Topic: Fatty acid Catabolism Part II

B.Sc (H) Zoology

Sem IV

Paper: Biochemistry of Metabolic Processes

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Oxidation of Fatty acids

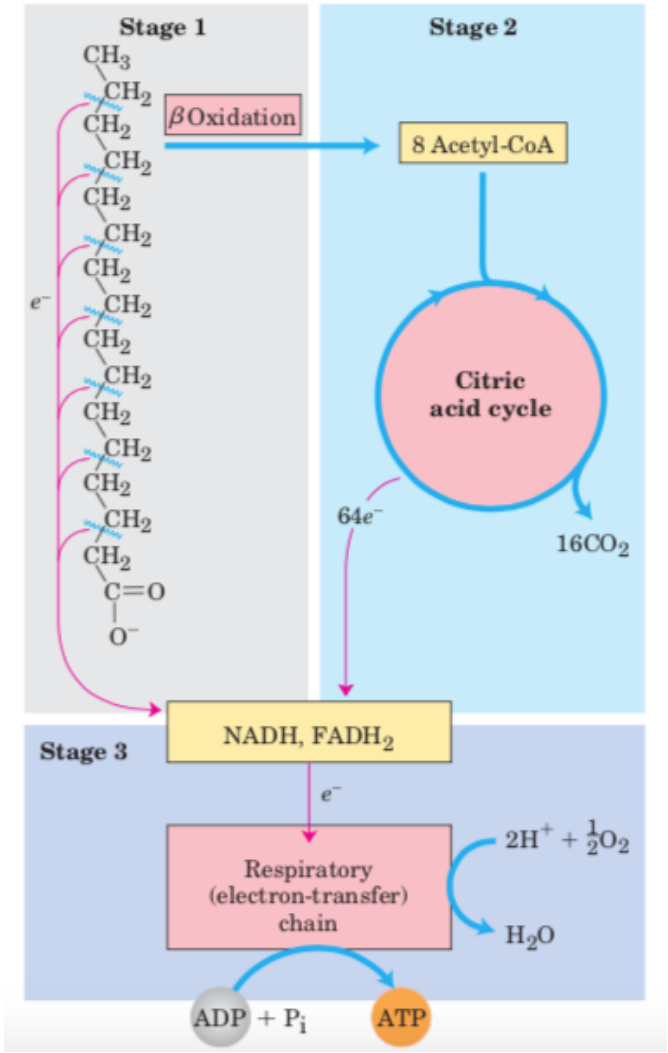
- Fatty acid oxidation takes place in three steps:

Stages of fatty acid oxidation.

Stage 1: A long-chain fatty acid is oxidized to yield acetyl residues in the form of acetyl-CoA. This process is called beta oxidation.

Stage 2: The acetyl groups are oxidized to CO_2 via the citric acid cycle.

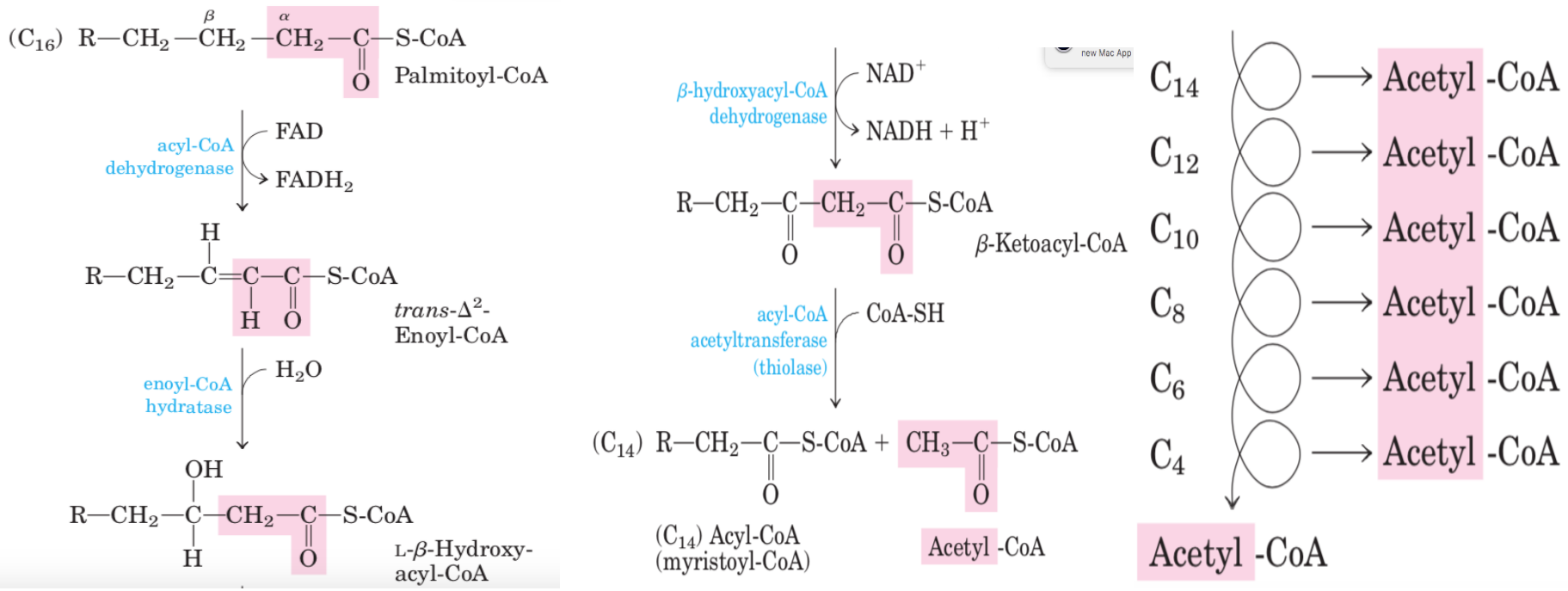
Stage 3: Electrons derived from the oxidations of stages 1 and 2 pass to O_2 via the mitochondrial respiratory chain, providing the energy for ATP synthesis by oxidative phosphorylation.



- In the first stage—beta oxidation—fatty acids undergo oxidative removal of successive two-carbon units in the form of acetyl-CoA, starting from the carboxyl end of the fatty acyl chain. For example, the 16-carbon palmitic acid (palmitate at pH 7) undergoes seven passes through the oxidative sequence, in each pass losing two carbons as acetyl-CoA. At the end of seven cycles the last two car- bons of palmitate (originally C-15 and C-16) remain as acetyl-CoA. The overall result is the conversion of the 16-carbon chain of palmitate to eight two-carbon acetyl groups of acetyl-CoA molecules. Formation of each acetyl-CoA requires removal of four hydrogen atoms (two pairs of electrons and four H^+) from the fatty acyl moiety by dehydrogenases.

- In the second stage of fatty acid oxidation, the acetyl groups of acetyl-CoA are oxidized to CO_2 in the citric acid cycle, which also takes place in the mitochondrial matrix. Acetyl-CoA derived from fatty acids thus enters a final common pathway of oxidation with the acetyl-CoA derived from glucose via glycolysis and pyruvate oxidation.
- The first two stages of fatty acid oxidation produce the reduced electron carriers NADH and $FADH_2$, which in the third stage donate electrons to the mitochondrial respiratory chain, through which the electrons pass to oxygen with the concomitant phosphorylation of ADP to ATP. The energy released by fatty acid oxidation is thus conserved as ATP.

The β Oxidation of Saturated Fatty Acids Has Four Basic Steps



The Beta-oxidation pathway. (a) In each pass through this four-step sequence, one acetyl residue (shaded in pink) is removed in the form of acetyl-CoA from the carboxyl end of the fatty acyl chain— in this example palmitate (C_{16}), which enters as palmitoyl-CoA. **(b)** Six more passes through the pathway yield seven more molecules of acetyl-CoA, the seventh arising from the last two carbon atoms of the 16-carbon chain. Eight molecules of acetyl-CoA are formed in all.

- This first step is catalyzed by three isozymes of **acyl-CoA dehydrogenase**, each specific for a range of fatty-acyl chain lengths: very-long-chain acyl-CoA dehydrogenase (VLCAD), acting on fatty acids of 12 to 18 carbons; medium-chain (MCAD), acting on fatty acids of 4 to 14 carbons; and short-chain (SCAD), acting on fatty acids of 4 to 8 carbons. All three isozymes are flavoproteins with FAD as a prosthetic group.
- In the second step of the ß -oxidation cycle, water is added to the double bond of the *trans*-2- enoyl-CoA to form the L stereoisomer of ß**-hydroxyacyl- CoA (3-hydroxyacyl-CoA)**. This reaction, catalyzed by **enoyl-CoA hydratase**.
- In the third step, L- ß -hydroxyacyl-CoA is dehy- drogenated to form ß **-ketoacyl-CoA**, by the action of ß **-hydroxyacyl-CoA dehydrogenase**; NAD^+ is the electron acceptor. This enzyme is absolutely specific for the L stereoisomer of hydroxyacyl-CoA.
- The fourth and last step of the ß -oxidation cycle is catalyzed by **acyl-CoA acetyltransferase**, more com- monly called **thiolase**, which promotes reaction of ß - ketoacyl-CoA with a molecule of free coenzyme A to split off the carboxyl-terminal two-carbon fragment of the original fatty acid as acetyl-CoA. The other product is the coenzyme A thioester of the fatty acid, now short- ened by two carbon atoms. This reaction is called thiolysis, by analogy with the process of hydroly- sis, because the ß -ketoacyl-CoA is cleaved by reaction with the thiol group of coenzyme A.

- The last three steps of this four-step sequence are catalyzed by either of two sets of enzymes, with the en- zymes employed depending on the length of the fatty acyl chain. For fatty acyl chains of 12 or more carbons, the reactions are catalyzed by a multienzyme complex associated with the inner mitochondrial membrane, the **trifunctional protein (TFP)**. TFP is a heterooctamer of alpha4 ß 4 subunits. Each subunit contains two activities, the enoyl-CoA hydratase and the ß - hydroxyacyl- CoA dehydrogenase; the ß subunits contain the thiolase activity. This tight association of three enzymes may al- low efficient substrate channeling from one active site to the next, without diffusion of the intermediates away from the enzyme surface. When TFP has shortened the fatty acyl chain to 12 or fewer carbons, further oxida- tions are catalyzed by a set of four soluble enzymes in the matrix.
- As noted earlier, the single bond between methyl- ene (—CH2—) groups in fatty acids is relatively stable. The beta-oxidation sequence is an elegant mechanism for destabilizing and breaking these bonds. The first three reactions of beta oxidation create a much less stable C—C bond, in which the alpha carbon (C-2) is bonded to *two* carbonyl carbons (the beta-ketoacyl-CoA intermediate). The ketone function on the beta carbon (C-3) makes it a good target for nucleophilic attack by the—SH of coenzyme A, catalyzed by thiolase.

- The four beta oxidation steps are repeated to yield acetyl CoA and ATP
- In one pass through the beta-oxidation sequence, one mol- ecule of acetyl-CoA, two pairs of electrons, and four pro- tons (H^+) are removed from the long-chain fatty acyl–CoA, shortening it by two carbon atoms. The equation for one pass, beginning with the coenzyme A ester of our example, palmitate, is

$$\text{Palmitoyl-CoA} + \text{CoA} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \longrightarrow \text{myristoyl-CoA} + \text{acetyl-CoA} + \text{FADH}_2 + \text{NADH} + \text{H}^+$$

- Following removal of one acetyl-CoA unit from palmi- toyl-CoA, the coenzyme A thioester of the shortened fatty acid (now the 14-carbon myristate) remains. The myristoyl-CoA can now go through another set of four beta- oxidation reactions, exactly analogous to the first, to yield a second molecule of acetyl-CoA and lauroyl-CoA, the coenzyme A thioester of the 12-carbon laurate. Alto- gether, seven passes through the beta-oxidation sequence are required to oxidize one molecule of palmitoyl-CoA to eight molecules of acetyl-CoA. The overall equation is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{FAD} + 7\text{NAD}^+ + 7\text{H}_2\text{O} \longrightarrow 8\ \text{acetyl-CoA} + 7\text{FADH}_2 + 7\text{NADH} + 7\text{H}^+ \qquad (17\text{–}3)$$

- In hibernating animals, fatty acid oxidation provides metabolic energy, heat, and water—all essential for survival of an animal that neither eats nor drinks for long periods. Camels obtain water to supplement the meager supply available in their natural environment by oxidation of fats stored in their hump.
- The overall equation for the oxidation of palmitoyl- CoA to eight molecules of acetyl-CoA, including the electron transfers and oxidative phosphorylations, is

$$\text{Palmitoyl-CoA} + 7\text{CoA} + 7\text{O}_2 + 28\text{P}_\text{i} + 28\text{ADP} \longrightarrow 8\ \text{acetyl-CoA} + 28\text{ATP} + 7\text{H}_2\text{O}$$

- **Acetyl-CoA Can Be Further Oxidized in the Citric Acid Cycle**

- The acetyl-CoA produced from the oxidation of fatty acids can be oxidized to CO2 and H2O by the citric acid cycle. The following equation represents the balance sheet for the second stage in the oxidation of palmitoyl-CoA, together with the coupled phosphorylations of the third stage:

$$8\ \text{Acetyl-CoA} + 16O_2 + 80P_i + 80\text{ADP} \longrightarrow 8\text{CoA} + 80\text{ATP} + 16CO_2 + 16H_2O$$

The overall equation for the complete oxidation of palmitoyl- CoA to carbon dioxide and water:

$$\text{Palmitoyl-CoA} + 23O_2 + 108P_i + 108\text{ADP} \longrightarrow \text{CoA} + 108\text{ATP} + 16CO_2 + 23H_2O$$

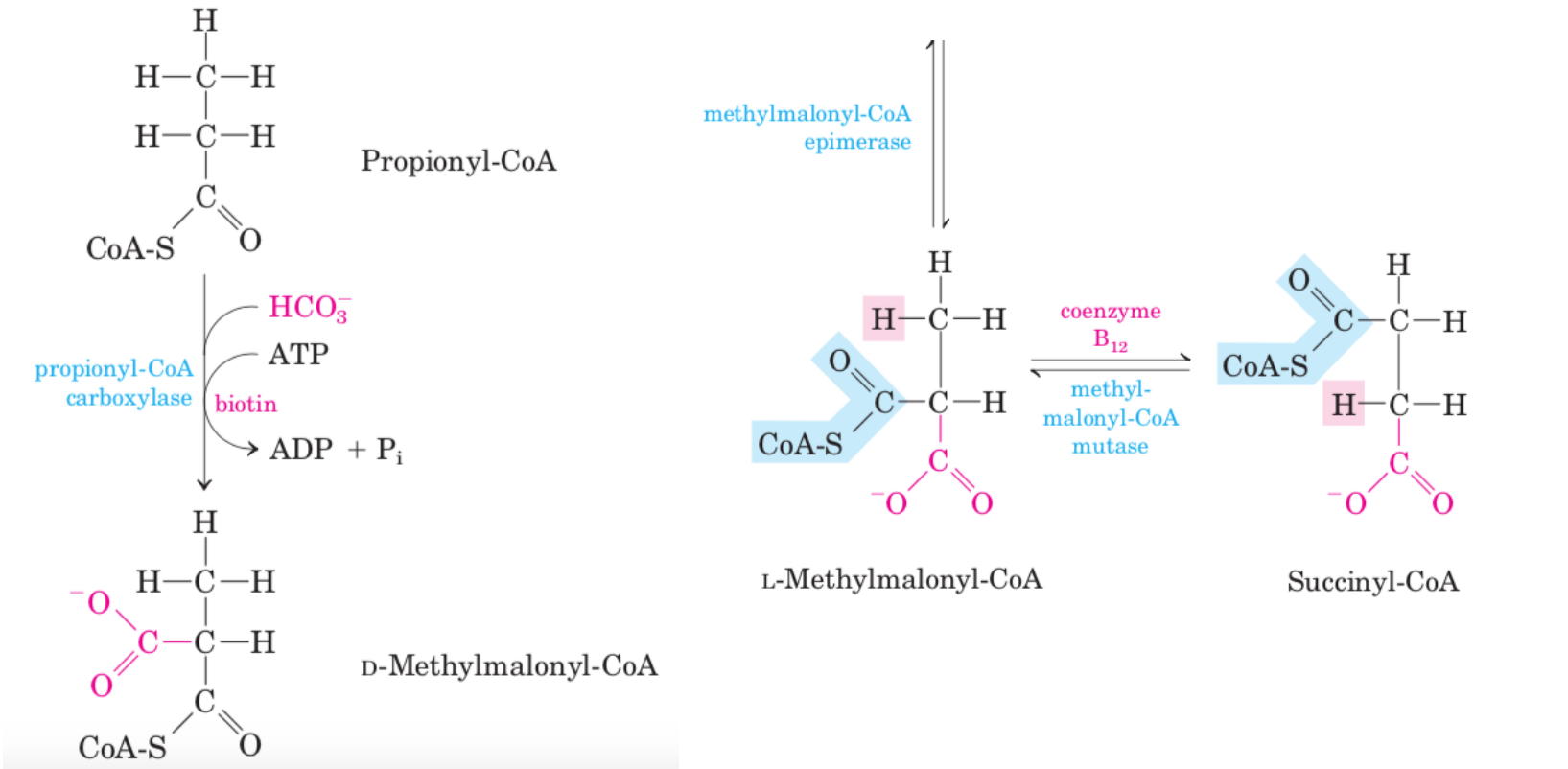
TABLE 17–1 Yield of ATP during Oxidation of One Molecule of Palmitoyl-CoA to CO_2 and H_2O

Enzyme catalyzing the oxidation step	Number of NADH or $FADH_2$ formed	Number of ATP ultimately formed*
Acyl-CoA dehydrogenase	7 $FADH_2$	10.5
β-Hydroxyacyl-CoA dehydrogenase	7 NADH	17.5
Isocitrate dehydrogenase	8 NADH	20
α-Ketoglutarate dehydrogenase	8 NADH	20
Succinyl-CoA synthetase		8†
Succinate dehydrogenase	8 $FADH_2$	12
Malate dehydrogenase	8 NADH	20
Total		108

*These calculations assume that mitochondrial oxidative phosphorylation produces 1.5 ATP per $FADH_2$ oxidized and 2.5 ATP per NADH oxidized.

Complete Oxidation of Odd-Number Fatty Acids Requires Three Extra Reactions

- Although most naturally occurring lipids contain fatty acids with an even number of carbon atoms, fatty acids with an odd number of carbons are common in the lipids of many plants and some marine organisms. Cattle and other ruminant animals form large amounts of the three-carbon **propionate** ($CH_3—CH_2—COO^-$) during fermentation of carbohydrates in the rumen. The propi- onate is absorbed into the blood and oxidized by the liver and other tissues.
- Long-chain odd-number fatty acids are oxidized in the same pathway as the even-number acids, beginning at the carboxyl end of the chain. However, the substrate for the last pass through the beta-oxidation sequence is a fatty acyl–CoA with a five-carbon fatty acid. When this is oxidized and cleaved, the products are acetyl-CoA and **propionyl-CoA**. The acetyl-CoA can be oxidized in the citric acid cycle, of course, but propionyl-CoA enters a different pathway involving three enzymes.



Oxidation of propionyl-CoA produced by beta oxidation of odd-number fatty acids. The sequence involves the carboxylation of propionyl-CoA to D-methylmalonyl-CoA and conversion of the latter to succinyl-CoA. This conversion requires epimerization of D- to L-methylmalonyl-CoA, followed by a remarkable reaction in which substituents on adjacent carbon atoms exchange positions.

- Propionyl-CoA is first carboxylated to form the D stereoisomer of **methylmalonyl-CoA** by **propionyl-CoA carboxylase**, which contains the cofactor biotin.
- The D-methylmalonyl-CoA thus formed is enzymatically epimerized to its L stereoisomer by **methylmalonyl- CoA epimerase**
- The L-methylmalonyl- CoA then undergoes an intramolecular rearrangement to form succinyl-CoA, which can enter the citric acid cycle. This rearrangement is catalyzed by **methyl- malonyl-CoA mutase**, which requires as its coenzyme **5'deoxyadenosylcobalamin,** or **coenzyme B12**, which is derived from vitamin B12 (cobalamin).

Reference:

- Chapter 17: • Cox, M.M and Nelson, D.L. (2008).Lehninger’s Principles of Biochemistry. VEdition, W.H. Freeman and Co., New York.