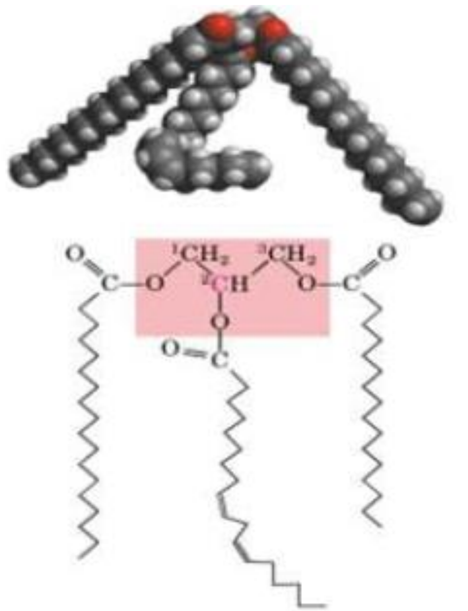
TRIACYLGLICEROL METABOLISM; LIPOLYSIS

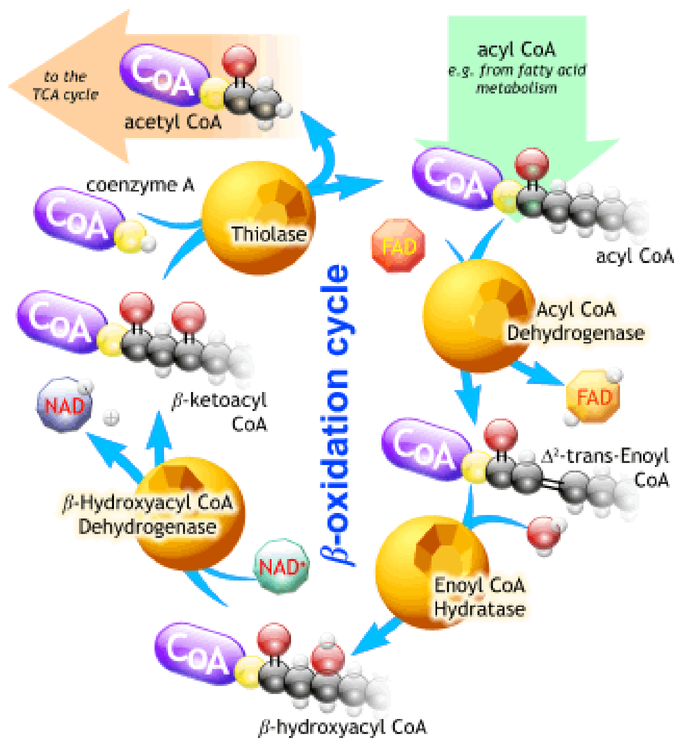
OXIDATION OF FATTY ACIDS

KETOGENESIS



1-Stearoyl, 2-linoleoyl, 3-palmitoyl glycerol, a mixed triacylglycerol

Triacylglycerols – esters of fatty acids and glycerol



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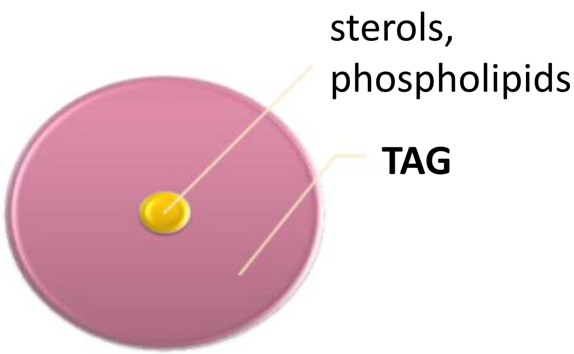
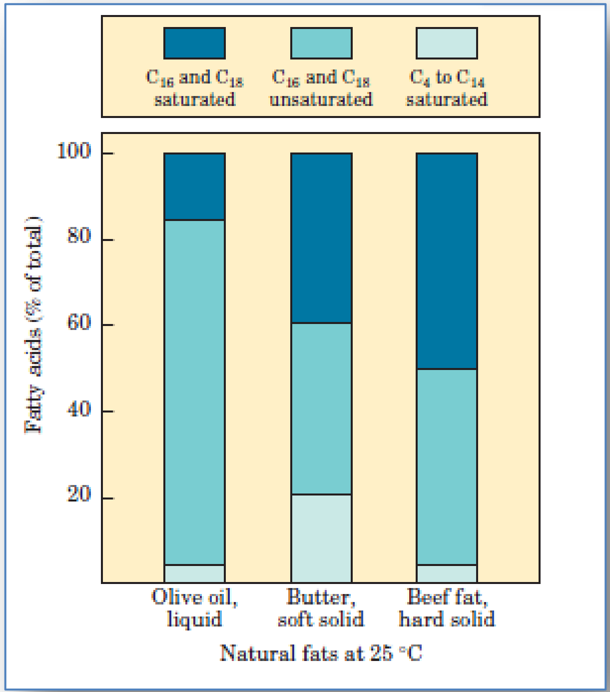
FATTY ACID SOURCES

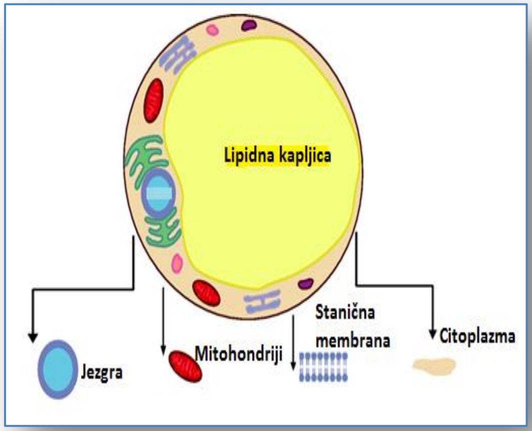
- **food** – **TAG, cholesterol, phospholipids**
    - 40% of energy comes from food lipids (recomended - up to 30%)
- **lipid reserves** – mobilization from **fat tissue (adipocytes)**
- ***de novo* fatty acids biosinthesis**
    - conversion of excess carbohydrates from food - **liver**
    - conversion of amino acids from food - **skeletal muscles**



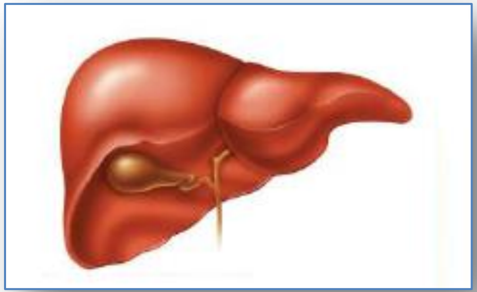
Food lipids



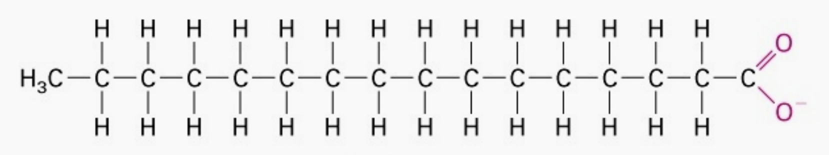
Food fatty acids



Adipocyte

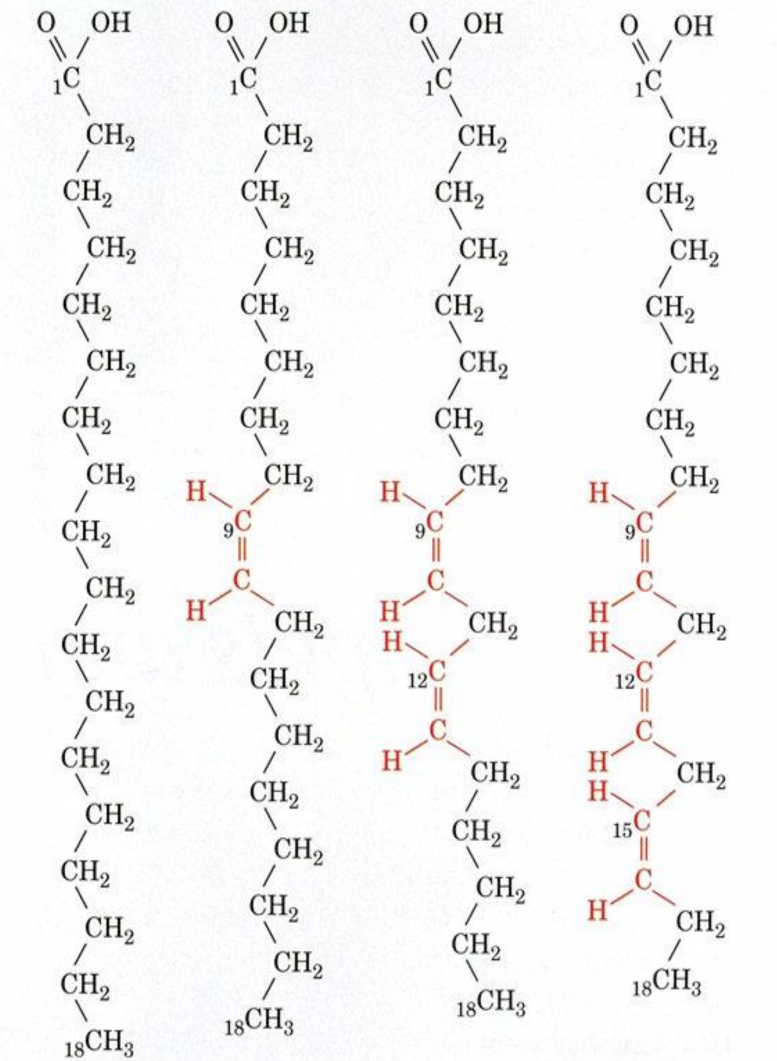


FATTY ACIDS



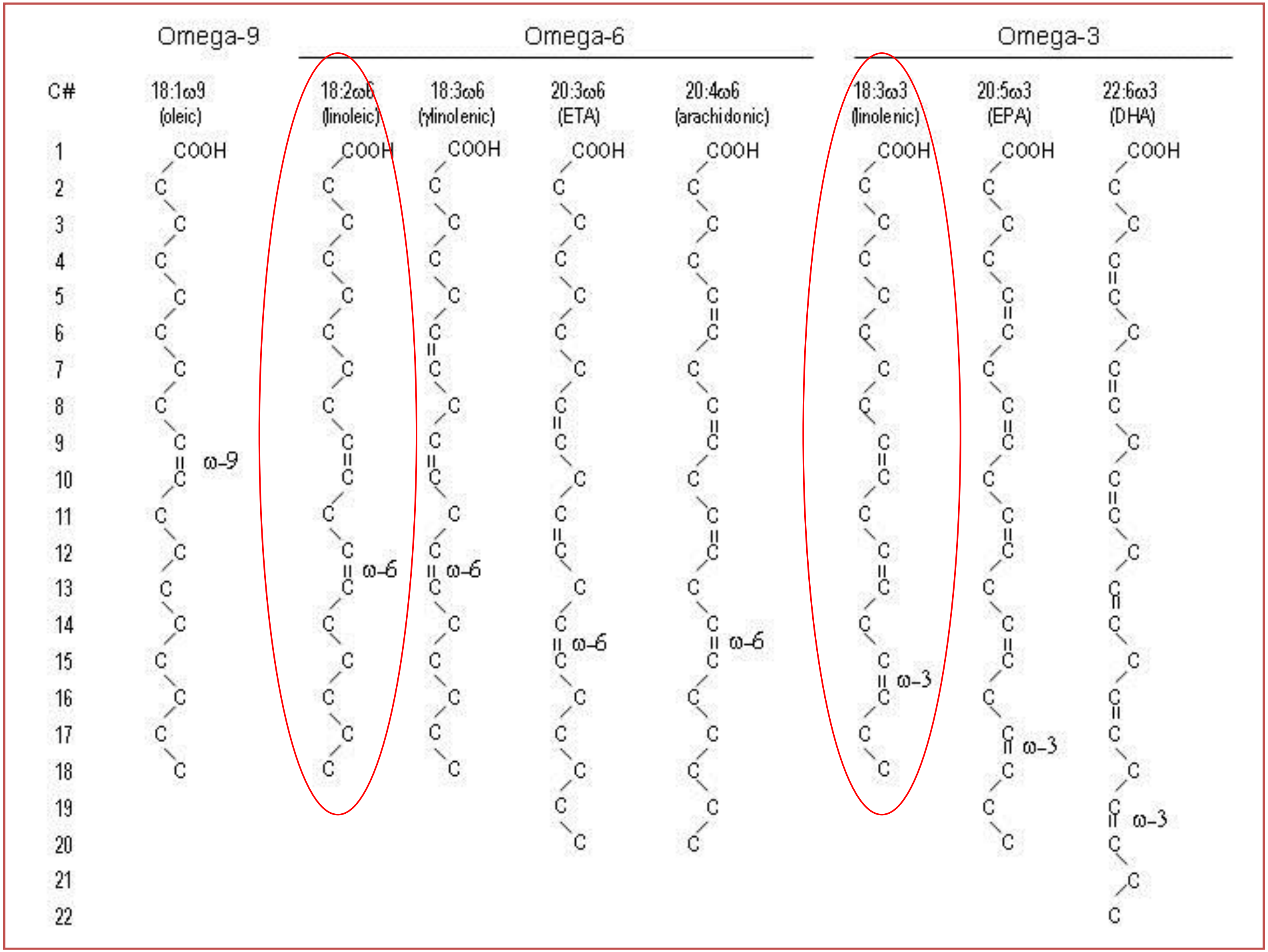
Palmitic acid (16:0)

Acid	Stearic	Oleic	Linoleic	α-Linolenic
# of carbons	18	18	18	18
Degree of unsaturation	18:0	18:1	18:2	18:3
Structure (all double bonds are *cis*)				



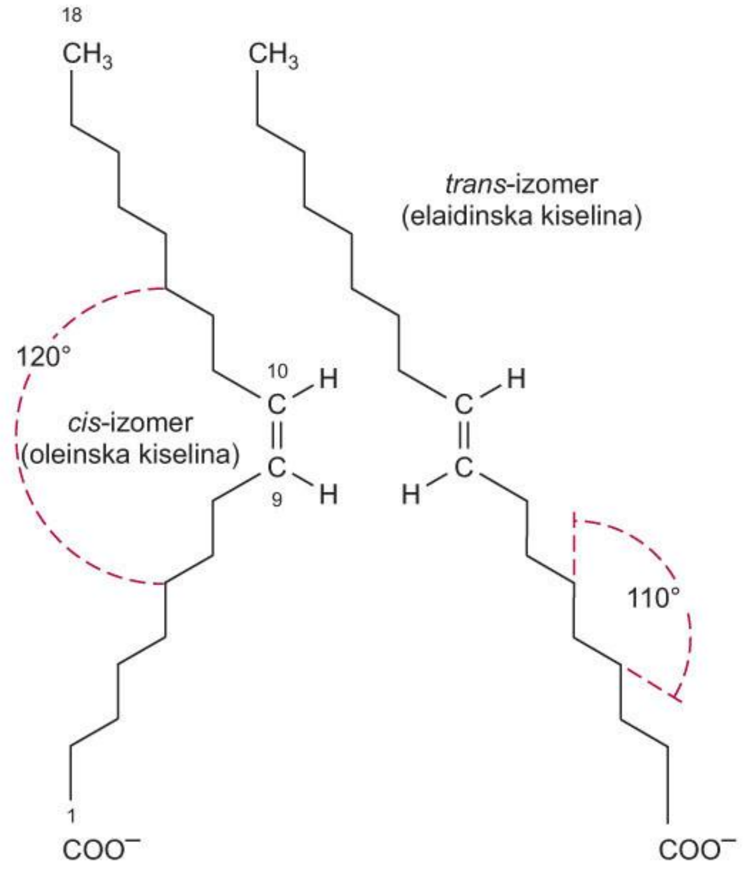
Stearic acid **Oleic acid** **Linoleic acid** **α-Linolenic acid**

ω3, ω6 i ω9 fatty acids



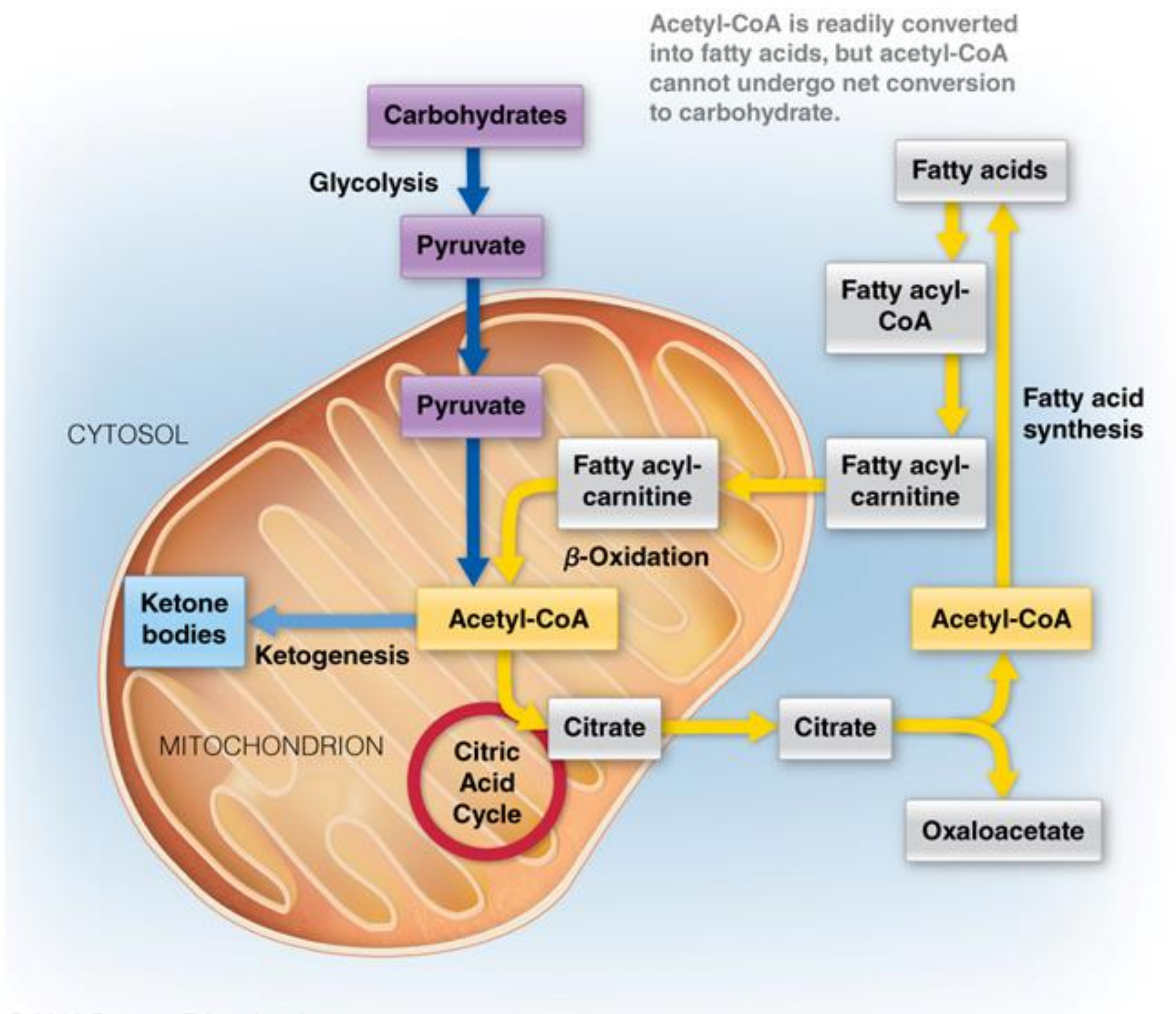
Essential fatty acids: linoleic (18:2) and α-linolenic (18:3)

- all natural fatty acids have double bonds in *cis* configuration

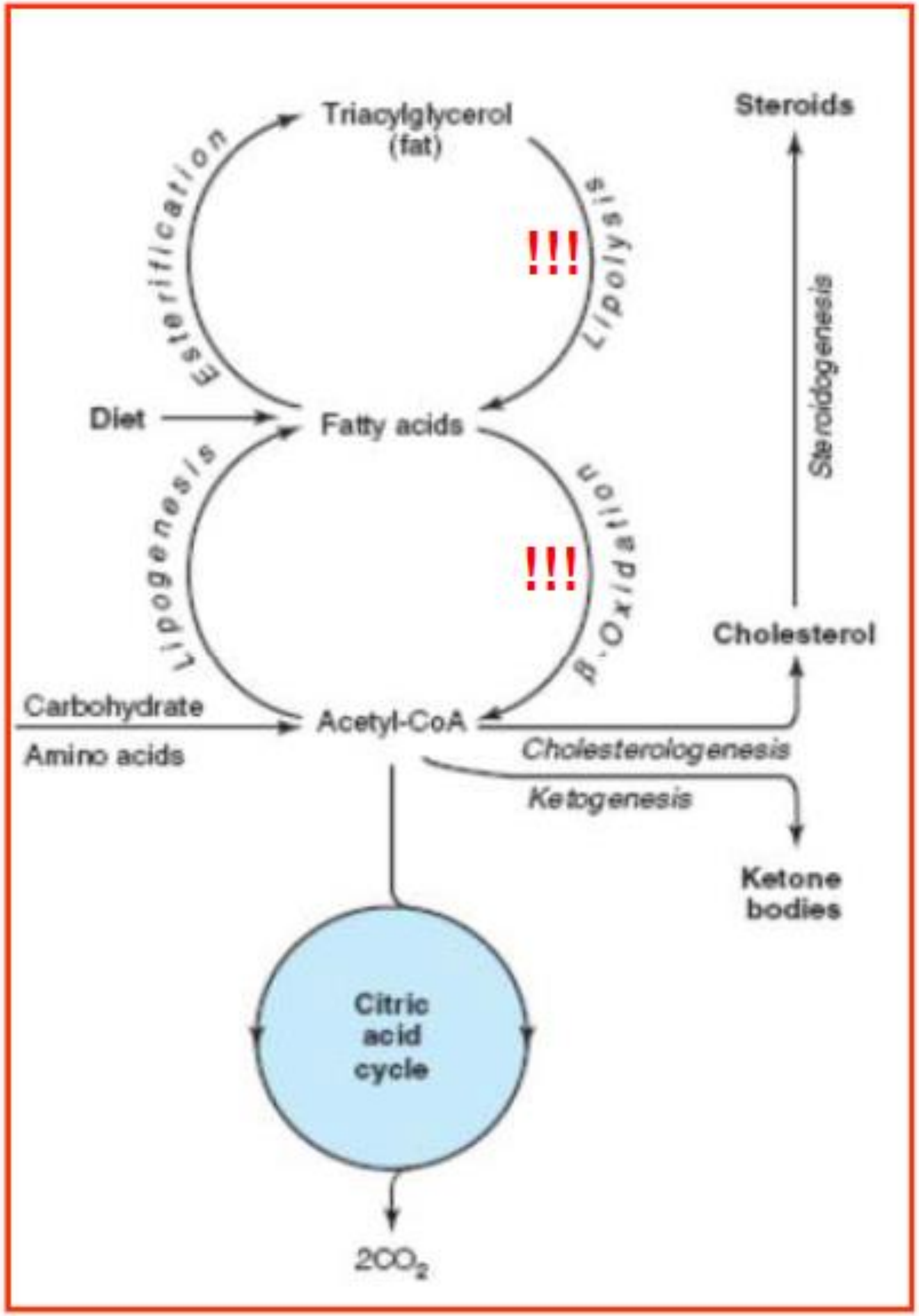


Slika 15-5. *Cis-trans*-izomerija masnih kiselina Δ^9 18:1 (oleinska i elaidinska kiselina).

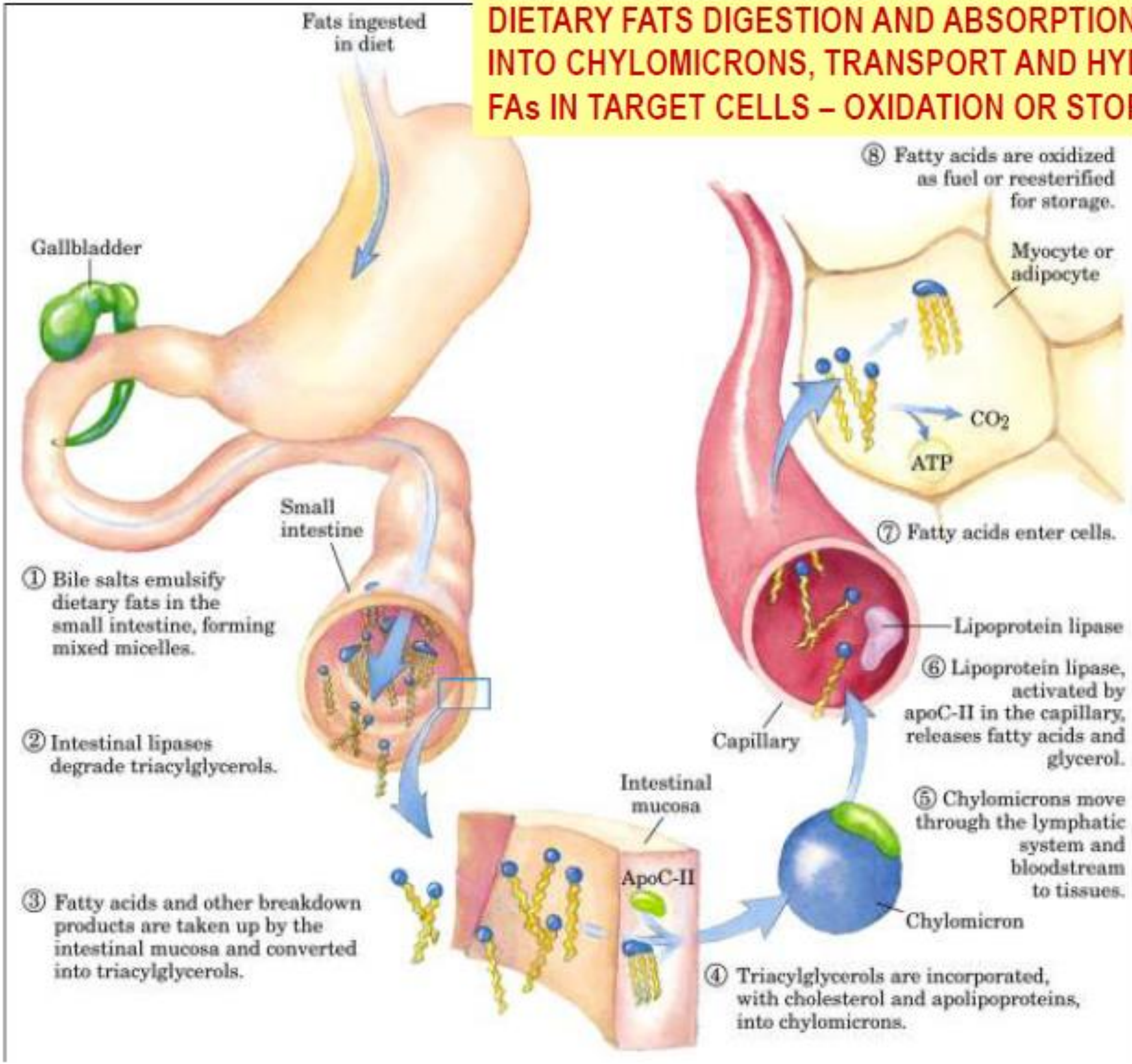
Acetyl-CoA - important intermedier linking carbohydrate and fatty acid metabolism



METABOLISM OF TRIACYLGLYCEROLS

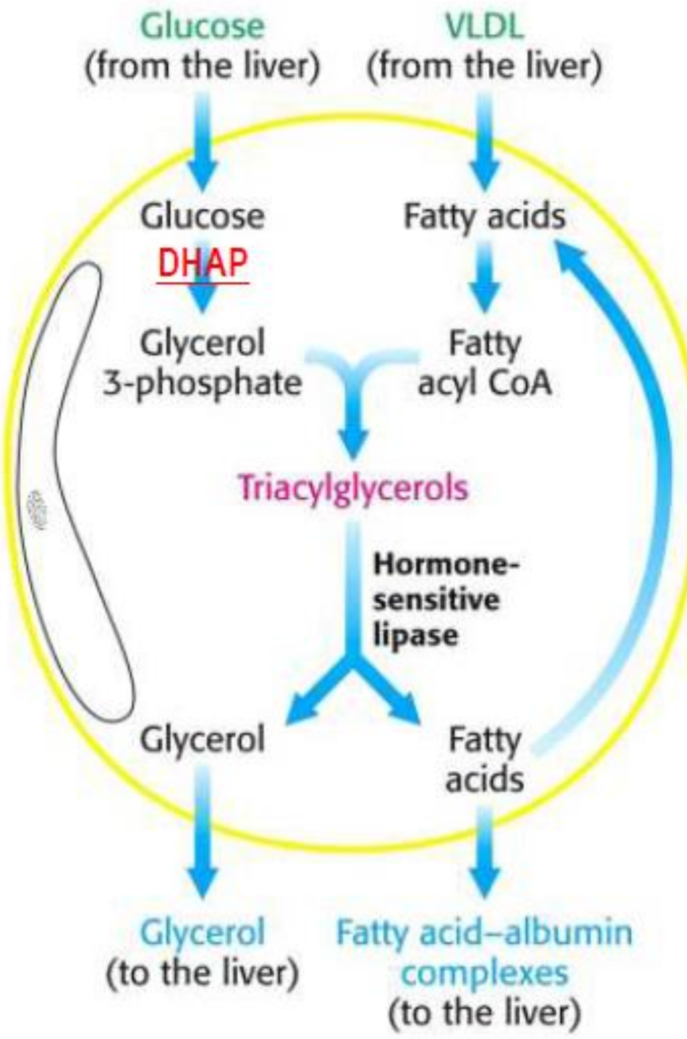


DIETARY FATS DIGESTION AND ABSORPTION, INCORPORATION INTO CHYLOMICRONS, TRANSPORT AND HYDROLYSIS; FAs IN TARGET CELLS – OXIDATION OR STORAGE



FATES OF TAGS IN ADIPOCYTES

ADIPOCYTE



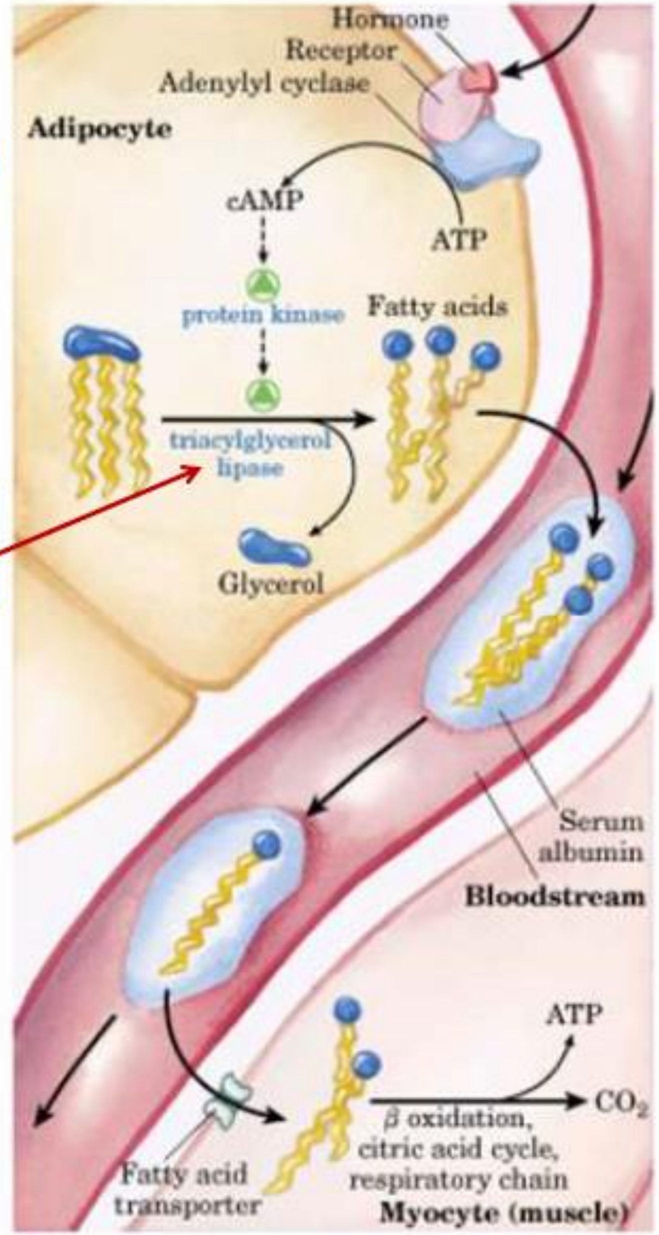
STORAGE

OR

MOBILIZATION (LIPOLYSIS)

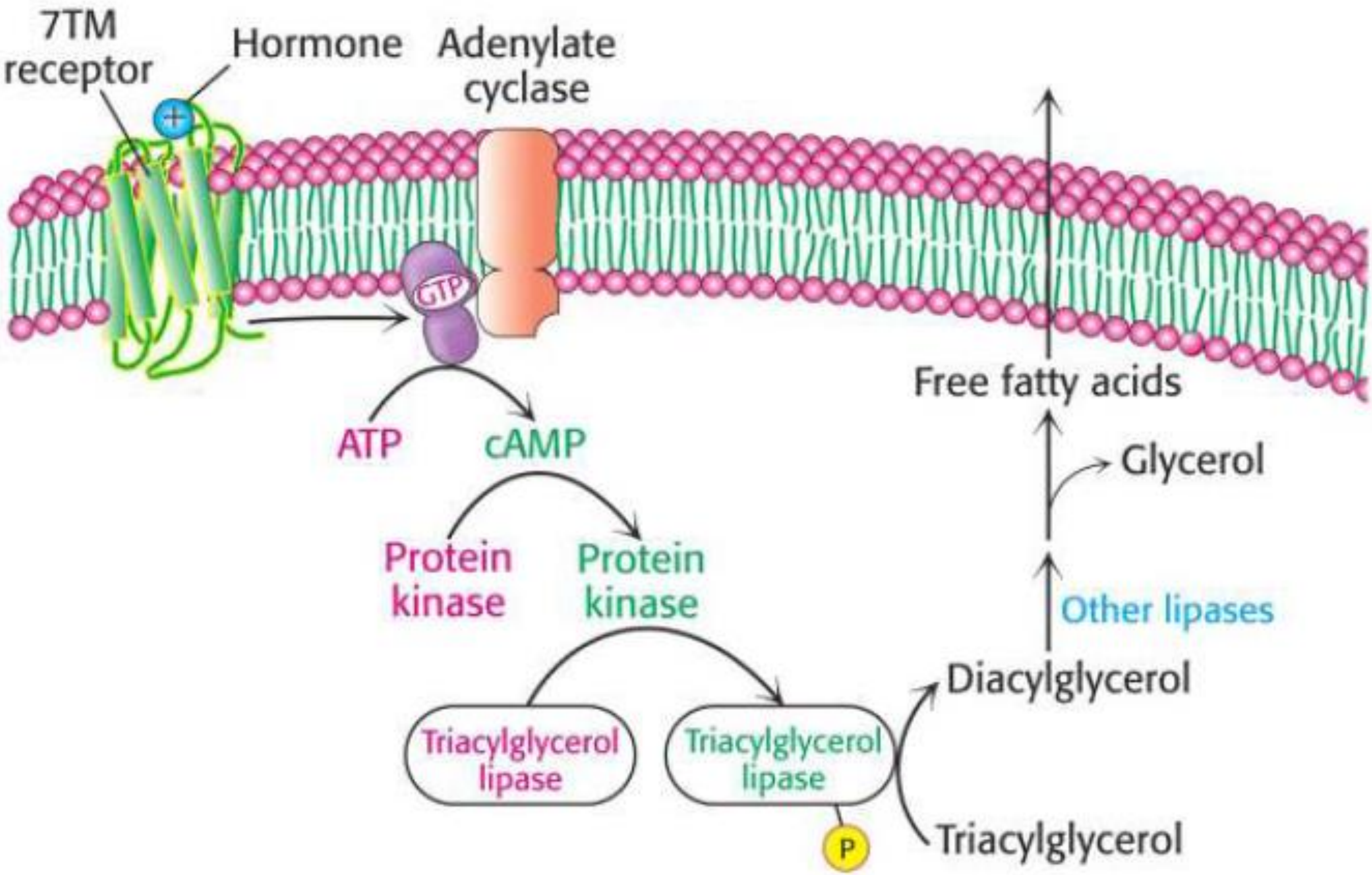
MOBILIZATION OF TRIACYLGLYCEROLS STORED IN ADIPOSE TISSUE

HORMONE-SENSITIVE LIPASE



EPINEPHRINE, NOREPINEPHRINE,
(glucagon, ACTH, TSH)

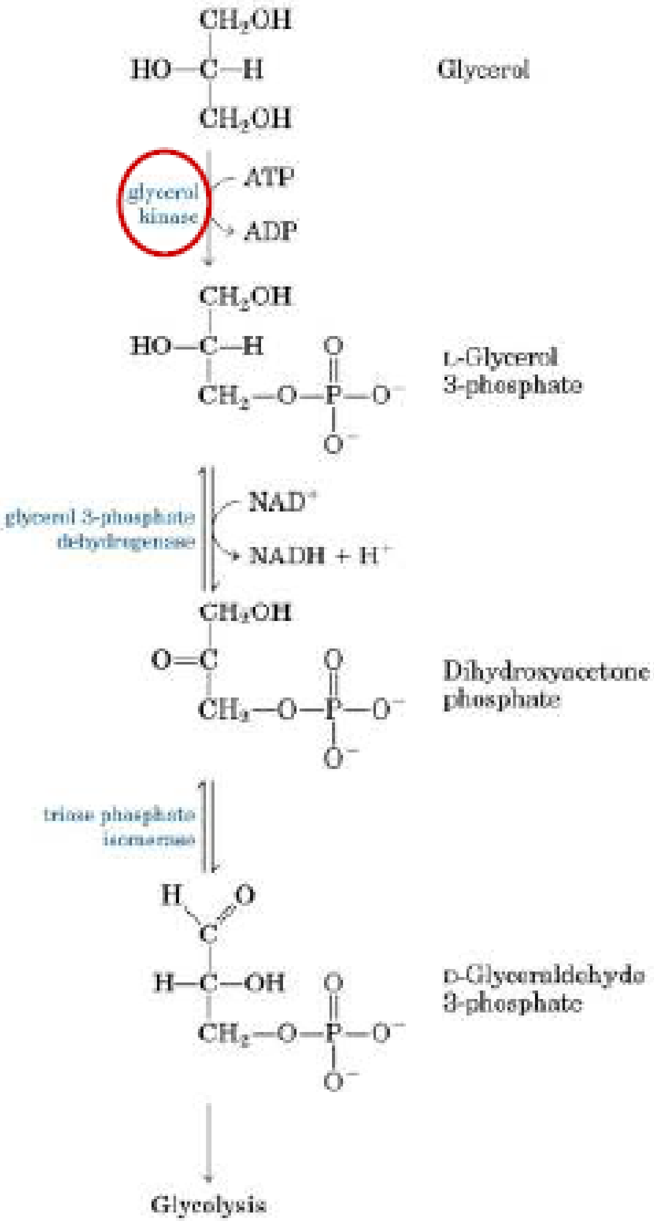
ADIPOCYTE: Epinephrine, norepinephrine, glucagon and ACTH stimulate adenylate cyclase and activate lipase

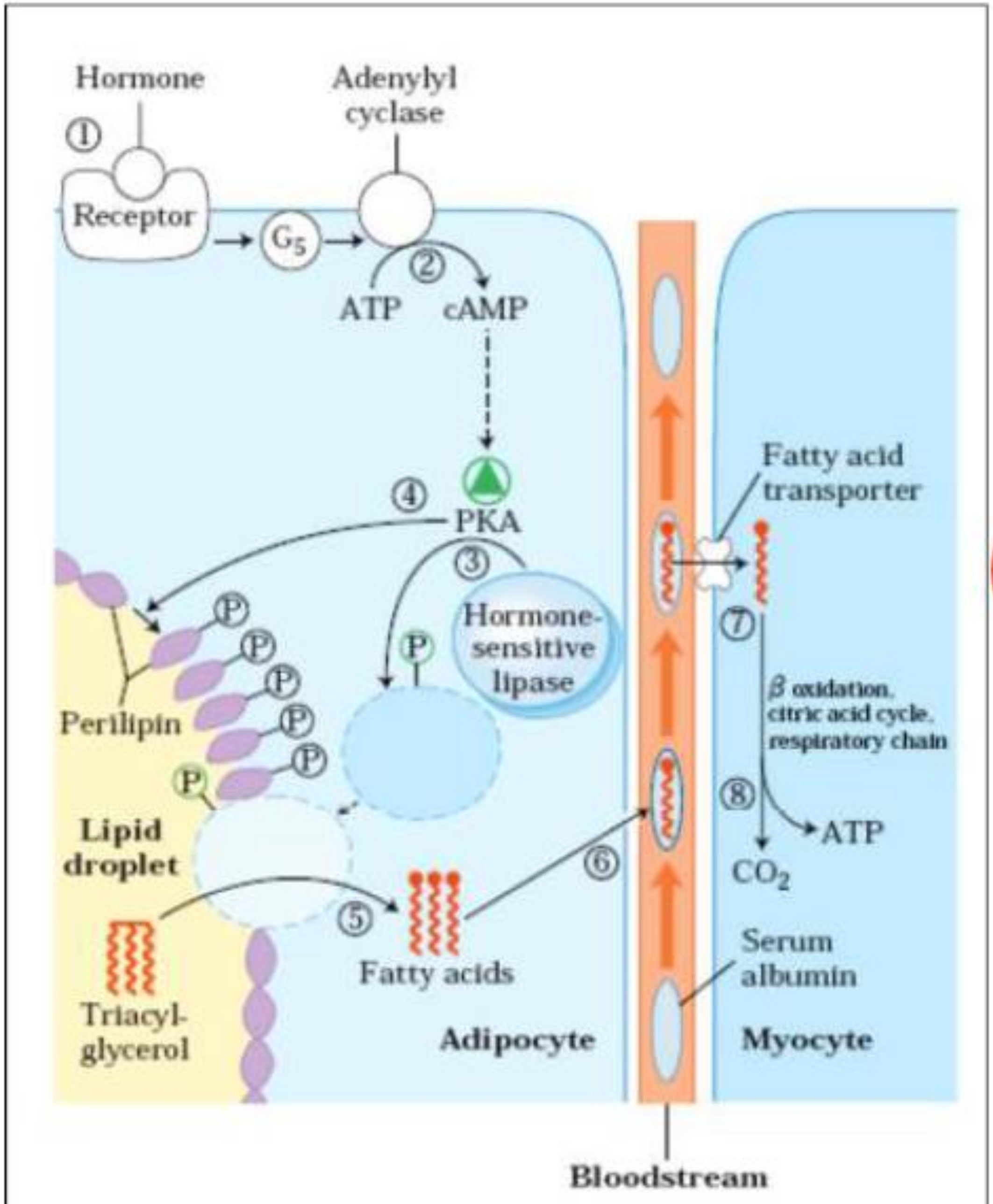


IN LIVER:

ENTRY OF GLYCEROL INTO THE GLYCOLYTIC PATHWAY

Only 5% of energy released by TAGs degradation (oxidation) originates from glycerol (95% from FAs oxidation).





Mobilization of TAGs from adipocytes stimulated by hormone action:

- adrenaline, noradrenaline (glucagon, ACTH, TSH);

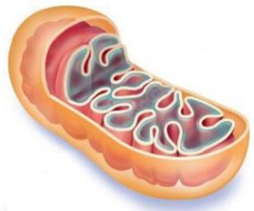
- Phosphorylation of perilipin and hormon-senitive lipaze by PKA

- Insulin inhibits mobilization of free FAs from adipose tissue (result: lowering of FA concentration in plasma)

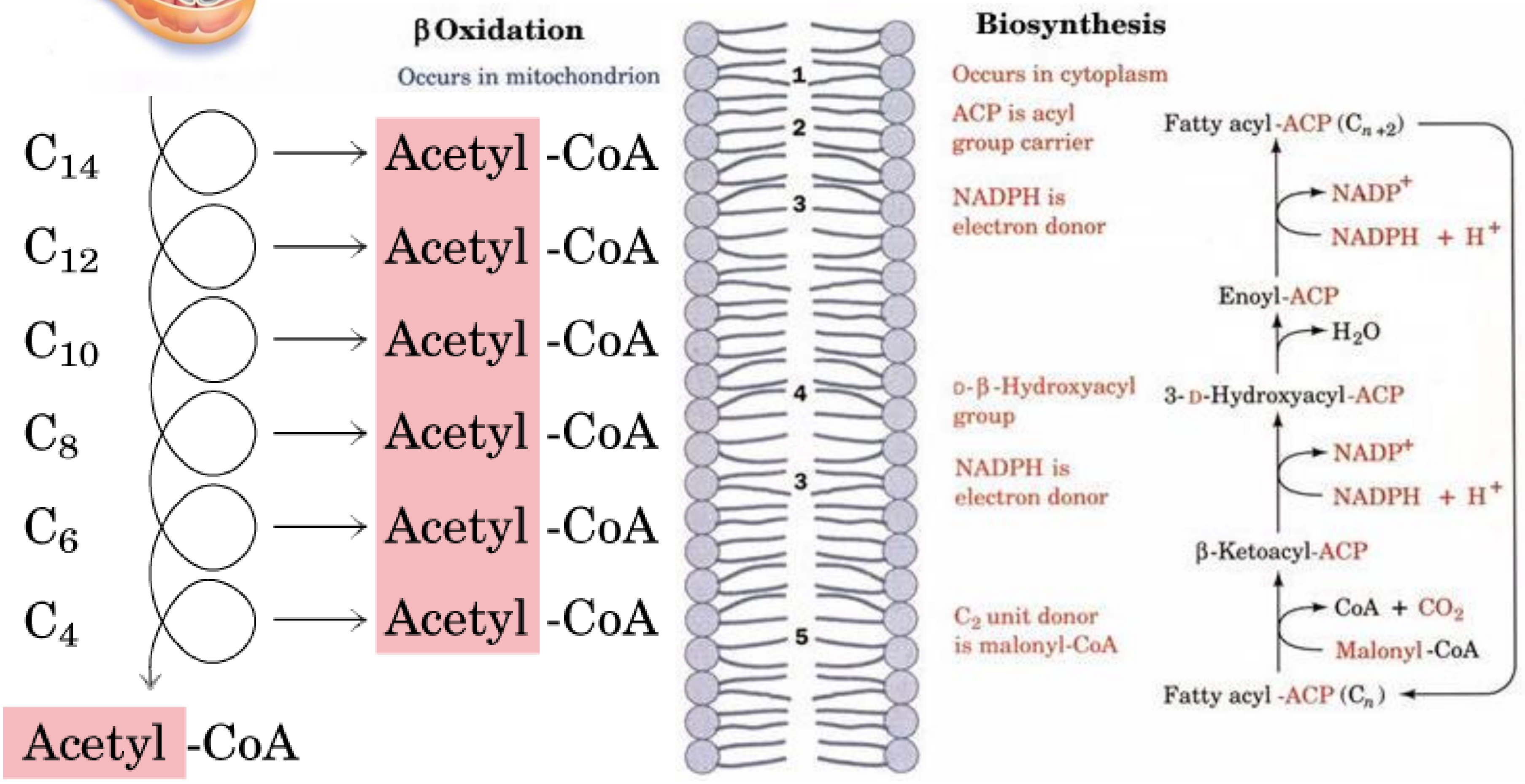
- inzulin stimulates synthesis of FAs and acylglycerols.

FATTY ACID METABOLISM

β-oxidation of fatty acids - MITOCHONDRION



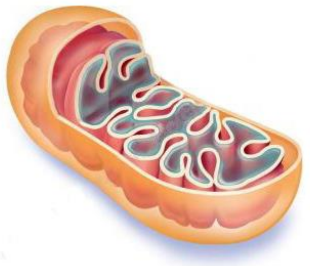
Fatty acid biosynthesis - CYTOPLASM



β-OXIDATION OF FATTY ACIDS

Enzymes catalyzing reactions of β-oxidation are located in

mitochondrial matrix!



- **FA oxidation:** liver, kindneys, skeletal and heart muscle

BEFORE β-OXIDATION IN MITOCHONDRIA, FATTY ACIDS ARE FIRST:

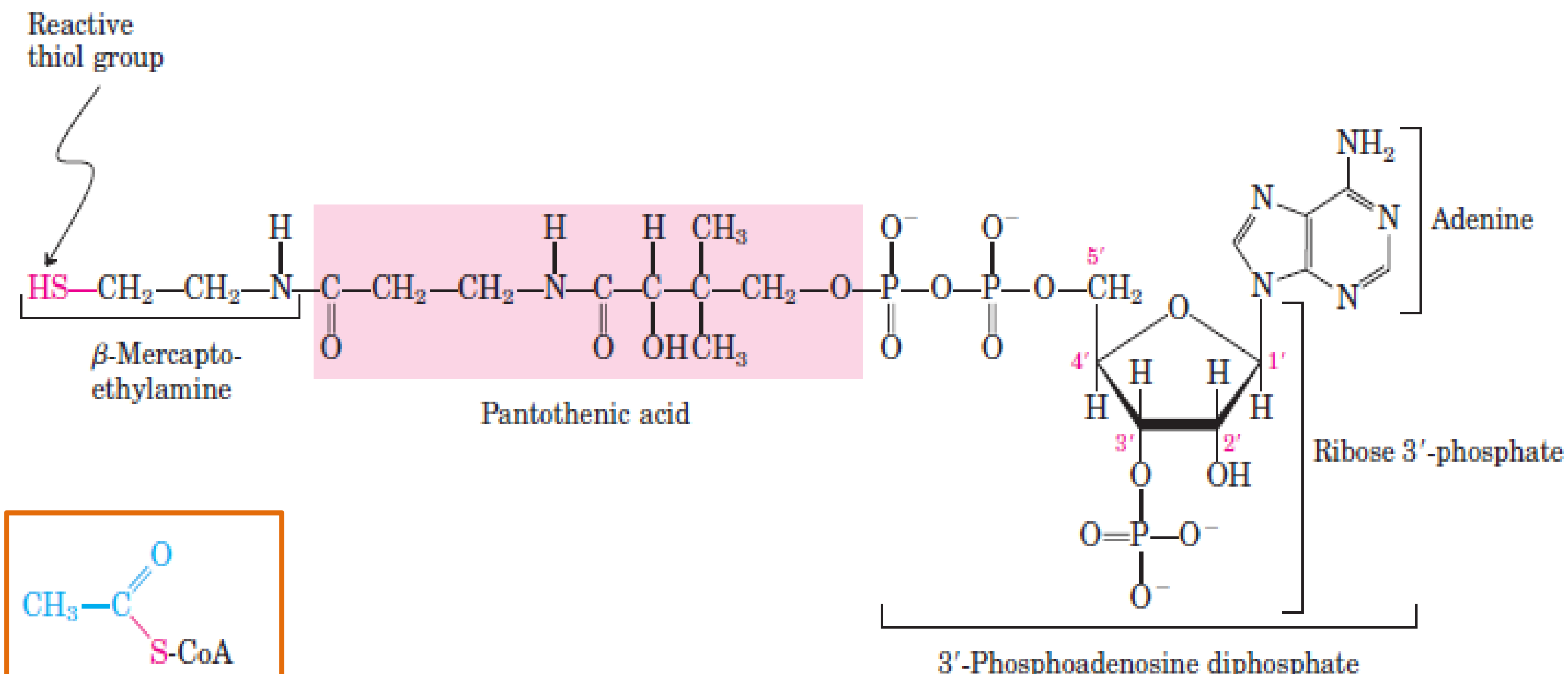
1) ACTIVATED by bonding to coenzyme A*

2) TRANSPORTED FROM CYTOSOL TO MITOCHONDRIA by carnitine

- this counts for for FAs having **14 and more C-atoms**, while FAs having **less than 12 C-atoms** enter the mitochondria **without the carnitine transport shuttle!**

* to overcome the relative stability of the C-C bond in FA

REMINDER: activation by bonding to CoA



Coenzyme A

Acetyl-CoA

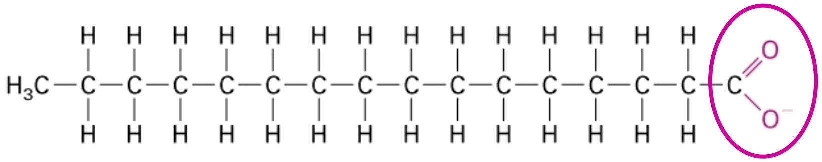
Acetyl-CoA is a thioester

- biological meaning of thioester: **coenzyme A is a thiol** that functions as a „carrier” of **acetyl** or **acyl** group in biochemical reactions!

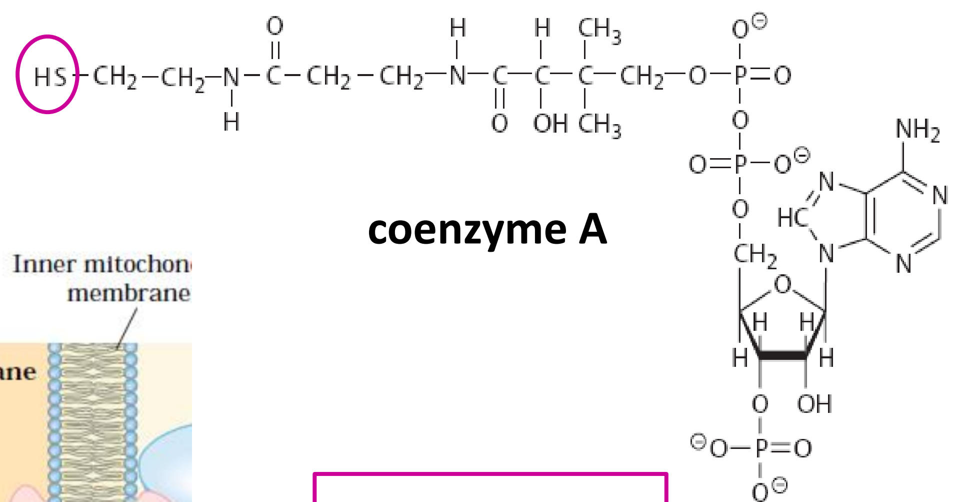
FATTY ACID ACTIVATION - FATTY ACYL-CoA FORMATION

***Acyl-CoA* synthetase** catalyzes formation of **thioester bond** *(esterification!)* between **–COOH** group of fatty acid and **–SH** group of CoA

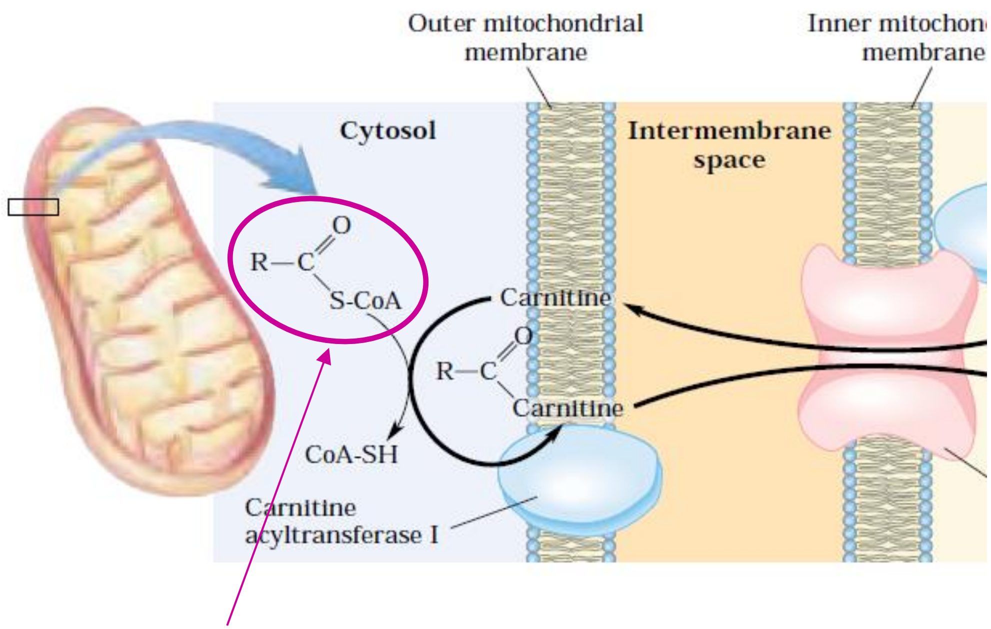
→ **formation of ACYL-CoA** (eg. palmitoyl-CoA)

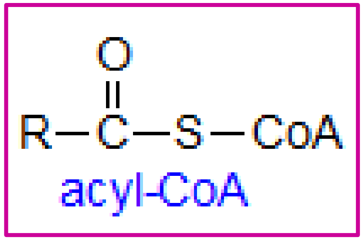


palmitate



coenzyme A





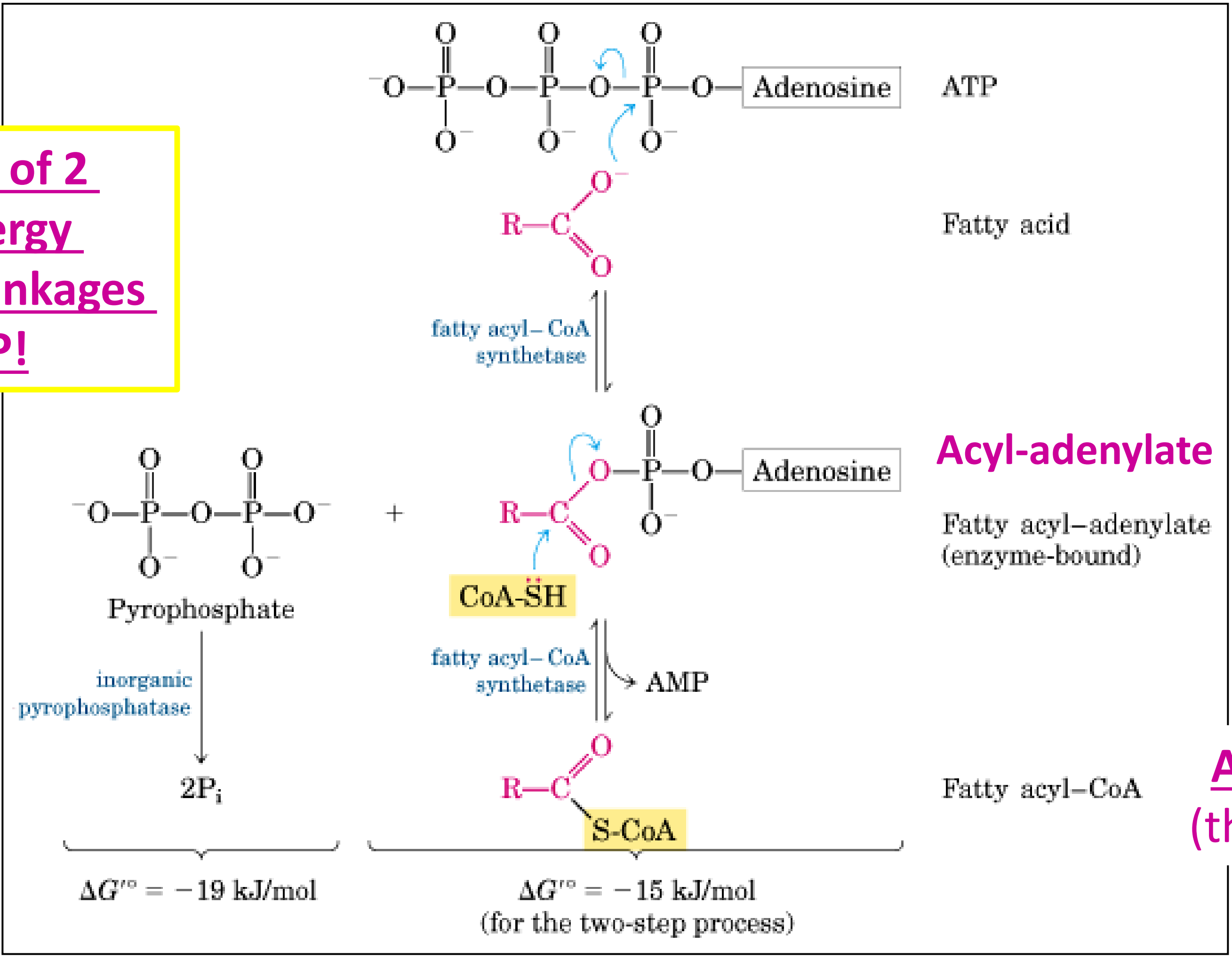
***Acyl-CoA** is formed at the cytoslolic side of the outer mitoch. membrane!*

FATTY ACID ACTIVATION: formation of acyl-CoA catalyzed by *acyl-CoA synthetase*

fatty acid + CoA + **ATP** $\rightleftharpoons$ acyl-CoA + **AMP** + **PP_i**

- the ONLY step in FA oxidation that requires ATP!

Cleavage of 2 high energy anhydride linkages in ATP!



Acyl-adenylate

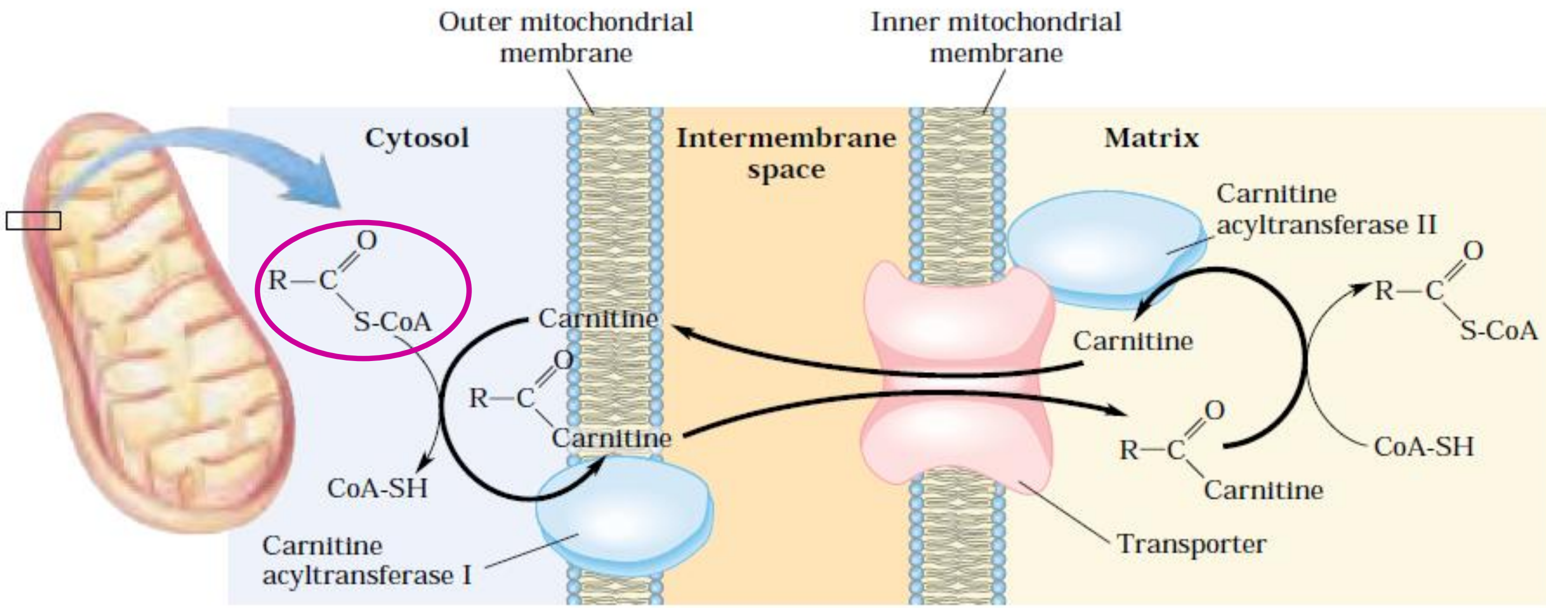
mixed anhydride of carboxylic and phosphoric acid

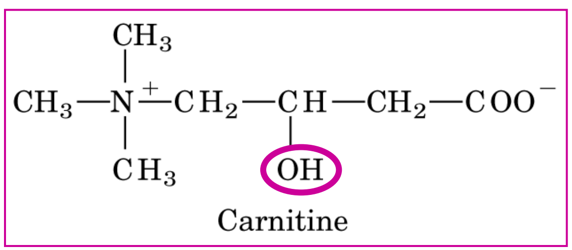
Acyl-CoA (thioester!)

inorganic pyrophosphatase:
- PP_i hydrolysis enables reaction

fatty acid + CoA + **ATP** $\longrightarrow$ acyl-CoA + AMP + **$2P_i$** ; **$\Delta G'° = - 34$ kJ/mol**

ACTIVATED FATTY ACID (ACYL-CoA) ENTERS MITOCHONDRIA VIA ACYL-CARNITINE/CARNITINE TRANSPORTER (CARNITINE SHUTTLE)

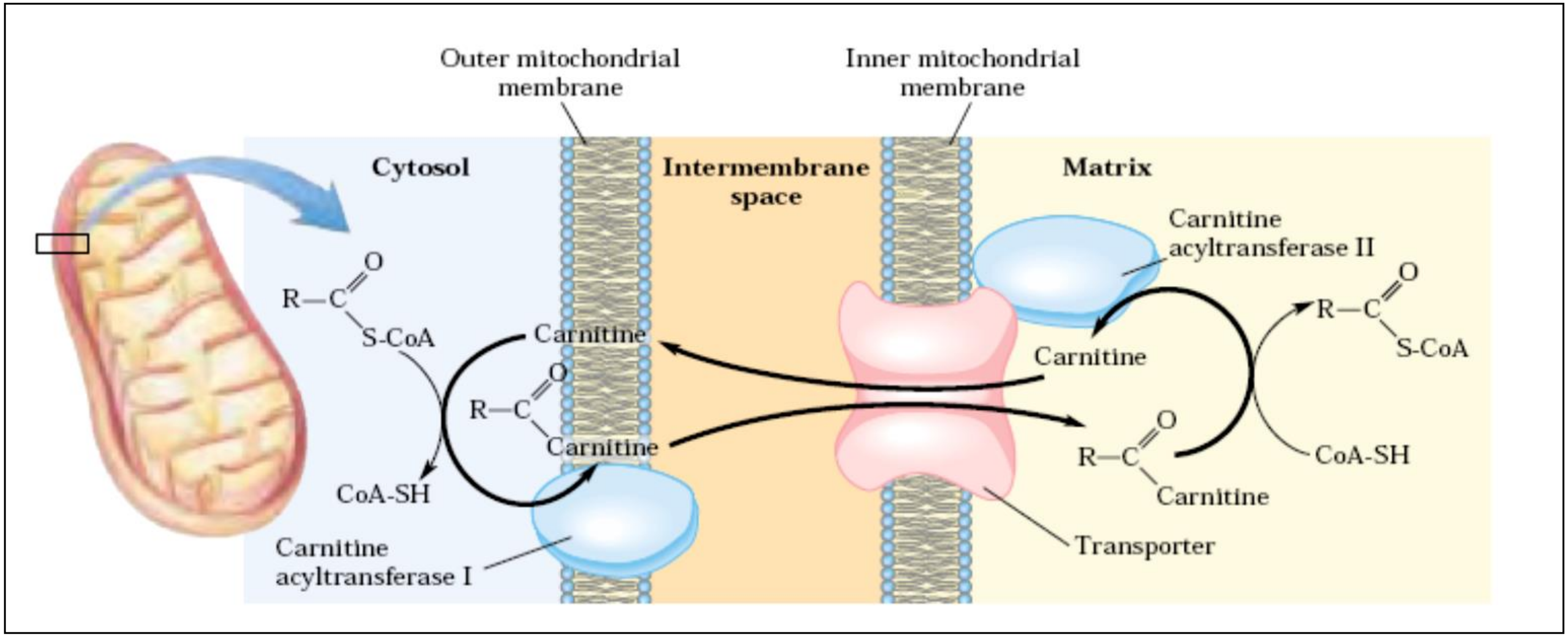




β-hidroxy-γ-trimethylammonium butyrate

1. Acyl-CoA esters are attached to –OH group of carnitine → formation of acyl-carnitine: ***carnitine acyltransferase I***
2. Transported to the matrix via acyl-carnitine/carnitine transporter
3. Acyl group is linked to CoA in mitochondria, reaction catalyzed by ***carnitine acyltransferase II***

Carnitine acyltransferase I → **key regulatory enzyme, inhibited by malonyl-CoA**

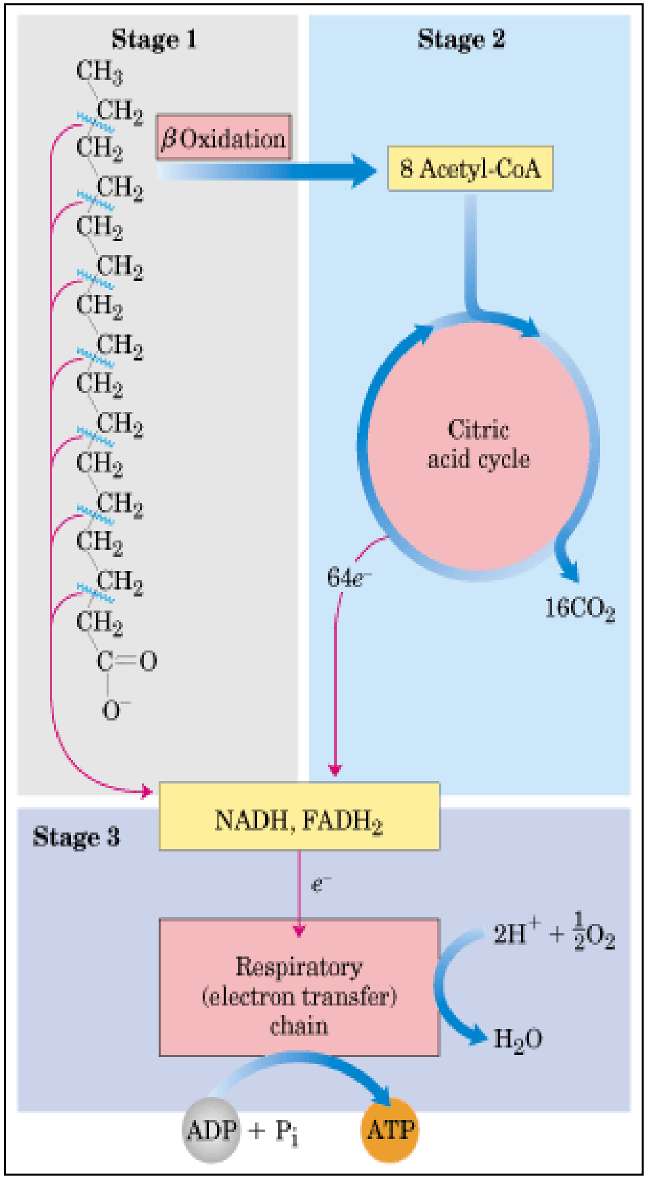


- transport of activated FA to mitochondria via **carnitine shuttle** is the **regulation step** of FA oxidation

- FA transport to mitochondria via carnitine shuttle links two separate pools of coenzyme A and of fatty acyl-CoA: **cytosolic** and **mitochondrial**

  - cytosolic CoA i acyl-CoA are important for membrane lipid synthesis

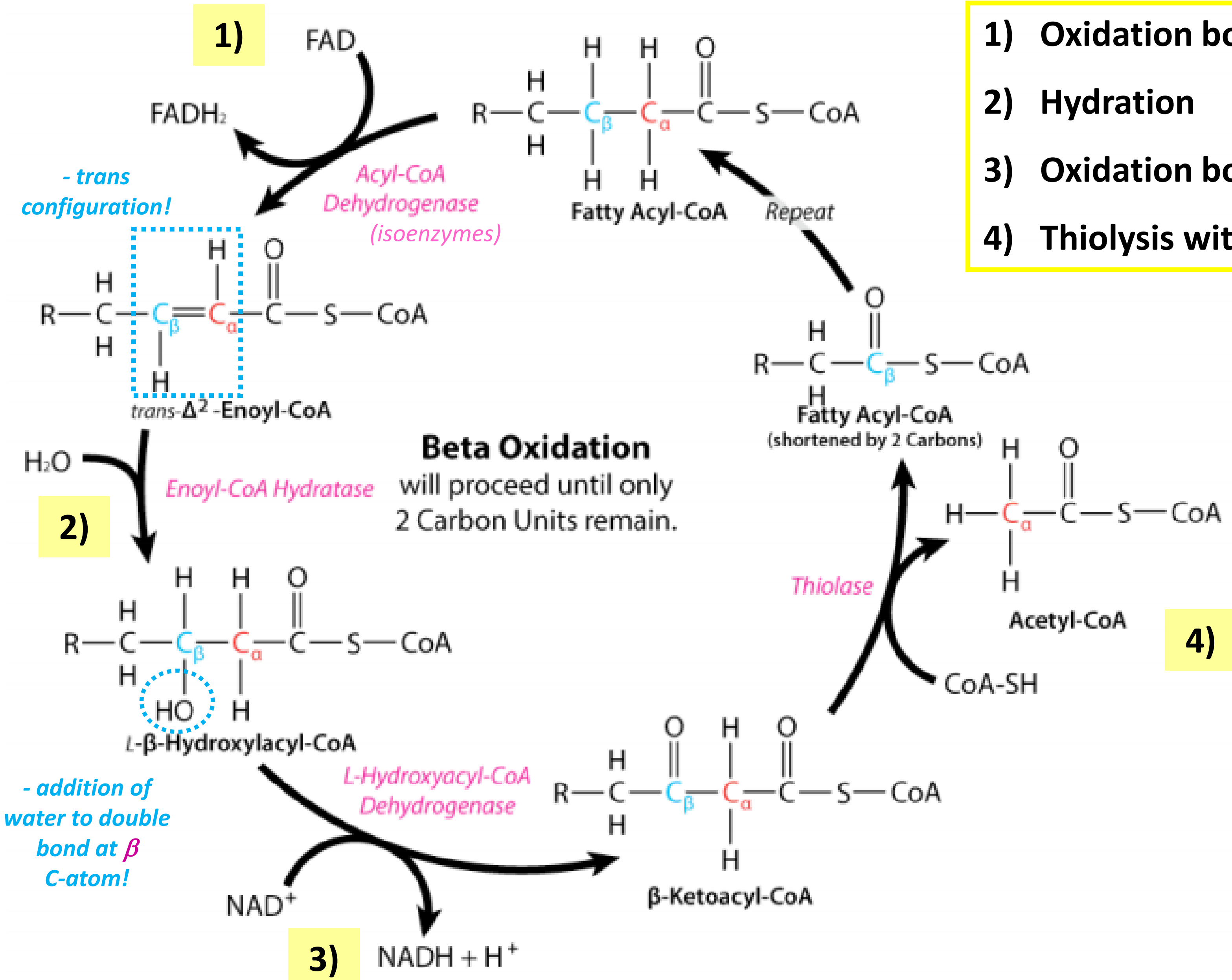
  - mitochondrial CoA i acyl-CoA are mostly used for **oxidative degradations** (eg. oxidative decarboxylation of pyruvate, oxidation of FAs and some AAs)

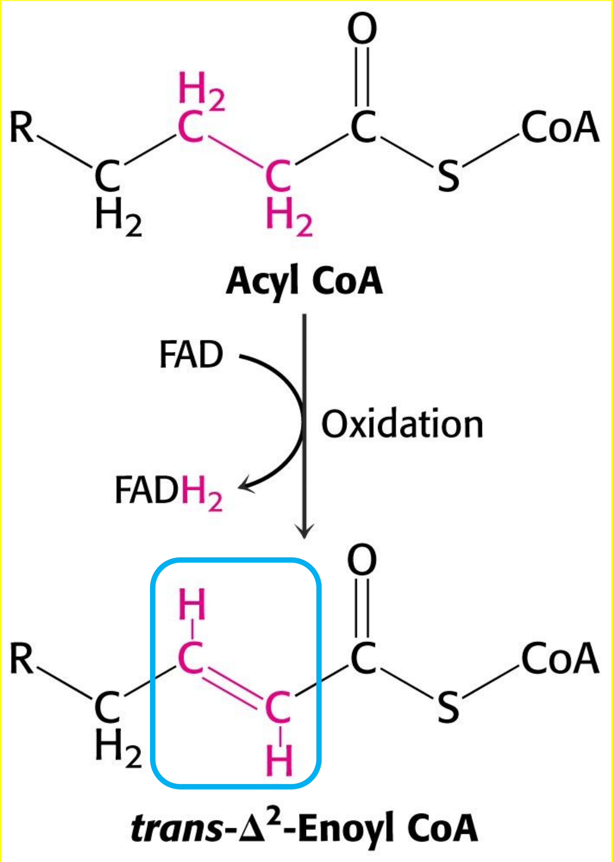


- ✓ **β-oxidation of saturated FAs**
- ✓ β-oxidation of unsaturated FAs
- ✓ β-oxidation of odd number Fas
- ✓ oxidation of branched and long-chained FA

β-oxidation of saturated fatty acids

1) **Oxidation bound to FAD**
2) **Hydration**
3) **Oxidation bound to NAD^+**
4) **Thiolysis with CoA**





1) Oxidation bound to FAD

Acyl-CoA dehydrogenase **-** isoenzymes specific for different FA chain length

(3 isoenzymes: for long-, middle- and short-chained FA)

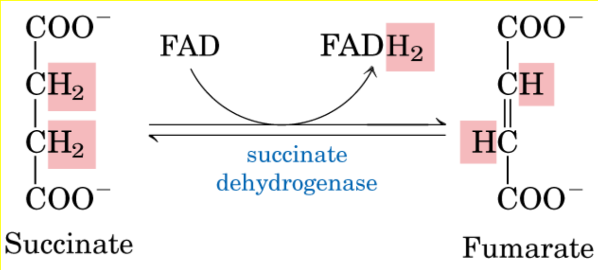
- introducing ***trans*-double bond**

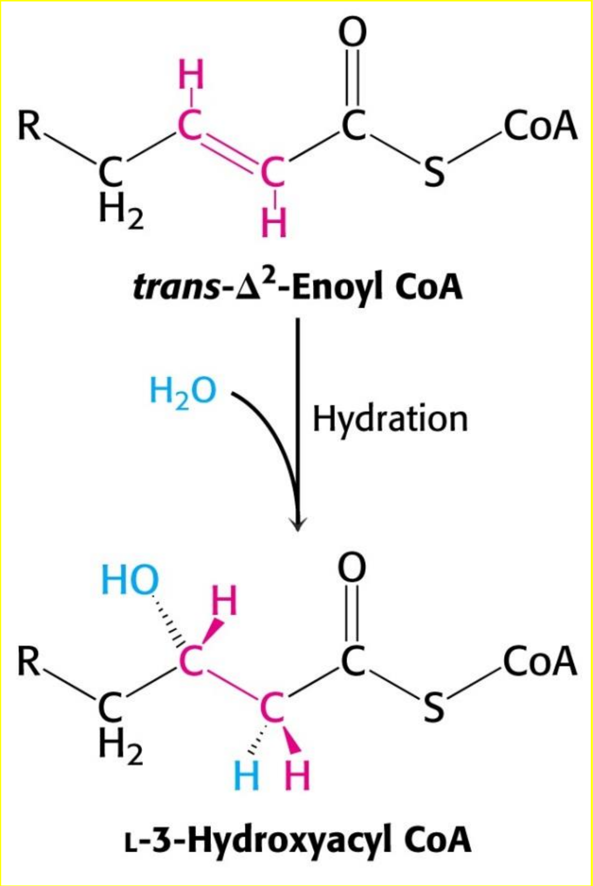
- flavoproteins with FAD as a prosthetic group

- flavoprotein in mitoch. respiratory chain - sinthesis of 1,5 ATP per el. pair from 1 mol. $FADH_2$

- *analogy with the reaction catalyzed by **succinyl-CoA dehydrogenase** from CAC:*

- enzyme bound to inner mitoch. membrane
- double bond introduced betwwen α and β C-atoms
- FAD is electron acceptor



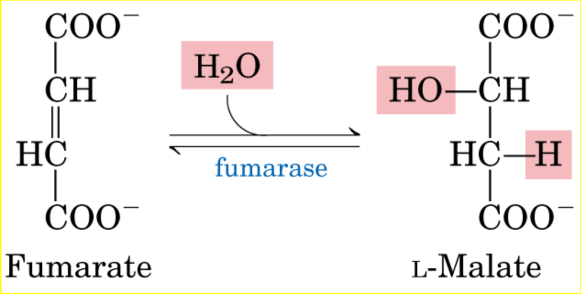


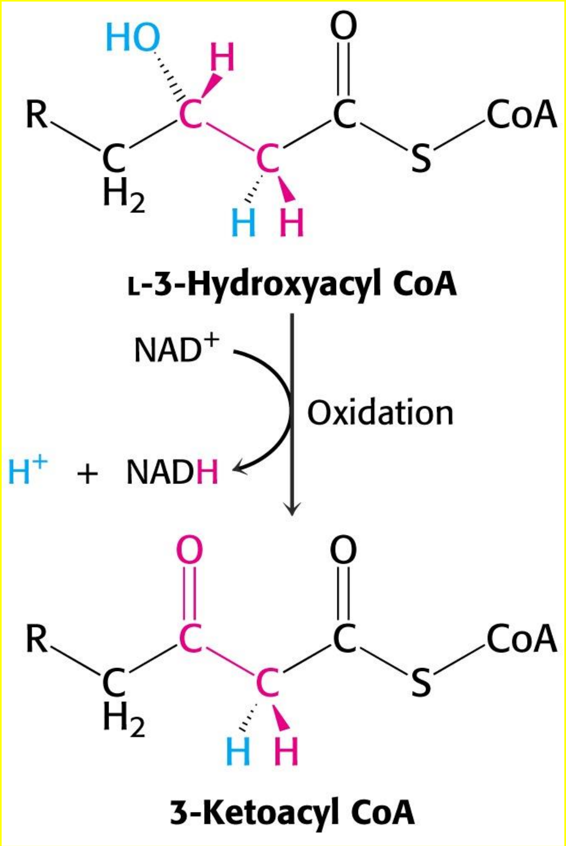
2) Hydration

Enoyl-CoA hydratase - addition of water to the double bond **of Δ²-*trans*-Enoyl CoA** and formation of L-stereoisomer **β(3)-Hydroxyacyl-CoA**

- *analogy with the reaction catalyzed by **fumarase** from CAC:*

- addition of water to double bond between α and β C-atoms



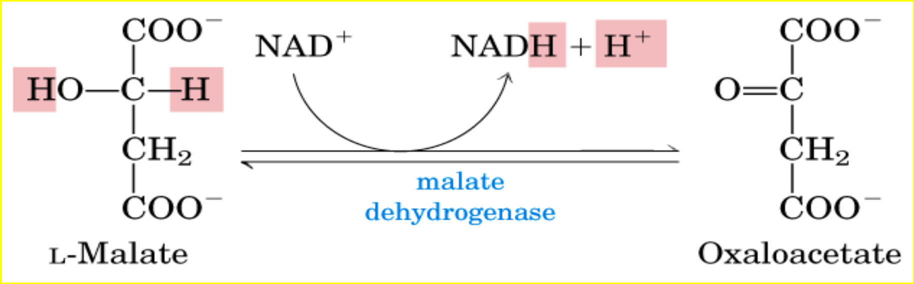


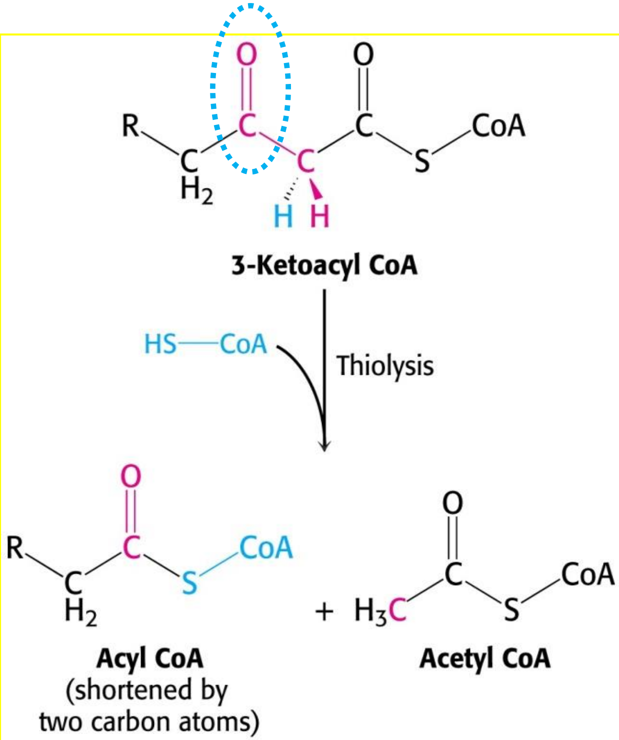
3) Oxidation bound to NAD^+

L-β-hydroxyacyl-CoA dehydrogenase

- specific for L-stereoisomer
- NAD^+ is electron acceptor, which it donates to the carier (NADH-dehydrogenase) in the respiratory chain; synthesis of 2,5 ATPs

- *analogy with the reaction catalyzed by **malate dehydrogenase** from CAC:*





4) Thiolysis with CoA
- breaking of β-ketoacyl-CoA in reaction with thiol group from CoA

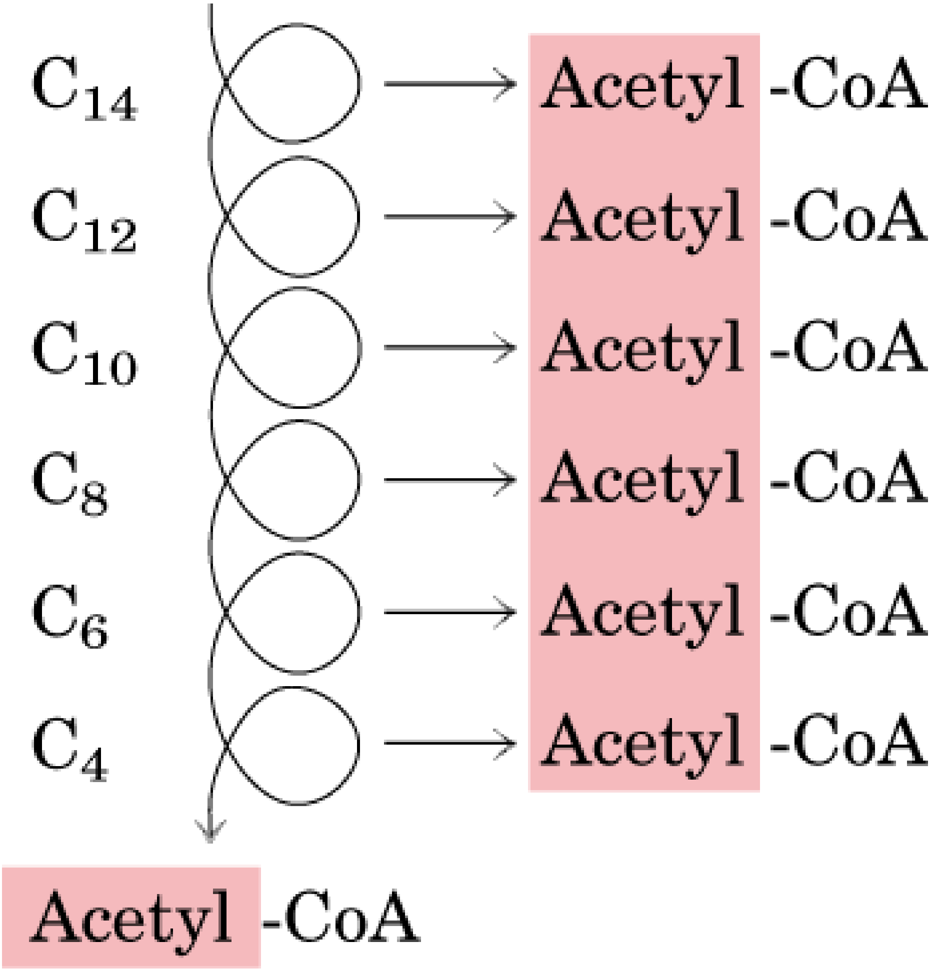
<u>Thiolase</u>
(3-ketoacyl-CoA thiolase** or **acyl-CoA acetyltransferase)

- *the first three reactions of β-oxidation create unstable C-C bond in which α-C atom is bonded to **two** carbonyl carbons (β-ketoacyl-CoA intermediate)*
- *the ketone group on the **β-C atom (C-3)** makes it a good target for nucleophilic attack by the thiol (-SH) group of coenzyme A*

- **<u>thiolase</u>** breaks the bond at the position 2,3 → formation of <u>two products</u>:

1) **<u>acetyl-CoA</u>** and 2) **<u>acyl-CoA</u>** shortened by 2 C-atoms

β-OXIDATION OF FATTY ACIDS



Each turn (round) of β-oxidation produces acetyl-CoA, NADH and $FADH_2$

Stoichometry of palmitate oxidation

In every cycle of FA oxidation, formation of acetyl-CoA, NADH and $FADH_2$!

1st cycle of oxidation:

Palmitoyl-CoA + **CoA** + FAD + NAD^+ + H_2O → myristoyl-CoA + **acetil-CoA** + $FADH_2$ + NADH + H^+

NET equation of palmitate oxidation (total 7 cycles of oxidation):

$$CH_3(CH_2)_{14}\overset{\overset{\displaystyle O}{\|}}{C}\text{-S-CoA} + 7\ \text{CoA-SH} + 7\ FAD^+ + 7\ NAD^+ + 7\ H_2O \longrightarrow$$

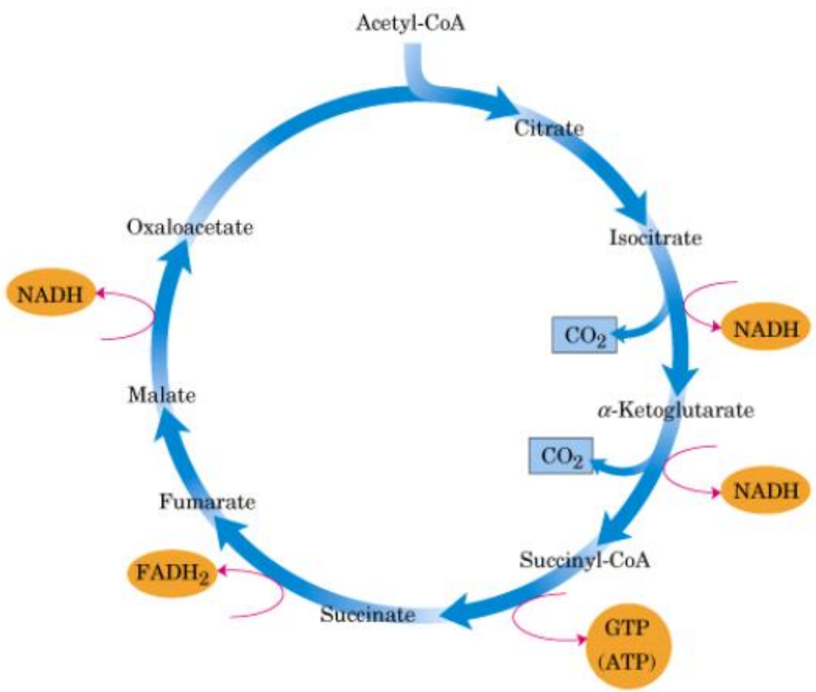
$$\longrightarrow 8\ CH_3\overset{\overset{\displaystyle O}{\|}}{C}\text{-S-CoA} + 7\ FADH_2 + 7\ NADH + 7\ H^+$$

$FADH_2$ → 1.5 ATP

NADH → 2.5 ATP

1 cycle = 4 ATPs for every C2-unit;
7 cycles = 28 ATPs

Acetyl-CoA is further oxidized in CAC



1 acetyl-CoA → $FADH_2$, 3 NADH, GTP } 10 ATP

→ 2 CO_2

1 acetyl-CoA in CAC = 10 ATP-a
<u>8 acetyl-CoA = 80 ATP-a</u>

✓ **<u>Total reaction of 2nd and 3rd phase of oxidation (CAC and respiratory chain):</u>**

8 acetil-CoA + 16 O_2 + 80 P_i + 80 ADP → 8 CoA + 80 ATP + 16 CO_2 + 16 H_2O

✓ **<u>Total reaction of complete oxidation od palmitate:</u>** **80 ATP + 28 ATP** (from 7 cycles):

Palmitoil-CoA + 23 O_2 + 108 P_i + 108 ADP → CoA + 108 ATP + 16 CO_2 + 23 H_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.

†GTP produced directly in this step yields ATP in the reaction catalyzed by nucleoside diphosphate kinase (p. 578).

- because the **activation of palmitate** to palmitoyl-CoA breaks both phosphoanhydride bonds in ATP, **the energetic cost of activating a fatty acid is equivalent to 2 ATPs**, and **the net gain per molecule of palmitate is 106 ATPs**!

Total: 106 ATP

TAGs

are less oxidized than carbohydrate molecules;

⇒ when they are degraded (oxidized) they release more energy 38.9 kJ/g of fat compared with 17.2 kJ/g of carbohydrate

1 molecule of palmitic acid (3300 kJ/mol) → 106 molecules of ATP → 106/16= 6.6 per C atom
1 glucose molecule (2880 kJ/mol) → 30 (32) molecules of ATP → 30 (32)/ 6=5 (5.3) per C atom

(-4) CH_4 (+4) CO_2

• store energy more efficiently than glycogen (because glycogen binds a substantial amount of water, the anhydrous triacylglycerols store an equivalent amount of energy in about one-eighth of glycogen's volume)

FIGURE 23–16 Scanning electron micrograph of human adipocytes. In fat tissues, capillaries and collagen fibers form a supporting network around spherical adipocytes. Almost the entire volume of these metabolically active cells is taken up by fat droplets.

long-chain fatty acids (12-18 *C* atoms)
medium-chain fatty acids (4-14 *C* atoms)
short-chain fatty acids (4-8 atoms)


- **FA oxidation ensures metabolic energy, heat and water for hibernating animals**

- grizzly bears hibernate for 5-7 months and maintain a body temperature 32-35°C and expend about 6,000 kcal/day!

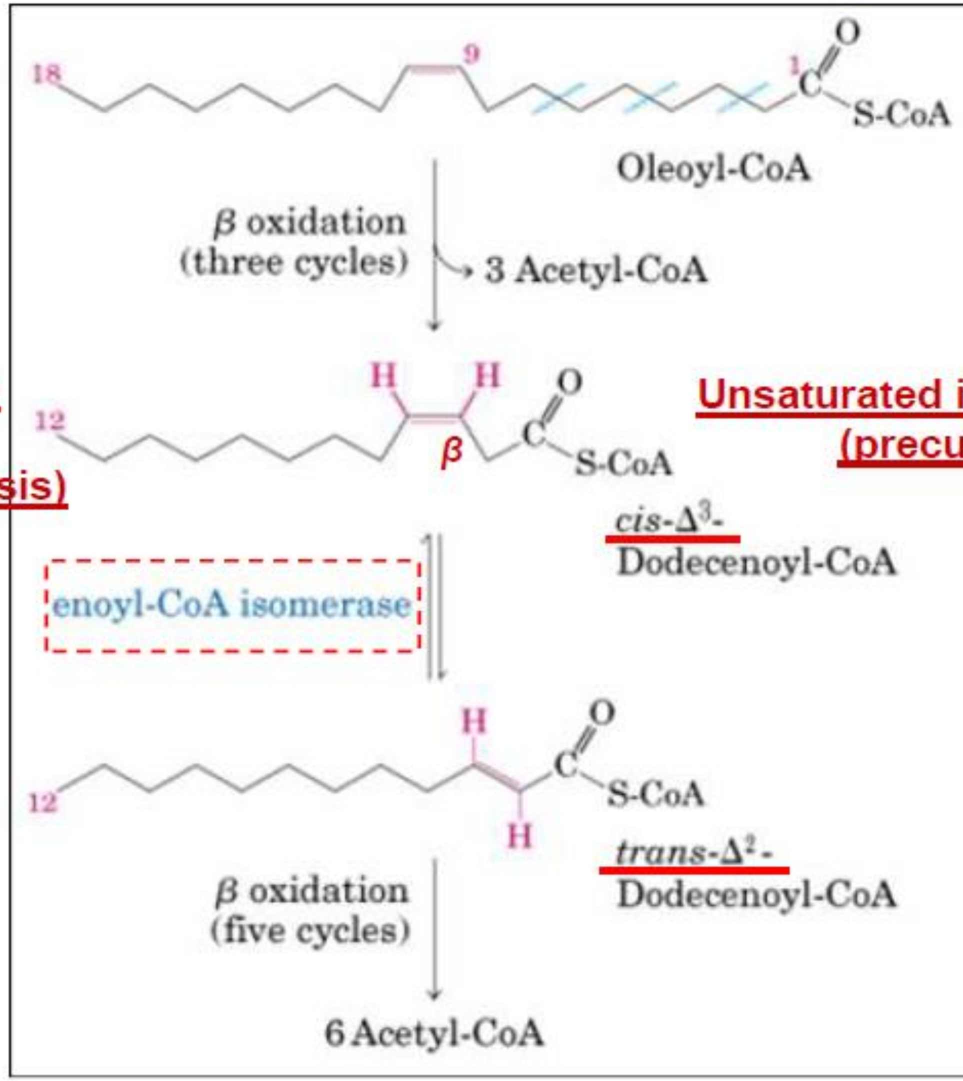
- **water*is formed in FA oxidation** (and lost through respiration)**, while glycerol formed by TAG degradation is used for gluconeogenesis**

- **kidneys reapsorb urea which is recycled and used for amino acid formation and preservation of body proteins**

- camels have long-termed storage of water due to the oxidation of fatty tissue in their humps

***($NADH + H^+ + \frac{1}{2} O_2 \rightarrow NAD^+ + H_2O$)**

Mitochondrial β-oxidation of monounsaturated FA



NO 1st β-oxidation step required (no $FADH_2$ synthesis)

Unsaturated intermediate (precursor)

enoyl-CoA isomerase acts only upon ***trans***-double bond!

Unsaturated fatty acids are *cis* isomers in nature:
- activity of **isomerase** is required;
- 1st oxidation reaction is <u>not needed</u>, there is no electron transfer to FAD, thus 1 $FADH_2$ less is produced

C 18:1 ili *cis*-Δ^9 (ω-9)

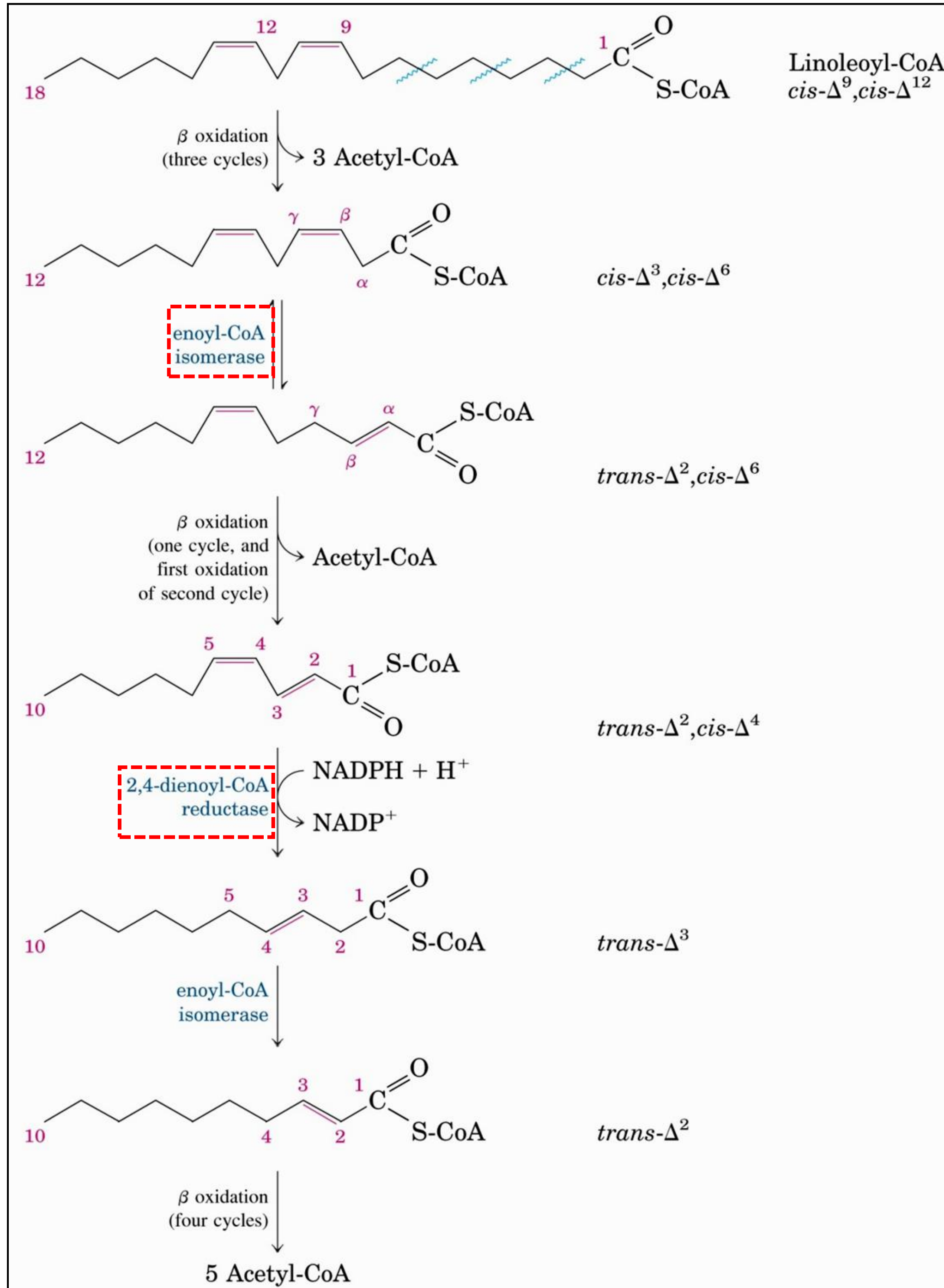
oleic acid

Mitochondrial β-oxidation of polyunsaturated FA

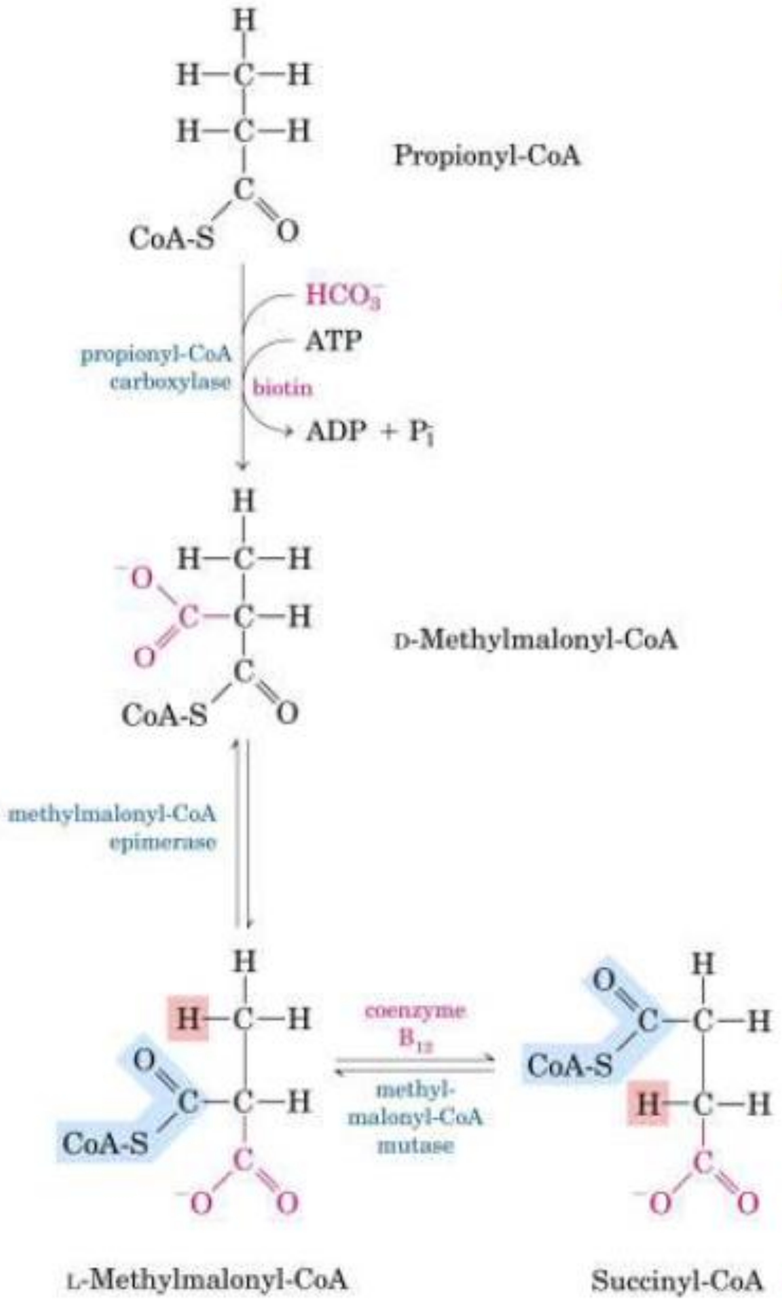
- **besides** isomerase **,** **additional** reductase is required for further rearrangements of double bonds

- reduction with NADPH
- from 2 double bonds (C2-3 i C4-5) one is formed (C3-4)

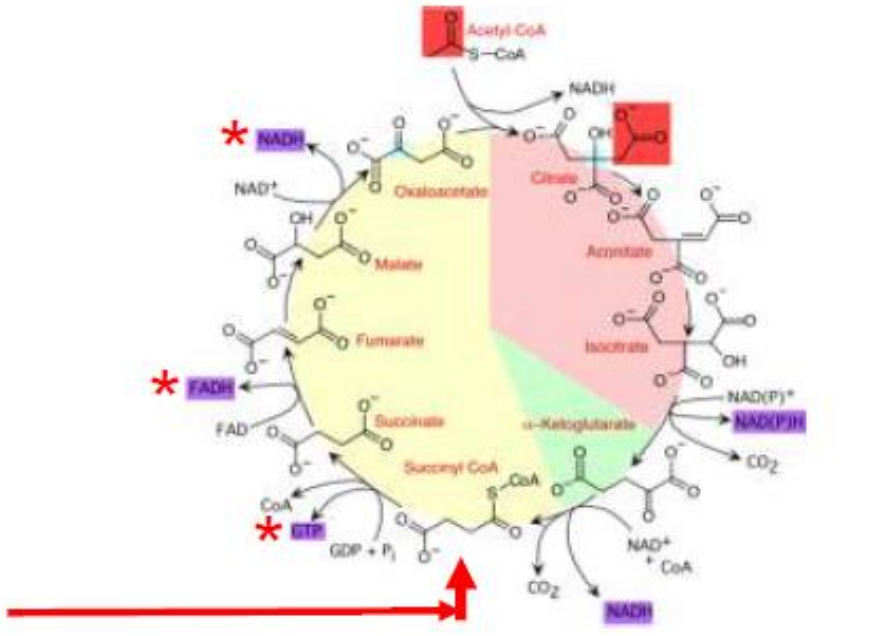
- ***odd numbered C-atoms*** *with double bond (eg. Δ⁹): only isomerase is needed;*
- ***even numbered C-atoms*** *(eg. Δ¹²): isomerase and reductase!*



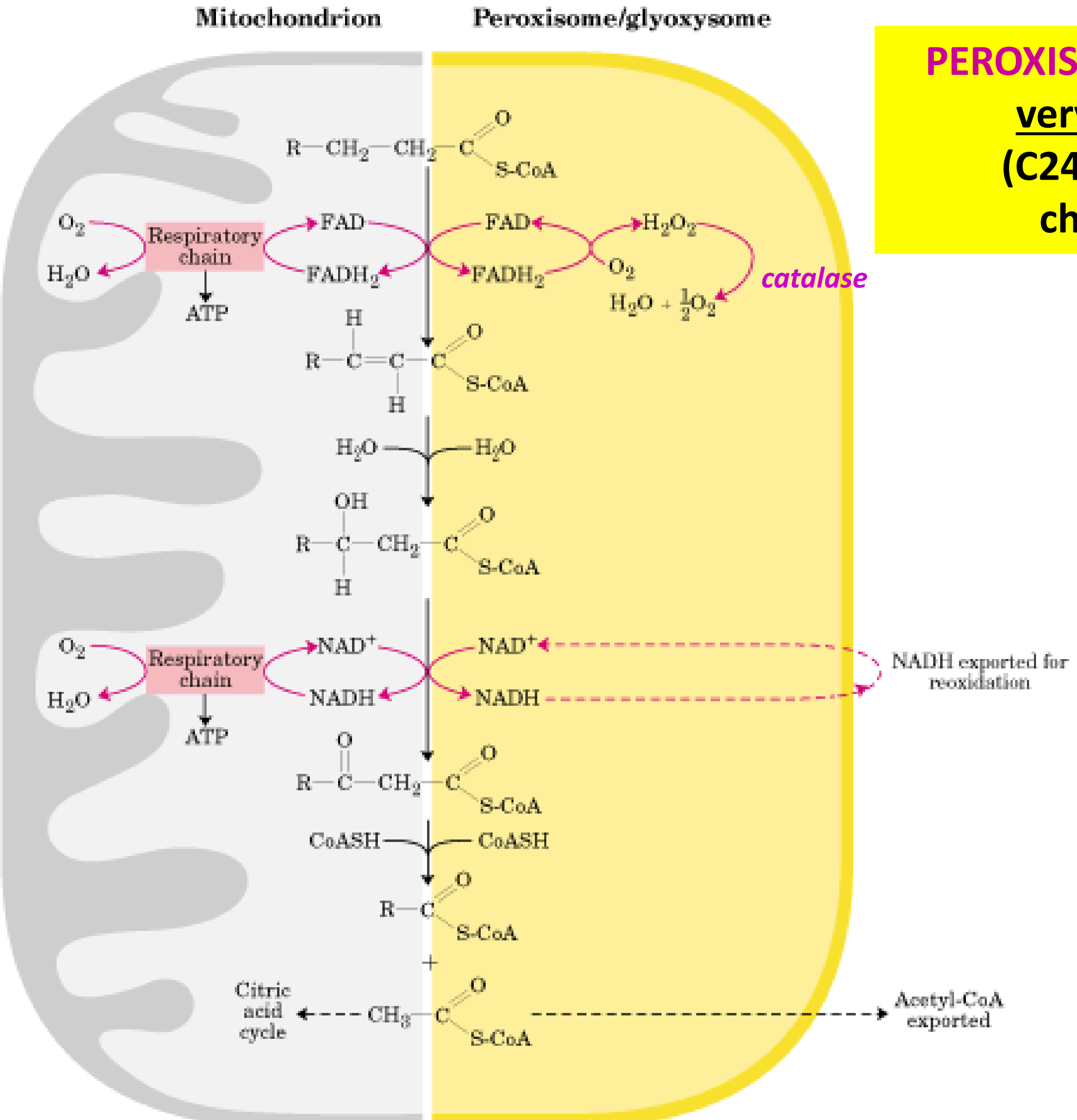
β-oxidation of odd C-number FAs



Last turn (round) of *β*-oxidation produces acetyl-CoA and PROPIONYL-CoA which is converted to succinyl-CoA



PEROXISOMAL β-oxidation of very long-chain FAs (C24, C26 and more), chain shortening



X-linked adrenoleucodistrophy - unability to degrade very long chained FAs due to defficiency of the peroxysomal transport protein

- mixture of olive oil and rapeseed oil;
TAGs of oleic (C18:1) and eruic acid (C22:1) in ratio 4:1

Zellweger (cerebro-hepato-renal) sydrom - defficiency of functional peroxysomes or complete deficiency of peroxysomes in tissues

Lorenzo Odone was born on May 29, 1978 to Michaela and Augusto Odone.
At age 6, in 1984, he was diagnosed with the childhood cerebral form of ADL.
He far outlived his prognosis, surviving to age 30. He died on May 30, 2008, one day after his 30th birthday.

In 1992 director George Miller turned the story of the Odones and their struggle to find a cure for ALD into the movie, "Lorenzo's Oil" starring Susan Sarandon and Nick Nolte.

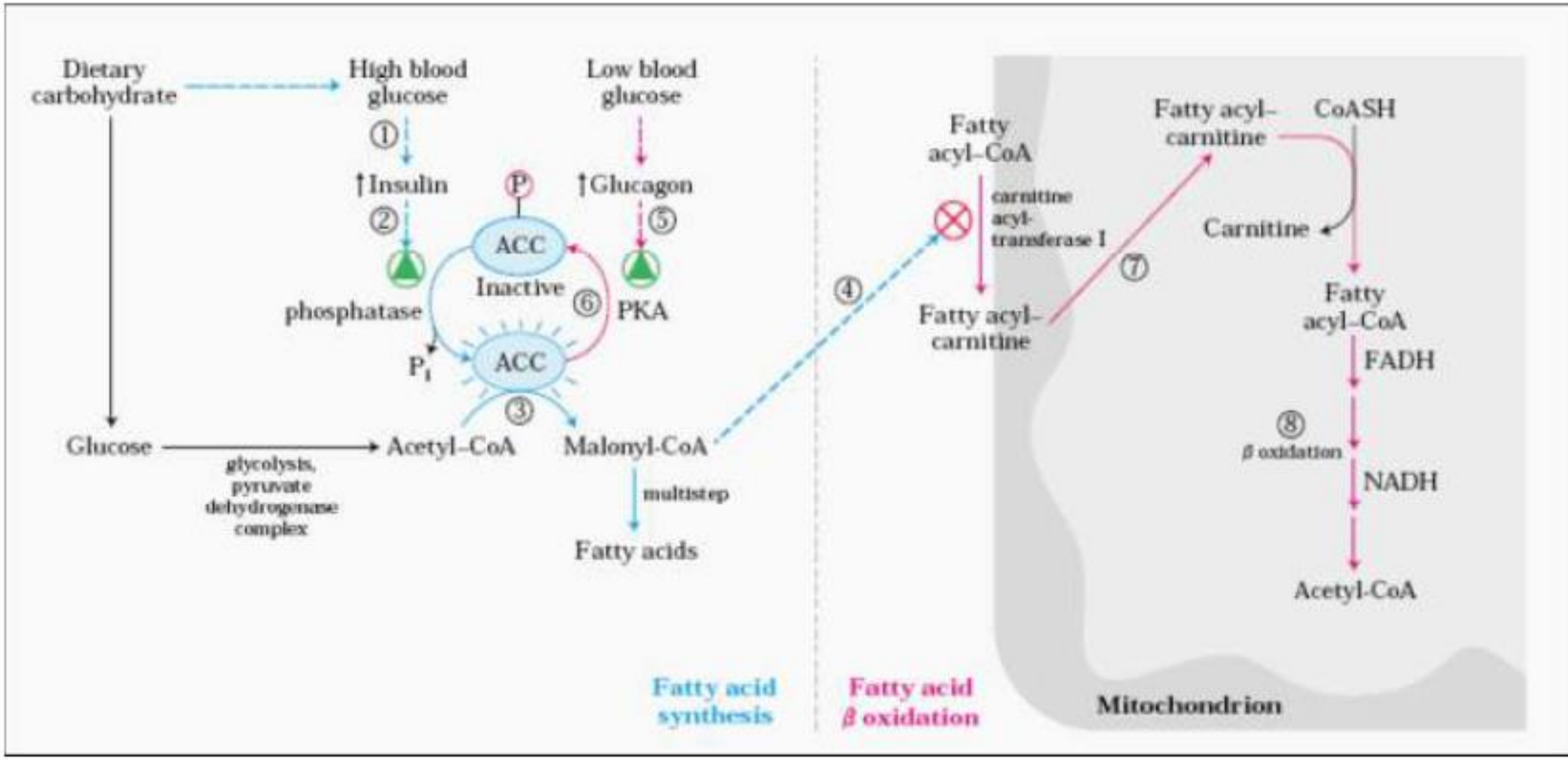
X-linked adrenoleukodystrophy (X-ALD)

Childhood cerebral ALD is one of the most common forms of X-linked ALD, comprising approximately 30% of all patients with X-ALD. Onset of childhood cerebral ALD occurs between the ages of 2 and 10. Up to the point of onset, development is normal. The most common initial symptoms are difficulty in school, behavioral disturbance, impaired vision, or impaired hearing. After initial neurological symptoms appear, the health of the patients deteriorates rapidly. Further symptoms may include dementia, poor coordination, seizures, hyperactivity, difficulty with speech, and headaches. The average time between the initial symptoms and a vegetative state or death is approximately 2 years, although it can range anywhere from 6 months to 20 years.

The peroxisome is a cellular compartment that is responsible for the breakdown of certain types of fatty acids (very long chain fatty acids). In X-ALD, this ability is impaired, resulting in the accumulation of very long chain fatty acids. This leads to the breakdown of the myelin sheath, resulting in the neurologic problems characteristic of leukodystrophies. The gene that is defective in X-ALD is called *ABCD1*, and encodes a protein called ALDP (which stands for ALD protein). This protein resides in the wall of the peroxisome, and is involved in the breakdown of fatty acids. However, its exact role in this process is currently unclear.

Michaela and Augusto, devastated by Lorenzo's diagnosis, decided to research ALD even though neither had a scientific or medical background. They eventually learned that ALD leaves the body unable to break down big fat molecules, either ones the body makes itself or ones that enter the body through food. After much hard work they helped develop an oil made from olive and rapeseed, which they named "Lorenzo's Oil." The oil, if started early in boys with ALD but no symptoms, is now known to have some benefit in preventing the form of ALD that Lorenzo had.

Coordinated regulation of mitochondrial β-oxidation and synthesis of fatty acids



Key enzymes : acetyl-CoA-carboxylase (ACC) and carnitine acyltransferase I

β-OXIDATION OF FATTY ACIDS

1. Enzymes for FA oxidation are in **mitochondria**; FAs are mobilized from cytosol, activated (Acyl-CoA) and transferred into mitochondrial matrix via **carnitine shuttle**.

2. Oxidation of FAs through **4 repeating steps (reactions)**:

   oxidation – hydration – oxidation – thyolysis

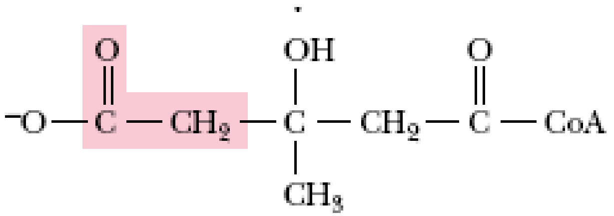
   and in every cycle foming **acetyl-CoA, $FADH_2$ i NADH**.

3. FAs oxidation regulation:

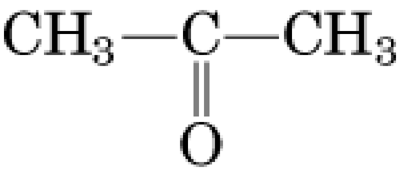
   - ***carnitine-acyltransferase I*** (carnitine shuttle) inhibits **malonyl-CoA** (FAs biosynthesis);

   - ↑**$NADH/NAD^+$** and ↑**acetyl-CoA** inhibit enzymes of oxidation with NAD^+ and thyolysis.

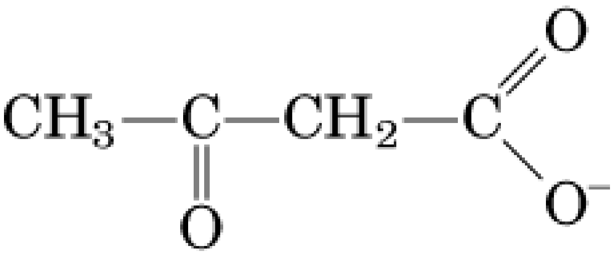
KETOGENESIS - KETONE BODIES



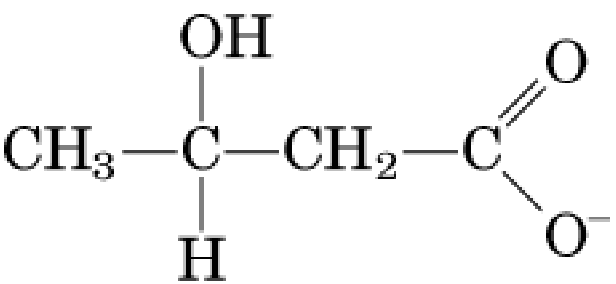
β-Hydroxy-β-methylglutaryl-CoA (HMG-CoA)



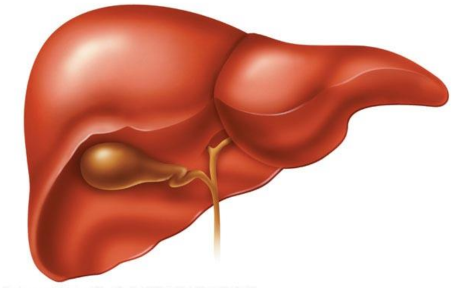
Acetone



Acetoacetate

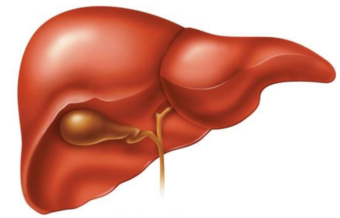


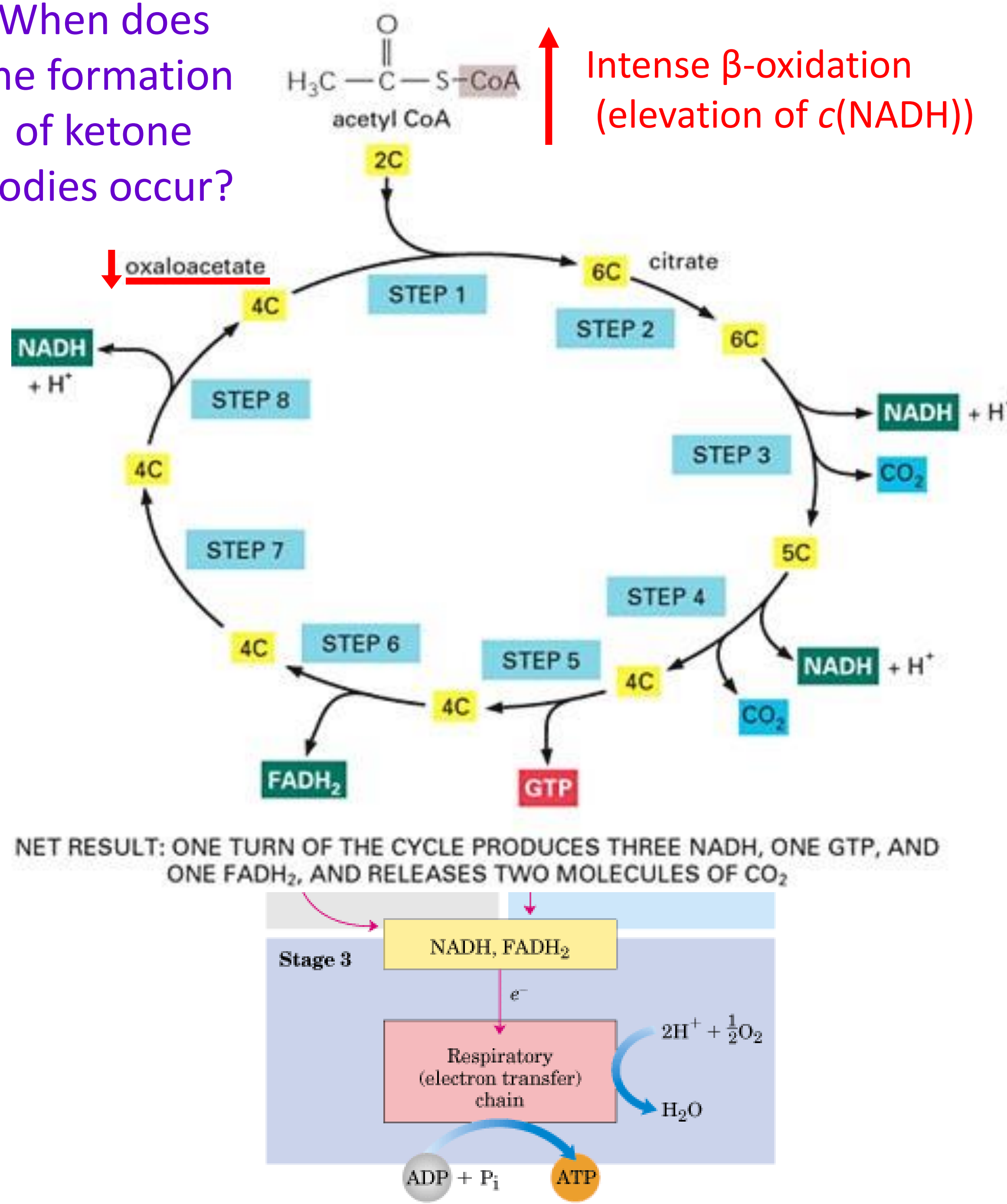
D-β-Hydroxybutyrate



IN LIVER (mitochondria)

When does the formation of ketone bodies occur?





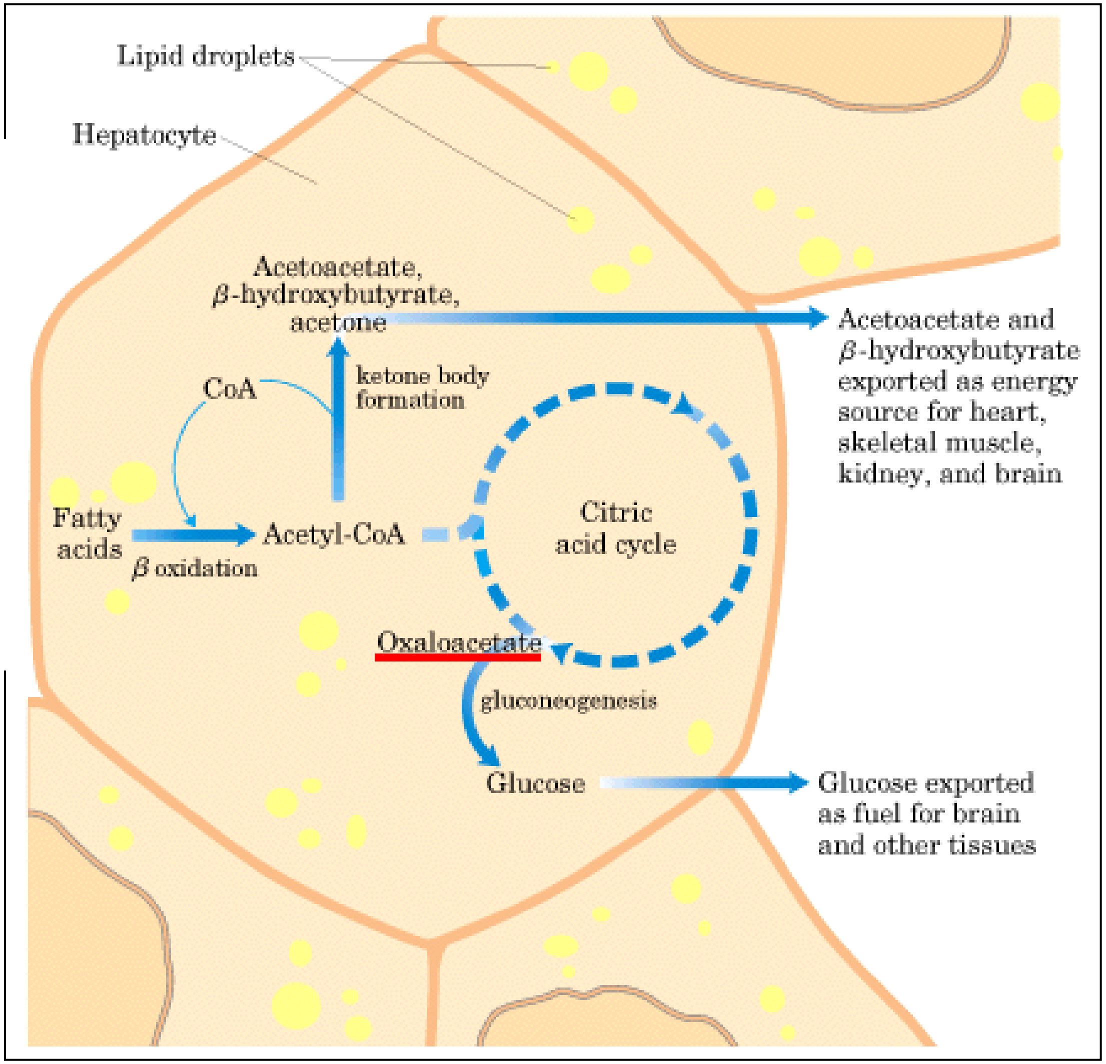
IN LIVER (mitochondria)

table 17–2

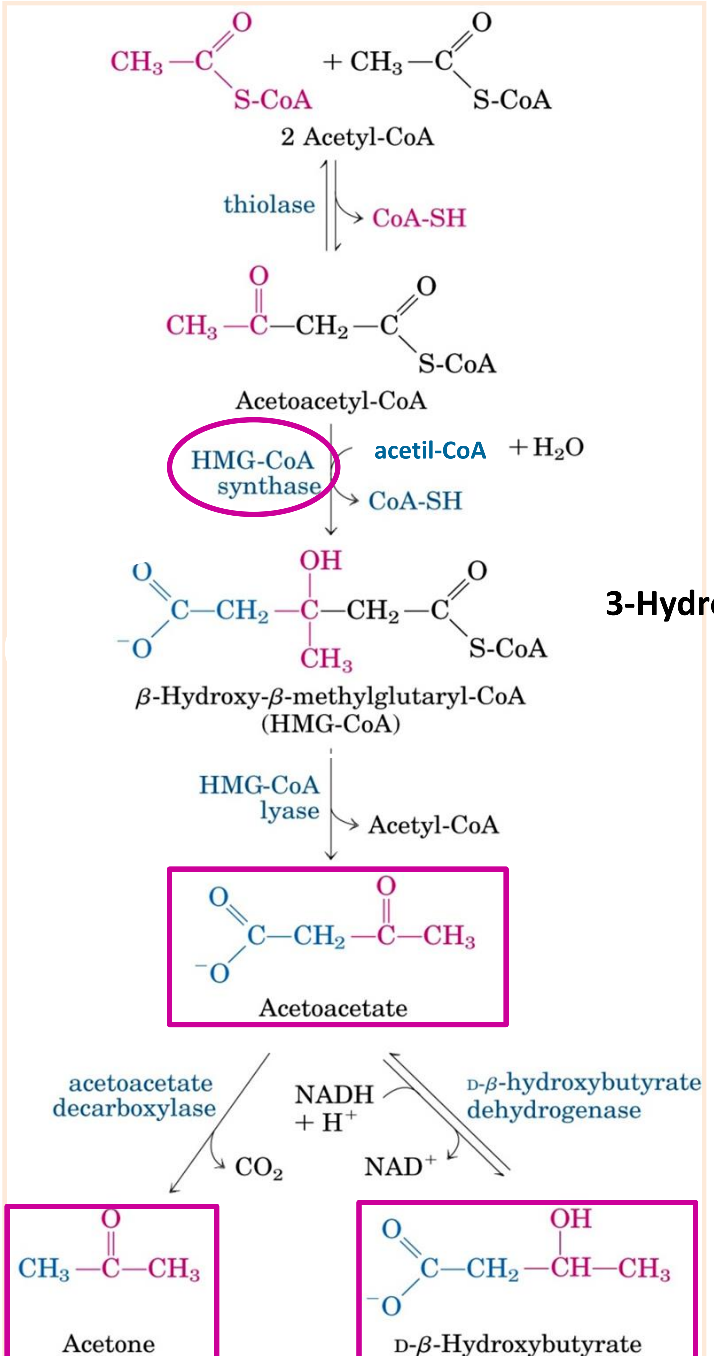
Ketone Body Accumulation in Diabetic Ketosis

	Urinary excretion (mg/24 h)	Blood concentration (mg/100 mL)
Normal	≤125	<3
Extreme ketosis (untreated diabetes)	5,000	90

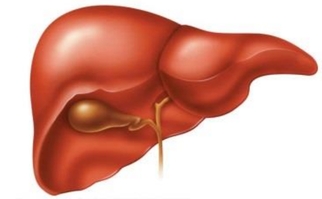
Ketoacidosis, coma



Formation of ketone bodies in the liver and transport to extrahepatical tissues



KETOGENSIS occurs primarily in <u>**mitochondria** of liver cells</u>



- <u>1st reaction</u>: formation od acetoacetate - inverse reaction of the last β-oxidation reaction!

3-Hydroxy-3-methylglutaryl-CoA
(<u>cytosol</u>)

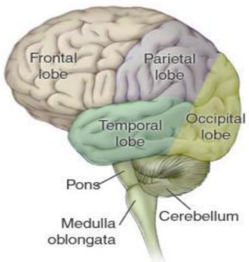
↓

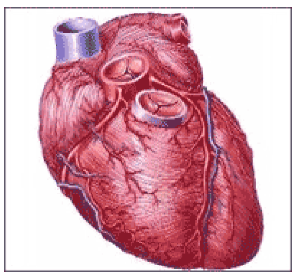
cholesterol
biosynthesis

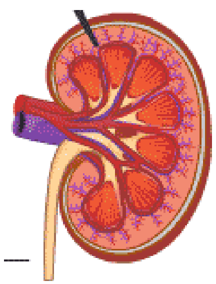
HMG-CoA in **mitochondria** of hepatocytes is involved in biosynthesis of **ketone bodies**, while in **cytosol** in biosynthesis of **cholesterol**

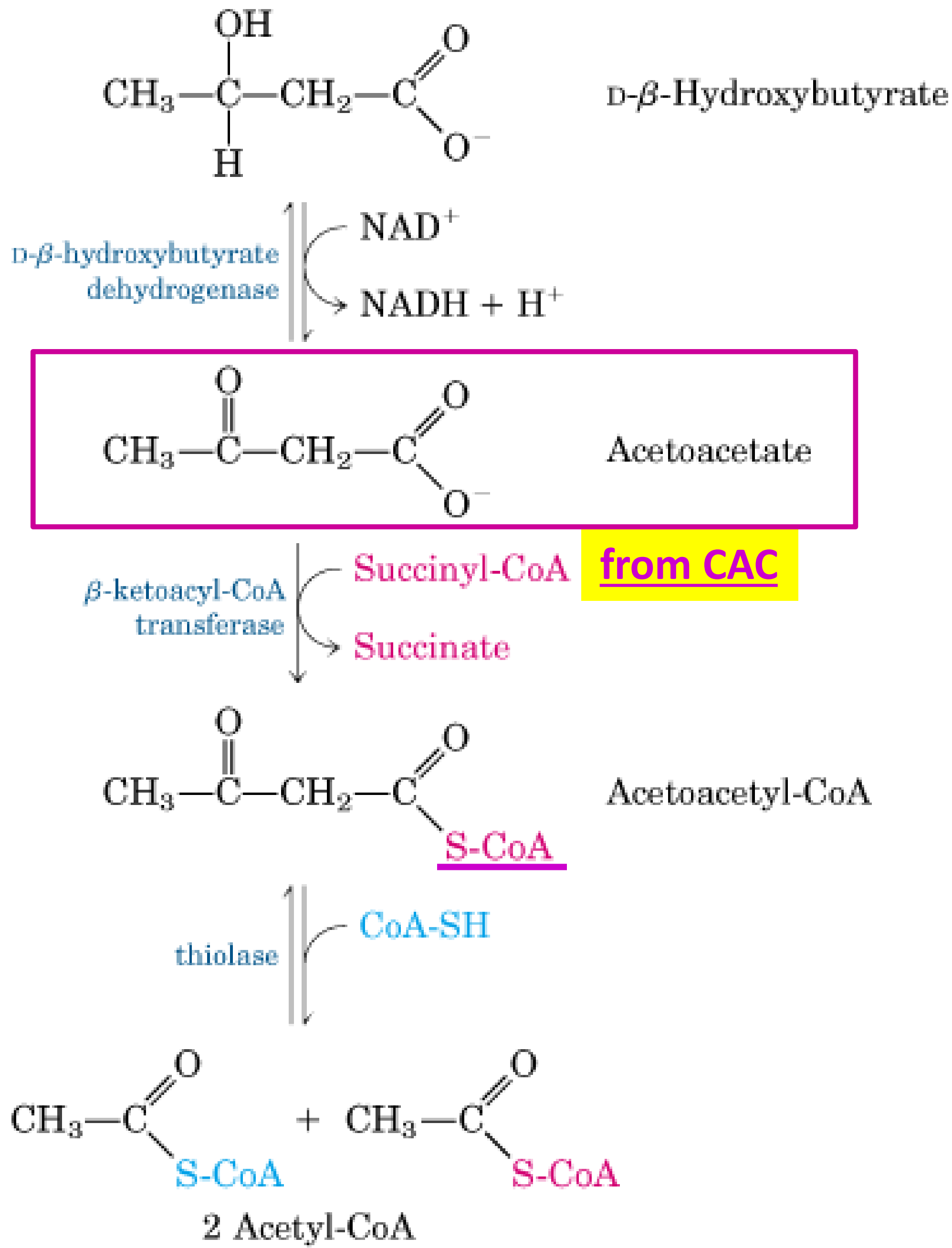
- *mitochondrial* **HMG-CoA syntase** and cytosolic **HMG-CoA synthase** are isoenzymes!

Ketone bodies to acetyl-CoA (in extrahepatical tissues)

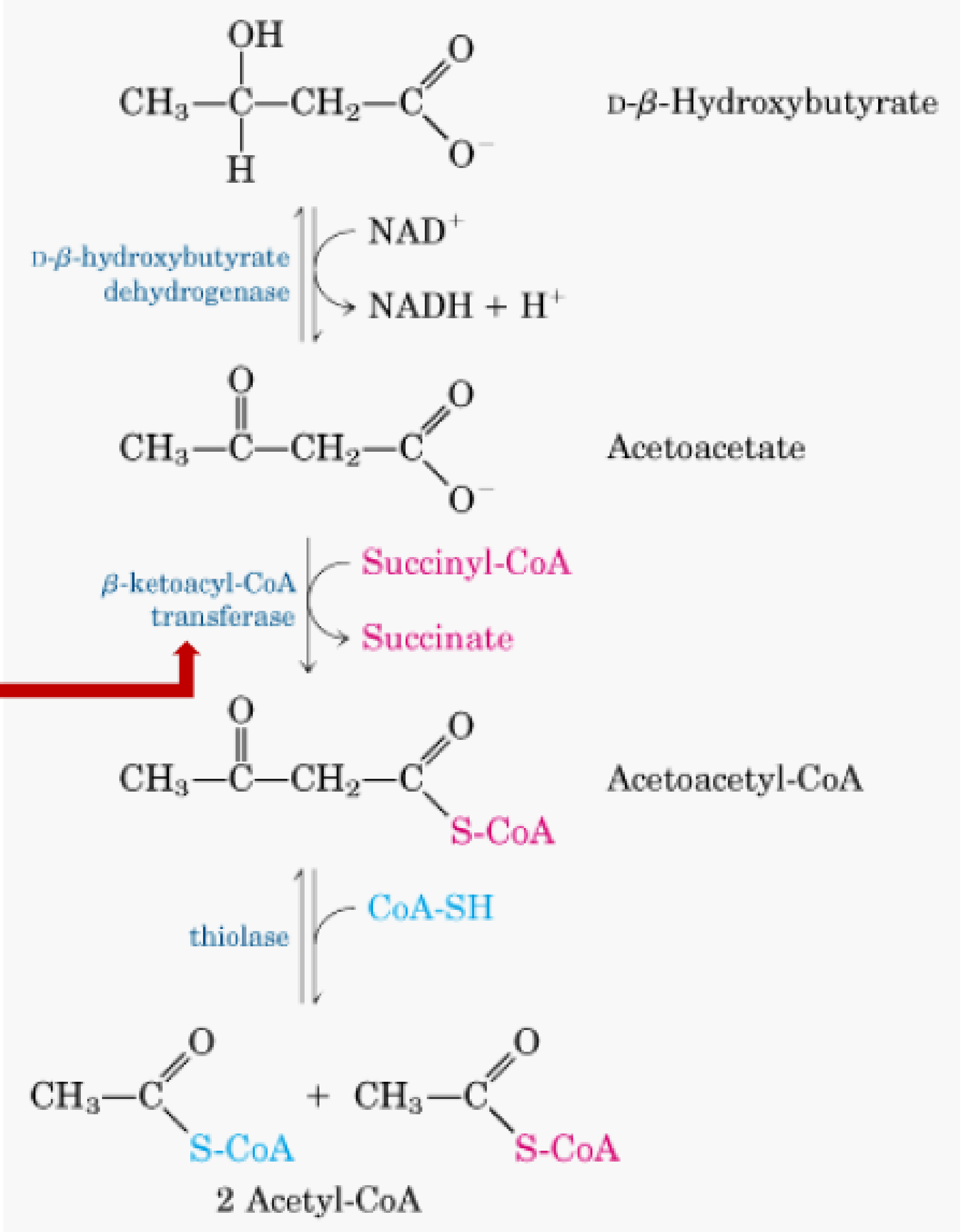








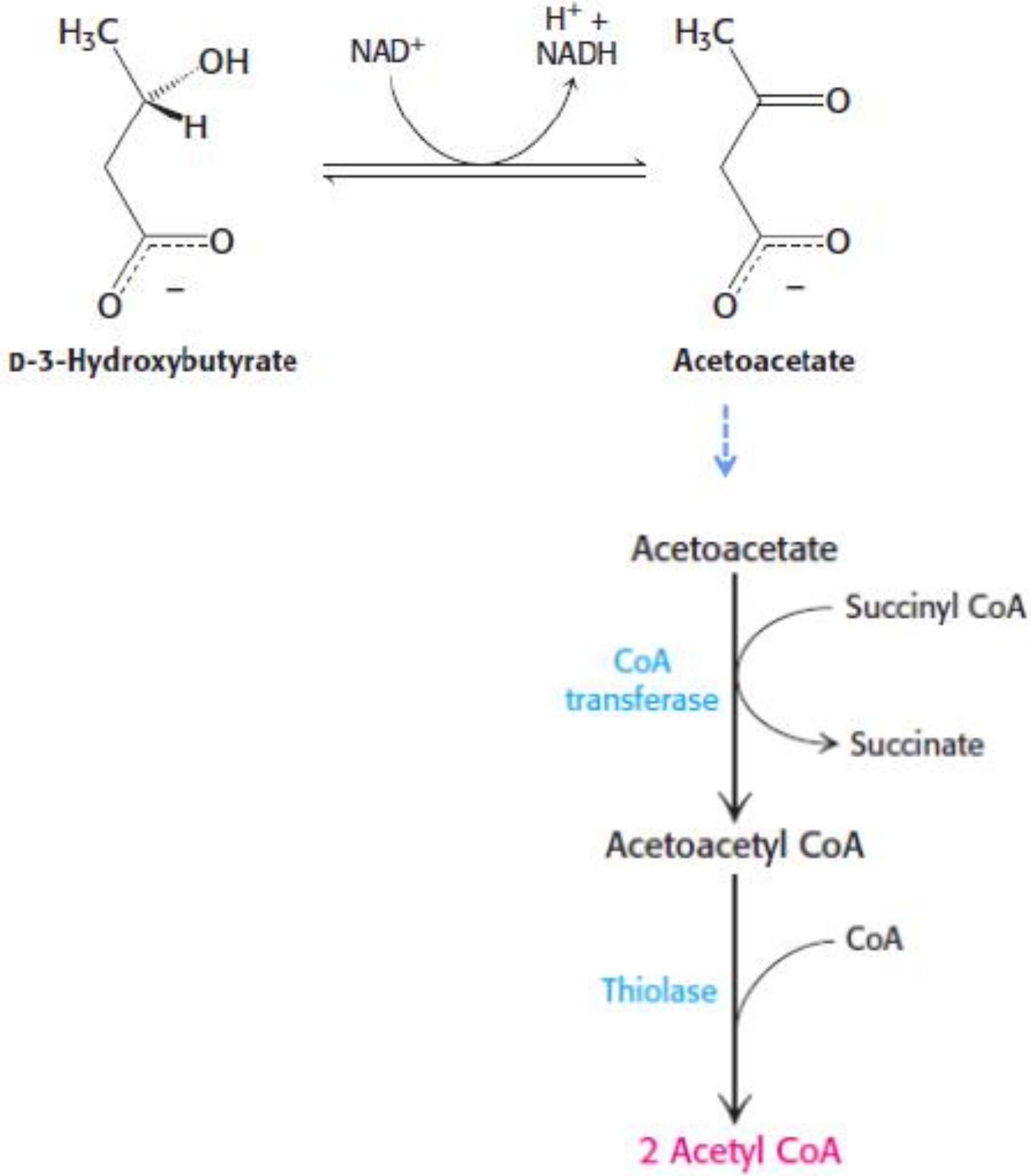
Utilization of ketone bodies as a fuel in extrahepatic tissues – degradation to acetyl-CoA



Expression of the enzyme in extrahepatic tissue, but not in the liver!

Liver does not use ketone bodies as a source of energy!

Red blood cells do not use ketone bodies (lack of mitochondria!)

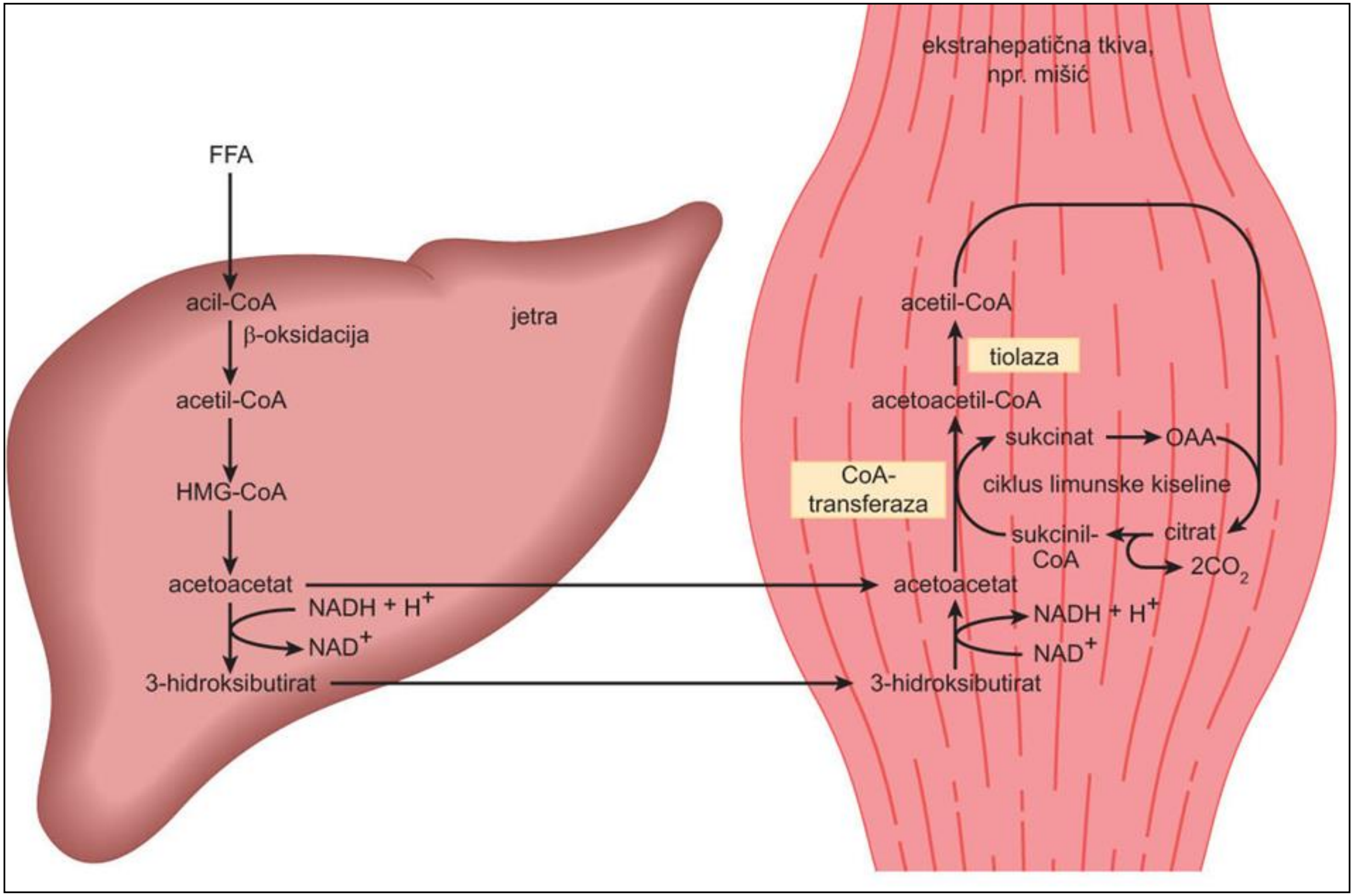


Net production of ATP by degradation of hydroxybutyrate (22.5 ATP) and acetoacetate (20 ATP)

<u>Take into account</u>:

- Formation of NADH in degradation of hydroxybutirate!

Ketogenesis in the liver and transport to extrahepatical tissues



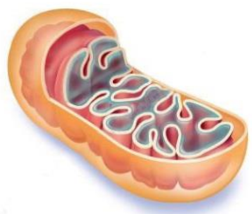
*CoA-transferase = **succinyl-CoA-acetoacetate-CoA-transferase** - all tisues EXCEPT liver!*

DIFFERENT PATHWAYS OF DEGRADATION AND BIOSINTHESIS OF FATTY ACIDS

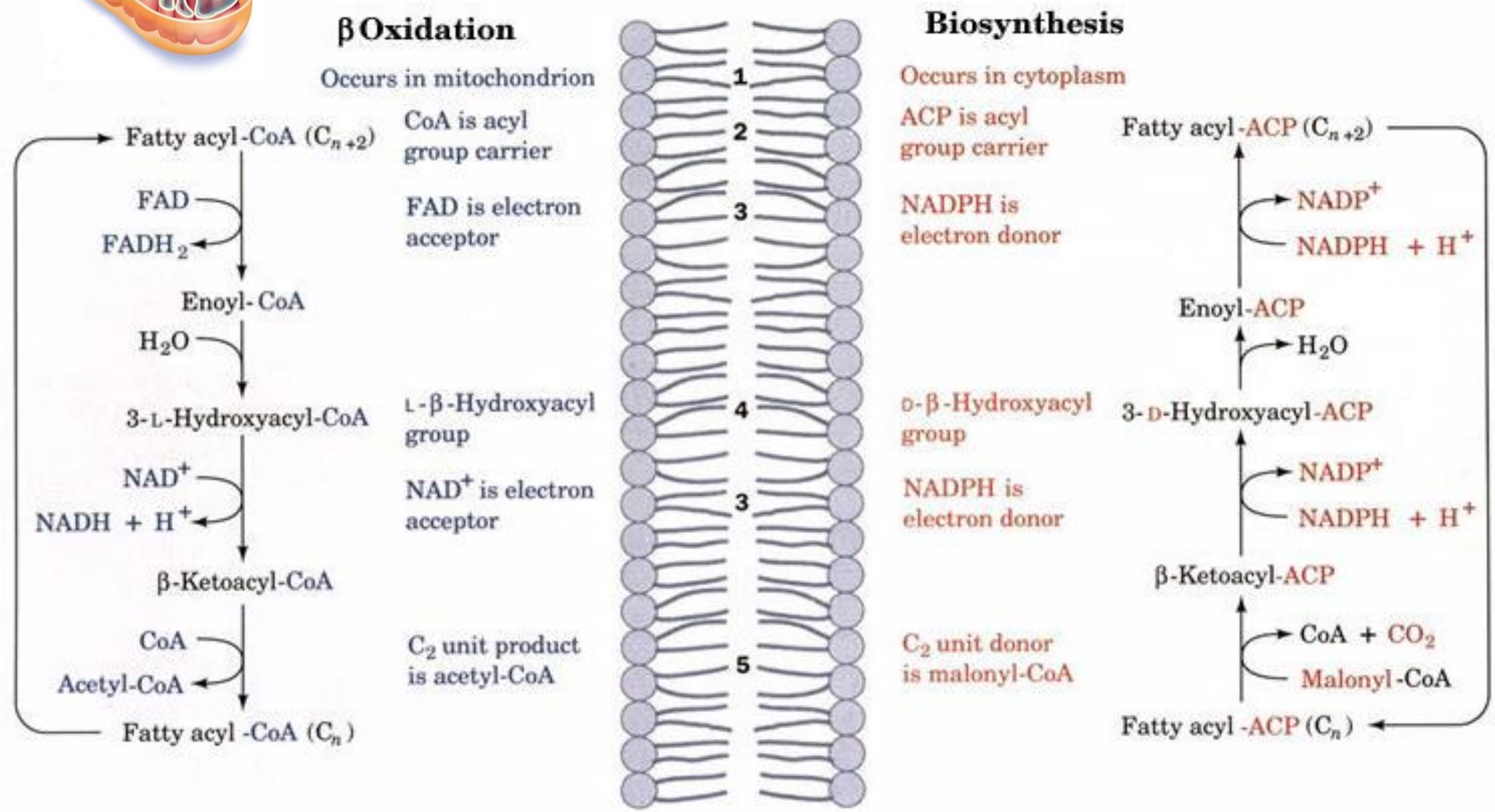
	FAs DEGRADATION	FAs BIOSYNTHESIS
intermediates	thioester bound to **CoA**	covalently bound to -SH group of **ACP** *(acyl carrier protein)*
localization	mitochondria	cyosol
enzymes	separate enzymes	enzymes as a part of a polypeptide chain - **fatty acid synthase**
cofactors	NAD^+ i FAD	NADPH

FATTY ACIDS METABOLISM

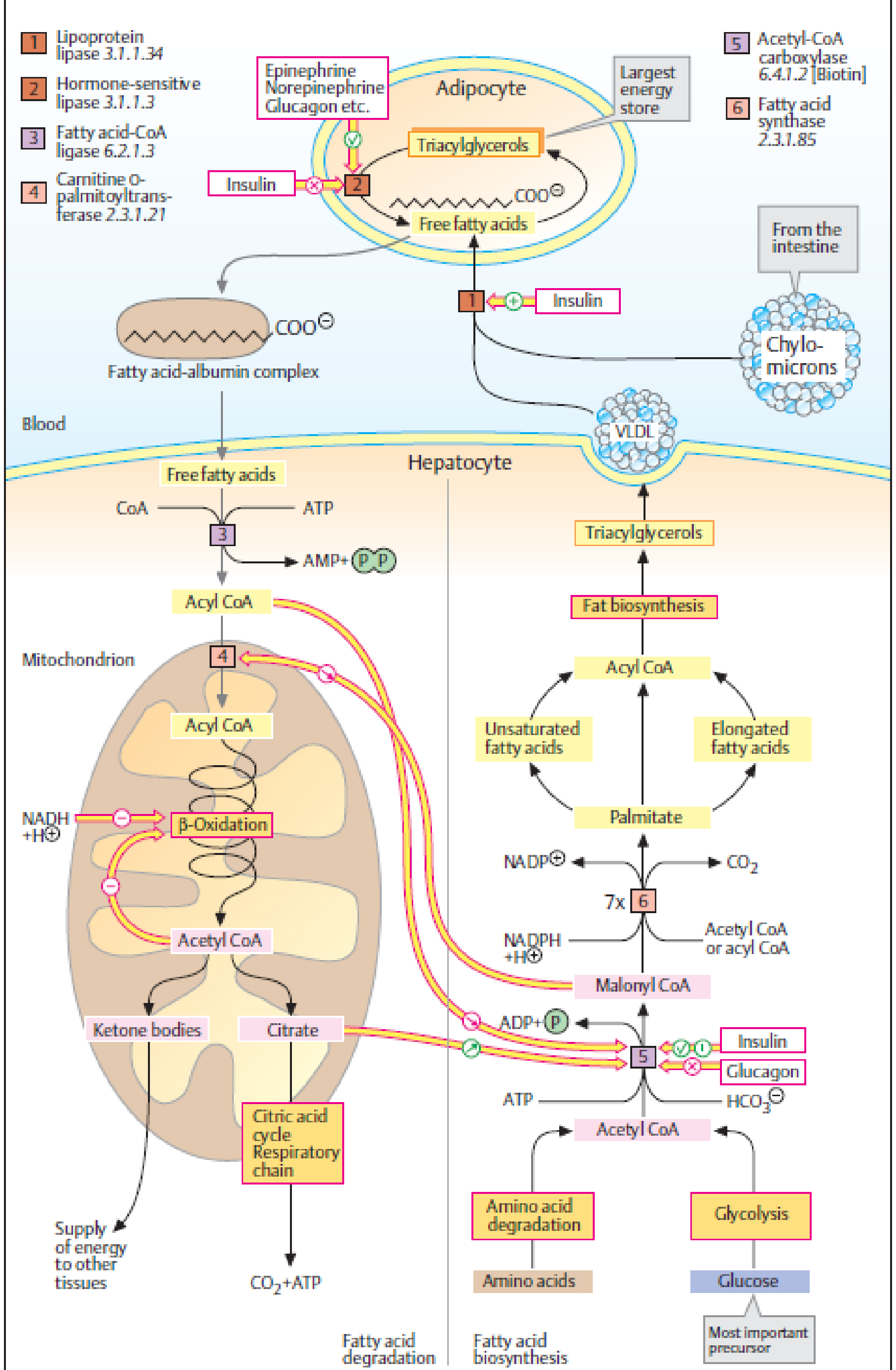
β-oxidation in mitochondria

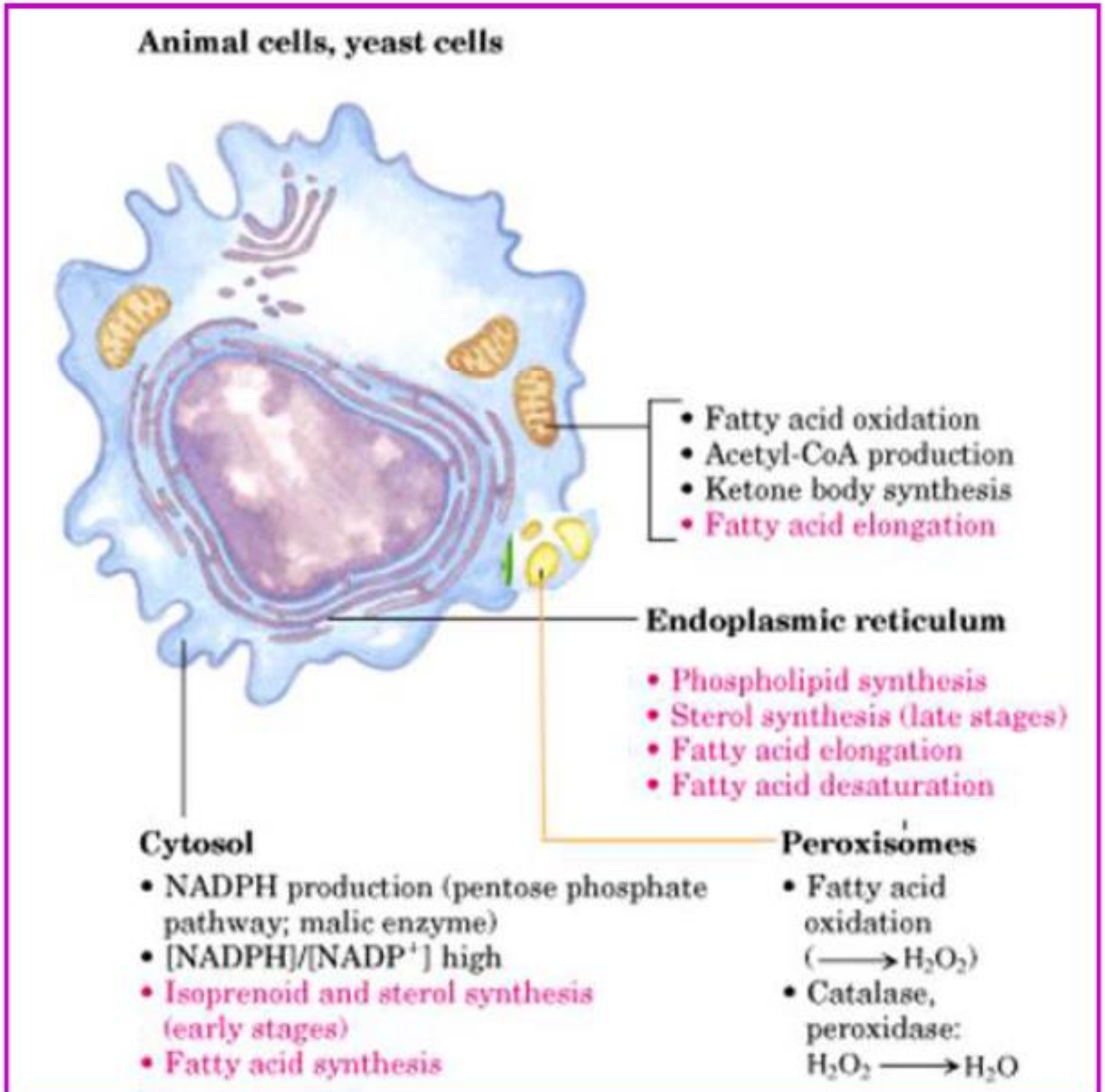


Biosynthesis in cytosol



A. Fat metabolism




Animal cells, yeast cells
• Fatty acid oxidation
• Acetyl-CoA production
• Ketone body synthesis
• Fatty acid elongation
Endoplasmic reticulum
• Phospholipid synthesis
• Sterol synthesis (late stages)
• Fatty acid elongation
• Fatty acid desaturation
Cytosol
• NADPH production (pentose phosphate pathway; malic enzyme)
• [NADPH]/[NADP⁺] high
• Isoprenoid and sterol synthesis (early stages)
• Fatty acid synthesis
Peroxisomes
• Fatty acid oxidation (⟶ H_2O_2)
• Catalase, peroxidase: H_2O_2 ⟶ H_2O

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Ppt presentations: Prof. Vukelić, Prof. Kalanj Bognar, Prof. Karmelić, Prof. Flögel (PMF, FBF)