فريق طوفان الأقصى

METABOLISM

Modified N.

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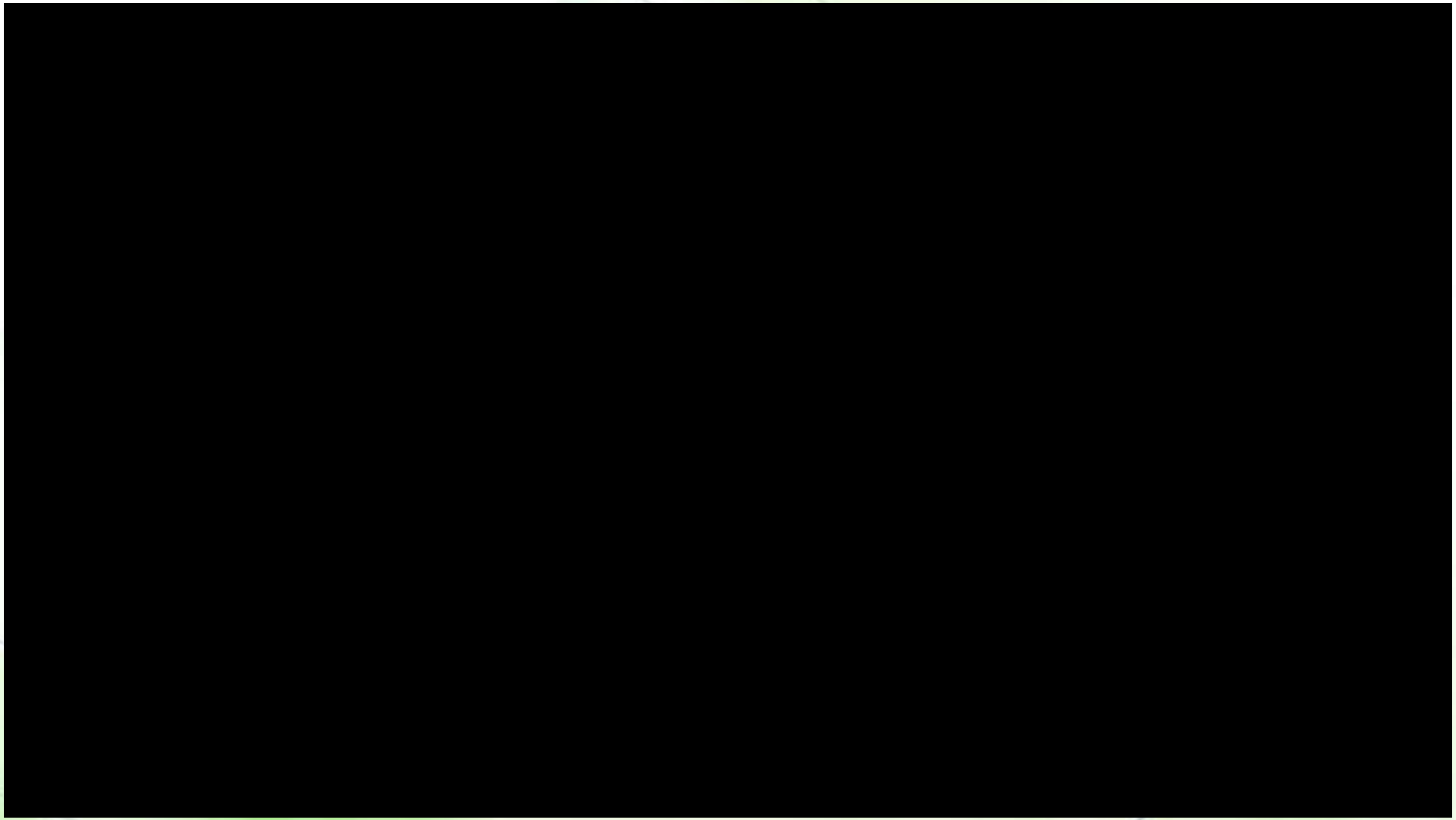
Ketoacyl synthase (KS)
Malonyl/acetyltransferase (AT)
Dehydrase (DH)
Enoyl reductase (ER)
Ketoacyl reductase (KR)
Thioesterase (TE)
Acyl carrier protein (ACP)



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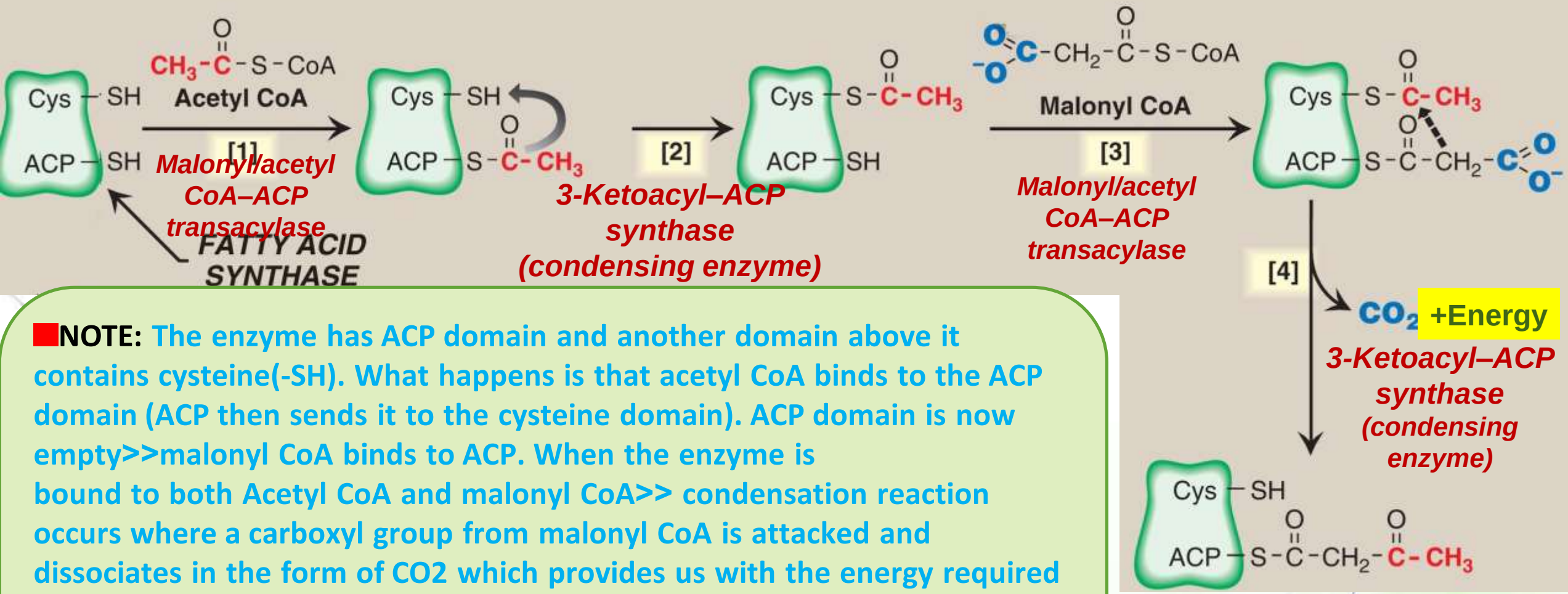
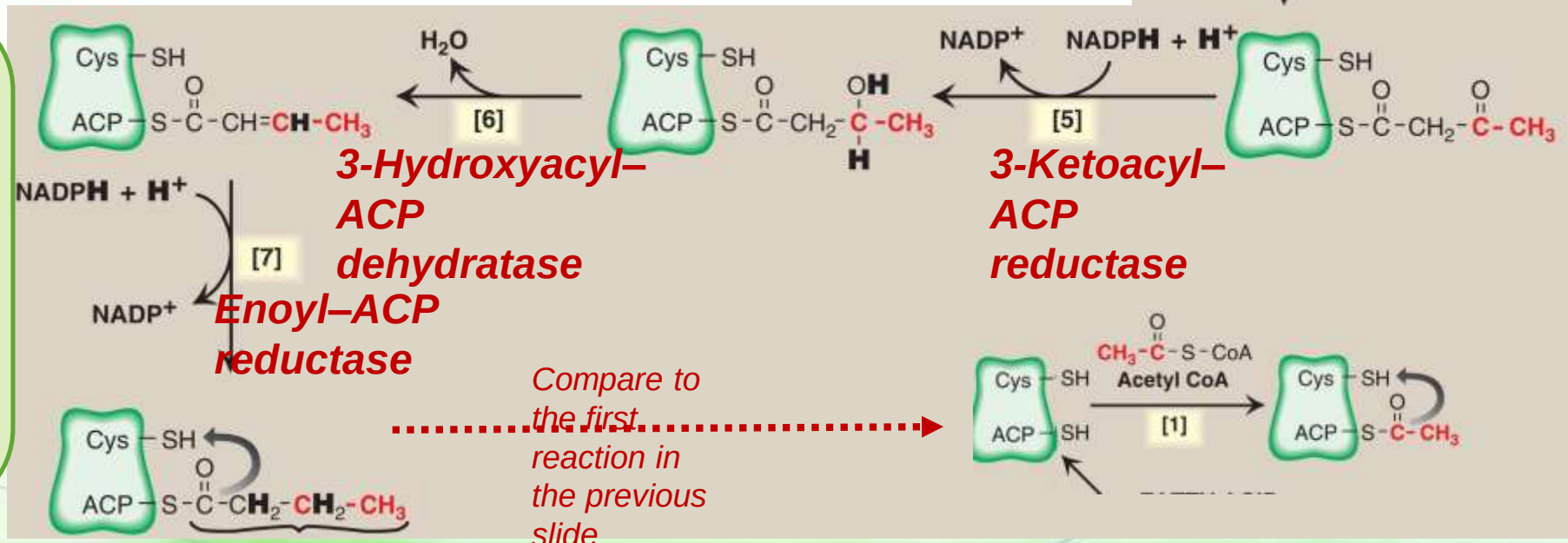


NOTE: The enzyme has ACP domain and another domain above it contains cysteine(-SH). What happens is that acetyl CoA binds to the ACP domain (ACP then sends it to the cysteine domain). ACP domain is now empty>>malonyl CoA binds to ACP. When the enzyme is bound to both Acetyl CoA and malonyl CoA>> condensation reaction occurs where a carboxyl group from malonyl CoA is attacked and dissociates in the form of CO2 which provides us with the energy required for the condensation reaction>> condensation of 2 carbons from malonyl CoA with 2 carbons from acetyl CoA>> the product contains 4 carbons.

Condensation, reduction, dehydration, reduction

NOTE: The 4-carbon molecule is now attached to the ACP domain where 3 reactions occur (reduction >> dehydration >> reduction) and these reduction reactions require NADPH as a source of electrons for the redox reactions >> reduction of the oxygen of the carbonyl group to OH >> dehydration (remove H2O) >> reduction (adding hydrogen atoms). Then this 4-carbon molecule is attached to the cysteine domain >> empty ACP domain >> binds another malonyl CoA >> repeat the previous steps.

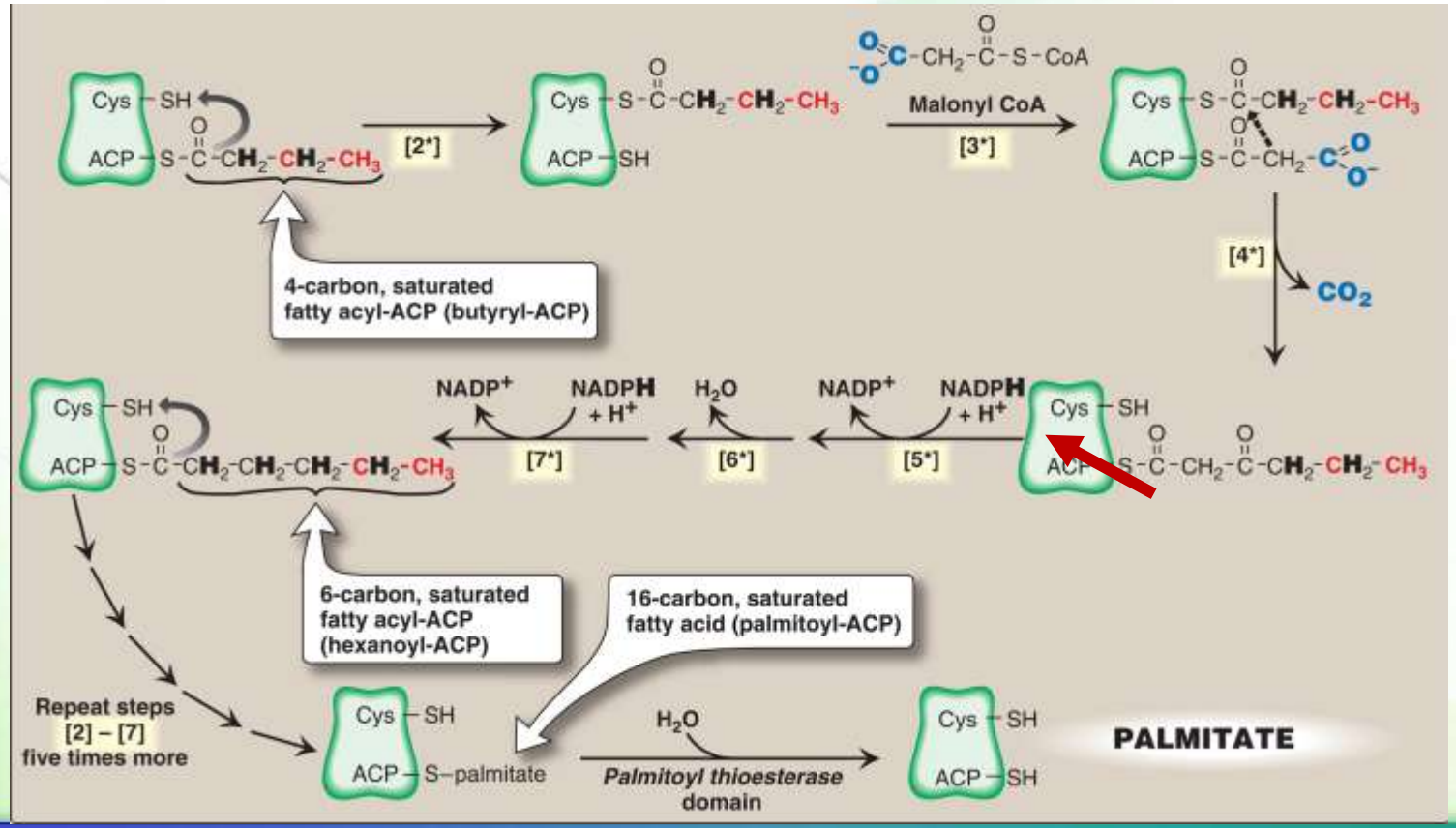
each round 2 carbons are added until we reach 16 carbons 2 carbons from acetyl CoA and 14 from malonyl CoA molecules producing palmitate. Each round we use malonyl CoA which is synthesized from acetyl CoA as we mentioned before.



The lactating mammary gland terminates lengthening the chain EARLY.

REMEMBER:

- ***We use NADPH in anabolism reactions (synthesis).**
- ***We use NADH in breakdown reactions (degradation).**



NOTE: The lactating mammary gland terminates lengthening the chain (elongation) EARLY, that's why milk contains a lot of medium chain fatty acids. In lactating glands, elongation is terminated early before the production of palmitate.

The final product (palmitate) is released by thioesterase which breaks the thioester bond between the palmitate and thiol group of the ACP domain then the fatty acid is finally synthesized.

The stoichiometry of palmitate synthesis

- **Stoichiometry of palmitate synthesis:**

  Acetyl-CoA + 7 malonyl-CoA + 14 NADPH + $14H^+$ ⟶

  palmitate + $7CO_2$ + $14NADP^+$ + 8CoA + $6H_2O$

- **Malonyl-CoA synthesis:**

  7 Acetyl-CoA + $7CO_2$ + 7ATP ⟶

  7 malonyl-CoA + 7ADP + $7P_i$ + $7H^+$

- **Overall stoichiometry of palmitate synthesis:**

  8 Acetyl-CoA + 14 NADPH + 7ATP + $7H^+$ ⟶

  palmitate + $14NADP^+$ + 8CoA + $6H_2O$ + 7ADP + $7P_i$

Sources of molecules

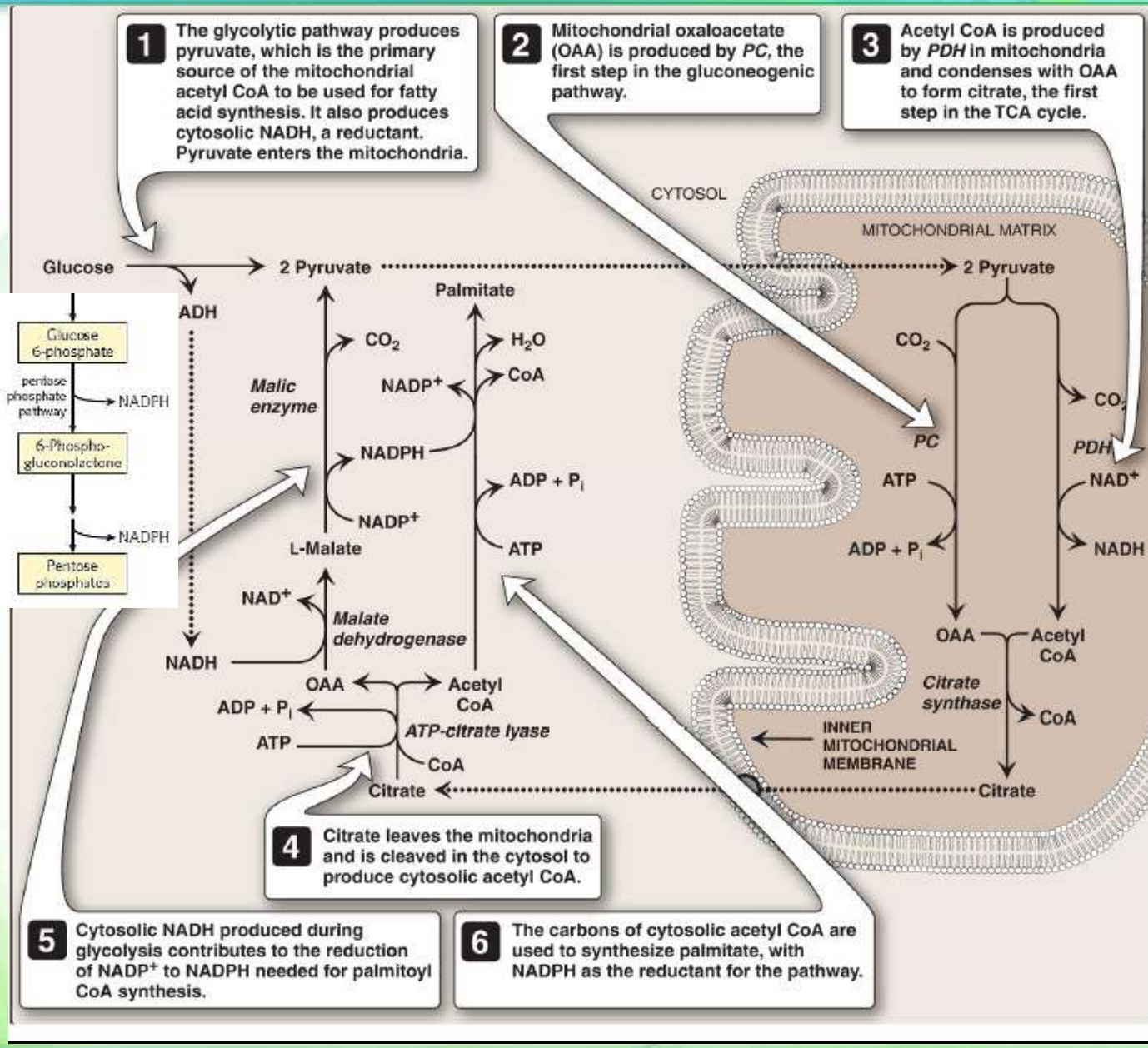
- Acetyl CoA
  - Pyruvate

NOTE: from pyruvate that enters the mitochondria and gets converted to citrate which move to the cytosol and then breaks down into OAA and acetyl CoA.

- NADH (for *oxaloacetate to malate)*
  - Glycolysis

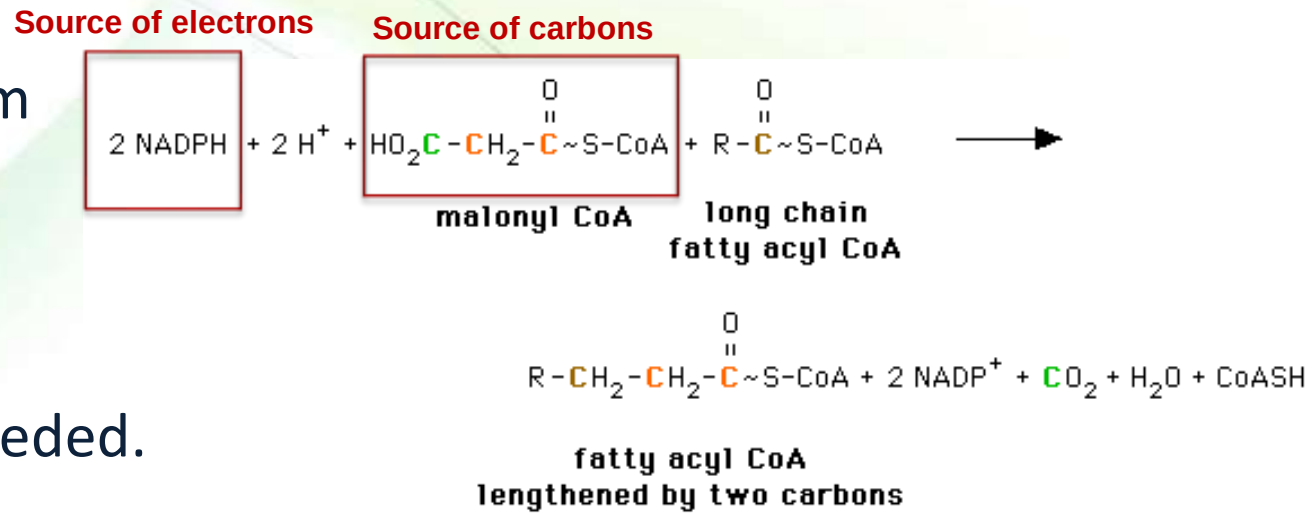
(for converting OAA to malate): from glycolysis in cytosol. Malate can either enter the mitochondria by a transporter or get convertedto pyruvate.

- NADPH:
  - Pentose phosphate pathway
  - Malate to pyruvate



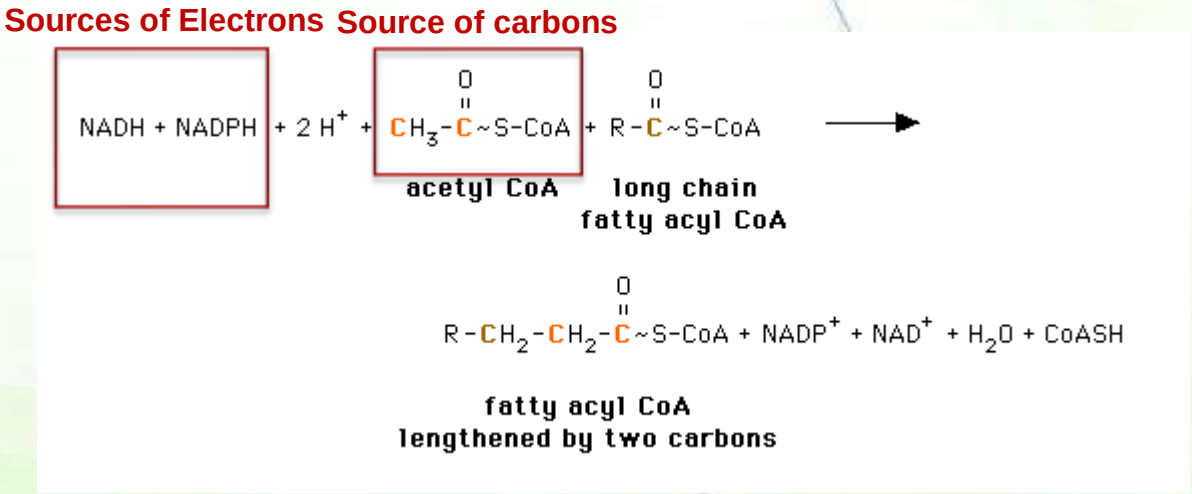
Further elongation

- Location: smooth endoplasmic reticulum
- Different enzymes are needed.
- Two-carbon donor: Malonyl CoA
- Source of electrons: NADPH
- No ACP or multifunctional enzyme is needed.



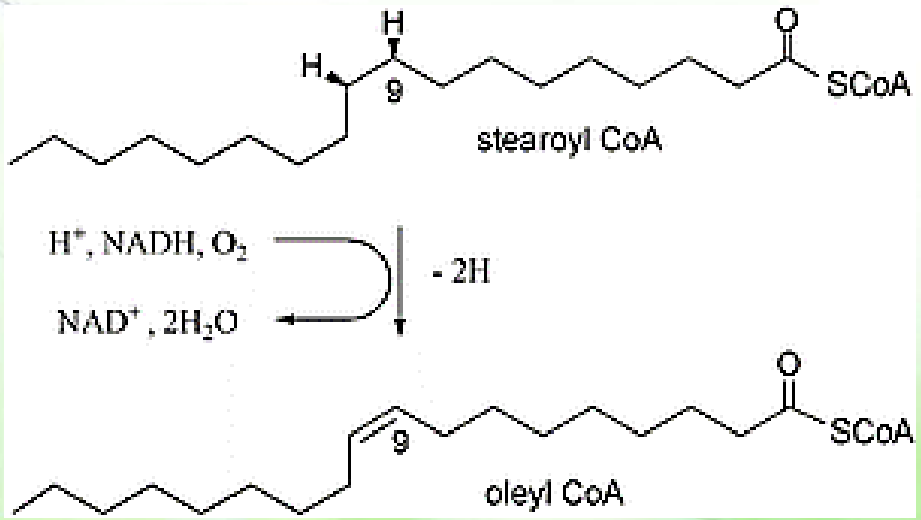
- Note: the brain has additional enzymes allowing it to produce the very-long-chain fatty acids ([VLCFA] over 22 carbons)

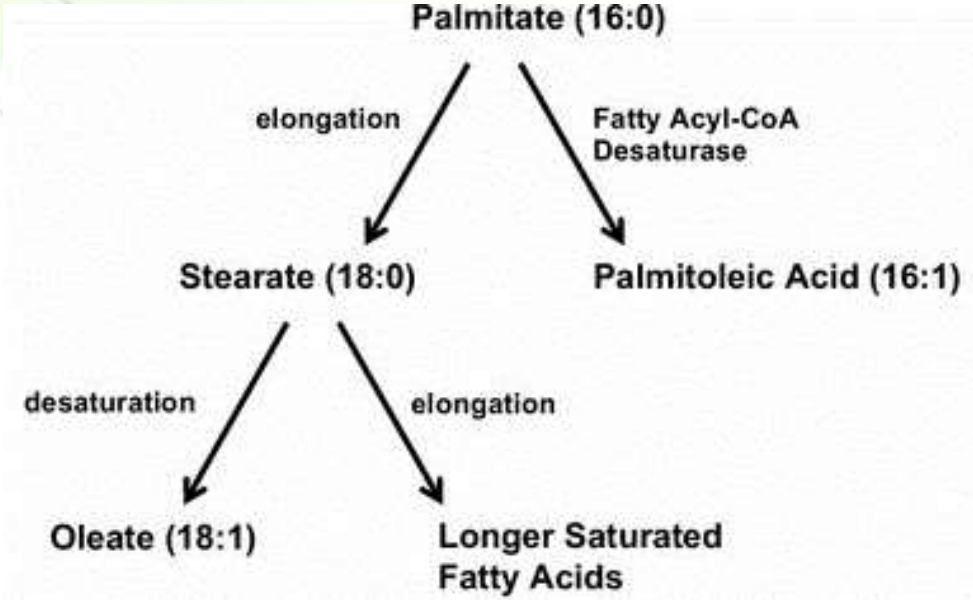
- Location: mitochondria
- Two-carbon donor: Acetyl CoA
- Source of electrons: NADPH and NADH
- Substrates: fatty acids shorter than 16

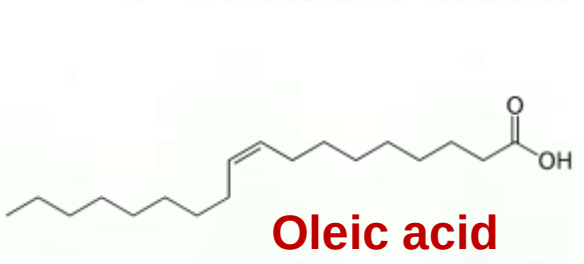


Chain desaturation

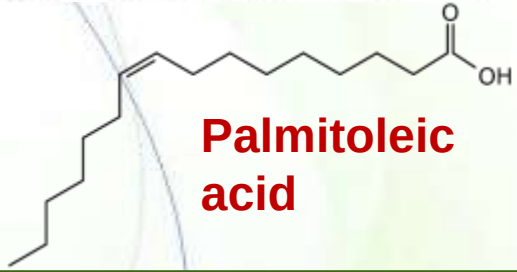
- Enzymes: fatty acyl CoA desaturases
- Substrates: long-chain fatty acids
- Location: smooth endoplasmic reticulum
- Acceptor of electrons: oxygen (O_2), cytochrome b5, and its flavin adenine dinucleotide (FAD)-linked reductase
- Donor of electrons: NADH
- The first double bond is inserted between carbons 9 and 10, producing oleic acid, 18:1(9), and small amounts of palmitoleic acid, 16:1(9).







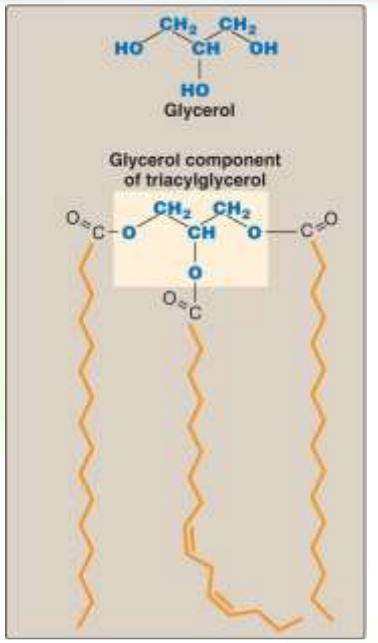
Oleic acid



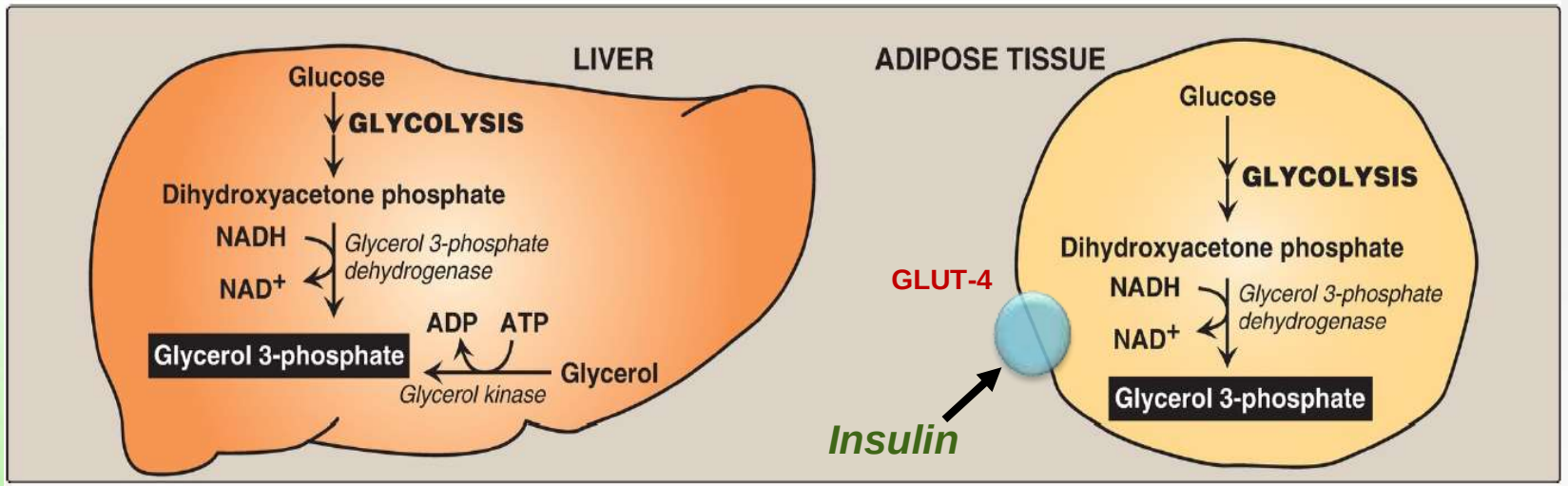
Palmitoleic acid

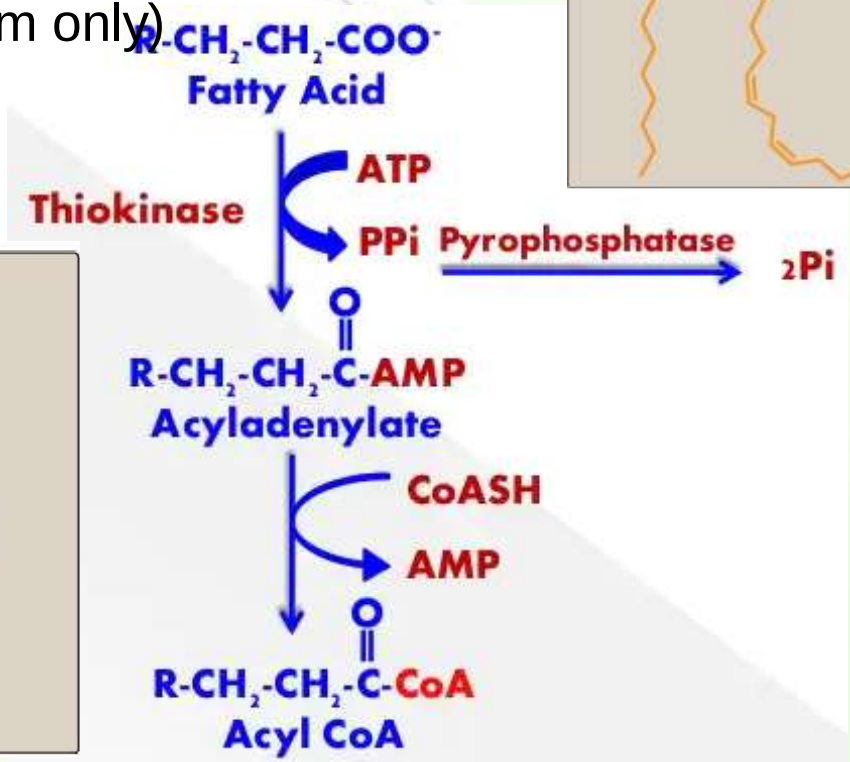
Humans have carbon 9, 6, 5, and 4 desaturases but cannot introduce double bonds from carbon 10 to the ω end of the chain. Therefore, the polyunsaturated ω-6 linoleic acid and ω-3 linolenic acid are essential.

Triacylglycerol structure and synthesis



- The fatty acid on carbon 1 is typically saturated, that on carbon 2 is typically unsaturated, and that on carbon 3 can be either.
- Synthesis involves three steps:
  - Glycerol 3-phosphate synthesis
    - Liver (2 mechanisms) vs. adipose tissue (one mechanism only)
  - Activation of fatty acids
  - Synthesis of triacylglycerol



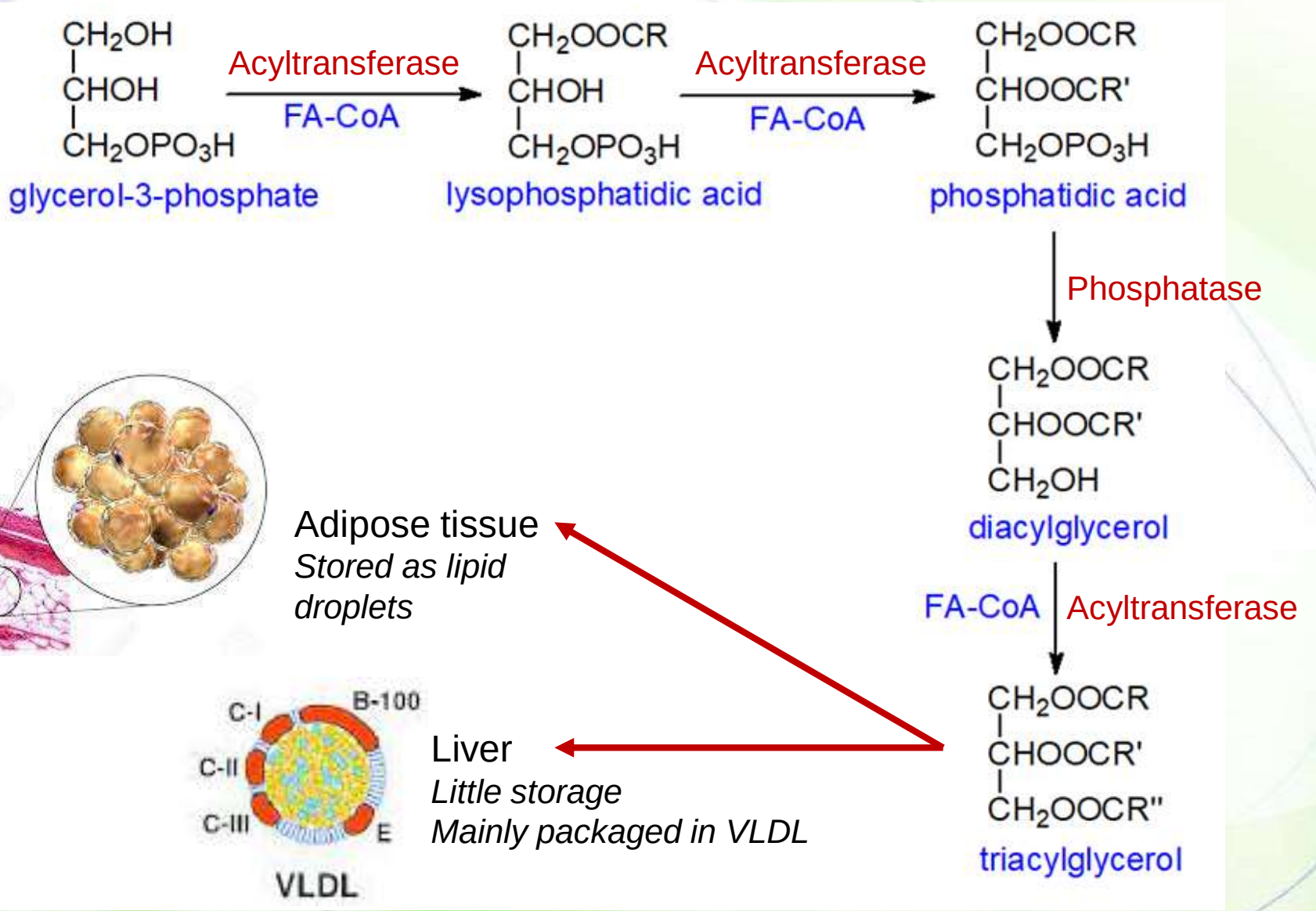


- **Additional information:**

Storage of FAs in adipocytes and liver by binding them to glycerol to produce triacylglycerol molecule in 3 steps:

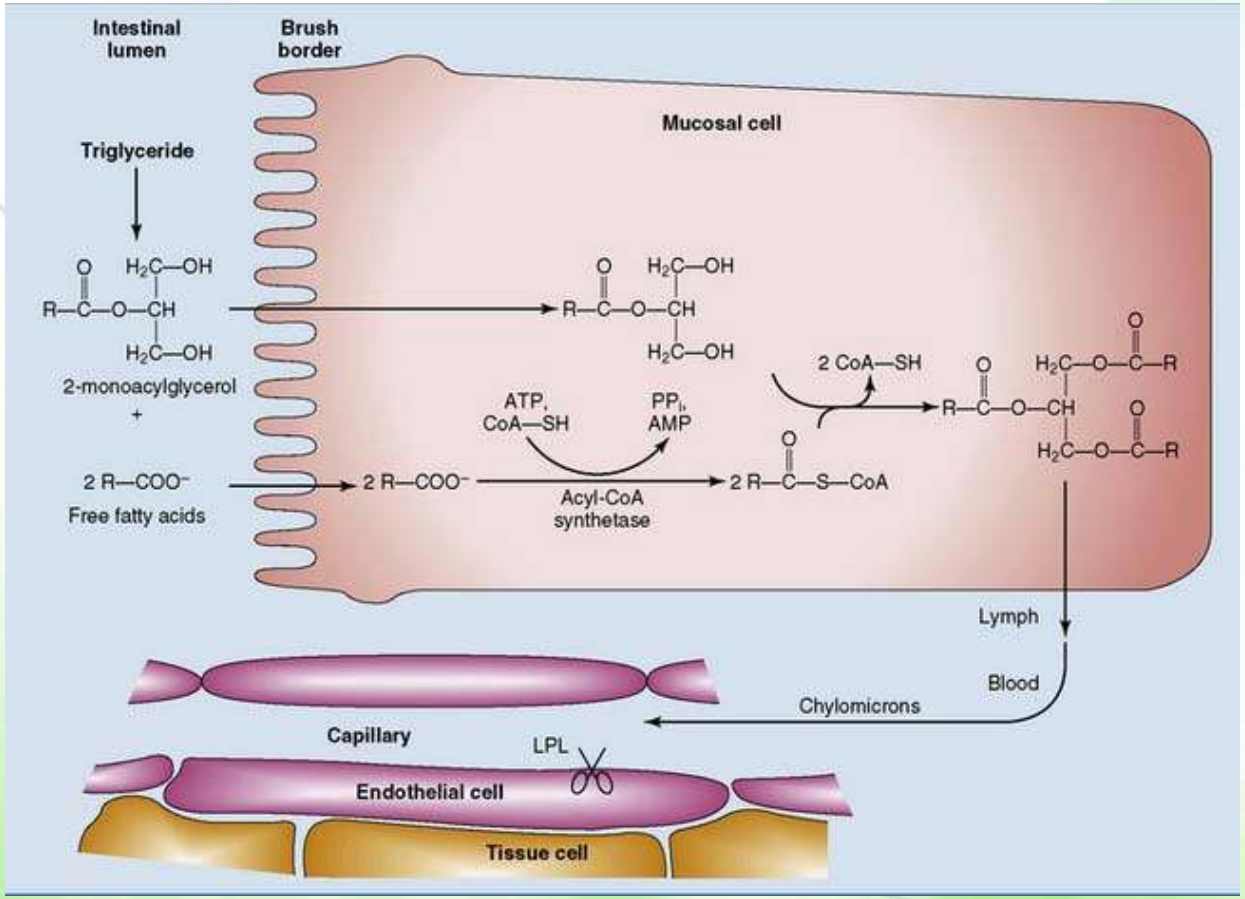
- STEP1: synthesis of glycerol-3-p (two mechanisms)
- from glucose>>DHAP>> glycerol-3-P
- phosphorylation of glycerol by glycerol kinase (only in liver, that's why adipocytes can't have glycerol-3-P or TAGs unless there is enough glucose.
- STEP2: activation of fatty acids by adding CoA>> fatty acyl-CoA (high energy molecules).
- STEP3: synthesis of triacylglycerol using the energy stored in the bond between CoA and carboxyl group of the fatty acid (a condensation reaction between FA and glycerol)

Synthesis of triacylglycerol



Intestinal mucosal cells

- In addition to these two pathways (as in the liver), TAG is synthesized via the MAG pathway in the intestinal mucosal cells.



NOTE: TAGs are also synthesized in enterocytes (intestines). After eating TAG, fatty acids are released to the lumen then to enterocytes and TAGs are resynthesized in the intestines then packaging them in chylomicrons to deliver them to lymphatics.

Additional information:

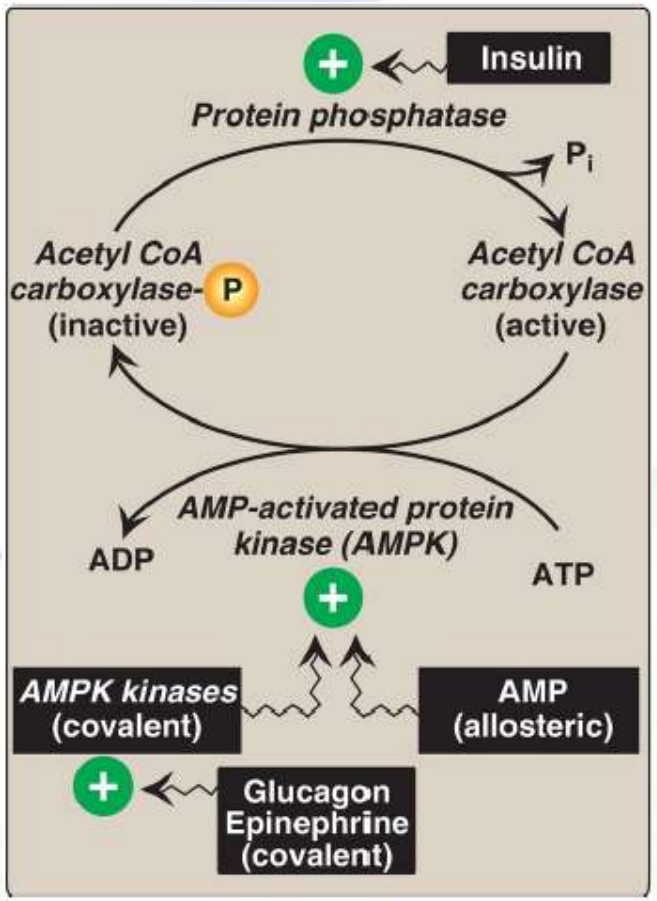
Although TAG is synthesized in both liver and adipose tissue, TAG is synthesized for different purposes in the two places. The purpose of adipose tissue is storing energy so TAG will be synthesized in well-fed state, but the liver purpose is to provide peripheral tissues with source of energy like glucose and TAG so it will be synthesized in starvation state also.

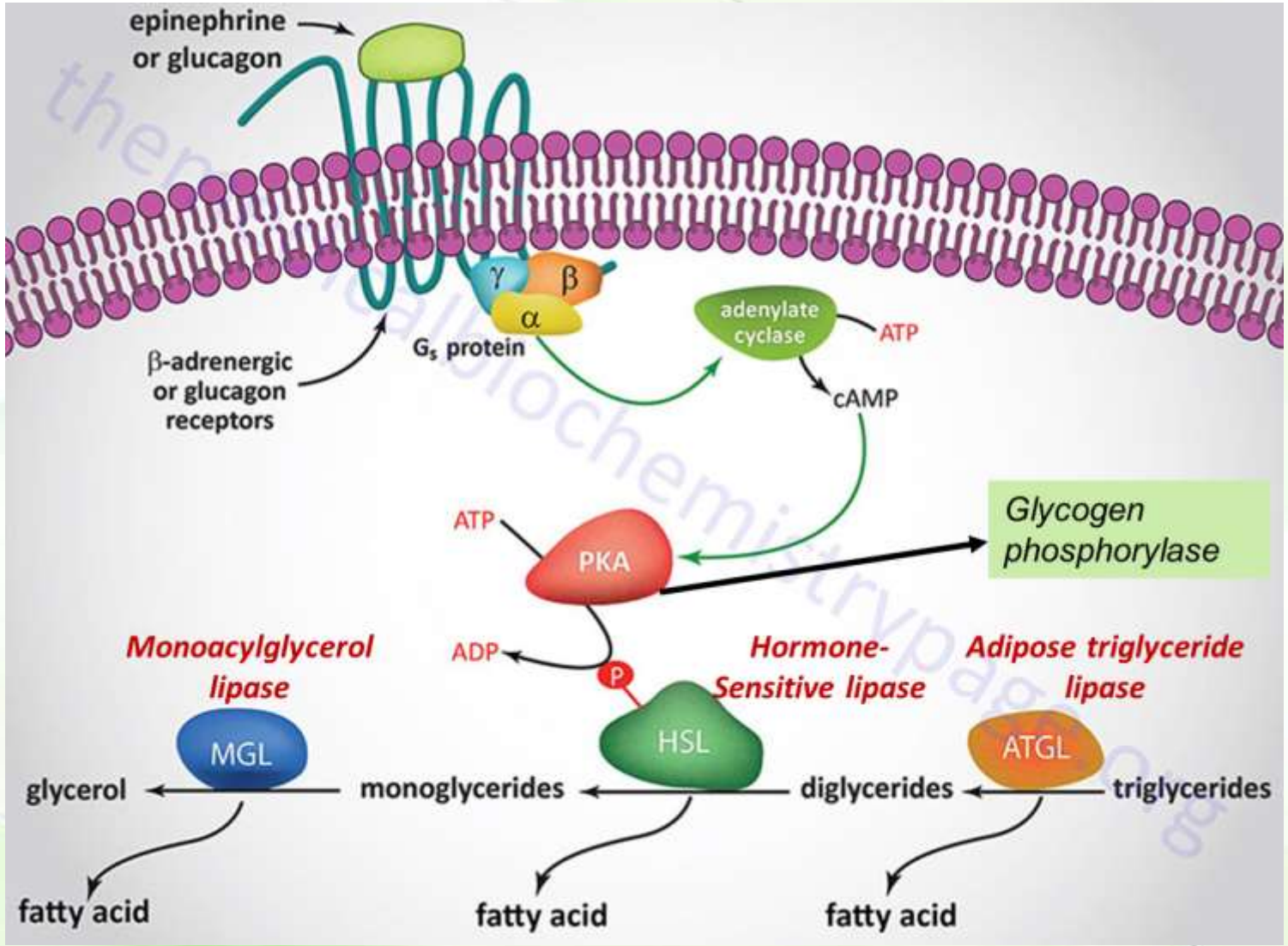
Liver has two mechanisms to provide glycerol in the TAG synthesis, one depends on DHAP and one depends directly on glycerol, in well-fed state we prefer to use DHAP pathway to produce glycerol, when glucose level is low >> DHAP level is also low >> we will use the glycerol pathway also to ensure enough TAG for the peripheral tissue.

Release of fatty acids from TAG

As an introduction to our concept, lipids are rich in energy and they are the best energy storage molecules (long term storage molecules), and the most common type of lipids is TAG (triacylglycerol) either we take it from food or synthesize and store it in our body.
As we know, TAG is composed of glycerol and 3 fatty acids, so we need to break down this TAG to have free fatty acids and then break down these fatty acids to produce energy, so how this process occurs?

Hormonal regulation





Note: Acetyl CoA carboxylase (ACC) is inhibited by the same signaling pathway

Additional information:

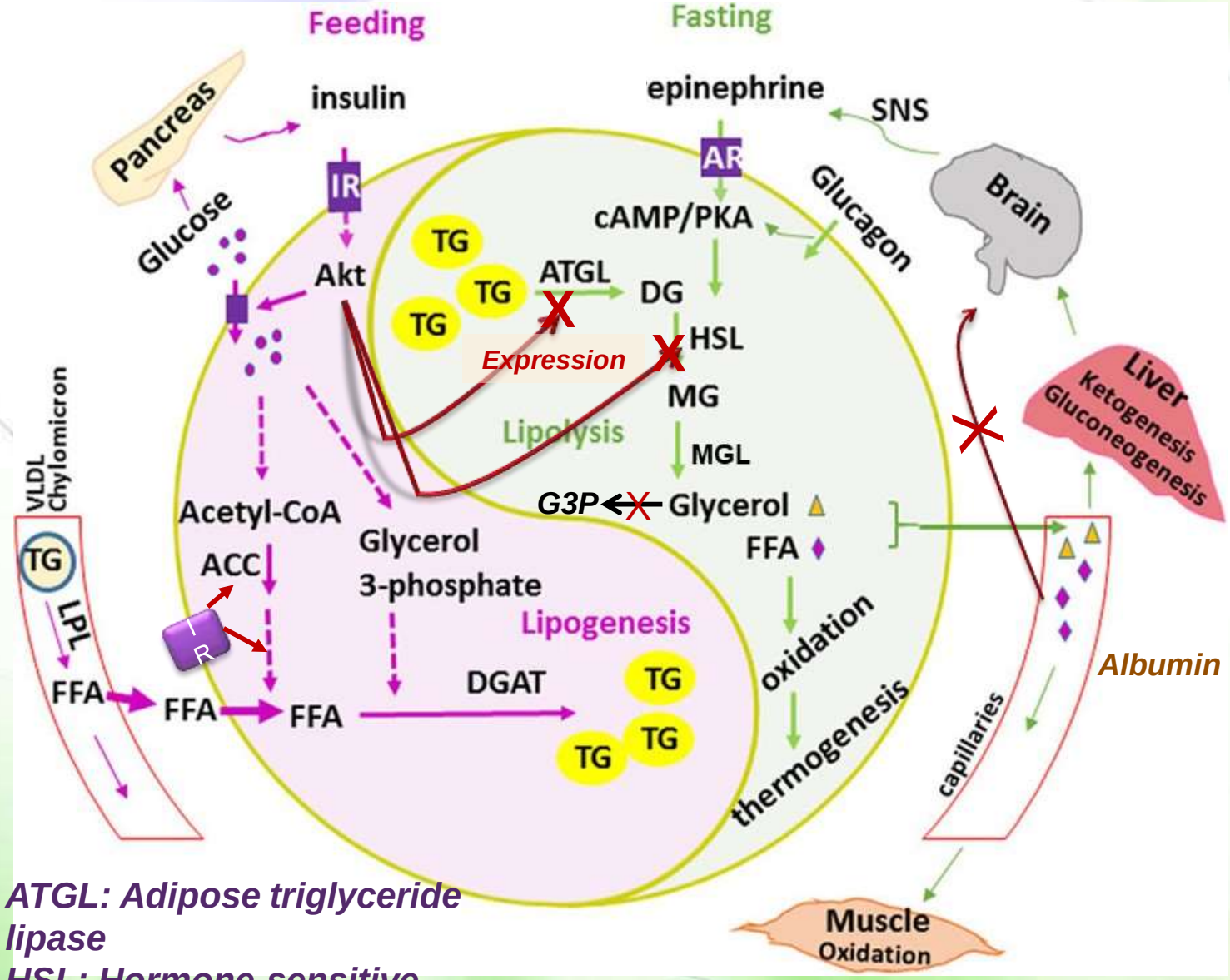
- as you can see in the figure above, this is the mechanism of releasing fatty acids from TAG, how it starts?

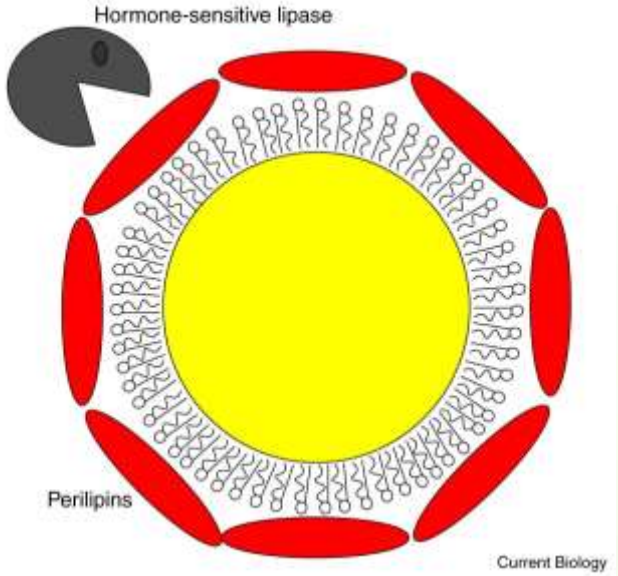
- The body sends signals to the liver and adipocytes that it (the body) needs fatty acids which are part of TAG in the adipocytes, so the glucagon hormone (or the epinephrine in the muscles) binds to its receptor and activates it >> the receptor activates G protein >> activating adenylate cyclase >> increasing cAMP in adipocytes >> cAMP activates PKA that activates the enzymes which are responsible of releasing fatty acids from the TAG, especially the HSL (hormone sensitive lipase).

- Then the mechanism happens as follow:
- Triglycerides become diglycerides by an enzyme called ATGL (adipose triglyceride lipase) and releasing 1 fatty acid >> diglycerides become monoglycerides by an enzyme called HSL and releasing 1 fatty acid >> monoglycerides become glycerol by an enzyme called MGL (monoacylglycerol lipase) and releasing the last fatty acid.

- HSL is under regulation of protein kinase A (PKA) which is activated by cAMP and its level increases when there is epinephrine or glucagon.

Perilipin





Perilipin (in red) coats fat droplets blocking HSL. It is phosphorylated by PKA releasing it.

ATGL: Adipose triglyceride lipase
HSL: Hormone-sensitive lipase

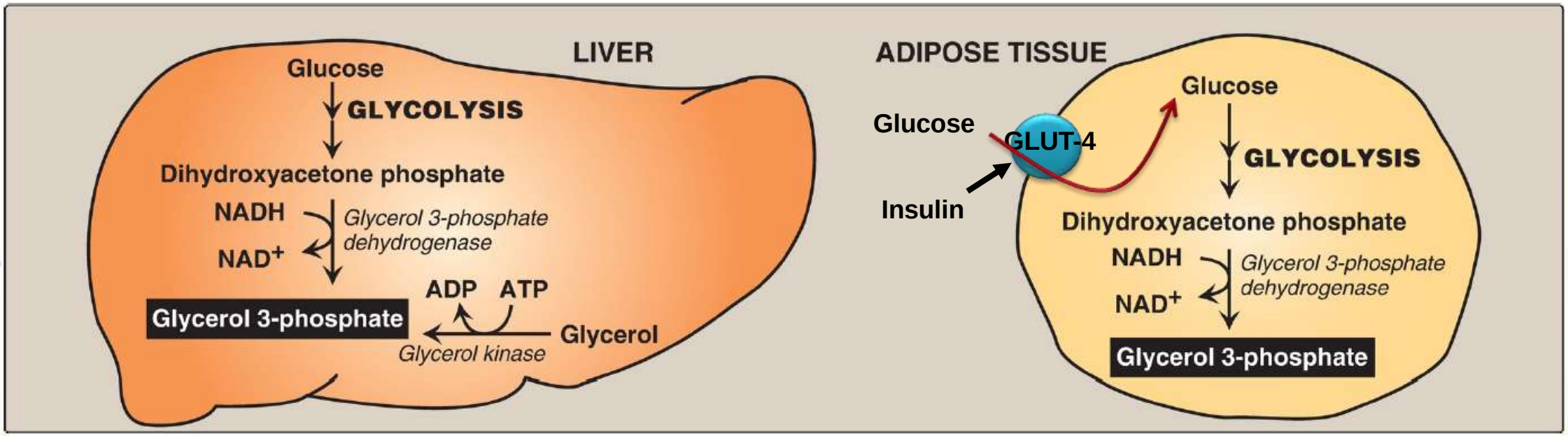
- **Additional information:**
- In case of fasting, glucagon and epinephrine work to produce energy by activating HSL …

- In well-fed state, insulin binds to its receptor and inhibits synthesis of ATGL and inhibits the activity of HSL by activation of phosphatase, which release the phosphate groups, also insulin activate the entry of fatty acids to cells to make TAG.

- look at the figure above, Perilipin (in red), it is a structure that coats fat droplets in adipocytes, which blocks the activity of HSL, so perilipin is phosphorylated by PKA to release fats.

- Now, we have 3 free fatty acids which will bind to albumin and go to peripheral tissues and the glycerol will go with blood to the liver and then the liver will build TAG again and put it in VLDL and send it to the peripheral tissues.

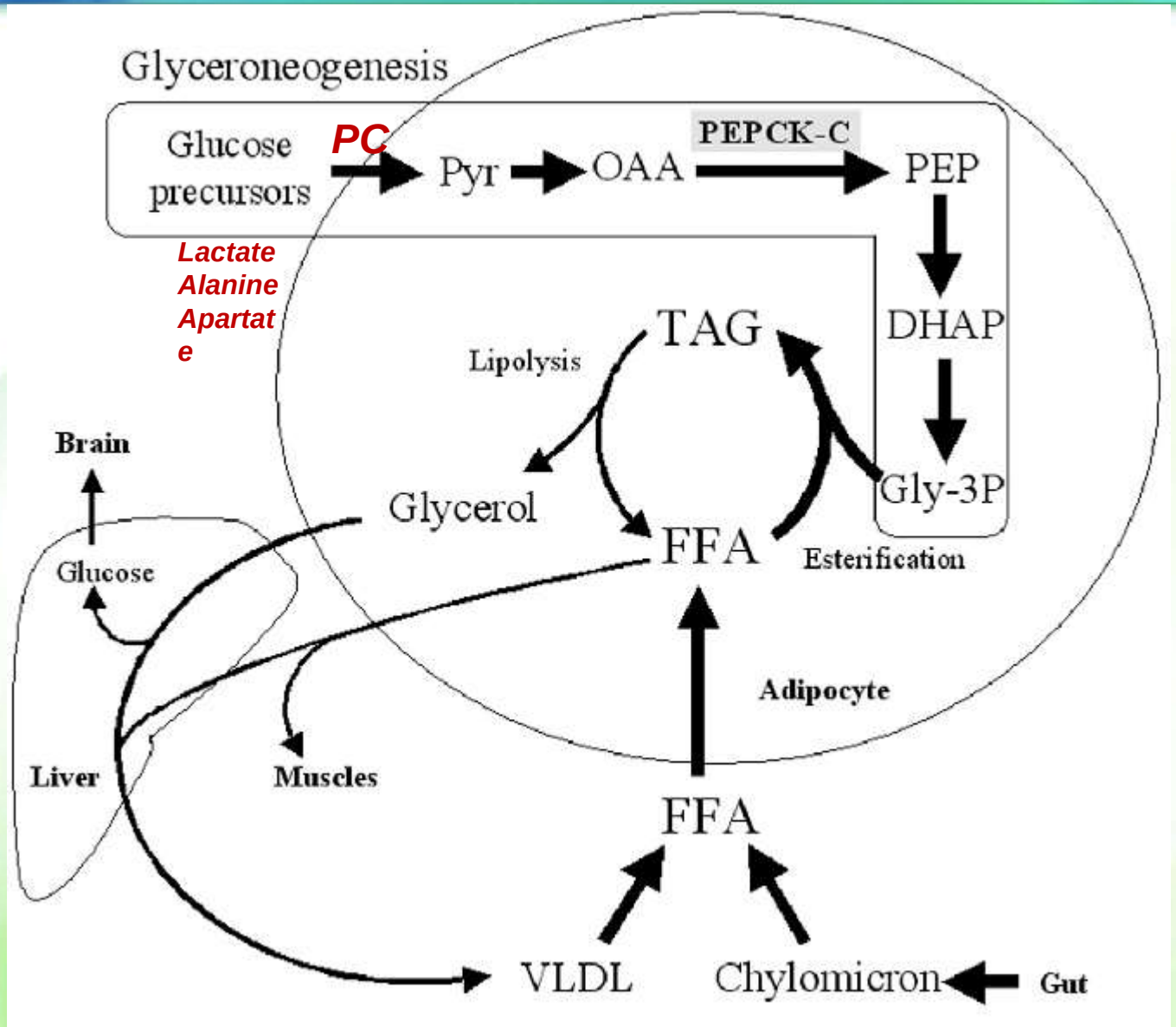
Formation of glycerol in liver and adipose tissues



Glyceroneogenesis

Notes:

- **Purpose: regulating the levels of FAs in blood.**
- **Glycerol leaves the adipocytes into the liver.**
- **Failure in regulating glyceroneogenesis may lead to Type 2 diabetes.**



PC: Pyruvate carboxylase

PEPCK:* phosphoenolpyruvate *carboxykinase

Additional information:

Recent studies proved that we have synthesis of glycerol in adipocytes by a process called Glyceroneogenesis.

when we release fatty acids from TAG at adipocytes, the glycerol goes to the liver and the fatty acids go with albumin, but 50% of fatty acids will re-esterificate (resynthesizing TAG by binding fatty acids with glycerol by ester bonds), but the question here is why to do this process?

the body regulates the amount of free fatty acids in the blood, so without this process the amount of free fatty acids in the blood will be high and this is not good for the body (it is a protective mechanism by the adipocytes).

But as we mentioned before, the glycerol left to the liver, so how we can make re-esterification (TAG synthesis), and we have starvation so we don't have glucose to make glycerol? what is the solution?

- The solution is (Glyceroneogenesis): formation of glycerol from pyruvate in adipocytes.
- Cells take lactate and amino acids from Krebs cycle and convert them into pyruvate >> pyruvate is converted to OAA (oxaloacetate) >> OAA is converted to phosphoenolpyruvate PEP >> PEP is converted to glyceraldehyde-3-phosphate >> glyceraldehyde-3-phosphate is converted to dihydroxy acetone phosphate >> dihydroxy acetone phosphate is converted into Glycerol-3-phosphate which is used as a back bone for fatty acids, (gluconeogenesis).
- Simply the cells take the glycerol from the "gluconeogenesis" pathway, and this what we call Glycroneogenesis.

The enzyme phosphoenolpyruvate carboxykinase PEPCK-C is responsible of converting OAA into PEP, and it is regulated by:

- Activated by monounsaturated and polyunsaturated fatty acids.
- It gets inhibited by high carbohydrate diet or glucose.

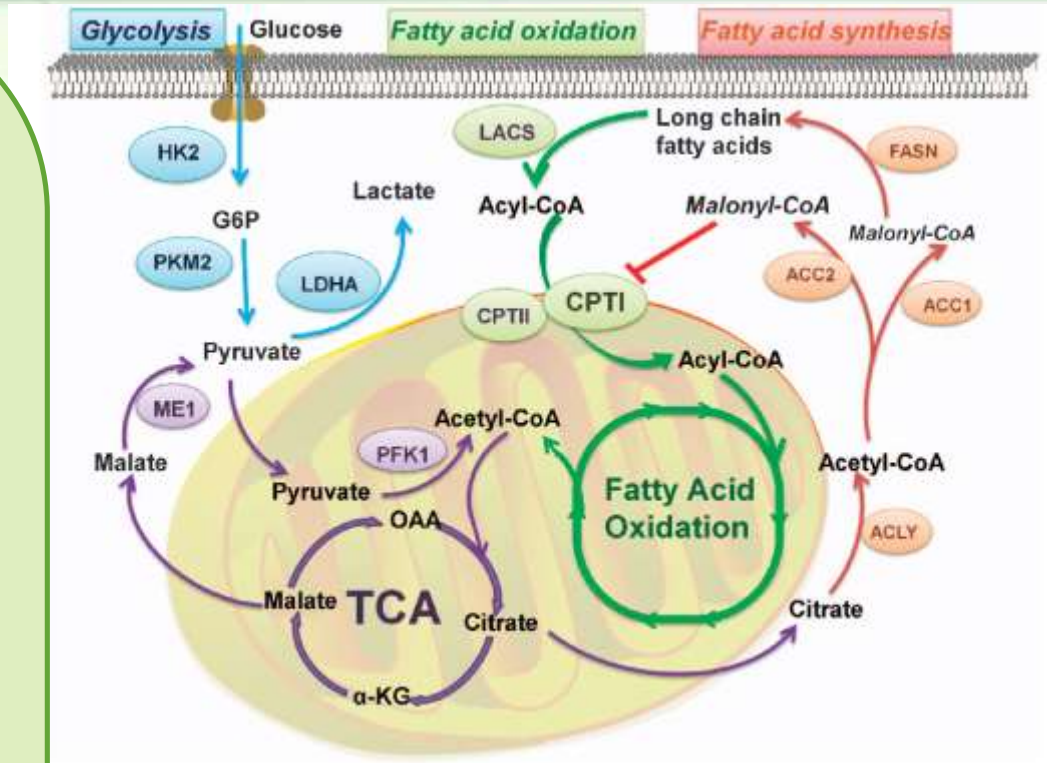
Fatty acid β-oxidation

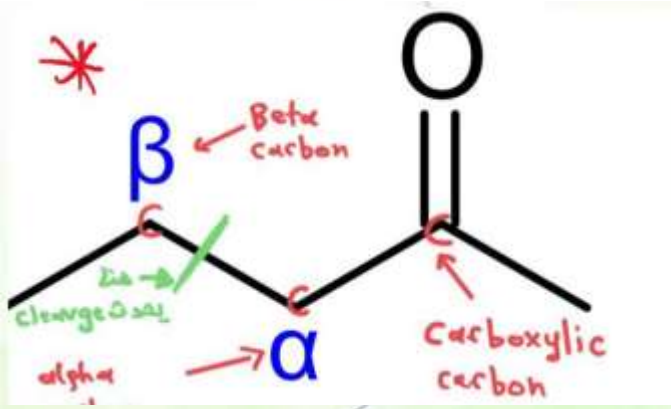
NOTE: Means breaking down of fatty acids ,we call it beta oxidation because it happens on the beta carbon
Look at the picture below: ✳

Previously, we talked about degradation of TAG which is the process for cleavage and separation of fatty acids from glycerol in a TAG molecule... Now we get these fatty acids and we want to break them down

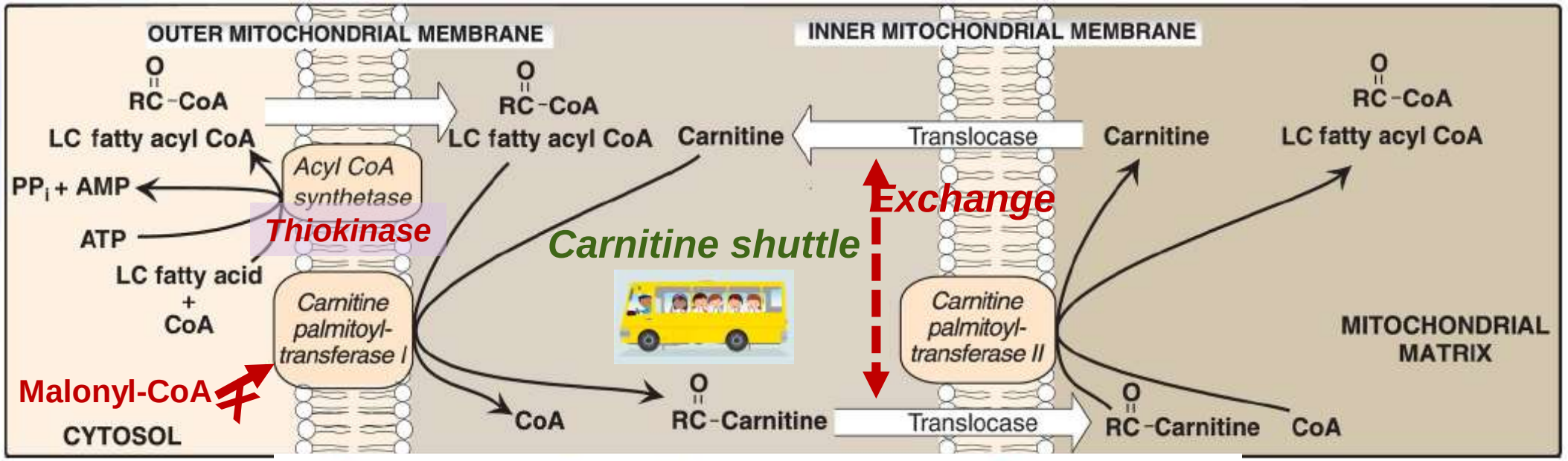
The idea here is when you have beta oxidation, you have the release of 2 carbons in a form of Acetyl CoA and this takes place in the mitochondria (mitochondrial matrix), fatty acid synthesis takes place in the cytosol (there is a separation of these two opposite pathways).

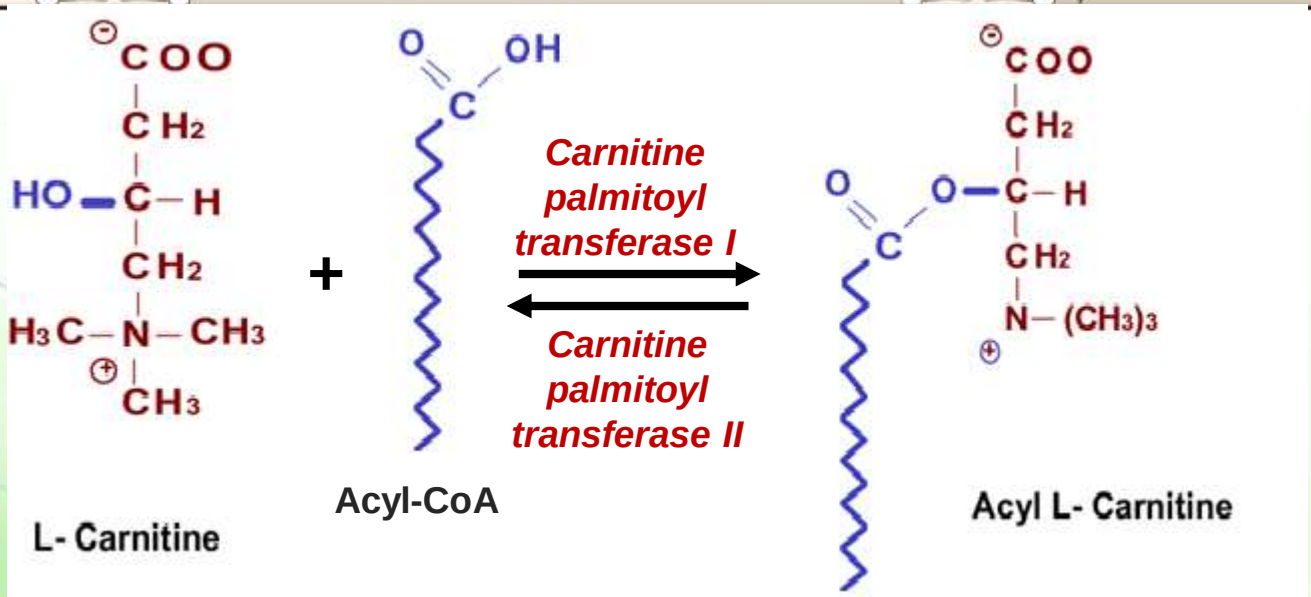
So we have the production of Acetyl CoA from the breaking down of fatty acids, and this Acetyl CoA goes to the Kreb's cycle (you know the pathway) .





LCFA is mitochondrial





The complement in this slide: Now, we reach a stage that we have free fatty acids (palmitic acid) in the cytosol, so we need to transport them to the mitochondria (to the matrix of mitochondria) in which the beta oxidation (B-oxidation) takes place.

Firstly, we need to activate the fatty acids which means we need to make the fatty acids high energy bond molecules, by binding it to CoA so it becomes fatty acyl – CoA, through an enzyme called acyl- CoA synthetase (or thiokinase) which is located on the outer membrane of the mitochondria, and this molecule (fatty acyl – CoA) enters to the intermembranous space.

This process needs energy and we take this energy from hydrolyzing ATP to AMP and you will have the release of pyrophosphate and hydrolyze it so delta G becomes low

* Note: by converting ATP to AMP, it is like hydrolyzing 2 ATP molecules.

The complement in this slide: But as we know, the inner membrane has a high impermeability, so we can not proceed to the matrix.

because of that, the fatty acyl – CoA binds to carnitine (a structure you can imagine it as a car) by an enzyme located on the outer membrane called CPT 1 (carnitine palmitoyl transferase 1), this enzyme cleaves the CoA and replace it with carnitine, now we have shuttle, the fatty acyl-carnitine enters to the matrix through translocase.

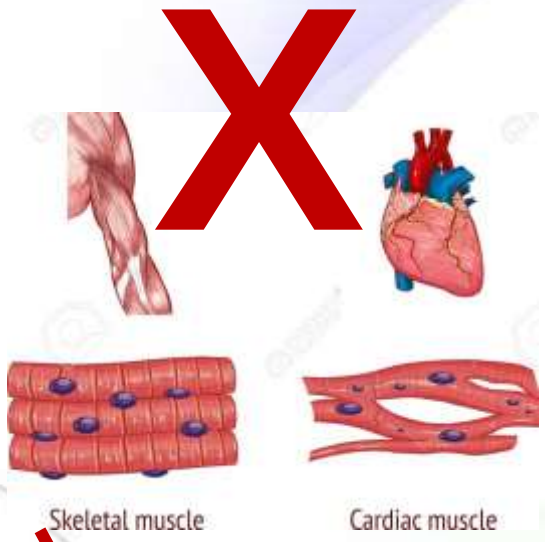
in the matrix, we will have exchange process, the carnitine is replaced with another CoA by an enzyme called CPT 2 (carnitine palmitoyl transferase 2) on the inner membrane of the mitochondria and the resulting free carnitine will return to the intermembranous space but on one condition which is entry of another fatty acyl – carnitine and we call that carnitine shuttle system.

* Note: here we are talking about long chain fatty acids, small and medium chain fatty acids have easy entry to the matrix without these processes.

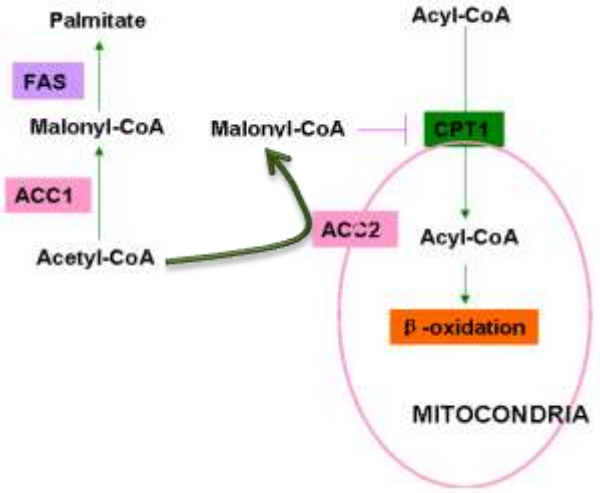
CPT 1 is inhibited by malonyl – CoA.

The malonyl – CoA (which is an intermediate for the first reaction of fatty acid synthesis) is an inhibitor for the degradation of fatty acids, that makes a sense!!! Because you don't want to get fatty acids into mitochondria if you have enough malonyl CoA (the rate limiting step in fatty acid synthesis), it means there is enough energy in the cells and there is no need to break down more fatty acids so, it is illogical to make synthesis and degradation for the lipids at the same time.

More on carnitine…sources



contains ~97% of all carnitine in the body. No ACC1, no FA synthesis but contains a mitochondrial ACC2.



L- methionine L-lysine

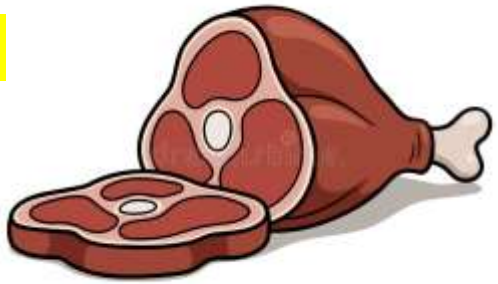
S-adenosyl-methionine

Protein-lysine

Protein-6-N-trimethyllysine —Lysosomal hydrolysis→ 6-N-trimethyllysine (TML)

O_2 + 2-oxoglutarate; TMLD, Fe^{2+}; CO_2 + Succinate

Do not memorize the pathway



NAD+; HTMLA, PLP; NADH; Glycine

4-trimethilaminobutyroaldehyde (TMABA)

NAD+; TMABA DH; NADH+ H+

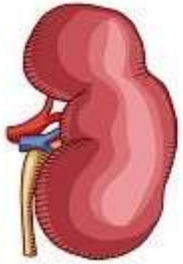
Butyrobetaine (BB)

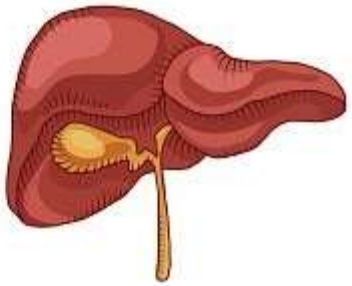
BBD, Fe^{2+}; O_2

L-carnitine

Succinate + CO_2

2-oxoglutarate





- **The complement in this slide:** We have two sources for the carnitine:

- Synthesis: the Methionine and the Lysine are the precursors of carnitine.
The synthesis of carnitine occurs in the liver and the kidney, and b – oxidation occurs mainly in the muscles because it is the most tissues need energy, so the carnitine leaves the liver and the kidney to the cardiac and skeletal muscles because these muscles don't synthesize carnitine.

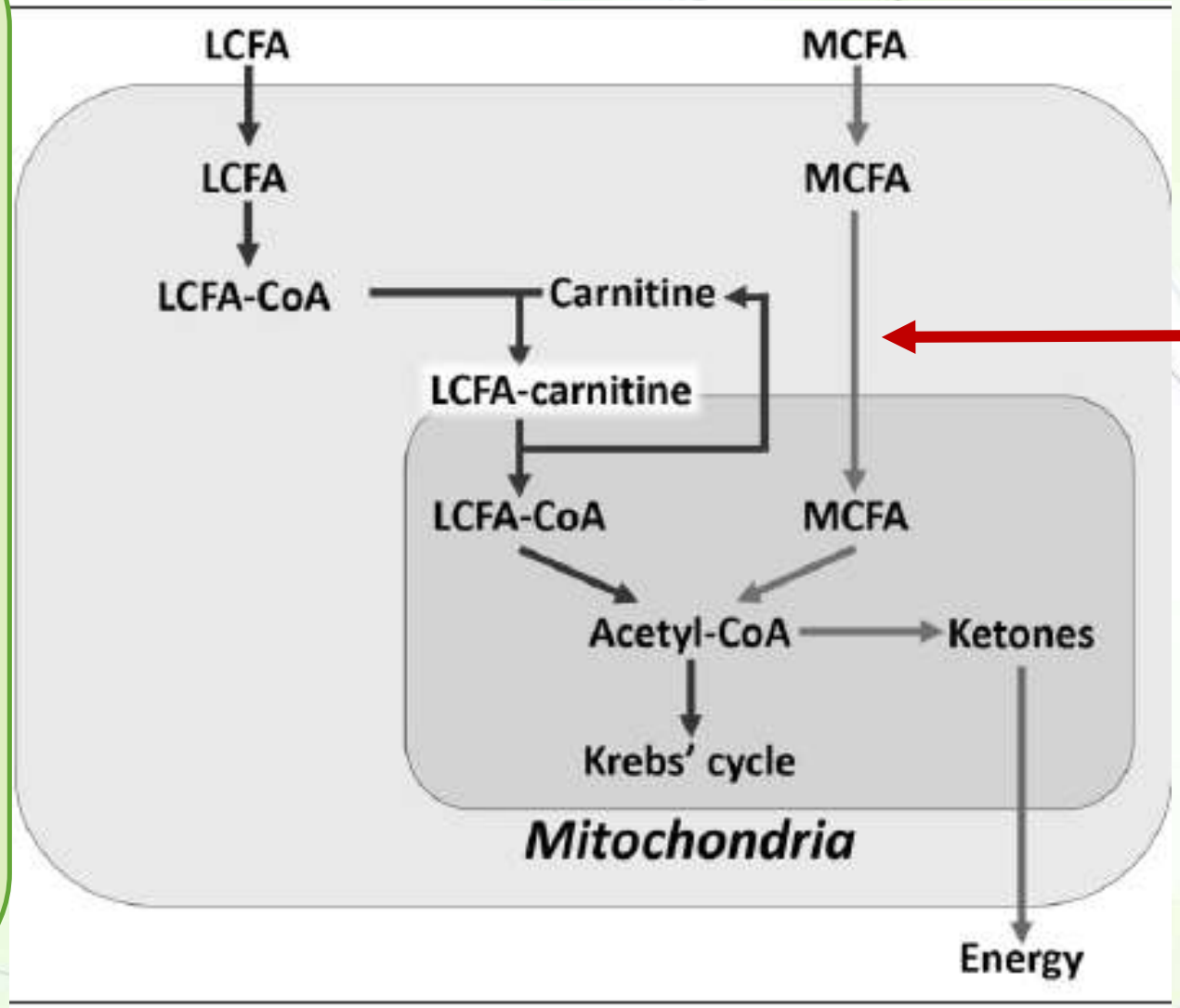
- Meat: 97% of carnitine is present in the cardiac and skeletal muscles, these muscles depend on the fatty acids as a source of energy.

Carnitine deficiencies

- Primary carnitine deficiency
  - Defects in a membrane transporter: No uptake of carnitine by cardiac and skeletal muscles and the kidneys, causing carnitine to be excreted.
    - Treatment: carnitine supplementation.
- Secondary carnitine deficiency
  - Taking valproic acid (antiseizure) → decreased renal reabsorption
  - Defective fatty acid oxidation → acyl-carnitines accumulate → urine
  - Liver diseases → decreased carnitine synthesis
  - CPT-I deficiency: affects the liver; no use of LCFA, no energy for glucose synthesis during fasting → severe hypoglycemia, coma, and death
  - CPT-II deficiency: affects the liver, cardiac muscle, and skeletal muscle
    - Treatment: avoidance of fasting and adopting a diet high in carbohydrates and low in fat but supplemented with medium-chain TAG.

SCFAs and MCFAs

NOTE: And in case of carnitine deficiencies , the largest dependent becomes on small and medium chain fatty acids (SCFAs , MCFAs) because their entry to the matrix does not depend on CPT 1,2 so SCFA and MCFA enter to the matrix easily and will be oxidized , unlike long chain fatty acids (LCFA) .

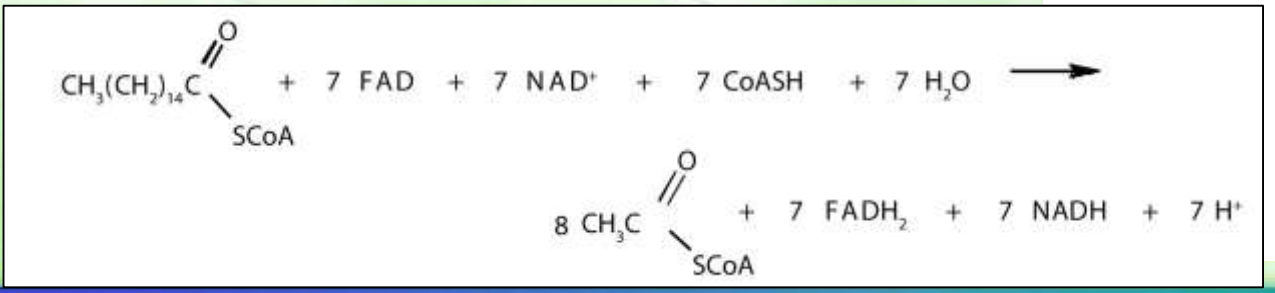


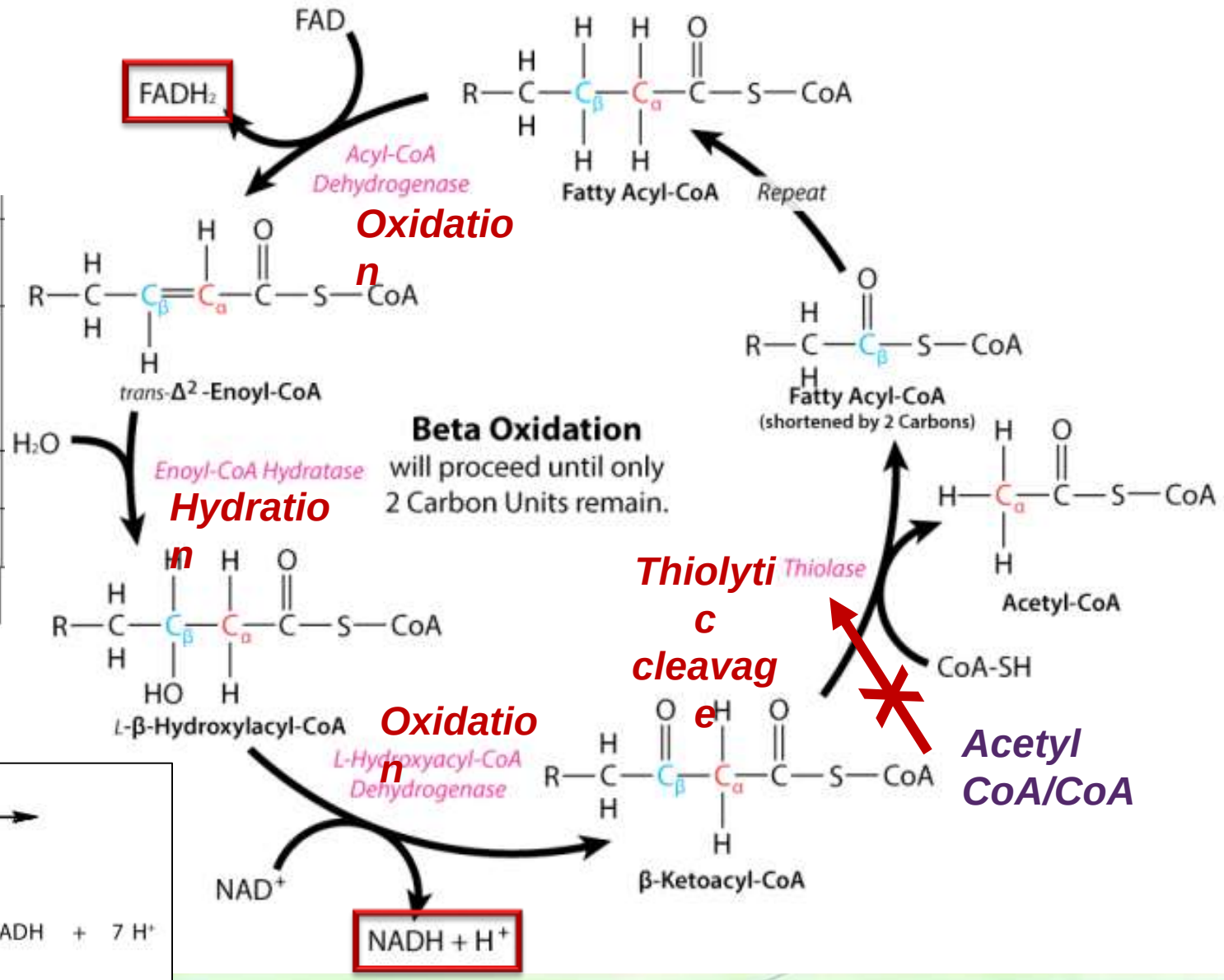
Note: No regulation of entry like that of CPTI by malonyl CoA

β-Oxidation of fatty acids

- **Number of cycles: (n/2)-1**

Mechanism	ATP Yield
1. β-oxidation 7 cycles 7 $FADH_2$ and 7 NADH are generated when oxidized by ETC	7$FADH_2$- 7 x 1.5 = 10.5 7 NADH- 7 x 2.5 = 17.5
2. From 8 acetyl CoA 1 acetyl CoA =3 NADH + 1 $FADH_2$ + 1 GTP Oxidized by citric acid cycle, each acetyl CoA provides ATP	10 x 8 = 80
Total Energy from one mole of palmitoyl CoA	**108**
Energy utilized for activation (Formation of Palmitoyl CoA)	2
Net yield of oxidation of one mole of palmitate	108-2 =**106**



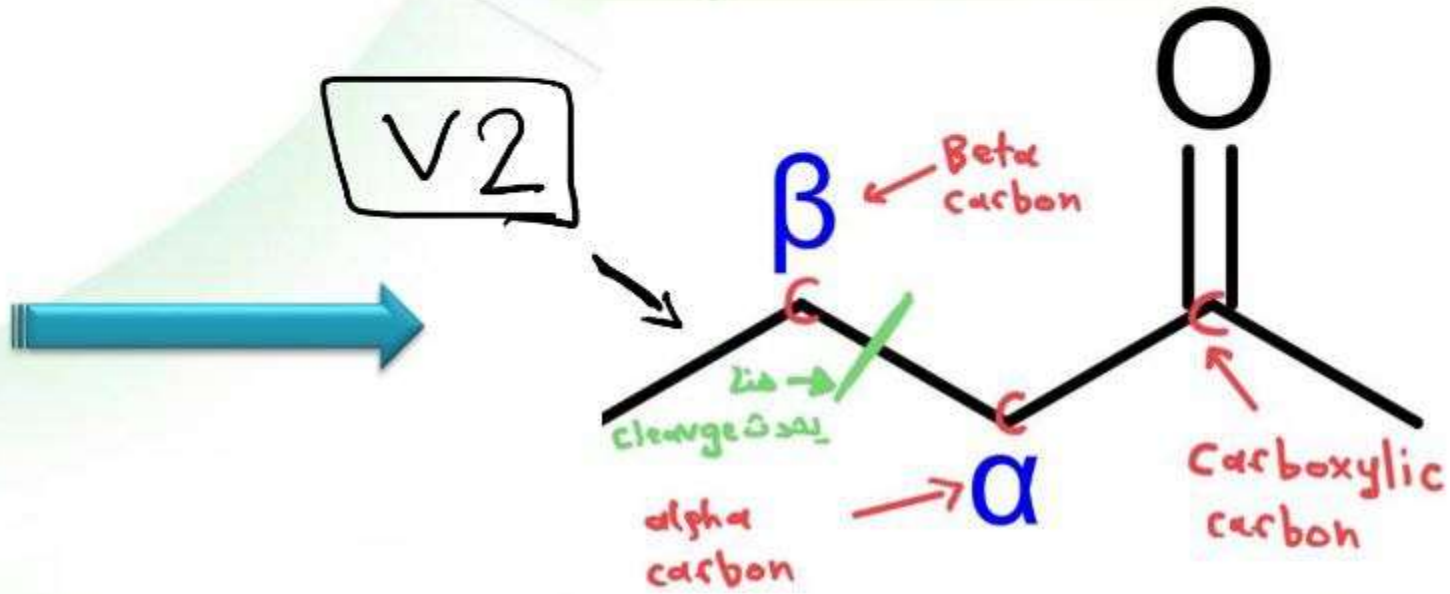


- **The complement in this slide:** We start with FA molecule
  - The first reaction is oxidation reaction by dehydrogenase(acyl CoA dehydrogenase), you have the production of FADH2 (the result is a compound with double bond) and the FADH2 enters the ETC in mitochondrial matrix ,so here we used electron carrier (FAD > $FADH_2$)
  - Hydration reaction (now you will get a compound with a hydroxy/ group that is attached to fatty acid)
  - Another oxidation reaction by another dehydrogenase, you have the production of NADH. (Hydroxyl to keto group),and here we used another electron carrier (NAD^+ > NADH) so the FA synthesis: reduction-dehydration-reduction (the reverse reaction)
  - Thiolytic cleavage (CoA attacks the fatty acid by using its terminal reactive group "Thio/ group SH-"(Breaking the bond between alpha and beta carbons) and it results in the releasing of Acetyl CoA (2carbons).

- **The complement in this slide:** So how many ATP molecule can we produce from the FA?
- We should know how many acetyl CoA molecule produced ,those molecules will go to Krebs cycle
- Every cycle we have 3 NADH + 1 FADH2 +1 ATP (from GTP) + the products from FA oxidation =1NADH+1FADH2 SO EVERY CYCLE WE WILL GET
- 4 NADH + 2 FADH2 +1 ATP (from GTP)
- THE TOTAL ATP= 14-2=12 ATP MOLECULES FOR EACH CYCLE
- These 2 ATP MOLECULES Because we activate the FA(by binding it to CoA) IT requires hydrolysis of ATP to AMP(it seems like the hydrolysis two times of ATP to ADP)
- REMEMBER THAT:
- 1FADH2= 1.5 ATP
- 1 NADH = 2.5 ATP

اللهم انصر أهل غزة وثبت أقدامهم
اللهم احرس أهل غزة بعينك التي لا تنام
اللهم كن لاهل غزة عونا ونصيرا
اللهم انا لانملك لفلسطين الا الدعاء
فيارب لا ترد لنا دعاء ولا تخيب لنا رجاء
وأنت أرحم الراحمين

V1: (alpha C IS CARBOXYL GROUP).



Slide 23

V3 : Slide 19

VLDL instead of chylomicron