

Introduction to Lipid Metabolism

Roles of Lipids

Lipids have a wide variety of roles in biological systems. These roles are a consequence of their chemical and physical properties. Fatty acids and their derivatives (especially triacylglycerols) can act as highly concentrated **energy storage molecules**. The high energy density (*i.e.* the relatively large amount of energy released per unit of mass) of fat stores is due to three main factors. 1) The completely reduced carbons of fatty acids have a higher energy content than the partially oxidized carbons of carbohydrates and proteins. 2) The fortuitous fact that the reduced carbons have covalent bonds to light atoms (hydrogen rather than to the heavier oxygen) means that the fully reduced hydrocarbon compounds are lighter than the partially oxidized carbohydrates. 3) Lipids are hydrophobic molecules and therefore fat stores contain little water, which would add to the weight of the molecules without adding to the energy content.

Because layers of lipids are good **insulators**, and because adipose tissue has limited metabolic activity, fat stores can reduce the exchange of heat between an organism and its environment. This insulation is important for mammals living in cold climates, and is especially important for marine mammals, which would otherwise rapidly lose their body heat to the surrounding water.

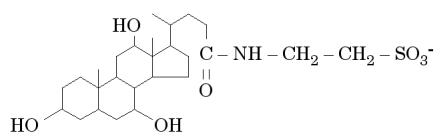
As we have already seen, **membranes** are composed of fatty acid derivatives. These compounds form hydrophobic barriers that separate cells from their surroundings and which subdivide cells into multiple compartments that allow more finely tuned control of metabolism. Lipids are also used as **signaling molecules**, such as prostaglandins and steroids, and as **enzyme cofactors**.

Digestion of lipids

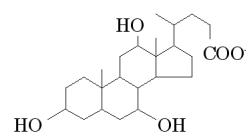
The majority of lipids in a normal diet are present in the form of triacylglycerols. Digestion of these compounds begins in the stomach, which contains acid-stable lipases that release some free fatty acids from dietary triacylglycerols. However, the stomach is not capable of efficiently cleaving triacylglycerols, because these hydrophobic molecules tend to aggregate, and the lipases are only capable of hydrolyzing the triacylglycerols at the surface of the aggregates. In addition, the stomach has a small surface area to volume ratio, and therefore many of the triacylglycerols are not accessible to the enzymes.

The small intestine has mechanisms for emulsifying lipids. The process begins by dispersing the lipid aggregates mechanically as a result of the muscles of the small intestine forcing the partially digested material through the relatively small spaces of the intestinal lumen. In addition, the intestine contains bile acids and bile salts, detergents that break up the lipid aggregates into smaller micelles.

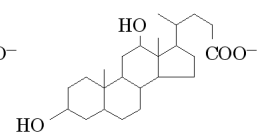
Examples of bile acids



Taurocholate

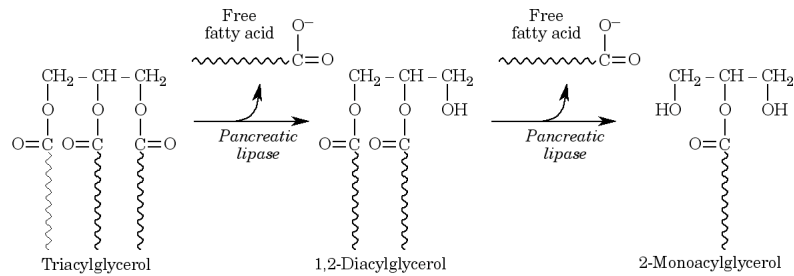


Cholate



Deoxycholate

Finally, the small intestine also contains a variety of digestive enzymes produced in the pancreas. These enzymes include **pancreatic cholesteryl ester hydrolase**, which releases free cholesterol from cholesteryl esters, **pancreatic lipase**, which releases free fatty acids from the 1- and 3-positions of triacylglycerols, and several **phospholipases**, which release free fatty acids from phospholipids. The monoacylglycerols, partially hydrolyzed phospholipids, and free fatty acids act as additional detergents and assist in further disrupting the larger lipid aggregates.



Absorption of fatty acids

Once the micelles of free fatty acids, 2-monoacylglycerols, and bile acids become small enough, they can be absorbed from the intestinal lumen into the body. Inside the body the fatty acids are esterified to re-form triacylglycerols. These triacylglycerols combine with lipoproteins released by the intestines to produce chylomicrons, which act as serum transport particles for triacylglycerols.

Lipid transport

Lipid transport is a continuously varying process. During the absorption of nutrients from the diet, lipids must be transported to the tissues for use. When lipids are not being absorbed, they must be transported from adipose stores to maintain metabolism. Finally, cholesterol redistribution from one tissue to another requires movement of cholesterol through the blood stream.

Lipids are hydrophobic and exhibit very limited solubility in aqueous media such as the blood. Analysis of blood indicates that plasma contains triacylglycerol, phospholipids, cholesterol, and free fatty acids.

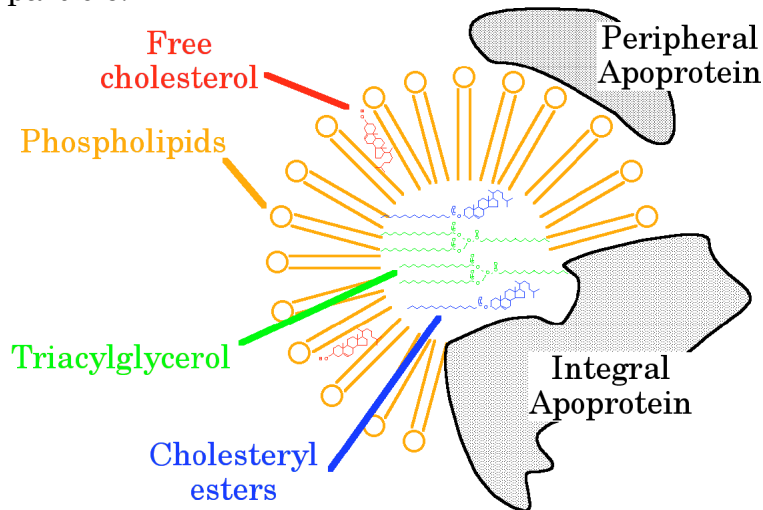
Free fatty acid levels in the blood are usually quite low (less than 5% of the total plasma lipids). The levels of free fatty acids depend on the rate of their release by adipose tissue. Most free fatty acids are actually bound to serum albumin. A sodium-dependent active transporter mediates transport of the free fatty acids into cells. Uptake of fatty acids is largely a function of fatty acid concentration in plasma; the relative levels of β -oxidation and esterification to form triacylglycerol or phospholipids depend on the status of the cell.

Transport and use of lipids other than free fatty acids requires specialized mechanisms to overcome their insolubility. One option would be to simply form micelles, and allow these to move freely. However, most lipids are insufficiently soluble to allow favorable micelle formation. In addition, actual lipid transport requires a greater degree of control than would result from release of individual

lipid molecules. Actual lipid transport involves specialized particles combining the lipids with specific proteins that allow the control of lipid movement.

Lipoproteins

Lipoproteins consist of a mixture of protein, phospholipid, cholesterol, and triacylglycerol. The proportions of each vary depending on the specific type of particle.



Lipid is less dense than protein or water. Initial studies on lipid transport separated the different transport forms on the basis of density, with the density differential being largely the result of differing protein content. Lipoproteins are considered to fall into four major classes:

1. Chylomicrons (the least dense form)
2. VLDL (very low density lipoproteins)
3. LDL (low density lipoproteins)
4. HDL (high density lipoproteins)

In addition, there are two minor classes: IDL (intermediate density lipoproteins, which are intermediate between VLDL and LDL), and chylomicron remnants, which are the residual protein and lipid after the completion of triacylglycerol extraction from chylomicrons.

The proteins present in lipoproteins are called apolipoproteins or simply apoproteins. (The prefix “apo-” means without, with apolipoprotein referring to the protein without the lipid.) The apoproteins play a major role in the regulation of cellular interactions with the lipoproteins. Some apoproteins are permanent parts of the particles; others are capable of transferring from one lipoprotein to another.

Apoproteins are divided into classes. The Apo-A forms (comprised of several different gene products) are found in chylomicrons and HDL. The Apo-C forms (especially Apo-C-II) and Apo-E are found in HDL, VLDL, and chylomicrons; these apoproteins are released as part of HDL, and are transferred to VLDL and

chylomicrons while in circulation. Apo-B-100 is found in VLDL and LDL, while Apo-B-48 is found in chylomicrons.¹

Apoproteins interact with cell surface receptors to allow transport of lipids into cells. In addition, some of the apoproteins modulate (either activate or inhibit) enzyme activities related to lipids, and assist in the transfer of the lipids from one lipoprotein to another, or from the lipoprotein to the cell.

Chylomicrons

Intestinal absorption of fatty acids results in triacylglycerol synthesis in the intestine. The triacylglycerols are then incorporated into chylomicrons in endoplasmic reticulum and Golgi apparatus of the intestinal cells. The chylomicrons then leave the cells by an exocytotic process, enter the lymph system and slowly enter the bloodstream. (Diffusion into blood is a slow process for the particles.)

As synthesized, chylomicrons contain only Apo-A and Apo-B-48. Mature chylomicrons also contain Apo-C and Apo-E; however, these apoproteins appear to be added during circulation, probably by transfer from HDL. Chylomicrons have a short half-life in circulation (less than 60 minutes in humans); note, however, that entry into circulation takes a long time, and chylomicron levels are elevated for ~12 hours after a meal.

Lipoprotein lipase

Removal of fatty acids from chylomicrons and from VLDL requires *lipoprotein lipase*, an enzyme located on the capillary walls. Lipoprotein lipase requires Apo-C-II and phospholipid as activators; VLDL and chylomicrons have Apo-C-II, allowing the lipoprotein lipase to hydrolyze the triacylglycerols in these particles. Heart lipoprotein lipase has a lower K_m for triacylglycerol than does the adipose tissue isozyme; as a result, the heart enzyme is always active, while the rate of triacylglycerol cleavage by adipose tissue depends on the level of substrate. Thus the heart can always obtain substrate, while the adipose tissue only removes fatty acids from circulation when circulating lipid levels are elevated.

During lactation, the mammary gland lipoprotein lipase is highly active (due to both high levels of enzyme and low K_m) in order to support milk production at the expense of storing lipids in the adipose tissue.

Insulin increases lipoprotein lipase levels in adipose tissue; this is one mechanism for increasing triacylglycerol storage in adipose tissue.

Chylomicron remnants

The action of lipoprotein lipase depletes the chylomicron of TAG. The process occurs rapidly; interaction of the chylomicron with the lipase results in loss of ~90% of the lipid before the particle dissociates. In addition, the action of lipoprotein lipase

¹ Apo-B-100 is a very large protein, containing 4536 amino acids. The “100” does **not** refer to the size in kD; instead, Apo-B-48 is 48% of the size of Apo-B-100. Both Apo-B-100 and Apo-B-48 are produced from the same gene. Apo-B-48 is produced in the intestine; it is shorter than Apo-B-100 because of a differential editing of the mRNA.

results in the dissociation of Apo-C-II from the particle, with the released Apo-C-II going back to HDL particles. Without Apo-C-II, the lipoprotein is no longer a substrate for lipase, and is called a chylomicron remnant. In contrast, the Apo-E remains with the remnant; Apo-E acts as the ligand for the chylomicron remnant receptor in liver.

VLDL

VLDL is synthesized and released by the liver. VLDL is used to transport triacylglycerol from the liver to other tissues. As with chylomicrons, triacylglycerols from VLDL are hydrolyzed by lipoprotein lipase. Apo-C and B-100 are the major apoproteins in VLDL. As with chylomicrons, after the majority of the triacylglycerols have been removed from the VLDL, the Apo-C dissociates. The loss of the triacylglycerol means that the remnants of the VLDL, called IDL (intermediate density lipoprotein) have a higher density (due to a higher protein to lipid ratio) and a higher ratio of cholesterol to other lipids.

LDL

IDL is converted to LDL, largely by the liver, by removal of additional triacylglycerol. In addition to its formation from VLDL, some LDL is produced and released by the liver. LDL is a major transport form of cholesterol and cholesteryl esters. The relative rates of VLDL and LDL release by the liver depend on the availability of cholesterol. If the regulatory pathways signal the liver to increase its cholesterol output, then the liver increases its LDL production.

LDL has specific cell surface receptors. It is internalized by receptor-mediated endocytosis. The receptor-LDL complex is transported to lysosomes, for degradation of the particle, while most of the LDL receptors are recycled to the cell surface. The amount of LDL receptor is regulated by the cellular requirement for lipids, with the primary regulatory lipid being cholesterol.

High levels of LDL cholesterol are associated with elevated risk of heart disease. LDL cholesterol is the “bad cholesterol” of the popular literature.

HDL

The intestine and the liver release HDL. HDL particles contain Apo-C and Apo-E, which can be transferred to VLDL and chylomicrons to allow the metabolism of those particles. HDL also contains Apo-A-I, which functions as an activator of Lethicin:cholesterol acyltransferase (LCAT) LCAT transfers acyl chains from phospholipids to cholesterol. This releases monoacyl phospholipids, and concentrates cholesterol from both tissues and other lipoproteins.

Apo-A is the ligand for the HDL receptor. HDL binds its receptor in liver and transfers accumulated cholesterol and cholesteryl esters to the liver for processing. The HDL is then either released or degraded. Some steroid hormone biosynthetic tissues also have HDL receptors, and use these receptors as a mechanism for obtaining cholesterol from circulation. (The HDL is not internalized, except by the liver.)

High levels of HDL are associated with reduced risk of heart disease, possibly due to increased cholesterol scavenging by HDL, and therefore lower LDL and total plasma cholesterol levels. (HDL cholesterol is the “good cholesterol” of the popular literature.) Exercise is associated with an increase in HDL levels.

Females have higher HDL until menopause; this is strongly correlated with lower risk of heart disease, and an increase in risk as HDL levels fall after menopause. The precise reason for this gender-based difference is poorly understood. Although estradiol levels have been proposed to be involved, a recent large clinical trial suggested that estrogen supplementation in post-menopausal women resulted in an increased incidence in heart disease.

(Note: HDL levels are always much lower than LDL levels; the above discussion refers to relative values.)

Fatty liver

The liver has an important role in a wide variety of metabolic processes, including lipid metabolism. Lipid accumulation in the liver results in a condition called fatty liver, and can eventually lead to irreversible damage to the organ. Fatty liver can occur as a result of elevated free fatty acids in circulation; if the fatty acid release from lipoproteins or from the adipose tissue exceeds liver VLDL export, the fatty acids build up in the liver. This is most commonly observed in individuals with poorly controlled diabetes mellitus.

Fatty liver can also occur due to inhibition of VLDL production. Some liver toxins work at least in part by this mechanism, as does a severely protein-deficient diet, and deficiencies in essential fatty acids and in some vitamins.

Side note: Serum levels

In the United States blood component values are reported using units of mg/dL (milligrams per deciliter). The rest of the world uses millimolar, which is a more convenient unit in most respects. The major problem with mg/dL is that comparison of the values requires knowing the molecular weight of the compounds. For example, are there more glucose or cholesterol molecules in circulation? 100 mg/dL of glucose is the middle of the normal range, while 200 mg/dL total cholesterol is at the upper limit of normal. Looking at the numbers, one would assume a greater cholesterol concentration. However, conversion of the values to mM reveals that glucose is present in slightly great amount (5.6 mM *versus* 5.2 mM)

Cholesterol levels are typically reported as both total serum cholesterol and as LDL and HDL cholesterol (which together comprise the major repositories of cholesterol in circulation).

Nutrient storage

Fatty acids are stored in adipose tissue in the form of triacylglycerols, while cholesterol is stored in the form of cholesteryl esters in a variety of tissues. These molecules are essentially entirely hydrophobic, and therefore tend to remain present as aggregates (called lipid droplets) within tissues.

Different tissues contain different amounts of fuel available for use during fasting.² The fuel is present in three major forms: carbohydrate, protein, and fat. The table below summarizes the distribution of this fuel among the tissues of the body.

Fuel reserves of “typical” 70 kg individual			
Organ	Available energy (kcal)		
	Glucose or glycogen	Triacylglycerols	Degradable Protein
Brain	8	0	0
Blood	60	45	0
Liver	400	450	400
Muscle	1200	450	24,000
Adipose tissue	80	135,000	40

(modified from Stryer (1995) *Biochemistry*, 4th Ed.)

The carbohydrate stores, predominately glycogen with small amounts of circulating glucose, contain sufficient energy to support metabolism for about one day. In principle, the various protein stores could provide fuel for a prolonged fast (one to two weeks); in practice, most of the proteins involved have functional roles (in the form of enzymes, contractile proteins, and structural molecules). However, some protein degradation is often necessary to support gluconeogenesis, since acetyl-CoA, the main product of lipid breakdown, cannot be used as substrate for glucose synthesis. (Note that the brain and blood do not contain “degradable protein”; these tissues obviously contain protein, but in general this protein is exempt from degradation for fuel.) The fat stores of adipose tissue provide the major energy reservoir for the animal.

As mentioned above, triacylglycerol has a much higher energy density than protein or carbohydrate. The standard figures quoted for dietary calculations (*i.e.* fat yielding ~9 kcal/g and protein or carbohydrate yielding ~4 kcal/g), apply to the dry weight of the compounds. *In vivo*, metabolism of protein or carbohydrate yields only about 1 kcal/g of stored substrate due to the large amount of water associated with these compounds. In contrast, triacylglycerol is hydrophobic, and therefore little water is associated with fat stores; metabolism of the fat stored in adipose tissue yields nearly the full 9 kcal/g. This is good news for individuals attempting to carry their energy stores with them: the weight of glycogen equivalent in energy to the normal fat stores of a 70 kg man would be about 100 kg! On the other hand, in contemplating weight loss, each kilogram corresponds to 8000 kcal, enough energy to maintain normal metabolism for several days.

Side note: calories, Calories, and joules

We have been using “joules” as our unit of energy throughout this course. For some calculations joules have advantages. However, when discussing the energy content

²Fasting is a technical term that applies to the few hours between meals as well as to a prolonged period without food.

of food, many sources use “Calories”. The terminology used is that a “Calorie” (*i.e.* written with a capital “C”) is a kilocalorie. The distinction is important: one calorie is the amount of energy required to raise the temperature of 1 gram of water by 1°C, while one kcal is the energy required to raise the temperature of 1 kilogram of water by 1°C.

Misunderstanding of the difference between calories and Calories has led some people to proclaim that drinking a soda should result in weight loss. The soda has 180 Calories, while the energy required to raise the temperature of 355 ml of the liquid from its initial temperature of 4°C to body temperature of 37°C is 11,715 calories. Naively comparing 11,715 calories to 180 Calories presents the appearance of an energy deficit.

To avoid this potential problem, the Table above explicitly uses “kcal”, rather than the equivalent, but potentially misleading “Calories”. To convert calories to joules, multiply by 4.184 (the ratio of the gas constants 8.3145 and 1.9872).

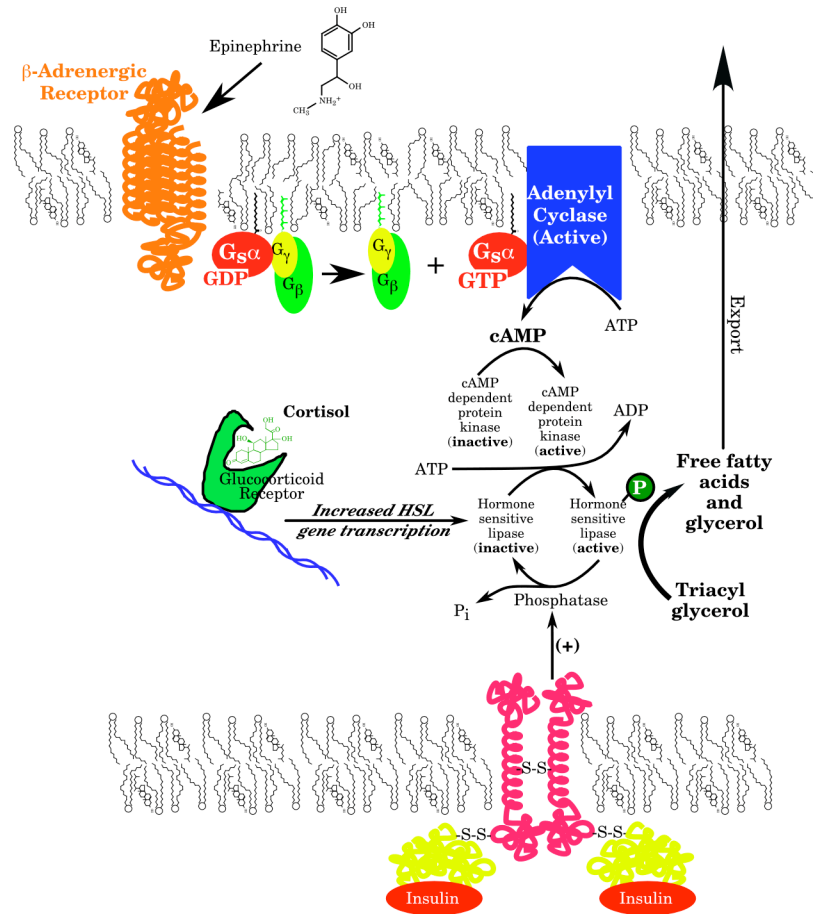
Utilization of lipid stores

The first step in the metabolism of fat stores is the release of free fatty acids from the adipose tissue. This release is a regulated process, with three major stimulators (epinephrine, cortisol, and growth hormone), and one major inhibitor (insulin). The regulatory hormones epinephrine, cortisol, and insulin are known to alter the activity of the hormone-sensitive lipase, the enzyme that hydrolyzes triacylglycerol from the lipid droplets to release the free fatty acids and glycerol into circulation. (The mechanism for the growth hormone effect is poorly understood, and may be confined to decreasing the effect of insulin.)

Hormone-sensitive lipase activity is increased by phosphorylation. Epinephrine increases cAMP production, which in turn increases phosphorylation of the enzyme, and therefore increases the activity of the enzyme. Cortisol acts by increasing the transcription of the hormone-sensitive lipase; cortisol and epinephrine thus act via different mechanisms to increase triacylglycerol breakdown.

Insulin inhibits triacylglycerol breakdown by increasing the activity of a protein phosphatase that reverses the cAMP-dependent phosphorylation of the hormone-sensitive lipase. Insulin also decreases cAMP levels, and decreases hormone-sensitive lipase gene transcription. Adenosine seems to also inhibit triacylglycerol breakdown, probably by decreasing cAMP production.

Caffeine and thyroid hormone both indirectly stimulate triacylglycerol breakdown. Caffeine inhibits phosphodiesterase, and therefore increases the half-life of cAMP, and also acts as an adenosine antagonist. Thyroid hormone makes the cell more sensitive to the effects of epinephrine.



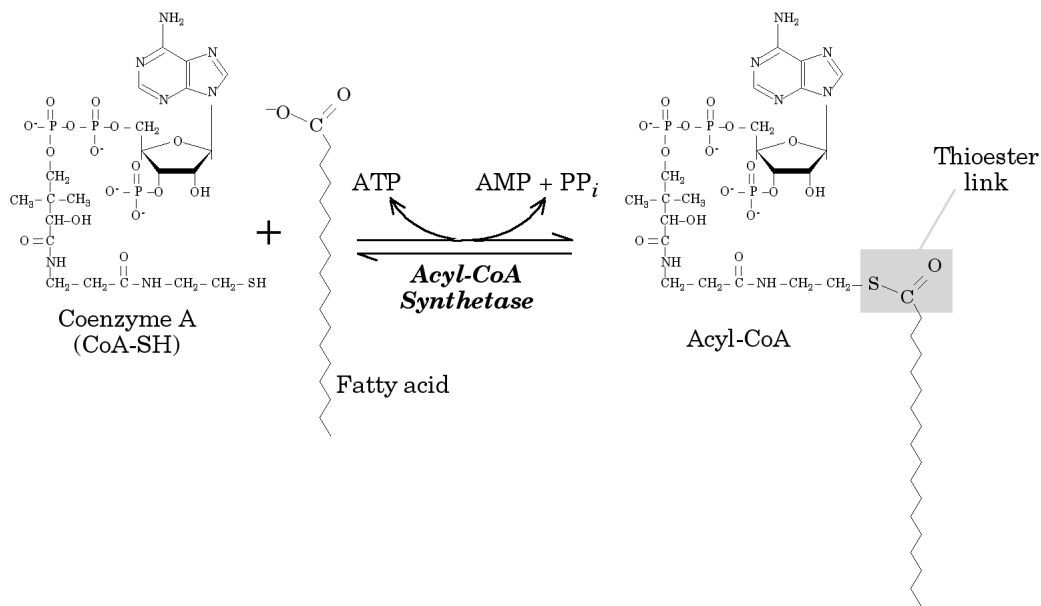
The fatty acid breakdown pathway

The reactions involved in the actual breakdown of free fatty acids occur in the mitochondria. While short chain fatty acids (about 10 carbons or shorter) 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



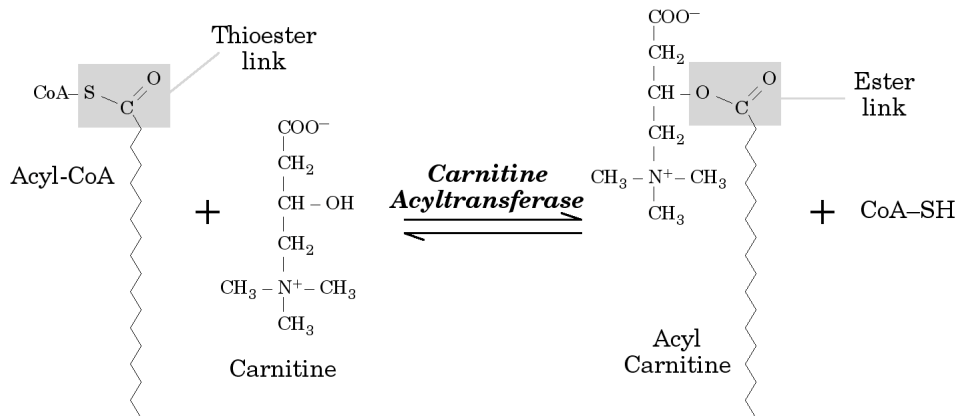
In the drawing above, the coenzyme A structure is given explicitly. This is relatively rarely done; coenzyme A participation in the reaction is limited to the altered chemistry it introduces in the thioester and to the improved ability for enzymes to bind the more complex coenzyme A structure rather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

Translocation

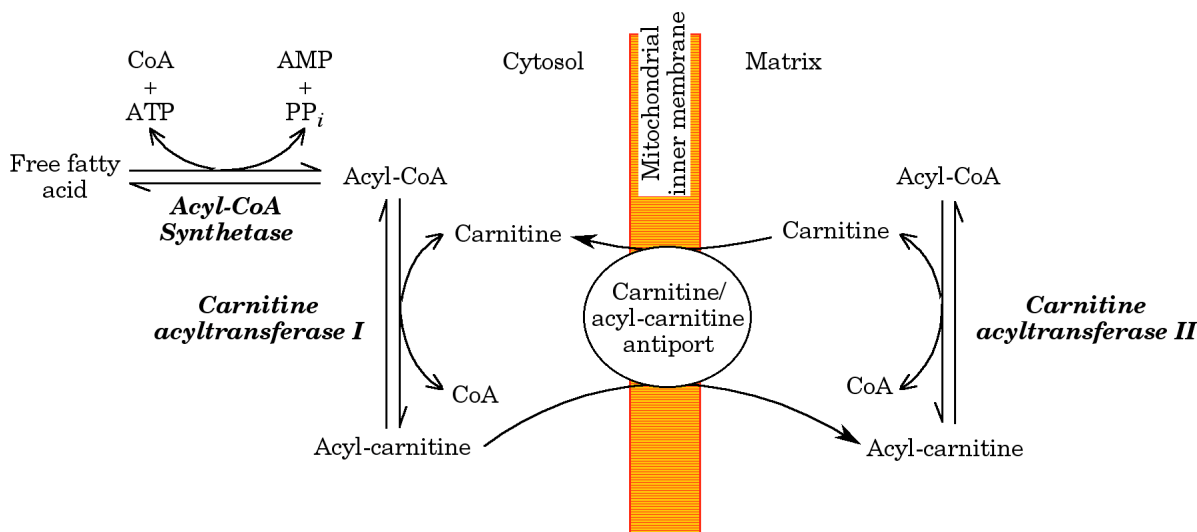
Once activated by conjugation to coenzyme A, the acyl-CoA must be transported into the mitochondria. Entry of the activated fatty acid into the mitochondria is a multistep process. In order to maintain separate cytoplasmic and mitochondria pools of coenzyme A, the transport process uses a separate small 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 acids, and because only the acyl-carnitine can enter the mitochondria, carnitine acyltransferase I acts as a major control point for fatty acid breakdown.

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.

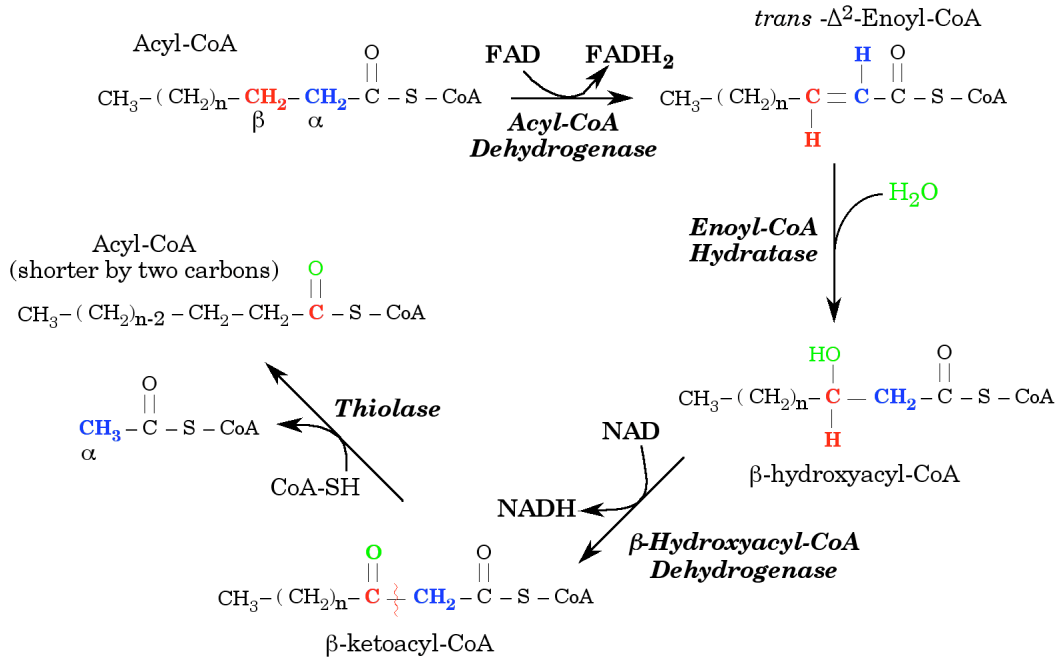


Acyl-carnitine is a ligand for a specific transporter, the carnitine/acyl-carnitine antiport. Once inside the mitochondrion, carnitine acyltransferase II reforms the Acyl-CoA. (Note that both of the carnitine acyltransferase reactions are readily reversible; no energy is added or lost during the transport 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 β and α carbons (the blue and red carbons, respectively, in the pathway drawing). Direct cleavage of an unsubstituted carbon-carbon bond is extremely difficult. 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 some of those found in the TCA cycle.

Acyl-CoA dehydrogenase oxidizes the β - α 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 FADH_2 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.

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.

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

Thiolase (also called Acyl-CoA:acetyltransferase) cleaves the α -ketoacyl-CoA, releasing an acyl-CoA two carbons shorter, and acetyl-CoA. The thiolase reaction forms a thioester bond between the α -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 α -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 α -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 α -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. Both glucose and fatty acids are ultimately converted into acetyl-CoA. 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, the activation step is followed by eight spirals of the □-oxidation pathway, resulting in nine acetyl-CoA, eight NADH, and eight FADH₂. 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 the energy per unit mass.

Special cases

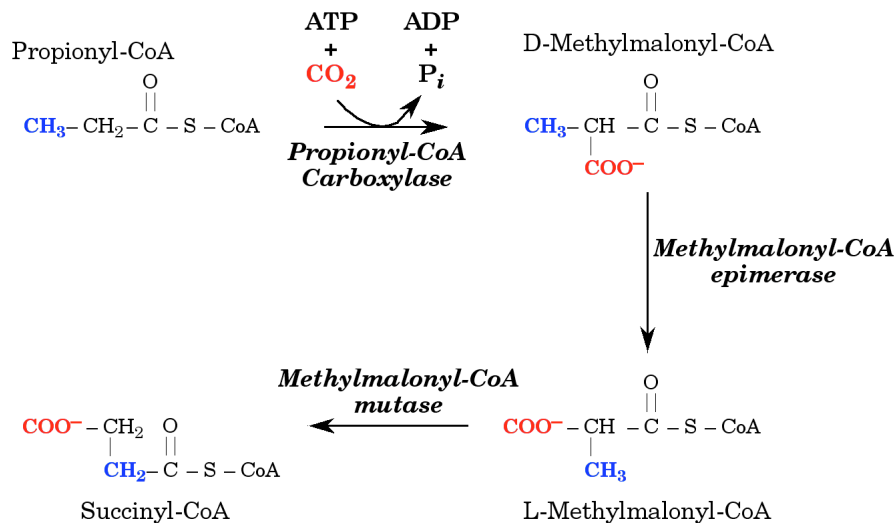
The β -oxidation pathway discussed above applies to nearly all fatty acids and their derivatives. However, some fatty acids contain odd-numbers of carbons or sites of unsaturation. These compounds require additional reactions to complete their breakdown.

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.

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



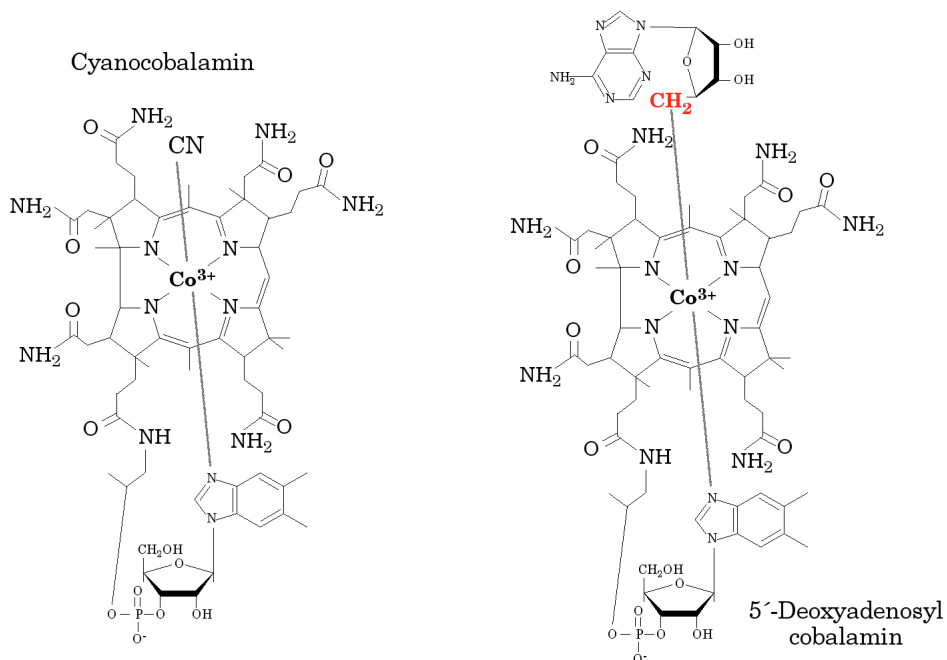
The reactions involved in converting propionyl-CoA to succinyl-CoA are useful for more than merely completing the metabolism of odd chain fatty acids; metabolism of some amino acids and of some other compounds also results in propionyl-CoA production.

Side note: vitamin B12

Methylmalonyl-CoA mutase is one of two known vitamin B₁₂-dependent enzymes in humans (the other is methionine synthase). 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 β -carbon to the methyl carbon.

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 disorder associated with Vitamin B₁₂ deficiency.

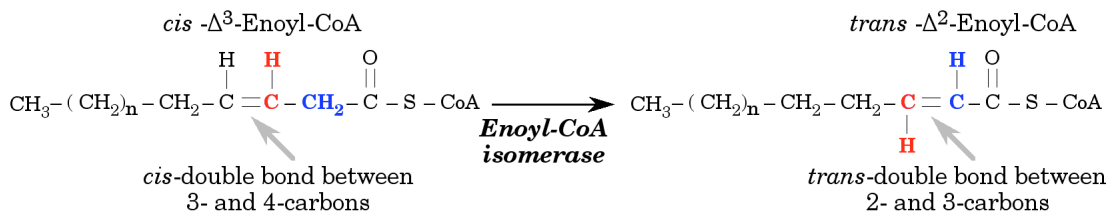
Although the cobalamin ring structure similar in general appearance to the porphyrin structure of heme and chlorophyll, cobalamin contains a corrin ring, not a porphyrin. In addition, the cobalamin contains a cobalt ion rather than the iron typically present in heme or the magnesium found in most chlorophyll derivatives. Vitamin B₁₂ is frequently called cyanocobalamin, although the cyanide group is actually 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.



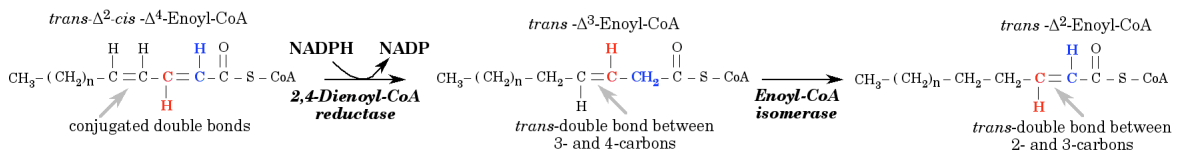
Unsaturated fatty acids

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) Odd-numbered double bonds: If the original double bond is in an odd-numbered position, normal \square -oxidation will eventually result in the presence of a double bond at the 3-position. For example, three \square -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 **enoyl-CoA isomerase**. The product of this process is *trans*- Δ^2 -enoyl-CoA, which is identical to the first \square -oxidation intermediate.



2) Even-numbered double bonds: If the original double bond is in an even-numbered position, normal \square -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 \square -oxidation intermediate by enoyl-CoA isomerase as described above.



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 \square -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 \square -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.

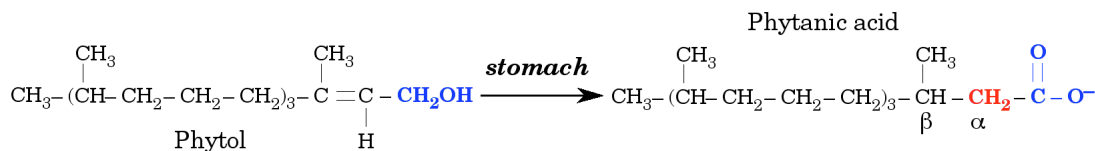
Long chain fatty acids diffuse into peroxisomes unassisted (neither carnitine nor activation of the fatty acid is necessary). Once in the peroxisome, a peroxisomal acyl CoA synthetase activates the long chain fatty acids. Acetyl-CoA produced in the peroxisomes is released to the cytoplasm (the peroxisomes 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*.

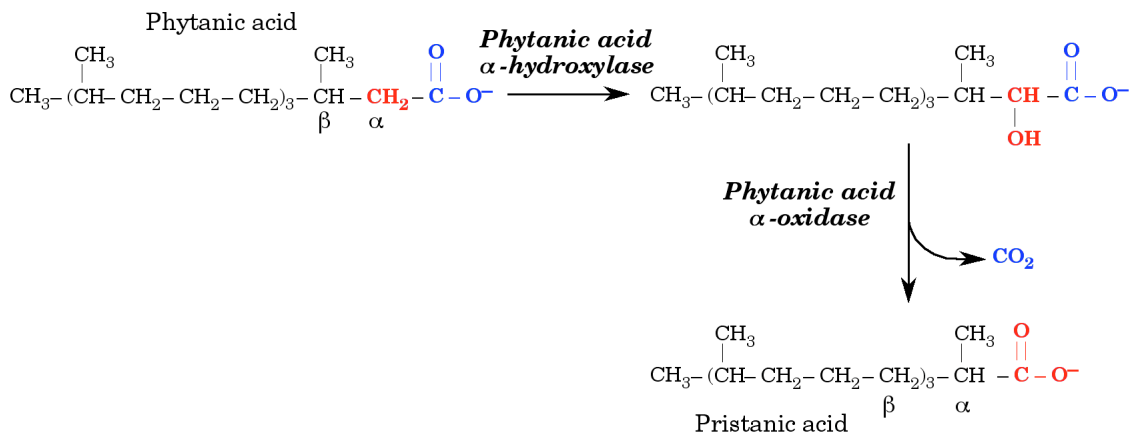
α -Oxidation

The α -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 α -carbon is odd-numbered; if the α -carbon is branched, it is impossible to form the α -carbon ketone required for normal α -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 α -carbon. The presence of the α -methyl group means this compound is not a substrate for α -oxidation. Instead, phytanic acid must be subjected to ω -oxidation. The ω -oxidation process involves the addition of a hydroxyl group to the α -carbon by **phytanic acid α -hydroxylase**, followed by an oxidative decarboxylation reaction, catalyzed by **phytanic acid α -oxidase**.



The product of this two-enzyme process, pristanic acid, is branched at the β -position, rather than the α -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 β -carbons) will release the three-carbon propionyl-CoA, which, as noted earlier, can be converted to succinyl-CoA.

The β -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.

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.