Metabolism: Fats and Fatty Acids

In the modern Western world, which is fat and getting fatter, there is a tremendous amount of interest in the metabolism of fat and fatty acids. Fat is the most important energy storage form of animals, storing considerably more energy per carbon than carbohydrates, but its insolubility in water requires the body to package it specially for transport. Surprisingly, fat/fatty acid metabolism is not nearly as tightly regulated as that of carbohydrates. Neither are the metabolic pathways of breakdown and synthesis particularly complicated, either.

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Movement of dietary fat

Before we discuss the breakdown and synthesis of fat, let us first discuss the movement of dietary fat and oil (triglycerides - Figure 6.82) in the body. Upon consumption of triglycerides in the diet, they first are solubilized in the digestive system by the churning action of the stomach and the emulsifying properties of the bile acids.

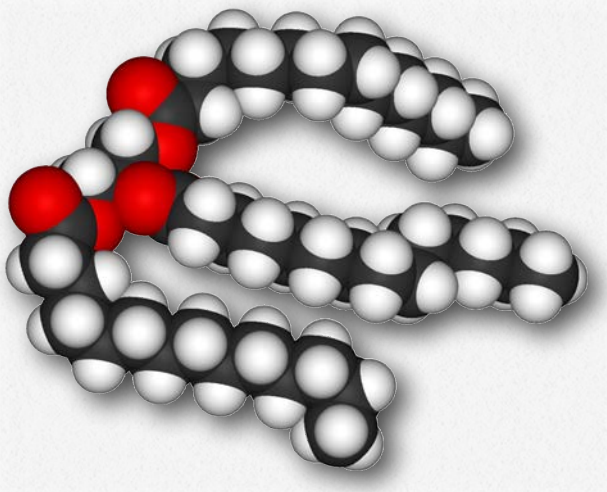

Figure 6.82 - Trimyristin - A triacylglyceride

Upon passing into the lumen of the intestine, the triglycerides are acted on first by enzymes known as lipases that use water twice on each triglyceride to release two fatty acids, leaving behind a monoacylglyceride. As shown in Figure 6.83, the fatty acids and the monoacylglyceride are moved across the intestinal wall into the lymph system where they are reassembled back into a triglyceride. In the lymph system triglycerides and other insoluble lipids are packaged into lipoprotein complexes called chylomicrons that enter the blood stream and travel to target cells. The journey of lipids in the body after leaving the digestive system is long and is discussed in more depth HERE.

In the body, fat is stored in specialized cells known as adipocytes. When these cells receive appropriate signals, they begin the breakdown of fat into glycerol and fatty acids.

Breakdown of fat

Breakdown of fat in adipocytes requires catalytic action of three enzymes. The first

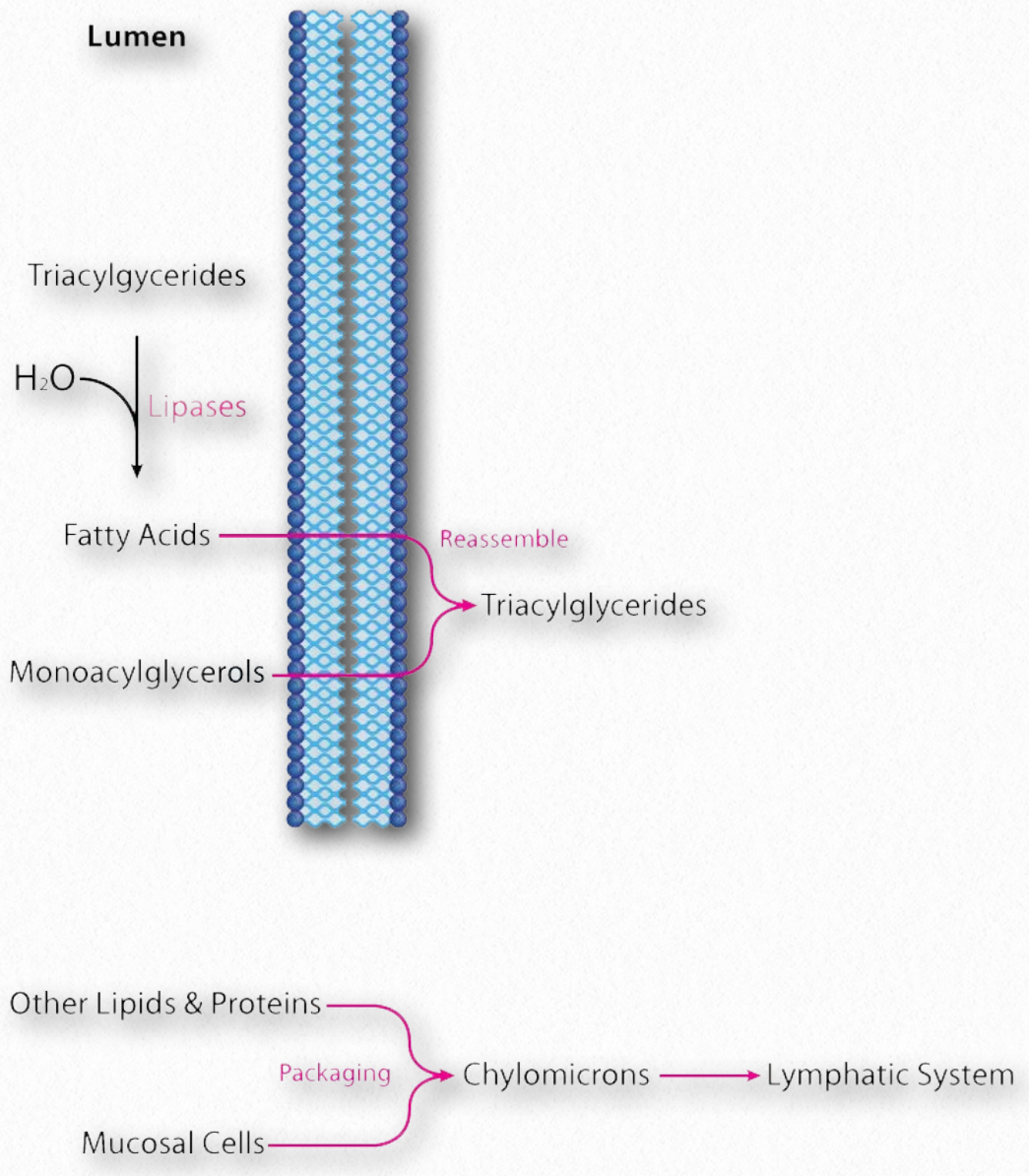

Figure 6.83 - Movement of dietary triglycerides

Image by Aleia Kim

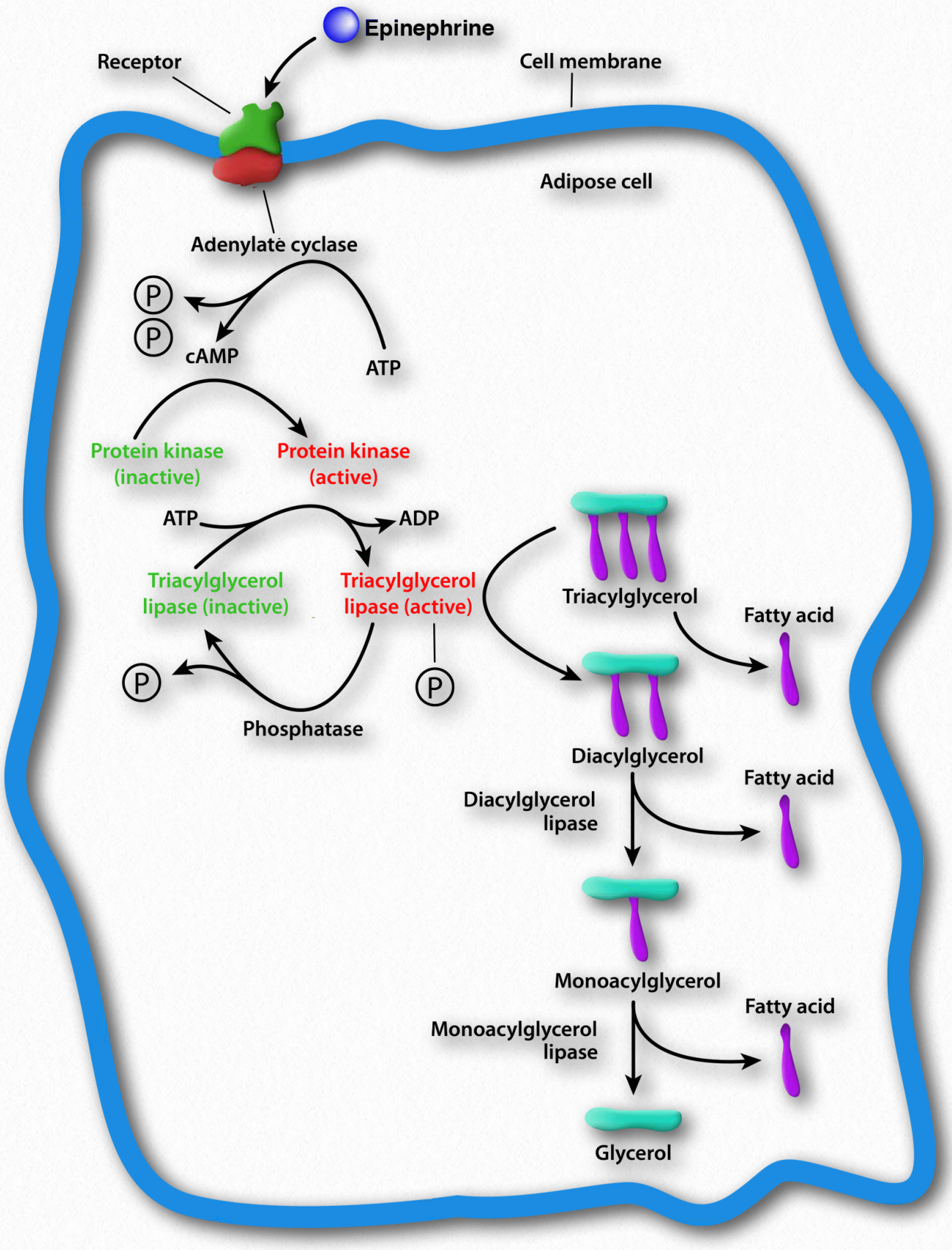

Figure 6.84 - Breakdown of fat in adipocytes

Image by Pehr Jacobson

of these is controlled by binding of hormones to the cell membrane (Figure 6.84). It is the only regulated enzyme of fat breakdown and is known as hormone sensitive triacylglycerol lipase. It removes the first fatty acid from the fat. Diacylglyceride lipase removes the second one and monoacylglyceride lipase removes the third. As noted, only the first one is regulated and it appears to be the rate limiting reaction when active.

Epinephrine activation

As shown in Figure 6.84, activation of hormone sensitive triacylglycerol lipase (HSTL) is accomplished by epinephrine stimulation process and that it overlaps with the same activation that stimulates glycogen breakdown and gluconeogenesis.

This coordination is very important. Each of the pathways stimulated by the epinephrine signaling system aims to provide the body with more materials to catabolize for energy - sugars and fatty acids. The HSTL is inhibited

Synthesis of Phosphatidic Acid from Glycerol-3-phosphate

1. **Glycerol-3-phosphate** + Acyl-CoA <=> Monoacylglycerol phosphate + CoA-SH

2. Monoacylglycerol phosphate + Acyl-CoA <=> **Phosphatidic acid** + CoA-SH

by dephospohrylation and this is stimulated by binding of insulin to its cell membrane receptor.

Perilipin

A protein playing an important roles in regulation of fat breakdown is perilipin. Perilipin associates with fat droplets and helps regulate action of HSTL, the enzyme catalyzing the first reaction in fat catabolism. When perilipin is not phosphorylated, it coats the fat droplet and prevents HSTL from getting access to it. Activation of protein kinase A in the epinephrine cascade, however, results in phosphorylation of both perilipin and HSTL. When this occurs, perilipin loosens its tight binding to the fat droplet, allowing digestion of the fat to begin by HSTL.

Perilipin expression is high in obese organisms and some mutational variants have been associated with obesity in women. Another mutation reduces perilipin expression and is associated with greater lipolysis (fat breakdown) in women. Mice lacking perilipin eat more food than wild-type mice, but gain 1/3 less weight when on the same diet.

Fat synthesis

Synthesis of fat requires action of acyl transferase enzymes, such as glycerol-3 O-phosphate acyl transferase, which catalyzes addition of fatty acids to the glycerol backbone (reaction #1 above). The process requires glycerol-3-phosphate (or DHAP) and three fatty acids. In the first reaction, glycerol-3-phosphate is esterified at position 1 with a fatty acid, followed by a duplicate re-

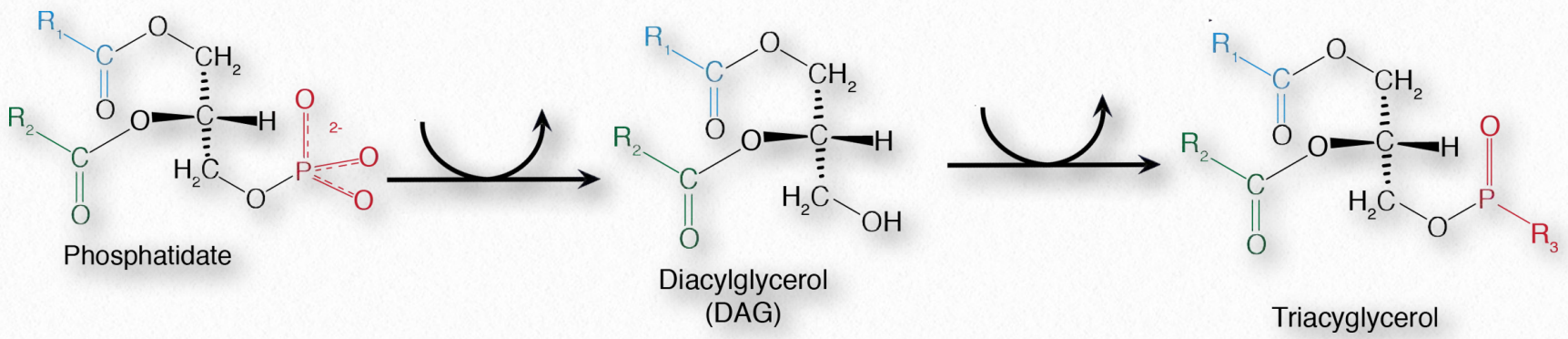

Figure 6.85 - Synthesis of fat from phosphatidic acid (phosphatidate)

Image by Penelope Irving

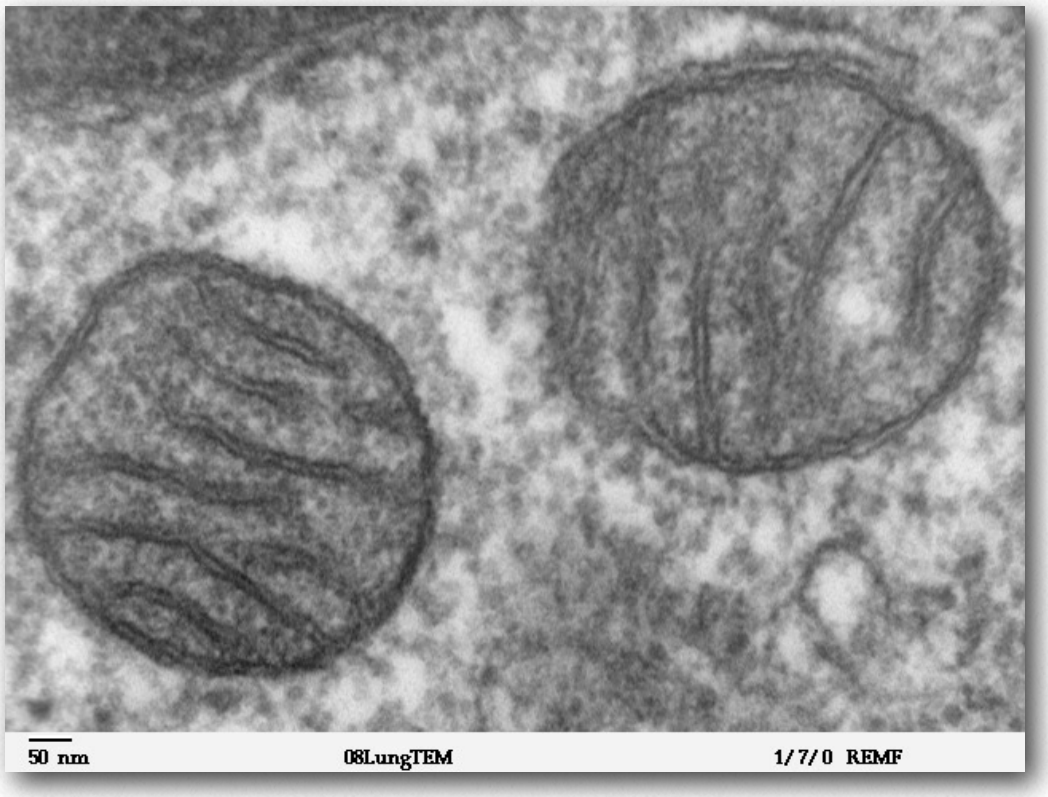

Figure 6.86 - Mitochondria - site of β-oxidation

action at position 2 to make phosphatidic acid (diacylglycerol phosphate). This molecule, which is an intermediate in the synthesis of both fats and phosphoglycerides, gets dephosphorylated to form diacylglycerol before the esterification of the third fatty acid to the molecule to make a fat.

Fatty acids released from adipocytes travel in the bloodstream bound to serum albumin. Arriving at target cells, fatty acids are taken up by membrane-associated fatty acid binding proteins, which help control cellular fatty acid uptake by transport proteins. Players in this process include CD36, plasma membrane-associated fatty acid-binding protein, and a family of fatty acid transport proteins (called FATP1-6).

Fatty acid oxidation

Upon arrival inside of target cells, fatty acids are oxidized in a process that chops off two carbons at a time

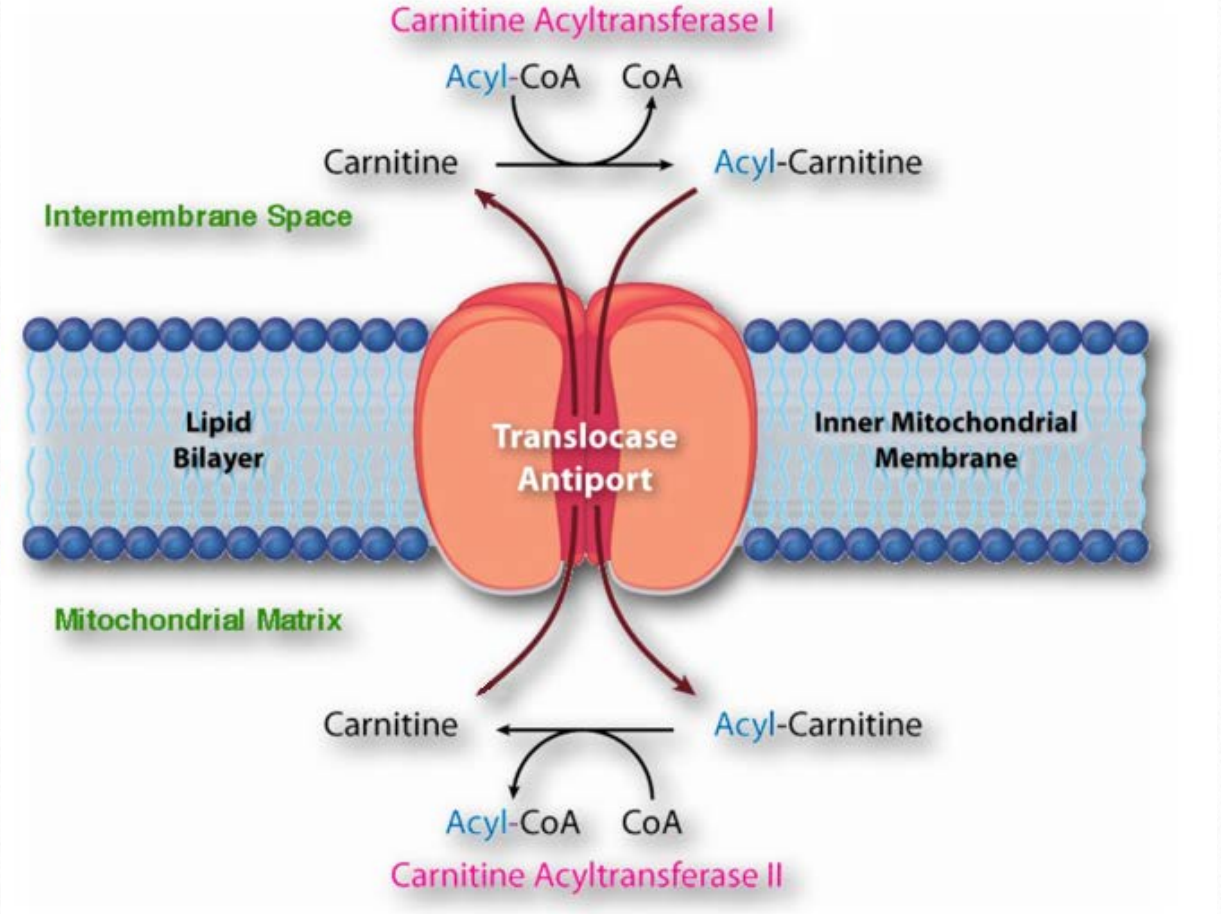

Figure 6.87 - Transport of fatty acid (acyl group) across mitochondrial inner membrane

Image by Aleia Kim

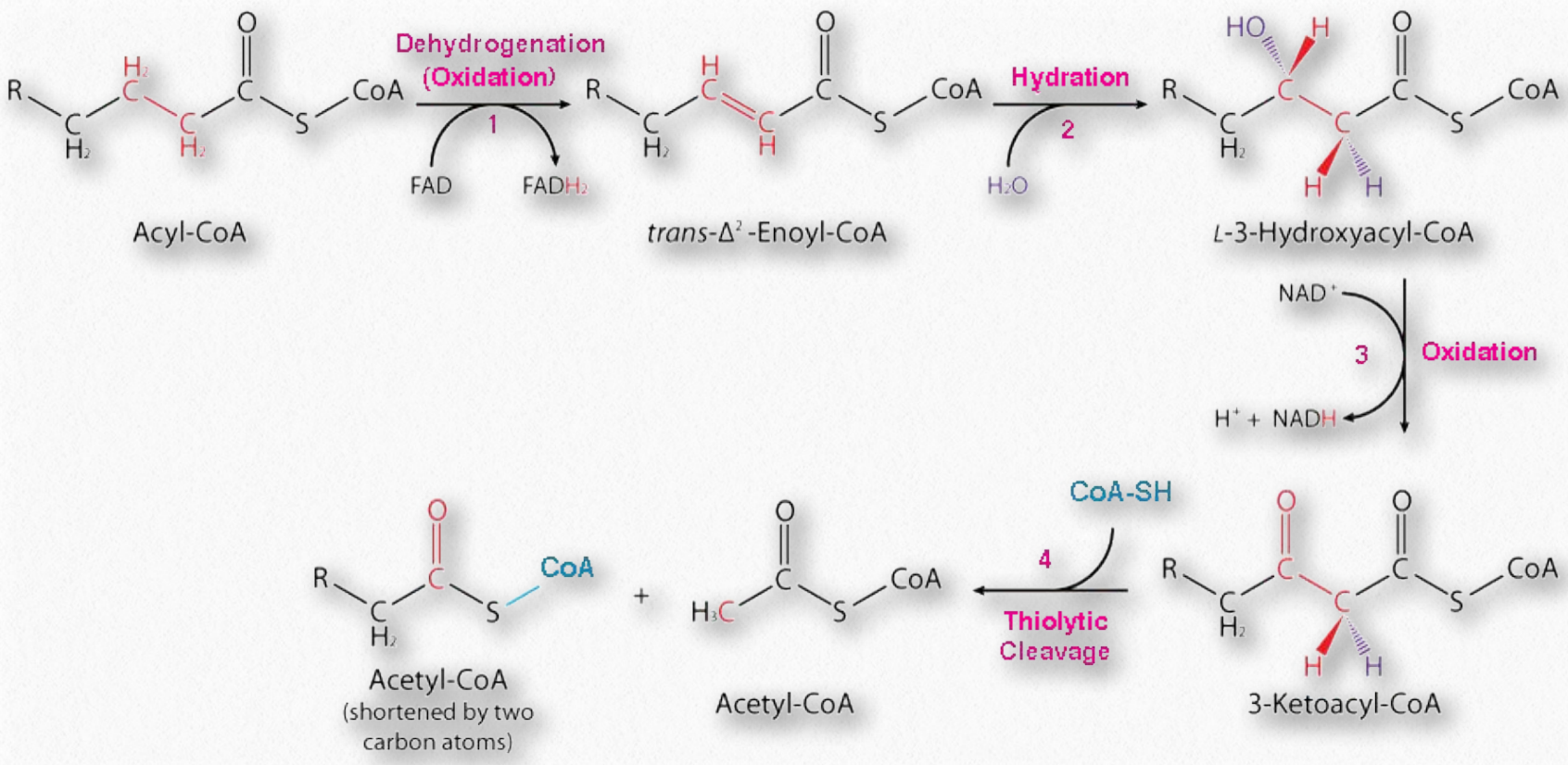

Figure 6.88 - Four reactions in β-oxidation

Image by Aleia Kim

to make acetyl-CoA, which is subsequently oxidized in the citric acid cycle. Depending on the size of the fatty acid, this process (called β-oxidation) will begin in either the mitochondrion (Figure 6.86) or the peroxisomes (see HERE).

Transport

To be oxidized in the mitochondrion, fatty acids must first be attached to coenzyme A (CoA-SH or CoA) and transported through the cytoplasm and the outer mitochondrial membrane. In the mitochondrion's intermembrane space, the CoA on the fatty acid is replaced by a carnitine (Figure 6.87) in order to be moved into the matrix. After this is done, the fatty acid linked to carnitine is transported into the mitochondrial matrix and in the matrix the carnitine is replaced again by coenzyme A. It is in the mitochondrial matrix where the oxidation occurs. The fatty acid linked to CoA (called an acyl-CoA) is the substrate for fatty acid oxidation.

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Steps

The process of fatty acid oxidation (Figure 6.88) is fairly simple. The reactions all occur between carbons 2 and 3 (with #1 being the one linked to the CoA) and sequentially include the following steps 1) dehydrogenation to create $FADH_2$ and a fatty acyl group with a double bond between carbons 2 and 3 in the *trans* configuration; 2) hydration across the double bond to put a hydroxyl group on carbon 3 in the L configuration; 3) oxidation of the

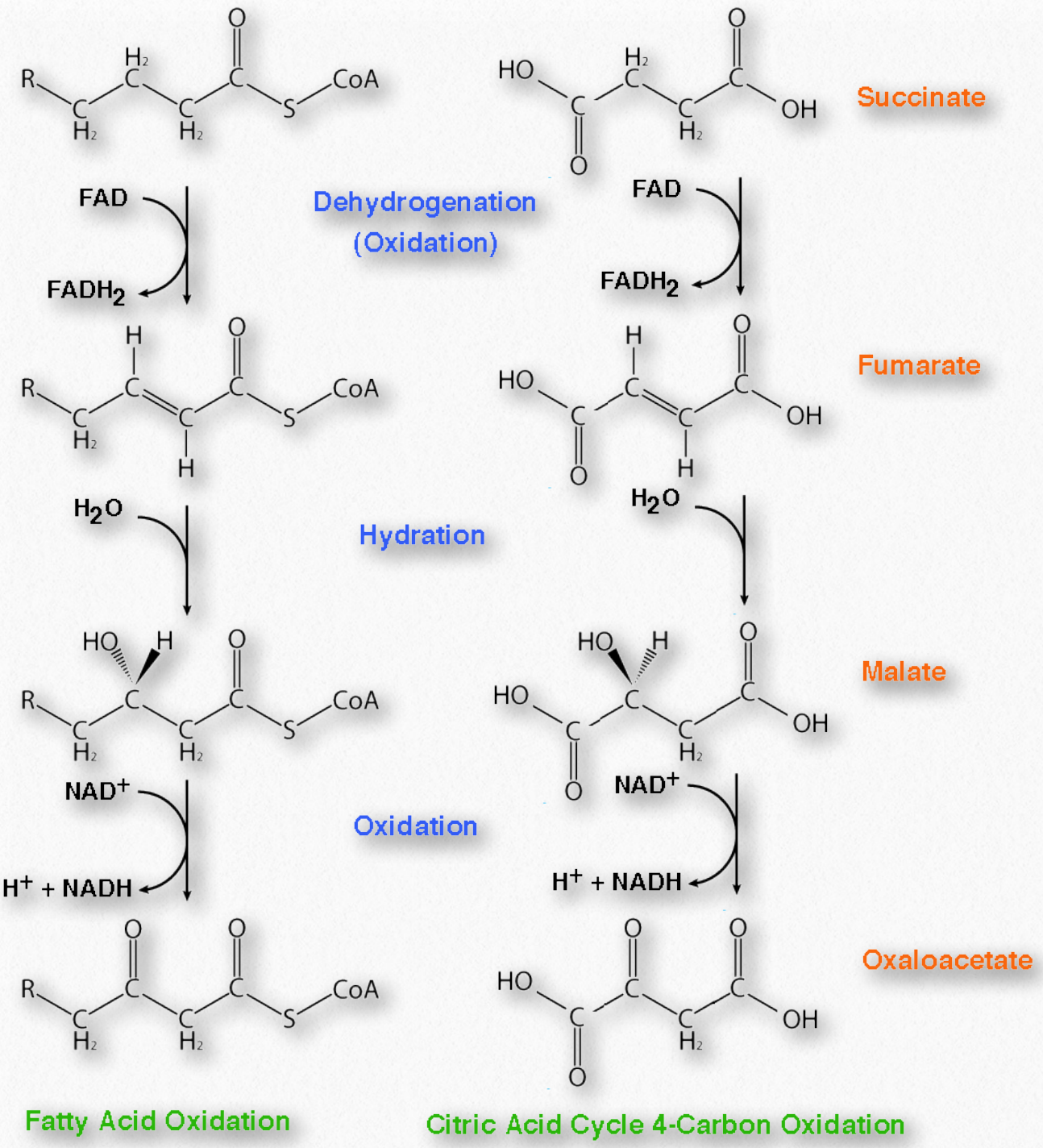

Figure 6.89 - Similar reactions for fatty acid oxidation and oxidation of 4-carbon compounds in the citric acid cycle

Image by Aleia Kim

which catalyzes the dehydrogenation in the first reaction and yields $FADH_2$. The enzyme comes in three different forms – ones specific for long, medium, or short chain length fatty acids. The first of these is sequestered in the peroxisomes of animals (see below) whereas the ones that work on medium and shorter chain fatty acids are found in the mitochondria. Action of all three enzymes is typically needed to oxidize a fatty acid. Plants and yeast perform β-oxidation exclusively in peroxisomes.

hydroxyl group to make a ketone; and 4) thiolytic cleavage to release acetyl-CoA and a fatty acid two carbons shorter than the starting one.

Enzymes of β-oxidation

Two of the enzymes of β-oxidation are notable. The first is acyl-CoA dehydrogenase, The most interesting of the acyl-CoA dehydrogenases is the one that works on medium length fatty acids. This one, which is the one most commonly deficient in animals, has been associated with sudden infant death syndrome. Reactions two and three in β-oxidation are catalyzed by enoyl-CoA hy-

dratase and 3-hydroxyacyl-CoA dehydrogenase, respectively. The latter reaction yields an NADH.

Thiolase

The second notable enzyme of β-oxidation is thiolase because this enzyme not only catalyzes the formation of acetyl-CoAs in **β**-oxidation, but also the joining of two acetyl-CoAs (essentially the reversal of the last step of β-oxidation) to form acetoacetyl-CoA – essential for the pathways of ketone body synthesis and cholesterol biosynthesis.

Similarity to citric acid cycle oxidation

It is worth noting that oxidation of fatty acids is chemically very similar to oxidation of the four carbon compounds of the citric acid cycle (Figure 6.89). In fatty acid oxidation, dehydrogenation between carbons 2 and 3 generates electrons which are donated to FAD to make $FADH_2$ and a *trans*-bonded intermediate is formed.

The same thing happens in the citric acid cycle reaction catalyzed by succinate dehydrogenase - the *trans*-bonded molecule is fumarate. Addition of water in the second step of fatty acid oxidation occurs also in the next step of the citric acid cycle catalyzed by fumarase to create malate. Oxidation of the hydroxyl on carbon 3 in β-oxidation is repeated in the citric acid cycle reaction catalyzed by malate dehydrogenase yielding oxaloacetate.

Oxidation of odd chain fatty acids

Though most fatty acids of biological origin have even numbers of carbons, not all of them do. Oxidation of fatty acids with odd numbers of carbons ultimately produces an intermediate with three carbons called

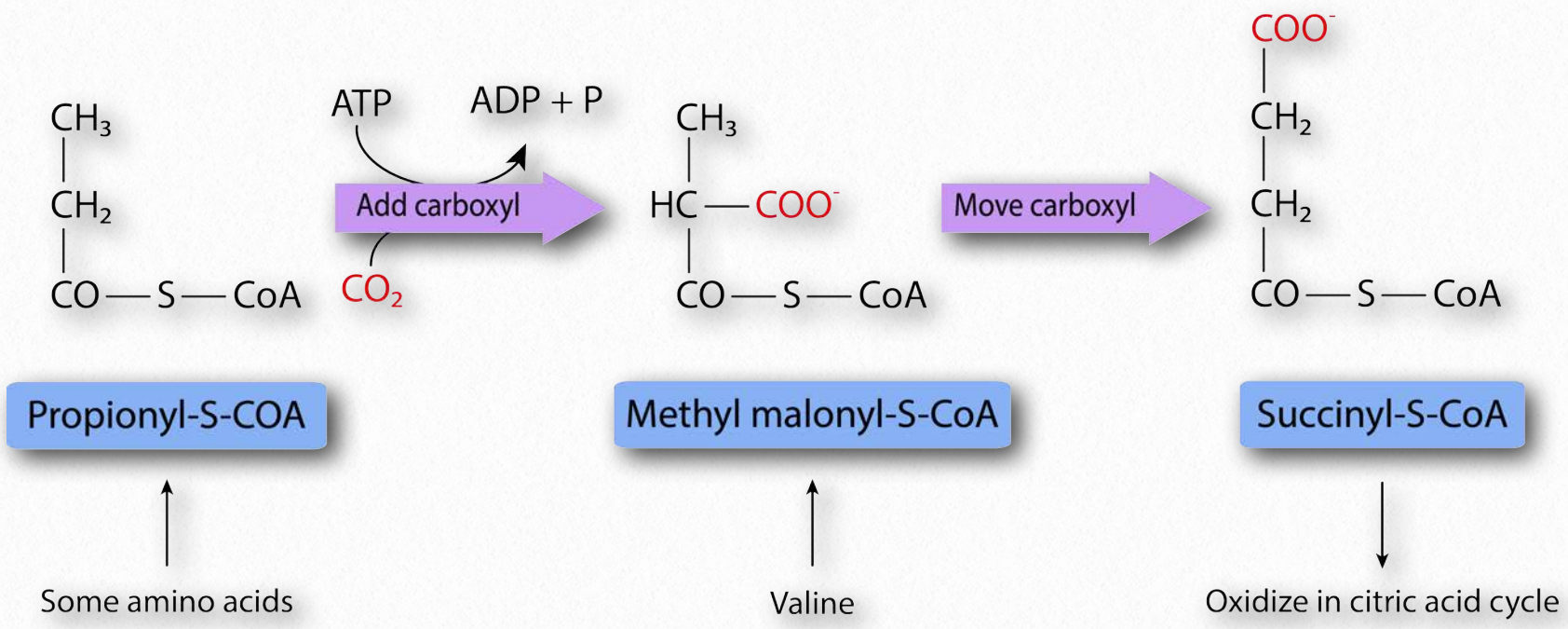

Figure 6.90 - Metabolism of propionyl-CoA

Image by Pehr Jacobson

In beta oxidation, it just occurred to me
The process all takes place 'tween carbons two and three
Some hydrogens are first removed to $FADH_2$
Then water adds across the bond, the H to carbon two
Hydroxyl oxidation's next, a ketone carbon three
Then thiolase catalysis dissects the last two C's
The products of the path, of course, are acetyl-CoAs
Unless there were odd carbons, hence propionyl-CoA

propionyl-CoA, which cannot be oxidized further in the β-oxidation pathway.

Metabolism of this intermediate is odd. Sequentially, the following steps occur (Figure 6.90) – 1) carboxylation to make D-methylmalonyl-CoA; 2) isomerization to L-methylmalonyl-CoA; 3) rearrangement to form succinyl-CoA. The last step of the process utilizes the enzyme methylmalonyl-CoA mutase, which uses the B_{12} coenzyme in its catalytic cycle. Succinyl-CoA can be metabolized in the citric acid cycle.

Peroxisomal oxidation

Long chain fatty acids (typically 22 carbons or more - Figure 6.91) have their oxidation initiated in the peroxisomes, due to the localization of the long acyl-CoA dehydrogenase in that organelle. Peroxisomal fatty acid oxidation is chemically similar to β-oxidation of mitochondria, but there are some differences in the overall process.

Differences

First, since there is no electron transport system in peroxisomes, the reduced electron carriers produced in oxidation there must have their own recycling process. Peroxisomes accomplish this by transferring electrons and protons from $FADH_2$ to O_2 to form hydrogen peroxide (H_2O_2). As a result of this, the lack of electron transport means no proton pump and, consequently, no ATP produced from $FADH_2$ for peroxisomal fatty acid oxidation, making it less efficient that mitochondrial β-oxidation.

Electrons from NADH produced in the third step of the fatty acid oxidation must be shuttled to the cytoplasm and ultimately to the mitochondrion for ATP generation. Peroxisomal oxidation is increased for individuals on a high fat diet. In addition to long chain fatty acids, peroxisomes are also involved in

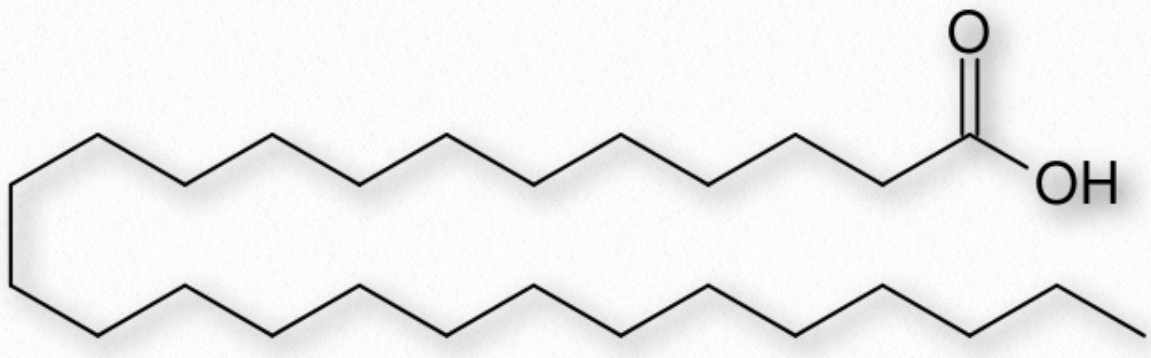

Figure 6.91 - Cerotic acid - A long chain fatty acid with 26 carbons

oxidation of branched chain fatty acids, leukotrienes, and some prostaglandins.

Unsaturated fatty acid oxidation

Unsaturated fatty acids complicate the oxidation process a bit (see below), primarily because they have *cis* bonds, for the most part, if they are of biological origin, and these must be converted to the relevant *trans* intermediates for β-oxidation. Sometimes the bond must be moved down the chain, as well, in order to be positioned properly. Two enzymes (described below) handle all the necessary isomerizations and moves necessary to oxidize all of the unsaturated fatty acids (Figure 6.92).

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Extra enzymes

As noted above, oxidation of unsaturated fatty acids requires two additional enzymes to the complement of enzymes for β-oxidation. If the β-oxidation of the fatty acid produces an intermediate with a *cis* bond between carbons three and four, *cis*-Δ3-enoyl-CoA isomerase will convert the bond to a *trans* bond between carbons two and three and β-oxidation can proceed as normal.

On the other hand, if β-oxidation produces an intermediate with a *cis* double bond between carbons four and five, the first step of β-oxidation (dehydrogenation between car-

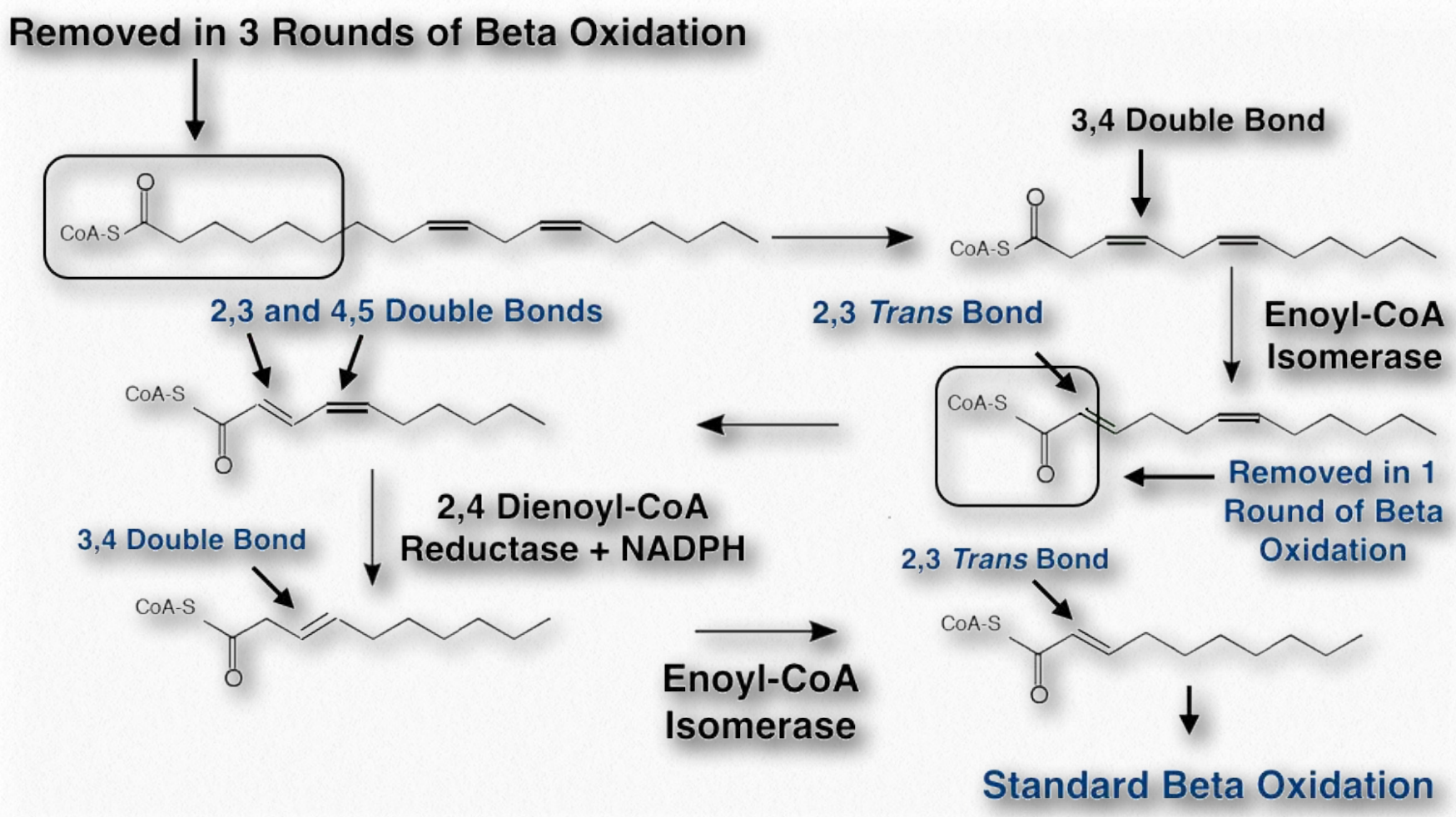

Figure 6.92 - Unsaturated fatty acid oxidation

bons two and three) occurs to produce an intermediate with a *trans* double bond between carbons two and three and a *cis* double bond between carbons four and five.

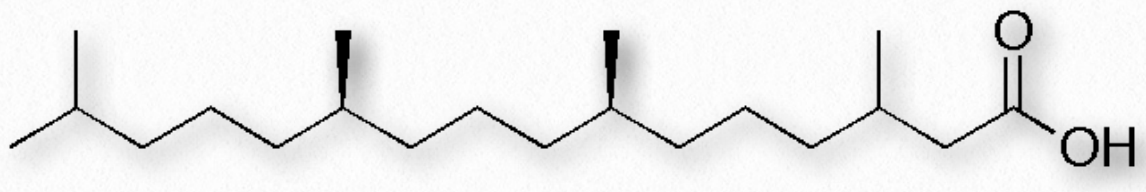

Figure 6.93 - Phytanic acid

2,4 dienoyl-CoA reductase

The enzyme 2,4 dienoyl CoA reductase reduces this intermediate (using NADPH) to one with a single *cis* bond between carbons three and four. The newly created *cis*-bonded molecule is then identical to the one acted on by *cis*-Δ3-enoyl-CoA isomerase above, which converts it into a regular **β**-oxidation intermediate, as noted above.

α-oxidation

Yet another consideration for oxidation of fatty acids is **α**-oxidation. This pathway, which occurs in peroxisomes, is necessary for catabolism of fatty acids that have branches in their chains. For example, breakdown of chlorophyll's phytol group yields phytanic acid (Figure 6.93), which undergoes hydroxylation and oxidation on carbon number two (in contrast to carbon three of β-oxidation), followed by decarboxylation and production of an unbranched intermediate that can be further oxidized by the β-oxidation pathway. Though **α**-oxidation is a relatively minor metabolic pathway, the inability to perform the reactions of the pathway leads to Refsum's disease where accumulation of phytanic acid leads to neurological damage.

ω-oxidation of fatty acids

In addition to **β**-oxidation and **α**-oxidation of fatty acids, which occur in the mitochondria and peroxisomes of eukaryotic cells respectively, another fatty acid oxidation pathway known as **ω**-oxidation also occurs in the smooth endoplasmic reticulum of liver and kidney cells. It is normally a minor oxidation pathway operating on medium chain fatty acids (10-12 carbons), but gains importance 1) when **β**-oxidation is not functional or 2) for production of long chain intermediates, such as 20-HETE (20-hydroxyeicosatetraenoic acid), that can function in signaling.

Steps in the process involve 1) oxidation of the terminal methyl group of the fatty acid to an alcohol; 2) oxidation of the alcohol to an aldehyde, and 3) oxidation of the aldehyde group to a carboxylic acid (Figure 6.94). The first oxidation is catalyzed by a mixed function oxidase, and yields 20-HETE if the starting material is arachidonic acid.

The last two reactions are catalyzed by alcohol dehydrogenase and each requires NAD^+. After the last oxidation, the fatty acid has carboxyl groups at each end and can be attached to coenzyme A at either end and subsequently oxidized, ultimately yielding succinate.

Regulation of fatty acid oxidation

Breakdown of fatty acids is controlled at different levels. The first is by control of the availability of fatty acids from the breakdown of fat. As noted above, this process is by regulating the activity of hormone-sensitive triacylglycerol lipase (HSTL) activity by epinephrine (stimulates) and insulin (inhibits).

A second level of control of fatty acid availability is by regulation of carnitine acyl transferase (Figure 6.87 - see HERE). This enzyme controls the swapping of CoA on an acyl-CoA molecule for carnitine, a necessary step for the fatty acid to be imported into the mitochondrion for oxidation.

The enzyme is inhibited by malonyl-CoA, an intermediate in fatty acid synthesis. Thus, when fatty acids are being synthesized, import of them into the mitochondrion for oxidation is inhibited. Last, the last enzyme in the β-oxidation cycle, thiolase, is inhibited by acetyl-CoA.

Fatty acid synthesis

Synthesis of fatty acids occurs in the cytoplasm and endoplasmic reticulum of the cell and is chemically similar to the reverse of the β-oxidation process, but with a couple of key differences (Figure 6.95). The first of these occur in preparing substrates for the reactions that grow the fatty acid. Fatty acid synthesis occurs in the cytoplasm of eukary-

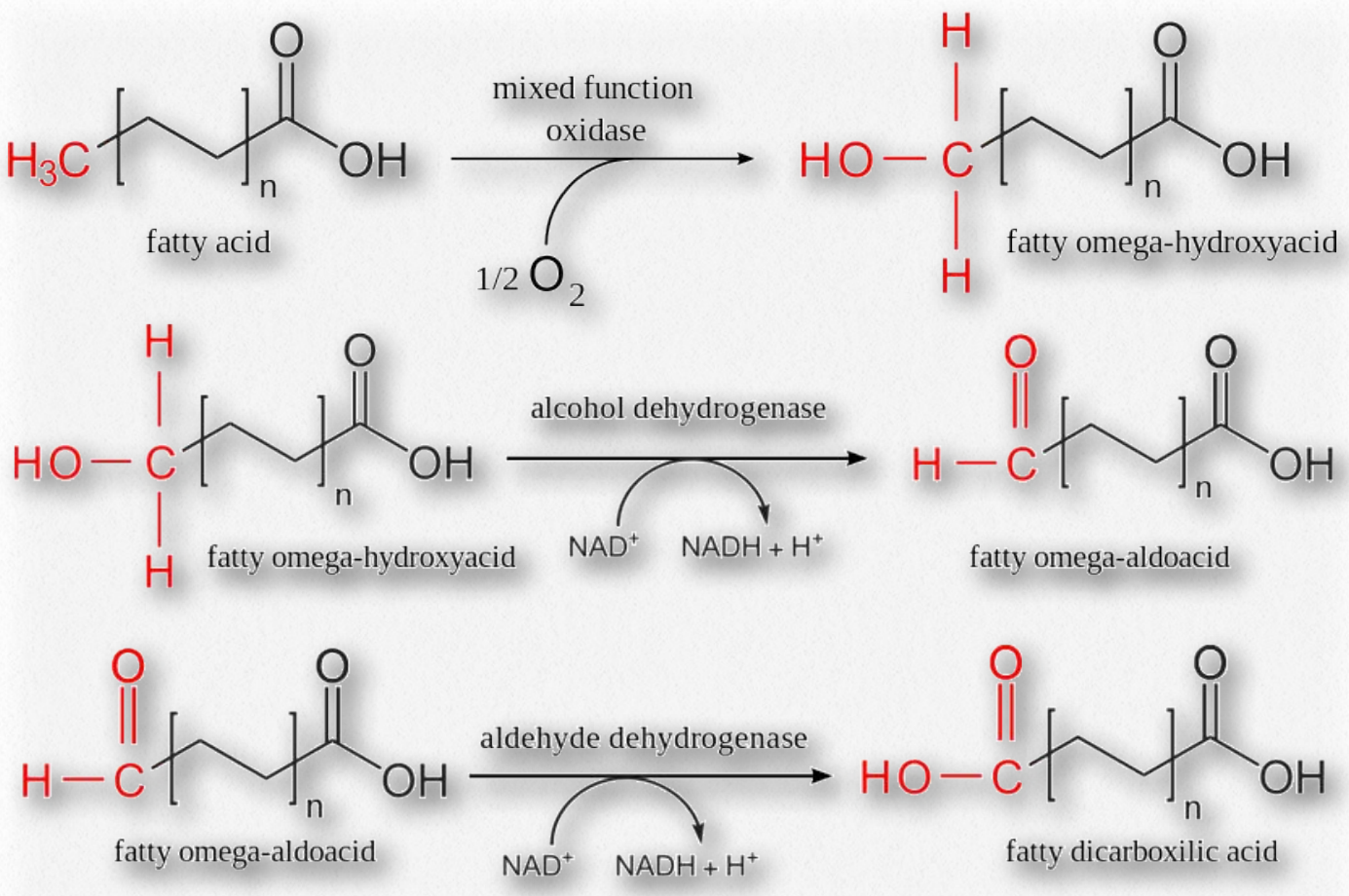

Figure 6.94 - ω Oxidation

Wikipedia

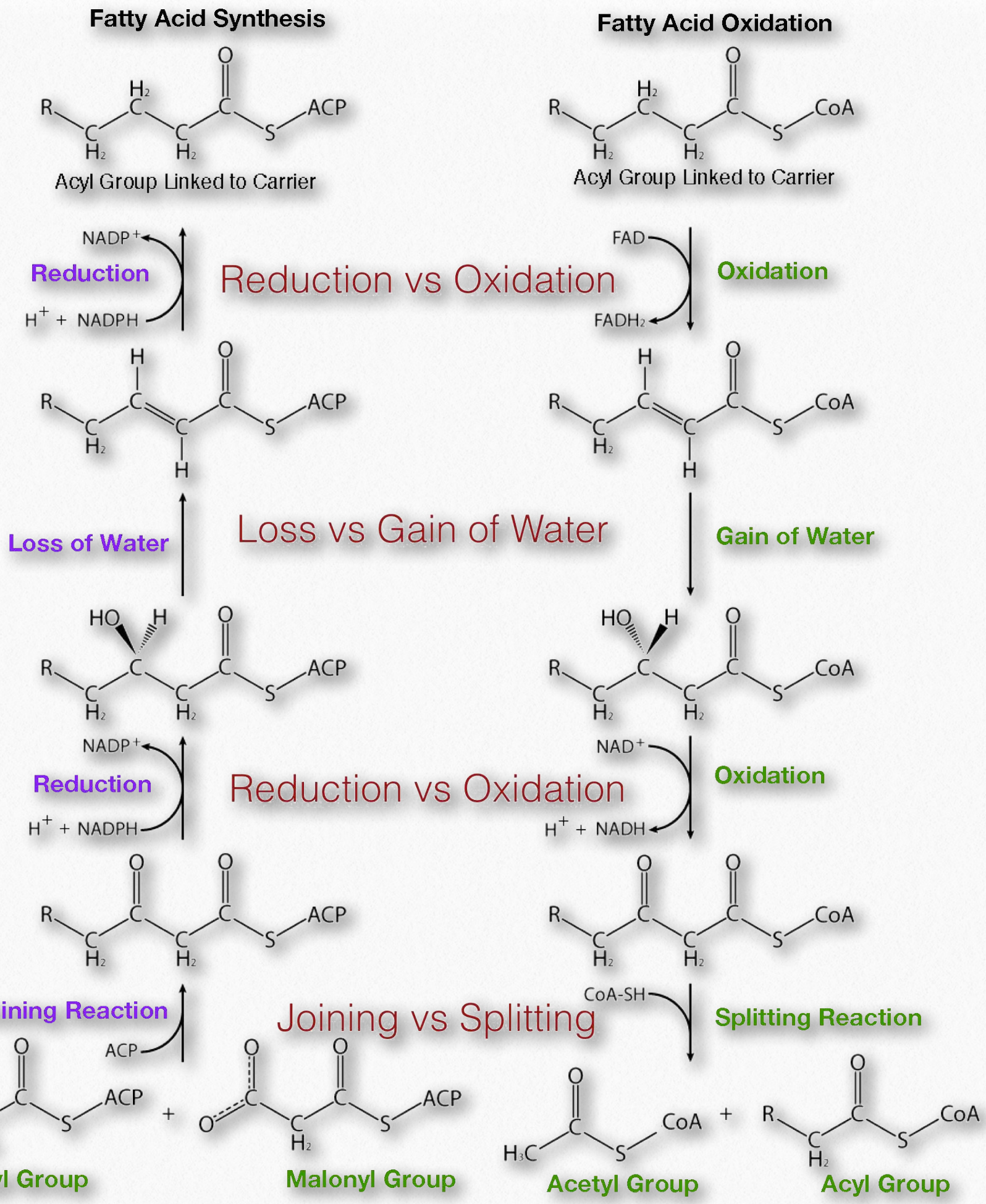

Figure 6.95 - Fatty acid synthesis is the reverse of fatty acid oxidation chemically

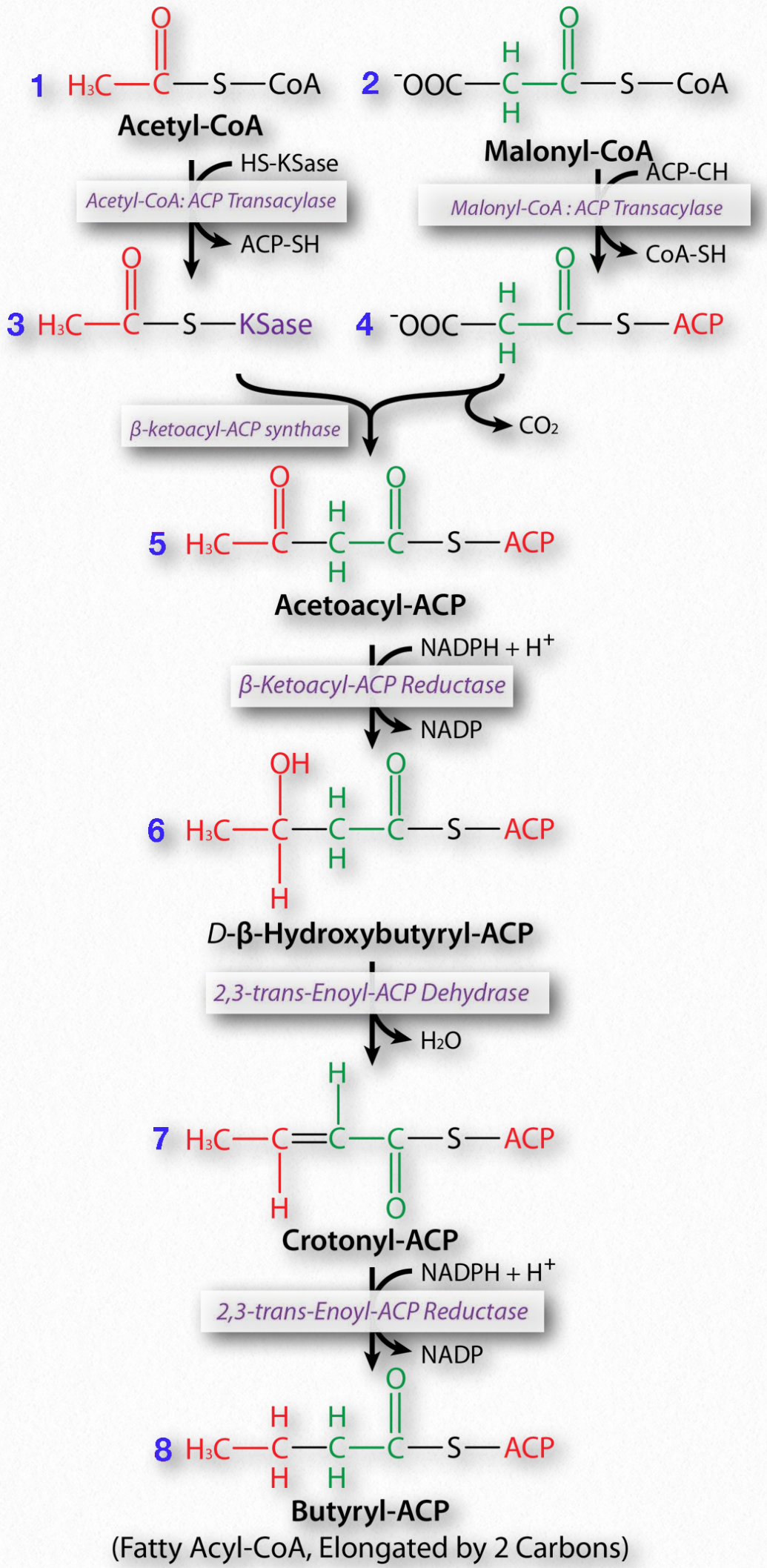

Figure 6.96 - One round of fatty acid synthesis

image by Aleia Kim

otic cells. Transport of acetyl-CoA from the mitochondrial matrix occurs when it begins to build up. This happens when the citric acid cycle slows or stops from lack of exercise.

Two molecules can play roles in moving acetyl-CoA to the cytoplasm – citrate and acetylcarnitine. Joining of oxaloacetate with acetyl-CoA in the mitochondrion creates citrate which gets transported across the membrane, followed by action of citrate lyase in the cytoplasm of the cell to release acetyl-CoA and oxaloacetate. Additionally, when free acetyl-CoA accumulates in the mitochondrion, it may combine with carnitine and be transported out to the cytoplasm.

Fatty acid synthase

In animals, six different catalytic activities necessary to fully make palmitoyl-CoA are contained in a single complex called Fatty Acid Synthase. As shown in Figures 6.96 and 6.97, these include 1) transacylases (MAT) for swapping CoA-SH with ACP-SH on acetyl-CoA and malonyl-CoA; 2) a synthase (KS) to catalyze addition of the two carbon unit from the three carbon malonyl-ACP in the first step of the elongation process; 3) a reductase (KR) to

reduce the ketone; 4) a dehydrase (DH) to catalyze removal of water; 5) a reductase (ER) to reduce the *trans* double bond and 6) a thioesterase (TE) to cleave the finished palmitoyl-CoA into palmitic acid and CoA-SH.

In the middle of the complex is a site for binding the ACP portion of the growing fatty acid chain to hold it as the other part of the fatty acid is rotated into positions around the enzyme complex for each catalysis. In bacteria, these six activities are found on separate enzymes and are not part of a complex.

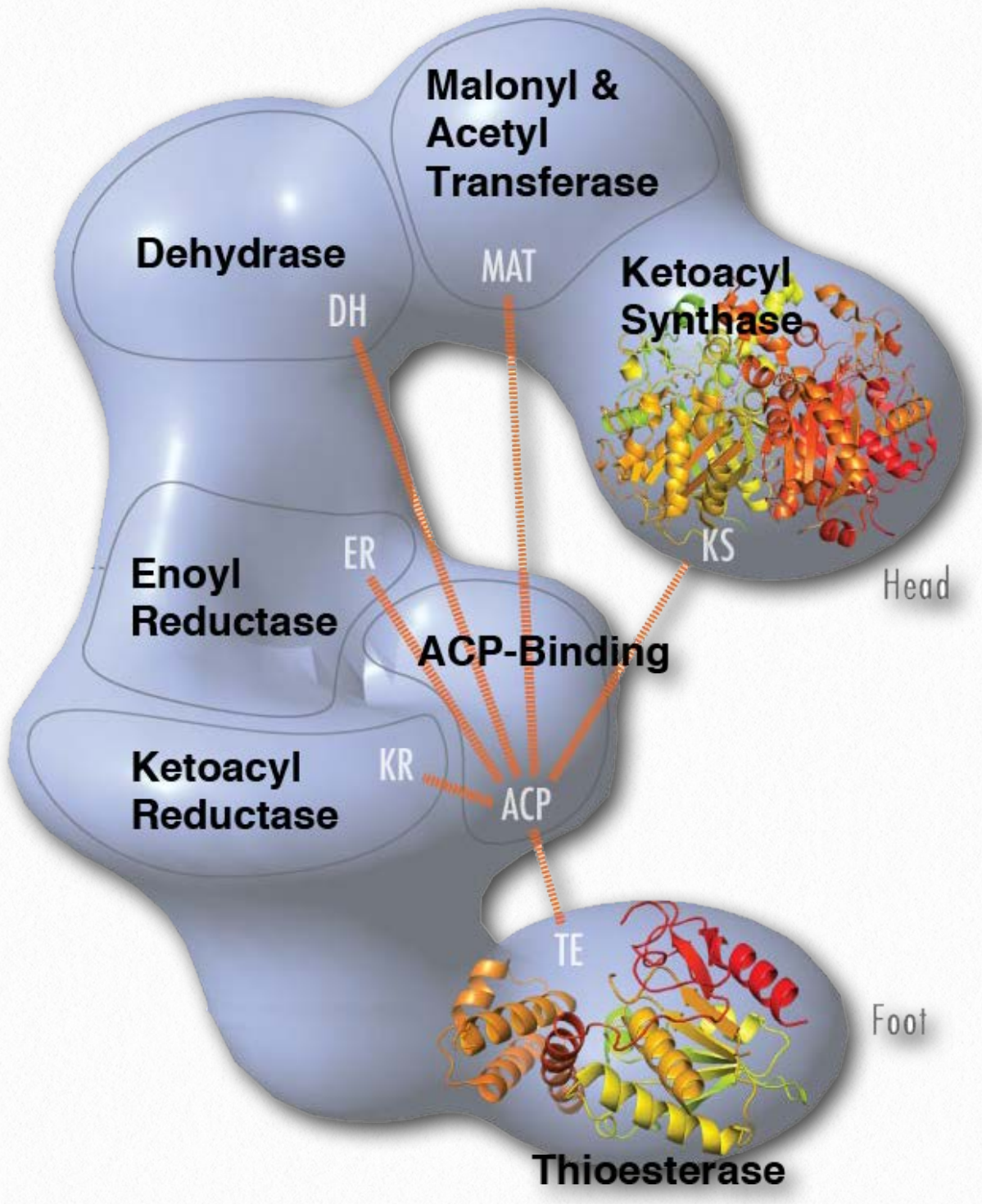

Figure 6.97 - Fatty acid synthase complex

Cytoplasmic reactions

The process of making a fatty acid in the cytoplasm starts with two acetyl-CoA molecules. One is converted to malonyl-CoA by adding a carboxyl group. This reaction is catalyzed by the enzyme acetyl-CoA carboxylase (ACC), the only regulated enzyme of fatty acid synthesis (see below) and the only one separate from the fatty acid synthase. Next, both acetyl-CoA and malonyl-CoA have their CoA portions replaced by a carrier protein known as ACP (acyl-carrier protein) to form acetyl-ACP (catalyzed by acetyl-CoA : ACP transacylase - MAT in Figure 6.97) and malonyl-ACP (catalyzed by malonyl-CoA : ACP transacylase - MAT in Figure 6.97). Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with malonyl-ACP splits out the car-

For fatty acid synthesis, I must reverse the path
Of breaking fatty acids, though you'll wonder 'bout the math

Each cycle of addition starts with carbons one two three
Yet products of reactions number carbons evenly

The reason is that CO_2 plays peek-a-boo like games
By linking to an Ac-CoA then popping off again

Reactions are like oxidations 'cept they're backwards here
Reduction, dehydration, then two hydrogens appear

The product of the process is a 16 carbon chain
The bonds are saturated. No double ones remain

For them desaturases toil to put in links of *cis*
In animals to delta nine, but no more go past this

And last there's making longer ones eicosanoidic fun
They're made by elongases in the *e. reticulum*

Kevin Ahern

boxyl group from malonyl-ACP that was added to it and creates the acetoacyl-ACP intermediate (catalyzed by **β**-ketoacyl-ACP synthase - KS on Figure 6.97) .

From this point forward, the chemical reactions resemble those of β-oxidation reversed. First, the ketone is reduced to a hydroxyl using NADPH (catalyzed by **β**-ketoacyl-ACP reductase - KR on Figure 6.97). In contrast to the hydroxylated intermediate of **β**-oxidation, the intermediate here (D-β- hydroxybutyryl-ACP) is in the D-configuration.

Dehydration

Next, water is removed from carbons 2 and 3 of the hydroxyl intermediate in a reaction catalyzed by 2,3-*trans*-enoyl-ACP dehydrase - DH on Figure 6.97. This yields a *trans* doubled-bonded molecule. Last, the double bond is hydrogenated to yield a saturated intermediate by 2,3-trans-enoyl-ACP reductase - ER on Figure 6.97. This completes the first cycle of synthesis.

Additional cycles involve addition of more two-carbon units from malonyl-ACP to the growing chain until ultimately an intermediate with 16 carbons is produced (palmitoyl-ACP). At this point, a thioesterase cleaves the ACP from the palmitoyl-ACP to yield palmitic acid and the cytoplasmic synthesis ceases.

Regulation of fatty acid synthesis

Acetyl-CoA carboxylase, which catalyzes synthesis of malonyl-CoA, is the only regulated enzyme in fatty acid synthesis. Its regulation involves both allosteric control and covalent modification. The enzyme is known to be phosphorylated

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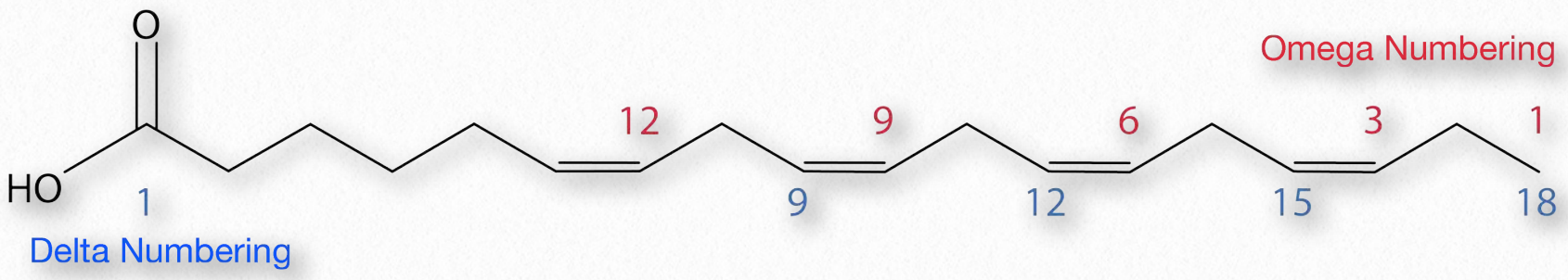


Figure 6.98 - Carbon numbering schemes for fatty acids

Image by Pehr Jacobson

by both AMP Kinase and Protein Kinase A.

Dephosphorylation is stimulated by phosphatases activated by insulin binding. Dephosphorylation activates the enzyme and favors its assembly into a long polymer, while phosphorylation reverses the process. Citrate acts as an allosteric activator and may also favor polymerization. Palmitoyl-CoA allosterically inactivates it.

Elongation past 16 carbons

Elongation to make fatty acids longer than 16 carbons occurs in the endoplasmic reticulum and is catalyzed by enzymes described as elongases. Mitochondria also can elongate fatty acids, but their starting materials are generally longer than 16 carbons long.

The mechanisms in both environments are similar to those in the cytoplasm (a malonyl group is used to add two carbons, for example), but CoA is attached to the intermediates, not ACP. Further, whereas cytoplasmic synthesis employs the fatty acid synthase complex, the enzymes in these organelles are separable and not part of a complex.

Desaturation of fatty acids

Fatty acids are synthesized in the saturated form and desaturation occurs later - in the endoplasmic reticulum. Reactions to elongate the fatty acid (with elongases) may also occur to make unsaturated fatty acids of varying lengths. Desaturases are named according to the location of the double bonds they introduce in fatty acids. The delta (Δ) system numbers the carbon at the carboxyl end as number 1 and the omega (ω) number system numbers the carbon at the methyl end as number 1 (Figure 6.98). Humans have desaturases named as Δ5, Δ6, and Δ9. A Δ9 desaturase, for example, could convert stearic acid into oleic acid, because stearic acid (see HERE) is a saturated 18 carbon fatty acid and oleic acid is an 18 carbon fatty acid with only one double bond - at position Δ9.

Polyunsaturated fatty acids

Polyunsaturated fatty acids require the action of multiple enzymes and (in some cases)

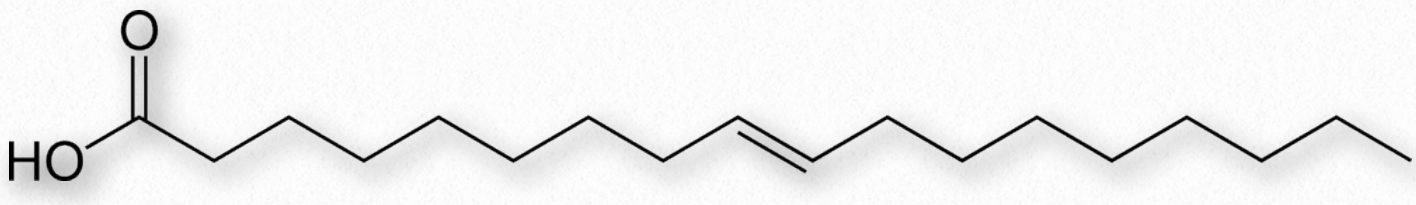


Figure 6.99 - Elaidic acid - A rare *trans* fatty acid in biology

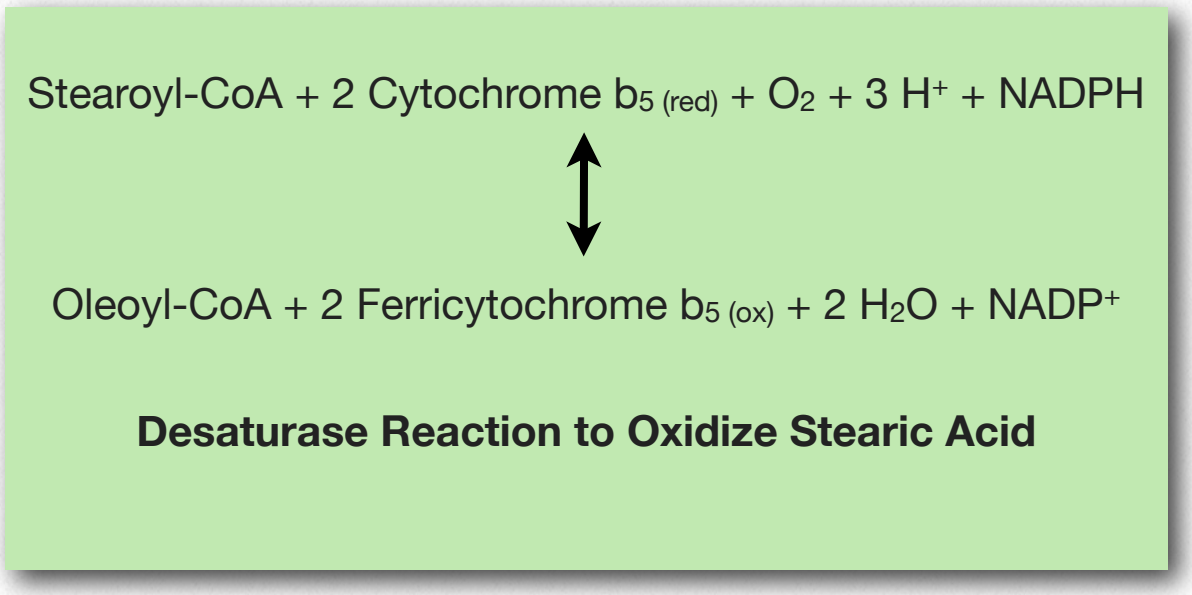
the action of elongases. Arachidonic acid, for example, is a 20 carbon fatty acid with four double bonds and its synthesis requires both an elongase (to increase the length of the fatty acid from 16 to 20) and multiple desaturases - one for each desaturated double bond.

Animals are limited in the fatty acids they can make, due to an inability of their desaturases to catalyze reactions beyond carbons Δ9. Thus, humans can make oleic acid, but cannot synthesize linoleic acid (Δ9,12) or linolenic acid (Δ9,12,15). Consequently, these two must be provided in the diet and are referred to as essential fatty acids.

Almost all desaturases make *cis*, not *trans* double bonds. There are a few minor exceptions to this, in cattle, for example (Figure 6.99). The *trans* fatty acids found in *trans* fat of prepared food are produced not by biological processes, but rather by the process of partial hydrogenation of unsaturated fats.

Unusual oxidation reaction

Removal of electrons and protons from a fatty acid to create a double bond is an oxidation reaction and these electrons, must have a destination. The path they take is a bit complex. It involves NAD(P)H, O_2, two membrane-bound cytochromes, the membrane bound desaturase, and the fatty acid.



Desaturase Reaction to Oxidize Stearic Acid

In the electron transfer, the O_2 is reduced to two molecules of H_2O. This reduction requires four electrons and four protons. Two electrons and two protons come from the fatty acid to form the double bond on it. Two electrons come from the NAD(P)H via the cytochromes and two protons come from the aqueous solution.

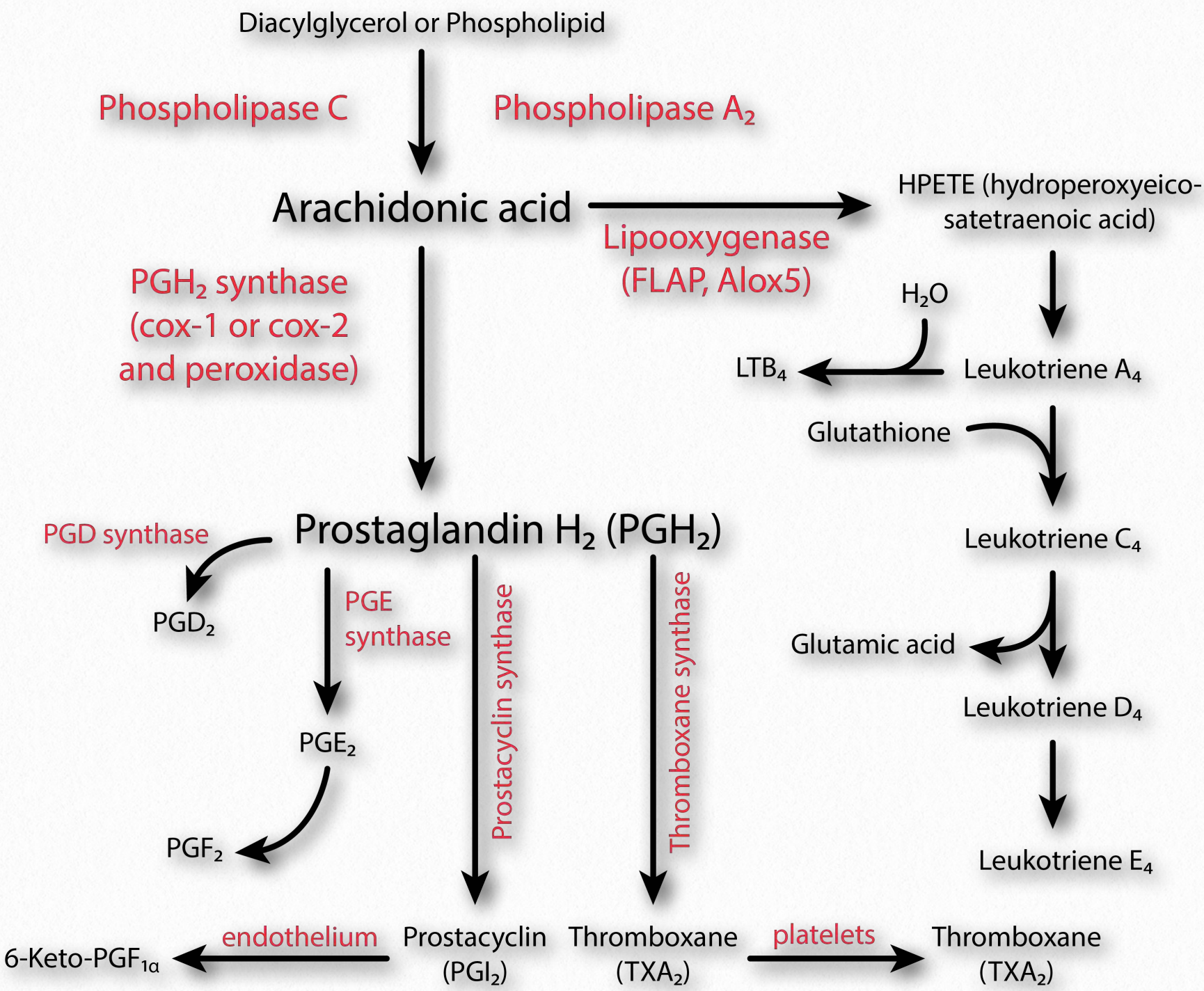


Figure 6.100 - Eicosanoid synthesis pathways

Image by Pehr Jacobson

Prostaglandin synthesis

The pathway for making prostaglandins and related molecules, such as the leukotrienes, prostacyclin, and thromboxanes is an extension of the synthesis of fatty acids (Figure 6.100).

Prostaglandins, known as eicosanoids because they contain 20 carbons, are synthesized in cells from arachidonic acid whenever it has been cleaved from membrane lipids. Prostaglandins are important for many physiological phenomena in the body, including swelling and pain and reduction of their levels is a strategy of some painkillers, such as aspirin (see below). Inflammation arising from bee stings, for example, occurs because bee (and snake) venom contains mellitin, an activator of PLA2 activity (Figure 6.100). There are two strategies for reducing prosta-

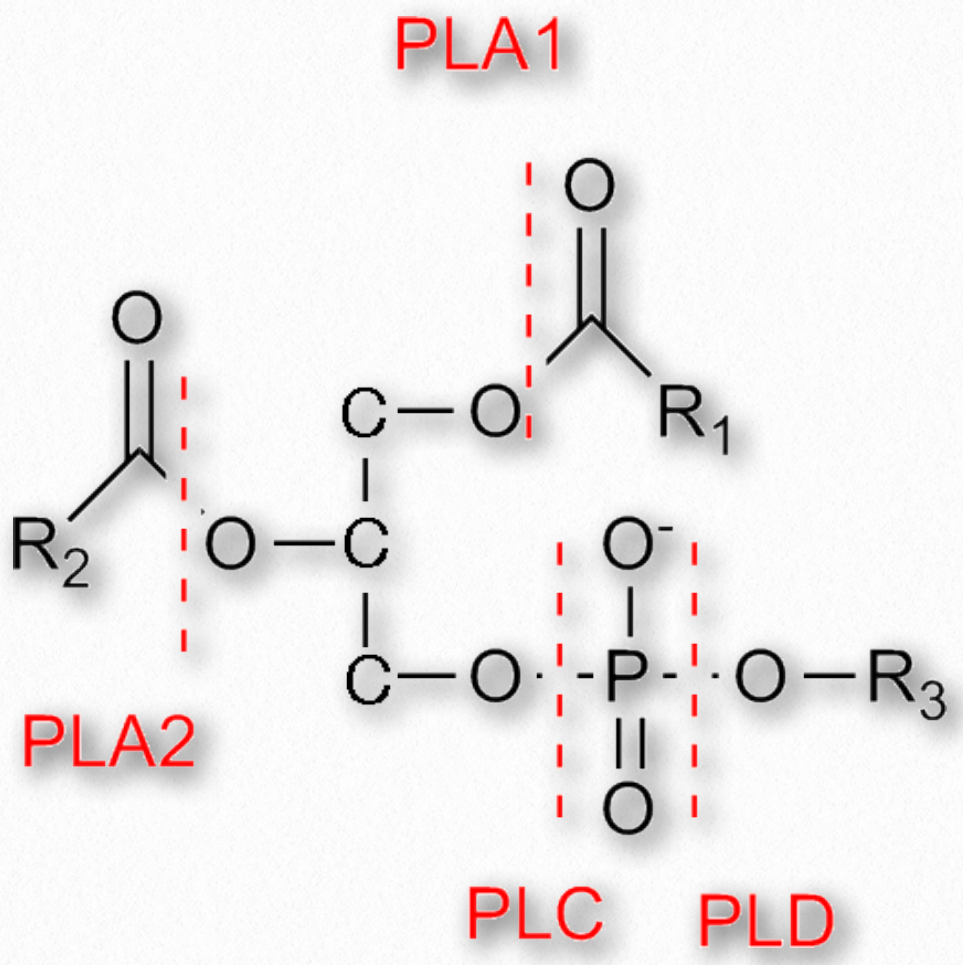


Figure 6.101 - Cleavage sites for four phospholipiases on a glycerophospholipid - phospholipases A_1 (PLA1), A_2 (PLA2), C (PLC), and D (PLD)

glandin production and the pain associated with it.

Phospholipase A_2

Action of phospholipase enzymes on glycerophospholipids produces fatty acids and either glycerol-3-phosphate or other substances. Figure 6.101 shows cleavage sites on phospholipids that are targeted by different phospholipases. Phospholipase A_1 (PLA1), for example, cleaves the fatty acid from position one of the glycerophospholipid and phospholipiase D (PLD) cleaves the R group from the phosphate part of the molecule.

Since the fatty acid on position #2 (where PLA_2 cuts) is most commonly unsaturated, PLA_2 is an important phosopholipase for hydrolyzing the unsaturated fatty acid known as arachidonic acid from glycerophospholipids. Release of arachidonic acid from membranes is necessary for synthesis of prostaglandins.

Inhibition of the release of arachidonic acid from membranes is the mechanism of action of steroidal anti-inflammatory drugs. They block action of phospholipase A_2 (PLA2 - Figure 6.101) which cleaves arachidonic acid from membrane lipids.

YouTube Lectures by Kevin HERE & HERE

Lipocortin

Lipocortin (also called annexin) is a protein that inhibits action of PLA2. Synthesis of lipocortin is stimulated by glucocorticoid hormones, such as cortisol, and is used in some treatments to reduce swelling/inflammation when it is severe and untreatable by non-steroidal drugs.

Second strategy

Synthesis of the prostanoid compounds (prostaglandins, prostacyclin, and thromboxanes) depends on conversion of arachi-

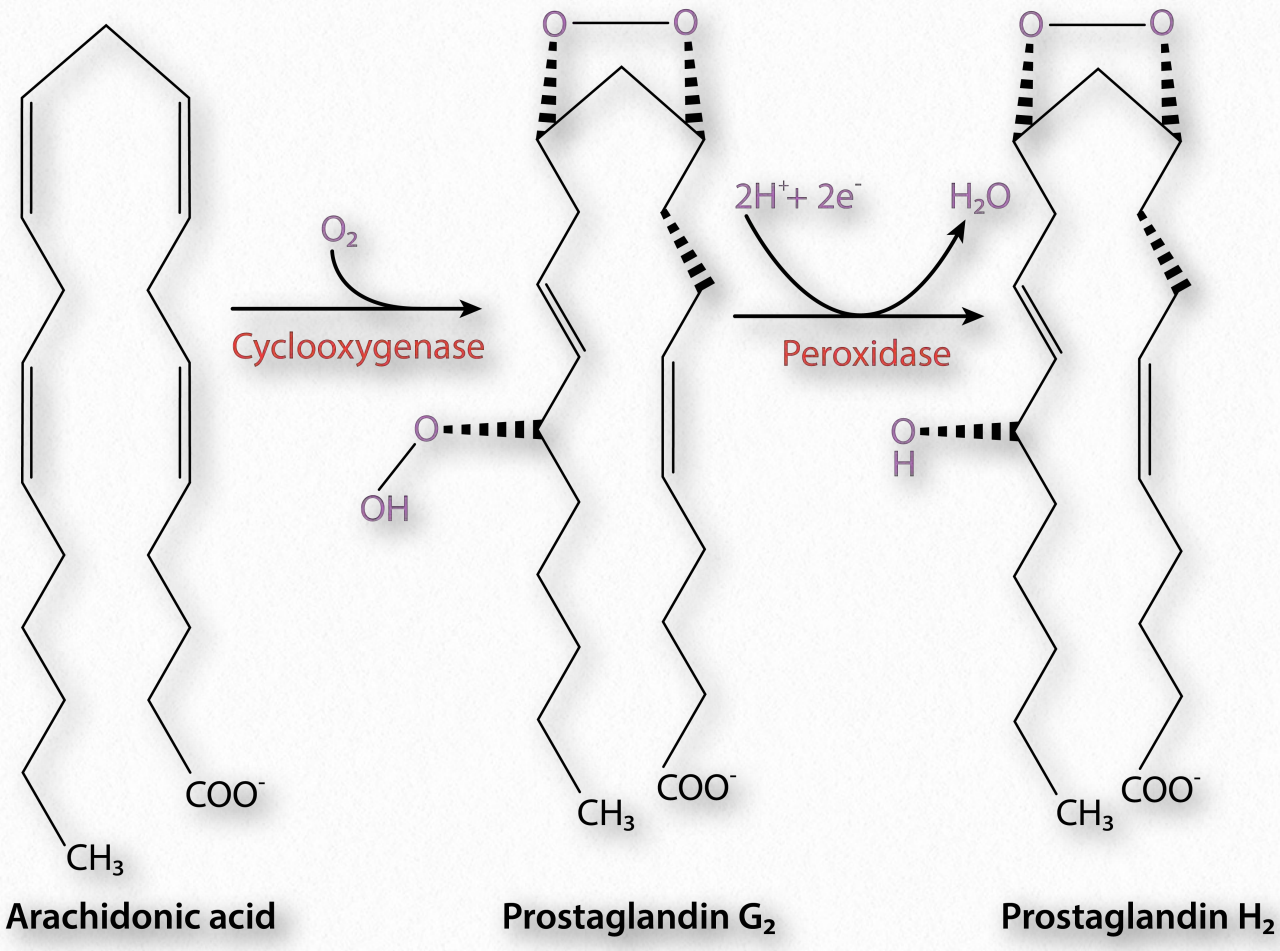


Figure 6.102 - Catalytic activity of cyclooxygenase and peroxidase in making prostaglandins

Image by Pehr Jacobson

donic acid to prostaglandins G_2 and H_2 by COX enzymes. A non-steroidal strategy for decreasing production of prostaglandins then is to inhibit the enzyme that catalyzes their synthesis from arachidonic acid (Figure 6.102). This enzyme is known as prostaglandin synthase, but is more commonly referred to as a cyclooxygenase (or COX) enzyme.

COX enzymes come in at least two forms in humans - COX-1, COX-2. A third form known as COX-3 has been reported as a splice variant of COX-1, but information about it is unclear. COX-1 and COX-2 are very similar in structure (70 kD and 72 kD, respectively, and 65% amino acid sequence homology), but coded by different genes.

COX-1 is synthesized constitutively whereas COX-2 displays inducible expression behavior and has a more specific pattern of tissue expression. COX-2 enzymes are expressed in increasing amounts in areas of growth and inflammation.

Non-steroidal drugs

Molecules inhibiting cyclooxygenases are known as non-steroidal anti-inflammatory drugs (NSAIDs). Molecules in this class include aspirin, ibuprofen, vioxx, and celebrex.

Targeting inhibitors

Some NSAID inhibitors, such as aspirin, bind to all types of COX enzymes. Newer COX inhibitors target the COX-2 enzyme specifically because it was believed to be a better target for relief of joint pain than COX-1 enzymes which are synthesized by most cells. COX-2 enzymes are found more specifically in joints so the thinking was that specific inhibition of them would not affect the COX-1 enzymes

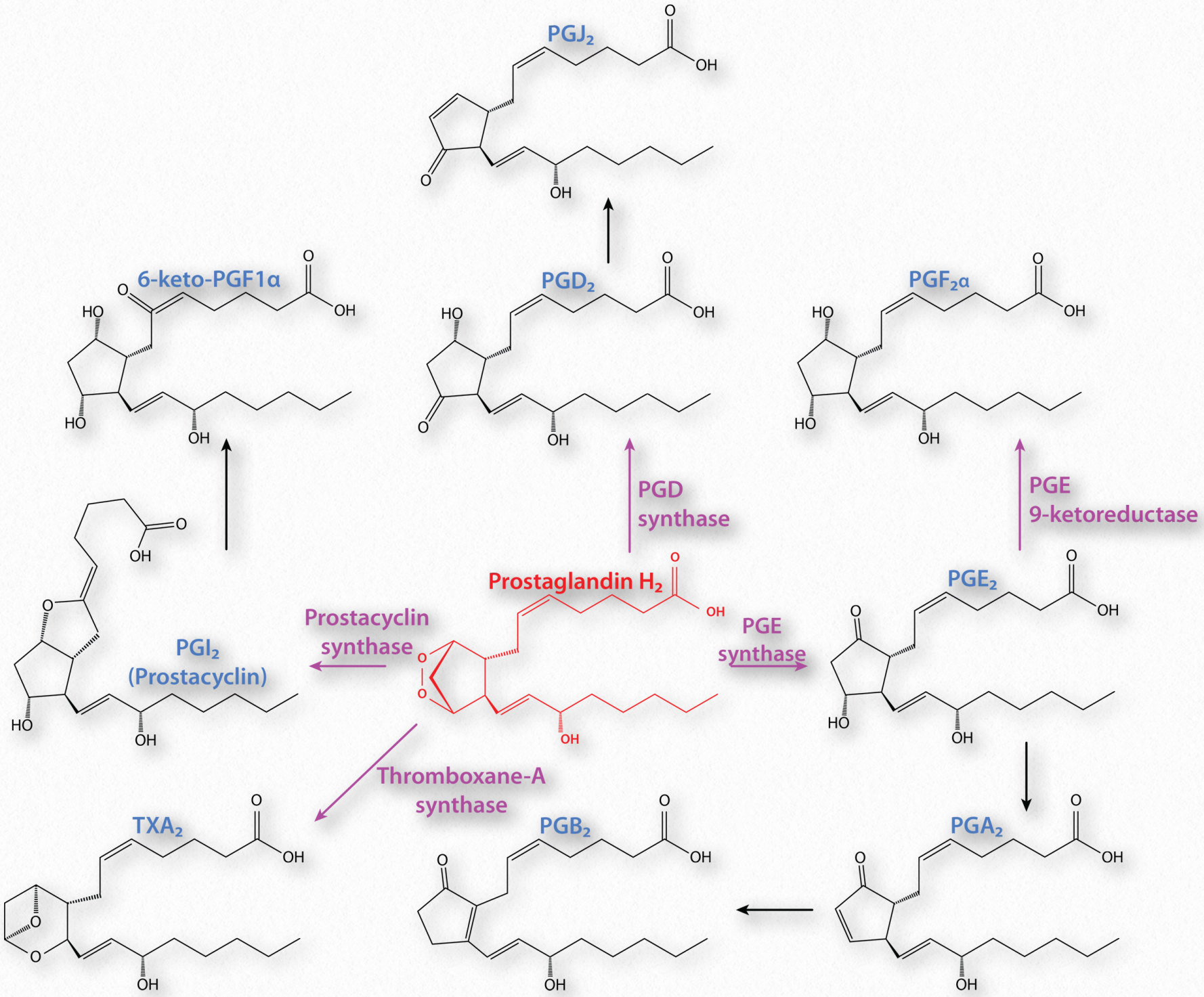


Figure 6.103 - Synthesis of prostaglandins from prostaglandin H_2 (red)

Image by Pehr Jacobson

which are important for producing prostaglandins that help maintain gastric tissue.

Numerous COX-2 - specific inhibitors were developed - celecoxib, etoricoxib, and rofecoxib (Vioxx), for example. Unfortunately, the COX-2 specific inhibitors are associated with some serious side effects, including a 37% increase in incidence of major cardiovascular events in addition to some of the gastrointestinal problems of NSAIDs.

Imbalance

The increased risk of heart attack, thrombosis, and stroke are apparently due to an imbalance between prostacyclin (reduced by inhibitors) and thromboxanes (not reduced by the inhibitors). Prostacyclin (made from

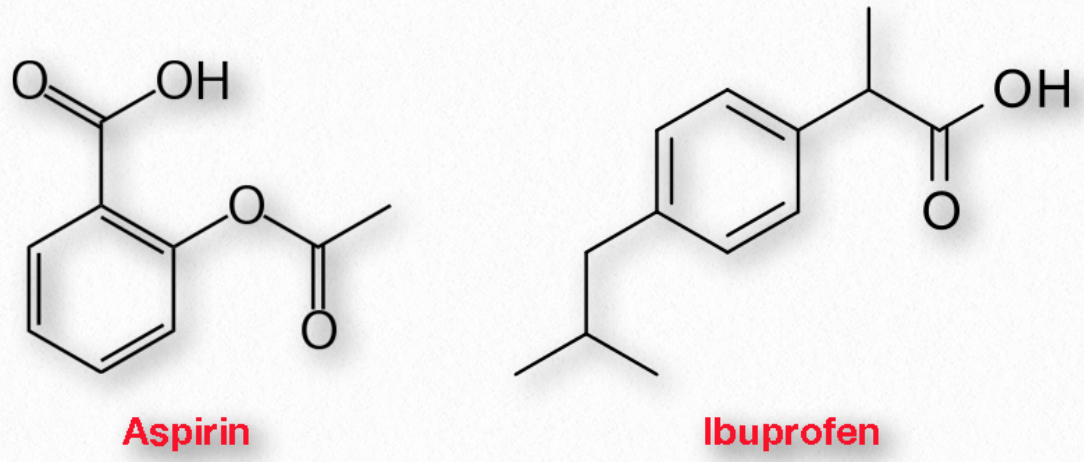


Figure 6.104 - Two NSAIDs

prostaglandin H_2 by prostacyclin synthase) is a special prostaglandin that inhibits activation of blood platelets in the blood clotting process and acts as a vasodilator. Thromboxanes counter prostacyclin, causing vasoconstriction and activating blood platelets for clotting. Due to imbalances in these opposite acting molecules resulting from COX-2-specific inhibition, Vioxx, was withdrawn from the market in September, 2004, due to health concerns.

Other compounds known to inhibit COX enzymes include some flavonoids, some components of fish oil, hyperforin, and vitamin D.

Connections to other pathways

There are several connections between fats and fatty acid metabolism and other metabolic pathways. Diacylglycerol (DAG - Figure 6.105), which is produced by removal of a phosphate from phosphatidic acid, is an intermediate in fat synthesis and also a messenger in some signaling systems. Phosphatidic acid, of course, is a branch intermediate in the synthesis of triacylglycerols and other lipids, including phosphoglycerides.

Fatty acids twenty carbons long based on arachidonic acid (also called eicosanoids) are precursors of the leukotrienes, prostaglandins, thromboxanes, and endocannabinoids.

Acetyl-CoA from β-oxidation can be assembled by the enzyme thiolase to make acetoacetyl-CoA, which is a precursor of both ketone bodies and the isoprenoids, a broad category of compounds that include steroid hormones, cholesterol, bile acids, and the fat soluble vitamins. In plants, acetyl-CoA can be made into carbohy-

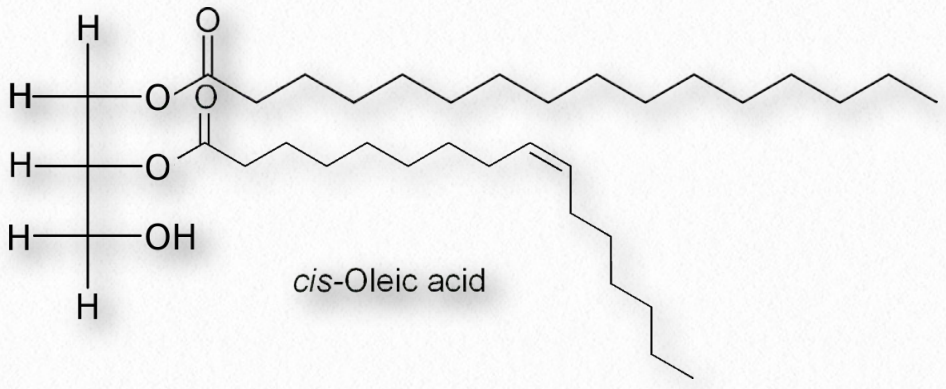


Figure 6.105 - Diacylglycerol

drates in net amounts via the glyoxylate cycle.

Fat, obesity, and hunger

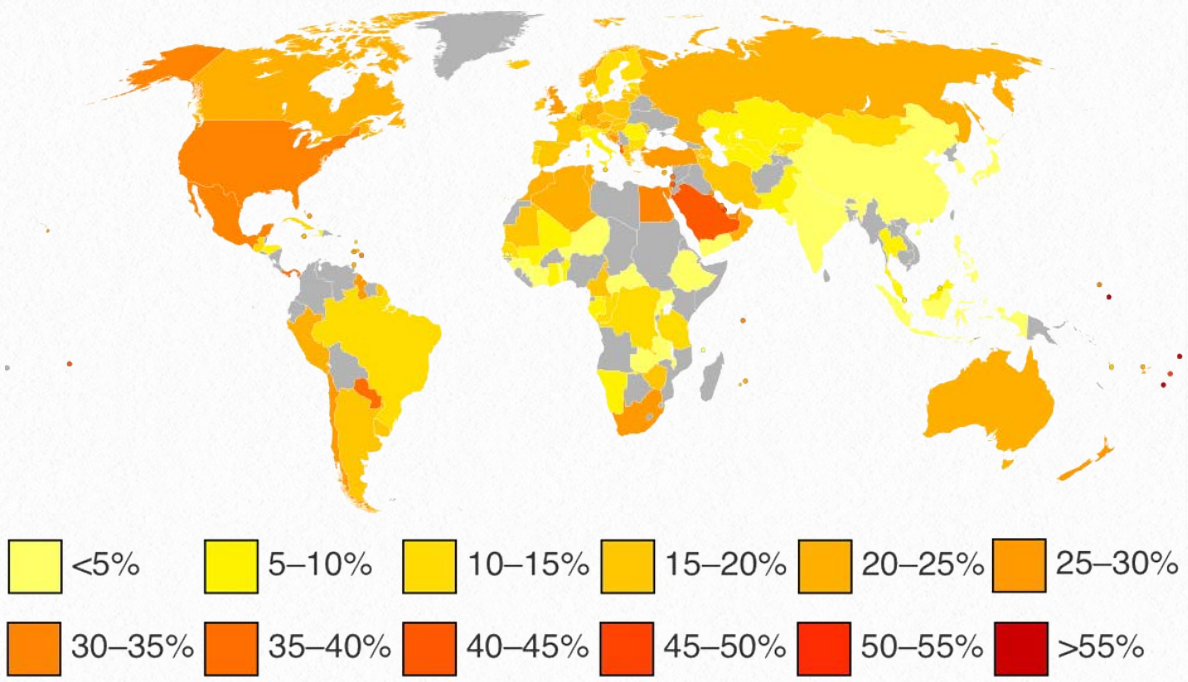
Obesity is an increasing problem in the western world. It is, in fact, the leading preventable cause of death worldwide. In 2014, over 600 million adults and 42 million children in the world were classified as obese, a condition when their body mass index is over 30 kg/m^2 (Figure 6.106). The body mass index of a person is obtained by dividing a person's weight by the square of their height. At a simple level, obesity arises from consumption of calories in excess of metabolic need, but there are many molecular factors to consider.

Adipokines

Adipokines are adipose tissue-synthesized cytokines. The class of molecules includes leptin (first discovered adipokine) and hundreds of other such compounds. These include adiponectin (regulates glucose levels and fatty acid oxidation), apelin (control of blood pressure, angiogenesis promotion, vasodilator release, increased water intake), chemerin (stimulation of lipolysis, adipocyte differentiation, link to insulin resistance), and resistin (links to obesity, type II diabetes, LDL production in liver), among others.

Resistin

Resistin is an adipokine peptide hormone with numerous associated negative health effects. Injection of the hormone into



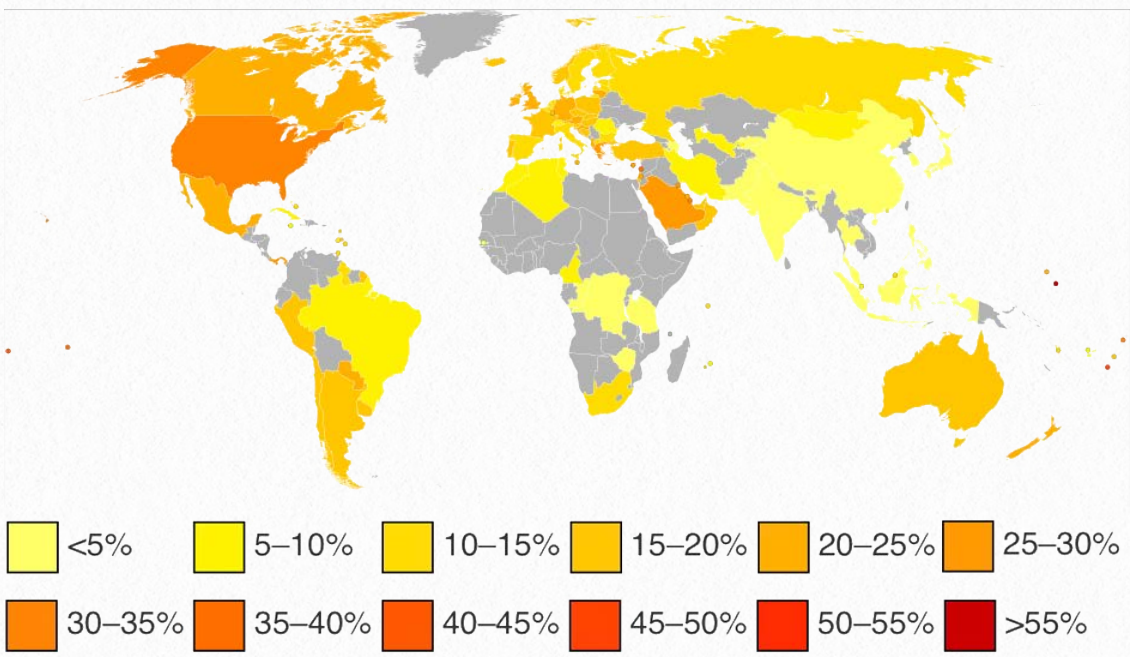


Figure 6.106 - Obesity worldwide - females (top) and males (bottom)

Wikipedia

mice results in increased resistance to insulin, a phenomenon of type 2 diabetes.

Resistin is linked to increased inflammation and serum levels of it correlate with increased obesity, though direct linkage of it to obesity is controversial. Resistin stimulates production of LDLs in the liver, supporting increased levels in the arteries. Resistin also adversely impacts the effects of statin drugs used to control levels of cholesterol in the body.

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Leptin

Leptin is a peptide hormone (adipokine) made in adipose cells that negatively impacts hunger and regulates energy balance. It is countered by ghrelin, also known as the hunger hormone. Both hormones act in the hypothalamus where hunger is controlled. When leptin levels are higher due to higher levels of body fat, hunger is suppressed, but when levels of leptin are lower (less body fat), then appetite increases.

Notably, leptin is also made in places besides adipose tissue and leptin receptors are found in places besides the hypothalamus, so the hormone has other effects in the body. When sensitivity to leptin changes, increased obesity can result. In mice, deletion of leptin function by mutation results in mice with voracious appetites and extreme obesity. Deletion of the leptin receptor gene in mice results in the same phenotype. Eight humans with leptin mutations all suffer from extreme obesity in infancy.

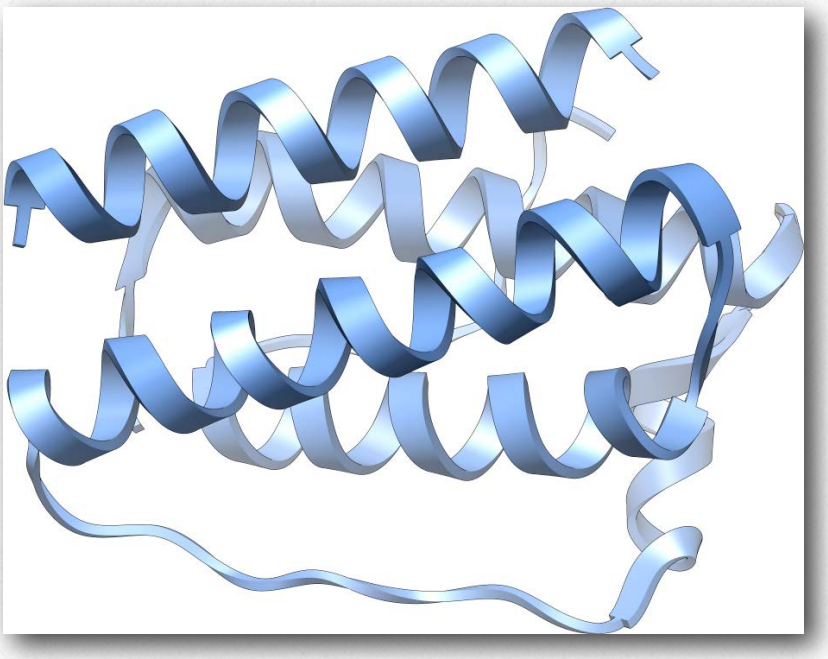

Figure 6.107 Leptin

Wikipedia

Physiology

Leptin is produced primarily by cells in white adipose tissue, but is also made in brown adipose tissue, ovaries, skeletal muscle, stomach, mammary epithelial cells and bone marrow.

Leptin levels

Leptin levels in the body are highest between midnight and early morning, presumably to suppress appetite. Though it is produced by fat cells, levels of leptin in humans do not strictly reflect levels of fat. For example, early in fasting, leptin levels fall before fat levels fall. Sleep deprivation can reduce leptin levels, as can increasing levels of testosterone and physical exercise.

Increasing estrogen, however, increases leptin levels. Emotional stress and insulin can increase leptin levels. Obesity increases leptin levels, but doesn't fully suppress appetite. Leptin resistance in these individuals is an important consideration, lessening the effects of the hormone on appetite.

Blocking leptin action

In the medial hypothalamus, leptin stimulates satiety and in the lateral hypothalamus, leptin inhibits hunger. Lesions in the lateral hypothalamus that block the ability to sense hunger result in anorexia (there are other causes of anorexia, though) and lesions in the medial hypothalamus cause excess hunger (no satiety). Neuropeptide Y is a potent hunger promoter whose receptors in the arcuate nucleus can be bound and blocked by leptin. Leptin levels are more sensitive to decreasing food intake than increasing food intake meaning that in humans the hormone plays a bigger role with respect to appetite than to levels of fat in the body.

At the molecular level, binding of leptin to the Ob-Rb receptor causes down-regulation of synthesis of endocannabinoids, whose normal function is to increase hunger. High fructose diets have been associated with reduced levels of leptin and of leptin receptor.

Ghrelin

Ghrelin is a peptide hormone made by cells in the gastrointestinal tract when the stomach is empty. Stretching of the stomach reduces the expression of the hormone. Ghrelin exerts its effects on the central nervous system to increase appetite and it is an unusual peptide in being able to cross the blood-brain barrier. The ghrelin receptor in the brain is found on the same cells as the leptin receptor (arcuate nucleus). Leptin can counter the ghrelin effect by decreasing hunger.

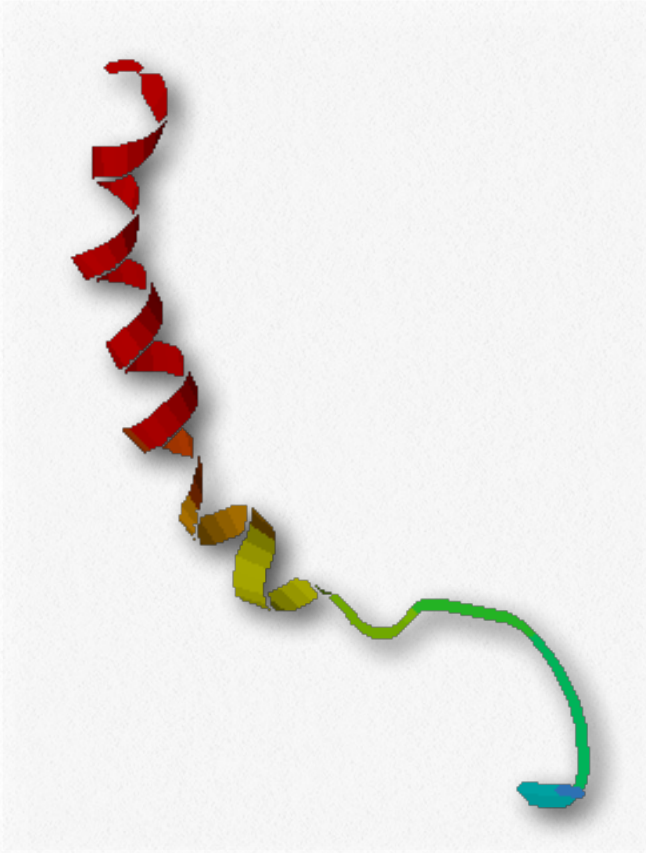

Figure 6.108 **Neuropeptide Y**

Behavioral effects

Activation of ghrelin occurs after processing the zymogen form of the hormone (preproghrelin) followed by linkage of an octanoic acid to a serine at position 3. Circulating levels of ghrelin increase before eating and decrease afterwards. There appears to be a

dose dependence for ghrelin on the amount of food consumed. Ghrelin increases food seeking behavior and there is a negative correlation between levels of ghrelin and weight.

Neuropeptide Y

Neuropeptide Y is a neuropeptide neurotransmitter produced by neurons of the sympathetic nervous system. It acts as a vasoconstrictor and favors growth of fat tissue. It appears to stimulate food intake, fat storage, relieve anxiety/stress, reduce pain perception, and lower blood pressure. Blockage of neuropeptide Y receptors in the brain of rats decreases food intake.

Stress effects

In mice and monkeys, repeated stress and high fat, high sugar diets stimulate neuropeptide Y levels and cause abdominal fat to increase.

High levels of neuropeptide Y may also help individuals to recover from post-traumatic stress disorder and to reduce the fear response. It may also protect against alcoholism. Mice lacking the ability to make neuropeptide Y have a higher voluntary consumption of alcohol and are less sensitive to its effects. The neuropeptide Y receptor is a G-protein-coupled receptor in the 7-transmembrane domain family.

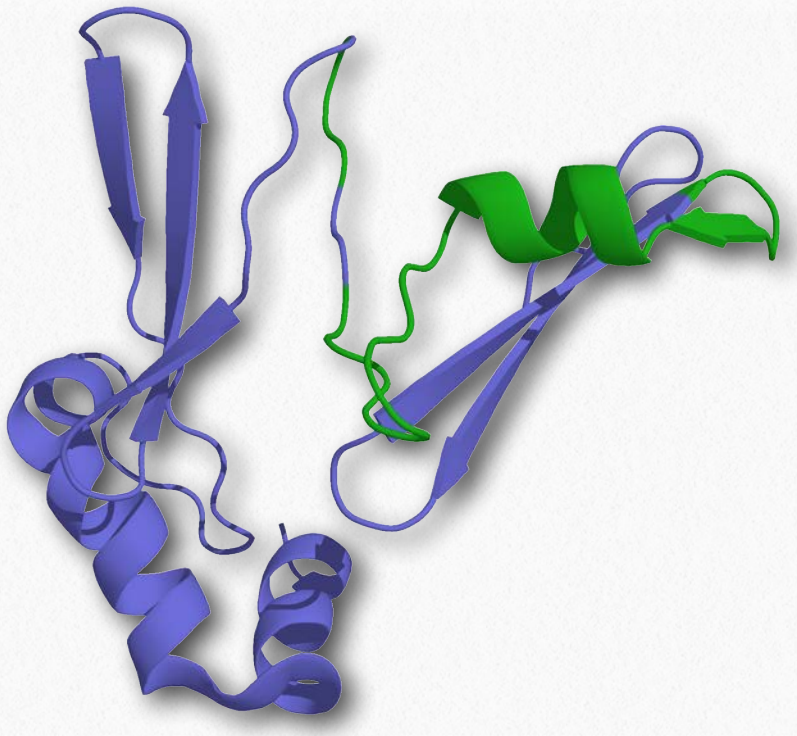

Figure 6.109 - Pre-proghrelin

Graphic images in this book were products of the work of several talented students. Links to their Web pages are below

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When Acids Get Oxidized

To the tune of "*When Johnny Comes Marching Home*"

Metabolic Melodies Website HERE

The fatty acids carried by
CoA, CoA
Are oxidized inside the
mi-to-chon-dri-ay

They get to there as you have seen
By hitching rides on carnitine
Then it goes away
When acids get oxidized

Electrons move through membranes, yes
It's true, it's true
They jump from complex I onto
Co-Q, Co-Q

The action can be quite intense
When building proton gradients
And its good for you
When acids get oxidized

The protons pass through complex V
You see, you see
They do this to make lots of
A-TP, TP

The mechanism you should know
Goes through the stages L-T-O
So there's energy
When acids get oxidized

Recording by Tim Karplus
Lyrics by Kevin Ahern

When Acids Are Synthesized

To the tune of "*When Johnny Comes Marching Home*"

Metabolic Melodies Website HERE

The 16 carbon fatty acid, palmitate
Gets all the carbons that it needs from acetate
Which citric acid helps release
From mitochondri - matrices
Oh a shuttle's great
When acids are synthesized

Carboxylase takes substrate and it puts within
Dioxy carbon carried on a biotin
CoA's all gain a quick release
Replaced by larger ACPs
And it all begins
When acids are synthesized

A malonate contributes to the growing chain
Two carbons seven times around again, again
For saturated acyl-ates
There's lots of N-A-DPH
That you must obtain
When acids are synthesized

Palmitic acid made this way all gets released
Desaturases act to make omega-threes
The finished products big and small
Form esters with a glycerol
So you get obese
When acids are synthesized

Recording by Tim Karplus
Lyrics by Kevin Ahern