Lecture 33: Fatty Acid Oxidation, Electron transport, ATP synthesis

Fatty Acid Oxidation (β-Oxidation):

A. Formation of Acyl-CoA (Cytosol): The fatty acids in the cytosol are coupled to coenzyme A to form acyl-CoA. The activation reaction is catalyzed by acyl-CoA synthetase and involves the hydrolysis of ATP to AMP, i.e., the equivalent of two high energy ATP molecules (60 kJ/mol). The released pyrophosphate is hydrolyzed to inorganic phosphate, making the overall ΔG negative for the reaction (indirect coupling).

It is only necessary to utilize ATP once in the activation of the fatty acid.

B. Transport into mitochondria: The acyl-CoA is transported into the mitochondrial matrix for oxidation. This location is ideal for funneling the products of β-oxidation (NADH and FADH₂) to E. transport.

C. β-Oxidation (Mito. matrix): Acyl-CoA is shortened 2 carbons at a time from the carboxyl end of the fatty acid using the following steps:

1. Formation of trans α-β double bond by acyl-CoA dehydrogenase, an FAD enzyme.

2. Addition of water to the newly formed double bond to generate the alcohol by enoyl-CoA hydratase.

3. Oxidation of the alcohol by NAD⁺ to give the ketone, catalyzed by 3-oxoacyl-CoA thiolase (thiolysis).

4. Cleavage reaction by β-ketoacyl-CoA thiolase (thiolysis), generates acetyl-CoA and an acyl-CoA that is two carbons shorter. The CoA enters the TCA cycle.

5. Steps 1-4 are repeated until only acetyl-CoA remains. The last cycle produces two acetyl-CoA.

Synthesis of fatty acids. Fatty acids can be synthesized from acetyl CoA, two carbons at a time. Pyruvate that is obtained from glycolysis can be used to make fatty acids, after its conversion to acetyl-CoA. Unfortunately, humans lack the ability to generate pyruvate from acetyl-CoA. Consequently, pyruvate dehydrogenase is tightly regulated to reduce “carbon leakage" from pyruvate to acetyl-CoA because once the carbon from glucose becomes acetyl-CoA, it cannot be returned to glucose.
Electron Transport:
- The energy captured in glycolysis, TCA cycle, and fatty acid oxidation on NADH and FADH₂ is converted to a proton gradient across the inner mitochondrial membrane.
- *The energy stored in this gradient is used to produce ATP.*
- In most organisms the electrons from NADH and FADH₂ are deposited on oxygen, reducing it to water. Note that the oxygen only serves as a final acceptor of electrons in this process.
- In many organisms other compounds besides oxygen can serve as electron sinks, allowing organisms to perform 'oxidative' phosphorylation in the absence of O₂.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>NAD⁺/NADH</th>
<th>FAD/FADH₂</th>
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<tbody>
<tr>
<td>Glycolysis</td>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
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<tr>
<td>TCA cycle</td>
<td>Pyruvate dehydrogenase</td>
<td>Succinate dehydrogenase</td>
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<td>Isocitrate dehydrogenase</td>
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<td>α-ketoglutarate dehydrogenase</td>
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<td></td>
<td>Malate dehydrogenase</td>
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<td>Fatty Acid Ox,</td>
<td>Hydroxyacyl-CoA dehydrogenase</td>
<td>Acyl-CoA dehydrogenase</td>
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<td>Within above</td>
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<tr>
<td>pathways</td>
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<tr>
<td>Electron Transport</td>
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<tr>
<th>The oxidation of NADH releases a lot of energy:</th>
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<tr>
<td>Oxidation of NADH</td>
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<tr>
<td>Reduction of oxygen</td>
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<tr>
<td>Tot. Reaction</td>
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</table>

~ 50% is used to make ATP: (NADH = 3 ATP)
Key Components in Electron Transfer:
1. Inorganic carriers of electrons
   a) Iron-sulfur centers (e.g. succinate dehydrogenase)

\[ \text{Fe}^{2+} \rightleftharpoons \text{Fe}^{3+} \]

2. Organic Carriers of electrons:
   Coenzyme Q. Coenzyme Q is a non-polar electron carrier that diffuses freely in the fluid mitochondrial membrane. R group is non-polar.
   - Can participate in one or two electron redox transactions, two electron reduction shown on the right

**Electron Transport: Gibbs Energy & Flow**

- NADH → Complex I
- Succinate → Complex IIA [FADH2]
- Fatty acid ox. → Complex IIB [FADH2]
- Complex IIA = succinate dehydrogenase (TCA cycle)
- Complex IIB = acyl-CoA dehydrogenase (fatty acid oxidation)

\[ \text{Complex II} \rightarrow \text{Complex III} \rightarrow \text{Cytochrome C} \rightarrow \text{Complex IV} \]

\[ \Delta G < 0 \text{ for every step} \]

\[ 2\text{H}^+ \rightarrow \text{H}_2 \]

\[ \text{Reduction of Coenzyme Q (ubiquinol)} \]

**Glucose**
- Cytosol → Acyl-CoA
- Pyr → Outer membrane

**Inner membrane**
- Cytochrome C
- FeS
- IIa
- III
- IV
- IIb

**Mito Matrix**
- FADH
- FAD
- NAD
- NADH

**Oxygen**
- \[ \text{O}_2 \rightarrow 2\text{H}_2\text{O} \]

**Gibbs Energy**
- \[ \text{NADH} \rightarrow \text{NAD}^+ \sim 10 \text{protons} \]
- \[ \text{FADH}_2 \rightarrow \text{FAD} \sim 6 \text{protons} \]
Complex I: NADH-CoQ oxidoreductase
- Four protons/NADH are pumped from the inside (matrix) to the intermembrane space.

Complex II: Succinate-CoQ oxidoreductase
- Succinate dehydrogenase of the citric acid cycle is part of this complex.
- Two electrons from FADH₂ are transferred to CoQ via Fe-S clusters, generating CoQH₂.
- Does not pump any protons.

Complex III: CoQH₂-cytochrome c oxidoreductase
- Transfers electrons from CoQH₂ to cytochrome c one electron at a time.
- Four protons are pumped/NADH or FADH₂

Cytochrome C: Shuttles one electron from III to IV.

Complex IV: Cytochrome c oxidase
- Accepts 4 e⁻, one at a time from cytochrome c.
- Donates a total of four electrons/O₂.
- Site of oxygen reduction to water.
  i) Produces 2 water molecules/O₂ molecule.
  ii) Pumps an additional two protons/NADH or FADH₂.

Energy Stored in the Proton Gradient
The energy 'stored' in a concentration gradient can be considered to consist of two parts: \( \Delta G_{\text{TOTAL}} = \Delta G_{\text{CONC}} + \Delta G_{\text{ELEC}} \)

Defining the reaction direction from inter-membrane space (out) to the matrix (in):

i) The Gibbs energy due to a concentration difference across a sealed membrane. The Gibbs energy is:

\[
\Delta G = \Delta G^0 + RT \ln \frac{[X_{\text{IN}}]}{[X_{\text{OUT}}]} = (\mu_{X_{\text{IN}}}^{\text{0}} - \mu_{X_{\text{OUT}}}^{\text{0}}) + RT \ln \frac{[X_{\text{IN}}]}{[X_{\text{OUT}}]}
\]

Since the standard chemical potential \( \mu_0 \) for the species \([X]\) is the same on both the inside and the outside of the membrane:
This is the amount of energy that is released when the concentration gradient moves towards equilibrium.

ii) Movement of a charged particle through a voltage difference. The free energy associated with moving a particle of charge \( Z \), through a voltage difference \( \Delta \Psi (= \Delta V) \), is:

\[
\Delta G_{\text{ELEC}} = ZF \Delta \Psi
\]

- \( Z \) = the charge on the transported ion (+1 in the case of the proton)
- \( F \) = Faraday's constant, 96,494 C/mol. e⁻=coulomb
- \( \Delta \Psi \) is the voltage difference across the membrane, in volts. This voltage difference is often referred to as the membrane potential: \( \Delta \Psi = V_{\text{IN}} - V_{\text{OUT}} \)

The total Gibbs free energy is the sum of these two terms:

\[
\Delta G_{\text{TOTAL}} = RT \ln \frac{[X_{\text{IN}}]}{[X_{\text{OUT}}]} + ZF \Delta \Psi
\]

Example Calculation: Typical values across the inner mitochondrial membrane are:
\([H+]_{\text{IN}}/\text{mol} = 0.1 \text{ (pH=6.5 outside, 7.5 inside), voltage difference } -0.15 \text{ V, inside negative.}
\Delta G = (8.31)(300)\ln(0.1) + (1)(96,000)(-0.150)
= -5.7kJ/mol - 14.4kJ/mol
= -20kJ/mol

\( \text{mechanism requires } 3H^+ / ATP \text{ synth.} \)
ATP Synthesis (Oxidative Phosphorylation):
ATP synthesis is attained by coupling the free energy of a proton gradient to the chemical synthesis of ATP. The enzyme that accomplishes this coupling is called ATP-synthase (also known as $F_{0}F_{1}$ATPase)
3 $H^{+}$ transported = 1 ATP synthesized

Structural Features:
1. The $F_{0}$ Complex
   - Membrane-spanning, multi-protein complex.
   - Responsible for coupling the movement of three protons to 120° rotations of the $\gamma$-subunit.
2. The $F_{1}$ Complex
   - Attached to $F_{0}$, it protrudes into the mitochondrial matrix.
   - Composed of five different subunits: $\alpha_{3}\beta_{3}\gamma\delta\varepsilon$
   - The $\gamma$ subunit is the shaft at the center of the $\alpha_{3}\beta_{3}$ disk. $\gamma$ rotates 120° every time 3 protons pass through the complex.
   - The $\beta$ subunits are asymmetric due to their interactions with the $\gamma$-subunit.
   - One conformation of the $\beta$ subunit has very low affinity for both ADP and ATP. Everything is released.
   - One conformation of the $\beta$ subunit has high affinity for ADP and P$i$.
   - One conformation of the $\beta$ subunit makes ATP lower in energy than ADP+$P_{i}$.

How the motor works:
- Every time three proton move through the complex, the $\gamma$ subunit rotates 120°.
- The rotation of $\gamma$ subunit changes the conformation of the $\beta$-subunits such that the Gibbs energy of the bound ADP + $P_{i}$ becomes higher than the energy of ATP, thus ATP forms spontaneously from the bound ADP and $P_{i}$.
- The newly-formed ATP is released with the transport of three additional protons.
- The actual synthesis, or formation of the bond between ADP and $P_{i}$, is catalyzed by conformational changes of the $\beta$-subunit that occur as a consequence of the rotation.
- Since all three $\beta$ subunits are functioning at the same time, the transport of 9 protons in a complete cycle produces 3 ATP.

<table>
<thead>
<tr>
<th>NADH</th>
<th>~10 protons pumped</th>
<th>~3 ATP</th>
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<tr>
<td>FADH$_{2}$</td>
<td>~6 protons pumped</td>
<td>~2 ATP</td>
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</table>
Summary of Metabolism:
- Carbohydrates
- Fatty Acids
- Amino Acids

Anaerobic Metabolism: (No O₂)
NAD⁺ is required as the electron acceptor in glycolysis. How is NAD⁺ regenerated when oxygen is present?

What happens to glycolysis if NAD⁺ cannot be regenerated?
Glycolysis off, No ATP produced.

Alternative electron acceptors can be used to regenerate NAD⁺:
- pyruvate → lactate
- pyruvate → ethanol.

Other Fates of Pyruvate
- Pyr can be converted to Acetyl CoA, a one way reaction in humans.
  - acetyl CoA can be oxidized by the TCA cycle.
  - acetyl CoA can be used to synthesize fatty acids, which then make triglycerides.
- Pyruvate can be converted to alanine in a one-step transaminase reaction.
- Pyruvate can be used to make oxaloacetate, to replace the carbons that are removed from the TCA cycle by anabolic processes, e.g. amino acid synthesis (oxaloacetate is also the first step in glucon eogenesis).

Cooperation between muscle and liver during exercise (Cori cycle).
- During intense exercise muscle cannot get sufficient oxygen to regenerate NAD⁺, it can only do glycolysis.
- Pyruvate is reduced to lactate, to regenerate NAD⁺ for glycolysis.
- The lactate travels to the liver, where it is oxidized to pyruvate, used to make more glucose, which is then returned to the muscle.