Lipids 1: Fatty Acids and the Beta-Oxidation Pathway

Functions of Lipids:
- Components of cell membranes.
- Concentrated energy stores.
- Metabolic fuel.
- Vitamin precursors.
- Hormones.
- Intracellular messengers.

Properties of Lipids:
- Low solubility in water.
- Can be neutral or amphipathic—both hydrophobic and hydrophilic (at different regions).

Functions of Fatty Acids:
- Structural components of various membrane and storage lipids.
- Metabolic fuel.
- Source of carbons for biosynthesis.
- Precursors for hormones.

Fatty Acid Structure
- General structure: \( \text{CH}_3(\text{CH}_2)_n\text{COO}^- \) (n=0-24).
- May contain double bonds.
- Low solubility in water.
- COOH ionizes at pH 7 ** therefore a regular FA by itself amphipathic.

Fatty Acid Nomenclature (Saturated)
- C12 = n-Dodecanoate = Laurate
- C14 = n-Tetradecanoate = Myristate
- C16 = n-Hexadecanoate = Palmitate
- C18 = n-Octadecanoate = Stearate

Unsaturated Fatty Acids
- Have double bonds in hydrocarbon chain.
- Lower melting temperature.
- Maintain membrane fluidity (more double bonds, more fluid).
- Double bonds in unsaturated FAs are usually cis, which makes them kinked and shortened (more fluid).
- Hydrogenation of polyunsaturated fatty acids or conversion to trans configuration causes fatty acids to "harden."
  - Trans form has a higher melting point (behaves like a saturated fatty acid).

Common Unsaturated Fatty Acids:
- Palmitoleate 16:1(9)
Fatty Acid Catabolism

- Fatty acids are transported to tissues (e.g. muscles) where they are **activated** and enter the mitochondria.
- Fatty acids are catabolized in 2-carbon units in mitochondria via **beta-oxidation**.
- Oxidation reactions occur in discrete steps coupled to FADH2 and NADH production and generation of ATP.
- Acetyl CoA units generated from fatty acids enter the TCA cycle.
- **Different enzymes catabolize short, medium, and long chain fatty acids.**

Activation of Fatty Acids by linkage to Coenzyme A (See pg. 38).

- **Catalyzed by** Acyl-CoA Synthetase
- *Hydrolysis of PPi drives reaction in the forward direction.
- Fatty Acid + ATP + HS-CoA ---> Acyl-S-CoA +AMP + PPi
  - Step 1 :
    - Fatty Acid + ATP <----> RCO-PO4-Ribose-Adenine (Acyl AMP) + PPI
    - *Acyl AMP is a high-energy intermediate
  - Step 2:
    - Acyl AMP + HS-CoA <---->Acyl-CoA + AMP

The Carnitine Shuttle -- Moves Fatty Acids into Mitochondria

- **See pg. 38 for diagram.
- Activated fatty acid (Acyl-CoA) is transported into the **intermembrane space** of the mitochondria as RCO-Carnitine via the action of Carnitine Acyltransferase I (CPT1).
- From the intermembrane space, the RCO-Carnitine is transported into the mitochondrial **matrix** via Carnitine Acyltransferase II (CPT2) and HS-CoA (from a free pool in the matrix) replaces Carnitine to reform RCO-S-CoA.
- **Carnitine is a zwitterionic alcohol** synthesized from Lys and Met in the liver and kidney.

Carnitine Palmityltransferase (Acyltransferase) Deficiencies -- **Autosomal recessive** for CPT I or CPT II.

- Patients with CPT I or II deficiencies cannot mobilize FAs into the mitochondria, and therefore cannot utilize beta-oxidation.
- Symptoms (triggered by exercise / fasting):
  - Muscle pain / stiffness.
  - Myoglobinuria (urine is pink).
  - May include enlarged liver.
- Treatment:
  - High carb diet / low fat diet.
  - Addition of medium-chain triglycerides to diet.
    - **Medium-chain FAs can enter the mitochondria without the carnitine shuttle (see pg. 40).**

Beta-Oxidation of Fatty Acyl-CoA

- Oleate 18:1(9)
- Linoleate 18:2(9,12)
- Linolenate 18:3(9,12,15)
- Arachidonate 20:4(5,8,11,14)
Recurring Steps: (Acyl chain shortened by 2 carbons, FADH2, NADH and Acetyl-CoA generated. ATP generated).
- Oxidation by FAD (Acyl-CoA dehydrogenase **chain specific** LC = 14-18, MC = 6-12, SC = 4-6)
  - **This is the RATE LIMITING STEP.**
  - Generates FADH2 --> goes to ETC.
  - Forms trans-Δ2-enoyl CoA.
- Hydration (Enoyl-CoA hydratase or "crotonase")
- Oxidation by NAD+ (β-hydroxy acyl-CoA dehydrogenase)
  - Generates NADH --> goes to ETC.
  - β-carbon is oxidized, hence "β-oxidation."
- Cleavage (thiolysis) by CoA (β-ketothiolase).
  - HS-CoA is added to end of chain (shortened by two carbons).
  - Acetyl-CoA is generated, and it enters the TCA cycle.

### Beta Oxidation Cycle

\[
\begin{align*}
\text{Oxidation} & : \quad \text{FAD} & \quad \text{FADH}_2 \\
\text{Hydration} & : \quad \text{H}_2\text{O} \\
\text{Oxidation} & : \quad \text{NAD}^+ & \quad \text{NADH} + \text{H}^+ \\
\text{Cleavage} & : \quad \text{HS-CoA} \\
\text{(in MCAD, process stops at <12 carbon length)} & \end{align*}
\]

Genetic Defects in FA Catabolism

- Defects in the different Acyl-CoA Dehydrogenase Enzymes (MC, LC, SC) are particularly significant in pediatric cases. Because there are different enzymes for different chain lengths, a defect in a certain enzyme corresponds to an inability to break down a specific length of fatty acid chain.
**MCAD -- Medium Chain Acyl-CoA Dehydrogenase Deficiency:**
- Beta-oxidation of medium chain fatty acids (6-12 carbons) is deficient due to defect in medium chain acyl-CoA dehydrogenase.
- Characteristics:
  - Onset of symptoms within first 2 years of life.
  - Precipitated by fasting >12 hours or infection.
  - Symptoms include: vomiting, lethargy, coma, hypoglycemia.
  - Urine contains MCFA esters of glycine and carnitine.
  - Can cause long-term disability or fatality if untreated.
  - Treatment: IV glucose, high carb diet, and avoid fasting.

β-oxidation Summary
- Overall equation: (example).
  - Palmitoyl CoA (16C) + 7FAD + 7NAD+ + 7CoASH + 7H2O -->(7 cycles)---> 8 Acetyl-CoA + 7FADH2 + 7NADH + 7H+
- Energy "Balance Sheet":

<table>
<thead>
<tr>
<th>Stage</th>
<th>NADH</th>
<th>FADH2</th>
<th>GTP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acylation</td>
<td></td>
<td></td>
<td></td>
<td>-2(PPi)</td>
</tr>
<tr>
<td>Beta-Oxidation (7 rounds)</td>
<td>2.5</td>
<td>1.5</td>
<td></td>
<td>4x7 = 28</td>
</tr>
<tr>
<td>Acetyl-CoA (8 molecules)</td>
<td>7.5</td>
<td>1.5</td>
<td>1</td>
<td>10x8 = 80</td>
</tr>
</tbody>
</table>

TOTAL ATP = 108 - 2 = 106

Beta-Oxidation of Other FAs
- Odd-carbon fatty acids still yield Acetyl-CoA, but are left with a Propionyl-CoA (3C) that is converted and enters the TCA cycle as Succinyl-CoA, yielding 2 fewer NADH than Acetyl-CoA.
- Mono- and polyunsaturated fatty acids require additional accessory enzymes to complete beta-oxidation. **See pg. 47-48.
  - **Cis-Δ3-Enoyl-CoA Isomerase** converts double-bond cis configurations to trans configurations that can be metabolized by enoyl-CoA hydratase.
  - **2,4-Dienoyl-CoA Reductase** is used for polyunsaturated FAs to change two double bonds into one via the reduction of NADPH to NADP+. The resulting molecule can be isomerized by **Cis-Δ3-Enoyl-CoA Isomerase** and continue through the beta-oxidation pathway.

Fatty Acids are Also Oxidized in Peroxisomes
- Very long chain (VLC) fatty acyl-CoAs (20-26C) are oxidized in peroxisomes.
- Oxidation cycle ends with an octanyl-CoA (transported to mitochondria for complete catabolism).
- **The first step of the oxidation cycle uses molecular oxygen as the electron receptor.**
  - VLC Acyl-CoA DH Step uses O2 --> H2O2 -->(catalase)---> H2O + 1/2O2
- **Zellweger Syndrome** -- rare hereditary disorder affecting infants.
  - Problems with prenatal development, enlarged liver, high levels of Fe and Cu in blood, muscle and vision abnormalities. Death by age 6.
  - **Mutations in the PXR1 gene product -- a receptor found on the surface of
peroxisomes.

- PXR1 receptor is vital for the import of enzymes into the peroxisomes.
- Not only can peroxisomes not perform beta-oxidation, they can't clear Fe and Cu from the blood.

**Symptom = accumulation of VLCFAs.**