



الأيض (١)

Metabolism (1)

BCH 340

Lecture 12: Biosynthesis of fatty acids

Intended learning outcomes (ILOs)

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

- Understand the process of *de novo* fatty acid synthesis.
- Explain the pathway of fatty acid elongation in both endoplasmic reticulum and mitochondria.
- Describe the mechanism of fatty acid desaturation.

Introduction

- Fatty acid synthesis is a complex biochemical process that occurs primarily in the **cytoplasm** of cells.
- There are several pathways of fatty acid synthesis, each involving different enzymes and substrates. They include:
 - *De novo* lipogenesis
 - Fatty acid elongation
 - Fatty acid desaturation

De novo synthesis of fatty acids

- *De novo* synthesis of fatty acids is a complex biochemical process that takes place in the **cytoplasm** of certain cells, notably in the **liver, adipose tissue,** and **mammary glands** during lactation
- It is the primary pathway for the synthesis of fatty acids from **non-lipid precursors**, such as carbohydrates and proteins.

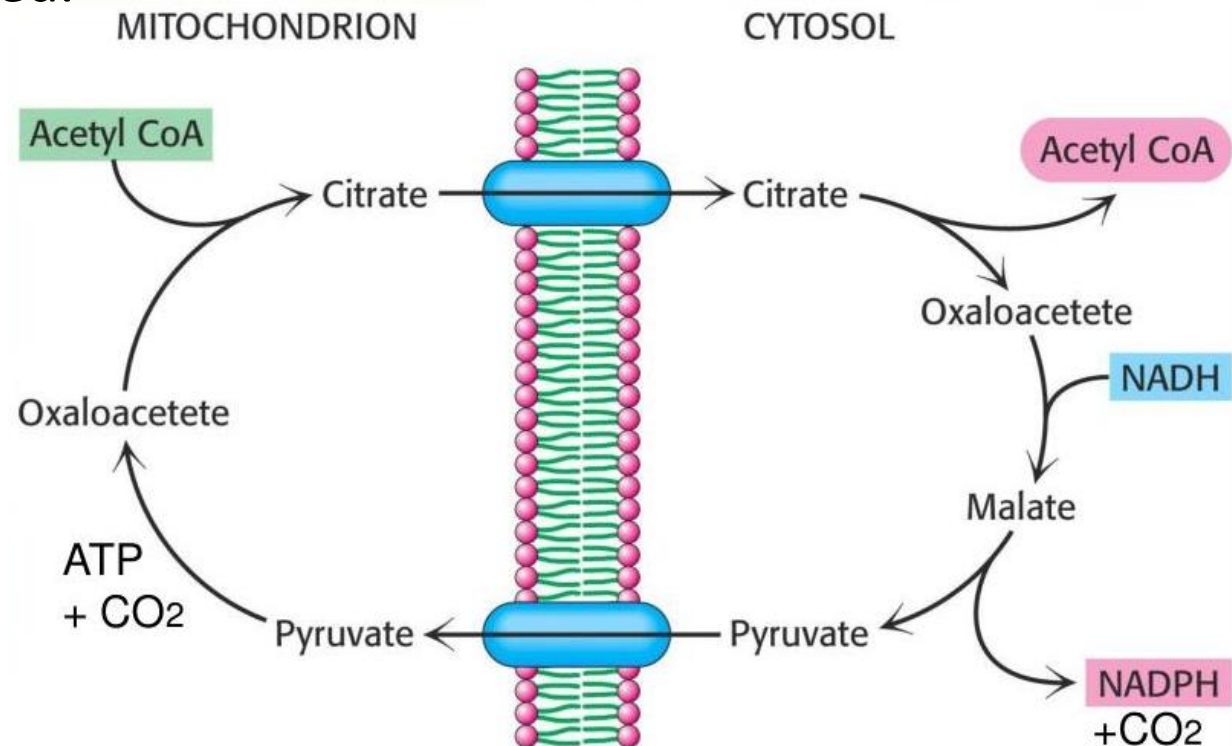
1. Acetyl-CoA formation:

- The process starts with the formation of **acetyl-CoA** (**starting material** for *de novo* synthesis of fatty acids).
- Acetyl-CoA is generated from various non-lipid sources including glucose and amino acid metabolism.

De novo synthesis of fatty acids (cont.)

2. Transportation of acetyl-CoA:

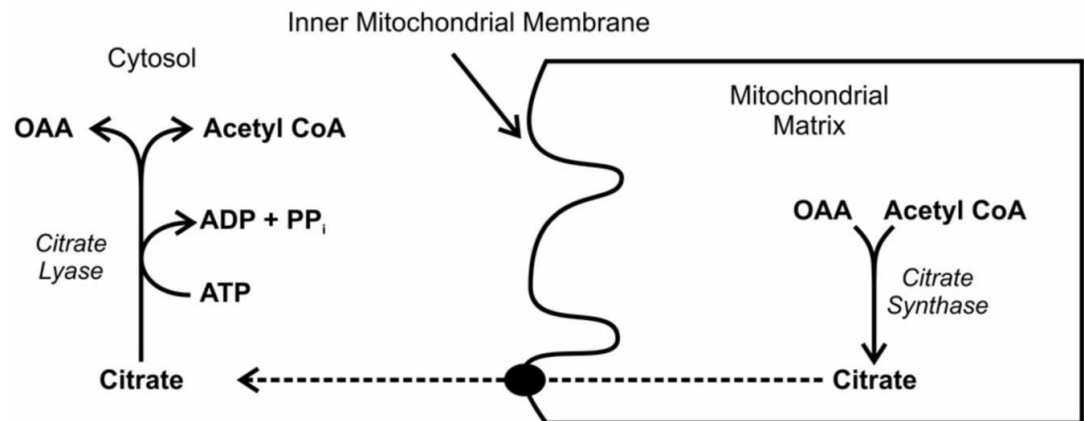
- The molecules of acetyl-CoA used in this pathway is generated within the **mitochondria**. Therefore, they must be **transported out** to the cytoplasm where the enzymes involved in fatty acid synthesis are located.



De novo synthesis of fatty acids (cont.)

2. Transportation of acetyl-CoA (cont.):

- i. Acetyl-CoA is first converted into citrate in the mitochondria through the action of the enzyme **citrate synthase**.
- ii. Citrate is then transported out of the mitochondria into the cytoplasm by a specific transporter called the **citrate transporter** (citrate shuttle).
- iii. Once in the cytoplasm, citrate is cleaved by **ATP citrate lyase** to yield acetyl-CoA (used for fatty acid synthesis) and oxaloacetate.



De novo synthesis of fatty acids (cont.)

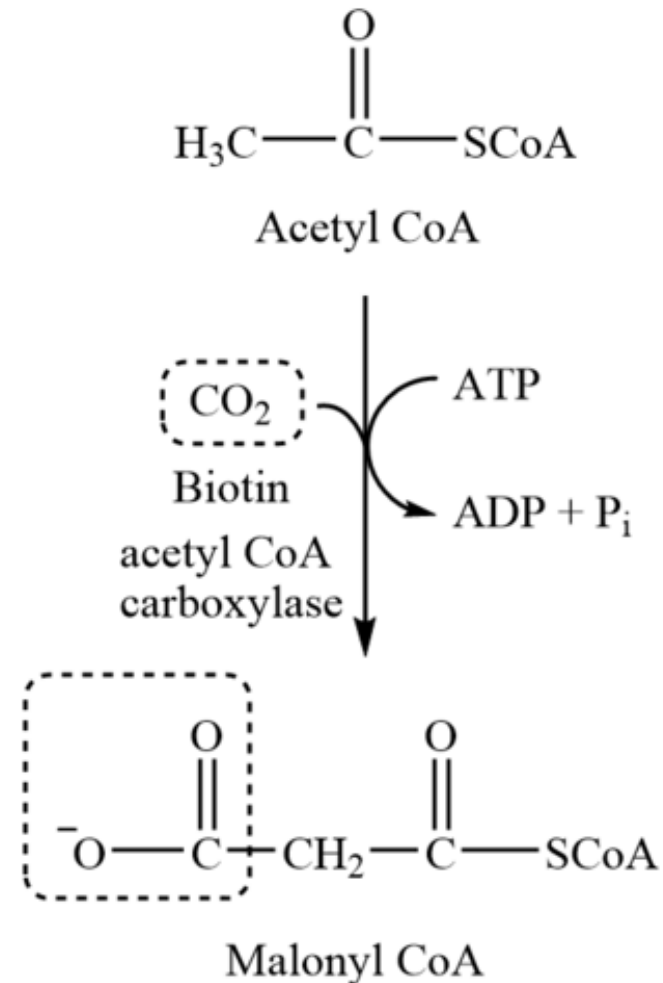
2. Transportation of acetyl-CoA (cont.):

- iv. The oxaloacetate is then converted into malate by the **cytosolic** enzyme **malate dehydrogenase**. From this point, malate can follow two possible routes:
- It can be transported **directly** back into the mitochondria via the malate- α -ketoglutarate transporter, where it is reconverted to oxaloacetate by **mitochondrial** malate dehydrogenase, completing the shuttle.
 - It can be converted to **pyruvate** in the cytosol by the malic enzyme, generating NADPH, which is essential for fatty acid and lipid biosynthesis. The resulting pyruvate then enters the mitochondria, where it can be converted back into oxaloacetate or acetyl-CoA, depending on the cell's metabolic needs.

De novo synthesis of fatty acids (cont.)

3. Formation of malonyl-CoA:

- In the cytoplasm, acetyl-CoA is carboxylated to form malonyl-CoA, which is catalyzed by the enzyme **acetyl-CoA carboxylase**.
- This reaction is a rate-limiting and a **committed step** in fatty acid synthesis.
- Acetyl-CoA carboxylase, is a multi-subunit enzyme that is found in most organisms, **from bacteria to humans**.
- It requires biotin, ATP, and Mg^{2+} as co-factors.

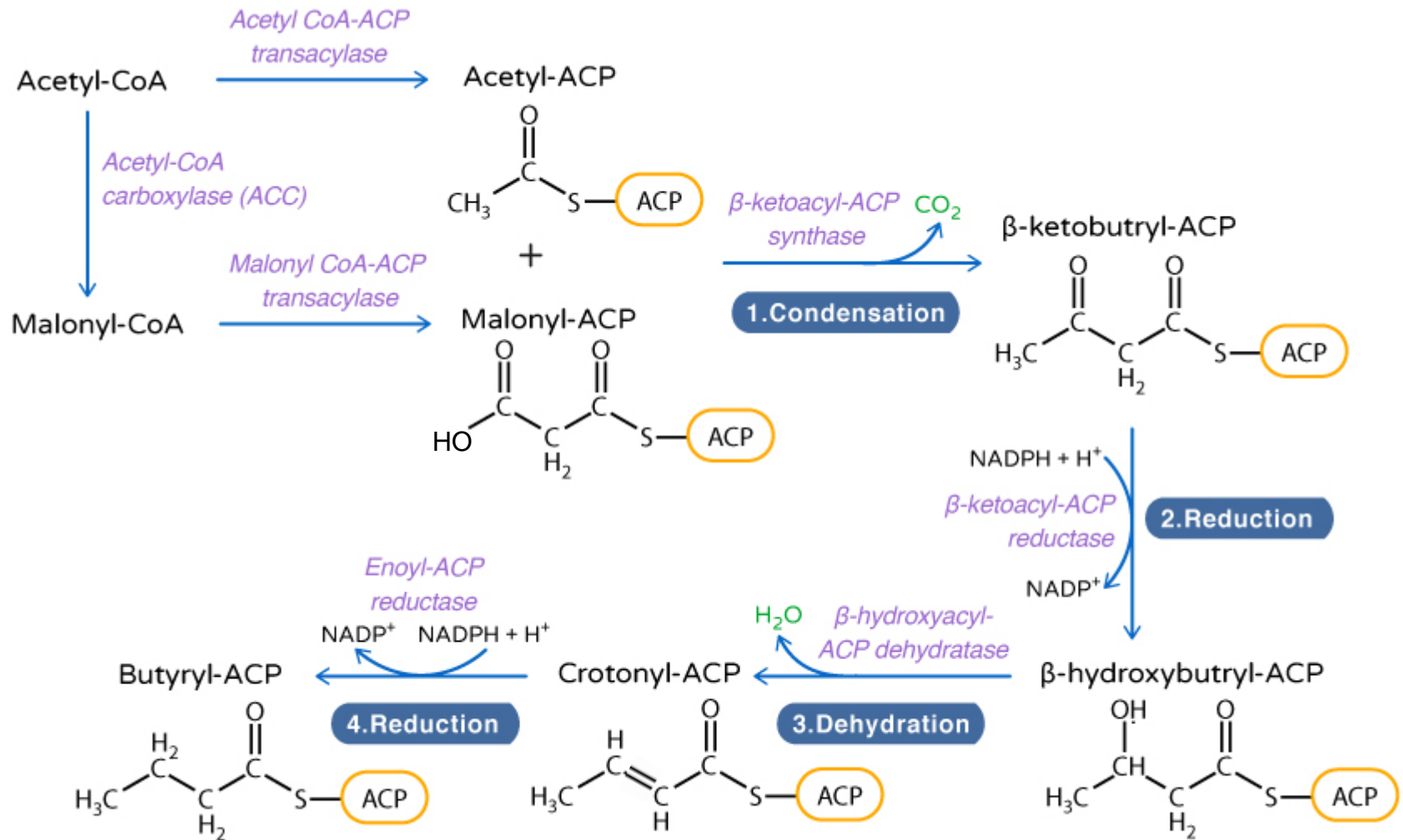


De novo synthesis of fatty acids (cont.)

4. Fatty acid chain elongation:

- Elongation of fatty acids involves the **stepwise addition** of malonyl-CoA (**activated carbon donor**) to the growing fatty acid chain.
- This process is mediated by **fatty acid synthase** (a multi-enzyme complex) and involves **a series of enzymatic reactions** that produce long-chain saturated fatty acid.
- These reactions involve the following:
 - Condensation
 - Reduction (1st)
 - Dehydration
 - Reduction (2nd)

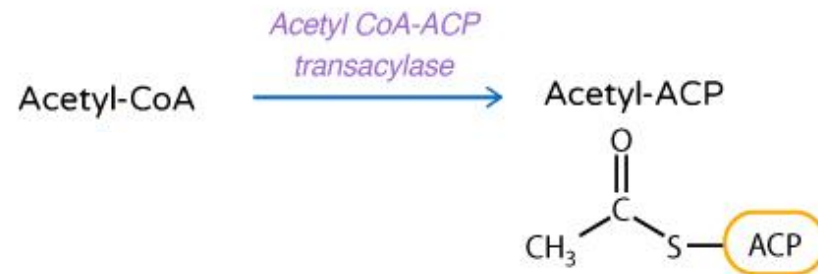
De novo synthesis of fatty acids: elongation of fatty acids



De novo synthesis of fatty acids (cont.)

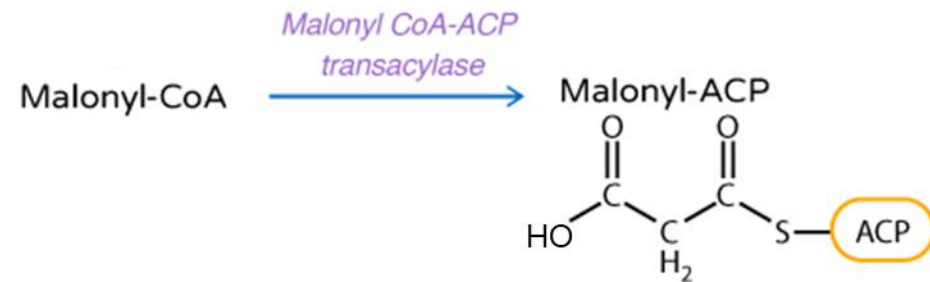
Initiation of fatty acid chain elongation:

- First, **acetyl CoA-ACP transacylase** (a component of the fatty acid synthase complex), attaches a **priming molecule of acetyl-CoA** to the acyl carrier protein (ACP) and releases CoA. This results in the formation of **acetyl-ACP**.
- The acetyl group attached to the ACP is then transferred to the active site of the adjacent enzyme within the fatty acid synthase complex, which is **β -ketoacyl-ACP synthase**.
- This transfer initiates the elongation cycle of fatty acid synthesis, where the growing fatty acid chain is extended by **the addition of two-carbon units** (from **malonyl-CoA**) in each cycle.



De novo synthesis of fatty acids (cont.)

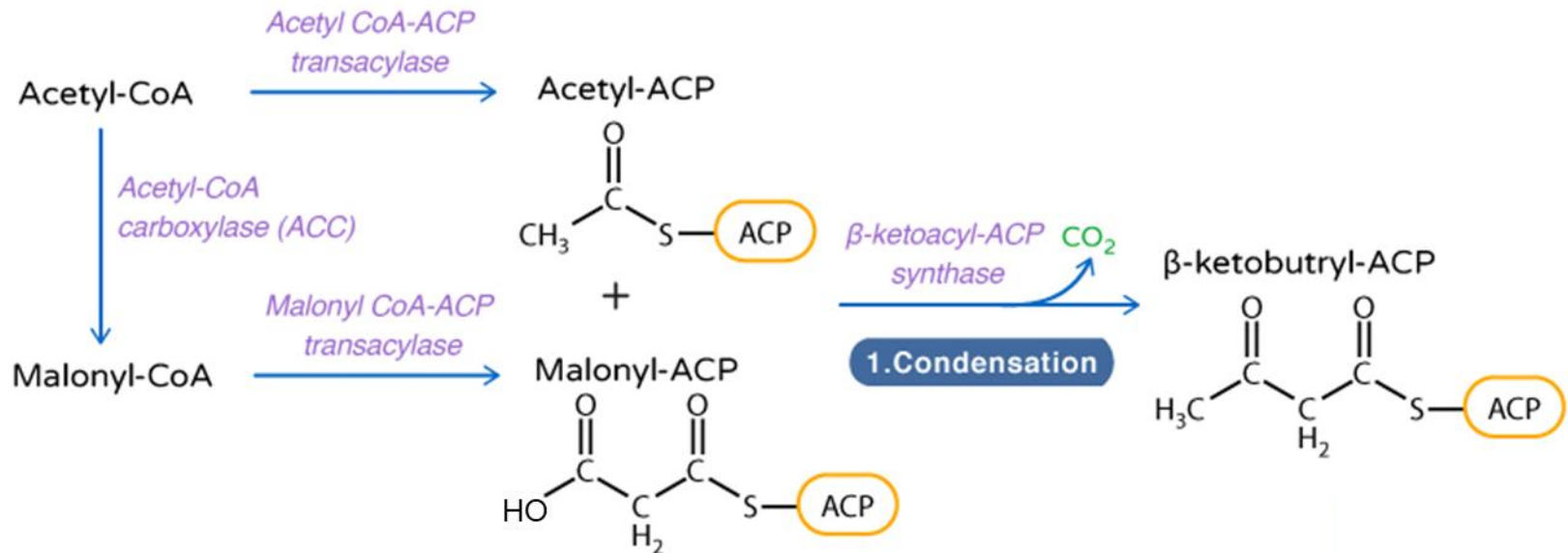
- Acyl carrier protein (ACP) **carries** the growing fatty acid chain as it is synthesized, **shuttling** it between the different catalytic (enzymatic) sites within the fatty acid synthase complex.
- A malonyl group is transferred from malonyl-CoA to ACP, resulting in the formation of **malonyl-ACP**. This reaction catalyzed by **malonyl transacylase**.



De novo synthesis of fatty acids (cont.)

Condensation:

- The enzyme **β -ketoacyl-ACP synthase** catalyzes the condensation of the growing acyl group (attached to ACP) with a two-carbon unit from **malonyl-ACP**, releasing CO_2 and forming β -ketoacyl-ACP.
- This reaction elongates the fatty acid chain by two carbons.



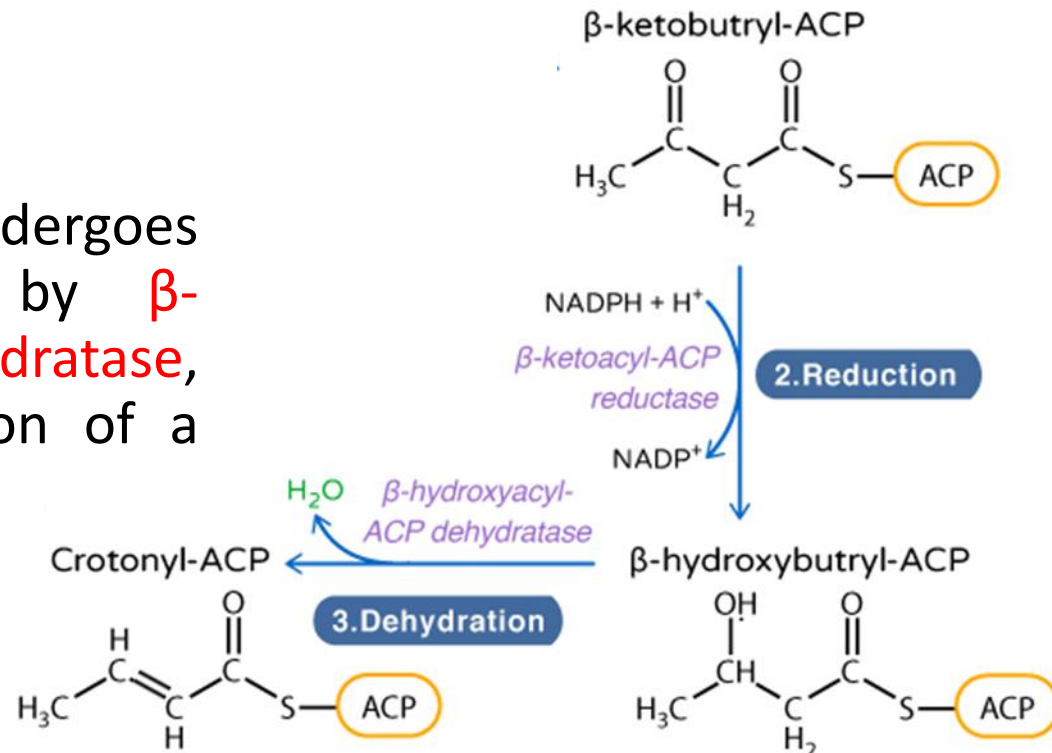
De novo synthesis of fatty acids (cont.)

Reduction (1st):

- The β -ketoacyl-ACP is then reduced by **NADPH-dependent β -ketoacyl-ACP reductase**, leading to the formation of a β -hydroxyacyl-ACP.

Dehydration:

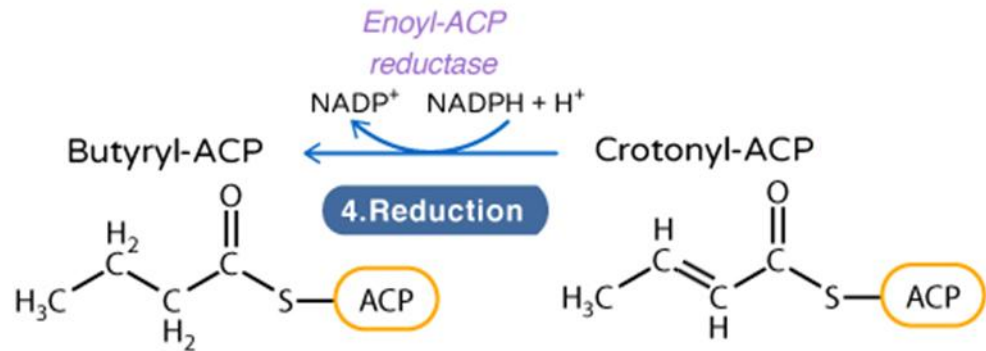
- The β -hydroxyacyl-ACP undergoes dehydration catalyzed by **β -hydroxyacyl-ACP dehydratase**, resulting in the formation of a *trans*-2-enoyl-ACP.



De novo synthesis of fatty acids (cont.)

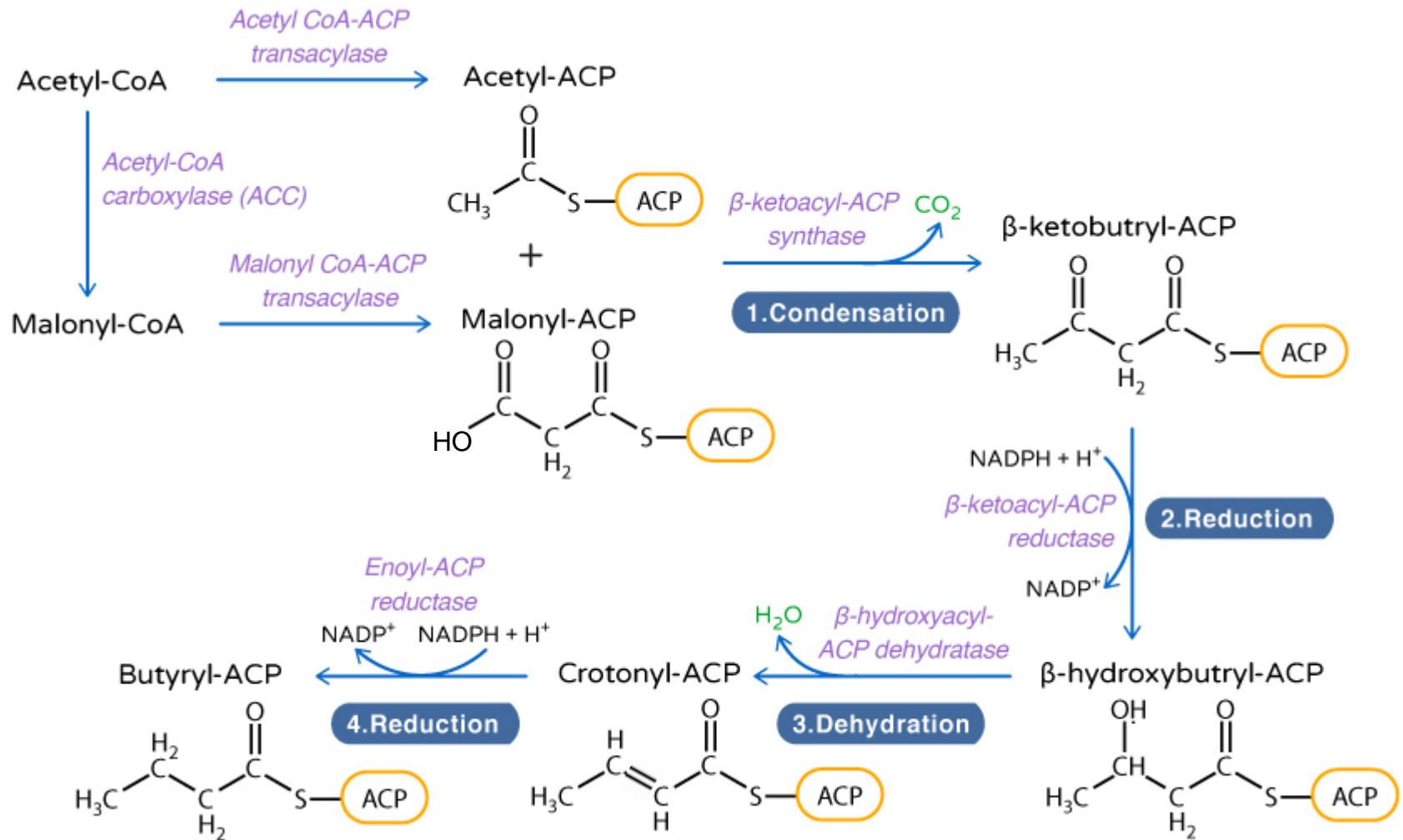
Reduction (2nd):

- Finally, the *trans*-2-enoyl-ACP is reduced by **NADPH-dependent enoyl-ACP reductase**, yielding a fully saturated acyl-ACP.



- Through repeated cycles of condensation, reduction, and dehydration, the fatty acid chain is elongated **until it reaches the desired length**, after which it can be **released** from the fatty acid synthase complex and utilized for various cellular functions.

De novo synthesis of fatty acids: elongation of fatty acids

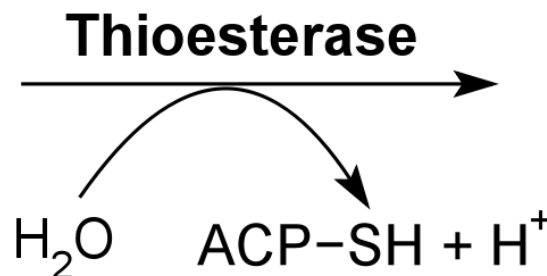
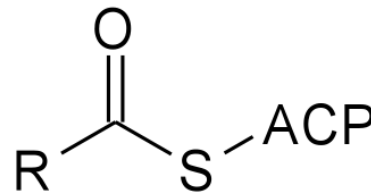


De novo synthesis of fatty acids (cont.)

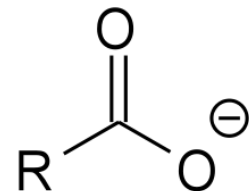
Release of the fatty acid:

- When the fatty acid reaches 16 carbon atoms long, a **thioesterase domain** (within the fatty acid synthase complex) cleaves and releases the fatty acid from the ACP.
- The resulting 16-carbon saturated fatty acid (palmitic acid) is the **final product** of the fatty acid synthase complex (this process can also produce shorter chains of fatty acids).

Fatty Acyl ACP



Fatty Acid



De novo synthesis of fatty acids (cont.)

Release of the fatty acid (cont.):

- Palmitic acid serves as a **precursor** for the synthesis of longer-chain fatty acids and other lipid molecules within the body.
- The newly synthesized fatty acid can undergo further **elongation** and **insertion of double bonds** in separated processes of fatty acid elongation and desaturation (carried out by a different set of enzymes).

Fatty acid synthase

- Fatty acid synthase is a **multi-enzyme complex** that catalyzes the synthesis of long-chain fatty acids from malonyl-CoA.
- This enzyme is typically found in organisms ranging from bacteria to mammals.
- In mammals, fatty acid synthase functions as a **dimer** that is composed of **multiple enzymatic domains** that work together to sequentially add two-carbon units donated by malonyl-CoA onto a growing fatty acid chain.
 - This process involves several steps (the 4 elongation steps), ultimately leading to the elongation of the fatty acid chain.

Fatty acid synthase (cont.)

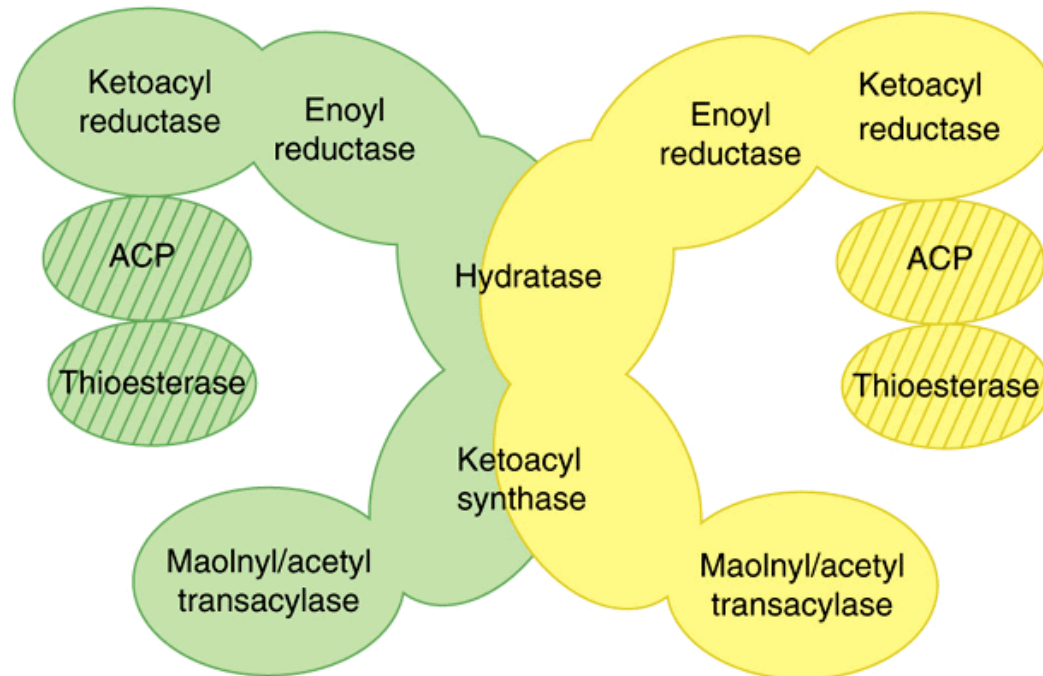
- Typically, fatty acid synthase consists of multiple protein subunits organized into distinct functional domains:
 - Condensation domain
 - Acyl carrier protein
 - β -Ketoacyl synthase
 - β -Ketoacyl reductase
 - Dehydratase
 - Enoyl reductase

https://www.youtube.com/watch?v=Dc3_LLXsguw

Fatty acid synthase structure and domains



Sequence of enzyme domains in primary structure of fatty acid synthase monomer



Fatty acid synthase homodimer

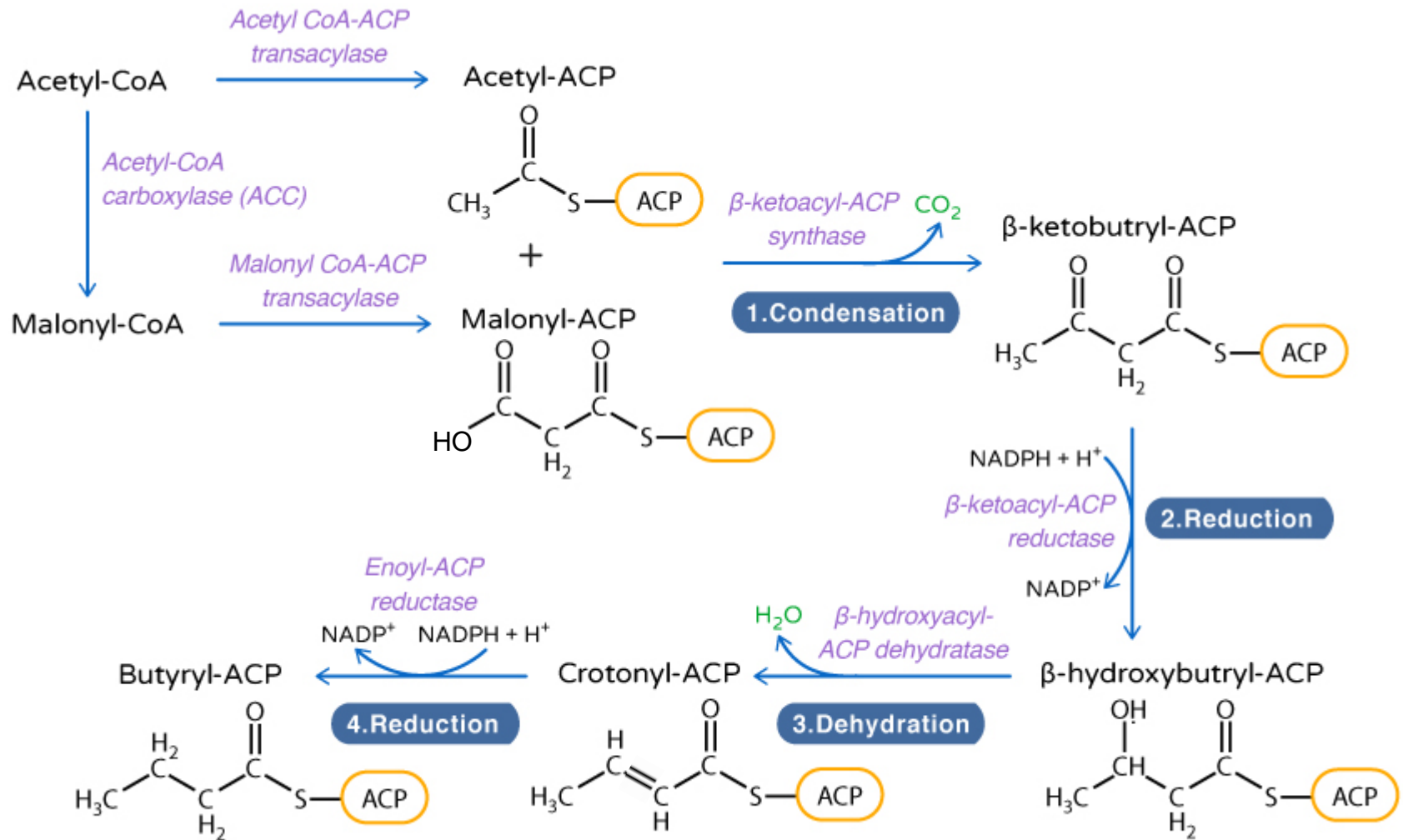
Fatty acid synthesis: energy costs

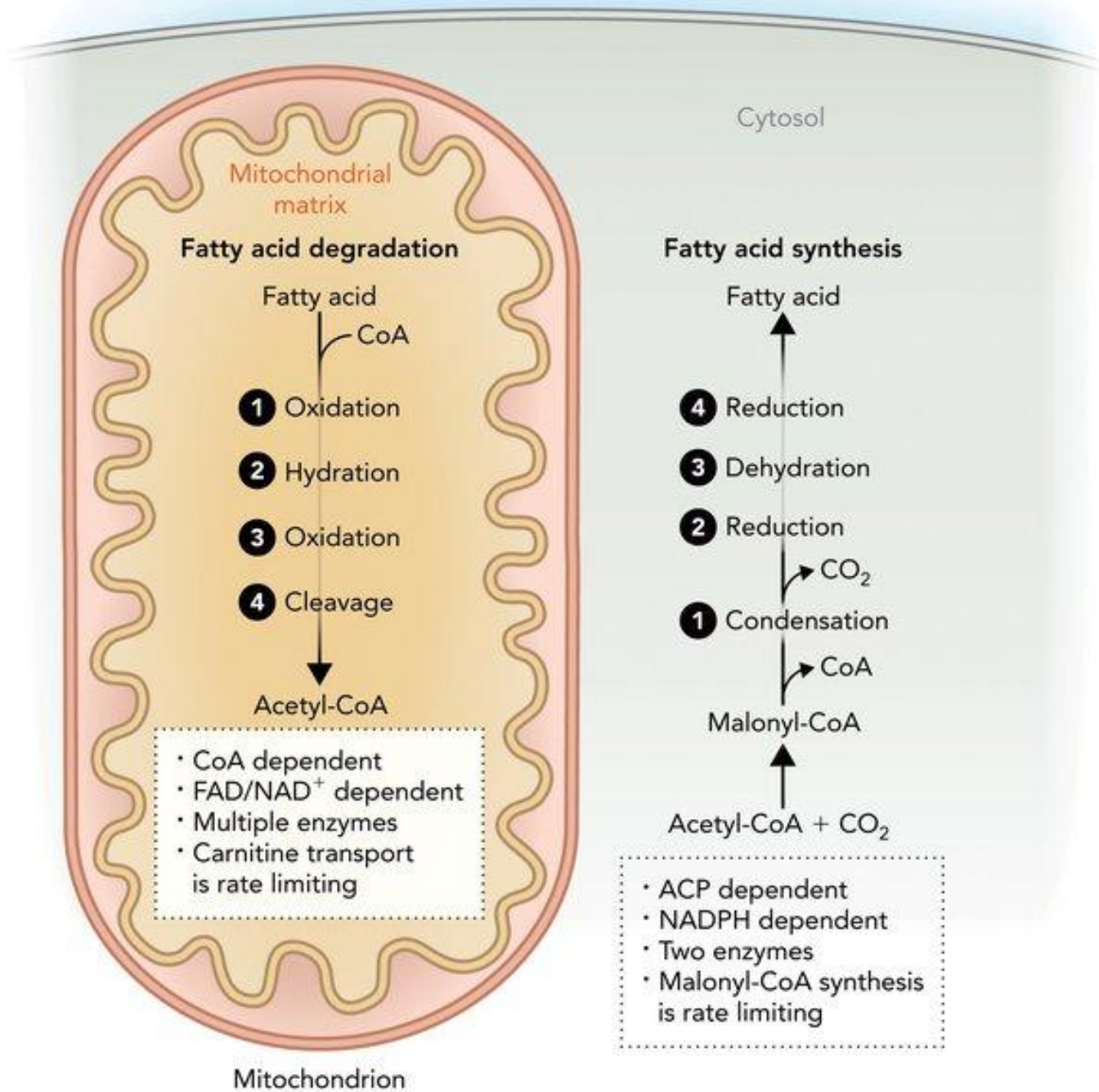
- Typically, the synthesis of one molecule of palmitic acid (a 16-carbon saturated fatty acid) via the fatty acid synthase pathway consumes the following:

	No. of molecules
Acetyl-CoA	8
Malonyl-CoA	7
ATP	7
NADPH	14

- Each round of elongation involves the condensation of one molecule of malonyl-CoA with the growing acyl-ACP chain, releasing CO₂. This is followed by two reduction steps (both requiring NADPH) and one dehydration step, resulting in the extension of the fatty acid chain by two carbons per cycle. Additionally, ATP is consumed by acetyl-CoA carboxylase during the formation of malonyl-CoA.

De novo synthesis of fatty acids: elongation of fatty acids

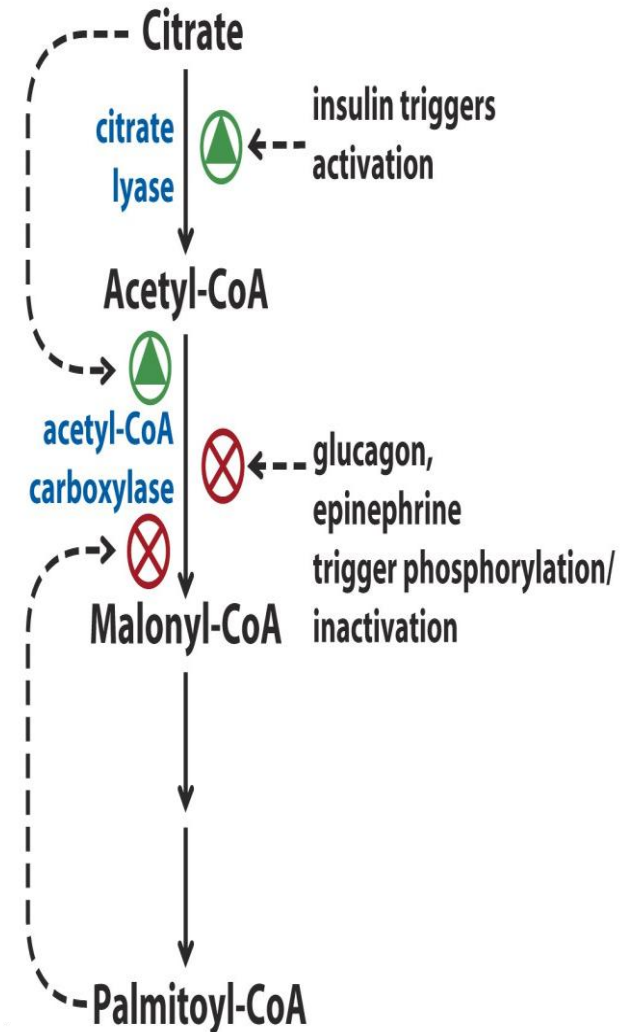




Regulation of fatty acid synthesis

Citrate:

- Citrate **allosterically activates** acetyl-CoA carboxylase, promoting the conversion of acetyl-CoA to malonyl-CoA, the first committed step in fatty acid synthesis.
- High levels of citrate indicate an abundance of TCA cycle intermediates and energy, signaling that fatty acid synthesis **can proceed**.



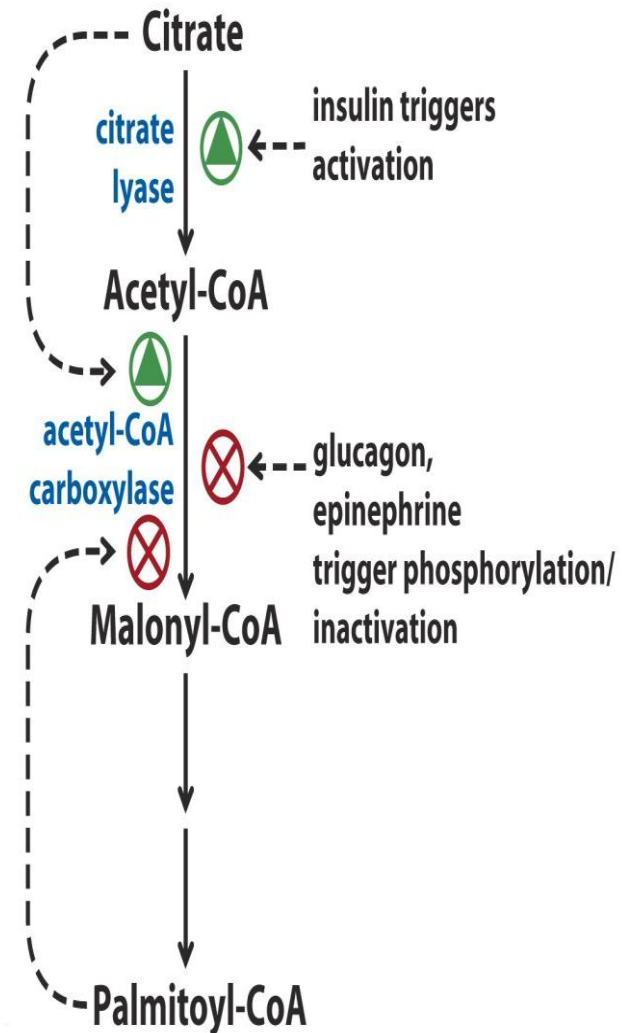
Regulation of fatty acid synthesis (cont.)

Malonyl-CoA:

- Malonyl-CoA has a unique role in fatty acid metabolism, acting both as a **building block** for fatty acid synthesis and as an **inhibitor** of fatty acid oxidation.

Palmitic acid:

- Palmitic acid (end product of fatty acid synthesis) regulates the *de novo* synthesis of fatty acids through **feedback inhibition**.



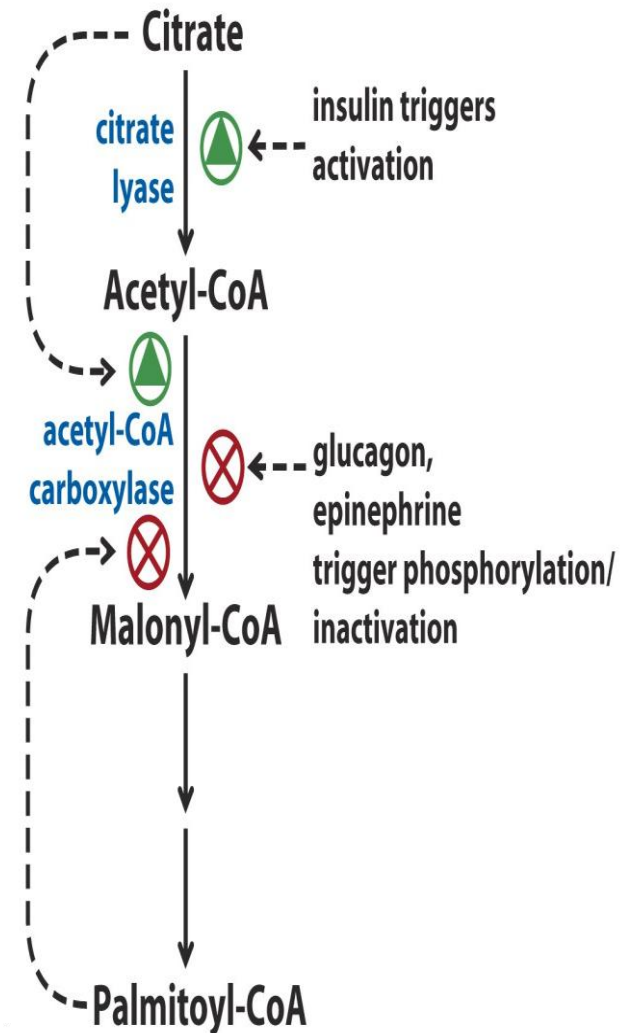
Regulation of fatty acid synthesis (cont.)

Insulin:

- Insulin **promotes the uptake of glucose** into cells, where it can be used as a substrate for fatty acid synthesis.
- It also **activates key enzymes** involved in fatty acid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase.

Glucagon:

- Glucagon **prevents fatty acid synthesis** (by inhibits acetyl-CoA carboxylase), while promoting β -oxidation of fatty acids.



Further processing: chain elongation

Microsomal chain elongation

- Within mammalian cells, elongation of fatty acid (the synthesis of **longer-chain** fatty acids) can occur in both **microsomes** and **mitochondria**.

Microsomal fatty acid chain elongation:

- It refers to the process of elongating fatty acids that occurs within the **endoplasmic reticulum** (particularly in microsomes) of cells.
- This process involves a series of enzymatic reactions that **add two-carbon units** to the fatty acid chain, extending it further.
- Medium-chain saturated fatty acids as well as monounsaturated fatty acids (like oleic acid (C18:1)) can serve as **substrates for fatty acid chain elongation**.

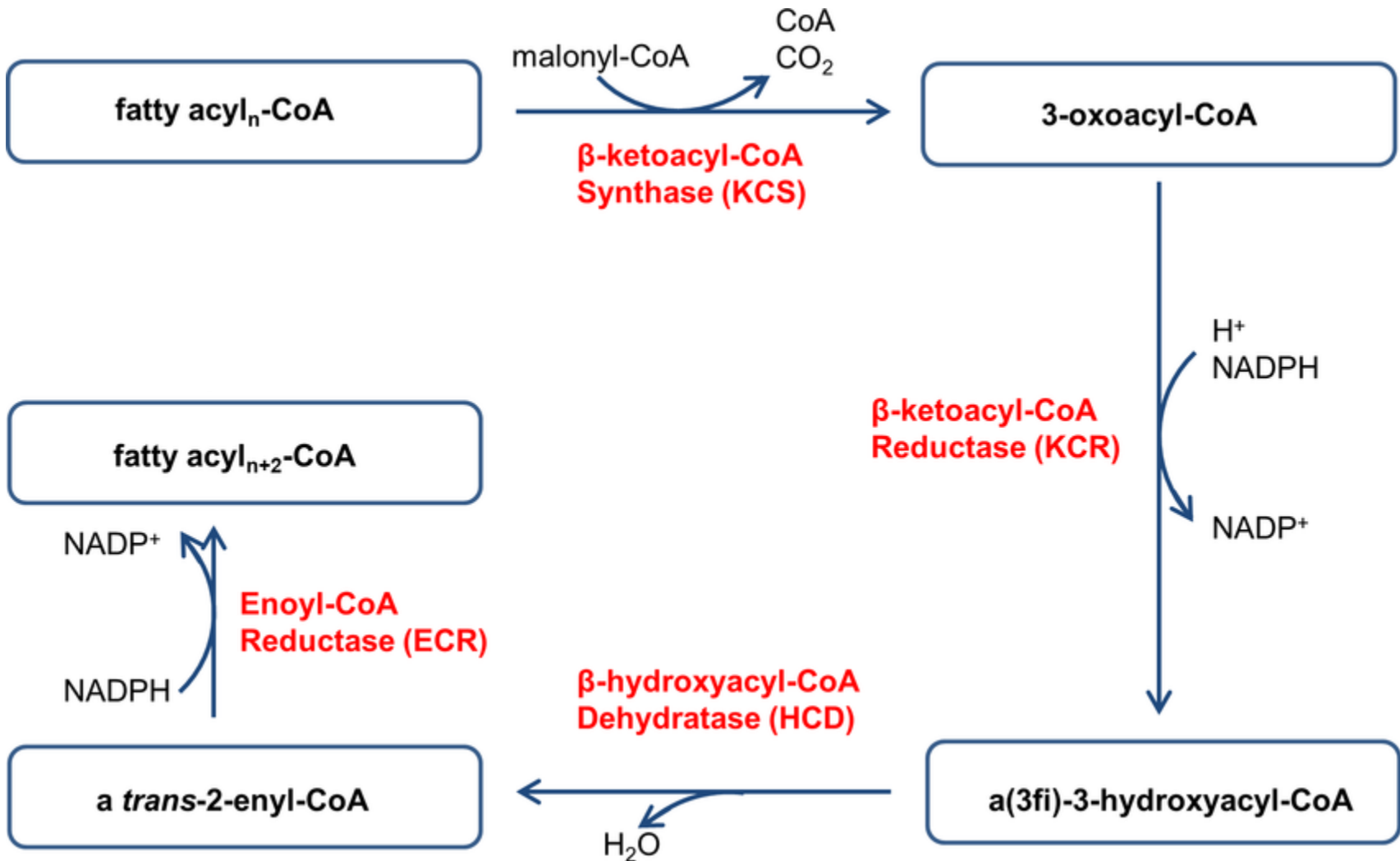
Microsomal chain elongation (cont.)

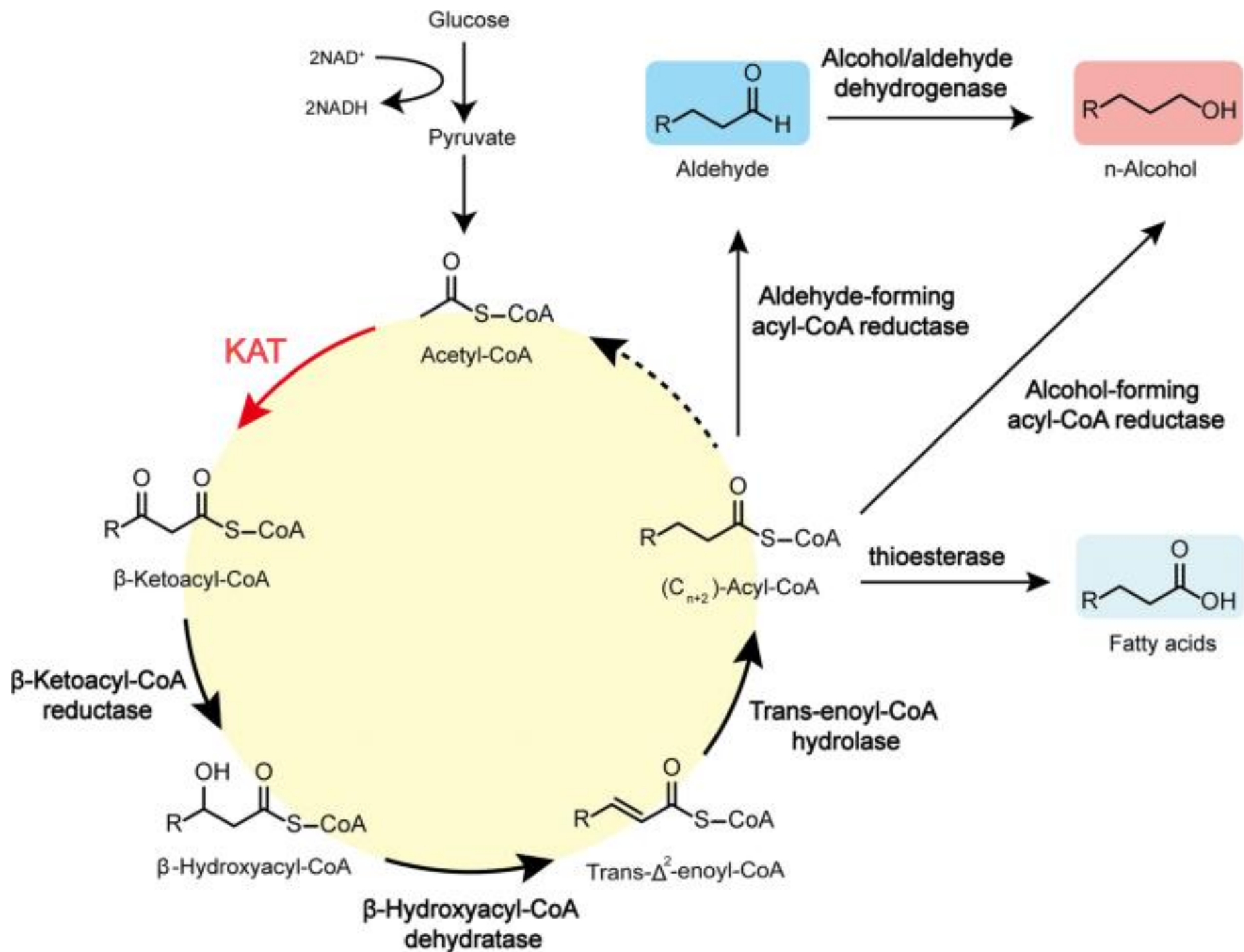
- In the microsomal elongation pathway, fatty acids are first **activated by esterification to coenzyme A**, forming fatty acyl-CoA. These fatty acyl-CoA molecules serve as substrates for further elongation reactions.
- Similar to the process in fatty acid synthesis, **malonyl-CoA serves as the donor of two-carbon units** in the microsomal elongation pathway.
 - This involves a series of reactions where malonyl-CoA is added to the growing fatty acyl-CoA chain, resulting in elongation by two carbons per cycle.

Microsomal chain elongation (cont.)

- Unlike the fatty acid synthase complex (where multiple enzymatic activities are present within a single protein complex), microsomal fatty acid elongation **involves separate enzymes**.
 - Each enzyme catalyzes a specific step in the elongation process, allowing for greater regulation and specificity.
- Reduction reactions involved in this process require **NADPH as the reducing agent**, providing the necessary reducing power for the conversion.

Elongation of fatty acids





Mitochondrial chain elongation (cont.)

- In mitochondria, the fatty acid elongation pathway involves a series of enzymatic reactions carried out by proteins located in the **mitochondrial inner membrane**.
- Mitochondrial fatty acid elongation **specifically elongates fatty acids that are:**
 - Destined for mitochondrial membranes.
 - Specific metabolic pathways within the mitochondria.

Mitochondrial chain elongation (cont.)

- Palmitic acid (16-carbon saturated fatty acid), serves as a **starting material** for the synthesis of various **longer-chain fatty acids** within the mitochondria.
- In mitochondrial chain elongation, successive additions of two-carbon units in the form of acetyl-CoA (NOT malonyl-CoA) to the existing palmitic acid, producing a longer-chain fatty acid.
- This process occurs by a **simple reversal** of β -oxidation, except for the substitution of FAD-linked acyl-CoA dehydrogenase with NADPH-linked α,β -unsaturated acyl-CoA reductase.
 - The elongation process requires both **NADH** and **NADPH** as reducing agents.

Mitochondrial chain elongation (cont.)

1. Condensation:

- First, **thiolase** enzyme catalyzes the condensation reaction between an palmitoyl-CoA and acetyl-CoA, forming a β -ketoacyl-CoA.

2. Reduction (1st):

- The β -ketoacyl-CoA is reduced by **α,β -unsaturated acyl-CoA reductase** using **NADH as a reducing agent**. This reduction converts the β -keto group into a hydroxyl group, forming a L- β -hydroxyacyl-CoA.

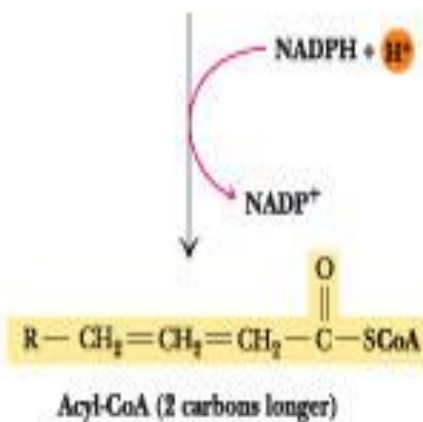
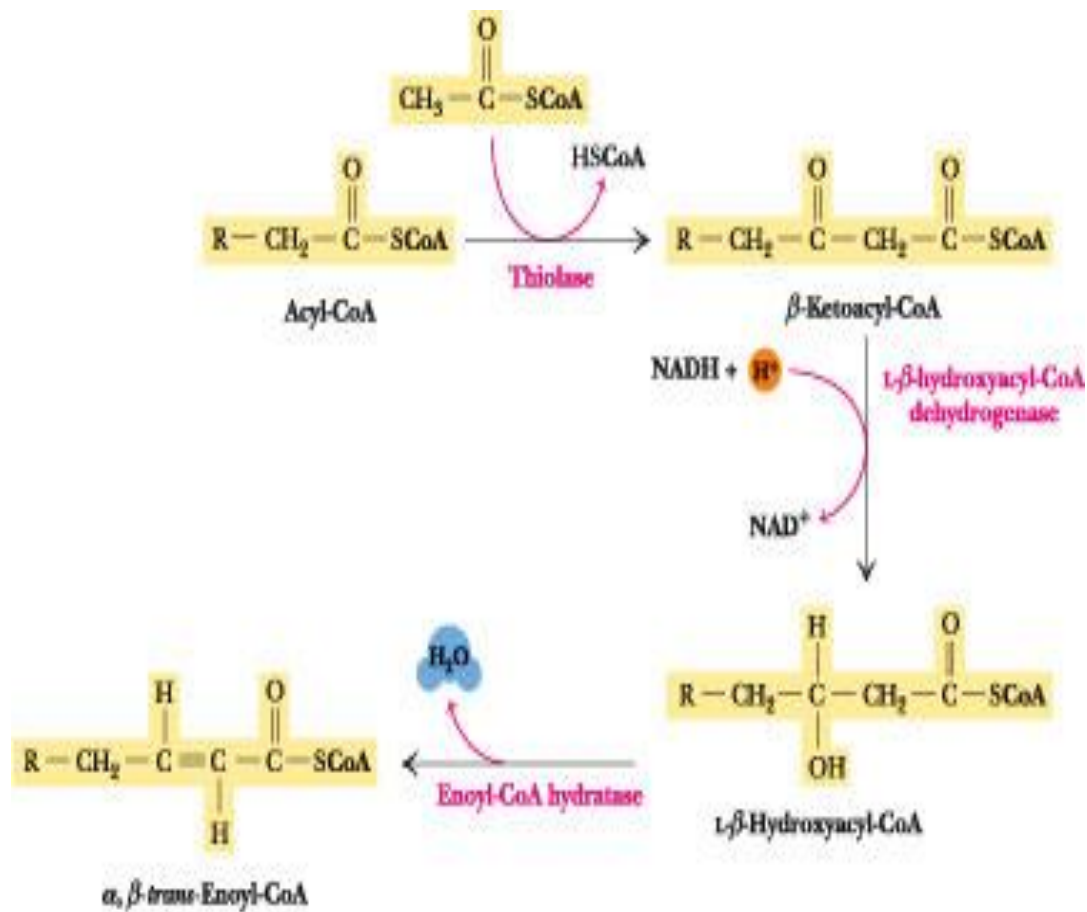
Mitochondrial chain elongation (cont.)

3. Dehydration:

- The removal of a water molecule from the β -hydroxyacyl-CoA results in the formation of α - β -unsaturated acyl-CoA. This reaction is catalyzed by enoyl-CoA hydratase.

4. Reduction (2nd):

- The α - β -unsaturated acyl-CoA undergoes reduction by enoyl-CoA reductase, using NADPH as a reducing agent. This reduction introduces a double bond between the α and β carbons, resulting in the formation of a two-carbon longer acyl-CoA.
- The product formed in step 4 undergoes repeated rounds of elongation by re-entering the cycle at step 1, where it condenses with another molecule of acetyl-CoA, continuing the chain elongation process.



Further processing: desaturation

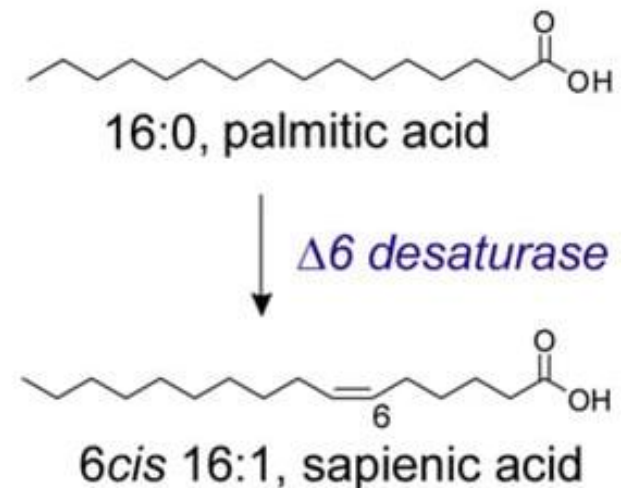
Desaturation of fatty acids

- Desaturation of fatty acids is a biochemical process that involves the **introduction of double bonds** into the fatty acid chains.
- This process typically occurs in the **endoplasmic reticulum** of cells and is catalyzed by enzymes known as **desaturases**.
- Desaturase enzymes are specific to the position of the double bond they introduce.
 - For example, **Δ^9 -fatty acid desaturase** introduces a double bond between the 9th and 10th carbon atoms counting from the carboxylic acid end of the fatty acid chain.

Desaturation of fatty acids (cont.)

Formation of monounsaturated fatty acids:

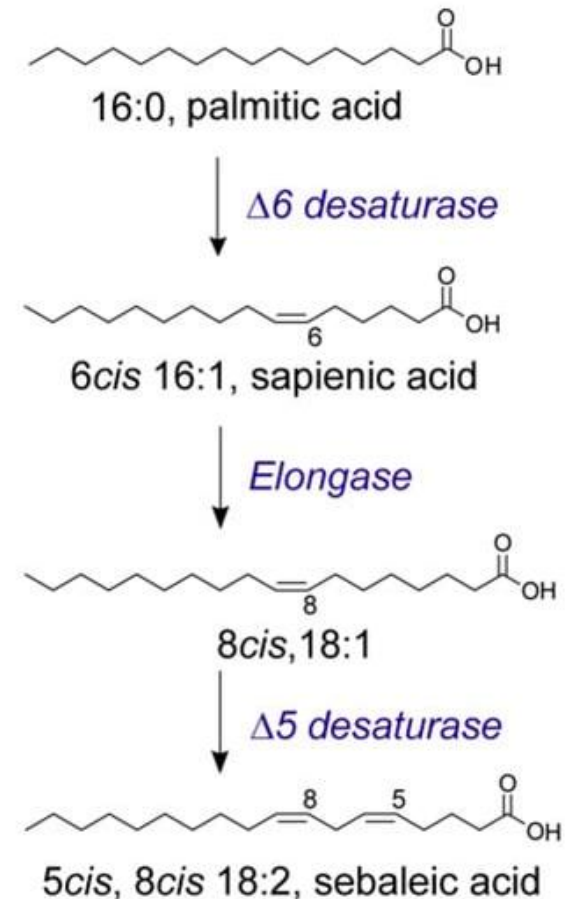
- The addition of a double bond results in the formation of a monounsaturated fatty acid.
 - For instance, the desaturation of palmitic acid (16:0) by Δ^6 -desaturase produces sapienic acid (16:1 Δ^6).
 - The desaturation of palmitic acid by Δ^9 -desaturase produces a different fatty acid, palmitoleic acid (16:1 Δ^9).

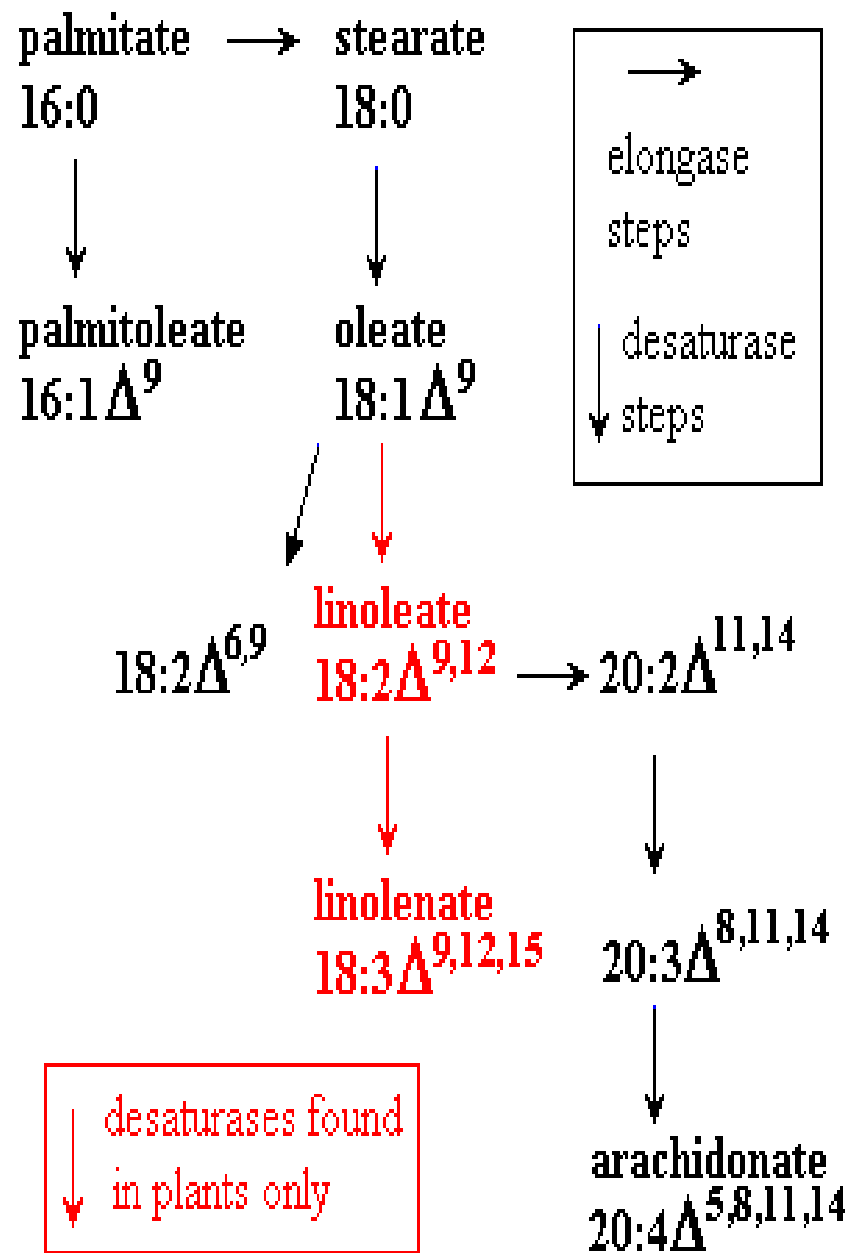


Desaturation of fatty acids (cont.)

Further desaturation:

- Further desaturation results in the formation of polyunsaturated fatty acids.
- This process requires the action of various **desaturases** that are specific to the positions of the additional double bonds, as well as **elongase** enzymes if the fatty acid chain needs to be extended.





Summary

- Fatty acid synthesis encompasses several pathways, each governed by specific enzymes and substrates:
- *De novo* lipogenesis initiates the process by synthesizing fatty acids from acetyl-CoA and malonyl-CoA, mainly in the liver and adipose tissue.
- Fatty acid elongation extends fatty acid chains through successive additions of two-carbon units, facilitated by enzymes localized in the endoplasmic reticulum (and to a lesser extent in mitochondria).
- Fatty acid desaturation introduces double bonds into fatty acid chains, enabling the formation of unsaturated fatty acids crucial for membrane fluidity and signaling.