King Saud University
College of Sciences
Biochemistry Department

الأيض (١)
Metabolism (1)
BCH 340

Lecture 11: Fatty acid oxidation

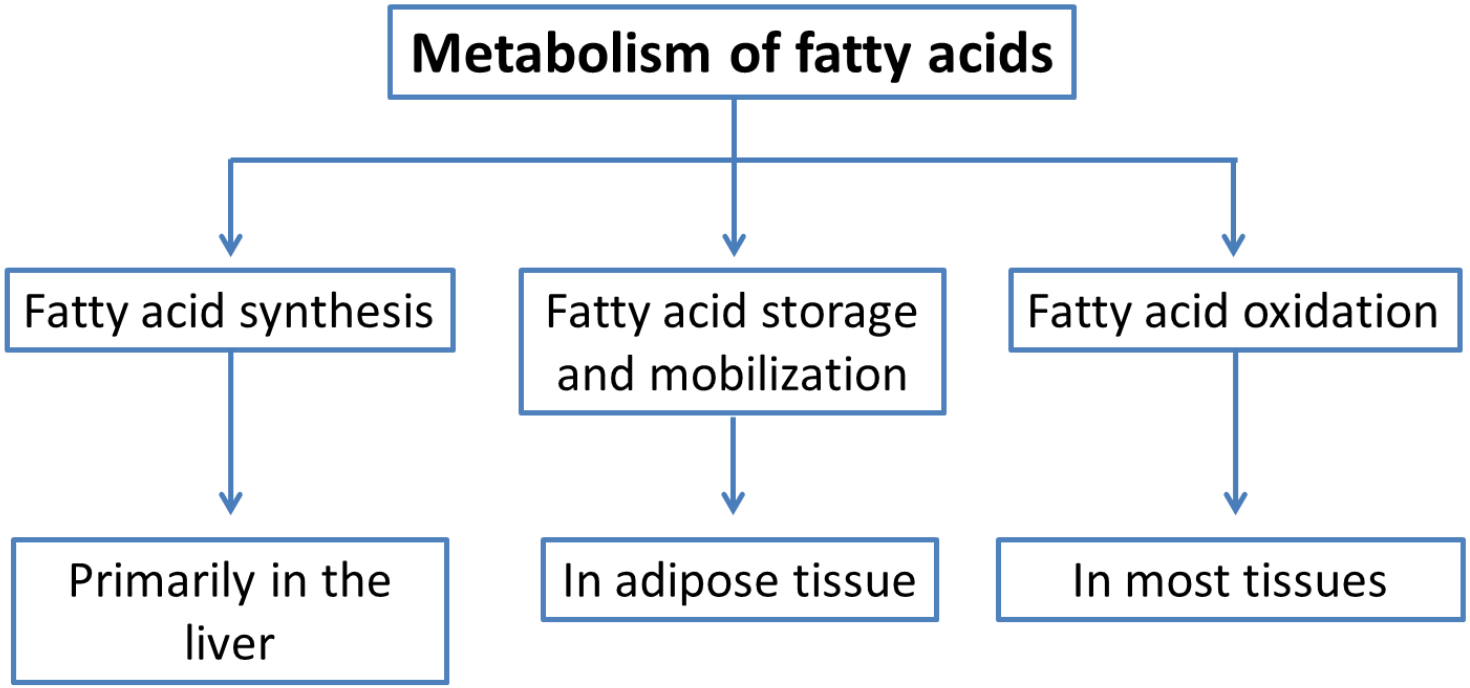
Intended learning outcomes (ILOs)

By the end of this lecture, students will be able to:

- Describe β-oxidation pathway.
- Differentiate between saturated and unsaturated fatty acid oxidation.
- Discuss different types of minor fatty acid oxidation pathways.

Fatty acid oxidation

- Fatty acid oxidation (β-oxidation) is the process by which fatty acids are broken down in the mitochondria to generate energy.
- Fatty acids oxidation is a major source of energy for many tissues, especially during periods of fasting or prolonged exercise when glucose levels are low.

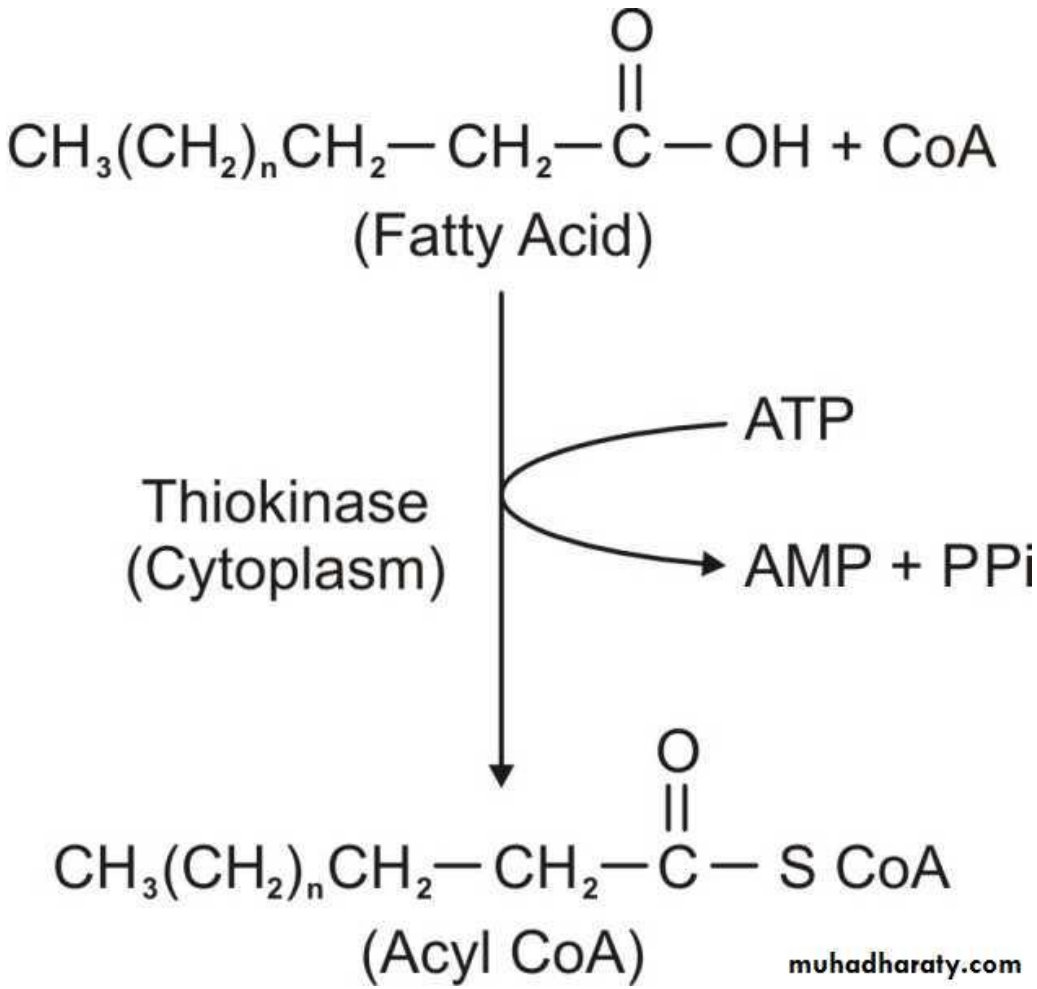


Activation and transport of fatty acid

Fatty acid activation:

- Before fatty acids can undergo oxidation, they need to be activated. This process occurs in the cytoplasm.
- Before they can enter the mitochondria for oxidation, fatty acids are activated by attaching coenzyme A (CoA) to form fatty acyl-CoA molecules in an ATP-dependent reaction.
- This reaction is catalyzed by an enzyme called fatty acyl-CoA synthetase (also known as fatty acid thiokinase).
- The resulting acyl-CoA can then be transported across the mitochondrial membrane into the mitochondrial matrix for further processing.

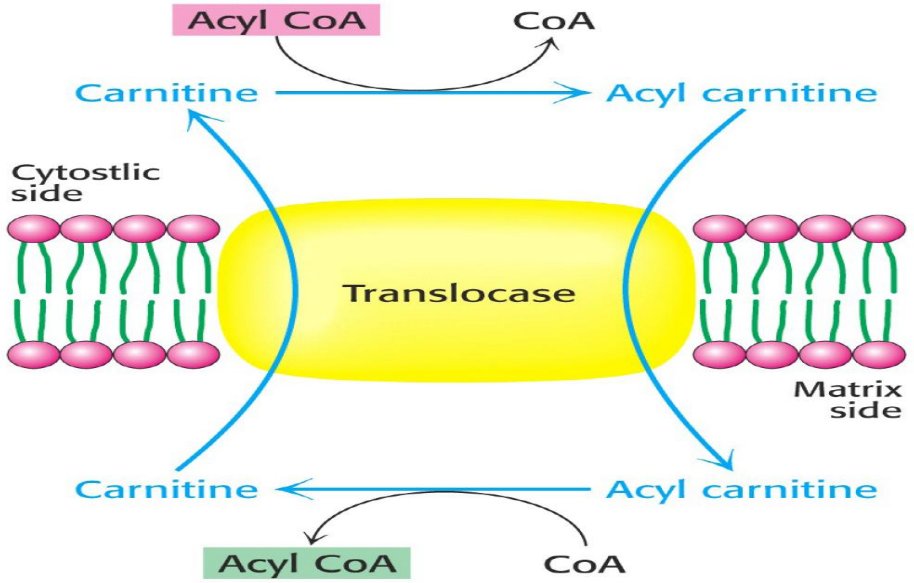
Activation of fatty acid by fatty acid thiokinase



Activation and transport of fatty acid (cont.)

Transport of activated fatty acyl-CoA:

- Once activated in the cytoplasm, long-chain fatty acyl-CoA molecules cannot directly enter the mitochondrial matrix (where β-oxidation takes place) due to their size and charge.
- The carnitine shuttle facilitates the transport of fatty acyl-CoA into the mitochondrial matrix.



Activation and transport of fatty acid (cont.)

Transport of activated fatty acyl-CoA (cont.):

- The fatty acyl-CoA is first converted into fatty acylcarnitine by carnitine palmitoyltransferase I (CPT-I) on the outer mitochondrial membrane.
- Fatty acylcarnitine is then transported across the mitochondrial membrane by a carnitine-acylcarnitine translocase.
- Once inside the mitochondrial matrix, another enzyme, carnitine palmitoyltransferase II (CPT-II), catalyzes the transfer of the fatty acyl group back to CoA, producing acyl-CoA once again.
- Acyl-CoA can then undergo β-oxidation to generate ATP through the TCA cycle and oxidative phosphorylation.

Fatty acid oxidation

- Fatty acid oxidation occurs in the mitochondrial matrix, where each cycle of the process consists of four main steps:

1. Oxidation:

- The fatty acyl-CoA is oxidized by FAD-dependent acyl-CoA dehydrogenase to form trans-Δ^2-enoyl-CoA. This reaction produces FADH2.

2. Hydration:

- Water is added to the trans-Δ^2-enoyl-CoA by enoyl-CoA hydratase, resulting in the formation of L-3-hydroxyacyl-CoA.

Fatty acid oxidation (cont.)

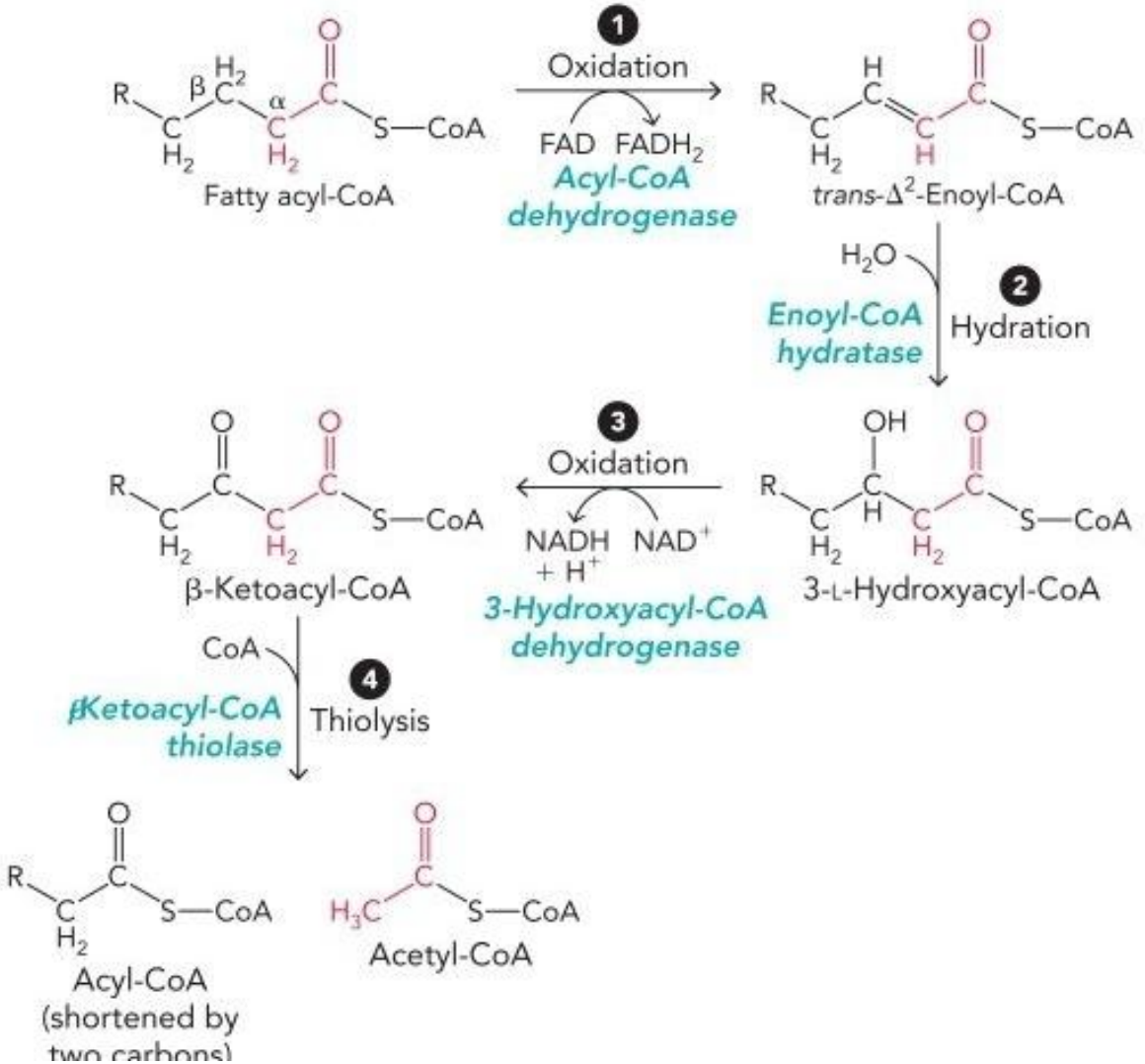
3. Oxidation (2nd oxidation):

- The L-3-hydroxyacyl-CoA is oxidized by NAD^+-dependent hydroxyacyl-CoA dehydrogenase to form β-ketoacyl-CoA. This reaction produces NADH.

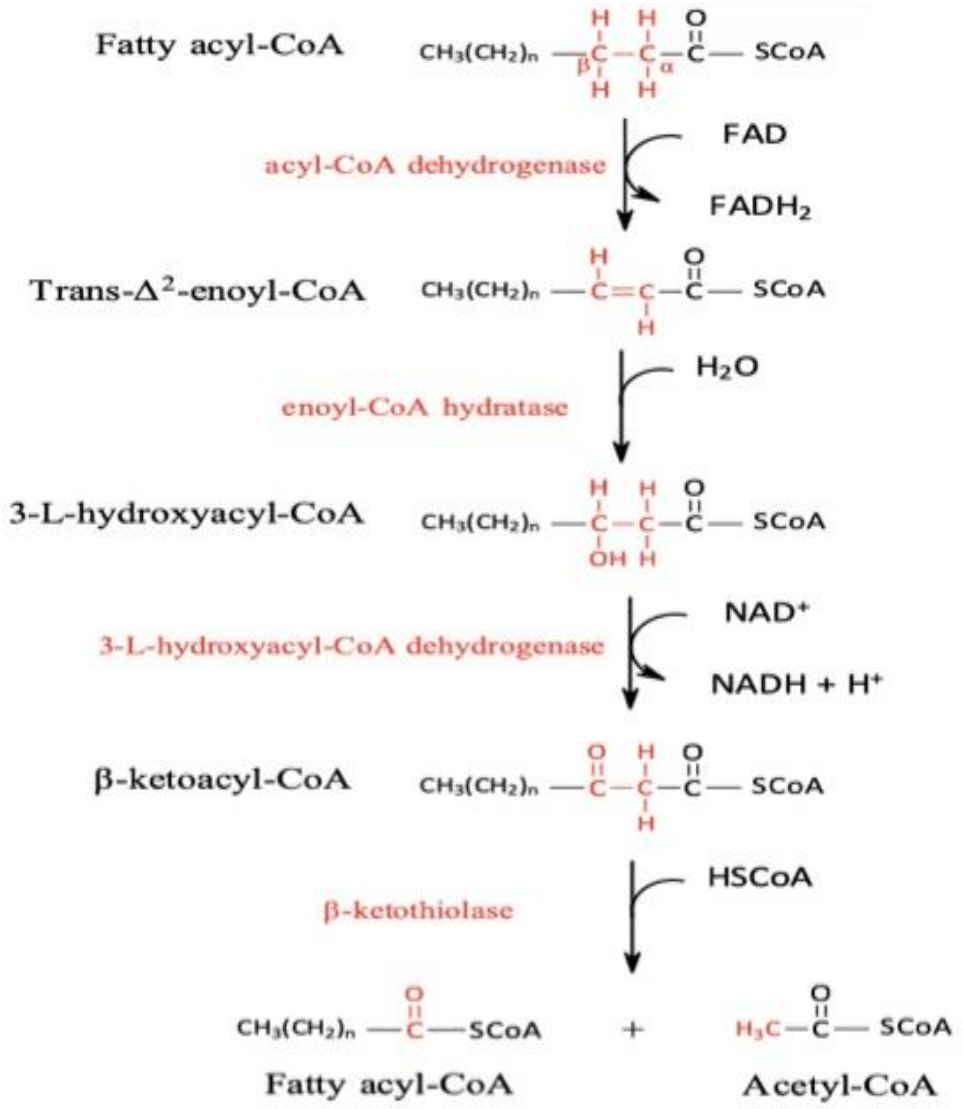
4. Thiolysis:

- The β-ketoacyl-CoA is cleaved by β-ketothiolase, resulting in the formation of acetyl-CoA and a new, shorter fatty acyl-CoA molecule.
- This shorter fatty acyl-CoA then undergoes another cycle of β-oxidation, starting the process again.

Fatty acid oxidation (β-oxidation)



Fatty acid oxidation (β-oxidation)



Fatty acid oxidation (cont.)

- The process of β-oxidation continues iteratively, with each cycle shortening the fatty acyl-CoA chain by two carbons and producing one molecule of FADH2, NADH, and acetyl-CoA.
- The acetyl-CoA molecules can then enter the TCA cycle, where they undergo further oxidation to produce reducing equivalents (NADH and FADH2) and GTP.
- The reducing equivalents generated during β-oxidation feed into the electron transport chain, where they drive ATP synthesis through oxidative phosphorylation, ultimately producing energy for the cell.

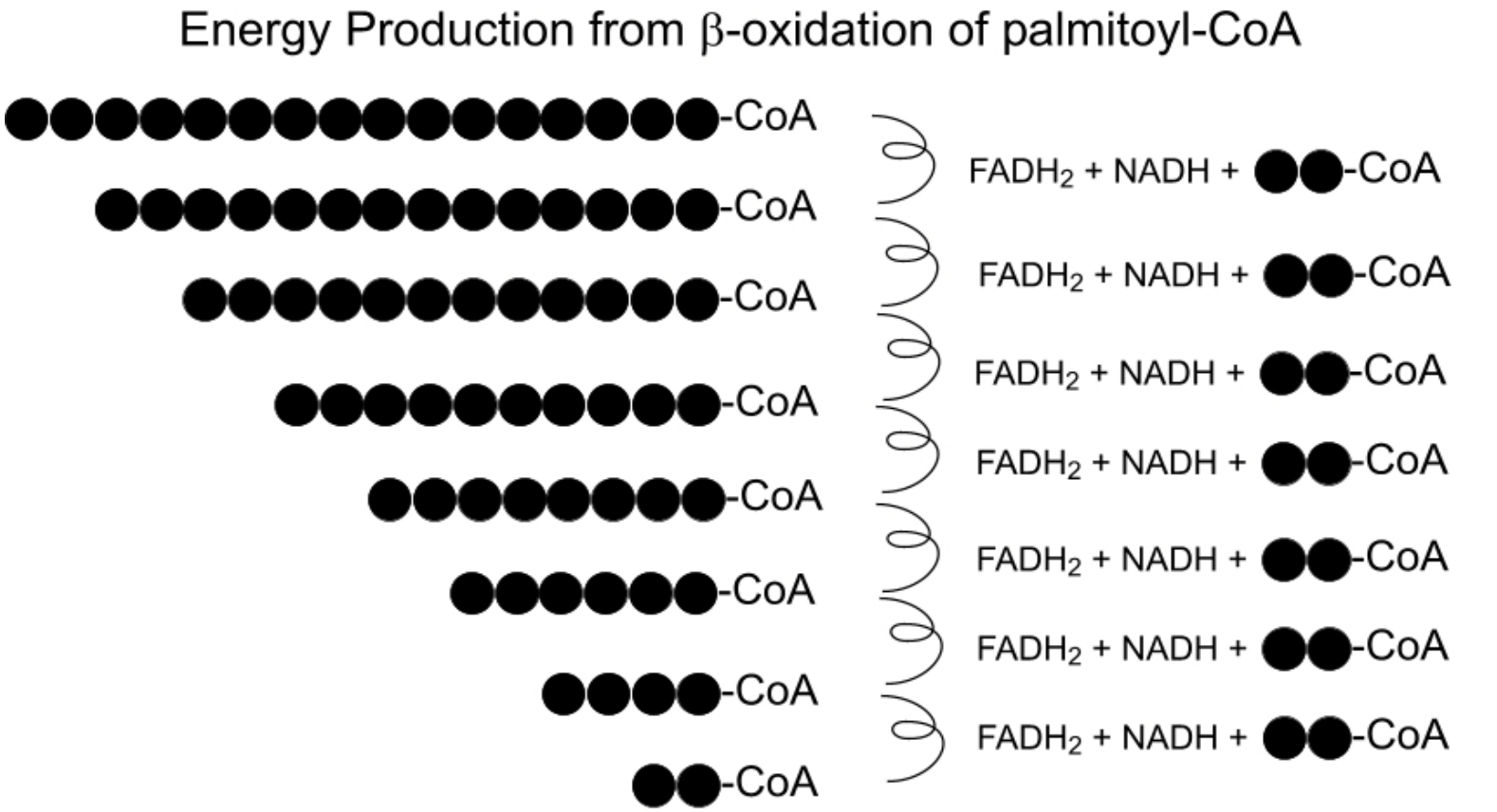
β-oxidation: energy production

- The amount of energy produced from β-oxidation can vary depending on factors such as:
  - The length of the fatty acid chain.
  - The number of double bonds.
- However, on average, β-oxidation of one molecule of a saturated fatty acid containing 16 carbon atoms generates around 129 ATP molecules.

Assignment:

Q1: Calculate the total energy generated from the complete oxidation of stearic acid ($C_{18}H_{36}O_2$).

Summary of β-oxidation: Palmitoyl-CoA



Total: palmitoyl-CoA ------> 7 FADH2 + 7 NADH + 8 AcCoA

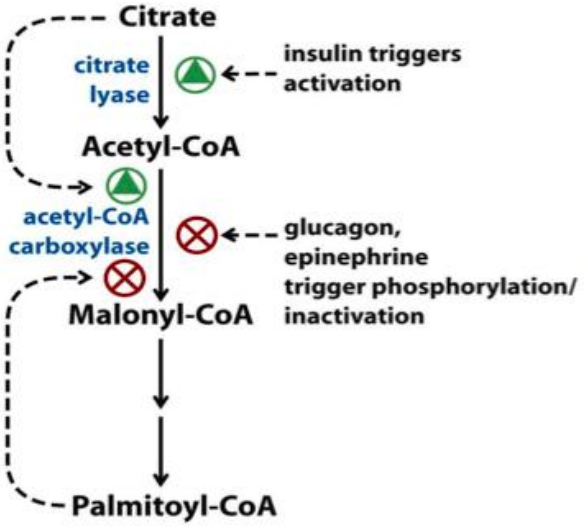
In ATP terms: (7 x 2) + (7 x 3) + (8 x 12) = 131 - 2 for charging fatty acid --> 129 ATPs net

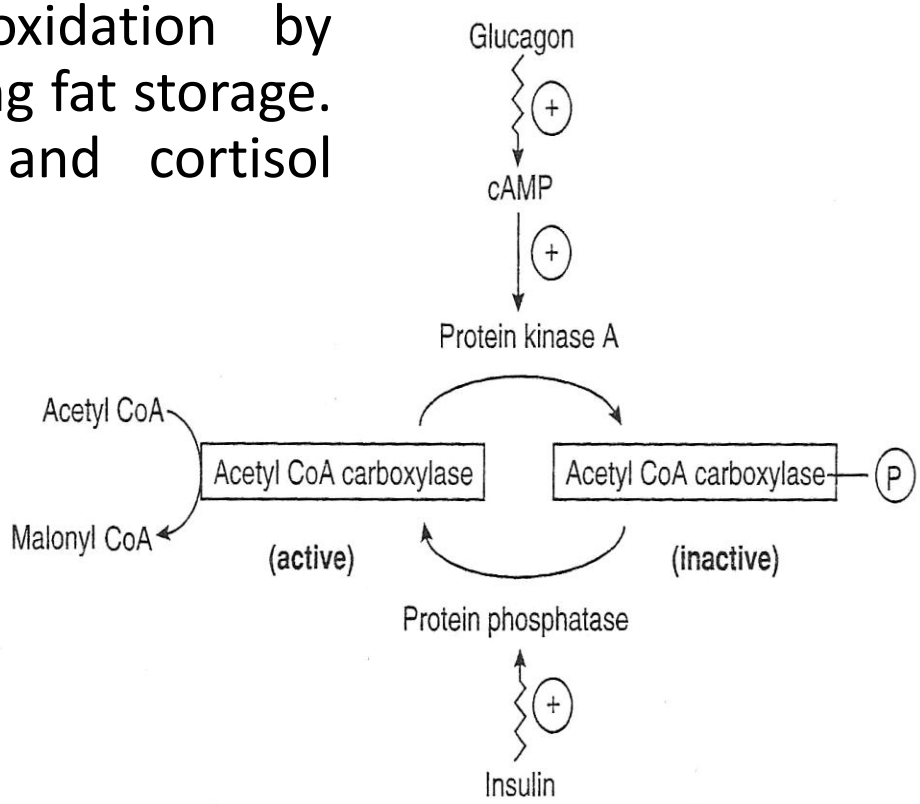
Regulation of fatty acid oxidation

- Fatty acid oxidation is tightly regulated to ensure energy balance and metabolic homeostasis in the body.

1. Hormonal regulation:

- Insulin inhibits fatty acid oxidation by inhibiting lipolysis and promoting fat storage. While glucagon, adrenaline, and cortisol stimulate it.

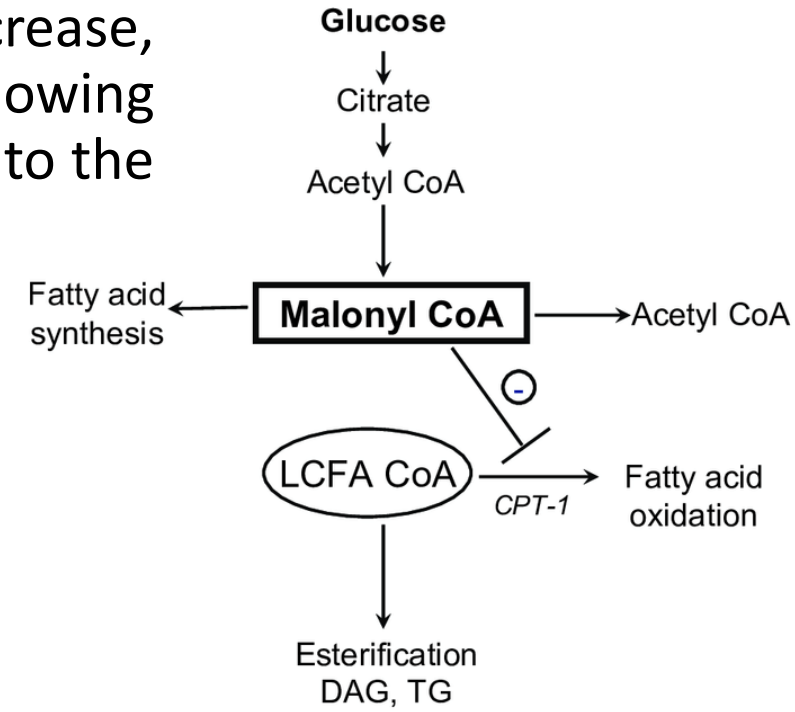




Regulation of fatty acid oxidation

2. Malonyl-CoA:

- Malonyl-CoA (a precursor for fatty acid synthesis) inhibits the rate-limiting step in carnitine shuttle system that is catalyzed by carnitine palmitoyltransferase I.
  - When energy demands are high, malonyl-CoA levels decrease, relieving this inhibition and allowing for increased fatty acid entry into the mitochondria.



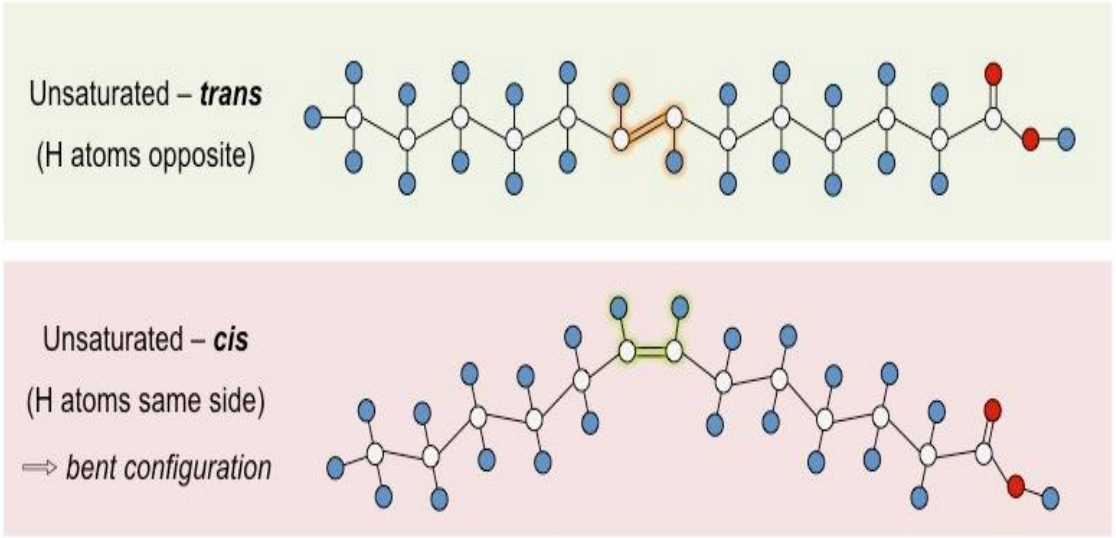
Regulation of fatty acid oxidation

3. Cellular energy level:

- Fatty acid oxidation is often upregulated when cellular energy levels are low.
  - This is mediated by the AMP-activated protein kinase (AMPK) pathway, which is activated in response to an increase in the AMP to ATP ratio.
  - AMPK activates pathways that stimulate fatty acid oxidation while inhibiting pathways that consume ATP.

Oxidation of unsaturated fatty acid

- Unsaturated fatty acids contain one or more double bonds in their carbon chain.
- The presence of double bonds alters the β-oxidation process compared to saturated fatty acids due to the presence of *cis* double bonds, which create a kink in the fatty acid chain.
  - This kink affects the action of the enzymes involved in β-oxidation.

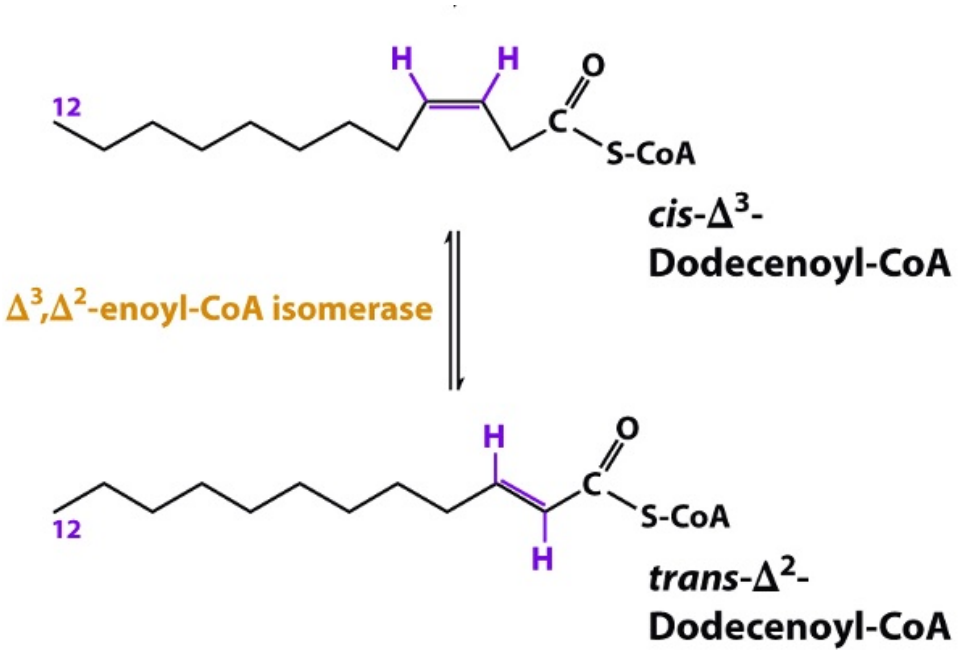


Oxidation of unsaturated fatty acid (cont.)

Introduction of a *trans* double bond:

- The presence of a *cis* double bond in unsaturated fatty acids requires additional enzymatic steps to rearrange the double bond to a *trans* configuration before β-oxidation can proceed.

- This is necessary because the enzymes involved in β-oxidation act on *trans* double bonds.
- The enzyme responsible for this is called enoyl-CoA isomerase.



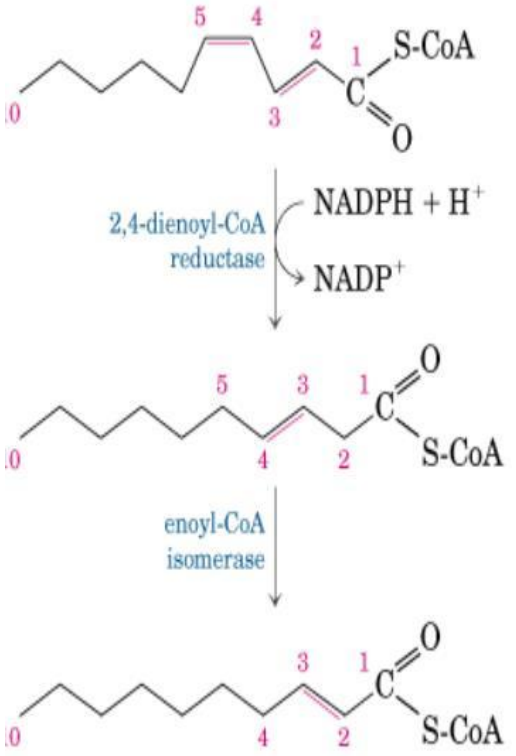
Oxidation of unsaturated fatty acid (cont.)

Action of 2,4-dienoyl-CoA reductase:

- In the case of unsaturated fatty acids with more than one double bond (such as di-unsaturated or polyunsaturated fatty acids), an additional enzyme called 2,4-dienoyl-CoA reductase is required.

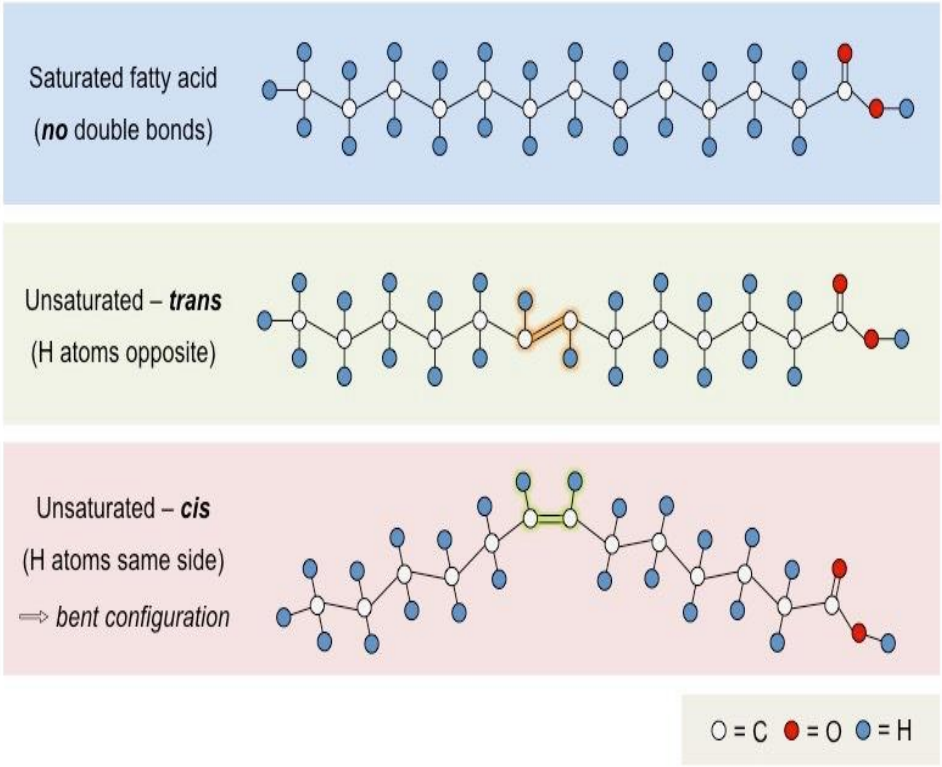
- This enzyme reduces the conjugated double bonds between positions 2 and 4 in the molecule to form a single *trans*-Δ^3 double bond.

- This *trans*-Δ^3 double bond is then converted by enoyl-CoA isomerase to a *trans*-Δ^2 double bond, a configuration that can continue through β-oxidation.



Oxidation of unsaturated fatty acid (cont.)

- Once the double bonds have been rearranged or reduced, β-oxidation proceeds similarly to that of saturated fatty acids, with the successive removal of two-carbon units until the entire fatty acid molecule is converted into acetyl-CoA molecules.



Oxidation of odd-numbered fatty acid

- The oxidation of odd-numbered fatty acids (found in plants and marine organisms) follows a similar pathway to even-numbered ones in β-oxidation, until the last round.

Activation step:

- Just like with even-numbered fatty acids, odd-numbered ones are activated in the cytosol by converting them into their corresponding acyl-CoA forms using ATP in a reaction catalyzed by acyl-CoA synthetase.

Oxidation of odd-numbered fatty acid (cont.)

β-Oxidation:

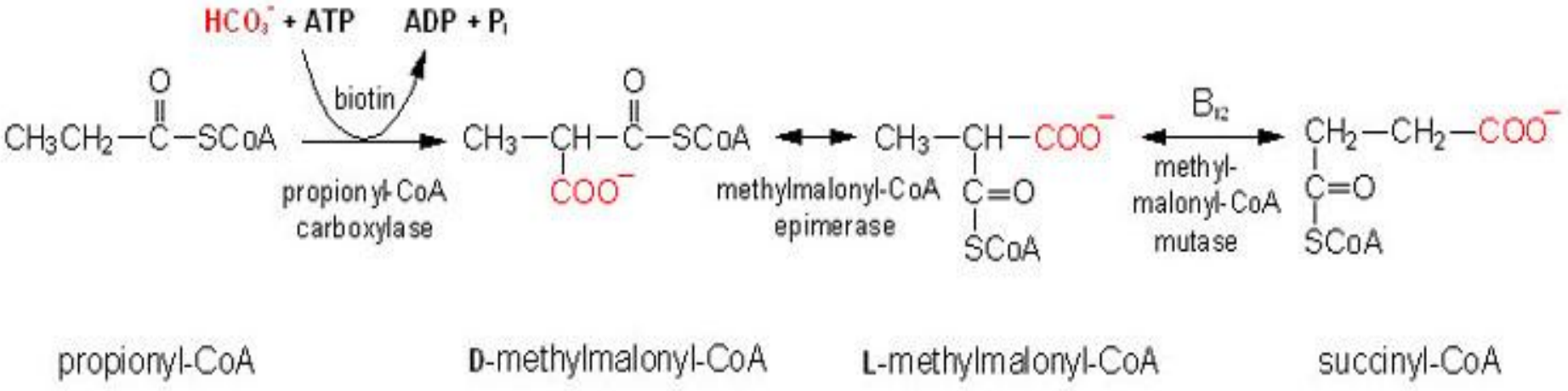
- The acyl-CoA is transported into the mitochondria (were β-oxidation occurs). This process involves the sequential removal of two-carbon units from the acyl-CoA molecule, forming acetyl-CoA.

- When the fatty acid chain reaches an odd number of carbons, after several rounds of β-oxidation, the last cycle will yield a three-carbon propionyl-CoA molecule instead of the usual two-carbon acetyl-CoA molecule.

Oxidation of odd-numbered fatty acid (cont.)

Conversion of Propionyl-CoA:

- The propionyl-CoA undergoes conversion via several enzymatic steps into succinyl-CoA, which can then enter the TCA cycle to produce energy.



Peroxisomal fatty acid oxidation

- While mitochondria are the primary site for fatty acid oxidation in eukaryotic organisms, peroxisomes also play a role in this process.
- Peroxisomes are organelles found in eukaryotic cells that are involved in various metabolic processes, including:
  - Breakdown of fatty acids.
  - Detoxification of harmful substances.
- One of their key functions is to carry out flavin-dependent oxidation reactions, which generate hydrogen peroxide as a byproduct.
- Peroxisomes also contain high concentrations of catalase, an enzyme that catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen ($2H_2O_2 \rightarrow 2H_2O + O_2$).

Peroxisomal fatty acid oxidation (cont.)

- The fatty acid oxidation in peroxisomes shares similarities with the same process that occurs in mitochondria.
- Some of the key differences include that:
  - β-oxidation in peroxisomes handle very long-chain fatty acids, while mitochondrial β-oxidation primarily deals with medium and long-chain fatty acids.
  - Peroxisomal fatty acid oxidation employs different enzymes, such as acyl-CoA oxidase (which catalyzes the first step), whereas in mitochondrial β-oxidation, this step is catalyzed by acyl-CoA dehydrogenase.

Peroxisomal fatty acid oxidation (cont.)

- Acyl-CoA oxidase is a key enzyme involved in the β-oxidation of fatty acid within peroxisomes.
- It primarily acts on very long-chain fatty acyl-CoA molecules, catalysis the removal of two hydrogen atoms from the acyl-CoA substrate.
- These hydrogen atoms are transferred to the enzyme's cofactor FAD, forming FADH2.
  - The reduced FADH2 is then reoxidized by molecular oxygen, producing hydrogen peroxide:

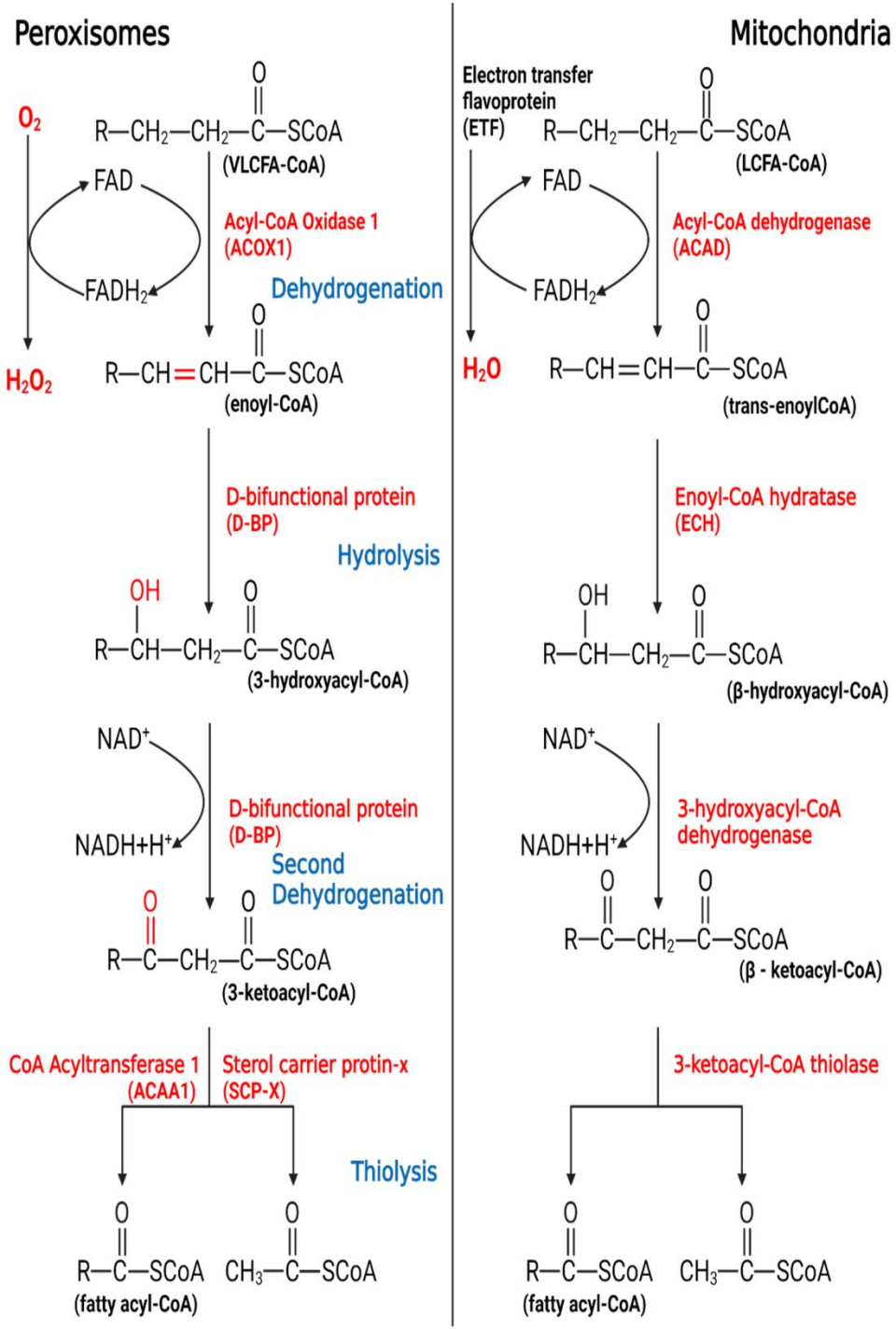
$$FADH_2 + O_2 \rightarrow FAD + H_2O_2$$

  - To prevent oxidative damage, the peroxisomal enzyme catalase rapidly decomposes H_2O_2 into water and oxygen:

$$H_2O_2 \rightarrow H_2O + \frac{1}{2} O_2$$

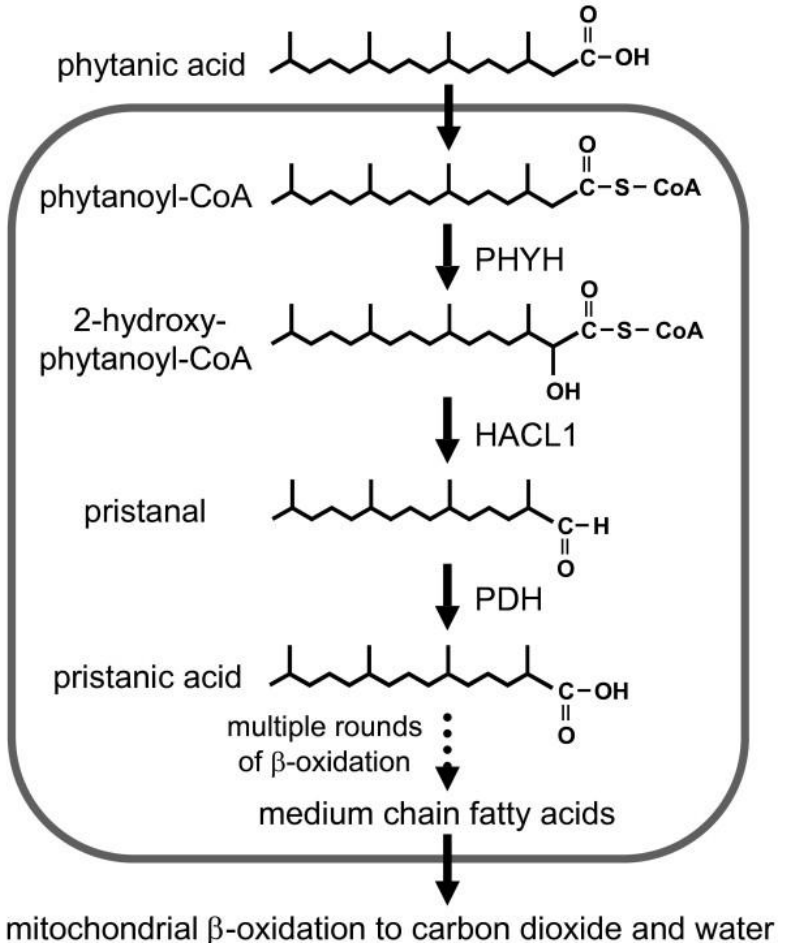
Peroxisomal fatty acid oxidation (cont.)

- Within peroxisomes, fatty acids are oxidized to shorter chain fatty acids (such as octanoyl-CoA).
- These shorter chain fatty acids can then be transported to the mitochondria, where further β-oxidation occurs, ultimately yielding acetyl-CoA, which can enter the TCA cycle to generate energy through aerobic respiration.


Peroxisomes
Mitochondria
O2
R—CH2—CH2—C—SCoA
(VLCFA-CoA)
FAD
FADH2
Acyl-CoA Oxidase 1
(ACOX1)
Dehydrogenation
H2O2
R—CH=CH—C—SCoA
(enoyl-CoA)
D-bifunctional protein
(D-BP)
Hydrolysis
OH
R—CH—CH2—C—SCoA
(3-hydroxyacyl-CoA)
NAD+
NADH+H+
D-bifunctional protein
(D-BP)
Second
Dehydrogenation
R—C—CH2—C—SCoA
(3-ketoacyl-CoA)
CoA Acyltransferase 1
(ACAA1)
Sterol carrier protin-x
(SCP-X)
Thiolysis
R—C—SCoA
(fatty acyl-CoA)
CH3—C—SCoA
Electron transfer
flavoprotein
(ETF)
R—CH2—CH2—C—SCoA
(LCFA-CoA)
FAD
FADH2
Acyl-CoA dehydrogenase
(ACAD)
H2O
R—CH=CH—C—SCoA
(trans-enoylCoA)
Enoyl-CoA hydratase
(ECH)
OH
R—CH—CH2—C—SCoA
(β-hydroxyacyl-CoA)
NAD+
NADH+H+
3-hydroxyacyl-CoA
dehydrogenase
R—C—CH2—C—SCoA
(β - ketoacyl-CoA)
3-ketoacyl-CoA thiolase
R—C—SCoA
(fatty acyl-CoA)
CH3—C—SCoA

α-oxidation of fatty acids (minor pathway)

- α-oxidation is a specialized pathway for the oxidation of fatty acids, particularly those with a methyl group at the β-carbon position, such as phytanic acid.
- This process occurs primarily in peroxisomes.
- This pathway helps in ensuring proper metabolism and removal of these fatty acid derivatives from the body.
- This pathway is particularly significant in brain tissue where it helps in the metabolism of certain dietary fatty acids.



α-oxidation of fatty acids (cont.)

- In α-oxidation, one carbon atom is removed at a time from the α position of the fatty acid chain.
- This process doesn't require CoA and doesn't generate ATP.

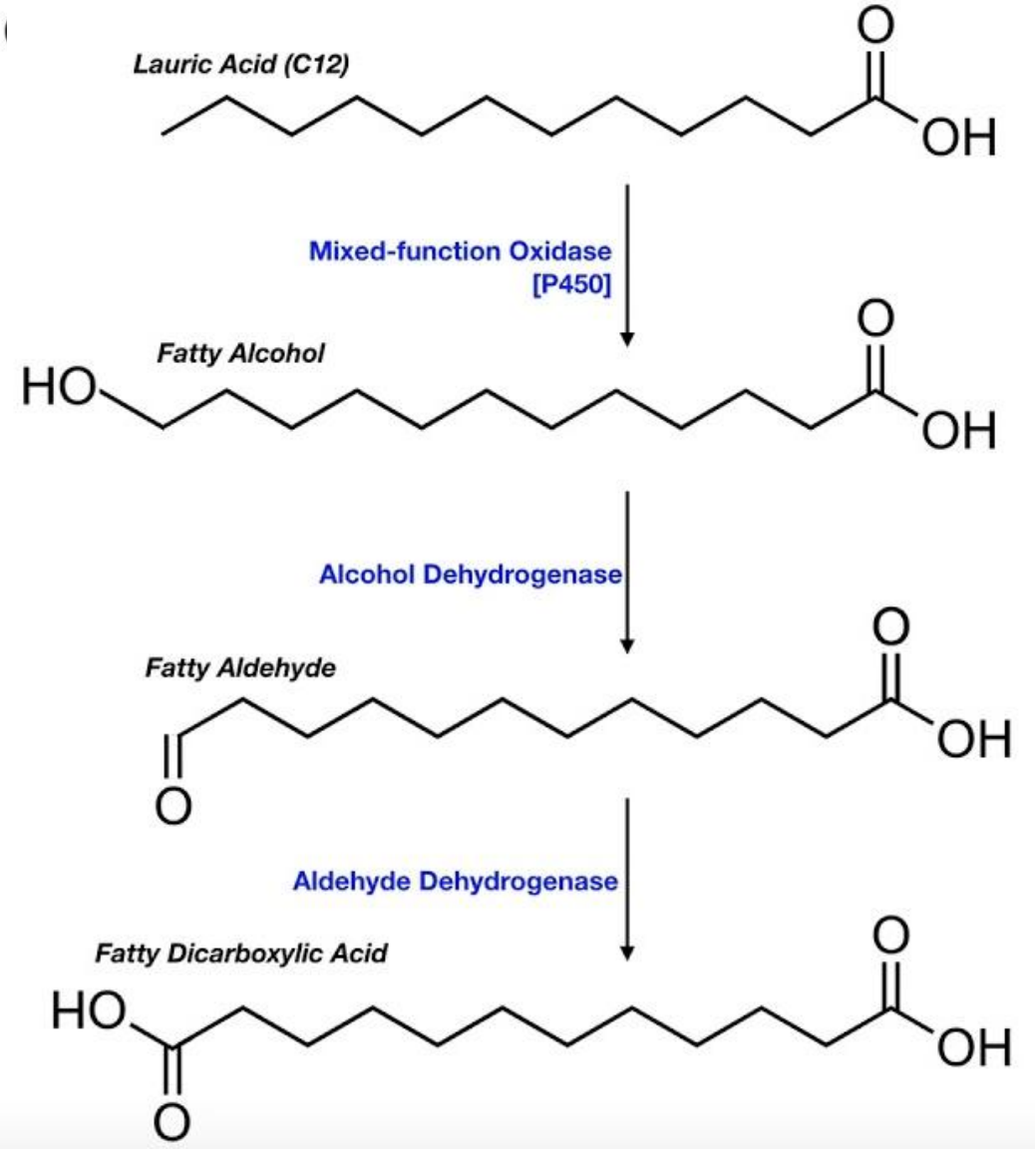
Refsum's disease:

- It is also known as phytanic acid storage disease, is a rare inherited metabolic disorder.
- It's characterized by the body's inability to break down phytanic acid, resulting in its accumulation in tissues and organs.

ω-oxidation of fatty acids (minor pathway)

- ω-oxidation is a minor fatty acid oxidation pathway that primarily occurs in the smooth endoplasmic reticulum of certain tissues, particularly the liver.
- This pathway becomes more active when β-oxidation is impaired or during metabolic conditions such as ketosis, where medium-chain fatty acids are mobilized from adipose tissue.
- In ω-oxidation, cytochrome P450 enzymes catalyze the oxidation of the ω-carbon (the terminal methyl group) of the fatty acid chain. This reaction introduces an oxygen atom, converting the methyl group into a hydroxyl group.
- The resulting ω-hydroxy fatty acid can then undergo further oxidation to form a dicarboxylic acid, which may subsequently enter the β-oxidation pathway in mitochondria or peroxisomes.

ω-oxidation of fatty acids (minor pathway)



Fates of acetyl-CoA