Fatty Acid Catabolism

- Caloric value of fatty acids
- Dietary triglycerides and lipases
- Lipoproteins (overview)
- Fatty acid β-oxidation
  - *activation
  - *transport to mitochondrial matrix
  - *Four-reaction pathway and repeated cycles
  - *β-oxidation of fatty acids with odd number of carbons
  - *β-oxidation of unsaturated fatty acids
  - *regulation of β-oxidation
  - *β-oxidation in peroxisomes
- Ketone bodies
- Energy calculations for complete oxidation of fatty acids

Caloric Value of Fats and Carbohydrates

- Calories obtained by complete oxidation:
  - Carbohydrates: 4 kcal/g

(Carbohydrates have to stored with water)

(Each g of glycogen is hydrated with 2 g water)

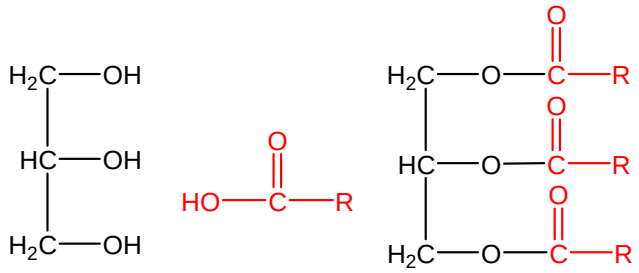
  - Hydrated carbohydrates: 1.3 kcal/g
  - Fat: 9 kcal/g (fats are not hydrated)
  - Proteins: 4 kcal/g

Fuel Reserves and Energy Needs

- An average human has the following fuel reserves:
  - ~1600 kcal as glycogen in liver and muscle
  - ~24,000 kcal as protein
  - ~130,000 kcal as fat
- Typically, humans need ~ 2000-2500 kcal/day
- Thus, fat reserves will last for ~2 months
- Average body weight is ~ 160 lb
- Fat weight is ~ 20 % (~ 32 lb)
- Same amount of energy stored as hydrated glycogen would weigh ~ 220 lb.

Fats Provide Energy During Fasting & Starvation

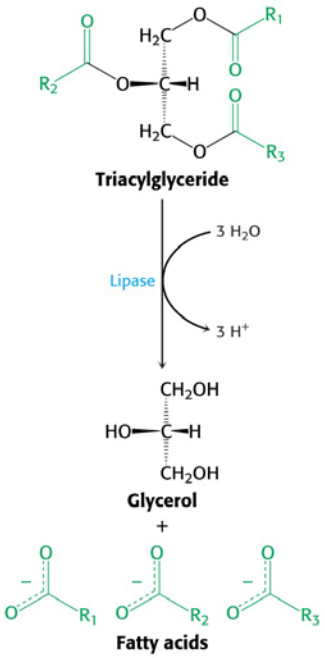
- Fasting: the first source of energy is stored glycogen.
- Starvation: glycogen stores depleted within 24 hours.
- Brain needs glucose as fuel. Two sources:
  - Proteins → Amino acids → glucose.
  - Triglycerides → Glycerol → glucose.
  - No glucose from fatty acids (PDH is irreversible).
- Prolonged starvation:
  - Proteins preserved for muscle activity.
  - Fatty acids converted to ketone bodies.
  - Brain fuel shifted to ketone bodies.



glycerol fatty acid triacylglycerol

Triacylglycerols (triglycerides, TAG or TG) are the most abundant dietary lipids, & the form in which most of the carbon energy is stored.

Each triacylglycerol has a **glycerol** backbone esterified with **3 fatty acids**. Most TG are **mixed**, with different fatty acids varying in chain length & double bonds.



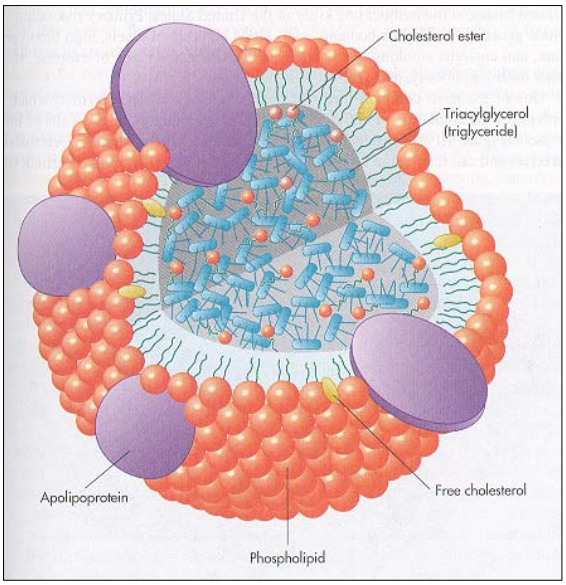
Lipases

- **Lipases** hydrolyze triacylglycerols, releasing one fatty acid at a time and forming diacylglycerols, monoacylglycerol & finally, glycerol.
- **Pancreatic lipase** hydrolyzes dietary TG, **lipoprotein lipase** acts on lipoprotein triglycerides, **hormone-sensitive lipase** is responsible for mobilization of adipose stores of TG.
- The obesity-management / weight-reduction prescription drug **Xenical** is an inhibitor of pancreatic lipase. It prevents absorption of dietary TG.

Digestion of Triglycerides

- TG are digested in the small intestine
- Mixed with bile salts secreted by gall bladder and dispersed into micelles.
- Lipids in micelles are accessible to pancreatic enzymes.
- Pancreatic lipase forms MAG and FFA.
- MAG and FFA are absorbed by mucosal cells lining the small intestine.
- Inside the cells, MAG and FFA are condensed back to TG.
- Intestinal cells assemble TG into chylomicrons.
- CM are released into the blood for transportation to all parts of the body.

Schematic Representation of a Lipoprotein Particle



From: Biochemistry By Peck Ritter

Overview of Lipoproteins (section 26.3.1 in Stryer)

- **Free fatty acids** have detergent properties in solution. A protein, serum **albumin**, transports fatty acids in the blood. TG are transported on **lipoproteins.**
- Lipoproteins are classified based on their **density**, which is determined by the **lipid:protein ratio**.
- **Chylomicrons** (CM): Dietary lipids are packaged into particles called Chylomicrons. They are the largest lipoproteins and also the least dense. They have the highest lipid: protein

Overview of Lipoproteins (continued)

- The **cholesterol-rich lipoproteins** are **LDL** and **HDL**. Plasma cholesterol is usually either on LDL or HDL.
- **LDL** particles are derived from VLDL by the action of lipoprotein lipase. HDL are side-products in the process.
- LDL particles have a greater lipid: protein. Their lipid is mostly cholesterol. LDL particles are the 'bad' cholesterol.
- **HDL** cholesterol is the 'good' cholesterol. HDL particles are lipid-poor and protein-rich and are the most dense of all lipoproteins.
- LDL transports cholesterol to the peripheral tissues including the arteries. HDL participates in **reverse cholesterol transport**; it transports cholesterol to the liver.

Fatty Acids

- FA are the direct source of energy from lipids.
- FA are oxidized by a process called β-oxidation.
- When excess fats or calories are consumed in the diet, they are condensed into triglycerides.
- TG are stored in specialized fat-storage cells called adipocytes.
- Adipose tissue accumulates subcutaneously and in different body cavities of obese people.
- Liposuction removes this adipose tissue.

Sources of Fatty Acids

1. Dietary lipids processed by the intestinal cells
2. Newly synthesized lipids, mostly by the liver
3. Lipids mobilized from adipocyte stores.

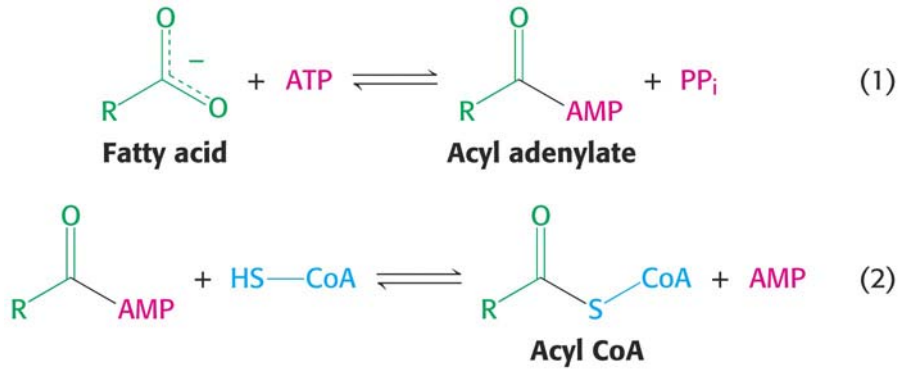
Mobilization of Adipose Fatty Acids

- During fasting or starvation, stored fuel needs to be utilized.
- the body secretes hormones such as epinephrine and glucagon.
- These hormones release the second messenger cAMP which activate hormone-sensitive lipase.
- HSL hydrolyzes stored TG to release FA.
- The mobilized fatty acids are released into the blood-stream where they associate with albumin and cirulate to various tissues in need of fuel.

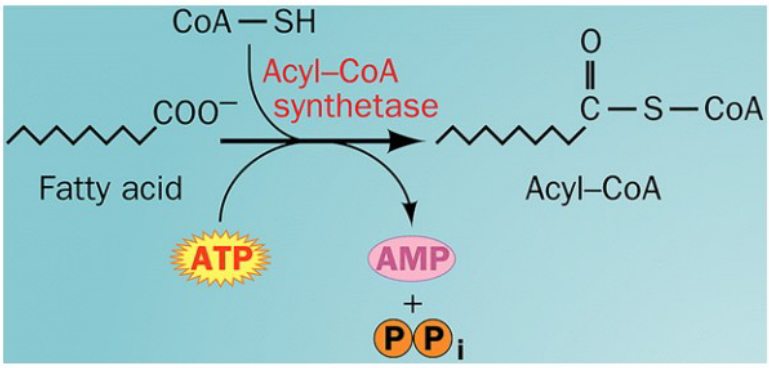
Fatty Acid Catabolism

- Most naturally occurring fatty acids have an even number of carbon atoms.
- The pathway for catabolism of fatty acids is referred to as the β-oxidation pathway, because oxidation occurs at the β-carbon (C-3).
- The first step of β-oxidation is activation of a fatty acid to a fatty acyl-CoA in the cytosol by enzyme on outer mitochondrial membrane.
- Enzymes of the β-Oxidation Pathway are in the mitochondrial matrix.
- Fatty acyl-CoA formed in the cytosol is transported across the inner mitochondrial membrane by **carnitine**.

- Fatty acid activation is catalyzed by **Acyl CoA Synthetase**:
- Two steps: Formation of mixed anhydride with AMP and Transfer of acyl group to CoA.
- Forward reaction favored by hydrolysis of ATP to AMP + PPi.
- PPi is rapidly hydrolyzed by a pyrophosphatase to 2Pi.



Activation of Fatty acid to Acyl CoA



Acyl-CoA synthetase present in outer mitochondrial membrane; FA acylation occurs in the cytosol.

Specific isoenzymes exist for different fatty acids

Import of FA into Mitochondria

- Fatty acyl CoA entry into mitochondrial matrix: 3-steps

1. Fatty acyl group transferred from CoA to -OH of carnitine by carnitine acyltransferase I in the cytosol (transesterification)
2. Fatty acyl-carnitine enters matrix by facilitated diffusion through a specific transporter in inner mitochondrial membrane. This is called the acyl carnitine/carnitine transporter or the carnitine translocase.
3. In matrix, acyl group is transferred to mitochondrial CoA (second transesterification) by carnitine acyltransferase II

- There are two separate pools of CoASH: a cytosolic pool is used for FA biosynthesis; mitochondrial pool used for FA (also aa and pyruvate) degradation.
- Entry of acyl-CoA into mitochondrial matrix is a rate-limiting step of β-oxidation.

Stages of FA oxidation

1. In mitochondrial matrix, successive 2-C units are removed from the carboxyl end of the fatty acyl chain in the form of acetyl-CoA in a repeated sequence of 4 reactions.

eg. 16-C palmitate goes through 7 passes of the sequence, and a total of 8 acetyl-CoA's are generated.

2. The acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle.

3. Stages (1) and (2) generate reduced electron carriers NADH and $FADH_2$, which donate e^- to the mitochondrial respiratory chain for ATP generation.

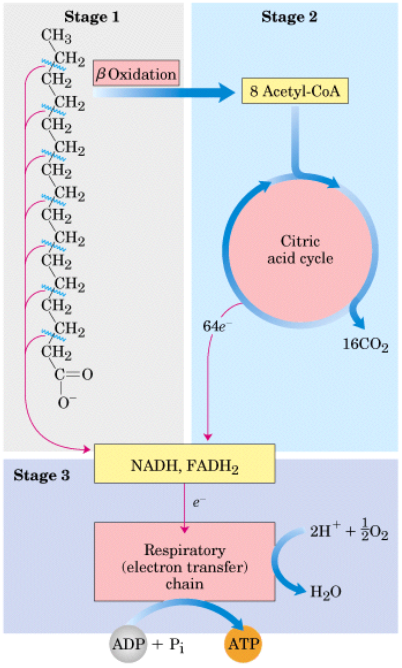


Figure from: Lehninger Principles of Biochemistry by Nelson and Cox

Reactions of β-oxidation

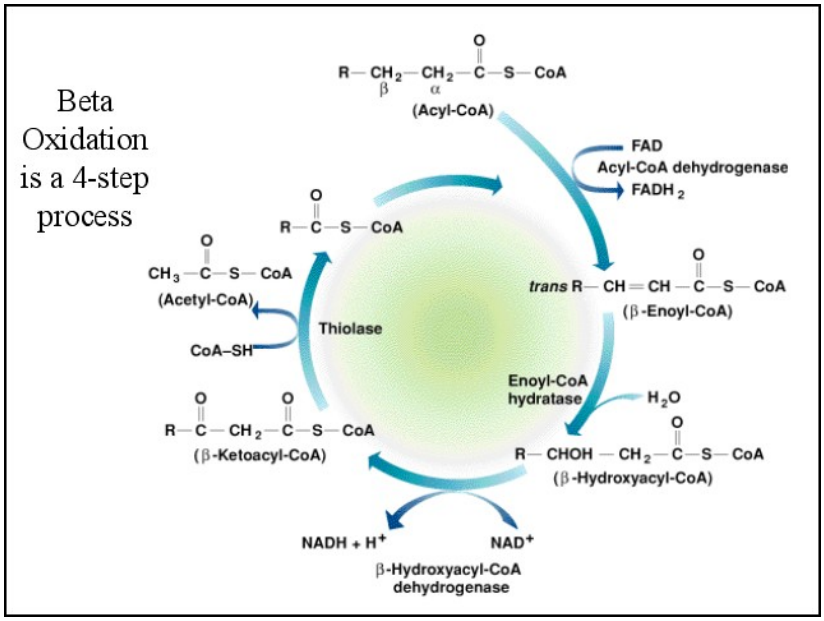
1. Dehydrogenation of fatty acyl CoA to produce a *trans* double bond between the α and β carbons (or C-2 & C-3).
   - the product is trans-Δ^2-enoyl-CoA
   - reaction catalyzed by acyl CoA dehydrogenase.
   - the electron acceptor is FAD
   - the reaction is analogous to succinate dehydrogenase
2. Addition of water across double bond of trans-Δ^2-enoyl-CoA.
   - reaction catalyzed by enoyl-CoA hydratase
   - product: L-β-hydroxyacyl CoA (3-hydroxyacyl CoA)
   - reaction analogous to fumarase
3. Dehydrogenation of L-β-hydroxyacyl CoA.
   - product is to β-ketoacyl-CoA.
   - enzyme: β-hydroxyacyl CoA dehydrogenase
   - cofactor: NAD reduced to NADH + H^+
   - reaction analogous to malate dehydrogenase

Final step of β-oxidation

- The bond between C2 and C3 in acyl CoA is relatively stable. The oxidation of C3 to a carbonyl group results in:
  - decreased stability of the C2-C3 bond (Cα–Cβ bond)
  - susceptibility of C3 (β carbon) to nucleophilic attack

4. The final step of β-oxidation cycle is thiolysis of C2-C3 (Cα-Cβ) bond by nucleophilic attack on C2 (β carbon) by the –SH group of a new CoASH.
   - products are acetyl CoA and acyl CoA (shorter by 2 C)
   - Enzyme: Thiolase (acyl-CoA acetyltransferase)
   - cofactor is CoASH

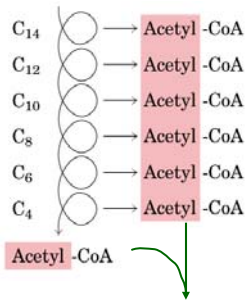
Beta Oxidation is a 4-step process



Repeating cycles of β-oxidation

One cycle of β-oxidation results in:
removal of 2 Carbons as acetyl CoA
formation of one NADH + H^+
reduction of one FAD to $FADH_2$

Reaction of one β-oxidation cycle:
palmitoyl CoA + CoA + FAD + NAD^+ + H_2O → myristoyl CoA + acetyl CoA + $FADH_2$ + NADH + H^+



8 acetyl CoA

Further cycles of β-oxidation:
Myristoyl-CoA can enter another β-oxidation cycle. 7 passes through the 4-reaction sequence results in complete oxidation of 1 molecule of palmitoyl-CoA to 8 acetyl CoAs

Net reaction of complete oxidation of palmitoyl CoA:
palmitoyl CoA + 7 CoA + 7 FAD + 7 NAD^+ + 7 H_2O →
8 acetyl CoA + 7 $FADH_2$ + 7 NADH + 7 H^+

Total yield of ATP from Palmitate

Pathway		ATP	NADH	FAD	TOTAL ATP
activation		-2	0	0	
b-oxidation		0	7	7	
TCA		8	24	8	
		6	31	15	
ATP Harvested		6	77.5	22.5	**106**

Fatty Acids with Odd-Numbered Carbon Atoms

For a fatty acid with an **odd number of carbons**, the final round of β-oxidation yields acetyl-CoA & **propionyl-CoA**.

Propionyl-CoA is converted to the Krebs cycle intermediate **succinyl-CoA**, by a pathway involving **vitamin B_{12}**.

Fatty Acids with Double Bonds

Most double bonds of naturally occurring fatty acids have cis configuration. As carbons are removed 2 at a time, a double bond may end up in the wrong configuration or wrong position for the action of Enoyl-CoA Hydratase.

Monounsaturated FA: The enzyme Enoyl-CoA Isomerase converts the cis- Δ^3 double bond to a trans-Δ^2 double bond.

Polyunsaturated FA: First, as above, Enoyl-CoA Isomerase converts the cis- Δ^3 double bond to a trans-Δ^2 double bond. Next, 2,4-Dienoyl-CoA Reductase converts the trans-Δ^2, cis-Δ^4 intermediate to trans-Δ^3. Then Enoyl CoA Isomerase converts the trans-Δ^3 double bond to trans-Δ^2.

Peroxisomes

- Peroxisomes found in some animal and plant cells.
- Like mitochondria, peroxisomes are self-replicating. High fatty diet results in increased peroxisome budding.
- Peroxisomes function to rid the body of toxic substances and metabolites.
- Numerous in liver where toxic byproducts accumulate.
- Major site of oxygen utilization. Contain oxidative enzymes, such as catalase and amino acid oxidase.
- Catalase causes the dismutation of H_2O_2 to H_2O and O_2.
- Zellweger syndrome is due to absence of functional peroxisomes and results in early death.

β-oxidation in Peroxisomes

- Preferred FA are 20- to 26-chain and branched FA. (once chains are shortened they can be exported to mitochondria)
- Carnitine derivative not required for entry into peroxisome.
- $FADH_2$ is reoxidized by passing its electrons directly to O_2 forming hydrogen peroxide (H_2O_2).
- H_2O_2 is highly toxic and is immediately broken down to H_2O and O_2 by catalase.
- The NADH formed by β-hydroxyacyl CoA DH cannot be reoxidized in peroxisomes and has to be exported to the cytosol for oxidation.
- TCA cycle enzymes are absent. Acetyl CoA formed by peroxisomal β-oxidation is transported to the cytosol, some of it may enter the mitochondria and join the Kreb's Cycle

Adrenoleukodystrophy (ADL)

- Due to defect in peroxisomal enzyme
- Gene is on X-chromosome, so males are affected; females are carriers without symptoms
- Accumulation of very long chain fatty acids in brain and adrenal cortex.
- myelin sheath, insulation surrounding neurons is damaged.
- Symptoms: behavioral changes such as abnormal withdrawal or aggression, poor memory. visual loss, learning disabilities, seizures etc.
- See Movie 'Lorenzo's Oil'

Regulation of Fatty Acid Oxidation

FA metabolism is under hormonal regulation. When fuel levels are low, Epinephrine and Glucagon stimulate mobilization of fat and glycogen reserves. Insulin, which is secreted during the fed-state, is anti-lipolytic (it inhibits β-oxidation).

The transport of FA into mitochondria is allosterically regulated. This is the rate-limiting step in β-oxidation. Carnitine Palmitoyl Transferases I and II are inhibited by **malonyl-CoA**, an intermediate of fatty acid synthesis. Thus fatty acid oxidation is diminished under conditions favoring fatty acid synthesis.

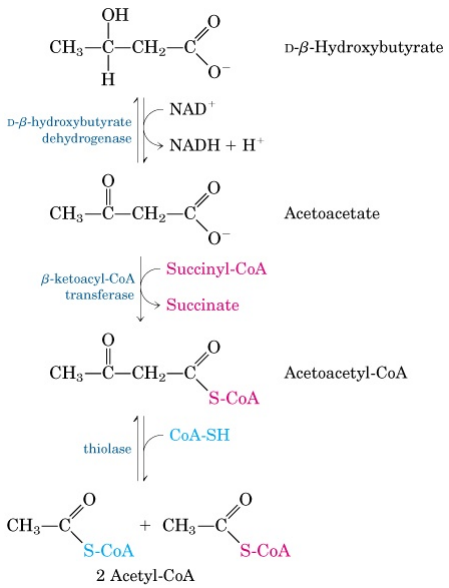
The two final steps in the β-oxidation cycle are also regulated. 3-hydroxyacyl-SCoA dehydrogenase is inhibited by **NADH**. Thiolase is regulated by feed-back inhibition by **acetyl CoA**.

Fate of excess acetyl CoA

- Entry of acetyl CoA into citric acid cycle depends on availability of oxaloacetate (for formation of citrate)
- Oxaloacetate is formed by pyruvate carboxylase from pyruvate (glycolysis product). Its availability is dependent on glycolysis and carbohydrate supply.
- Oxaloacetate is depleted during gluconeogenesis.
- If organism has a high intake of fat, low intake of carbohydrate, or is unable to metabolize glucose (diabetes), or during fasting/starvation, [acetyl CoA] >> [oxaloacetate]
- Acetyl CoA is diverted to formation of Ketone Bodies.
- Ketone Bodies are formed when capacity of TCA cycle is exceeded.

Use of Ketone Bodies as Fuel

- Ketone bodies are formed in the liver mitochondria
- Acetoacetate and β-hydroxybutyrate are transported by the blood to extrahepatic tissues
- Acetone is produced in smaller amounts and is exhaled.
- Ketone bodies are converted to acetyl CoA and oxidized by the TCA cycle for energy in skeletal and cardiac muscle and brain.
- The brain prefers glucose as fuel but can adapt to using ketones when glucose is not available.
- Oxidation of Ketones by other tissues facilitates the continued oxidation of FA in the liver.



Reconversion of ketones to acetyl CoA in extrahepatic tissues

Ketone Bodies Are Formed During Diabetes

- **During diabetes or starvation**:
- Glucose cannot enter cells and is not available for oxidation
- Gluconeogenesis depletes intermediates of the TCA cycle; TCA cycle pauses.
- FA biosynthesis is inhibited (activated by insulin)
- Malonyl CoA (first step of FA synthesis) is not formed.
- β-oxidation is not inhibited (at carnitine acyl transferase).
- Insulin is anti-lipolytic; in its absence FA oxidation continues unchecked.
- Acetyl CoA is in excess and ketones are formed
- Uncontrolled diabetes results in diabetic ketoacidocis. The decrease in blood pH because impairs several tissue functions and can be fatal.