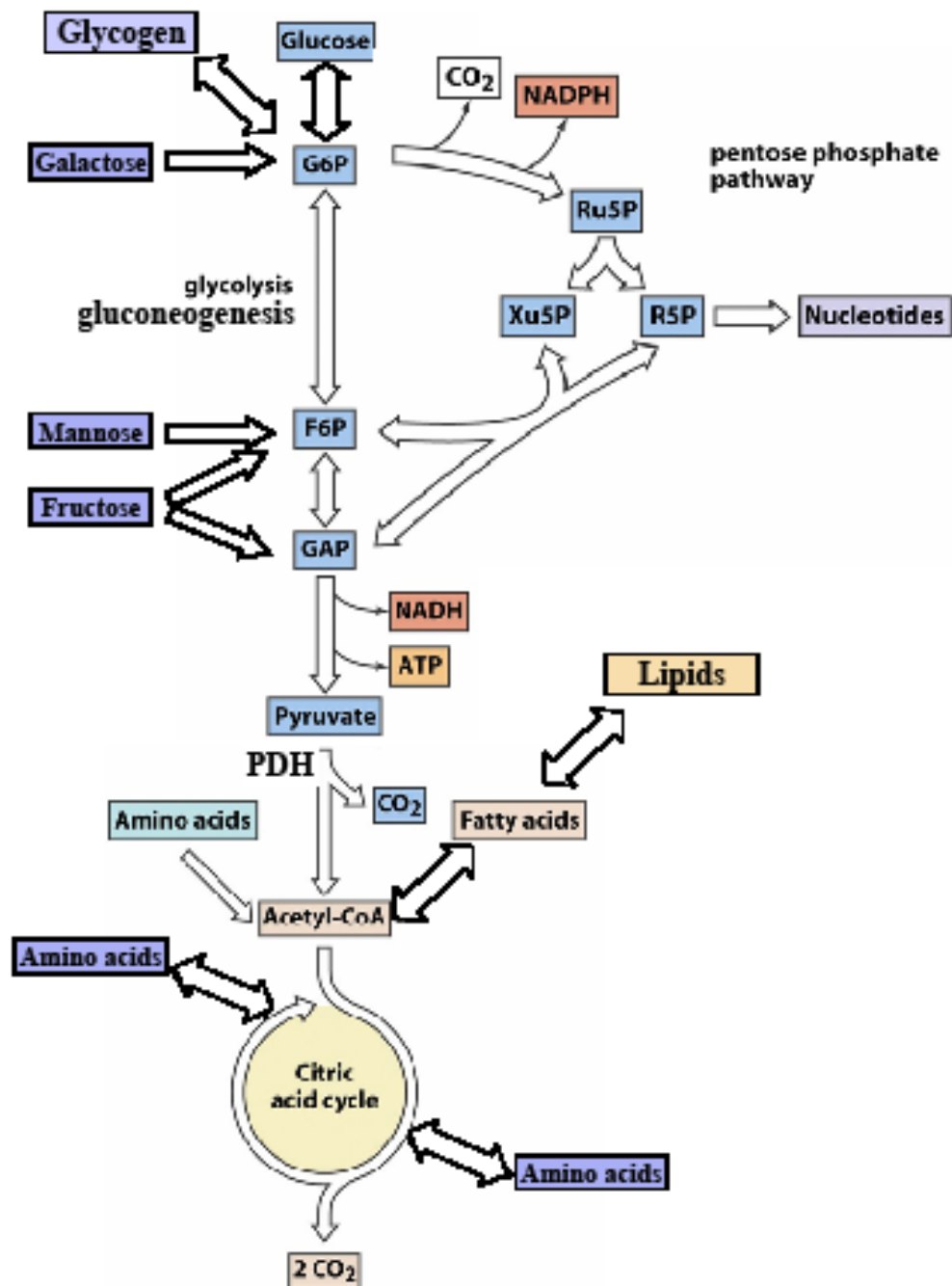


# Overview about metabolism so far....



# Fatty acids: Review

Fatty acids are fuel molecules that yield large amounts of energy when degraded in a series of reactions in the mitochondria. Other important functions of fatty acids include:

- 1) serving as building blocks of Phospholipids and glycolipids:
- 2) acting (as derivatives) as hormones and intracellular messengers:
- 3) modifying proteins through covalent attachment, and so affecting their cellular targeting and functioning.

Fatty acids contain a long hydrocarbon chain and a terminal carboxylate group. Most contain between 14 and 24 carbon atoms. The chains may be saturated or contain double bonds. The complete oxidation of fatty acids yields about 9 kcal/g, which is over 2 times the energy that can be obtained from the same weight of carbohydrate.

# Fatty acid breakdown

- Fatty acids are an important energy source. Oxidation of fatty acids generates the high-energy compounds reduced NAD (NADH) and reduced flavine adenine dinucleotide (FADH<sub>2</sub>) and yields acetyl CoA, which is the substrate for the TCA cycle
- Fatty acid breakdown (also called  $\beta$ -oxidation) brings about the oxidation of long - chain fatty acids with the production of energy in the form of ATP
- Fatty acids are converted into their acyl CoA derivatives and then metabolized by the removal of two-carbon acetyl CoA units from the end of the acyl chain

## Basic Concepts

### 1. OXIDATION FUNCTION

- To break down fatty acids to acetyl-CoA to provide fuel for the TCA cycle

### 2. OXIDATION LOCATION

Mitochondria of all tissues

### 3. CARNITINE SHUTTLE

Transfers fatty acids from cytoplasm to mitochondria for oxidation

Inhibited by malonyl-CoA

# key enzymes in fatty acid metabolism

1. **Fatty acyl CoA synthetase** - enzyme catalyzing the "priming" reaction in fatty acid metabolism which converts free fatty acids in the cytosol into fatty acyl-CoA using the energy available from ATP and PPI hydrolysis. When the energy charge in the cell is low, the fatty acyl-CoA is used for fatty acid oxidation inside the mitochondria, however, when the energy charge is high, the fatty acyl-CoA is used to synthesize triacylglycerols or membrane lipids.
2. **Carnitine acyltransferase I** - catalyzes the committed step in fatty acid oxidation which links fatty acyl-CoA molecules to the hydroxyl group of carnitine. The activity of carnitine acyltransferase I is inhibited by malonyl CoA, the product of the acetyl-CoA carboxylase reaction, which signals that glucose levels are high and fatty acid synthesis is favoured.

**3. Acetyl CoA carboxylase** - catalyzes the commitment step in fatty acid synthesis using a biotin-mediated reaction mechanism that carboxylates acetyl CoA to form the C3 compound malonyl CoA. The activity of acetyl CoA carboxylase is regulated by both reversible phosphorylation (the active conformation is dephosphorylated) and allosteric mechanisms (citrate binding stimulates activity, palmitoyl-CoA inhibits activity).

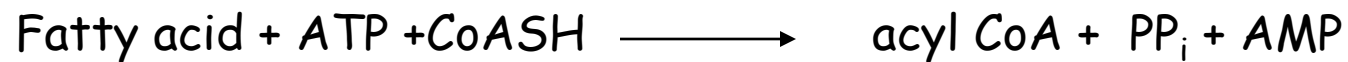
**4. Fatty acid synthase** - this large multi-functional enzyme is responsible for catalyzing a series of reactions that sequentially adds C2 units to a growing fatty acid chain covalently attached to the enzyme complex. The mechanism involves the linking malonyl-CoA to an acyl carrier protein, followed by a decarboxylation and condensation reaction that extends the hydrocarbon chain.

## Activation of fatty acids:

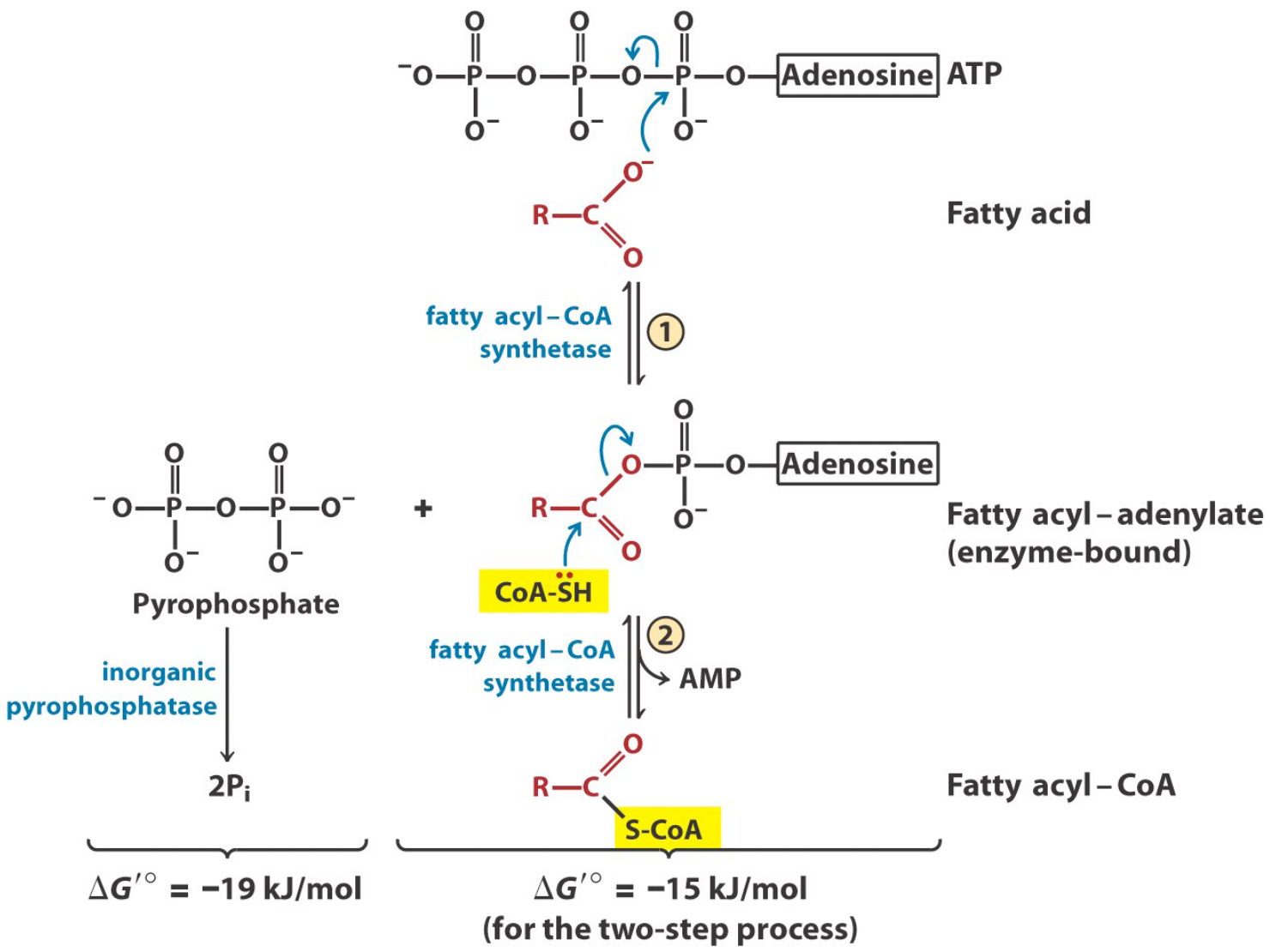
-Fatty acid breakdown occurs in the cytosol of prokaryotes and in the mitochondrial matrix of eukaryotes

-Before entering the **mitochondrial matrix**, the fatty acid is activated by forming a thioester link with CoA; The reaction is catalyzed by **acyl CoA synthase** (fatty acid thiokinase) which is present on the outer mitochondrial membrane, and uses a molecule of ATP.

-The overall reaction is irreversible due to the subsequent hydrolysis of  $PP_i$  to two molecules of  $P_i$



# Fatty acids need activation by CoASH for degradation



-The illustration shows the two step fatty acyl-CoA reaction catalyzed by medium chain fatty acyl CoA synthetase.

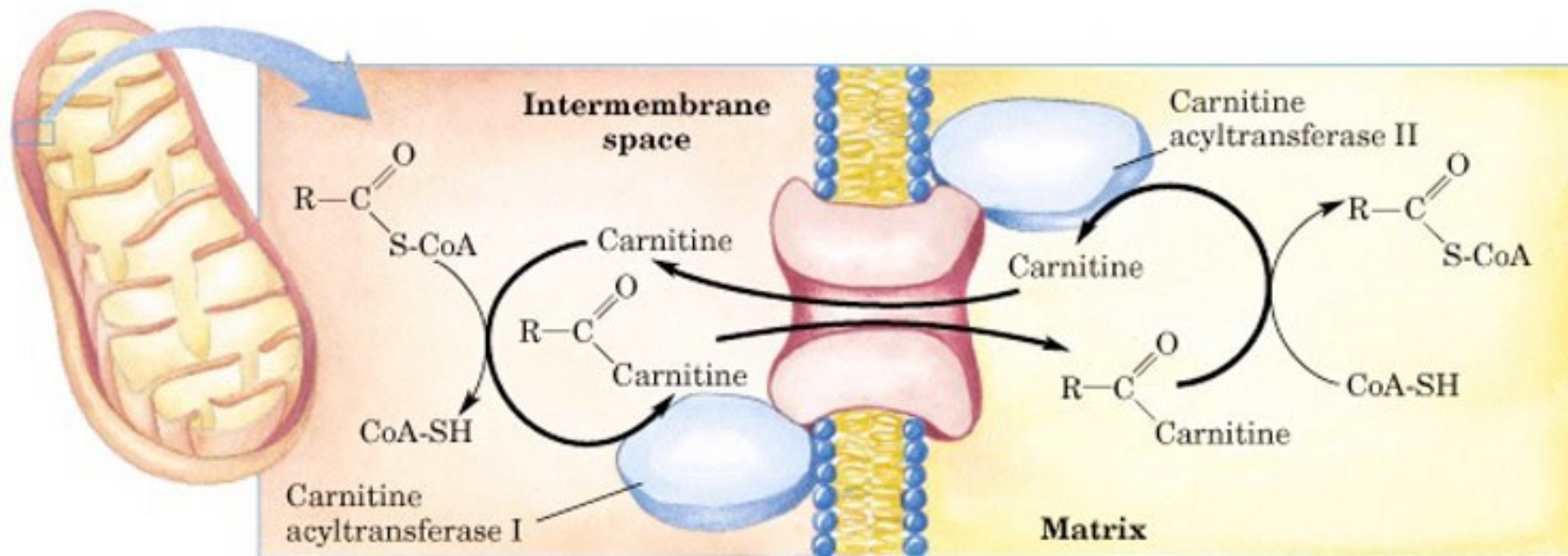
-In the first step, the carboxylate ion of the fatty acid attacks a phosphate in ATP to form an acyl-adenylate intermediate and release pyrophosphate (PPi) which is quickly hydrolyzed by the enzyme **inorganic pyrophosphatase** to form  $2 P_i$ .

-In the second step of the fatty acyl CoA synthetase reaction, the fatty acyl-adenylate intermediate is attacked by the thiol group of CoA to form the thioester fatty acyl-CoA product and release AMP.



# Transport of acyl CoA into mitochondria

- Small and medium-chain acyl CoA molecules ( $\sim 10$  C atoms) are readily able to cross the **inner mitochondrial membrane** by diffusion
- Longer chain acyl CoAs don't readily cross the membrane, and require a specific transport mechanism
- This is achieved by the conjugation of the long acyl CoAs to carnitine molecules
- This is catalyzed by **carnitine acyltransferase I**
- The CoA gp is removed and substituted with a carnitine molecule

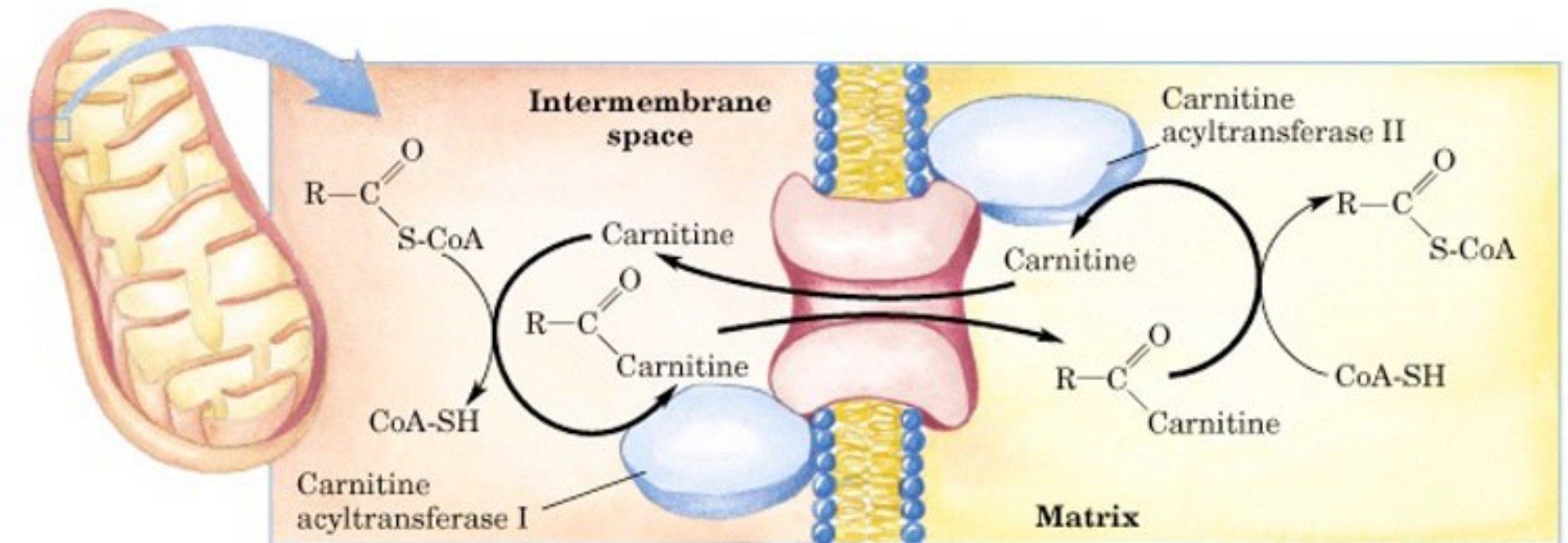


-The acylcarnitine is then transported across the membrane by a **carnitine/acylcarnitine translocase**.

-This integral membrane transport protein transports acylcarnitine molecules into the mitochondrial matrix and free carnitine molecules out

-Once inside the matrix, the acyl gp is transferred back on to CoA, releasing free carnitine, by the enzyme **Carnitine acyltransferase II** which is located on the matrix side of the inner mitochondrial membrane

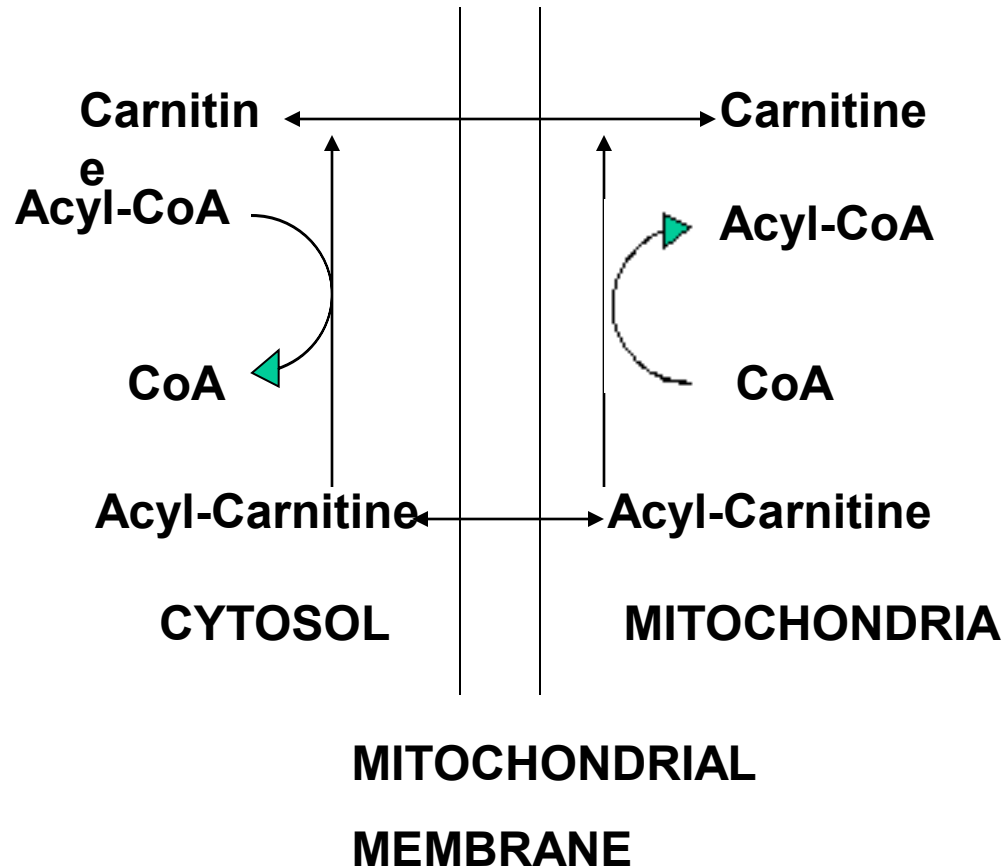
\*\* Carnitine mediates transfer of fatty acyl gps from the cytosol to the mitochondrial matrix where they are oxidized



# Transfer reaction:

-On the outer surface of the inner mitochondrial membrane, the enzyme **carnitine palmitoyl transferase I (CPT I)** catalyzes the transfer of the acyl gp from CoA to carnitine

-The fatty acyl group is translocated across the membrane to the inner surface, where the enzyme **CPT II** catalyzes the transfer of the acyl gp to CoA drawn from the matrix CoA pool



## Sources of carnitine

1. **Dietary** sources include red meat and dairy products
2. **Synthesis.** Carnitine ( $\beta$ -hydroxy- $\gamma$ -trimethylammonium butyrate) may be synthesized in the body from the amino acid lysine

**Carnitine deficiency (in humans\*)** may be classified as:

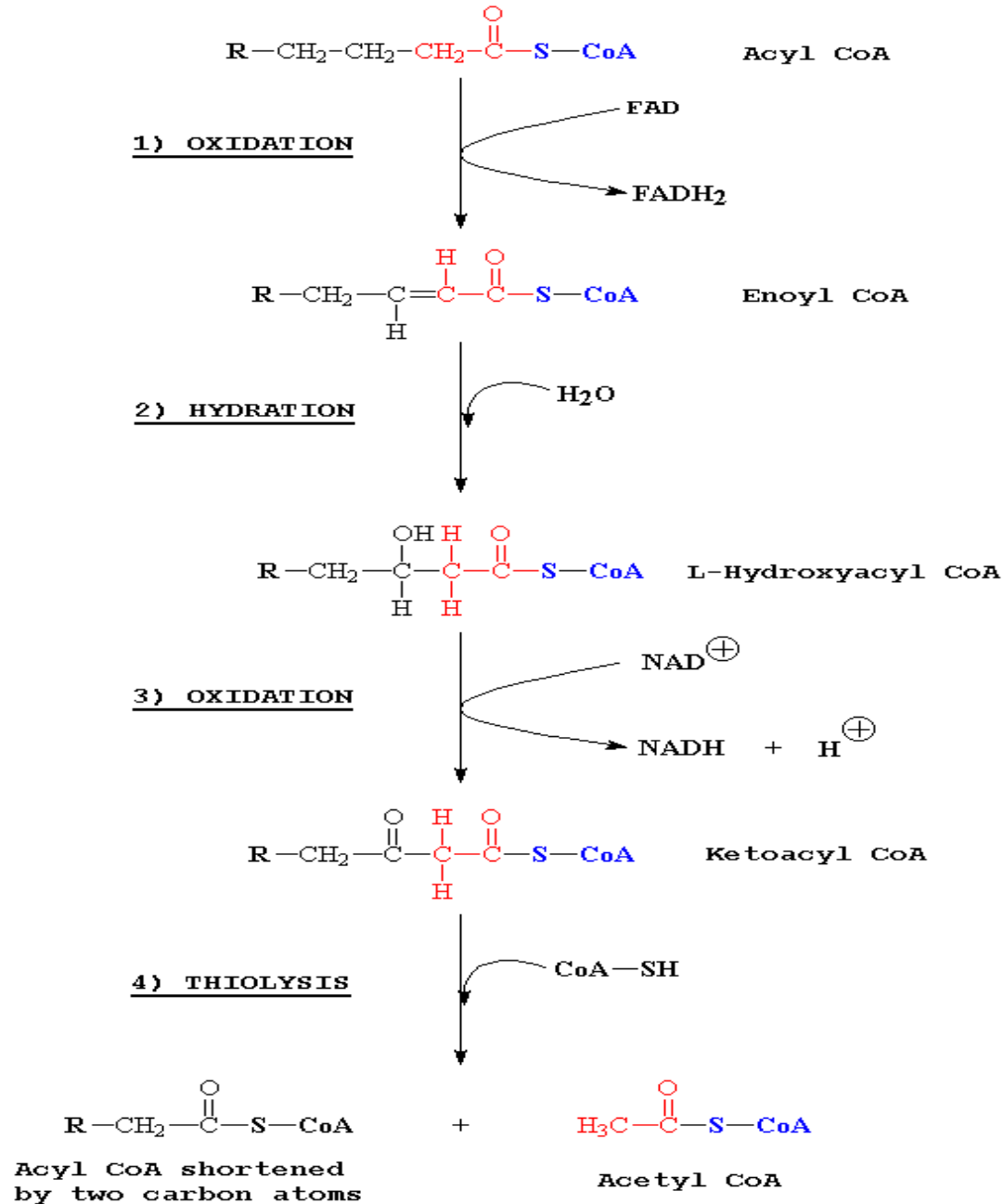
1. **Systemic**, in which levels are reduced in all tissues
2. **Myopathic**, in which levels are reduced only in muscle, including the heart

# $\beta$ -oxidation pathway

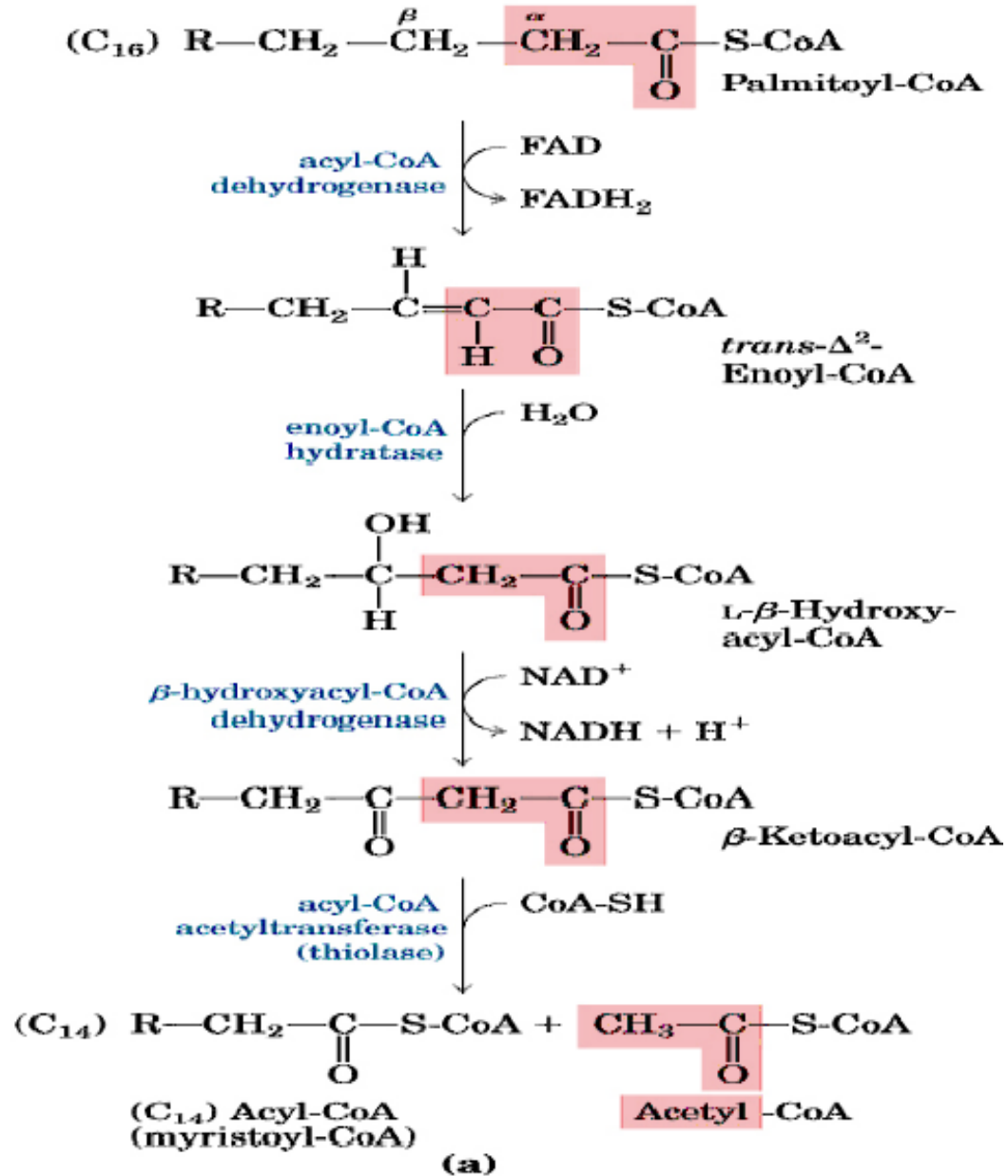
The individual reactions involved in the degradation of fatty acids by  $\beta$ -oxidation are as follows:

1. **Oxidation** of the fatty acyl CoA to enoyl CoA forming a trans  $\Delta^2$ -double bond on the fatty acyl chain and producing  $\text{FADH}_2$  (catalyzed by **acyl CoA dehydrogenase**)
2. Hydration of the two trans  $\Delta^2$ -enoyl CoA to form 3-hydroxyacyl CoA (catalyzed by **enoyl CoA hydratase**)
3. **Oxidation** of 3-hydroxyacyl CoA to 3-ketoacyl CoA producing NADH (catalyzed by **hydroxyacyl CoA dehydrogenase**)
4. Cleavage of thiolytic, of 3-ketoacyl CoA by a second CoA molecule, giving acetyl CoA and an acyl CoA shortened by two carbon atoms (catalyzed by  **$\beta$ -ketothiolase**)

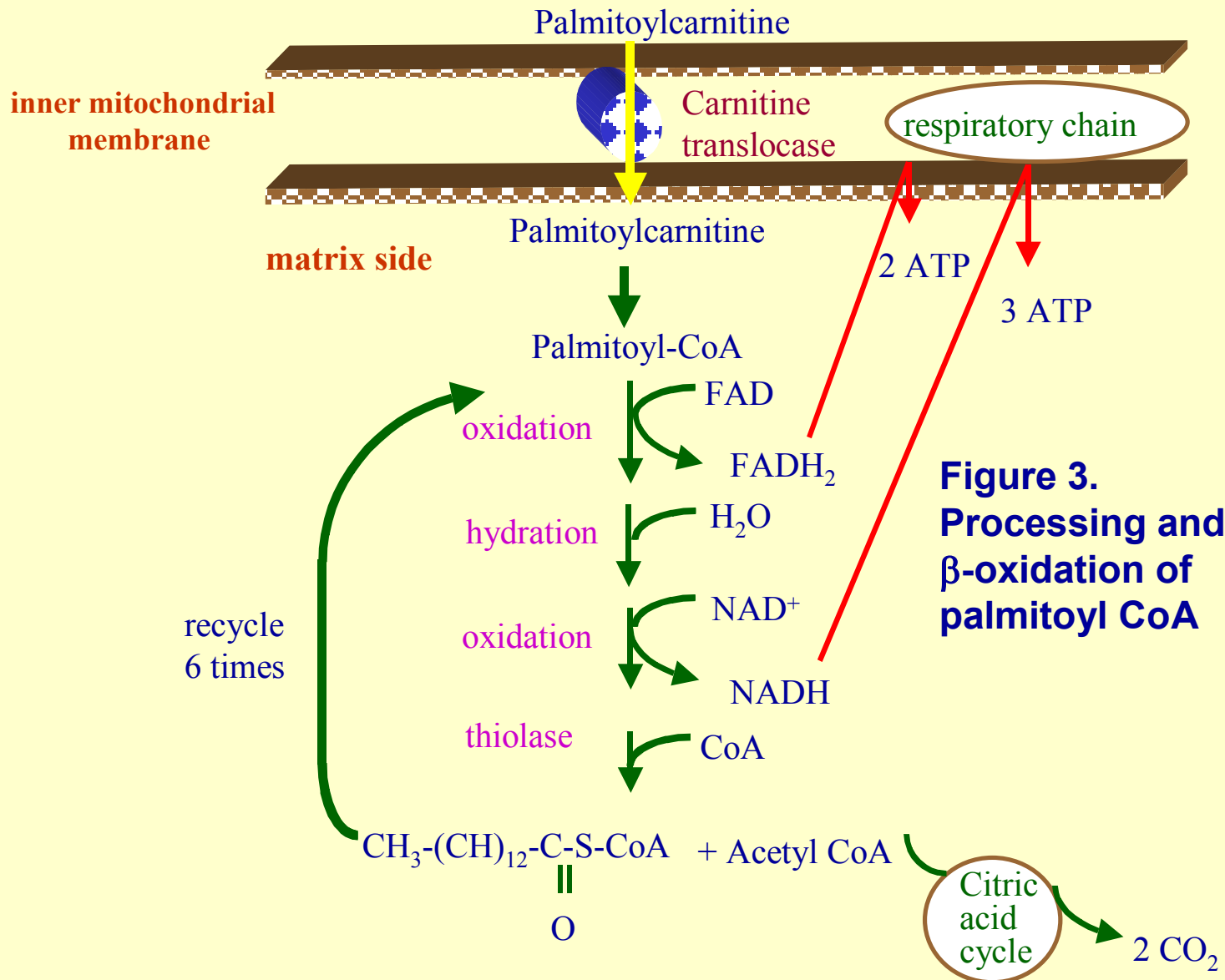
BETA-OXIDATION REACTION SEQUENCE



# Fatty acids undergo $\beta$ -oxidation

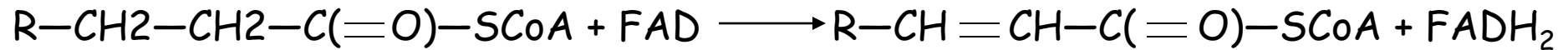


# $\beta$ -Oxidation of palmitate

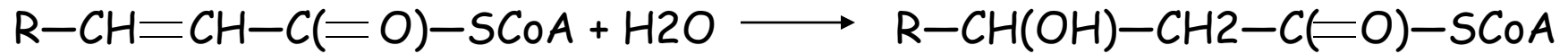




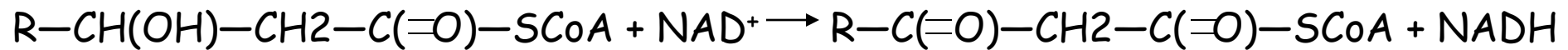
### Oxidation:



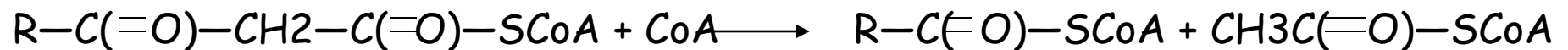
### Hydration:



### Oxidation:



### Cleavage:



\*\*\*What oxidation actually accomplishes is the removal of a C-2 unit as acetyl-CoA from the carboxyl end of the fatty acid. This keeps happening until the fatty acid is completely converted to acetyl-CoA.

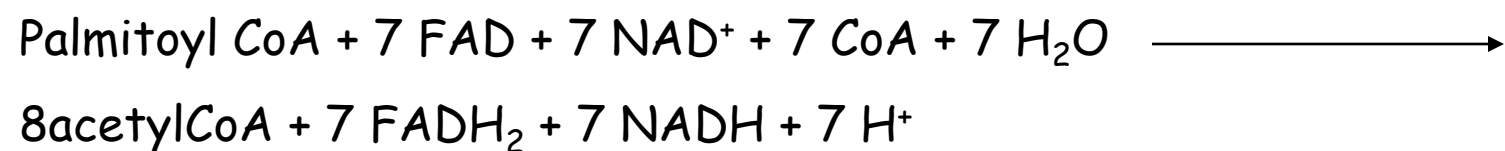
-Thus, the breakdown of individual fatty acids occurs as a repeating sequence of 4 reactions: **Oxidation** by (FAD), **Hydration**, **Oxidation** (by NAD<sup>+</sup>) and **thiolysis**

-The overall effect is to remove 2-C units sequentially in the form of acetyl CoA from the fatty acid chain

-Cleavage of the  $\Delta^2$  (or  $\beta$ ) bond of the fatty acyl chain gives fatty acid breakdown its alternative name  **$\beta$ -oxidation**

-The shortened acyl CoA then undergoes further cycles of  $\beta$ -oxidation until the last cycle, when the acyl CoA is with four carbon atoms is split into 2 molecules of acetyl CoA

-Thus a C16 saturated acyl CoA, such as palmitoyl CoA, would be completely degraded into 8 molecules of acetyl CoA by 7 rounds of degradation, leading to the overall equation:



# OXIDATION OF UNSATURATED FATTY ACIDS

-They require some additional processing before they can be degraded completely by  $\beta$ -oxidation

-If a fatty acid already has a double bond in it, the scheme by which the fatty acid is oxidized depends on where the double bond ends up after several of the C-2 fragments have been removed by normal  $\beta$ -oxidation

-As the oxidation machinery chews off 2-carbon fragments, it nibbles down to one of two possible situations depending on whether the first double bond started out at an *even*- or an *odd*-numbered carbon when counting from the carboxylate end. If the double bond is on an odd-numbered carbon (as in *cis*  $\Delta^9C_{18:1}$ ), it is metabolized slightly differently than a fatty acid in which the unsaturation is on an even-numbered carbon (as in *cis*  $\Delta^{12}C_{18:1}$ )

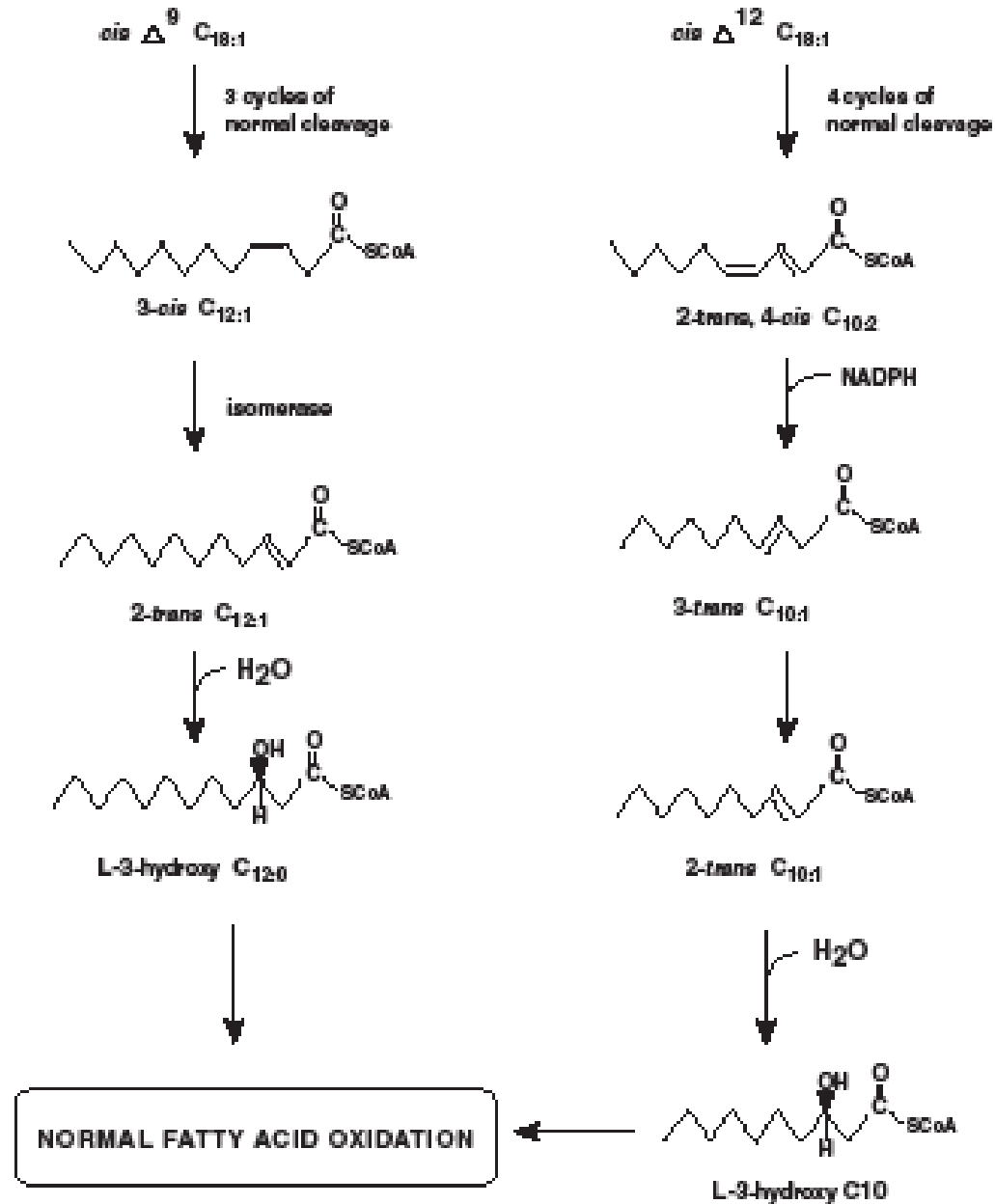
-If the double bond is on an odd carbon,  $\beta$  oxidation removes 2-carbon fragments until it gets to the structure with a 3-*cis* double bond [R-CH=CH-CH<sub>2</sub>-C(=O)-SCoA]. A new double bond can't be placed between C-2 and C-3 because there's already a double bond at C-3

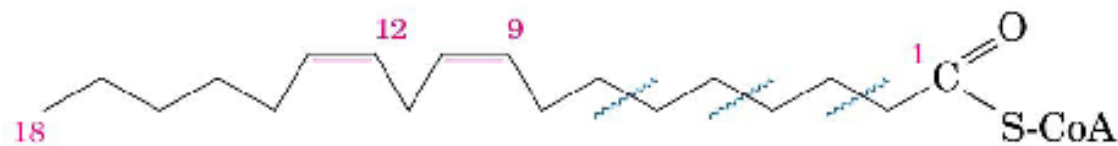
-In this situation, the activity of an isomerase simply moves the double bond from C-3 to C-2 and at the same time makes sure that the configuration is *trans*

-From this point on, the metabolism is just like normal oxidation (hydration, oxidation, cleavage)

\*\*\*If the double bond starts out at an even carbon,  $\beta$  oxidation runs its normal course until the structure *cis*-2-R-CH=CH-C(=O)-SCoA is reached. The double bond is on an OK carbon (C-2), but it's in the wrong configuration

-The double bonds in unsaturated fatty acids are invariably of the *cis* configuration, but  $\beta$ -oxidation introduces the double bond at C-2 in the *trans* configuration

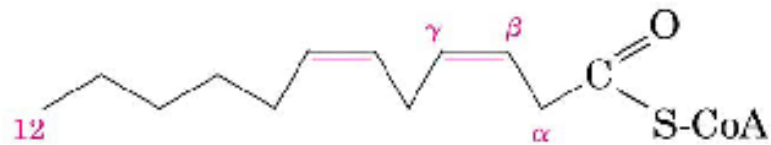




Linoleoyl-CoA  
*cis*- $\Delta^9$ ,*cis*- $\Delta^{12}$

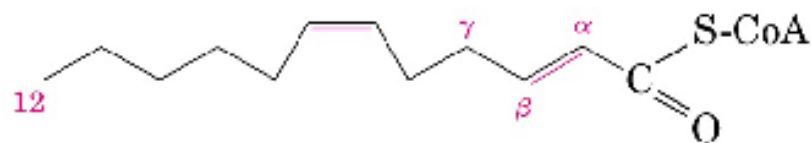
$\beta$  oxidation  
(three cycles)

→ 3 Acetyl-CoA



*cis*- $\Delta^3$ ,*cis*- $\Delta^6$

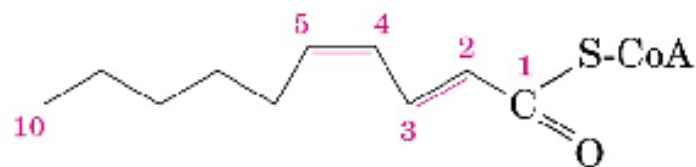
enoyl-CoA  
isomerase



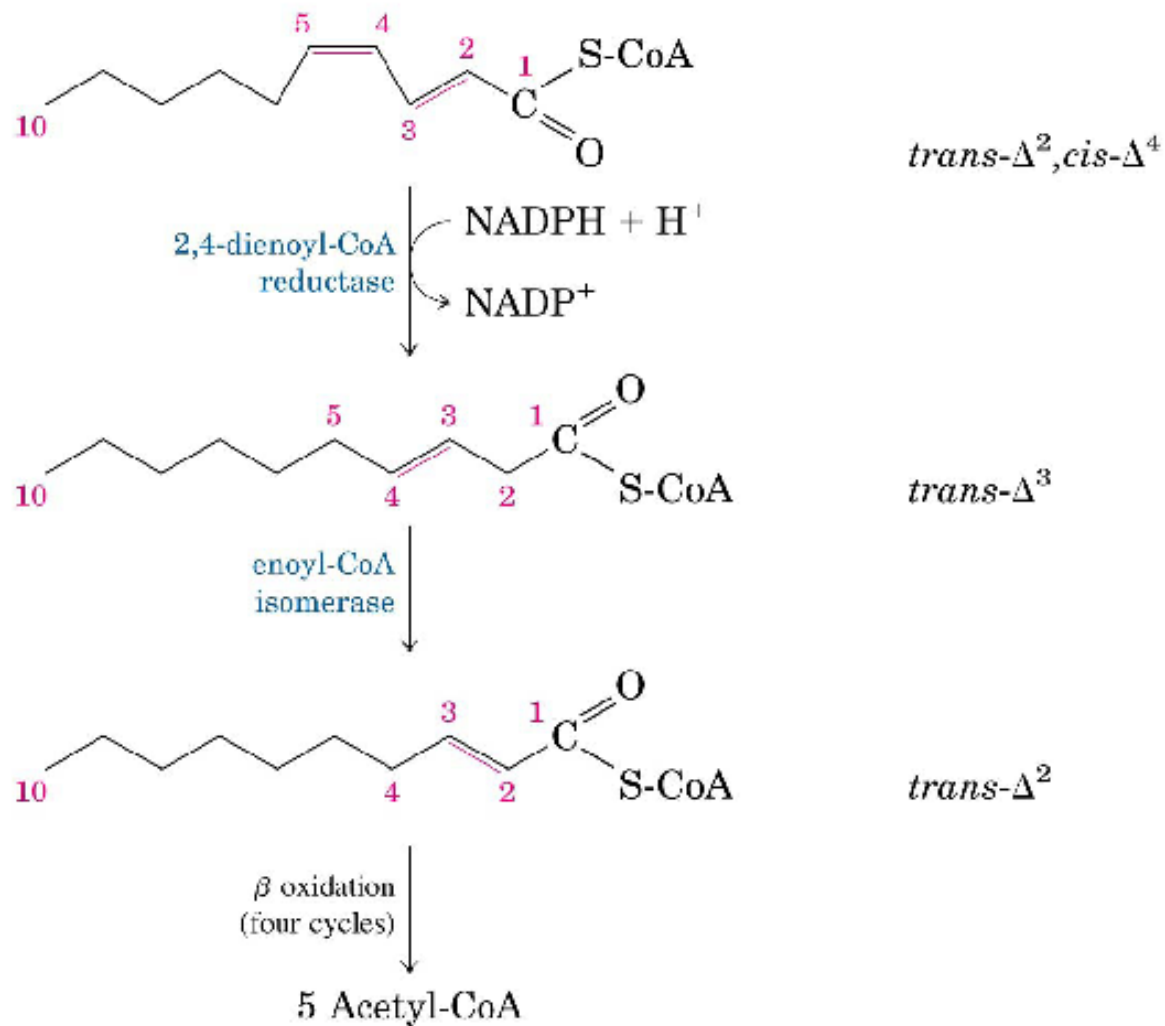
*trans*- $\Delta^2$ ,*cis*- $\Delta^6$

$\beta$  oxidation  
(one cycle, and  
first oxidation  
of second cycle)

→ Acetyl-CoA



## Polyunsaturated fatty acids further need reductases



## $\alpha$ -oxidation of fatty acids:

- This involves the oxidation of long-chain fatty acids to 2-hydroxyl fatty acids which are constituents of brain lipids, followed by oxidation of fatty acid with one less carbon
- Removal of one carbon from the carbonyl end of a fatty acid has been demonstrated in microsomal fractions from brain tissue

## $\omega$ -oxidation:

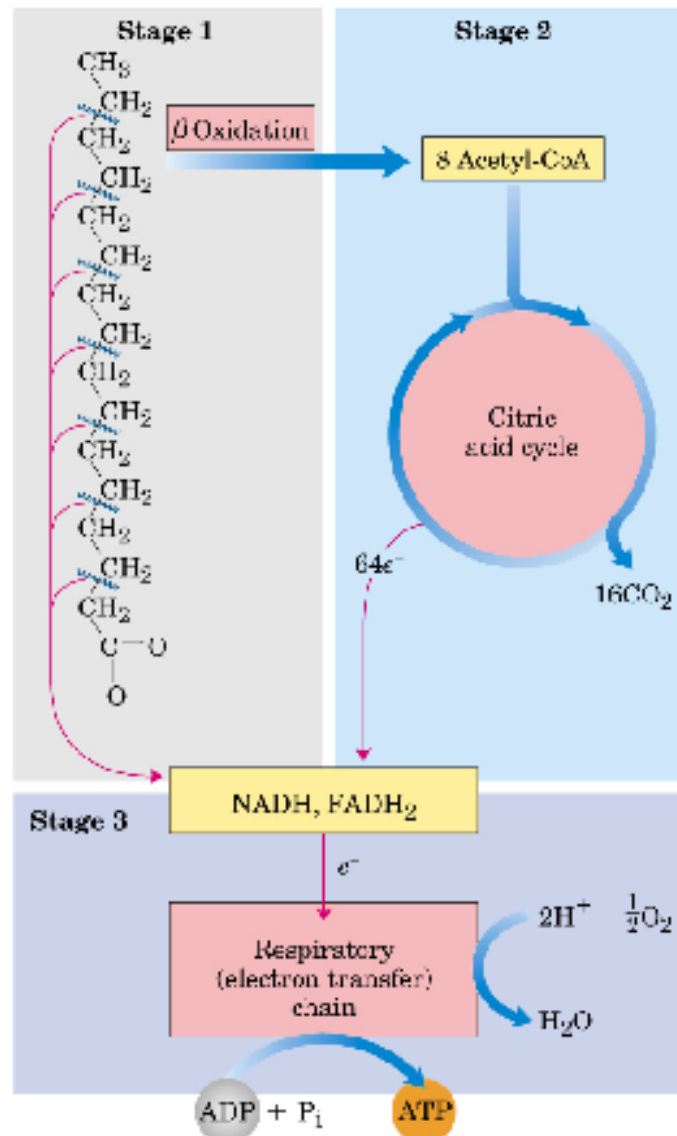
- Involves oxidation of the terminal methyl gp to form an  $\omega$ -hydroxy fatty acid
- This is a minor pathway observed with the liver microsomal preparations

## Regulation of $\beta$ -oxidation

- The major point of control is the availability of fatty acids
- The major source of free fatty acids in the blood is from the breakdown of **triacylglycerol** stores in adipose tissue which is regulated by the action of hormone-sensitive triacylglycerol lipase
- Fatty acid breakdown and synthesis are coordinately controlled so as to prevent a futile cycle



# Fatty acid $\beta$ -oxidation releases energy through the TCA cycle and the electron transport chain



## **$\beta$ -OXIDATION ATP YIELD**

$C_{16}$ fatty acid $\longrightarrow$ $16CO_2$	129 ATP
$C_{16}$ fatty acyl-CoA $\longrightarrow$ $16CO_2 + CoA$	131 ATP

### **Breakdown into steps:**

Activation of fatty acid to fatty acyl-CoA	-2 ATP
7 $FADH_2$ made from forming double bond at C-2 ( $7 \times 2$ )	14 ATP
7 NADH made from oxidations during formation of 3-ketoacyl-CoA ( $7 \times 3$ )	21 ATP
8 acetyl-CoA ( $8 \times 12$ ) through TCA cycle	96 ATP

When calculating ATP yields from  $\beta$  oxidation, you have to be careful to notice whether you start with the fatty acid or with the fatty acyl-