CHAPTER 18 - FATTY ACID METABOLISM

It's time to begin talking about a subject which we would all like to avoid, that is, fat. Yes, it is important to understand how our bodies utilize fat, because in our diets somewhere between 20 - 50 % of our calories (depending upon the nature of our diets) come to us as fat. We have already discussed ad nausium how our bodies make energy from sugar; let us now begin a discussion of how our bodies make energy from fat.

Let's first talk about how our bodies deal with standard fat, that is, triacylglycerol. We're talking real fat here. On our grocery store shelves, we're talking olive oil, margerine, butter, LARD.

As an aside, there's nothing wrong with fat as a food source for our bodies. I remember when I was a wee little lad studying about the French trappers and explorers who traveled through North America. The food they brought with them was lard. This food sounds disgusting, but fat is the way that our bodies and animal bodies store the maximum amount of energy in the least amount of weight. This is why lard makes sense as a food source for hungry explorers, and this is why we make fat. I also recently learned that my grandfather, a product of the depression, would often want as a treat a lard sandwich. Seems disgusting to you or I, but not for some. And one last story – when my wife and I were rafting for our 20th anniversary, we visited a cabin lined with old magazines. I found an early advertisement for margarine, and the big ad push was that you should not just give your children plain bread, you should add calories to it in the form of margarine to help them to grow big and strong.

There are cosmetic reasons for storing food energy as fat. If we stored energy in the form of glycogen, for example, we would have to create much more glycogen by weight to store the same amount of energy than we would need in fat. So fat, when looked at in this light, is a method for us to lose weight.

Anyway, let's talk about how our bodies use fat by reviewing the structure and nomenclature of fats. A triacylglycerol consists of a glycerol "backbone" to which are attached three fatty acid chains. A fatty acid is a long chain alkane, typically between 14-24 carbon atoms long, with between 0-6 double bonds in normal systems, with a carboxylic acid at one end. The fatty acid is attached to the glycerol via an ester linkage between the carboxylic acid group of the fatty acid and the alcohol group on the ethanol.

When we eat our nice juicy steak, the "fat" on the steak is primarily triacylglycerol. The
fatty acids attached consist to a great extent of stearic acid (18:0), with smaller amounts of oleic (18:1) and other fatty acids. When triacylglycerols contain large amounts of fatty acids which do not have any double bonds, they are called saturated fats. We won't discuss the evils of saturated fat with you; I just wanted to point out this little bit of trivia. Another bit of trivia is ivory soap. Ivory soap is 99 and 44/100 ths pure, but pure what? That's right, the sodium salt of stearic acid. And I bet you can guess what the raw material is to make ivory soap, can't you, boys and girls?

Anyway, back to our story. We eat a nice steak, and overload our bodies with triacylglycerol. What does our body do with this fine stuff? During digestion, the triacylglycerols are digested in the small intestine into free fatty acids and 2- monoacylglycerols (primarily). The triacylglycerols are reassembled after absorption, and delivered in large lipoprotein particles called chylomicrons to the cells which line the walls of our capillaries; these are called endothelial cells.

The endothelial cells carry out the first step in triacylglyceride metabolism. The first step in the metabolism of triacylglycerols is the hydrolysis of the ester bonds to yield free fatty acids and glycerol. Chylomicrons bind to a membrane protein found on the outside of endothelial cells called lipoprotein lipase, the enzyme which catalyzes the release of fatty acids and glycerol from triacylglycerol. The reaction carried out is shown on the whiteboard. This enzyme exists in highest concentration in the endothelial cells near other types of cells which like to use fatty acids. There is a lot near adipose tissue. This is intelligent; the adipose tissue will reabsorb the fatty acids, make new triacylglycerol, and add it to its fat collection, increasing your dress size in the process. Curiously enough, there is also high activity of this enzyme around the heart. For all those people who say that fatty foods are bad for the heart... our heart actually prefers to eat fatty acids over sugar for energy.

If fatty acids are not needed near their site of liberation from triacylglycerol, they can circulate through the blood until they find a cell that wants them. Free fatty acids are not very water soluble (that's why our bar of soap dissolves very slowly), so they need to be bound to a specific carrier molecule to be transferred. The carrier molecule in our blood is albumin, the most common circulatory protein next to hemoglobin. Albumin is capable of binding many molecules of fatty acid to transfer them around the body.

**Lipoproteins**

The absorbed fatty acids, glycerol, and chylomicrons generated by the intestinal lining
cells are taken up in large part by the liver. The liver will then repackage the lipids as **Lipoproteins.** Lipoproteins are exactly as they sound - multimolecular complexes containing both protein and lipid. There are five different classes of lipoproteins, separated by their density. Every biochemistry book will include a table like the one found in Mathews on p. 631. Although the types of lipoproteins are separable by density, their composition and function vary greatly; the relationship between density and function is somewhat arbitrary. Here are the different classes of serum lipoproteins and their function.

1. **Chylomicrons** - Density is between 0.92 - 0.96 g/ml, or in other words, is less dense than water. Chylomicrons are made up of about 90% triacylglycerol. Chylomicrons are the way that our liver packages dietary fat to send around the body. These little globules of fat are held together by a little protein, and contain a minimum amount of other lipids.

2. **Very low density lipoprotein (VLDL)** Density is between 0.95 - 1.006 g/ml. VLDL is also synthesized in the liver as a mechanism to transport lipids around the body. VLDL contains about 50% triacylglycerol. As the VLDL moves about the body, triacylglycerol is stripped from the VLDL molecule, converting it first to **intermediate density lipoprotein (IDL)**, containing about 30% fat, and then to **low density lipoprotein (LDL)**, which is down to 4% triacylglycerol.

   As the VLDL loses triacylglycerol, other changes occur. The particle size becomes smaller, for one. In addition, the weight percent of other molecules in the VLDL increases as it loses triacylglycerol. Specifically, VLDL starts out with about 6% cholesterol and about 14% cholesterol esters, which are synthesized by enzymatic transfer of a fatty acid from phosphatidylcholine to cholesterol. As the particle loses triacylglycerol, not only does the relative percentage of cholesterol increase, but more cholesterol is converted to cholesterol ester, until the final LDL particle is about 8% cholesterol and over 40% cholesterol ester.

   LDL is the primary mechanism by which cholesterol is transported around the body. LDL is taken up by cells using a process called receptor-mediated **endocytosis.** The LDL interacts with a cell by way of an integral membrane protein at the cell surface called the **LDL receptor.** Upon binding, the LDL receptors migrate in the plasma membrane and aggregate. At the same time, a polymeric mesh of the protein **clathrin** forms underneath the aggregating receptors. Next, the plasma membrane invaginates, forming what is referred to as a **coated pit.** The coated pit pinches off and forms a spherical structure, now inside of the cell, called a **coated vesicle.** A lysozome fuses with the coated vesicle; the lysozome contains enzymes and acid that
break down the LDL, generating cholesterol and free fatty acids. After LDL breakdown, its freed cholesterol can be utilized.  

Tissues that have high amounts of the LDL receptor include the adrenal glands, the testes, and the ovaries. Why would these tissues have high receptor densities and the need to ingest lots of LDL? **Steroid hormone biosynthesis.**

4. **High density lipoprotein (HDL)** - Density of between 1.06 - 1.25 g ml. HDL particles contain high amounts of protein. The proposed function of HDL is to scavenge free cholesterol and cholesterol esters from places where they shouldn't be, such as on arterial walls.

In a simple minded fashion, the ratio of LDL to HDL in the blood can be used to predict the occurrence of coronary artery disease. If the ratio of LDL to HDL is high, there is the potential for lots of cholesterol and cholesterol ester transport to places where it doesn't belong. If the LDL to HDL ratio is low, there is enough HDL around to avoid cholesterol deposition.

For example, let’s take a look at my lipid profile. I was just at the doctor this morning to get my lipid profile retested. I have hypercholesterolemia and hyperlipidemia, which are… As a result, I am taking the anticholesterolemic drug Lipitor, which we will talk about when we get to steroid biosynthesis. I previously took the anticholesterol drug Zocor, but I experienced the muscle cramps and discomfort that are a common side effect of anticholesterolemic drugs. Lipitor is slightly more potent than Zocor, and so I take a smaller dose (20 mg per day rather than 40 mg per day) which I have tolerated fairly well.

I am also taking fish oil supplements to help to lower my triglycerides (show bottle). There have been extensive recent studies that have shown that taking fish oil supplements will lower serum cholesterol to a similar extent as anticholesterolemic drugs. Specifically, supplements containing omega-3 fatty acids EPA and DHA (show structures) are useful in lowering cholesterol (cite studies – perhaps distribute paper?). I was taking two of these per day before I had the pancreatitis attack, but since these are little fat pills, I thought it best on a low fat diet to discontinue these until my gall bladder was removed.

Returning to the lipoproteins, the structure of the lipoprotein particle is similar to a big micelle. The particles are essentially spherical. Polar constituent of the particle, specifically protein, phospholipid, and cholesterol, are found on the outside of the particle. If the molecule is amphipathic, then the hydrophilic part points out into the aqueous environment while the hydrophobic part points into the particle. The nonpolar constituents of the particle, specifically
triacylglycerol and cholesterol ester, are found in the center of the particle, hidden from solvent. This is why LDL increases its cholesterol content as cholesterol ester, because only a certain amount of cholesterol can fit around the circumference of the particle.

Let’s return to fat metabolism. When a chylomicron or VLDL particle passes a cell that needs energy, it will encounter lipoprotein lipase, which will hydrolyze the triacylglycerol in the lipoprotein into fatty acids and glycerol. Fatty acids and glycerol will get across the cell by passive diffusion. The molecule is primarily hydrophobic, and can get across a membrane bilayer at some reasonable rate, dependent upon the intra and extracellular fatty acid concentrations.

Fats are also generated at adipose tissue. If our bodies need food for some reason, either glucagon (in response to low blood glucose) or epinephrine (in response to stress) is released. These are sensed by adipose tissue receptors, which result in the synthesis of cyclic AMP. The cyclic AMP activates a protein kinase, which phosphorylates triacylglycerol lipase, activating it. This enzyme is also called hormone-sensitive lipase, because its activity is indirectly controlled by hormones. Hormone sensitive lipase will hydrolyze triacylglycerol to diacylglycerol, which in turn will be hydrolyzed to monoacylglycerol and glycerol. Only the triacylglycerol lipase is hormonally controlled; why is that? *Diacylglycerol is only generated when triacylglycerol is hydrolyzed.* The fatty acids that are generated diffuse into the bloodstream and are transported around the body bound to a protein called albumin, which is prolific in our blood. These are released at cells where fatty acid content is low (simple binding equilibria) and then enter the cells via passive diffusion.

Once in the cell, the fatty acids need to be activated. The activation occurs by attaching the fatty acid to coenzyme A by the action of the enzyme acyl-CoA ligase (also called acyl-CoA synthase or fatty acid thiokinase) (see powerpoint):

As with the reaction we saw in glycogen synthesis, the one catalyzed by glycogen synthase, this reaction is made essentially irreversible due to energetic coupling of the attachment of the fatty acid to CoA with the hydrolysis of pyrophosphate.

There are several different types of acyl-CoA ligases in our cells. The one which attacks
newly entering fatty acids is bound to the outside of the endoplasmic reticulum and the outer mitochondrial membrane. This is specific for long chain fatty acids, and uses both saturated and unsaturated fatty acids. There are two other acyl-CoA ligases which are specific for short and medium chain fatty acids. These are found in the mitochondrial matrix. Why are these in the matrix, you might ask? Because that's where fatty acid catabolism is carried out. At what point do the fatty acids get into the mitochondrial matrix, you might ask? In fact, before any additional metabolism can take place, the fatty acids have to get into the mitochondrial matrix. The fatty acyl-CoA cannot cross the inner mitochondrial matrix, so the fatty acid has to be converted to something else to get across the membrane into the mitochondria. The fatty acyl group is transferred from CoA to carnitine to form an acyl carnitine; the reaction is catalyzed by the enzyme carnitine acyltransferase (see powerpoint):

The enzyme carnitine acyltransferase I is found on the cytosolic side of the inner mitochondrial membrane, and catalyzes the transfer of the fatty acyl group from CoA to carnitine. The enzyme carnitine acyltransferase II is found in the mitochondrial matrix, and catalyzes the transfer of the fatty acyl group from carnitine back to CoA. The free carnitine can travel back and forth across the inner mitochondrial membrane. There is a specific carrier protein in the inner mitochondrial membrane which can transport carnitine, acetylcarnitine, and acyl carnitine. The direction of the reaction is entirely governed by the differences in fatty acyl CoA concentrations inside and outside of the mitochondria. Fatty acyl CoA is immediately degraded after being formed within the mitochondria, decreasing the fatty acyl CoA concentration and freeing up more CoA for combination with new fatty acids.

**Beta oxidation**

So after all this work, the fatty acid has finally reached its destiny. The fatty acid is now turned into energy by the process called **fatty acid oxidation**. The process of fatty acid oxidation was not understood until the 1950's but early experiments provided two important pieces of groundwork upon which later experiments were based. In the early 1900's, the German Biochemist Fritz Knoop fed some animal fatty acids which were attached at the methyl end to benzene. He then collected the urine to see what the final metabolic products were. When even chain fatty acids were fed to the animals, the result in the urine was phenylacetic acid. When odd chain fatty acids were fed to the animals, the resulting metabolite was benzoic acid. From
this result, Knoop concluded that fatty acid catabolism occurred in two carbon units. A second important discovery was made by the great enzymologist Fritz Lipmann. Al Lehninger had previously discovered that fatty acid oxidation required activation of the carboxylic acid group. Lipmann was the first to show that this activation occurred by attaching the fatty acid carboxyl group to coenzyme A.

Let's now discuss the presently accepted mechanism for fatty acid oxidation. At this point, we have a fatty acid attached to a CoA group, located in the mitochondrial matrix. Let us make the fatty acid linoleic acid (18:2) for demonstration purposes. The first reaction is the oxidation of the carbon-carbon bond between carbons 2 and 3 of the fatty acid to form a trans double bond. The enzyme which catalyzes the oxidation is *acyl-CoA dehydrogenase*:

This enzyme has a tightly bound FAD cofactor which is reduced to FADH$_2$ as the result of fatty acid oxidation. The reduced cofactor dissociates from the enzyme and donates its electrons to the electron transport chain at ubiquinone (resulting in the formation of 2 ATP).

The second reaction is catalyzed by the enzyme *enoyl-CoA hydratase* (or hydrase). This enzyme stereospecifically adds water across the double bond to form L-3-hydroxyacyl CoA.
The third step is the oxidation of the alcohol to a ketone to form a ketoacyl CoA. The oxidation is carried out by the enzyme 3-L hydroxyacyl-CoA dehydrogenase, and is specific for L-alcohols:

The oxidation of the alcohol to the ketone is coupled to the reduction of NAD+ to NADH. The reduced nicotinamide cofactor can enter electron transport to yield 3 ATP via oxidative phosphorylation.

The final step in this cycle is the cleavage and removal of an acetyl-CoA from the ketoacyl fatty acid. This reaction involves the attack of another molecule of coenzyme A at the newly formed keto group, forming a fatty acyl-CoA which is two carbon atoms shorter than the original fatty acids and liberating one molecule of acetyl-CoA. The enzyme which catalyzes this reaction is β-ketothiolase (or thiolase for short; mechanism on powerpoint):

Mechanism of β-ketoacyl-CoA thiolase

Let's review the energetics so far. We've removed two carbons from an 18-carbon fatty acid, and we've gained:

\[
\begin{align*}
1 \text{ FADH}_2 &= 2 \text{ ATP} \\
1 \text{ NADH} &= 3 \text{ ATP} \\
1 \text{ Acetyl-CoA} &= 3 \text{ NADH} = 9 \text{ ATP} \\
1 \text{ GTP} &= 1 \text{ ATP} \\
\text{Total} &= 17 \text{ ATP} (!)
\end{align*}
\]

Therefore, if you had a nice simple fatty acid like palmitic acid (16:0), it would undergo 7 cycles of oxidation to form 7 NADH, 7 FADH\(_2\), and 8 acetyl-CoA, which would yield 14, 21, and 96 ATP, respectively, equalling a total of 131 ATP. If you subtract the energy to form acyl-CoA from palmitic acid (which is equivalent to two equivalents of ATP), then the net yield of ATP from 1 palmitic acid (MW = 255) is 129. Since the yield from 1 glucose (MW = 180) is 36-38 ATP, you can see how the fatty acid is a better energy source by weight.

Let's return to our example on the board and fully oxidize this fatty acid. To remove 2 more carbons, just repeat the cycle and remove an acetyl CoA. Do the cycle 1 more time, remove 1 more acetyl CoA. Now, we have a problem. We have a cis double bond next to the site where under saturated conditions we would create a trans double bond, and common sense
would lead us to believe that the oxidation will not take place under these circumstances. Well, it doesn't. When a double bond is encountered, the acyl-CoA dehydrogenase won't work. At this point a different enzyme comes in to make the unsaturated fatty acid conform to the β-oxidation process. The enzyme is enoyl-CoA isomerase, and the reaction is the isomerization of the double bond from a cis double bond at the 3-position from the carboxyl end to a trans double bond at the 2 position from the carboxyl end (powerpoint).

This is the same product which would result from the β-oxidation by acyl-CoA dehydrogenase, so this compound will continue through fatty acid oxidation. The reduction of FADH$_2$, and therefore 2 ATP, are lost in the process.

This is the process for monounsaturated fatty acids, whose oxidation usually results in a delta-3 cis double bond. Polyunsaturated fatty acids provide a different type of problem which needs to be overcome. Let's for example, look at the oxidation of 18:2$^{\Delta 9,12}$ (linoleic acid). After three cycles of β-oxidation, the remaining fatty acyl-CoA has double bonds in the 3 and 6 positions. The enzyme enoyl-CoA isomerase changes the position of the double bond from Δ3 cis to Δ2 trans. One cycle of β-oxidation results in a fatty acid with a Δ4 cis double bond. This molecule is acted upon by acyl-CoA dehydrogenase to form a Δ2 trans, Δ4 cis diene. A new enzyme (new meaning not yet discussed), 2,4-dienoyl-CoA reductase, reduces one double bond and moves another - the final product has a Δ3 trans double bond. This is moves to a Δ2 trans double bond by enoyl-CoA isomerase, then enoyl-CoA hydratase, then 3-L-hydroxyacyl-CoA dehydrogenase, and finally thiolase.

**ODD-CHAIN FATTY ACIDS**

Most of the fatty acids we consume contain even numbers of carbon atoms. Occasionally our cells are required to metabolize an "odd-chain fatty acid" - one with an odd number of carbon atoms. Metabolism of an odd chain fatty acid proceeds exactly like the metabolism of other fatty acids until the product propionyl-CoA is reached. This three-carbon unit is not capable of undergoing another cycle of β-oxidation. The propionyl-CoA formed is metabolized by several different enzymes. The first step in metabolism of propionyl-CoA is carboxylation at C-2 of the propionyl group by the enzyme propionyl-CoA carboxylase. The product of the
reaction is D-methylmalonyl-CoA. The optical configuration at C-2 is then converted from D to L by the enzyme methylmalonyl-CoA epimerase. The carboxyl group is moved on the compound by the enzyme methylmalonyl-CoA mutase to form succinyl-CoA (powerpoint).

KETONE BODIES
When we've discussed fatty acid oxidation, we have assumed that the acetyl-CoA formed during β-oxidation is converted to energy in the citric acid cycle. Acetyl-CoA can have other fates in our cells, especially in liver cells. Acetyl-CoA can be metabolized to ketone bodies, which are nothing more than simple ketones (specifically acetoacetate, β-hydroxybutyrate, and acetone).

The reactions which form ketone bodies from acetyl-CoA occur only in the mitochondrial matrix. The first reaction is the condensation of two acetyl-CoA groups by the enzyme acetoacetyl-CoA thiolase to form acetoacetyl-CoA. Another acetate group is added from a different acetyl-CoA by the enzyme HMG-CoA synthase to form β-hydroxy-β-methylglutaryl-CoA (or HMG-CoA for short).

We will be discussing these two reactions later in the course, when we discuss the biosynthesis of steroids. These two reactions are the first steps in the synthesis of cholesterol from acetyl-CoA. Anyway, to return to ketone bodies, the enzyme HMG-CoA lyase removes a molecule of acetyl-CoA from HMG-CoA to leave acetoacetate, one of the ketone bodies. Acetoacetate can either be removed from the mitochondrial matrix as is, or two other things might happen. In the mitochondrial matrix, it may interact with the enzyme β-hydroxybutyrate dehydrogenase to result in β-hydroxybutyrate. Alternatively, there may be spontaneous decarboxylation of acetoacetate to yield acetone.

These processes occur primarily in the liver. This is because the enzyme HMG-CoA synthase exists in significant concentrations only in the liver. Ketone body production is functionally similar to gluconeogenesis. By making ketone bodies, the liver has made energy into a form which is easily transported around our bodies and in and out of cells (the ketone bodies are freely diffusible through cell membranes in their noncharged forms. The liver is incapable of using ketone bodies for energy, but the rest of our tissues, including the brain in a pinch, can use ketone bodies to generate energy.

How do our non-liver tissues use ketone bodies for energy, you might ask? The hardest one for our cells to use is β-hydroxybutyrate, so we'll discuss the process beginning with this ketone body. β-hydroxybutyrate is converted to acetoacetate by the enzyme β-hydroxybutyrate dehydrogenase (since acetoacetate is already acetoacetate, it doesn't need to see this first process). Acetoacetate is then added to Coenzyme A by the action of the enzyme β-oxoacid-CoA.
transferase. This is a CoA transferase; the CoA is transferred from a molecule of succinyl-CoA. The acetoacetyl-CoA formed by this reaction is converted into two molecules of acetyl-CoA by the enzyme acetoacetyl-CoA thiolase. The acetyl-CoA can then be used by the citric acid cycle of the host cell for energy.

Needless to say, all of these enzymes are found in the mitochondrial matrix. What regulates the forward and reverse reactions here? In this case, the reactions are entirely regulated by enzyme expression from the genes. In the liver, the enzyme HMG-CoA lyase is expressed, and therefore HMG-CoA can be converted to acetoacetate. Other cells do not have this enzyme. However, extrahepatic cells express the utilization enzyme β-oxoacid-CoA transferase, which is not expressed in the liver. Therefore, the liver makes the stuff, and other cells eat it.

REGULATION OF β-OXIDATION

We'll discuss regulation of liver processes later, after we discuss fatty acid synthesis. The regulation of liver pathways is relatively complex, and we'll need a wider knowledge base before we tackle this problem. I will take a minute to discuss the regulation of β-oxidation in cells like the heart, which primarily eat fatty acids, but don't make any. In the heart, regulation of β-oxidation is relatively straightforward. Fatty acids are metabolized by the heart to form acetyl-CoA. If energy is required by the heart, then the acetyl-CoA is converted into ATP by the citric acid cycle and oxidative phosphorylation. If energy is not required, the acetyl-CoA concentration in the cell builds up, which inhibits the enzyme thiolase in β-oxidation. This slows down the breakdown of the fatty acids.

There are several different metabolic diseases which result from deficiencies in acyl-CoA dehydrogenase. The most interesting one was called Jamaican vomiting sickness, which results from the eating of unripe ackee fruit. This contains the unusual amino acid hypoglycin A, which is metabolized to methylenecyclopropylacetyl-CoA. This molecule is a substrate for acyl-CoA dehydrogenase; however, a reactive intermediate is formed which supposedly reacts with FAD, making the enzyme useless.

The β-oxidation of fatty acids can also occur in the peroxizomes of cells. In particular, very long chain fatty acids are initially degraded in the peroxizomes, because they are not suitable substrates for the acyl-CoA synthases within the cytoplasm, and hence are not transported into the mitochondria directly. The long chain fatty acids will diffuse into cellular
peroxizomes, and be added to coenzyme A within the peroxizome. In the peroxizome, the β-
oxidation occurs until a shorter fatty acid is developed. The shorter products are then transferred
to carnitine for mitochondrial metabolism.

In the movie “Lorenzo’s Oil”, which I did not see, the disease X-adrenoleukodystrophy is
described. The disease is the destruction of the myelin sheath surrounding the central nervous
tissues by long chain fatty acids, which are accumulated in the body due to the lack of
peroxizomal fatty acid metabolism. The lack of metabolism may well be due to the lack of a
very long chain acyl-CoA synthase in the peroxizomes. The treatment of the disease is similar to
the treatment of phenylketonurics - avoid foods containing long chain fatty acids, especially
during development.

FATTY ACID BIOSYNTHESIS

As glucose can be both catabolized and anabolized in our liver, so can fatty acids both be
utilized for energy and synthesized in our liver cells. In the same way that different pathways
exist for glucose breakdown and synthesis, different pathways are in place for fatty acid
oxidation and synthesis. Some of the general differences between the two pathways are:

1. Fatty acid oxidation occurs in the mitochondrial matrix; fatty acid synthesis is a
cytoplasmic process. Therefore, each pathway uses entirely separate enzymes.

2. Each pathway requires an activated carboxylic acid group. However, in fatty acid
oxidation the activation occurs by the thioester linkage to coenzyme A, while in fatty acid
synthesis the activation is carried out by covalent attachment to sulfhydryl groups of an acyl
carrier protein (ACP for short).

3. Catabolic enzymes appear to work separately, while the enzymes involved in fatty acid
synthesis are organized in multienzyme complexes.

4. The acceptors for electrons in fatty acid oxidation are NAD and FAD; the donor in
fatty acid biosynthesis is NADPH.

We'll now discuss some of the specific aspects of fatty acid synthesis.

Sources of substrates

The main substrates for fatty acid synthesis are acetyl-CoA and NADPH. In what ways
are significant concentrations of these two substances generated in the cytoplasm of cells?

Acetyl-CoA could be formed by the action of pyruvate dehydrogenase complex;
however, this is a mitochondrial matrix enzyme. The acetyl-CoA formed by pyruvate
dehydrogenase cannot pass through the inner mitochondrial membrane. Acetyl-CoA in the cytoplasm is generated instead from citrate, which can be transported across the inner mitochondrial membrane. Citrate is converted into acetyl-CoA and oxaloacetate by the enzyme ATP-citrate lyase.

The cytoplasmic acetyl-CoA can be used for fatty acid synthesis. The oxaloacetate can be returned to the citric acid cycle in two ways. It can be simply reduced to malate at the expense of NADH, using the enzyme malate dehydrogenase:

The malate can be transported back across the inner mitochondrial membrane and used as a citric acid cycle intermediate. A second choice is to oxidatively decarboxylate malate in the cytoplasm to remake pyruvate, catalyzed by the enzyme malic enzyme:

In addition to making pyruvate, which can cross the mitochondrial membrane and make acetyl-CoA which can enter the citric acid cycle, a molecule of NADPH is made which can serve as a source of reducing equivalents for fatty acid synthesis. A second source for NADPH, which we discussed before, is the pentose phosphate pathway.

The first step in fatty acid synthesis is the carboxylation of acetyl-CoA by the enzyme acetyl-CoA carboxylase. This enzyme is not part of a multienzyme complex, and this is the only reaction in animal fatty acid synthesis which is not associated with the complex. The net reaction carried out by this enzyme is:

Let me discuss the E. coli enzyme first, which is a simpler enzyme system, and then progress to the mammalian enzyme. E. coli acetyl-CoA carboxylase has three different polypeptide components: biotin carboxyl carrier protein (BCCP for short; MW = 22,500), biotin carboxylase (a dimeric protein with subunit MW = 49,000) and carboxyltransferase (MW = 130,000). Biotin carboxyl carrier protein contains an active site biotin group, covalently bound to a lysine side chain. A carboxyl group is bound to the biotin, in similar manner to that seen in the enzyme pyruvate carboxylase. The carboxyl group is added by the action of the biotin carboxylase enzyme, using bicarbonate as a carbon dioxide source and requiring ATP hydrolysis for energy. The carboxyltransferase enzyme then transfers the carbon dioxide from biotin to acetyl-CoA to form the final product, malonyl-CoA.

In mammalian tissues, an entirely different form of acetyl CoA carboxylase is found. The rat liver enzyme, for example, is a dimer with a subunit molecular weight of 260,000. Each
subunit has one molecule of covalently bound biotin. In contrast to the procaryotic enzyme, all of the functions of acetyl CoA carboxylase can be carried out by a single polypeptide chain. However, the dimer has very low activity. The dimer, when exposed to citrate, polymerizes into a high molecular weight form consisting of 15 - 30 monomeric units. This active polymer is deactivated and depolymerized by exposure to either malonyl-CoA (the product of the reaction) or palmitoyl-CoA (the final product of fatty acid synthesis).

After formation of malonyl-CoA, the remainder of the reactions of fatty acid synthesis in mammalian cells are carried out by the fatty acid synthase complex. This complex contains seven different enzyme activities. The first reaction of fatty acid synthesis is the attachment of an acetyl group from acetyl-CoA to the acyl carrier protein. The enzyme which carries out this transfer is acetyl transacylase. The acetyl group is bound to ACP at a sulfhydryl group at the end of a covalently bound phosphopantetheine prosthetic group. The phosphopantetheine group is covalently bound to a ser hydroxyl on ACP. The structure of the prosthetic group looks like this:

The same group forms the binding site on coenzyme A; the only difference being an additional phosphate group linking the side chain to an adenosine nucleoside (powerpoint).

The enzyme acetyl transacylase transfers the acetyl group from a sulfhydryl group of ACP to a cys thiol group on the β-ketoacyl CoA synthase. After the acetyl group is properly placed on the enzyme which will add it to the growing fatty acid, the malonyl group from malonyl-CoA is moved onto the sulfhydryl binding site of ACP by the enzyme malonyl transacylase.

The next step in fatty acid synthesis is the condensation of the acetyl group onto the malonyl group. The reaction is catalyzed by the enzyme β-ketoacyl-ACP synthase, which is the same enzyme where the acetyl group is covalently bound. The end carboxyl group of malonyl-ACP is lost as carbon dioxide. Malonyl-CoA is used in this reaction instead of acetyl CoA because the energy generated by the loss of carbon dioxide helps to drive the condensation reaction. Also, the electronegative carboxyl group makes the central carbon of malonyl-ACP more nucleophilic and makes it into a better attacking group. The product of this reaction is acetoacetyl-ACP:

We've now discussed three reactions of fatty acid synthase. The next three reactions of fatty acid synthesis involves reduction of the ketone function to a methylene group. The enzyme β ketoacyl-ACP reductase reduces the ketone to an alcohol, β-3-hydroxybutyryl-ACP. The
enzyme β-3-hydroxyacyl-ACP dehydrase removes one molecule of water from the growing fatty acid chain, leaving crotonyl-ACP. Finally, crotonyl-ACP is reduced by the enzyme 2,3 transenoyl-ACP reductase to the product butyryl-ACP.

The final product at this point contains 4 carbon atoms. The fatty acid continues to grow at this point to a maximum length of 16 carbon atoms. The process is continued first by transfer of the butyryl group from ACP to the cys of β-ketoacyl-ACP synthase by the transacetylase. Another molecule of malonyl-CoA comes in, forming malonyl-ACP. The butyryl group condenses with the malonyl group, forming a 6-carbon unit which will be reduced at the β-keto group, etc.

The fatty acid will continue to enlarge until it is 16 carbon atoms long. At this point, no further additions can occur, and the palmitic acid is removed from the ACP by the action of a thioesterase.

Remember now that these enzymes are all just activity components of the fatty acid synthase complex. There are just two polypeptide chains in the complex, each of which has associated with it all of the enzymatic activities. I've given you a handout which describes what is believed to be the spatial orientation and sites of activity of the fatty acid synthase. The ACP site on one polypeptide chain works with the ketoacyl-ACP synthase activity of the second chain. The reason that fatty acids of different lengths can react with the enzymes is because there is some flexibility in the exact location of the condensing site, which depends upon the relative position of the two polypeptide chains and also the conformation of the phosphopantetheine group.

The synthesis of palmitate requires 8 molecules of acetyl-CoA, 14 molecules of NADPH, and 7 molecules of ATP. The ATP is used to form malonyl-CoA from acetyl-CoA, and the NADPH is used to reduce a ketone to an alcohol and finally a methylene group.

Our bodies have many more different types of fatty acids than just palmitate. To get longer or unsaturated fatty acids, different enzyme systems must be used. The elongation reaction is simply the addition of 2-carbon units to the carboxy terminus of the synthesized fatty acid. The 2-carbon units are provided by malonyl-CoA, and the reaction is driven by the release of carbon dioxide. The steps are the same, but the enzyme system is different than the fatty acid synthase. The only significant difference is that the fatty acid synthase is a cytoplasmic enzyme, while the elongation enzymes are all covalently bound to the endoplasmic reticulum, which is a
membrane system prevalent in the cytoplasm of most cells.

Mammalian cells have the capability of introducing one point of unsaturation into synthesized fatty acids. An enzyme complex associated with the endoplasmic reticulum will oxidize stearoyl-CoA to oleoyl-CoA. Desaturation requires the cooperative action of two enzymes, cytochrome b$_5$ reductase and stearoyl-CoA desaturase. Cytochrome b$_5$ reductase is a flavoprotein which transfers electrons from NADH by means of flavin to cytochrome b$_5$ heme group, reducing its iron from (III) to (II). Stearoyl-CoA desaturase uses the two electrons in 2 heme groups of two separate cytochrome b$_5$ molecules, along with 1/2 mole of oxygen as an electron acceptor, to reduce the Δ9 carbon-carbon single bond to a double bond with concurrent formation of water.

Our cells do not have the ability to synthesize polyunsaturated fatty acids from saturated fatty acids. The major polyunsaturated fatty acids in mammals are derived from exogenous linoleic (18:2) or linolenic (18:3) acid, which are termed the essential fatty acids. For example, arachidonic acid, which is 20:4$^{Δ5,8,11,14}$ is made starting from linoleic acid using this pathway:

Plants and bacteria have the ability to synthesize polyunsaturated fatty acids (corn oil is derived from polyunsaturated fatty acids, for example). These organisms use a different mechanism to produce points of unsaturation in fatty acids which we will not discuss in this course.

Regulation of fatty acid synthesis

We will only discuss regulation of fatty acid synthesis in mammalian liver, which much is known about, and also which is most relevant to our own experience.

Fatty acids are synthesized by our liver when the energy state of the liver is high. In this sense, fatty acid synthesis is similar to glycogen synthesis. However, our liver will only make as much glycogen as it can store. As we are all well aware, the liver is not the only site of fatty acid storage in our bodies. Fatty acids, after synthesis, can be released to the bloodstream and be deposited in adipose tissue.

In mammalian liver, there are several levels of regulation of fatty acid synthesis. One regulatory mechanism is controlling the concentration of crucial enzymes in the liver cell. In
fasted rats, the concentrations of fatty acid synthase and acetyl-CoA carboxylase are reduced 4-5 times the levels relative to fed rats. After allowing the rats to eat again, the concentrations of these enzymes increase. As another example, rats fed on fat-free diets have 14 times the amounts of fatty acid synthase than rats fed on normal diets.

These responses reflect the amount of protein in the cells, which reflects the amount of mRNA transcribed in the liver. This level of regulation requires hours or even days for a full response. Shorter term mechanisms of regulation exist as well. Palmitoyl-CoA is an inhibitor of mitochondrial citrate synthase, acetyl-CoA carboxylase, and glucose-6-phosphate dehydrogenase. High concentrations of malonyl-CoA inhibit cytoplasmic carnitine acyltransferase (why oxidize fatty acids if you are making them?). Acetyl-CoA carboxylase is sensitive to hormonal response; again we'll delay this discussion until the end of the semester. The energy charge also regulates; acetyl-CoA is an inhibitor of β-ketothiolase, and NADH inhibits 3-L-hydroxyacyl CoA dehydrogenase. And let us not forget the allosteric activation of acetyl-CoA carboxylase by citrate.

So, to summarize regulation:

1. Aggregation of acetyl-CoA carboxylase by citrate.
2. Palmitoyl-CoA inhibits citrate synthase, acetyl-CoA carboxylase, and glucose-6-phosphate dehydrogenase.
3. Malonyl-CoA inhibits palmitoyl acyltransferase I.
4. Acetyl-CoA carboxylase is subject to hormonal control.