Fatty Acid Oxidation

- **The β-oxidation pathway** degrades fatty acids two carbons at a time
- **Skeletal muscle, liver, kidney, heart** can use FA directly
- **Three stages:**
  1. Activation of fatty acids in the cytosol
  2. Transport into the mitochondria
  3. Degradation to two-carbon fragments (as acetyl CoA) in the mitochondrial matrix

**Activation of Fatty Acids**

![Diagram of fatty acid oxidation pathway]
• Fatty acids in the cytosol are activated by conversion to CoA thioesters by acyl-CoA synthetase (ATP dependent)
• Fatty acid attached to CoA-SH
• The PPi released is hydrolyzed by a pyrophosphatase to 2 Pi
• Net of two ATP equivalents are consumed to activate one fatty acid to a thioester

\[
R-\text{COO}^- + \text{HS-CoA} \xrightarrow{\text{Acyl-CoA synthetase}} R-\text{C-S-CoA} + \text{PP}_i
\]

\[
\Delta G''' = -32.3 \text{ kJ/mol}
\]

\[
\Delta G^{\text{synthesis}} = 31.5 \text{ kJ/mol}
\]

\[
\text{Net } \Delta G'' = -0.8 \text{ kJ/mol}
\]

2. Transport of Fatty Acyl CoA into Mitochondria
• Activated acyl-CoA freely diffuses through OMM into intermembrane space, however cannot pass IMM to be brought into mitochondrial matrix
• The carnitine shuttle system transfers fatty acyl CoA from the cytosol into the mitochondria
• Fatty acyl CoA is first converted to acylcarnitine (which can enter the mitochondria) and then back to fatty acyl CoA
• The β-oxidation cycle enzymes (mitochondrial) can then degrade the fatty acyl CoA

Sequence of Events:
1. At IMM, acyl group transferred to carnitine, a small organic molecule that carries the fatty acyl group into the mitochondrial matrix. Used as a SHUTTLE for fatty acyl groups.
   Compound = acylcarnitine
Enzyme = **carnitine acyltransferase I**

2. Acylcarnitine is transported across IMM via **protein translocase**

3. Once in the matrix, acyl group is transferred back to CoA-SH by **carnitine acyltransferase II**

4. Carnitine transported back to intermembrane space via translocase to get another molecule of acyl

5. Keep pools of CoA-SH separate
   - Cytosolic CoA-SH used for synthesis
   - Mitochondrial CoA-SH used for degradation

**ANIMATION:**


[Carnitine diagram]

**CARNITINE:**

- Diet: red meat, dairy, poultry, fish
- Body: made in liver and kidneys
- Enters cells by specific transporter
- Carnitine deficiencies:
  ▪ Symptoms:
    • Poor muscle tone
    • Muscle weakness
    • Brain dysfunction
    • Heart dysfunction
  ▪ Primary Deficiency: Rare disorder due to faulty transporter that allows carnitine into cells
  ▪ Secondary Deficiency: Poor dietary intake or metabolic diseases that deplete or limit stores
  ▪ Treatment: Pharmaceutical administration of carnitine to supplemental stores in the body; Can flood system and enough carnitine can enter if due to a faulty transporter
- NOTE: NO evidence exists that if normal, taking more supplements of carnitine does anything. Not bad – excess carnitine is not harmful but not necessarily beneficial.

The Reactions of β-oxidation
- One round of β-oxidation: 4 enzyme steps produce acetyl CoA from fatty acyl CoA
  - Each round generates one molecule each of:
    1 FADH$_2$ – oxidative phosphorylation
    1 NADH – oxidative phosphorylation
    1 Acetyl CoA – enters TCA cycle
    Fatty acyl CoA (2 carbons shorter each round)
  - Process continues until acyl-CoA is completely broken down to Acetyl-CoA groups
  - Cleavage occurs between the α and β carbons of the acyl-CoA (between 1$^{\text{st}}$ and 2$^{\text{nd}}$ C next to carbonyl)
  - # of acetyl-CoA made = $\frac{1}{2}$ the number of Carbons in the original fatty acid
  - # of cycles of β-oxidation = # of acetyl-CoAs made minus 1
  - Example:
    ▪ Palmitic acid = 16:0
    ▪ 8 moles of acetyl-CoA (enter TCA cycle)
    ▪ 7 rounds of β-oxidation
**β-OXIDATION, A SPIRAL PATHWAY:**

**Reaction 1:** Oxidation-reduction reaction catalyzed by *Acyl-CoA Dehydrogenase*
- Generates FADH₂
- Produces a double bond between carbon atoms 2 and 3

**Reaction 2:** Hydration reaction catalyzed by *Enoyl-CoA Hydrase*
- Catalyzes stereospecific hydration of the trans double bond produced in the 1st step of the pathway
- Forms 3-L-Hydroxyacyl-CoA

**Reaction 3:** Oxidation-reduction reaction catalyzed by *3-L-Hydroxyacyl-CoA Dehydrogenase*
- Generates NADH
- Oxidation of the hydroxyl in the β position (C3) to a ketone

**Reaction 4:** Bond cleavage reaction catalyzed by *Thiolase*
- Requires CoA-SH (Coenzyme A)
- Releases Acetyl-CoA – which enters the TCA cycle
- Acyl-CoA (2 carbons shorter) re-enters β-oxidation
**Table 18.1**

Reactions for fatty acid activation, transport, and the β-oxidation spiral

<table>
<thead>
<tr>
<th>Reaction Number</th>
<th>Reaction</th>
<th>Enzyme</th>
<th>Reaction Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fatty acid + CoASH + ATP $\rightarrow$ acyl-CoA + AMP + PP_i</td>
<td>Acyl-CoA synthetase</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>PP_i + H_2O $\rightarrow$ 2 P_i</td>
<td>Pyrophosphatase</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Carnitine + acyl-CoA $\rightarrow$ acyl-carnitine + CoASH (intermembrane space)</td>
<td>Carnitine acyltransferase I</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Acyl-carnitine + CoASH $\rightarrow$ acyl-CoA + carnitine (mitochondria)</td>
<td>Carnitine acyltransferase II</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Acyl-CoA + E-FAD $\rightarrow$ trans-Δ^2-enoyl-CoA + E-FADH_2^b</td>
<td>Acyl-CoA dehydrogenase</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>trans-Δ^2-Enoyl-CoA + H_2O $\rightarrow$ L-3-hydroxyacyl-CoA</td>
<td>Enoyl-CoA hydratase</td>
<td>4</td>
</tr>
<tr>
<td>7</td>
<td>L-3-Hydroxyacyl-CoA + NAD^+ $\rightarrow$ 3-ketoacyl-CoA + NADH + H^+</td>
<td>Hydroxyacyl-CoA dehydrogenase</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>3-Ketoacyl-CoA + CoASH $\rightarrow$ acyl-CoA + acyl-CoA^c</td>
<td>β-Ketothiolase</td>
<td>4</td>
</tr>
</tbody>
</table>

*a Reaction type: 1. oxidation-reduction; 2. group transfer; 3. hydrolysis; 4. nonhydrolytic cleavage (addition or elimination); 5. isomerization-rearrangement; 6. bond formation coupled to ATP cleavage.

^b E-FAD and E-FADH\textsubscript{2} refer to the cofactor flavin adenine dinucleotide covalently linked to the enzyme.

^c Acyl-CoA product is shortened by a C_2 unit.

Table 18.1 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons


**Fig. 19-9** -- The βOxidation Pathway of Fatty Acyl-CoA

**SUMMARY OF THE PRODUCTS OF EACH CYCLE:**

- Acyl-CoA which re-enters β-oxidation
- 1 Acetyl-CoA which enters TCA
- 1 FADH\textsubscript{2}
- 1 NADH
ATP Generation from Fatty Acid Oxidation

- Three things to keep in mind as sources of ATP
  - NADH and FADH$_2$ produced by β-oxidation cycles
  - Processing of Acetyl-CoA generated in β-oxidation cycles through TCA cycle and ox phos
  - How much ATP was USED in activating the FA for degradation
    - Remember – 2 ATP equivalents are used per mole of fatty acid; 1 ATP but 2 high energy bonds

The balanced equation for oxidizing one palmitoyl CoA by seven cycles of β–oxidation Net yield of ATP per palmitate oxidized to 16 CO$_2$

\[
\text{Palmitoyl CoA} + 7 \text{ HS-CoA} + 7 \text{ FAD} + 7 \text{ NAD}^+ + 7 \text{ H}_2\text{O} \xrightarrow{\text{β–oxidation}} 8 \text{ Acetyl CoA} + 7 \text{ FADH}_2 + 7 \text{ NADH} + 7 \text{ H}^+
\]

Each acetyl-CoA that enters TCA:
- 1 mole GTP
- 3 moles NADH
- 1 mole FADH$_2$

Therefore for palmitate: 80 ATP
- 8 GTP = 8 ATP
- 24 NADH = 60 ATP
- 8 FADH$_2$ = 12

ATP generated

| 8 acetyl CoA | 80 |
| 7 FADH$_2$ | 10.5 |
| 7 NADH | 17.5 |

108 ATP

ATP expended to activate palmitate -2

Net yield: 106 ATP

MUCH BETTER THAN GLUCOSE:
- Even for 3 glucose molecules, only get 96 ATP (18 carbons),
- Palmitate (16:0) gives 106!
- Fats are better energy stores that sugars/carbs!
- An important source of **metabolic water** for some animals:
- Water is ultimately formed through the process of electron transport & oxidative phosphorylation
  - Gerbils, Killer Whales (do NOT drink sea water)
  - Camel – hump is essentially a large deposit of fat
  - Fatty acid metabolism from fat store provides metabolic energy as well as needed water during periods when food and drinking water is not available!!
  - It has been found for every half pound of fat metabolized, a pint of water is produced!
  - Water also stored in specialized places near the stomach.

**Kangaroo rats**

- exhibit many of the most common desert animal specializations
- speciose and abundant - very successful
- do not use evaporative cooling, so water budgets can be easily calculated
- small, easily studied in the lab
- pioneering studies of Knut Schmidt-Nielsen at Duke University
- drink no water

**Oxidation water**

<table>
<thead>
<tr>
<th>Type of food</th>
<th>Water formed (g H₂O/g food)</th>
</tr>
</thead>
<tbody>
<tr>
<td>starch* (carbohydrate)</td>
<td>0.56</td>
</tr>
<tr>
<td>fat</td>
<td>1.07</td>
</tr>
<tr>
<td>protein</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*e.g. C₆H₁₂O₆ + 6 O₂ to 6 CO₂ + 6 H₂O*

**Water budget for a kangaroo rat**

<table>
<thead>
<tr>
<th>Water losses</th>
<th>Water gains</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ml</strong></td>
<td><strong>ml</strong></td>
</tr>
<tr>
<td>Urine</td>
<td>Oxidation</td>
</tr>
<tr>
<td>13.5</td>
<td>54.0</td>
</tr>
<tr>
<td>Feces</td>
<td>Absorbed water</td>
</tr>
<tr>
<td>2.6</td>
<td>6.0</td>
</tr>
<tr>
<td>Evaporation</td>
<td>Total</td>
</tr>
<tr>
<td>43.9</td>
<td>60.0 ml</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>Total</strong></td>
</tr>
<tr>
<td>60.0 ml</td>
<td>60.0 ml</td>
</tr>
</tbody>
</table>

β **Oxidation of Unsaturated Fatty Acids**
- Unsaturated FA are common in nature
- Degradation requires two other enzymes in addition to the β-oxidation pathway enzymes:
  1. Enoyl-CoA isomerase
  2. 2,4-Dienoyl-CoA-reductase

**Ketone Bodies Are Fuel That Are Synthesized in the Liver**

- Unbalanced metabolism of fats and carbohydrates changes the flow of nutrients in pathways
- Common factors in abnormal metabolic conditions:
  - Lack of carbohydrates
  - Impaired use of carbohydrates
  - Fasting
  - Starvation
  - Untreated diabetes
  - Atkins’ Diet

**Response to a Fast and Starvation**

The natural response to glucose and energy deficiency is involves two metabolic processes. Firstly the adrenal cortex secretes glucocorticoids to stimulate **gluconeogenesis**. Secondly growth hormone is secreted to accelerate **lipolysis** in adipose tissue to provide **fatty acids for oxidation**.

- Liver glycogen stores are depleted
- Fatty acids can be used by heart, kidney, skeletal muscle and liver
- Fatty acids are not used as fuel by the brain because they do not cross the blood-brain barrier
- Survival during starvation is mainly determined by the size of the stored triacylglycerol pool
- After several days of starvation, **acetyl-CoA** is made in abnormally high amounts:
  - Due to excessive fatty acid breakdown since glucose/glycogen is not available
  - Glucose is the primary source of fuel for the human brain, therefore the rate of gluconeogenesis has to increase
  - During fasting or carbohydrate starvation, **oxaloacetate** in liver is depleted from the TCA cycle because it is used for **gluconeogenesis** (making glucose for the brain)
  - Impedes entry of acetyl-CoA into the TCA cycle.
  - Excessive **Acetyl-CoA** is converted in liver mitochondria to **ketone bodies**
- Ketone bodies can be thought of as “soluble fats” – exported to cells that need it. They are transportable forms of fatty acids!
- There is a limited amount of mitochondrial CoA-SH so need to regenerate it for further fatty acid catabolism.

Ketone Bodies:
- β-Hydroxybutyrate
- Acetoacetate
- Acetone – expelled in breath
- Ketone bodies are acids – can cause lowering of blood pH leading to acidosis (ketosis); untreated can lead to coma and death

Ketone bodies can fuel brain cells during starvation
- Use of ketone bodies minimizes protein breakdown
- Able to cross blood-brain barrier
- Major energy source for brain during starvation
- β-Hydroxybutyrate and acetoacetate used as fuel

Acetone – expelled in breath

FORMATION OF KETONE BODIES:
1. **β-Ketothiolase.** The final step of the β-oxidation pathway runs backwards, condensing 2 acetyl-CoA to produce acetoacetyl-CoA, with release of one CoA.

2. **HMG-CoA Synthase** catalyzes condensation of a third acetate moiety (from acetyl-CoA) with acetoacetyl-CoA to form hydroxymethylglutaryl-CoA (HMG-CoA).

3. **HMG-CoA Lyase** cleaves HMG-CoA to yield acetoacetate plus acetyl-CoA.

4. **β-Hydroxybutyrate Dehydrogenase** catalyzes inter-conversion of the ketone bodies acetoacetate and β-hydroxybutyrate.

**Ketone Bodies Are Oxidized in Mitochondria of many tissues OTHER than liver**

- Liver **cannot** use ketone bodies because the activating enzyme required for ketone body utilization is absent in the liver.
- Acetyl-CoA regenerated and enter TCA cycle to make energy

**DIABETES:**

"Starvation of cells in the midst of plenty"

- Third leading cause of death in the U.S.
  - Two types: **Type 1 and Type 2**
- For glucose to get into cells, insulin must be present
- Causes of diabetes – Insufficient insulin is secreted or insulin does not stimulate its target cells
- Glucose builds up in the blood, overflows into urine, and passes out of body.
- Body loses main fuel source even though blood full of glucose

**Consequences:**

- Blood and urine [glucose] is **elevated**
- Glucose is abundant in blood, but uptake by cells in muscle, liver, and adipose cells is low
- Cells, metabolically starved, turn to gluconeogenesis and fat/protein catabolism
  - Processes of TAG hydrolysis, fatty acid oxidation, gluconeogenesis accelerated
  - **OAA is low, due to excess gluconeogenesis, so Acetyl-CoA from fat/protein catabolism does not go to TCA, but rather to ketone body production**
- Blood levels of **ketone bodies** are elevated
  - Lowered pH of blood
  - Increased elimination of water and electrolytes, causing dehydration and lowered blood volume
  - Acetone can be detected on breath
ATKINS DIET

- High protein/High Fat/Low to no carbohydrate diets
- In a carbohydrate free diet, newly ingested fatty acids are immediately oxidized by all tissues except brain
- When diet contains carbohydrates, ingested fatty acids are transported to fat cells for storage (Insulin signals fat storage)
- Effective because body fat is metabolized for energy
- Also effective because lots of water is lost (excessive urination)
- BUT: No idea of what long term effects of being in constant ketosis are or other side-effects
- Potentially Problematic Side Effects:
  - Dehydration – caused by excessive urination to rid body of acids
  - Electrolyte Imbalance – Loss of Na\(^+\) and K\(^+\) in urine
  - Difficulty in Concentration – Low fuel availability to brain
  - GI Problems – No fiber
  - Bad Breath – Acetone expulsion
  - Heartbeat Irregularities
  - Kidney Stress or Damage
    - Excessive urination to flush out toxins created by ketosis
    - Processes by-products of protein breakdown
  - Excessive proteins can cause Ca\(^{2+}\) loss from bones which can lead to osteoporosis – esp. bad for women
  - Acidosis and Death
  - Depression – Lack of carbohydrates can result in lowered serotonin production – a mood stabilizing compound in the brain
  - Workouts suffer – due to depleted energy stores

- Hard to stick with this diet – weight gain common afterwards
- If on this type of diet, be sure to drink lots of water and supplement with vitamins and salt
- **BE BALANCED!** Weight loss = Eat fewer calories and increase exercise
- 1 pound of fat = 3500 calories
- Cut 500 calories/day and lose 1 pound per week
- Safe weight loss that will last = 2 pounds per week
- EVERYTHING IN MODERATION to keep body in balance – eat all food groups

Integration of Metabolic Pathways:

Figure 20-1 Concepts in Biochemistry, 3/e © 2006 John Wiley & Sons
### Table 20.2
Metabolic profiles of major organs

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Fuel Stored</th>
<th>Fuel Used</th>
<th>Fuel Molecules Exported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>None</td>
<td>Glucose, ketone bodies</td>
<td>None</td>
</tr>
<tr>
<td>Skeletal muscle (at rest)</td>
<td>Glycogen</td>
<td>Fatty acids, glucose</td>
<td>None</td>
</tr>
<tr>
<td>Skeletal muscle (active)</td>
<td>None</td>
<td>Glucose, fatty acids, ketone bodies</td>
<td>Lactate, alanine</td>
</tr>
<tr>
<td>Heart muscle</td>
<td>None</td>
<td>Fatty acids, glucose, lactate, ketone bodies</td>
<td>None</td>
</tr>
<tr>
<td>Liver</td>
<td>Glycogen, triacylglycerols</td>
<td>Glucose, fatty acids, lactate, amino acids, glycerol</td>
<td>None</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Triacylglycerols</td>
<td>Fatty acids, glucose</td>
<td>Fatty acids, glycerol</td>
</tr>
</tbody>
</table>

---

**Figure 20-2** Concepts in Biochemistry, 3/e
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# DIABETES

<table>
<thead>
<tr>
<th></th>
<th>Insulin-dependent diabetes mellitus</th>
<th>Non-insulin dependent diabetes mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synonym</td>
<td>Type I</td>
<td>Type II</td>
</tr>
<tr>
<td>Age of onset</td>
<td>During childhood or puberty</td>
<td>After age 35</td>
</tr>
<tr>
<td>Nutrition status at time of onset</td>
<td>Frequently undernourished</td>
<td>Obesity</td>
</tr>
<tr>
<td>Prevalence</td>
<td>10-20% of diagnosed diabetics</td>
<td>80-90% of diagnosed diabetics</td>
</tr>
<tr>
<td>Genetic predisposition</td>
<td>Moderate</td>
<td>Very strong</td>
</tr>
<tr>
<td>Defect or deficiency</td>
<td>Beta cells are destroyed</td>
<td>Insulin resistance; not enough Beta cells</td>
</tr>
<tr>
<td>Ketosis</td>
<td>Common</td>
<td>Rare</td>
</tr>
<tr>
<td>Plasma insulin</td>
<td>Low to absent</td>
<td>Normal to high</td>
</tr>
<tr>
<td>Acute complications</td>
<td>Ketoacidosis</td>
<td>Hyperosmolar coma</td>
</tr>
<tr>
<td>Oral hypoglycemia drugs</td>
<td>Unresponsive</td>
<td>Responsive</td>
</tr>
<tr>
<td>Treatment with insulin</td>
<td>Always</td>
<td>Usually not required</td>
</tr>
</tbody>
</table>

**Type I (Insulin-Dependent) Diabetes (10% of cases)**
- Formerly called **juvenile diabetes**
- Caused by slow **autoimmune destruction of insulin-secreting pancreatic β-cells**
- Patients **require insulin** to live
- Life spans reduced due to complications resulting from the crude metabolic control provided by insulin injections
- Hyperglycemia leads to blindness
  - Degeneration of the retina
  - Cataract formation due to protein glucosylation
Type II

• Comprises > 90% of the diagnosed cases
• Affects almost 20% of individuals over age 65
• Characterized by an insensitivity to insulin (impaired recognition of insulin)
• Insulin levels are elevated, but affected individuals have low numbers of or defects in insulin receptors
• Transport of glucose into muscle, live and adipose tissue resudes significantly and despite abundant glucose, cells metabolically starved
• Respond by increased gluconeogenesis and catabolism of fat and protein

• Causes of Insulin Resistance in type II Diabetics:
  1. Defects in signal transduction
  2. Abnormal insulin or insulin receptors
  3. Defects in glucose transporters
  4. It is also thought that insulin resistance is caused by obesity as insulin response in type II diabetics dramatically improves with weight reduction.
  5. Postulated that in obese individuals there is a constant elevated level of insulin because of high carbohydrate intake.
     • Thought to cause beta cell destruction
     • Decrease the sensitivity of insulin receptors in the target organs
     • Increased insulin production resulting from overeating may suppress insulin receptor synthesis

Insulin Resistance

Complication of obesity and type 2 diabetes

★ Mildly increased plasma glucose
★ Normal or increased plasma insulin
  ➤ Tissue insensitivity to insulin

★ Several levels of defects

★ Pre-receptor: rare
  ➤ Defect: insulin receptor antibodies, abnormal molecule

★ Receptor:
  ➤ Decreased number or affinity of insulin receptors
Post-receptor: Most probable site of insulin resistance in diabetes
  - Defects in intracellular signal transduction, decreased activity of key enzymes such as pyruvate dehydrogenase or glycogen synthase

Glucose transport: To be established
  - Deficient or defective glucose transporters

- The primary treatment is exercise and diet.
  - The main goal with type II diabetes treatment is to keep the glucose concentration within the normal limits. In order to do this, the patient is asked to maintain a strict diet which is low in carbohydrates and weight reduction is encouraged by the doctor.

- Prevention:
  - Maintaining ideal body weight (weight management) and an active lifestyle may prevent the onset of type II diabetes in people at risk for the disease.

Drug Treatment:

1. **Sulfonylurea drugs** – enhance insulin secretion (e.g. glyburide)
2. **α-glucosidase inhibitors**: inhibits the digestion of ingested carbohydrates, thereby resulting in a smaller rise in blood glucose concentration following meals. Results in delayed glucose absorption. Effect is additive to that of sulfonylureas when used in combination.