Metabolism

Part III: Lipids

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Three of the four major classes of biomolecules are defined on the basis of similarities in their structures: proteins consist of amino acids; carbohydrates are polyhydroxyaldehydes or ketones; and nucleic acids are polynucleotides. Lipids are defined on the basis of similarities in their physical properties, they are the class of biomolecules that are soluble in nonpolar solvents such as benzene, chloroform, or ether. Lipids therefore exhibit a wide variety of structures characterized by the presence of hydrophobic groups.

Lipids are often divided into two classes. Complex lipids such as "fats" and "oils" contain fatty acids; simple lipids such as vitamin A or cholesterol do not.

Fatty Acids

Fatty acids are long-chain carboxylic acids such as stearic acid, $CH_3(CH_2)_{16}CO_2H$, that contain a nonpolar, hydrophobic hydrocarbon tail and a polar, hydrophilic carboxylic acid head. Fatty acids in terrestial organisms usually have an even number of carbon atoms, with chains of 16 to 18 being most common, although milk and butter fat have unusually high amounts of fatty acids with chain lengths between 4 and 12. Marine organisms have a greater abundance of fatty acids with an odd number of carbon atoms.

Fatty acids are divided into two classes, saturated fatty acids such as

lauric acid	$CH_3(CH_2)_{10}CO_2H$
myristic acid	$CH_3(CH_2)_{12}CO_2H$
palmitic acid	$CH_3(CH_2)_{14}CO_2H$
stearic acid	$CH_3(CH_2)_{16}CO_2H$

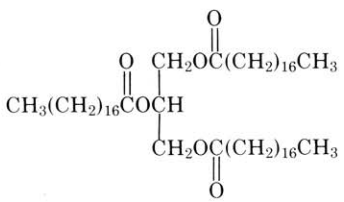
and unsaturated fatty acids such as

palmitoleic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7CO_2H$
oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7CO_2H$
linoleic acid	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$
linolenic acid	$CH_3CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7CO_2H$

in which the C=C double bonds are usually present in a cis orientation. The presence of a double bond between C_9 and C_{10} or C_{12} and C_{13} in these unsaturated fatty acids is indicated with the symbols Δ^9 and Δ^{12}.

Neutral Fats, Triglycerides, or Triacylglycerols

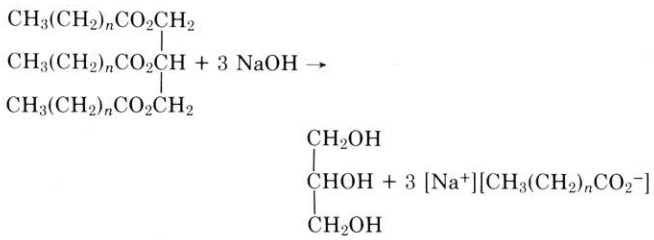
The concentration of free fatty acids in a cell is negligible, almost all of the fatty acids are bound to glycerol by ester linkages. The most abundant lipids are the triesters of glycerol with fatty acids that are typically divided into "fats" and "oils" on the basis of whether they are solids or liquids at room temperature.



These compounds were once called neutral fats, they are now called triglycerides, or more accurately, triacylglycerols. Their primary function is the long-term storage of biological energy.

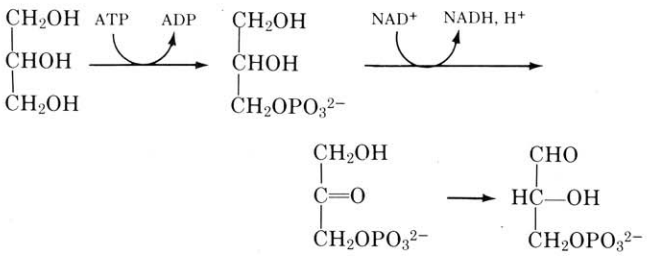
Saponification

One of the characteristic reactions of triacylglycerols is the alkaline hydrolysis of the ester linkage to give glycerol and sodium or potassium salts of the corresponding carboxylate ions.



This reaction is called saponification (from the Latin *saponis*, soap, and *facere*, to make) because it has been used for more than 2,000 years to make soap.

The first step in the catabolism of triacylglycerols involves an enzyme-catalyzed hydrolysis of the triester to glycerol and three fatty acids. The glycerol produced in this reaction is phosphorylated by glycerol kinase, oxidized to dihydroxyacetone phosphate by glycerol phosphate dehydrogenase, isomerized to glyceraldehyde 3-phosphate by triose phosphate isomerase, and finally injected into the glycolytic pathway.



Careful consideration of the reactions involved in glycolysis, the TCA cycle, electron transport, and oxidative phosphorylation suggests that a total of 22 ATP can be synthesized from the total oxidation of glycerol to CO_2 and H_2O.

Activation and Transport

Fats and oils are broken down to fatty acids and glycerol in the soluble portion of the cytoplasm. Oxidation of the fatty acids to CO_2 and H_2O, however, is catalyzed by enzymes found on the inner walls of the mitochondria. Before fatty acids can be metabolized, they must first be activated and then transported across a membrane into the mitochondria.

Fatty acids are activated by forming a thioester to coenzyme A.

$$CH_3(CH_2)_nCO_2H + HS\text{-}CoA \rightarrow CH_3(CH_2)_nCO\text{-}S\text{-}CoA$$

Because thioesters are themselves high-energy compounds, hydrolysis of ATP to ADP and the phosphate ion cannot provide quite enough energy to drive this reaction to completion. The energy released when ATP is hydrolyzed to AMP and the pyrophosphate ($P_2O_7^{4-}$) ion is used to form a mixed anhydride between the fatty acid and AMP.

$$CH_3(CH_2)_nCO_2 + ATP^{4-} + H_2O \rightarrow CH_3(CH_2)_nC(=O)\text{—}O\text{—AMP} + P_2O_7^{4-}$$

The energy released when the pyrophosphate ion is hydrolyzed to a pair of phosphate ions then drives the transfer of the fatty acyl group to form a thioester.

$$CH_3(CH_2)_nC(=O)\text{-}O\text{-AMP} + HS\text{-}CoA \rightarrow CH_3(CH_2)_nCO\text{-}S\text{-}CoA + AMP$$

$$P_2O_7^{4-} + H_2O \rightarrow 2\,HPO_4^{2-}$$

The fatty acyl CoA formed in this activitation step cannot be transported across the mitochondrial membrane because the coenzyme A fragment carries a significant net ionic charge due to the presence of phosphate linkages. The fatty acyl group is therefore transferred to the -OH group of a molecule of carnitine by an acyltransferase enzyme to form a zwitterion that carries no net electrical charge, and can therefore cross this membrane.

$$CH_3(CH_2)_nC(=O)\text{—}S\text{—}CoA + HO\text{—}CH(CH_2CO_2^-)(CH_2N(CH_3)_3^+) \rightarrow CH_3(CH_2)_nC(=O)\text{—}O\text{—}CH(CH_2CO_2^-)(CH_2N(CH_3)_3^+) + HS\text{—}CoA$$

Once the fatty acyl carnitine ester has been transported across the membrane, a second acyltransferase enzyme transfers the fatty acyl group back to coenzyme A in a regeneration step.

The β-Oxidation Spiral

Once inside the mitochondrion, the fatty acyl coenzyme A thioester enters the β-oxidation spiral shown in the figure, which degrades the fatty acid, two carbon atoms at a time.

Step 1: Oxidation

The first enzyme in this spiral is an acyl CoA dehydrogenase that uses a tightly bound FAD coenzyme to oxidize the fatty acyl thioester to form a trans C=C double bond. This reaction produces a conjugated, trans enoyl intermediate which is very different from the naturally occurring unsaturated fatty acids that contain cis C=C double bonds that are well insulated from the carboxylate group.

Step 2: Hydration

An enoyl CoA hydratase enzyme catalyzes the stereospecific addition of water across the C=C double bond to form a 3- or β-hydroxyacyl CoA derivative in the L configuration.

Step 3: Oxidation of the Alcohol

A 3-hydroxyacyl CoA dehydrogenase enzyme then uses an NAD^+ coenzyme to oxidize this alcohol to the corresponding ketone.

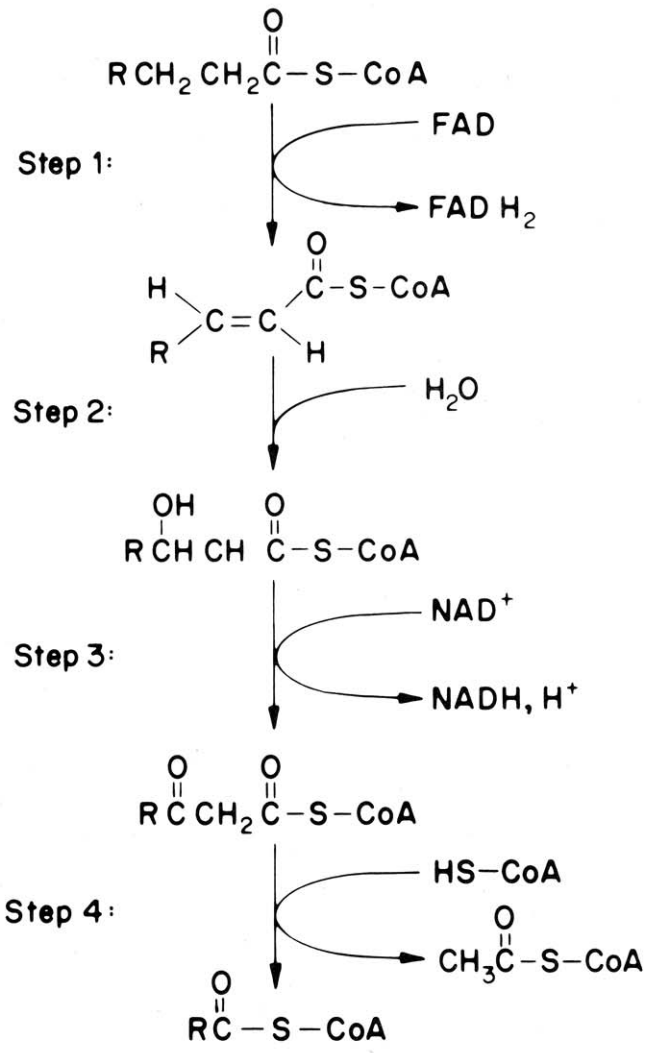
Step 4: Thiolysis

The β-ketothioester now reacts with coenzyme A to form acetyl CoA and a new fatty acyl thioester from which two carbon atoms have been cleaved. The β-oxidation spiral then begins again and continues until the last pass through the spiral converts a four-carbon fatty acyl group to two molecules of acetyl CoA.

The fourth step in the β-oxidation spiral is called thiolysis because the net effect is the addition of a thiol (HS-CoA) across a C—C single bond. This reaction can be understood by noting that β-diketones can be prepared by a Claisen condensation,

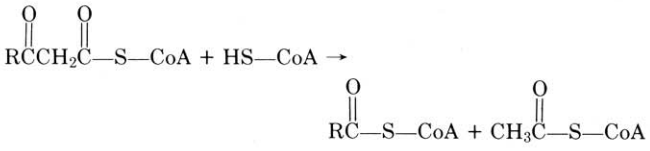
$$CH_3C(=O)CH_2CH_3 + CH_3C(=O)OCH_2CH_3 \rightarrow CH_3C(=O)CH_2C(=O)OCH_2CH_3 + CH_3CH_2OH$$

and carbonyl addition reactions such as Claisen condensa-



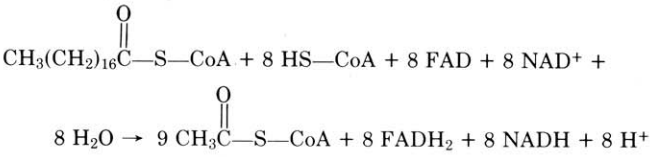
The β-oxidation spiral.

tions are reversible. Thiolysis of the β-ketothioester can be thought of as a retrograde Claisen condensation.



Fatty Acid Degradation as a Source of Biochemical Energy

Eight turns around the β-oxidation spiral are necessary to oxidize stearic acid to acetyl CoA. In the course of this sequence of reactions, eight FAD coenzymes are reduced to $FADH_2$, eight NAD^+ coenzymes are reduced to NADH, and nine molecules of acetyl CoA are produced.



The $FADH_2$ and NADH coenzymes are regenerated by electron transport coupled with oxidative phosphorylation. Two ATP are formed for each $FADH_2$ coenzyme recycled, and three ATP are produced each time an NADH coenzyme is oxidized. The acetyl CoA produced in this sequence of reactions is oxidized to CO_2 and H_2O in the TCA cycle, with the net effect of generating 12 ATP per acetyl CoA consumed.

The degradation of stearic acid therefore yields a total of 148 ATP.

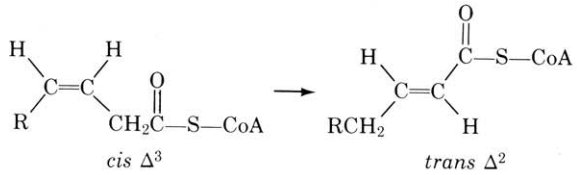
8 ($FADH_2$ → FAD)	8 × 2 ATP = 16 ATP
8 (NADH → NAD^+)	8 × 3 ATP = 24 ATP
9 (acetyl CoA → $CO_2 + H_2O$)	9 × 12 ATP = 108 ATP
	total = 148 ATP

However, one ATP was consumed in the activation of stearic acid, and a second ATP is consumed converting the AMP produced in the activation reaction to ADP, which can be cycled back through oxidative phosphorylation. Thus, a net total of 146 ATP are produced for each molecule of stearic acid consumed.

Under standard state conditions, 146 ATP corresponds to the capture of slightly more than 40% of the energy given off during the combustion of stearic acid. The β-oxidation spiral is therefore no more or less efficient at capturing energy than glycolysis or the TCA cycle. The oxidation of stearic acid to CO_2 and H_2O, however, gives off almost four times as much energy per mole, or almost 2½ times as much energy per gram, as the complete oxidation of glucose, and complex lipids are an excellent medium for the long-term storage of food energy.

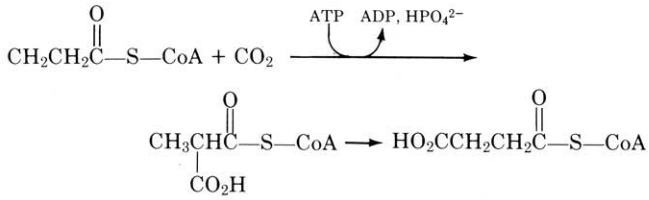
Degradation of Unsaturated Fatty Acids and Fatty Acids with an Odd Number of Carbon Atoms

The β-oxidation spiral cleaves two carbon atoms at a time from the carboxylic acid end of the fatty acid. Thus, when unsaturated fatty acids are catabolized, the third pass through the spiral leaves a cis Δ^3 instead of a trans Δ^2 intermediate. Metabolism of unsaturated fatty acids therefore requires an enoyl CoA isomerase enzyme, which not only shifts the position of the C=C double bond but also converts the naturally occurring cis isomer into the trans intermediate recognized by the enoyl hydratase enzyme.



Polyunsaturated fatty acids present another problem. After the isomerase enzyme operates on the first C=C double bond, two more passes around the spiral leaves a cis Δ^2 intermediate. The double bond is in the right position (Δ^2), and the enol hydratase enzyme adds water across this bond. But because the enzyme operates on a cis instead of a trans C=C bond, the D instead of L stereoisomer of 3-hydroxyacyl CoA is formed. An epimerase enzyme then inverts the configuration of the hydroxyl group at C_3, so that the product of this reaction can be recognized by the 3-hydroxyacyl dehydrogenase enzyme which catalyzes step 3.

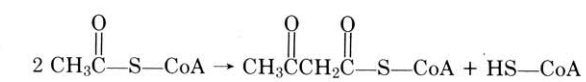
Fatty acids with an odd number of carbon atoms present yet another problem. Carbon atoms are cleaved from these fatty acids, two at a time, until the spiral leaves a three-carbon proprionyl CoA thioester. A carboxylase enzyme then catalyzes the addition of CO_2 at the expense of ATP to form methylmalonyl CoA, which is then isomerized by a mutase enzyme to form succinyl CoA which can enter the TCA cycle.



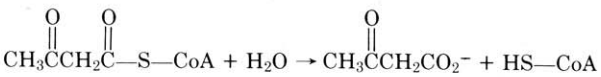
Ketone Bodies

When the degradation of carbohydrates and fatty acids is in balance, the acetyl CoA from fatty acids enters the tricarboxylic acid cycle where it is oxidized to CO_2 and H_2O. Low carbohydrate diets, or metabolic disorders such as diabetes, can lead to conditions where the amount of acetyl CoA produced from the degradation of fatty acids exceeds the ability of the TCA cycle to oxidize it.

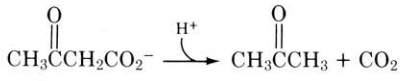
When this happens, the last step in the β-oxidation spiral is reversed, and two molecules of acetyl CoA condense to form acetoacetyl CoA.



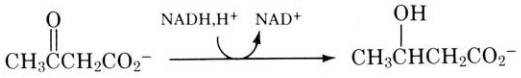
Acetoacetyl CoA then picks up a third acetyl CoA to form a 3-hydroxy-3-methylglutaryl CoA intermediate which decomposes to the acetoacetate ion and acetyl CoA. The net effect of these reactions is the hydrolysis of acetoacetyl CoA to acetoacetate and coenzyme A.



Acetoacetate can then undergo either decarboxylation to give acetone,



or reduction to give the 3-hydroxybutyrate ion.



Acetoacetate, acetone, and 3-hydroxybutyrate are often referred to as the *ketone bodies*. In animals, they are primarily generated within the mitochondria of liver cells. Acetyl CoA cannot cross the mitochondrial membrane, but these water-soluble ketone bodies easily diffuse through the membrane to enter the blood stream. They can then be transported to peripheral tissues such as the skeletal muscles where they can be activated to form acetoacetate CoA, cleaved to acetyl CoA, and then used as an alternative to glucose as a source of energy. The ketone bodies are important sources of energy even when carbohydrate and fatty acid metabolism are in balance. Under certain conditions, however, such as low carbohydrate diets or diabetes, they can become the principal source of biological energy.

Diabetes is the result of impaired transport of blood glucose across cell membranes into the skeletal muscles. Because glucose cannot be used as a fuel, the body turns to burning fat for energy. The acetyl CoA produced by the oxidation of fats cannot enter the TCA cycle because the level of oxaloacetate is depleted as the body tries to make more glucose to overcome the problem with the transport of blood glucose. The excess acetyl CoA is therefore converted to ketone bodies which appear in the blood stream and urine of diabetics.

Diabetes is treated with insulin, which increases the rate at which blood glucose is transported into the peripheral tissues, increases the concentrations of enzymes such as glucokinase, phosphofructokinase, and pyruvate kinase, which are involved in glycolysis, and inhibits the oxidation of fatty acids.

In recent years, low carbohydrate diets have become popular as a means of burning excess fat. Some have even gone so far as to recommend restricting carbohydrates in the diet until the urine tests positive for ketone bodies, in other words until the body produces the symptoms of ketosis that mimic diabetes. Although significant weight loss can occur, the long-term effects of artificially inducing a state of ketosis are unknown.