Fatty Acid Catabolism

Objectives:

I. Describe the digestion and absorption of lipids.
   A. What enzymes are involved?
      1. Lingual Lipase
      2. Gastric Lipase
      3. Pancreatic Lipases
   B. What non enzyme components are necessary?

II. Understand the role of the lipoproteins in triglyceride, phosphoglyceride, and cholesterol transport in the body.
   A. Have an understanding of the structure and function of the lipoproteins.
      1. What are the functions of the apolipoproteins?
         a) apoC-II
         b) apoC-III
         c) apoB-100
         d) apoE
               (1) Alzheimer’s Disease
      2. Compare and contrast the structure and function of the Chylomicrons, VLDL, LDL, & HDL
   B. Appreciate the roles of LDL, HDL, and cholesterol in heart disease.

III. Describe how fatty acids are mobilized (released) from the adipose tissue.

IV. How is glycerol metabolized?
    A. Tissue differences

V. Discuss the activation and transport of fatty acids into the matrix of the mitochondrion.
   A. How many high energy phosphate bonds are required to activate a fatty acid?

VI. Describe the degradation of fatty acids by β-oxidation.
   A. What is(are) the end product(s) of fatty acid β-oxidation?
   B. Describe the four recurring reactions of “main stream” β-oxidation.
      1. What cosubstrate(s)/coenzyme(s) are required for β-oxidation?
   C. Given the name or formula of a saturated even chain length fatty acid be able to predict:
      1. the number of turns through β oxidation required for complete cleavage.
      2. the number of moles of NADH produced from 1 mole of the fatty acid.
      3. the number of moles of FADH₂ produced from 1 mole of the fatty acid.
      4. the number of moles of acetyl-CoA obtained from 1 mole of the fatty acid.
      5. the ATP yield per mole of acetyl-CoA.
      6. the total ATP yield from its complete catabolism to CO₂ and H₂O.

VII. Describe the final steps in the oxidation of odd chain length fatty acids.
   A. What additional Vitamin(s) / Coenzyme(s) are required for the complete catabolism of odd chain length fatty acids?

VIII. Discuss the additional enzymes required for the complete β-oxidation of unsaturated fatty acids.
   A. When are they employed?
      1. initial location of the cis-double bond for utilization of only the Isomerase
      2. initial location of cis double bond for utilization of both the Dehydrogenase & Isomerase
   B. Energy yield from unsaturated fatty acid versus saturated fatty acid.
IX. Discuss the control points of $\beta$-oxidation.
   A. Allosteric control
   B. Hormonal control
X. Integrate $\beta$-oxidation of fatty acids with the metabolism of carbohydrates.
   A. Allosteric control
   B. Hormonal control
XI. Describe the degradation of branched chain fatty acids by $\alpha$-oxidation.
   A. What is the source of the branched chain fatty acids?
   B. Why is $\alpha$-oxidation necessary?
XII. Understand the relationship of ketone body production to $\beta$-oxidation.
   A. What are the three ketone bodies?
   B. For what reason are ketone bodies synthesized?
   C. How are ketone bodies synthesized?
   D. Where are ketone bodies synthesized?
   E. What tissues utilize ketone bodies?
      1. How are ketone bodies utilized?
   F. What are the consequences of ketosis?
XIII. Describe the disease state that results from abnormal hormonal regulation of glycolysis and $\beta$-oxidation.
XIV. Ask yourself “What If Questions”

Digestion

The metabolism of lipids begins with the digestion of dietary lipids. Digestion of lipids begins in the mouth with the salivary glands of the tongue secreting Lingual Lipase. This enzyme hydrolyzes short chain triacylglycerols to diacylglycerols and fatty acids. Upon reaching the stomach the gastric mucosa secretes Gastric Lipase. Like Lingual Lipase, Gastric Lipase primarily hydrolyzes short chain triacylglycerols to diacylglycerols and fatty acids, but this enzyme can also hydrolyze diacylglycerols to monoacylglycerols and fatty acids. Lingual Lipase and Gastric Lipase are called the “acidic lipases” because their pH optima are less than pH 3.0 and therefore both are functional at the pH of the stomach. Neither of these lipases require bile nor a co-lipase to function which renders them less efficient than the pancreatic lipases, at most 30% of lipid digestion occurs by the action of these two enzymes in the adult.

Entry of lipid containing chyme (partially digested food stuffs) into the small intestine triggers the gall bladder to release bile and triggers the pancreas to release Lipases, Phospholipases, and Ceramidase. Bile is synthesized by the liver and is a mixture of bile acids, bile esters, bile salts, and the phosphoglyceride phosphatidylcholine. Bile acts as an emulsifying agent. It breaks up the large globules of lipids present in the chyme into many very tiny droplets. Emulsification of lipids aids in their digestion. Lipids are water insoluble, the enzymes that digest lipids are dissolved in the water present in the digestive tract. Digestion occurs only at the lipid/water interface. The digestive enzymes are able to attack lipid molecules only where they are in contact with water. By breaking the lipids up into a large number of very tiny droplets, the surface area of the dietary lipid “glob” is greatly increased. The increased surface area dramatically multiplies the interface over which the digestive enzymes can work increasing the rate of the process.

The Lipases and Phospholipases released by the pancreas hydrolyze the ester bonds present in the dietary
triacylglycerols and phosphoglycerides. Lipases are specific for the triacylglycerols, whereas the phospholipases (A₁, A₂, B, C & D) digest the phosphoglycerides. Cerebrosidases and Gangliosidases hydrolyze the glycosidic bonds of the glycosphingolipids and Ceramidase hydrolyzes the amide bond between the fatty acid and sphingosine in the dietary sphingolipids. Products of lipid digestion are monosaccharides, modified monosaccharides, fatty acids, glycerol, polar alcohols, phosphate, sphingosine, and monoacylglycerols (one fatty acid attached by an ester bond to C-2 of glycerol). These products are absorbed by the cells lining the small intestine. {Metabolism of the monosaccharides has already been discussed. The modified monosaccharides that are absorbed from the GI tract are transported to the liver, activated by coupling to UTP, and used in protein and/or sphingolipid biosynthesis.} The short and medium length fatty acids (≤12 C’s) diffuse across the cell membranes and pass immediately into the blood. They are carried to the adipose and skeletal muscle bound to serum albumin. The polar molecules and the long chain fatty acids (≥12 C’s) are transported into the cells lining the small intestine by a secondary active transport mechanism. Once inside the cells, these lipid precursors are reassembled into triacylglycerols, phosphoglycerides and sphingolipids. The newly (re)assembled lipids, along with the absorbed dietary cholesterol are bundled with proteins to form macromolecular complexes - The LIPOPROTEINS. These lipoprotein complexes are released from the epithelial cells lining the small intestine into the Lymphatic System. From the lymph system they travel to the blood stream and once in the blood they are distributed to the skeletal muscle, adipose, and other tissues. At the tissues, the triacylglycerols are hydrolyzed into glycerol, fatty acids, and monoacylglycerols by Lipoprotein Lipase expressed on the surface of the endothelial cells lining the capillaries. Insulin stimulates the expression of Lipoprotein Lipase on the surface of the endothelial cells. These compounds are absorbed by the tissue cells and (re)synthesized into triacylglycerols. The liver deals with the remnants of the dietary lipoproteins.

Lipoproteins

Triacylglycerols, Phospholipids, Cholesterol, and Cholesteryl esters are water insoluble compounds that must be moved by the water based blood stream from the tissue of origin (small intestine for dietary lipids, liver for endogenous lipids) to the other tissues of the body for storage or utilization. They are carried in the blood as LIPOPROTEINS, macromolecular complexes of specific “carrier” proteins, the APOLIPOPROTEINS, and various combinations of triacylglycerols, phospholipids, cholesterol, and cholesteryl esters. There are nine different apolipoproteins. Different combinations of apolipoproteins unite with different mixtures of lipids to form the four main classes of lipoproteins. Each class of lipoprotein has a specific function, determined by its point of synthesis, lipid composition, and apolipoprotein content. In the lipoproteins the hydrophobic lipids are in the core of the particle with hydrophilic amino acid side chains and phospholipids on the surface. Different combinations of lipids and proteins produce particles of different densities, ranging from Chylomicrons (the least dense) to High-Density Lipoproteins (HDL).
CHYLOMICRONS are the largest in volume and least dense, containing a high proportion of triacylglycerols. They are synthesized by the epithelial cells lining the small intestine, secreted into the lymphatic system, and ultimately reach the blood. The apolipoproteins of chylomicrons include apoA-IV, apoB-48, apoC-II, apoC-III, and apoE. ApoC-II activates Lipoprotein Lipase on the surface of the endothelial cells in the adipose, heart, skeletal muscle, and mammary glands, allowing the release of fatty acids and glycerol into these tissues. Chylomicrons transport dietary lipids to the tissues. ApoC-III inhibits Hepatic Lipase in the liver capillaries preventing this organ from absorbing dietary fats until after the other tissues have removed what they need. The remnants of chylomicrons are moved to and taken up by the liver in a receptor mediated process involving apoE.

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>Function</th>
<th>Apolipoprotein</th>
<th>Triacylglycerol</th>
<th>Phospholipid</th>
<th>Cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron</td>
<td>Transports Exogenous Lipids</td>
<td>2% by mass apoA-IV, apoB-48, apoC-II, apoC-III, apoE</td>
<td>85%</td>
<td>9%</td>
<td>4%</td>
</tr>
<tr>
<td>Very-Low-Density Lipoprotein (VLDL)</td>
<td>Transports Endogenous Lipids from Liver</td>
<td>10% by mass apoB-100, apoC-I, apoC-II, apoC-III, apoE</td>
<td>50%</td>
<td>18%</td>
<td>19%</td>
</tr>
<tr>
<td>Low-Density Lipoprotein (LDL)</td>
<td>Transports Endogenous Lipids (Cholesterol) from Liver</td>
<td>23% by mass apoB-100</td>
<td>10%</td>
<td>20%</td>
<td>45%</td>
</tr>
<tr>
<td>High-Density Lipoprotein (HDL)</td>
<td>Transports Cholesterol to Liver</td>
<td>55% by mass apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, apo-D, apoE, Lecithin-Cholesterol Acyl Transferase</td>
<td>4%</td>
<td>24%</td>
<td>17%</td>
</tr>
<tr>
<td>Notes:</td>
<td>apoC-II Activates Lipoprotein Lipase</td>
<td>apoC-III Inhibits Hepatic Lipase &amp; Lipoprotein Lipase (slightly)</td>
<td>apoB-100 Binds to the LDL Receptor on Cells</td>
<td>apoE Binds to a Receptor on the Liver Cell and Triggers Clearance</td>
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</table>

In the liver, any fatty acids or lipids obtained from the chylomicron remnants as well as lipids synthesized
by the liver are packaged into **VERY-LOW-DENSITY LIPOPROTEIN** (VLDL). They are primarily triacylglycerols, with an intermediate amount of phospholipids and small amounts of cholesterol and cholesteryl esters, as well as, apoB-100, apoC-I, apoC-II, apoC-III, and apoE. They are released from the liver, travel to adipose and skeletal muscle where apoC-II activates **Lipoprotein Lipase** allowing the release of fatty acids and glycerol into these tissues. Most of the VLDL remnants are removed from the blood by the liver in a receptor mediated process involving apoE.

The loss of triacylglycerols converts some of the VLDL into **LOW-DENSITY LIPOPROTEIN** (LDL). LDL’s are very rich in cholesterol and cholesteryl esters and contain apoB-100 as the only lipoprotein. LDL’s carry cholesterol to extra-hepatic tissues that contain specific receptors for apoB-100 on their surfaces. These receptors mediate the uptake of the LDL from the blood. The cholesterol and cholesteryl esters are utilized by these tissues and apoB-100 is degraded and the amino acids utilized by the cell that absorbed the LDL.

**HIGH-DENSITY LIPOPROTEINS** (HDL) are synthesized by the liver and small intestine as small, protein rich particles that contain very little cholesterol and no cholesteryl esters. HDL’s contain apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, apoD, and apoE as well as the enzyme **Lecithin-Cholesterol Acyl Transferase** (LCAT), which catalyzes the formation of cholesteryl esters from phosphatidylcholine and cholesterol. LCAT on the surface of HDL particles converts the cholesterol and phosphatidylcholine of chylomicron and VLDL remnants to cholesteryl esters. These cholesterol-rich lipoproteins can deliver cholesterol to steroidogenic tissues such as the adrenal gland. However, most of the HDL’s return to the liver, where the cholesterol is unloaded and either recycled or converted into bile salts.

**Triacylglycerol Catabolism**

Immediately following a meal, under the influence of Insulin, every tissue of the body utilizes glucose as its primary energy source. Glucose is used to recharge cellular ATP and NADPH levels. The ATP and NADPH are used as the energy source and reducing equivalents for biosynthetic reactions. Glucose not immediately needed for energy is stored as glycogen or used as a precursor for the hetero-oligosaccharides that are coupled to proteins and sphingolipids. Once the glycogen stores have been replenished any remaining glucose is converted to fatty acids in the liver and adipose tissue and stored as triacylglycerols in the adipose. The triacylglycerols from the meal are transported by the Chylomicrons and immediately stored in the adipose and other tissues.

During the fast between meals many of the tissues switch from glucose to fatty acids as their primary energy source. The first pathway to be discussed is the main pathway that prepares fatty acids for entry into the TCA Cycle and ET/OxPhos.

Before fatty acids can be used for energy they must be released from adipose tissue, transported to the tissues that can/will utilize them, and activated for metabolism. Hormones control the release of fatty acids and glycerol from the adipose. Glucagon, epinephrine, and norepinephrine stimulates the release of fatty acids and glycerol from the adipose. These hormones via the Gs effector system activates cAMP Dependent Protein Kinase (protein kinase A or PKA). Active PKA phosphorylates two proteins in the adipose. It phosphorylates the protein **Perilipin** and the enzyme **Hormone Sensitive Lipase** (Diacylglycerol Lipase). In the dephosphorylated state, Perilipin coats the surface of the lipid droplet present in adipose cells and this protein coat restricts the access of Lipases to the surface of the lipid droplet. Perilipin when phosphorylated
by PKA, results in the release of protein CGI-58 from the surface of the lipid droplet. CGI-58 binds to Adipose Triacylglycerol Lipase (ATGL) and directs it to the surface of the lipid droplet. Hormone Sensitive Lipase when phosphorylated by PKA is active. The phosphorylated form migrates from the cytoplasm to the surface of the lipid droplet and binds to phosphorylated Perilipin on the surface of the lipid droplet.

Adipose Triacylglycerol Lipase catalyzes the hydrolysis of triacylglycerols in the adipose releasing the fatty acid from carbon one or carbon three of glycerol. Subsequent reactions catalyzed by Hormone Sensitive Lipase (Diacylglycerol Lipase) and Monoacylglycerol Lipase yields free fatty acids and glycerol from diacylglycerols and monoacylglycerols, respectively. The fatty acids and glycerol are transported from the adipose cell and enter the blood stream. Fatty acids are for the most part water insoluble and are carried to the peripheral tissues bound to SERUM ALBUMIN. Glycerol is water soluble and moves around the body dissolved in the blood plasma.

Glycerol is absorbed primarily by the liver, but other tissues can and do utilize glycerol.

Glycerol Metabolism

The absorbed glycerol is first phosphorylated by the action of Glycerol Kinase to form glycerol-3-phosphate. Adipose cells do not contain Glycerol Kinase. Glycerol-3-phosphate is then oxidized by the
action of Glycerol-3-phosphate Dehydrogenase to form dihydroxyacetone phosphate. In the liver, the dihydroxyacetone phosphate enters gluconeogenesis, in other tissues it enters glycolysis. (Why does glycerol enter gluconeogenesis in the liver? What hormones are present and controlling metabolism?)

At the peripheral tissues, fatty acids with 12 or fewer carbons enter the cell by simple diffusion across the cell membrane; fatty acids with more than 12 carbons require a transport protein to enter the cells. Once taken up by the cells they are utilized for energy.

Fatty Acid Activation and Transport

Before fatty acid catabolism can proceed within the cell, two events must occur. Fatty acids must be activated and they must be transported into the matrix of the mitochondria. The preparatory pathway(s) of fatty acid catabolism occur in the mitochondria matrix. The sequence of events is dependent upon the length of the fatty acid. Short chain fatty acids (≤ 8 C’s) and medium length fatty acids (> 8 C’s but ≤ 12
C’s) move through the cytoplasm, pass through the outer mitochondrial membrane most likely via the Porin Pores, and diffuse through the inner mitochondrial membrane. Once in the mitochondrial matrix these fatty acids are activated by coupling them to Coenzyme A to form an acyl-CoA; a fatty acid linked to CoA by a high energy thioester bond. Activation is catalyzed by the mitochondrial isoenzyme of Acyl-CoA Synthetase. The energy released by the hydrolysis of both high energy phosphate bonds of an ATP is necessary to form the high energy thioester bond between CoA and the fatty acid. The hydrolysis of ATP to AMP and two phosphates during the activation process is equivalent to the hydrolysis of 2 ATP to 2 ADP and 2 PO₄⁻³.

Activation of long chain fatty acids (> 12 C’s) occurs in the cytosol using the cytoplasmic form of Acyl-CoA Synthetase. The reaction catalyzed by the cytoplasmic isoenzyme is identical, substrate specificity is different. Once activated, the long chain fatty acids must be transported into the matrix of the mitochondria. Fatty acyl-CoA’s cannot diffuse across the inner mitochondrial membrane nor is there a transport protein for any molecule couled to CoA. Cytoplasmic and mitochondrial pools of coenzymes / cosubstrates do not mix. To get into the mitochondrial matrix the fatty acid is passed to the carrier molecule CARNITINE. Carnitine Acyltransferase I on the inner surface of the outer mitochondrial membrane transfers the fatty acid from CoA to carnitine forming fatty acyl carnitine and CoA-SH. The fatty acyl carnitine enters the matrix of the mitochondria by a specific transport protein. With the fatty acyl carnitine in the matrix of the mitochondria the fatty acid is transferred back to CoA-SH by the action of Carnitine Acyltransferase II on the inner aspect of the inner mitochondrial membrane. The free carnitine is transported back into the cytoplasm. The transport protein is an antiport, one carnitine goes out for every fatty acyl carnitine that enters.

**β-Oxidation**

Oxidation of straight chain saturated fatty acyl-CoA's into acetyl-CoA, the intermediate that enters the TCA-cycle, occurs by a pathway called β-OXIDATION. It’s called β-OXIDATION because all of the chemistry of the pathway involves the β-carbon (carbon 3) of the fatty acid. The first three steps of the pathway ready the molecule for cleavage and the last step is a β elimination reaction. Cycling the fatty acid through these four steps is all that is required to completely convert saturated fatty acids with an even number of carbon atoms into acetyl-CoA molecules. To catabolize fatty acids with an odd number of carbons, unsaturated fatty acids, and/or branched chain fatty acids additional enzymes are required.

In the first step of β-oxidation a trans double bond is introduced between the α and β carbons of the fatty acid to form a trans Δ²-enoyl-CoA. This is accomplished by the action of Acyl-CoA Dehydrogenase. Acyl-CoA Dehydrogenase is part of an enzyme complex that transfers electrons from the fatty acyl-CoA to CoQ of the electron transport chain. The hydrogens (re: electrons) removed from the fatty acyl-CoA by this oxidation are first accepted by FAD bound to Acyl-CoA Dehydrogenase forming FADH₂. The electrons on the FADH₂ of Acyl-CoA Dehydrogenase are passed to an FAD bound on Electron-Transferring Flavoprotein (ETF). From Electron-Transferring Flavoprotein the electrons are passed to ETF:Q Oxidoreductase, an iron-sulfur protein. Ultimately, the electrons are passed to Coenzyme Q to form CoQH₂ and the CoQH₂ passes the electrons to Complex III of ET/OxPhos.
This reaction is similar to the succinate dehydrogenase reaction of the TCA cycle (Complex II of Et/OxPhos); the enzyme is bound to the inner mitochondrial membrane, it introduces a trans double bond, FAD is the initial electron acceptor, and the electrons are ultimately passed to CoQ. Acyl-CoA Dehydrogenase is a second enzyme complex that bridges two pathways, in this case the bridge directly connects \( \beta \)-Oxidation to ET/OxPhos.

The \( \text{trans} \Delta^2 \)-enoyl-CoA formed in the first step is then hydrated to form L-3-hydroxyacyl-CoA (L-\( \beta \)-hydroxyacyl-CoA). This reaction is catalyzed by Enoyl-CoA Hydratase. The enzyme is specific for the formation of the L-enantiomer. This reaction is similar to the fumarase reaction of the TCA cycle.
The L-3-hydroxyacyl-CoA (L-β-hydroxyacyl-CoA) is then oxidized to a 3-ketoacyl-CoA (β-ketoacyl-CoA) by the action of L-3-Hydroxyacyl-CoA Dehydrogenase (β-Hydroxyacyl-CoA Dehydrogenase). NAD is the oxidizing agent for this reaction and it is reduced to NADH. This reaction is similar to malate dehydrogenase of the TCA cycle.

The last step is a β elimination reaction. Thiolase (Acyl-CoA Acetyltransferase) directs the sulfur of a CoA-SH molecule to attack the β carbon, oxidizing it to the carboxyl oxidation state and breaking the bond between the α and β carbon forming acetyl-CoA and a new fatty acyl-CoA two carbons shorter.

The shortened fatty acyl-CoA reenters β-oxidation. These four enzymes of β-Oxidation will break the entire fatty acid molecule into acetyl-CoA molecules, provided that the starting fatty acid is saturated and contains an even number of carbons. These acetyl-CoA molecules enter the TCA cycle for complete oxidation of the acetate moiety or they enter the pathway for ketone body biosynthesis.

The mitochondria contains three sets of β-oxidation enzymes. One set is specific for long chain fatty acids (≥ 14 C’s), one set is for medium length fatty acids > 8 C’s but ≤ 14 C’s, and one set is for short chain fatty acids (≤ 8 C’s). During the complete β-oxidation of a fatty acid the intermediate is passed from one set of enzymes to the next. This arrangement of enzymes increases the efficiency of β-oxidation.

For the complete β-oxidation of a saturated fatty acid with an even number of carbon atoms, \{(#C÷2)-1\} passes through the β-oxidation pathway are required (#C = number of carbon atoms in the fatty acid). The fatty acid produces \{(#C÷2)-1\} molecules of NADH, \{(#C÷2)-1\} molecules of FADH2 (CoQH2), and \{(#C÷2)\} molecules of acetyl-CoA. Each of the Acetyl-CoA molecules generates 3 NADH, 1 FADH2, and 1 GTP(ATP) when passed through the TCA cycle.

**QUESTION - How many ATP’s are produced by the complete oxidation to CO2 and H2O of a saturated fatty acid with an even number of carbons.**

The energy yield clearly depends upon the length of the fatty acid oxidized and whether it is saturated or unsaturated. Unsaturated fatty acids yield slightly less energy because they are slightly more oxidized. The ten carbon saturated fatty acid decanoate (caprate) will be used in this example to calculate energy yield. Decanoate was chosen because it has a molecular mass of 172 g/mol which is close to the molecular mass of glucose which is 180 g/mol.

During the four rounds through the spiral pathway of β-Oxidation, this fatty acid produces, 5 acetyl-CoA, 4 NADH, and 4 FADH2 (CoQH2).

The five acetyl-CoA’s produced by β-Oxidation require five rounds of the TCA cycle to be completely oxidized to 10 CO2. The five trips through the TCA Cycle produces 5 GTP (ATP), 15 NADH, and 5 FADH2 (CoQH2).

From β-oxidation and the TCA cycle a total of 19 NADH and 9 FADH2 (CoQH2) are produced.

When the NADH and FADH2 (CoQH2) are oxidized by ET/OxPhos
Sixty six ATP (gross) are obtained. Subtracting the 2 high energy phosphates necessary for the activation process yields a net of 64 ATP. Slightly greater than twice the amount of ATP obtained from glucose.

β-Oxidation as described above functions smoothly provided the fatty acid has an even number of carbon atoms, has no carbon-carbon double bonds, and it is unbranched. A fatty acid with an odd number of carbon atoms, an unsaturated fatty acid, and/or a branched chain fatty acid require additional enzymes for complete oxidation.

Odd chain fatty acids.

Fatty acids with an odd number of carbon atoms are obtained from plants, fungi, and bacteria. When an odd chain fatty acid is oxidized, the last trip through the β-oxidation spiral produces a molecule of propionyl-CoA. Propionyl-CoA is not recognized as a substrate by Acyl-CoA Dehydrogenase and it would be a useless waste product unless the cell can convert it to a compound that can enter the final common pathways. For entry into the final common pathways propionyl-CoA is first carboxylated by Propionyl-CoA Carboxylase to form D-methylmalonyl-CoA. This enzyme, like most carboxylases requires Biotin as a necessary prosthetic group. D-methylmalonyl-CoA is then converted to L-methylmalonyl-CoA by the action of Methylmalonyl-CoA Epimerase. In the final step of this pathway, L-methylmalonyl-CoA is converted to succinyl-CoA by the action of Methylmalonyl-CoA Mutase. Methylmalonyl-CoA Mutase requires Vitamin B₁₂ as a prosthetic group. This reaction is a 1-2 Hydride Shift. Carbon and hydrogen bonds are rearranged. Two reactions of this type occur in the cell and both use Vitamin B₁₂ as a necessary prosthetic group. The succinyl-CoA that is formed enters the TCA cycle for complete oxidation.

Unsaturated Fatty Acids

During the β-oxidation of unsaturated fatty acids two unique situations arise that requires the use of one or two additional enzymes for the complete β-oxidation of these fatty acids. If the double bond was originally at an odd carbon; for example between carbon 9 & 10 or between carbon 15 & 16, at some point during β-
oxidation an acyl-CoA intermediate is formed that is cis $\Delta^3$. The cis double bond is between the $\beta$ and $\gamma$ carbon, between carbon 3 and 4. This intermediate is not a substrate for Acyl-CoA Dehydrogenase nor is it a substrate for Enoyl-CoA Hydratase. The first of the two extra enzymes deals with this situation. Enoyl-CoA Isomerase catalyzes the isomerization of the cis 3-4 double bond (cis $\Delta^3$) to a trans 2-3 double bond (trans $\Delta^2$). The trans $\Delta^2$ enoyl-CoA is the normal substrate for Enoyl-CoA Hydratase and $\beta$-oxidation can continue.

The second unique situation arises when the double bond was at an even carbon (e.g. between carbon 12 & 13) in the original fatty acid. With a fatty acid of this type at some point during $\beta$-oxidation the intermediate contains a cis 4-5 double bond (cis $\Delta^4$). This intermediate is a substrate for Acyl-CoA Dehydrogenase and the enzyme introduces a trans 2-3 double bond (trans $\Delta^2$) into the intermediate to produce a trans $\Delta^2$, cis $\Delta^4$ intermediate. This intermediate contains conjugated double bonds. The trans $\Delta^2$, cis $\Delta^4$ intermediate is very stable because of the conjugated double bonds. Enoyl-CoA Hydratase does not have the “enzymatic power” to add water to the trans double bond of this system of conjugated double bonds. 2,4-Dienoyl-CoA Reductase is the second additional enzyme employed for the complete oxidation of unsaturated fatty acids and it deals with this situation. This enzyme uses NADPH as a reductant and adds a hydrogen atom to carbon 2 and a hydrogen atom to carbon 5, reducing the trans $\Delta^2$, cis $\Delta^4$ intermediate to a trans 3-4 double bond (trans $\Delta^3$). The trans $\Delta^3$ intermediate is still not a substrate for Enoyl-CoA Hydratase, but it is a substrate for Enoyl-CoA Isomerase, the first extra enzyme (discussed above). The isomerase converts the trans $\Delta^3$ intermediate into a trans $\Delta^2$ intermediate and $\beta$-oxidation continues.
β-Oxidation of linoleoyl-CoA (linoleate, 18:2Δ\(^9,12\)) illustrates the function of these two additional enzymes coupled with the four enzymes of “main stream” β-oxidation. After three rounds of β-oxidation of linoleoyl-CoA, an intermediate is formed that contains a cis 3-4 double bond rather than a trans 2-3 double bond. The enzymes of “main stream” β-oxidation cannot deal with this intermediate. The additional enzyme Enoyl-CoA Isomerase converts the cis 3-4 double bond into a trans 2-3 double bond and β-oxidation continues. β-oxidation precedes until a fatty acyl-CoA is formed with a cis 4-5 double bond and a trans 2-3 double bond. The trans 2-3 double bond was introduced by the Acyl-CoA Dehydrogenase. The second extra enzyme, 2,4-Dienoyl-CoA Reductase uses electrons donated by NADPH to reduce the resonance stabilized double bonds between carbon 2 & 3 and carbons 4 & 5 to a trans double bond between carbon 3 and 4. This trans 3-4 double bond is a substrate for the first extra enzyme Enoyl-CoA Isomerase. The isomerase converts the trans 3-4 double bond into a trans 2-3 double bond and now “main stream” β-oxidation can continue.
Control of β-Oxidation

β-Oxidation is controlled at two levels; hormonally and allosterically. Glucagon, epinephrine, and/or norepinephrine stimulates β-oxidation by activating protein kinase A. The protein kinase phosphorylates Perilipin and Hormone Sensitive Lipase (Triacylglycerol Lipase). Phosphorylation of these two proteins stimulates the hydrolase responsible for initiating the breakdown and release of fatty acids from the adipose tissue. Insulin inhibits Hormone Sensitive Lipase by stimulating dephosphorylation of the enzyme. Insulin, by a mechanism that has not been completely elucidated, activates Phosphoprotein Phosphatase 2A (Protein Phosphatase 2A) and this active phosphatase hydrolyzes the phosphate from Hormone Sensitive Lipase and Perilipin.

Carnitine Acyltransferase I is an allosteric enzyme and the overall rate limiting step of β-oxidation. This enzyme controls the rate at which long chain fatty acids are transported into the mitochondria for β-oxidation by controlling the rate at which acyl-caritnine is formed. Carnitine Acyltransferase I is allosterically inhibited by malonyl-CoA. Malonyl-CoA is a key intermediate in the biosynthesis of fatty acids. When the level of malonyl-CoA is elevated Carnitine Acyltransferase I is inhibited, preventing the futile cycle of newly synthesized fatty acids being immediately broken down by β-Oxidation. Within β-oxidation L-3-Hydroxyacyl-CoA Dehydrogenase (β-Hydroxyacyl-CoA Dehydrogenase) is allosterically inhibited by NADH and thiolase is inhibited by acetyl-CoA.
α-Oxidation is the pathway used to prepare branched chain fatty acids for β-oxidation. Green vegetables, dairy products, and the meat from herbivores contain branched chain alcohols and branched chain fatty acids. The branched chain alcohols are components of plant chloroplasts. They are oxidized to branched

α-Oxidation
chain fatty acids by the animal upon ingestion and digestion. Phytanic acid is an example of one of the branched chain fatty acids derived from chloroplastic alcohols. The methyl group on the β carbon (carbon 3) prevents this molecule from undergoing “normal” β-oxidation.

Phytanoyl-CoA Synthetase couples CoA to phytanoate and other branched chain fatty acids at the expense of 2 high energy phosphate bonds donated by ATP. A hydroxyl group is introduced on the α carbon of
phytanoyl-CoA by a reaction catalyzed by Phytanoyl-CoA Hydroxylase. The reaction requires O₂, α-ketoglutarate, and ascorbic acid (Vit C). It produces α-hydroxyphytanoyl-CoA, CO₂, and succinate. α-Hydroxyphytanoyl-CoA Lyase catalyzes an α elimination reaction releasing formyl-CoA (which spontaneously breaks down to CO₂ and CoA) and oxidizing the α-hydroxyl group to the aldehyde function group of pristanal. Aldehyde Dehydrogenase oxidizes pristanal to pristanic acid and Acyl-CoA Synthetase couples the acid to CoA to the acid to form 4,8,12-trimethyltridecanoyl-CoA. This molecules is now ready for β-oxidation.

During β-oxidation it will liberate 3 Acetyl-CoA, 3 Propionyl-CoA, and 1 Isobutryl-CoA. The Acetyl-CoA Enters the TCA cycle. The Propionyl-CoA is converted into Succinyl-CoA as described in the odd chain fatty acid section and the Succinyl-CoA enters TCA. The terminal carbon (the ω-carbon) of Isobutryl-CoA is oxidized to a carboxylic acid and the resulting methylmalonyl-CoA is converted to Succinyl-CoA as described in the odd chain fatty acid section and the Succinyl-CoA enters TCA.
Ketones are small packets of quick energy. They can be considered “water soluble lipids”. Once synthesized, ketone bodies are released from the liver into the bloodstream and absorbed by the heart, kidneys, and skeletal muscle. Ketone bodies are the preferred fuel source for heart and kidneys. Under normal conditions, the concentration of ketone bodies is vanishingly small in blood plasma ($1.5 - 3.0 \times 10^{-6}$ g/mL = 1.5 - 3 $\mu$g/mL). As fast as the liver synthesizes and releases ketone bodies, other tissues take them up and utilize them for energy. Large quantities of ketone bodies are synthesized by the liver and the blood concentration of these molecules increases dramatically under conditions of starvation and uncontrolled diabetes mellitus.

Ketone body biosynthesis or ketogenesis occurs in the mitochondrial matrix of the liver as follows:
1. Two molecules of acetyl-CoA are condensed to form acetoacetyl-CoA with the release of CoA. The enzyme Thiolase (Acyl-CoA Acetyltransferase) catalyzes this reaction. The acetoacetyl-CoA can, just as likely, come directly from the \( \beta \)-oxidation pathway since it is the penultimate product of the last cycle through the pathway. In \( \beta \)-oxidation Thiolase would cleave this precursor into two acetyl-CoA molecules.

2. The acetoacetyl-CoA then reacts with a third molecule of acetyl-CoA to form \( \beta \)-hydroxy-\( \beta \)-methylglutaryl-CoA, (HMG-CoA or 3-hydroxy-3-methylglutaryl-CoA) and CoA. HMG-CoA Synthase catalyzes this reaction.

3. The HMG-CoA is cleaved into acetoacetate and acetyl-CoA by the action of HMG-CoA Lyase. The net result of reaction 2 and 3 is the removal of CoA from acetoacetyl-CoA to form acetoacetate. Acetoacetate is the first of the three ketone bodies. Once formed acetoacetate may be released from the liver.

4. Usually the acetoacetate is reduced to \( \beta \)-hydroxybutyrate by the action of \( \beta \)-Hydroxybutyrate Dehydrogenase. NADH donates the electrons for this reduction. If the liver has excess acetyl-CoA
it will likewise have excess NADH from β-oxidation and the TCA cycle. This reaction helps regenerate NAD⁺ for the continued operation of β-oxidation and the TCA cycle. The second ketone body is β-hydroxybutyrate and this compound is released into the blood stream.

5. By a spontaneous decarboxylation reaction acetone is formed from acetoacetate. Acetone is the third and last ketone body. Acetone is a waste product, it cannot be utilized by the body and it is excreted by the lungs and by the sweat glands.

When ketone bodies are metabolized for energy in the heart, kidneys, skeletal muscle, etc.:

1. The β-hydroxybutyrate is first oxidized to acetoacetate by the action of β-Hydroxybutyrate Dehydrogenase. NAD is reduced to NADH during this reaction. This is the same enzyme that forms β-hydroxybutyrate in the liver. The reaction is run in the reverse direction in the other tissues.
2. Acetoacetate then reacts with succinyl-CoA to form acetoacetyl-CoA and succinate. The enzyme Succinyl-CoA Transferase (β-Ketoacyl-CoA Transferase) catalyzes the transfer of CoA from succinyl-CoA to acetoacetate.
3. The acetoacetyl-CoA is then cleaved into two acetyl-CoA molecules by the action of the enzyme Thiolase (Acyl-CoA Acetyltransferase).

Activation and cleavage of ketone bodies occurs in the matrix of the mitochondria. Thiolase is the last enzyme of the β-oxidation spiral, the enzyme that initiates ketogenesis, and the enzyme that cleaves ketone bodies.

**Succinyl-CoA Transferase = β-Ketoacyl-CoA Transferase**

Starvation / Diabetes Mellitus

During starvation the energy intake is significantly less than the energy requirements of the organism. To
make up the energy deficit the body utilizes a large amount of the stored triacylglycerols for energy. The body cannot store amino acids per se (as free amino acids), rather they are “stored” as muscle proteins. Starvation causes the hydrolysis of muscle proteins in order to liberate amino acids to meet energy and glucose requirements. Amino acids are used for ATP generation but in terms of mass, the largest proportion of amino acids are used as a carbon source for gluconeogenesis to supplement the glycerol released from triacylglycerols. The majority of the energy deficit is made up by triacylglycerol hydrolysis because:

1. the hormone glucagon is released from the pancreas when energy and glucose levels are low and this hormone mobilizes triacylglycerols.
2. more energy is stored as triacylglycerols than as muscle proteins.
3. the loss of too much muscle protein puts the organism at significant risk of death. If too many muscle proteins are hydrolyzed, when food becomes available again the organism is too weak to find it, capture it, and/or eat it.

With triacylglycerols as the major energy source and the major carbon source for gluconeogenesis the liver has an excess of acetyl-CoA that it converts to ketone bodies and releases into the blood. If the starvation state is brought about slowly (e.g. going from 2000 Cal/day to 800 to 1000 Cal/day), brain and nervous tissue can adjust to the low glucose levels and under these conditions up to 70% of the energy requirements of nervous tissue can be met by ketone bodies. However, the red blood cell always requires a constant supply of glucose.

Diabetes Mellitus

First some background information. Its important to remember that all cells require some glucose at all times. This glucose may not be the primary energy source of the cell, but it is needed as a precursor for other important cellular functions / biomolecules. GluT1 transporters allow the tissues to uptake a baseline amount of glucose, but to uptake glucose above these baseline levels insulin is required. Insulin, via its tyrosine protein kinase receptor, stimulates the synthesis of the GluT4 passive glucose transporter and the insertion of the transporter into cell membranes. Insulin dependent tissues in the absence of insulin have a limited ability for transporting glucose. The insulin independent tissues; nervous tissue, adrenal medulla, liver (GluT2 primary glucose transporter), and red blood cells; can transport all the glucose they need across their membranes in the absence of Insulin. Both insulin and glucagon are always present in the blood stream. Their concentrations, their ratios in the blood vary depending upon conditions. After a meal the insulin concentration increases and the glucagon concentration drops; during lean times the insulin concentration decreases and the amount of glucagon increases.

There are two types of Diabetes Mellitus. Type I or Juvenile Onset Diabetes is an autoimmune disease. The immune system of the affected individual attacks and destroys the β islet cells of the pancreas. These are the cells that synthesize and secrete Insulin. Therefore, there is a significant decrease or complete absence of functional insulin molecules. Type II or Adult Onset Diabetes is characterized by a decrease in the number or a decrease in the functionality of Insulin receptors. An adequate amount of Insulin is present, but the cells cannot / do not respond to it because of the decreased receptor number and/or functionality. Glucose uptake and utilization is impaired regardless of the actual blood glucose supply in both types of diabetes. The inability of cells to utilize glucose causes a characteristic set of signs and symptoms in Uncontrolled Diabetics. The individual with Uncontrolled Diabetes:
1. is always hungry, is always eating (polyphagia) because the cells think they are energy (glucose) starved.
2. is always thirsty, is always drinking (polydipsia) because the high blood glucose and ketone body concentrations cause the blood to be hypertonic and because the individual loses a large amount of water in their urine.
3. has high blood glucose concentrations (hyperglycemia) because the insulin dependent cells cannot absorb and utilize the glucose.
4. has glucose in their urine (glucosuria) because the kidney cannot reabsorb all of the glucose filtered from the blood plasma.
5. has high blood ketone body concentrations (ketonemia) because the liver is actively synthesizing and secreting ketone bodies in response to the perceived low blood glucose concentrations.
6. has a low blood pH (ketoacidosis) because the ketone bodies also contain carboxylic acid functional groups that ionize releasing protons (H⁺).
7. has ketone bodies in their urine (ketoneuria) because the kidney cannot reabsorb all of the ketone bodies filtered.
8. produces a large amount of dilute urine (polyuria) to “wash away” the excess glucose and ketone bodies.

Even though blood glucose levels are excessively high, the insulin dependent tissues believe that glucose is in short supply because there is no insulin available to aid in its uptake. The “perceived” lack of glucose stimulates the release of Glucagon. Glucagon stimulates glycogenolysis, gluconeogenesis, and triacylglycerol mobilization. The effects of glucagon raises blood glucose levels further, but the Insulin dependent tissues still believe that there is no glucose available and more Glucagon is released. A vicious cycle is established. Glycerol from triacylglycerols is used for gluconeogenesis and fatty acids are used in β-Oxidation to fuel the TCA cycle. The high rate of triacylglycerol turnover results in a high concentration of fatty acids in the blood and in the cell. This stimulates β-Oxidation which results in more acetyl-CoA in the liver than the TCA cycle can use. Excess acetyl-CoA is diverted to ketone body formation resulting in ketoacidosis, ketonemia, and ketoneuria. With the exception of hyperglycemia, the body exhibits all of the metabolic responses characteristic of starvation.