids with even numbers of carbons. However, the final β -oxidation spiral results in the production of the three-carbon compound propionyl-CoA, which cannot be metabolized in the same way as acetyl-CoA. Instead, propionyl-CoA is converted to succinyl-CoA, a TCA cycle intermediate, via a three enzyme pathway.⁴

Propionyl-CoA is a substrate for the biotin-dependent enzyme **propionyl-CoA carboxylase**, which uses the energy in ATP to add a carbon, resulting in the four-carbon compound D-methylmalonyl-CoA. The next reaction, catalyzed by **methylmalonyl-CoA epimerase**, reverses the stereochemistry at the chiral carbon of the substrate, resulting in L-methylmalonyl-CoA.

The final reaction in the pathway, catalyzed by **methylmalonyl-CoA mutase**, converts the branched chain compound L-methylmalonyl-CoA into succinyl-CoA, a TCA cycle intermediate. Unlike acetyl-CoA, succinyl-CoA can be used as a

⁴ In addition to odd-chain fatty acids, propionyl-CoA is produced from breakdown of isoleucine, methionine, and valine, and some threonine, and from the plant lipid phytol. Complete deficiency in the first enzyme of the pathway (propionyl-CoA carboxylase) results in a life-threatening disorder known as ketotic glycinemia.

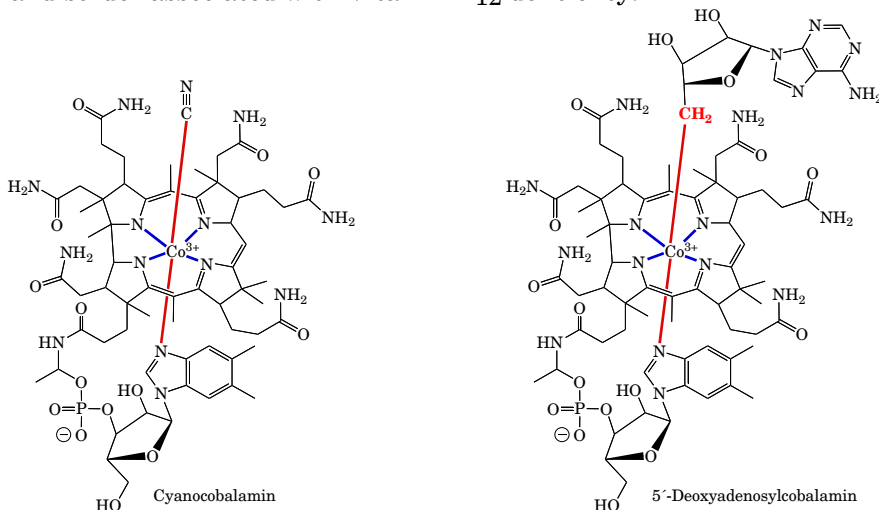
gluconeogenic substrate. Succinyl-CoA production can also be used to increase TCA capacity.



The reactions involved in converting propionyl-CoA to succinyl-CoA are useful for more than merely completing the metabolism of odd chain fatty acids; metabolism of some amino acids and of some other compounds also results in propionyl-CoA production.

Side note: vitamin B₁₂

Vitamin B₁₂ (cobalamin) is used only in animals and some microorganisms; because plants do not use this compound, strict vegetarians are at some risk for developing pernicious anemia, the potentially lethal disorder associated with Vitamin B₁₂ deficiency.



Methylmalonyl-CoA mutase is one of two known vitamin B₁₂-dependent enzymes in humans (the other is methionine synthase), although many more vitamin B₁₂-dependent enzymes are found in bacteria. Most vitamin B₁₂-dependent enzymes catalyze carbon-transfer reactions, where a group is moved from a first atom to second atom in exchange for a hydrogen derived from the second atom. In

the case of methylmalonyl-CoA mutase, the carbonyl of the thioester is moved from the branched α -carbon to the methyl carbon.

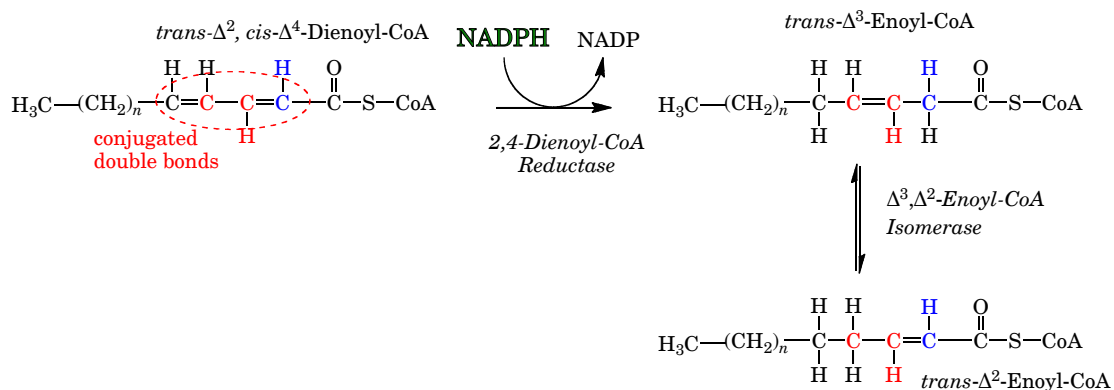
Although the cobalamin ring structure is similar in general appearance to the porphyrin structure of heme and chlorophyll, cobalamin contains a corrin ring, not a porphyrin. In addition, the cobalamin contains a cobalt ion rather than the iron typically present in heme or the magnesium found in most chlorophyll derivatives. The coenzyme form of Vitamin B₁₂ is the only known molecule in humans that may exhibit a covalent bond between a carbon and a metal ion. The carbon-cobalt bond forms readily; Vitamin B₁₂ is frequently called cyanocobalamin, due to the cyanide group that is frequently bound as an artifact of the purification procedure.

In methylmalonyl mutase, the active cofactor form of the vitamin is 5'-deoxyadenosylcobalamin. The methylene group of the 5'-deoxyadenosylcobalamin (shown in red) abstracts a hydrogen from the methyl group of the substrate to begin the catalytic process; this hydrogen is eventually returned to the substrate following the group transfer.

Unsaturated fatty acids

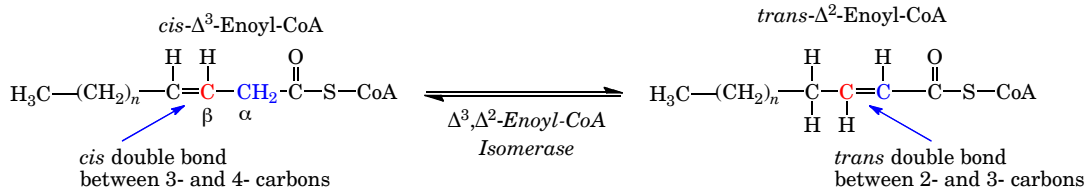
The reactions described above apply to saturated fatty acids. While unsaturated fatty acids are also metabolized using the β -oxidation pathway, the oxidation of the unsaturated carbons requires additional reactions. Depending on the position of the original double bond, the site of unsaturation presents one of two possible problems.

1) Even-numbered double bonds: If the original double bond is in an even-numbered position, normal β -oxidation will eventually result in the presence of a Δ^2 - Δ^4 conjugated intermediate. The enoyl-CoA hydratase cannot use the conjugated compound as a substrate. Instead, **2,4-dienoyl-CoA reductase** uses electrons from NADPH to reduce the two conjugated double bonds to a single double bond at the 3-position. This Δ^3 compound is then converted to the *trans*- Δ^2 -enoyl-CoA β -oxidation intermediate by enoyl-CoA isomerase as described above.

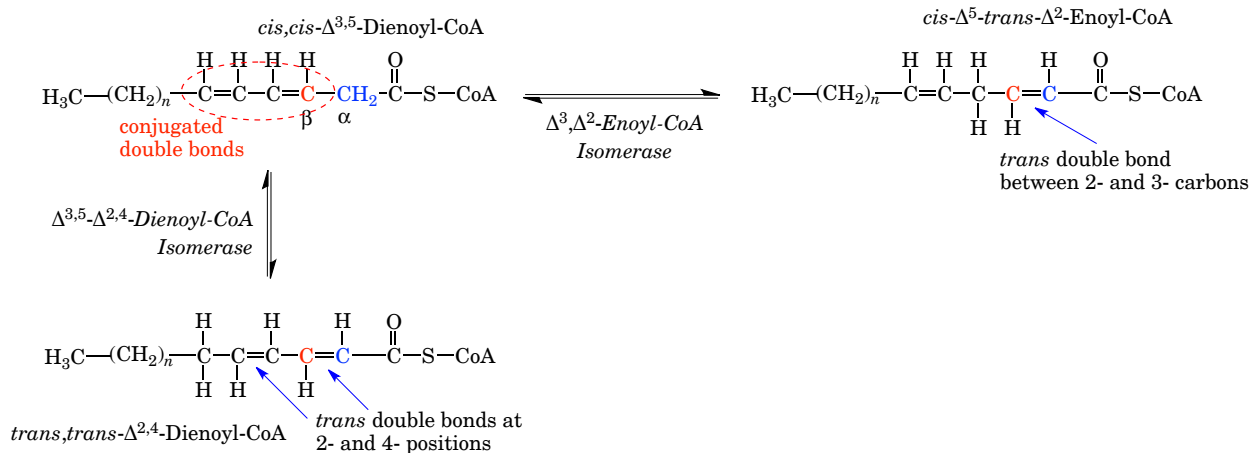


2) Odd-numbered double bonds: If the original double bond is in an odd-numbered position, normal β -oxidation will eventually result in the presence of a double bond at the 3-position. For example, three β -oxidation spirals for the Δ^9 fatty acid oleic acid will result in Δ^3 -enoyl-CoA. Acyl-CoA dehydrogenase, which normally oxidizes the bond between the 2-position and 3-position carbons, cannot use a Δ^3 -enoyl-CoA as a substrate. Instead, the double bond needs to be moved to the 2-position, a reaction catalyzed by Δ^3 , Δ^2 -enoyl-CoA isomerase. The product of this

process is *trans*- Δ^2 -enoyl-CoA, which is identical to the first β -oxidation intermediate.



2a) Odd-numbered double binds in compounds also containing even-numbered double bonds: the Δ^3,Δ^2 -enoyl-CoA isomerase is reversible. In fatty acids containing an odd-numbered double bond, the Δ^3,Δ^2 -enoyl-CoA isomerase may act unnecessarily, and create a conjugated double bond. This conjugated double bond is then the substrate for a second enzyme, $\Delta^{3,5}-\Delta^{2,4}$ -dienoyl-CoA isomerase, which moves the conjugated system from the 3,5 to the 2,4 positions. This conjugated system is then acted upon by the system that deals with even-numbered bonds.



Short-chain fatty acids

Fatty acids smaller than about 10 carbons can enter the mitochondrial matrix without needing assistance from carnitine pathway. Otherwise, they are metabolized normally.

Long-chain fatty acids

Fatty acids above a certain size (greater than 22 carbons) cannot enter the mitochondria. Instead, these compounds are metabolized in another type of subcellular organelle, the peroxisome. The peroxisomal β -oxidation pathways are basically similar to those of the mitochondria, except that the peroxisomal acyl-CoA dehydrogenase releases its electrons by forming hydrogen peroxide rather than by donation to the electron transport chain, because the electron transport pathway does not exist in the peroxisomes.

Peroxisomal β -oxidation stops with short acyl chains (4 to 8-carbons) because the peroxisomal thiolase will not cleave shorter acyl chains. The short chain acyl-CoA is converted to acyl-carnitine, which then goes to mitochondria to be metabolized to acetyl-CoA.

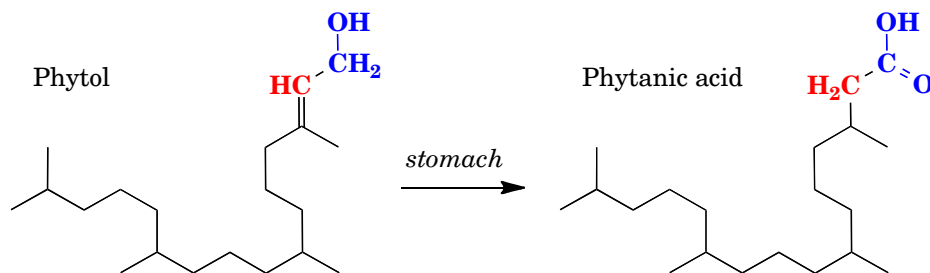
Long chain fatty acids diffuse into peroxisomes unassisted (neither carnitine nor activation of the fatty acid is necessary). Once in the peroxisome, a peroxisomal acyl CoA synthetase activates the long chain fatty acids. Acetyl-CoA produced in the peroxisomes is released to the cytoplasm (the peroxisomes do not perform the TCA cycle).

The function of the peroxisome pathway is not entirely clear. It only generates small amounts of energy. This pathway probably functions to prevent the accumulation of the relatively rare long-chain fatty acids; accumulation of long-chain fatty acids causes a rare disorder (X-adrenoleukodystrophy) that afflicted one of the characters in the movie *Lorenzo's Oil*.

α -Oxidation

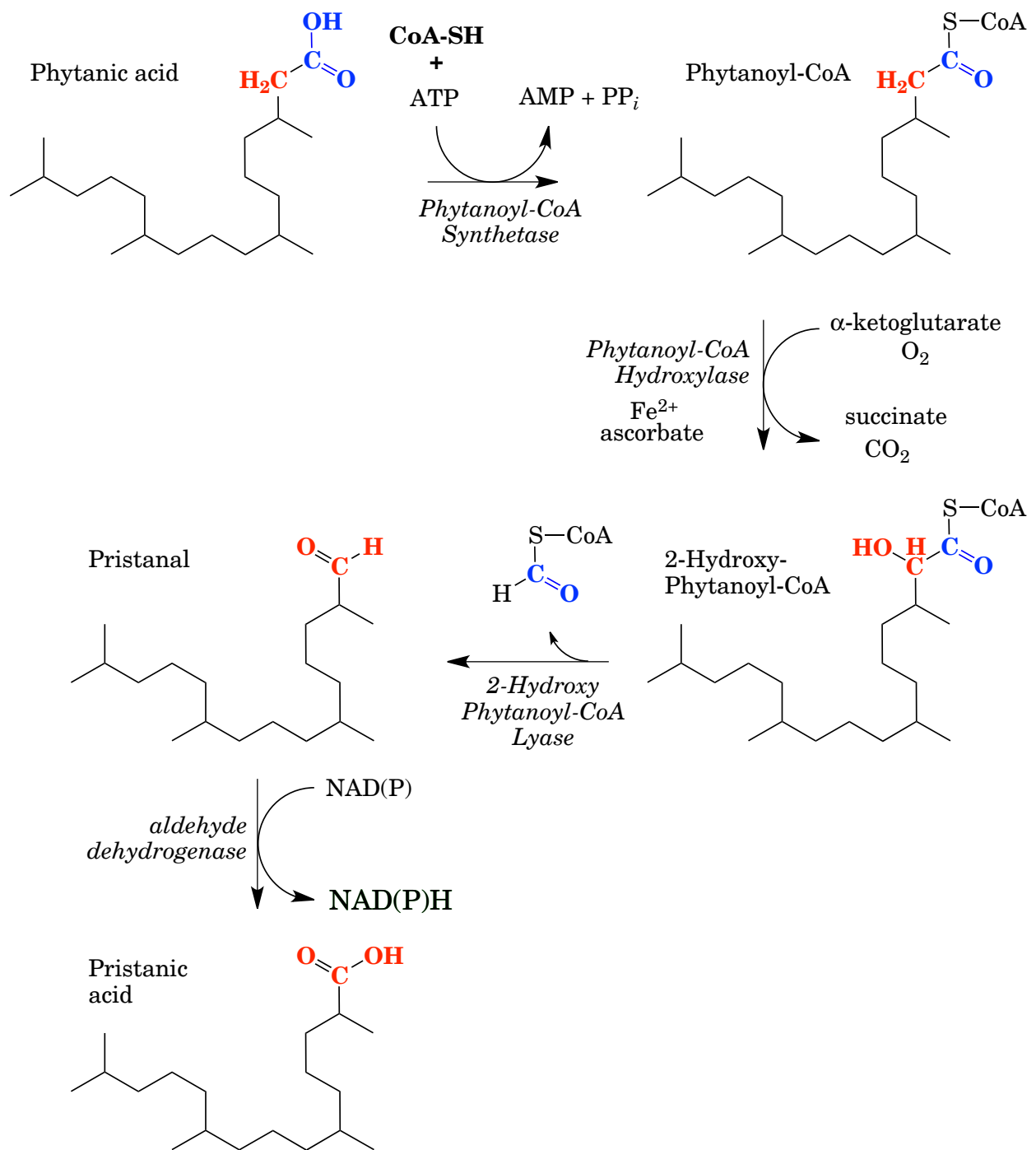
The β -oxidation pathway is capable of breaking down hydrocarbons that lack branch-points, and hydrocarbons with methyl-group branches at even-numbered carbons. If the branch is on the odd-numbered carbon, however, other reactions are necessary. (Note that the β -carbon is odd-numbered; if the β -carbon is branched, it is impossible to form the β -carbon ketone required for normal β -oxidation.)

Branched-chain fatty acids are relatively unusual. However, plants contain phytol (the long hydrocarbon side-chain of chlorophyll). Phytol is oxidized to phytanic acid in the stomach.



Note that phytanic acid contains a methyl group attached to the β -carbon. The presence of the β -methyl group means this compound is not a substrate for β -oxidation. Instead, phytanic acid must be subjected to α -oxidation. The α -oxidation process involves the addition of a hydroxyl group to the α -carbon by **phytanoyl-CoA α -hydroxylase**, followed by loss of the destabilized terminal carbon as formyl-CoA, and then oxidation of the aldehyde formed to a carboxylic acid.

The product of this two-enzyme process, pristanic acid, is branched at the α -position, rather than the β -position. This allows the β -oxidation pathway to proceed essentially normally. Note that some spirals of the β -oxidation pathway (*i.e.* the ones with branched α -carbons) will release the three-carbon propionyl-CoA, which, as noted earlier, can be converted to succinyl-CoA.



The α -oxidation pathway does not generate energy. It merely acts as a method for allowing the entry of β -branched hydrocarbons into the β -oxidation pathway. Alternatively, in some fatty acid metabolism disorders or dietary deficiencies in which metabolism of longer fatty acid is compromised, α -oxidation may act as a mechanism for partially degrading the fatty acid until β -oxidation can take over.

Summary

Lipids have a variety of uses. One of the most important uses is the storage of energy in a compact form.

Lipids must be solubilized to allow absorption from the diet. Solubilization involves the use of saponification enzymes (such various triacylglycerol lipases) and detergents such as bile acids, free fatty acids, and partially hydrolyzed phospholipids.

Lipids are stored as esters; the main storage form for fatty acids is the glycerol ester triacylglycerol. Hormone-sensitive lipase, the enzyme that releases free fatty acids from lipid stores, is controlled by epinephrine, cortisol, and insulin.

Degradation of free fatty acids is a multistep process. The free fatty acid is activated by the ATP-dependent formation of a thioester bond to coenzyme A. The acyl-CoA is then translocated into the mitochondria via the carnitine shuttle. Once inside the mitochondria, the bond between the α and β carbons of the acyl-CoA is activated and the cleaved in a sequential process called β -oxidation. The result is the release of several acetyl-CoA molecules from each acyl-CoA, followed by oxidation of the acetyl-CoA in the TCA cycle.

Fatty acids containing double bonds, odd-numbers of carbons, or branched β -carbons require somewhat modified pathways for metabolism.