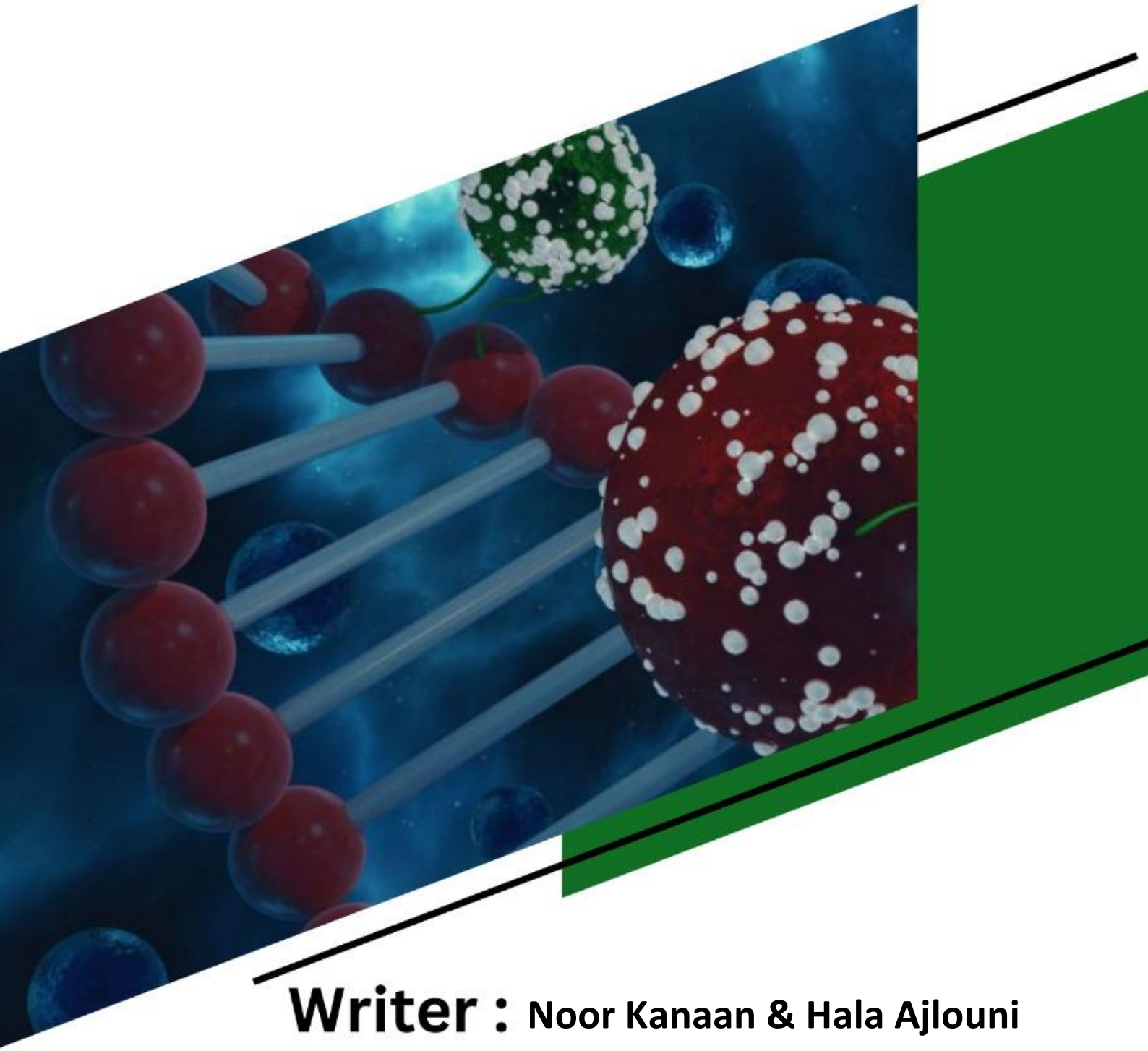
Doctor 021

METABOLISM

Sheet no. 21



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SYNTHESIS OF FATTY ACIDS:

In the first lecture we talked about FA in general and now we'll be talking about FA synthesis. We should know the important reactions and enzymes and their regulation.

Major sites of fatty acids synthesis are liver and adipose tissues (not only for FA synthesis but also for triacylglycerols storage).

Transport of fatty acids:

1. From the liver: packaging of fatty acids and triacylglycerols inside lipoproteins (VLDL: very low density lipoprotein) to be transported outside the liver.

2. From adipocytes: albumin is the carrier of fatty acids from adipocytes.

they are synthesized by:

(this is an overview of fatty acid synthesis, so you can read it again when you finish the lecture)

1. Production of malonyl acid CoA (3C): from acetyl CoA (2C) so one carbon is added to acetyl CoA in the form of CO_2 (bicarbonate form HCO_3).

2. Binding of acetyl CoA and malonyl CoA to the fatty acid synthase (a huge enzyme that has 6 enzymatic activities to make the reaction faster)

3. Condensation of acetyl CoA and malonyl CoA: FAS makes a condensation reaction in which malonyl CoA (3C) and acetyl CoA (2C) come together and produce a 4 carbon product (acyl CoA) + CO_2.

4. Elongation of acyl CoA by 2 carbons per round (3 reactions)

(reduction, dehydration, reduction) **will be explained later**

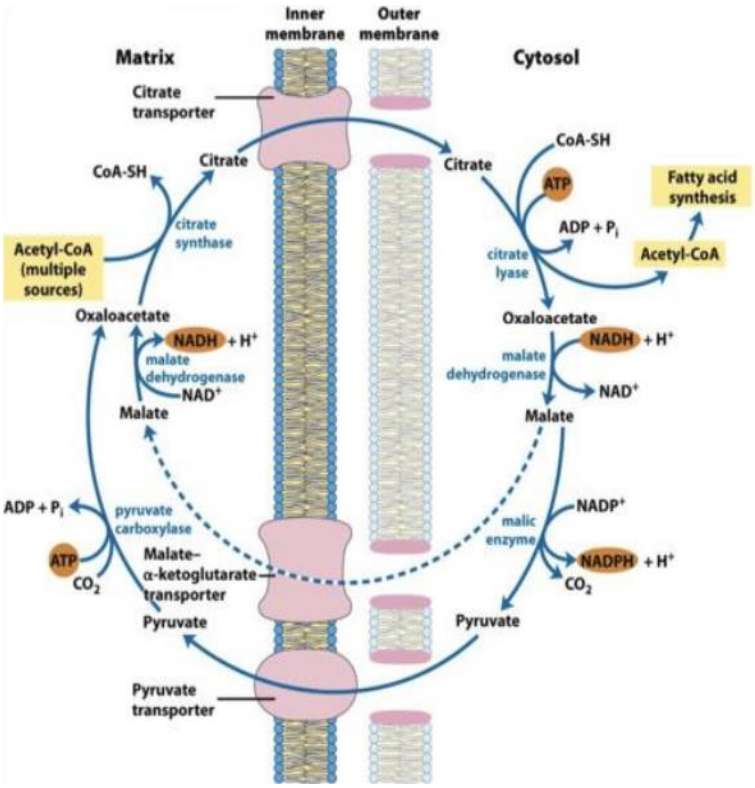
5. Binding of another malonyl CoA to FAS.

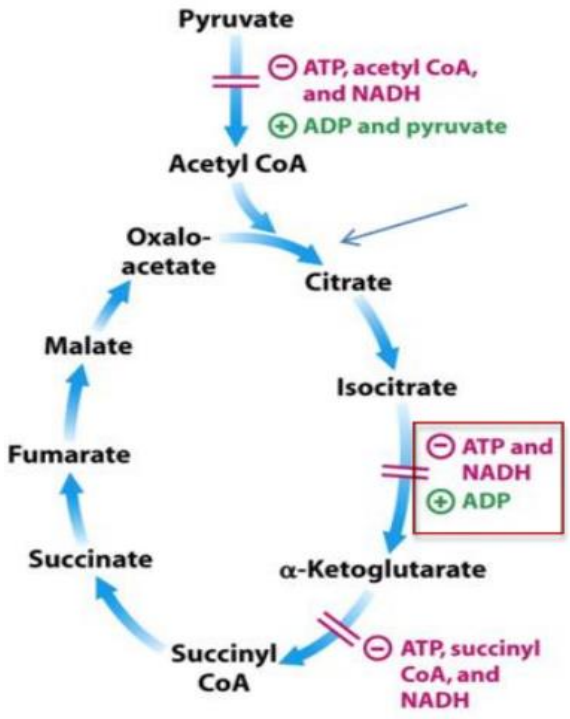
6.Repeat 3 (acyl CoA), 4, 5: after step 2 we have a 4 carbon molecule, repeat (3,4,5) each round there is addition of 2 carbons so the product is elongating (4 carbons, 6 carbons .. 16 carbons) this 16 carbon molecule is the palmitoyl CoA and it is still bound to the enzyme.

7. Release of the hydrocarbon chain by thioesterase (TE): the product which is released from the FAS is the palmitoyl (16C). Then the cell can do elongation and desaturation (introduction of double bonds) reactions to produce different fatty acids. (ex. Arachidonic acid, EPA, DHA).

MITOCHONDRIA TO CYTOPLASM TRANSPORT OF ACETYL-COA:

When ATP increases > ATP inhibits isocitrate dehydrogenase > citrate is transported into the cytosol > citrate is cleaved into oxaloacetate and acetyl CoA by ATP citrate lyase.





Glucose can be converted into fat (glucose > pyruvate > OAA > citrate > acetyl CoA > FA) **BUT** fat can't be converted to glucose; because when it degrades it gets converted to acetyl CoA that can never be converted to sugar.

SYNTHESIS OF MALONYL-COA: CARBOXYLATION RXN:

Acetyl CoA carboxylase (ACC) transfers a carbon from CO2 (as a bicarbonate) via biotin (vitamin B7) that is the cofactor of the carboxylation reaction, which is covalently bound to a lysyl residue of the ACC.

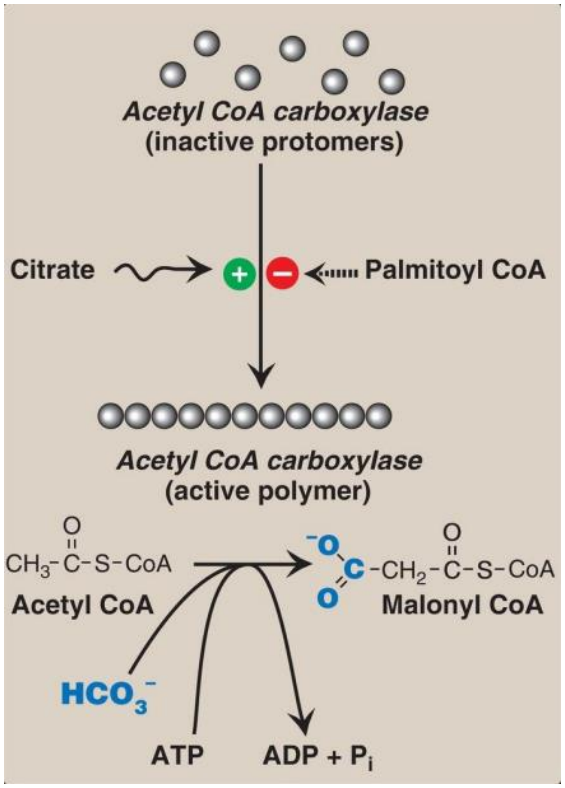
- ATP is needed.
- The reaction is a rate-limiting reaction.
- ACC is an allosteric enzyme.

Carboxylation reaction requires:

CO2, ATP (for condensation) , biotin as a cofactor (that binds to the active site of ACC to be activated).

Acetyl CoA carboxylase consists of different inactive subunits, so the get polymerized to form the enzyme complex (the active form). This reaction is the rate-limiting reaction (slow) because:

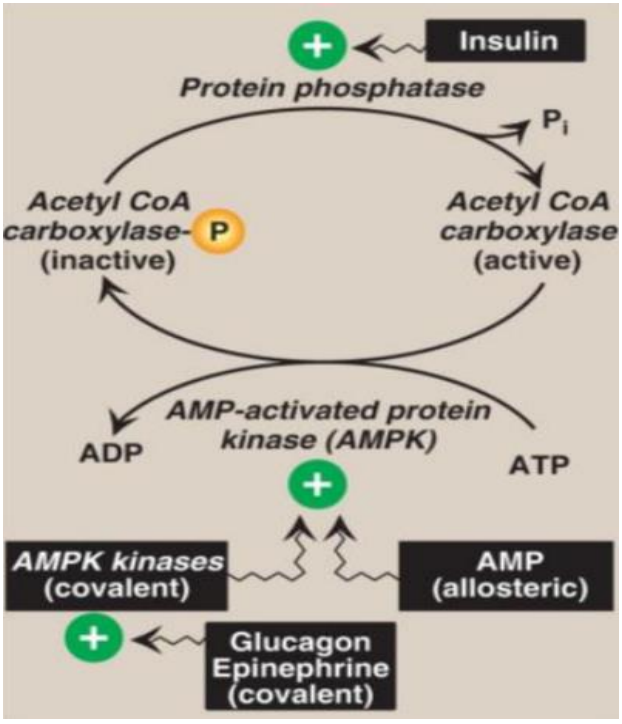
- It requires energy.
- It is highly regulated.



REGULATION OF ACC:

This reaction is regulated by citrate and palmitoyl CoA; citrate activates this reaction (when there is a high level of citrate this means that there is enough energy sources so this activates the fatty acid synthesis), palmitoyl CoA inhibits this reaction (enough fatty acids > STOP fatty acid synthesis).

ACC is also inactivated by phosphorylation by AMPK, which is activated by glucagon and epinephrine. High levels of glucagon means that there is starvation so there is no need for FA synthesis.



Revision of glucagon pathway:

glucagon activates the receptor which activates the synthesis of cAMP that activates protein kinase A which phosphorylates AMPK making it active and it phosphorylates ACC making it inactive.

High levels of glucagon > activate AMPK > inhibit ACC > inactivate FA synthesis

Insulin does the opposite; it activates the phosphatase (dephosphorylation) that inactivates the AMPK activating FA synthesis.

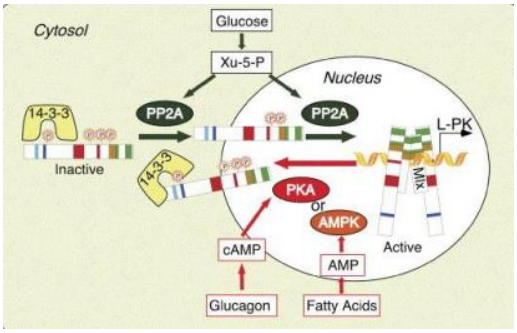
High levels of insulin > activate phosphatase > inactivate AMPK > activate FA synthesis

Regulation at the gene level:

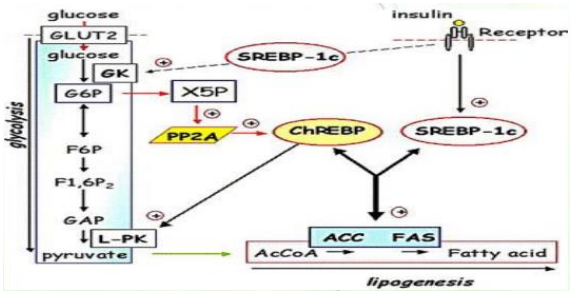
ACC synthesis is regulated by transcription factors:

***Transcription factors regulate more than one gene**

➢ The carbohydrate response element-binding protein (ChREBP)



- ChREBP is inactivated by phosphorylation by PKA and AMPK preventing its nuclear localization. In starvation, glucagon activates PKA and AMPK which phosphorylate ChREBP inactivating it and preventing its entry to the nucleus so there is no FA synthesis.



- it is dephosphorylated by phosphatase that is activated by insulin, so it enters the nucleus. It is dephosphorylated by excess glucose.

➢ The sterol regulatory element-binding protein-1c (SREBP-1c)

*Regulates the synthesis of cholesterol (steroids)

SREBP-1 is activated by insulin.

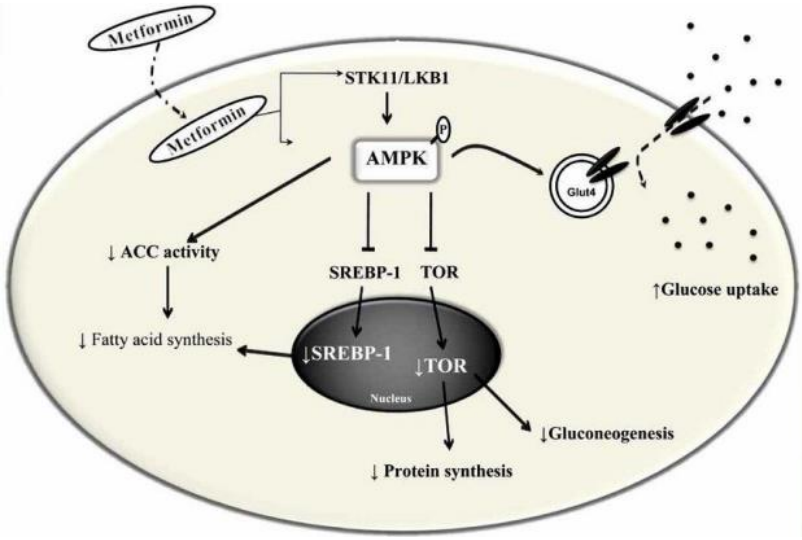
The same transcription factor regulates more than one gene. If you remember operons in bacteria, when bacteria synthesize all enzymes

responsible for the breakdown metabolism of lactose and tryptophan and others. These operons exist in eukaryotic cells as promoter proximal elements (PPE). Genes responsible for a specific pathway or metabolism are regulated by the same PPE (the same transcription factor binds to the PPE regions of all genes at the same time. (SREBP-1c) is activated by insulin > synthesis of FA and cholesterol and all cell demands for building and growth.

Fatty acid synthase, glucokinase, ATP citrate lyase and liver pyruvate kinase are similarly regulated (regulated by the same transcription factor).

Metformin (Glucophage) lowers plasma TAG through:

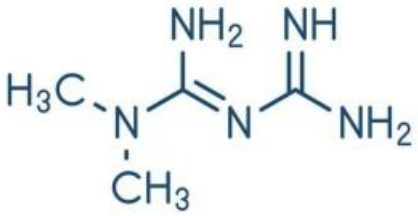
Activation of AMPK (inhibiting AMPK inhibitor) resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and fatty acid synthase expression (by decreasing ChREBP and SREBP-1c).



Given when there is susceptibility for diabetes; to lower the blood glucose level to prevent the resistance to insulin.

It lowers blood glucose by increasing AMPK-mediated glucose uptake by muscle.

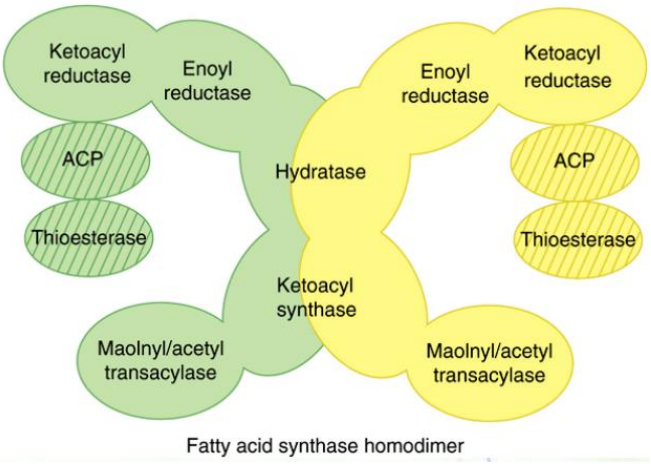
**The doctor mentioned statins as an example of beneficial drugs in addition to metformin and aspirin.



Statins are a group of medicines that can help lower the level of low-density lipoprotein (LDL) cholesterol in the blood which decreases heart attacks.

FATTY ACID SYNTHASE (FAS):

A multifunctional, homodimeric enzyme. Each FAS monomer is multicatalytic with six enzymatic domains and a domain for binding a phosphopantetheine-containing acyl carrier protein (ACP) domain (7domains= 6 enzymatic+1 binding) **in each monomer**.

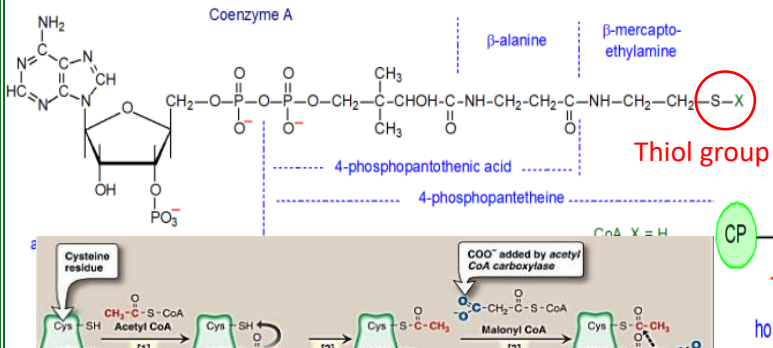


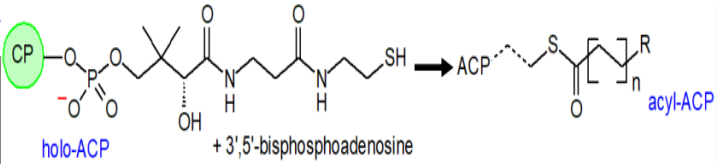
Fatty acid synthase homodimer

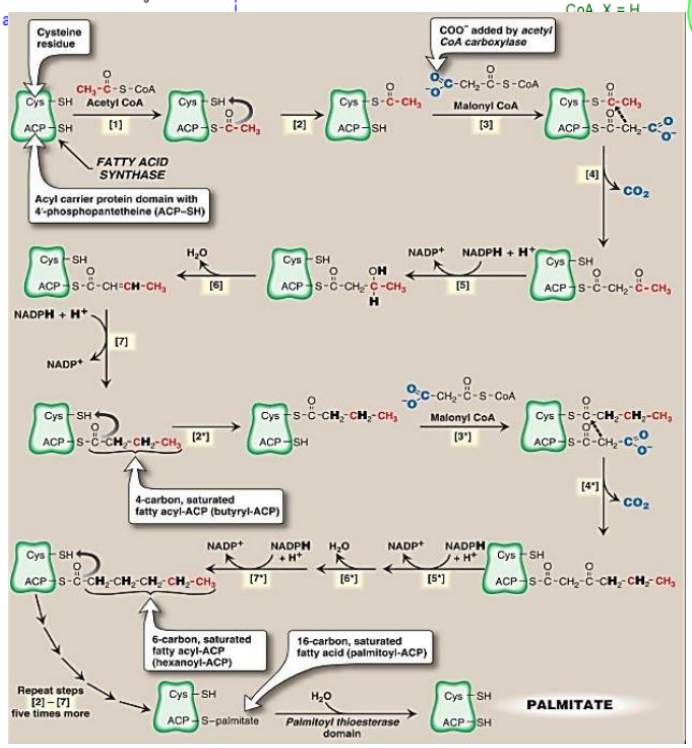
Binding domain for acyl molecule

*: It is a protein that binds to the enzyme and it binds to the fatty acyl molecule

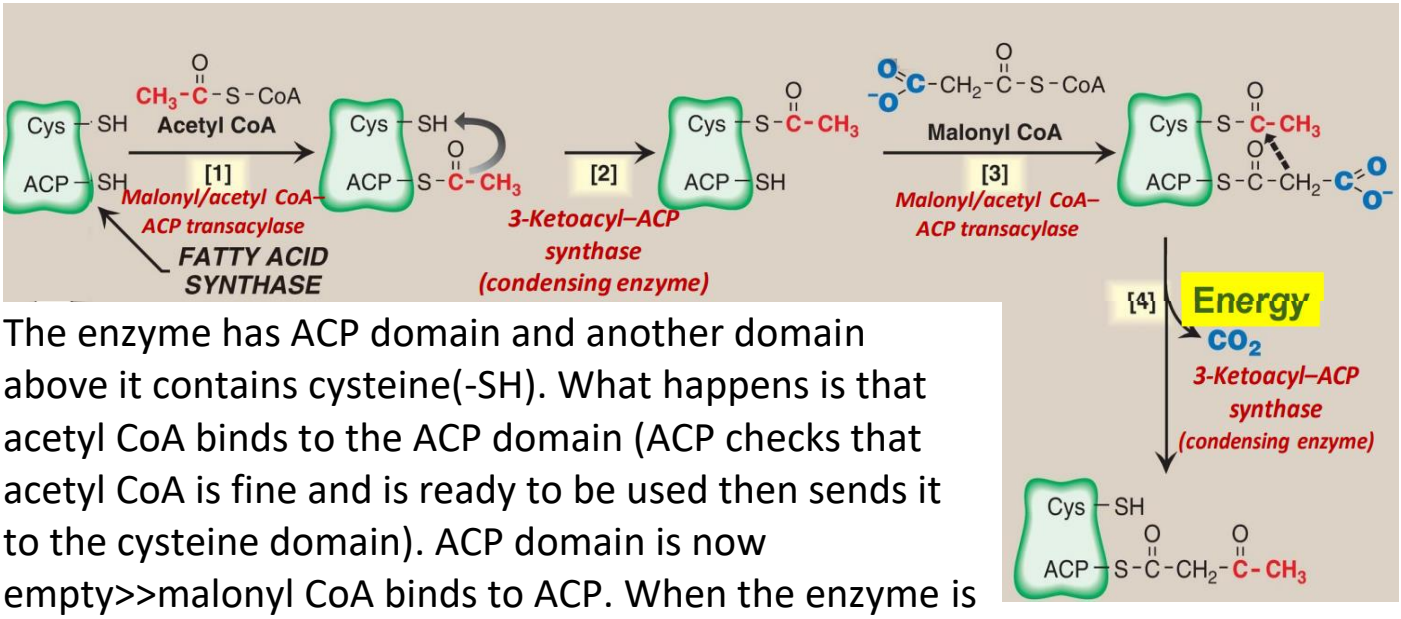
Phosphopantetheine, a derivative of pantothenic acid (vitamin B5), carries acyl units on its terminal thiol (—SH) group and presents them to the catalytic domains of FAS. The phosphate group of phosphopantetheine binds to the ACP.





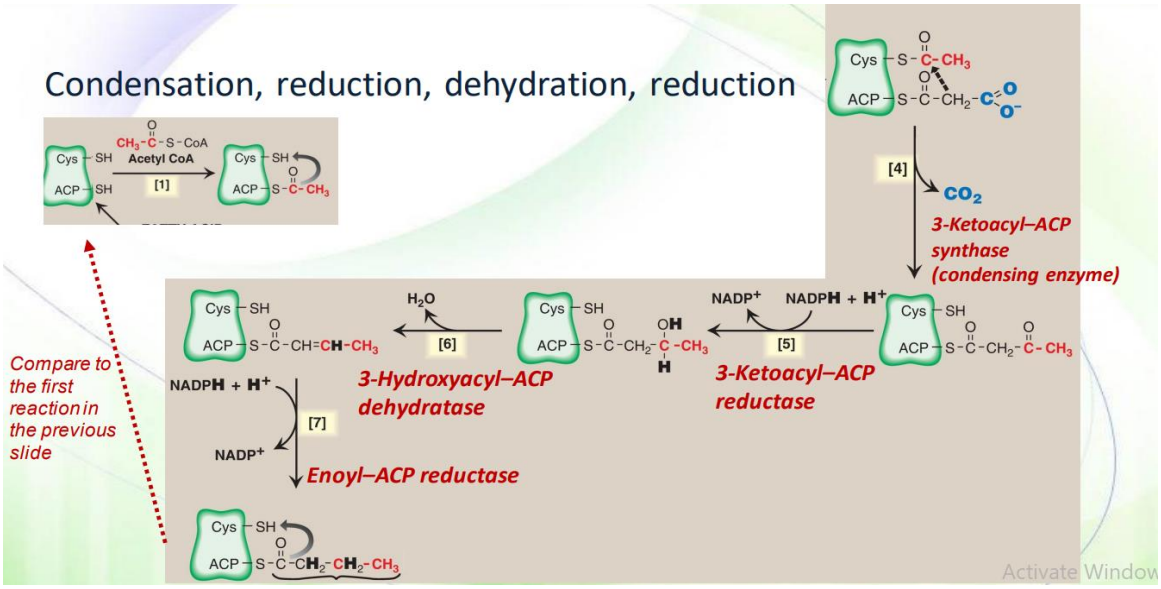


< This is the whole reaction



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 checks that acetyl CoA is fine and is ready to be used 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 dissociate 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.

*enzymes names are not required.



4 carbons are 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 double bond to OH >> dehydration(remove H2O)>>reduction(adding hydrogen atoms). This 4 carbon molecule is attached to the cysteine domain>>empty ACP domain>>binds another malonyl CoA>>repeat the previous steps.

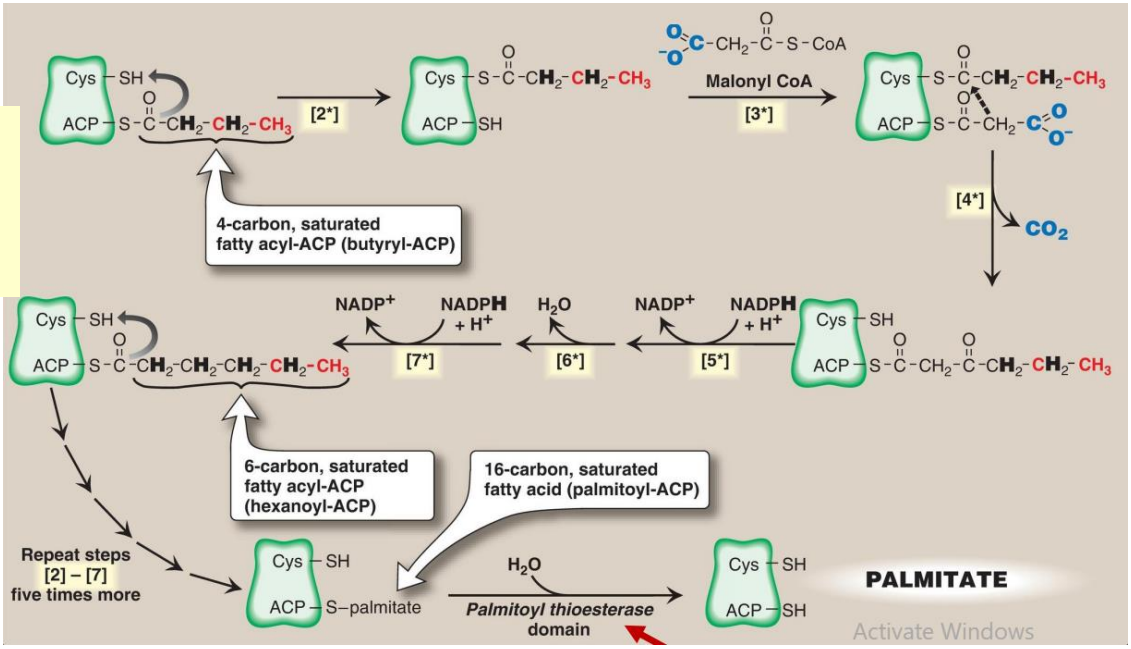
(each round 2 carbons are added until we reach 16 carbons (2 carbons from acetyl CoA and 14 from malonyl CoA producing palmitate). Each round we use malonyl CoA which is synthesized from acetyl CoA as we mentioned before.

REMEMBER:

*we use NADPH in anabolism reactions (synthesis).

*we use NADH in breakdown reactions (degradation).

The lactating mammary gland terminates lengthening the chain EARLY.



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; instead there will be production of stearate or butyrate (4C) etc.

*the doctor said stearate but it doesn't make sense because it has 18 carbons.

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

THE STOICHIOMETRY OF PALMITATE SYNTHESIS:

Stoichiometry of palmitate synthesis:

acetyl CoA + 7malonyl CoA + 14NADPH + 14H^+ → palmitate + 7CO2 + 14$NADP^+$ + 8CoA + 6H2O

malonyl CoA synthesis:

7acetyl CoA + 7CO_2 + 7ATP → 7Malonyl CoA + 7ADP + 7Pi + 7H^+

Overall stoichiometry of palmitate synthesis:

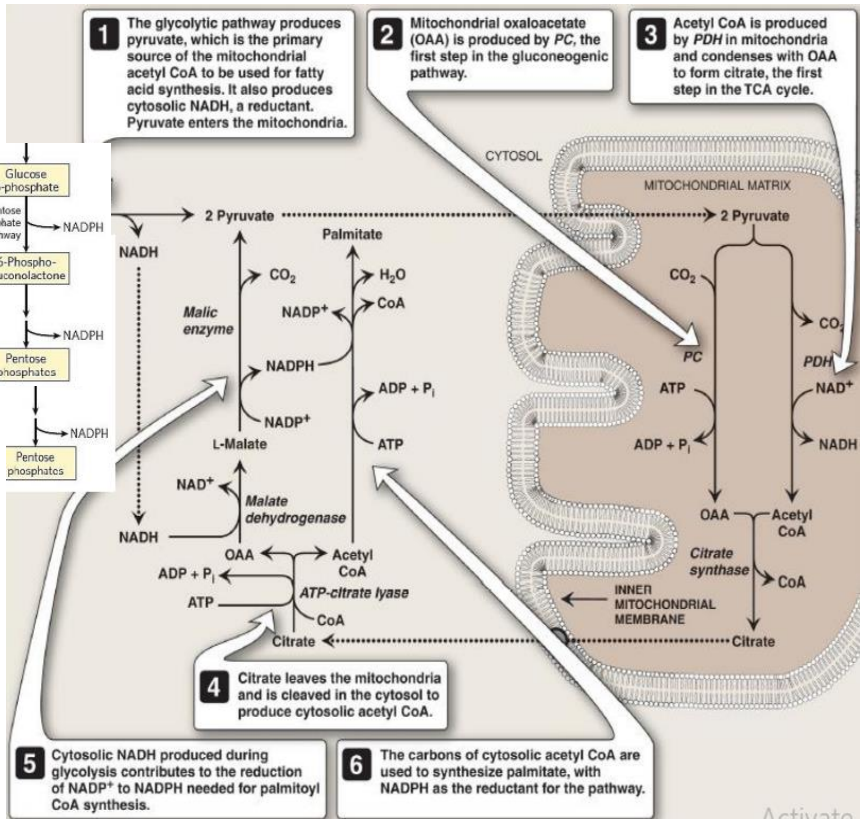
8Acetyl CoA + 14NADPH + 7ATP + 7H^+ → palmitate + 14$NADP^+$ + 8CoA + 6H2O + 7ADP + 7Pi

SOURCES OF MOLECULES:

1) Acetyl CoA: from **pyruvate** that enters the mitochondria and gets converted to citrate and then breaks down into OAA and acetyl CoA in the cytosol.

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

3) NADPH: from **pentose phosphate pathway (PPP)** or through the **conversion of malate to pyruvate**.



FURTHER ELONGATION:

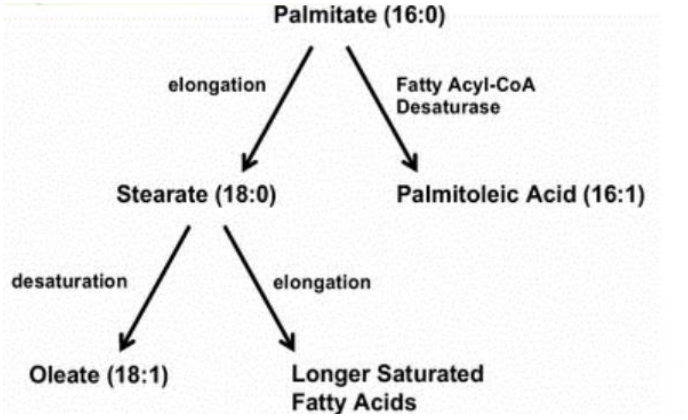
- 1st location: smooth endoplasmic reticulum.
- Different enzymes are needed (no need to know their names).
- Two-carbon donor: malonyl CoA.
- Source of electrons: NADPH.
- No ACP or multifunctional enzyme is need.

NOTE: the brain has additional enzymes allowing it to produce the very long chain fatty acid [VLCFA] over 22 carbons.

- 2nd location: mitochondria.
- Two-carbon donor: acetyl CoA.
- Source of electrons: NADPH and NADH.
- Substrates: fatty acids shorter than 16.

***That is all we need to know in slide 16 (reactions and enzymes are not required).**

CHAIN DESATURATION (INTRODUCING DOUBLE BONDS):



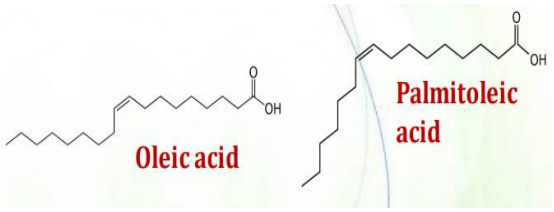
Enzymes: fatty acyl CoA desaturases

Substrates: long-chain fatty acids (e.g palmitate, stearate ..)

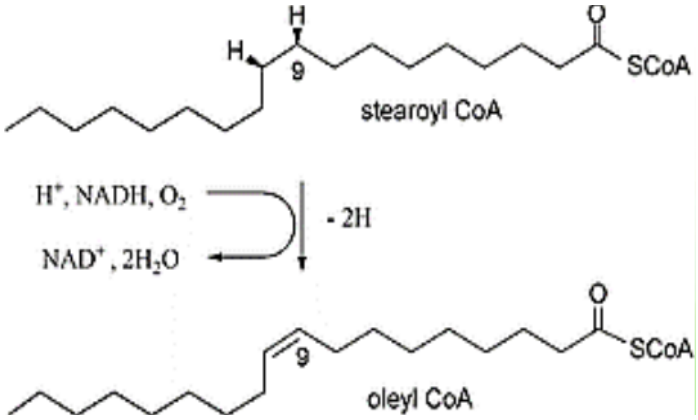
Location: smooth endoplasmic reticulum.

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).



VERY IMPORTANT!

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.

NOTE:

One of the strategies the cell regulates the metabolism > separation of the opposed reactions so they don't overlap.

- Fatty acid synthesis (in cytoplasm).

- Fatty acid degradation (in mitochondria).

TRIACYLGLYCEROL STRUCTURE AND SYNTHESIS:

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

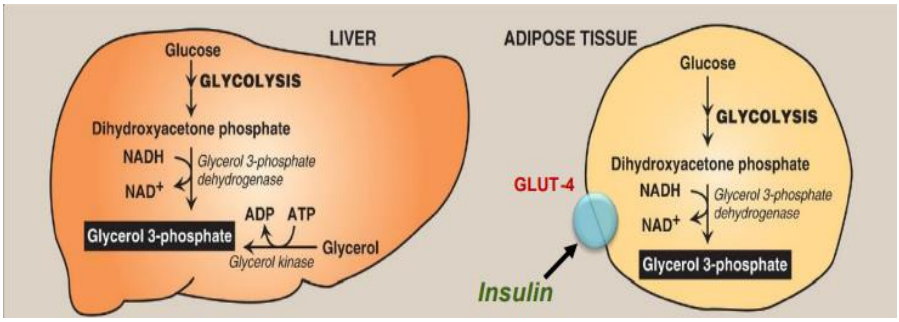
STEP1: (two mechanisms)

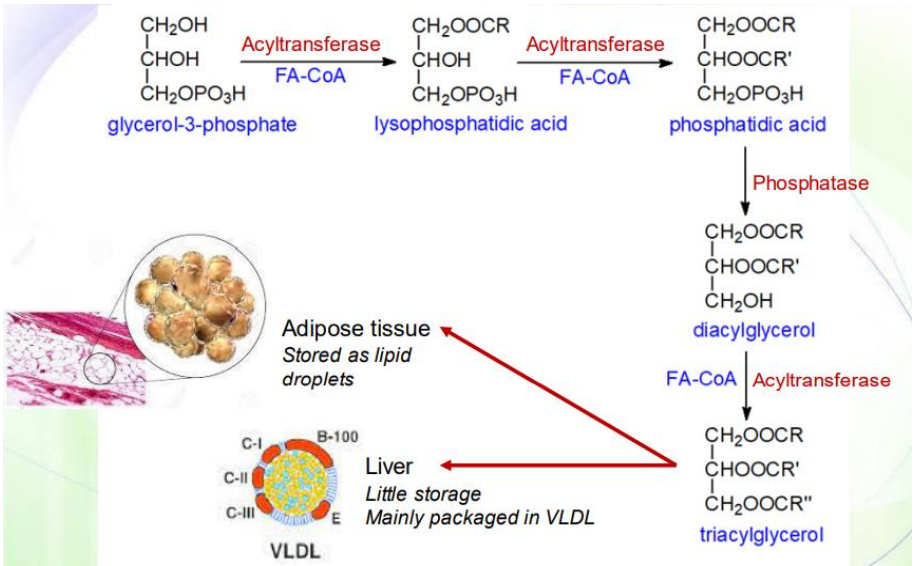
1) from glucose>>DHAP>> glycerol-3-P

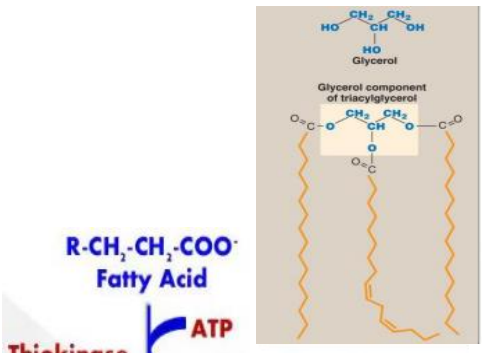
2) phosphorylation of glycerol by glycerokinase(only in liver, that's why adipocytes can't have glycerol-3-P or TAGs unless there is enough glucose (1).

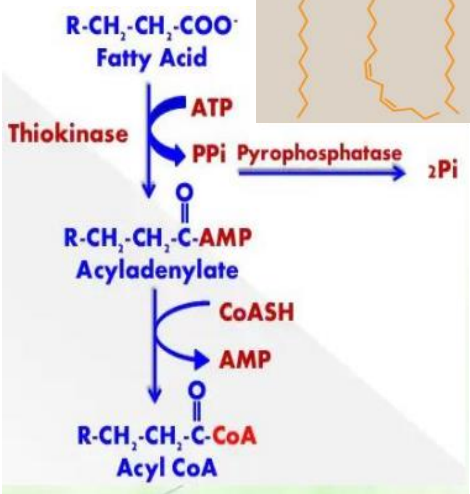
STEP2: activation of fatty acids by adding CoA>> fattyacyl CoA (high energy molecules).

STEP3: synthesis of triacylglycerol from the energy found in the bond between CoA and carboxyl group in fattyacyl (a condensation reaction between FA and glycerol).







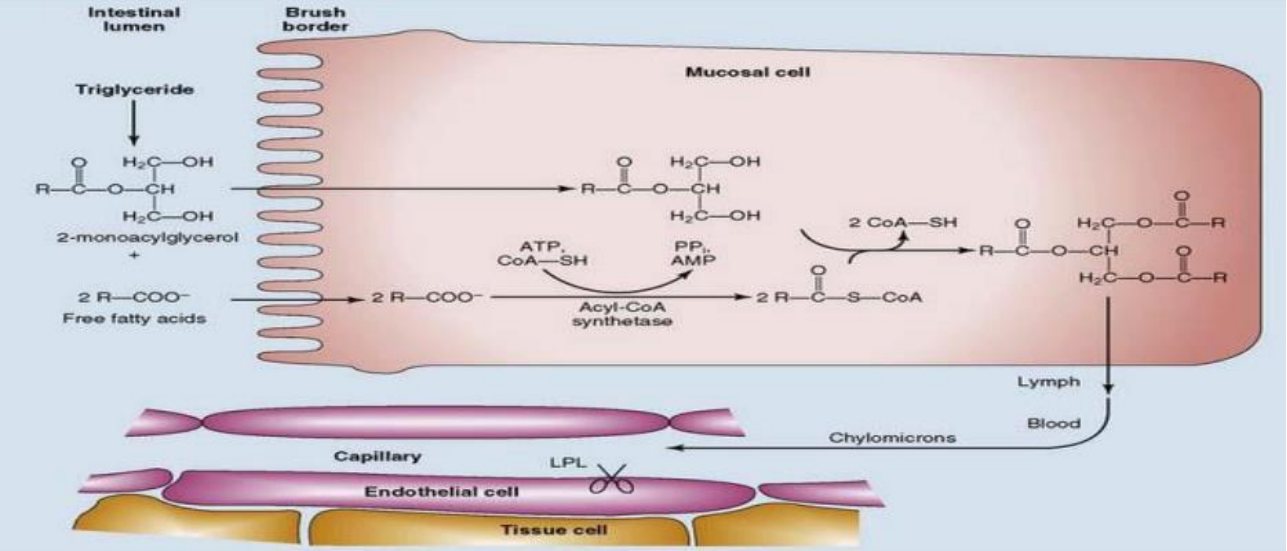


**Phosphatidic acid is the precursor of all glycerophospholipids.

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.

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.



Be a voice not an echo

V1