

Fatty Acid Metabolism II

Odd or even Oxidation - F. Knoop's experiment

Fatty Acid Oxidation (, and β -oxidation)

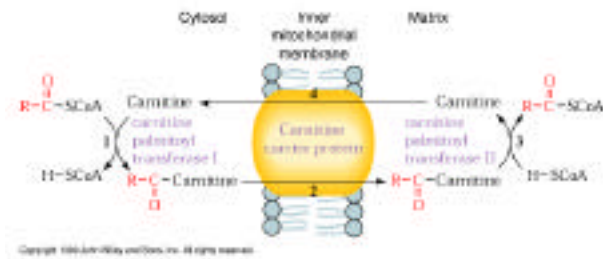
- Three phases to β oxidation
 - activation with Coenzyme A
 - transport into the mitochondria
 - oxidation of 2 carbon units

Activation Acyl CoA Synthetase aka thiokinase

- uses ATP (only energy requiring step)
- 2 part step forming acyl CoA and PPi (drives reaction)
- Mixed anhydride forms attack site for CoA

Transport

- FA greater than 12 C long need to be transported into mito
- three step reaction 2 transferases and a translocase
- carnitine replaces CoA
- has nearly the same E of hydrolysis and acylCoA
- little change of energy during translocation
- cell keeps separate pools of CoA



β -oxidation of fatty acids

occurs in the mitochondria (inner membrane)
subtracts 2 carbons from COO- end of β carbon of FA
four steps :

1. oxidation -> acyl-CoA dehydrogenase
 - FAD linked via electron transferring protein
 - feeds to site 2 in ETS
 - double bond formed between C 2 & 3
 - acyl-CoA dehydrogenase isozymes
3. hydration -> enoyl CoA hydratase
 - puts water across 2 carbon

4. oxidation -> β hydroxyacyl CoA dehydrogenase
 - NADH₂ linked oxidation of OH from last step
 - NADH₂ free to be oxidized at site 1 of ETS
 - specific for the L isomer

5. thiolase -> β keto thiolase
 - similar reaction to hydrolysis uses SH group of CoA
 - leads to new acylCoA (minus 2 carbons)

steps 1 thru 3 are similar to part of the TCA
 (succinate->fumarate->malate->oxalacetate)
 Repeat cycle until two or 3 Carbons (1 acyl CoA) are left

Energetics of β oxidation

palmitate (C₁₆) - CoA + 7 CoASH + 7 FAD + 7 NAD⁺ + 7 H₂O
 --> 8 acetyl CoA + 7 FADH₂ + 7 NADH

The net yield in ATP currency is:

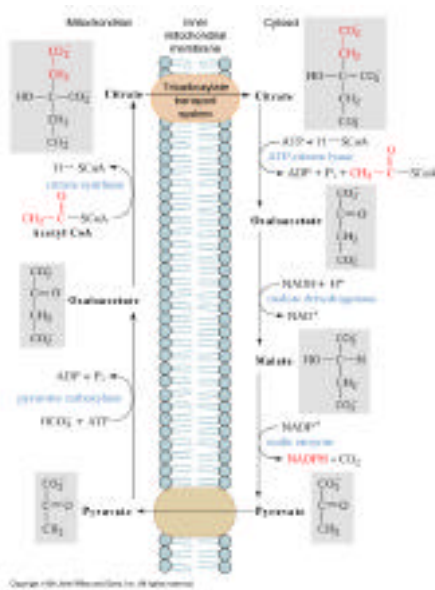
7 FADH - 14 ATP	8 Acyl-CoA	8 NADH = 72 ATP
7 NADH - 21 ATP		8 FADH = 16 ATP
		8 GTP = 8 ATP

with 100% efficiency - 131 ATP
 activation costs of 1 fatty acid - 2 ATP (?)
 total ATPs from palmitate 107 to 129

Biosynthesis of fatty acids

This pathway occurs in the cytosol. 2 carbon are added at a time to produce acetyl CoA. The precursors are from glucose and amino acids. This is distinct from β oxidation- it is a reductive process and uses NADPH. It takes place in the cytosol. A 3 carbon acid malonyl-CoA as the 2 carbon donor. The growing chain is attached to an acyl-carrier protein rather than Co-A. The enzymes are not shared between either

Acetyl CoA are produced by pyruvate carboxylase from both glycolytic and TCA intermediates. Acetyl CoA, which is generated by pyruvate dehydrogenase (PDH) in the mitochondrion cannot cross the inner mitochondrial membrane to reach the cytosol. Instead transport occurs by the tricarboxylate transport system.



Acetyl CoA transport

Acetyl CoA produced in mito - transported out as citrate

glucose \rightarrow pyruvate \rightarrow OAA and Acetyl CoA
 - pyruvate carboxylase and pyruvate dehydrogenase

OAA and Acetyl CoA \rightarrow Citrate (in mito)
 citrate transfer into cytosol and cleaved to Acetyl CoA and OAA by ATP citrate lyase - cost of ATP to transport

- OAA shuttled back into mito via MDH, Malic enzyme (NADPH)

- pyruvate transport into mito converted to OAA
 - produces both 1 NADPH /and Acetyl CoA

Biosynthesis-

Two phases - malonyl CoA formation and FA

complex

1) Production of malonyl CoA

Acetyl CoA Carboxylase active as a polymer (highly regulated step)

- 1st committed step in fatty acid synthesis (FAS)
- biotin cofactor - important point in activated one carbon carriers
- Highly regulated step

allosteric effectors and covalent modification citrate activates by increasing the V_{max} , where long chain FAs inhibit. AMP kinase inactivates (think of why) and insulin activates a phosphatase which relieves this inhibition by promoting the dephosphorylation of ACoA carboxylase

2) Fatty acid synthesis

transacylase

- the first reaction starts with a malonyl CoA and an ACoA
- transfer of acyl CoAs to ACP
- ACP - acyl carrier protein (derivative of CoA)
- long flexible arms w/ sulfhydryls
- allows flexibility to different active sites

condensation

- acetyl CoA + malonyl CoA
- loss of CO_2 drives reaction (from ATP hydrolysis)
- ACoA added onto malonyl CoA
- forms new 2 carbon acetoacetyl group

reductions

- NADPH required
- similar reactions to reversal of β oxidation
- end up with a new +2 carbon acyl - ACP group

continuation reactions

- the above is one full round of fatty acid synthase
- reactions continues 7 times until 16 carbon chain is formed (palmitate)
- termination - by thiolase

Two types of FAS systems bacterial and higher organisms. In *E. coli*, this takes place by several successive different enzymatic reactions. In eukaryotic cells, it takes place on one enzyme with several catalytic activities.

Reactions. The benefits of multienzyme systems:

- channeling of substrates - faster synthesis
- no loss of intermediates to side reactions

Source of reducing equivalents

pentose phosphate pathway

malic enzyme - 1 per acetyl CoA transport

Energetics of FA synthesis

$8 \text{ acyl CoA} + 7 \text{ ATP} + 14 \text{ NADPH} + 6\text{H}^+ \rightarrow$

$\text{palmitate (C}_{16}\text{)} + 8 \text{ CoASH} + 14 \text{ NADP}^+ + 6 \text{ H}_2\text{O} + 7 \text{ ADP/ Pi}$

The net yield in ATP currency is from mito is

- 8 ATP - citrate lyase
- 7 ATP - Acetyl CoA Carboxylase
- 7 rounds of FAS x NADPH \rightarrow 42 ATP

total of 57 ATP to make palmitate / 129 to break down palmitate

Fruity smelling breath - Ketone Bodies

Ketogenesis - Acetyl-CoA forms acetoacetate under high fatty acid oxidation and or prolonged starvation

- Acetoacetate can spontaneously decarboxylate into acetone OR reduce to β hydroxybutyrate
- These are collectively called ketone bodies
- Acetoacetate and β hydroxybutyrate serve as alternative fuel sources by the brain and muscle

Synthesis

- Acetoacetyl-CoA forms by the condensation of two acetyl-CoA - thiolase in reverse
- A third Acetyl CoA is added by another condensation reaction to form HMG-CoA (β -hydroxy- β -methylglutaryl-CoA) - part of cholesterol synthesis
- HMG CoA is lysed to form acetyl CoA and acetoacetate

Acetoacetate and β HG formed in the liver are transported in the bloodstream to peripheral tissues and there are converted into two acetyl CoA units. This is still inefficient because succinyl CoA is used and therefore is not available for STK formation of GTP in the kreb's cycle.