DOCTOR 2020 | JU

# METABOLISM


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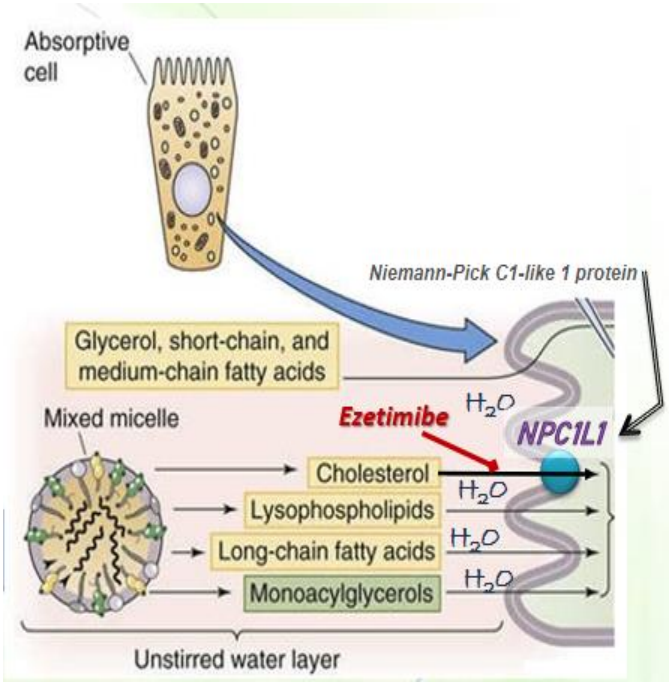
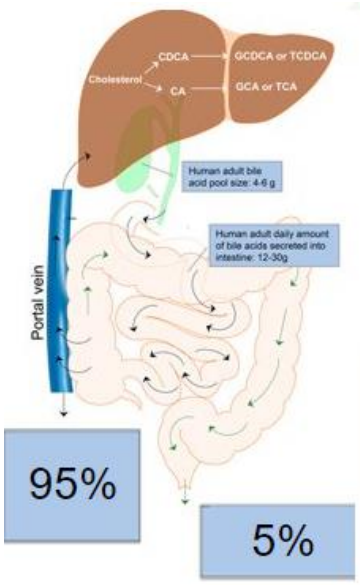
In the previous lecture we talked about lipid digestion and briefly talking we said that digestion starts in a minimal amount in the mouth via lingual lipases, then about 10-30% of digestion occurs in the stomach via gastric lipases, but the majority of digestion occurs in the intestine via pancreatic lipase, and remember the purpose from digestion is to Free the fatty acids from complex molecules like CE and TAG.

## Absorption by enterocytes



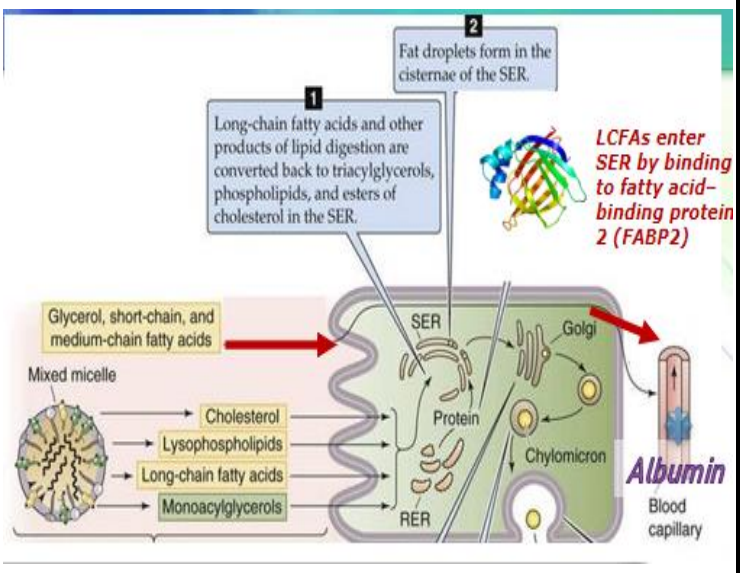

- Absorption takes place in the **apical** side of **enterocytes** with the help of unstirred water layer which prevents the lipid droplet aggregation.
- **Glycerol**, **short** and **medium fatty acids chains** are absorbed directly and rapidly into enterocytes so it's a **simple** proses.
- For the **complex** molecules like Cholesterol, long –chain fatty acid, monoacylglycerols and lysophospholipds get absorbed into enterocytes **not as rapid** as glycerol, short and medium fatty acids.

- **Micelles are formed in the lumen from FFA, free cholesterol, bile salts, and fat-soluble vitamins.**
- **Cholesterol is poorly absorbed and needs a specific carrier called NPC1L1** (Niemann-Pick C1-like 1 protein).
- **Note: it can be drug-targeted like Ezetimibe so we inhabit the absorption of cholesterol and thus lowering its concentration.**
- **Short- and medium-chain FF are directly absorbed.**
- **When bile acids reach the lumen 95% reabsorbed directly to the liver and 5% get out by feces.**

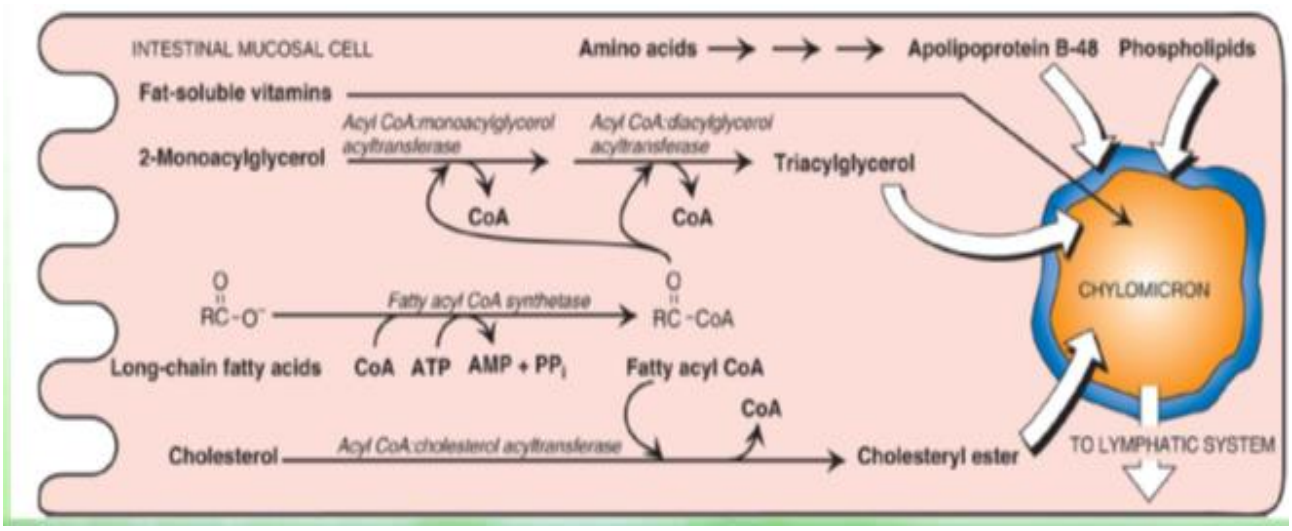


# Reformation of complex lipids

- **Now for Glycerol, short and medium fatty acids pass from the enterocytes to the blood capillary and bind to Albumin, but the other lipids enter the SER with the help for fatty acid-binding protein 2 (FABP2) which facilitate the transport of these lipids.**

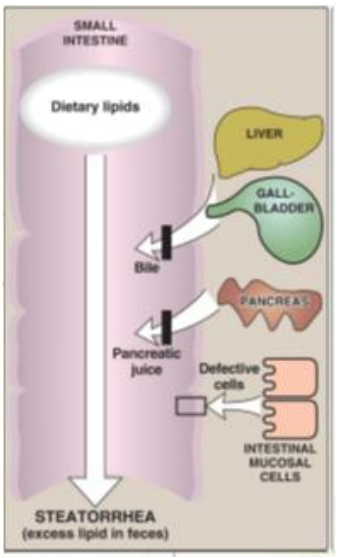



- **When long fatty acids chain enters the SER it gets activated by Fatty Acid CoA synthase which transport CoA to it, now the Fatty Acyl CoA has 2 fates either it gets attached to Cholesterol and forms Cholesterol Esters then get packed into Chylomicron or it get attached to 2 Monoacylglycerol and form Triacylglycerol then get packed into Chylomicron.**
- **Notice by this proses we Reform complex lipids!!**




**All enzymes above are required↑↑**

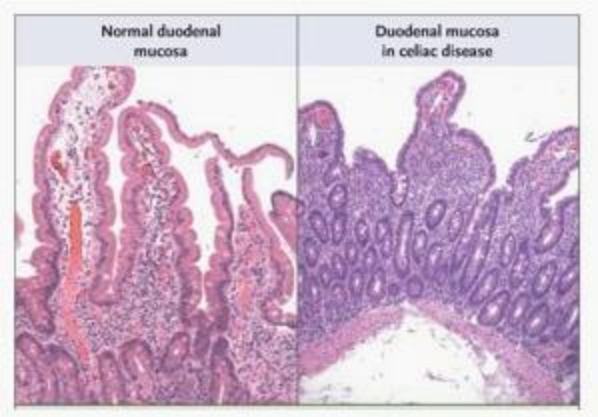
- **Notice that Chylomicron carry the dietary lipids.**
  - **What are Chylomicrons??**
  - **They are lipoproteins composed of different lipid molecules mainly from TAG and a B-48 Apolipoprotein**



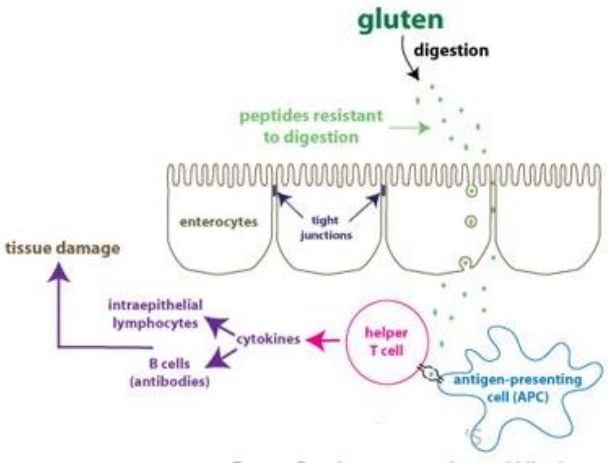

**Steatorrhea** is a problem in the absorption of the lipids so they get eliminated (excreted) by feces and its caused by:

1. **Short bowel disease**
2. **Liver or biliary tract disease**
3. **Pancreatic exocrine insufficiency**

## Celiac disease (CD)



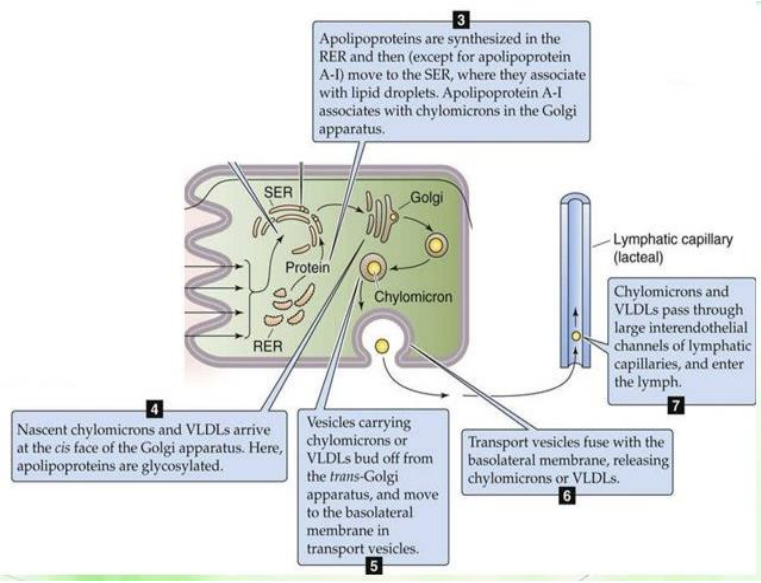

- **Fat malabsorption leading to steatorrhea.**
- **It is an autoimmune response to gliadin, a peptide found in gluten protein that present in (wheat, rye, and barley).**
- **Gliadin contains many proline and glutamine residues, making it resistant to digestion.**
  - **Gliadin causes an inflammatory response leading to an autoimmune response against transglutaminase enzyme and as result from inflammation the epithelial cells will be damaged and leads reduction of nutrient absorption**
- **Lab tests indicate the presence of anti-tissue transglutaminase (anti-tTG) antibodies.**
- **Tissue biopsy reveals the absence of villous surface epithelial cells resulting in decreased nutrient absorption.**

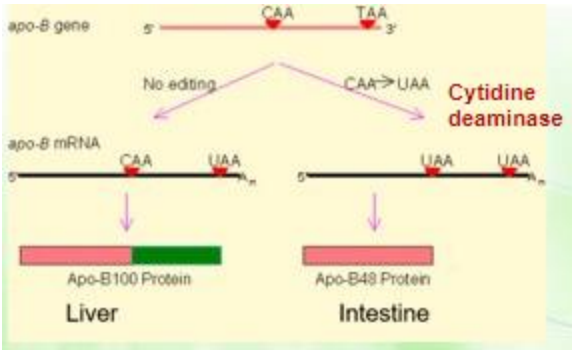
# Formation and release of chylomicrons

- **TAG and cholesteryl esters are packaged in chylomicrons made of phospholipids, nonesterified cholesterol, and apolipoprotein B-48.**

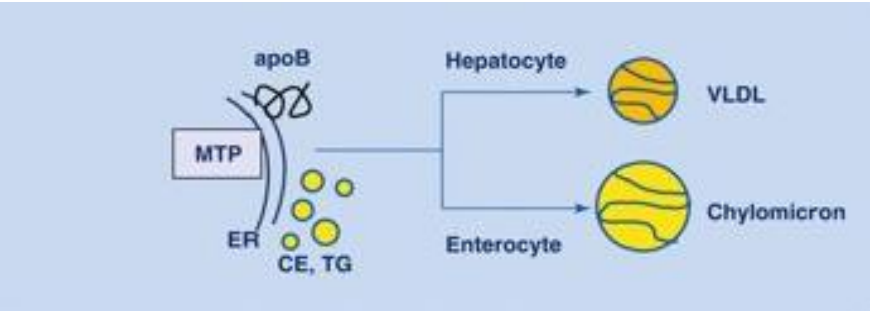
  - **Now the complex lipids will be transported from SER to Golgi apparatus, in Golgi Apolipoprotein B-48 get glycosylated and Chylomicron get packed into vesicles and get released from the cell into the Lymphatic capillary which drains in the blood.**
  - **Apo B -48 is produced from the same gene that produce Apo B-100 which is part of VLDL & LDL so how the same gene produces different proteins?**




  - ➢ **Through RNA editing in the enterocytes cells (intestine) we have Cytidine Deaminase, which modify the Apo B-gene from the middle and change the CAA nucleotides into a stop codon UAA, thus the translation stops here resulting in shorter protein which is Apo B-48, but remember cells without Cytidine Deaminase like liver cells will have the longer protein Apo-B 100 protein.**




- **Microsomal triglyceride transfer protein (MTP) is essential for the assembly of all TAG-rich apo B–containing particles in the ER (chylomicrons).**

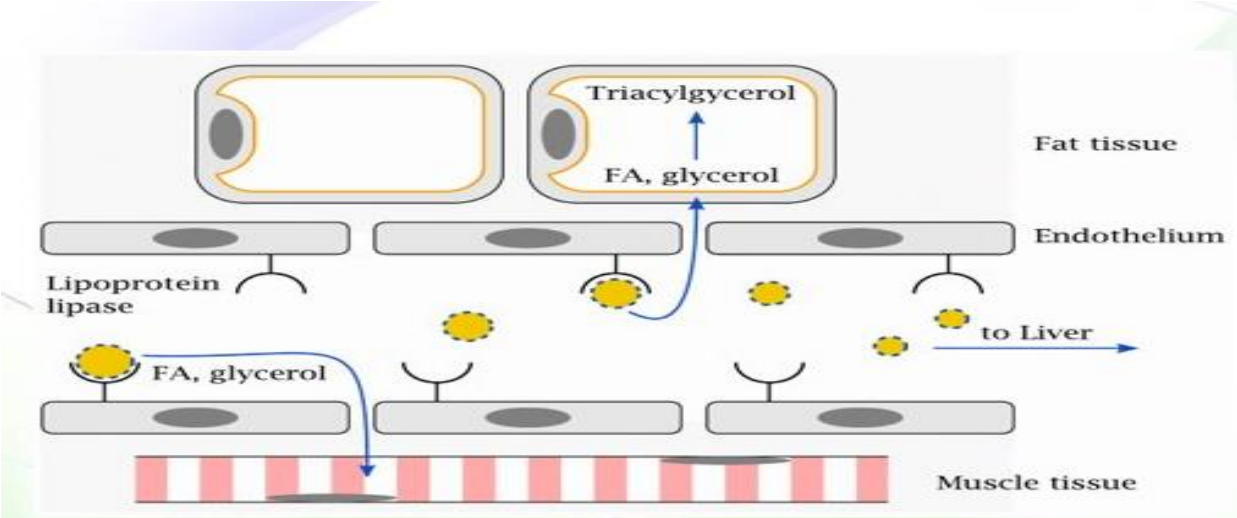


## Fates of TAGs in chylomicrons

- As chylomicrons travel in blood it gets transformed into chylomicron remnants (smaller lipoprotein) and fatty acid get released this transformation occurs via **lipoprotein lipase**.
- TAGs in chylomicrons are hydrolyzed in the bloodstream by **lipoprotein lipases** that are anchored in capillary walls.

➢ The resulting fatty acids have two possible fates:

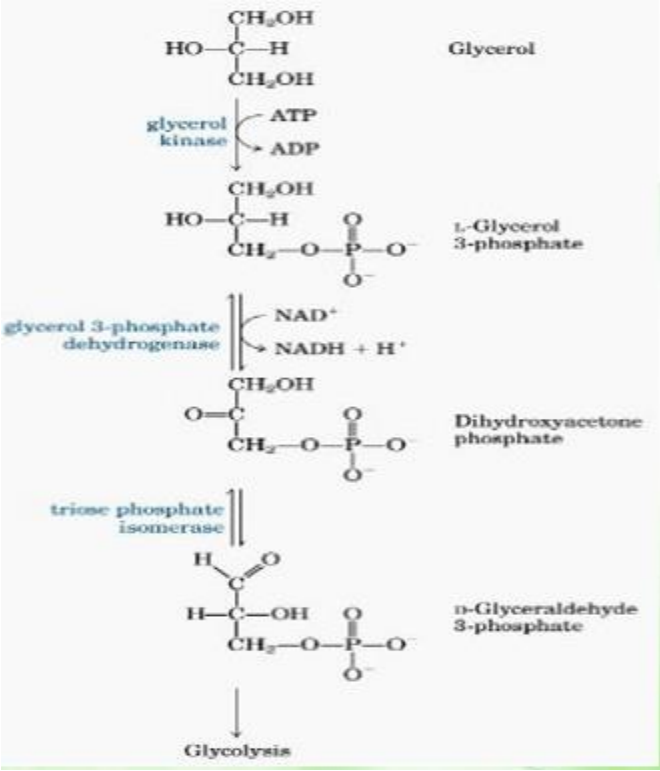
- When energy is in good supply, they are converted back to TAGs for storage in adipose tissue.
- When cells need energy, the fatty acid carbon atoms are oxidized into Acetyl-CoA.




✓ **Familial chylomicronemia** (type I hyperlipoproteinemia) is a rare, autosomal-recessive disorder caused by a **deficiency** of **LPL** (lipoprotein lipase) or its **coenzyme apo C-II** resulting in **fasting chylomicronemia** and **severe hypertriacylglycerolemia**, which can cause pancreatitis.

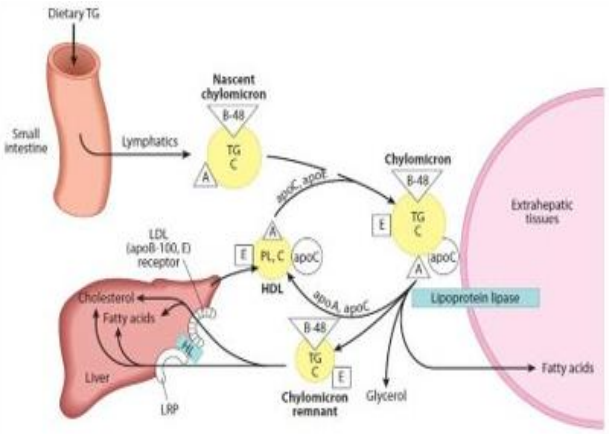
## Fate of glycerol

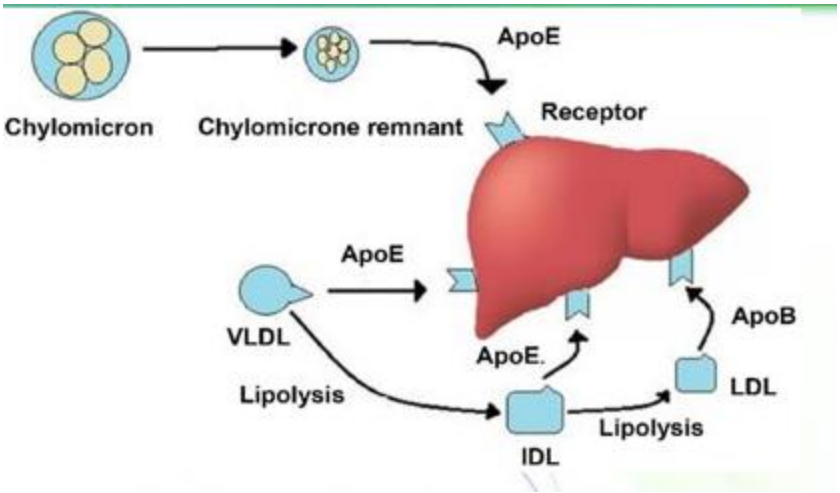
- **Glycerol is carried in the bloodstream to the liver or kidneys, where it is phosphorylated and then converted to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate (DHAP) for either glycolysis or gluconeogenesis or synthesis of TAG.**




## Fate of chylomicrons

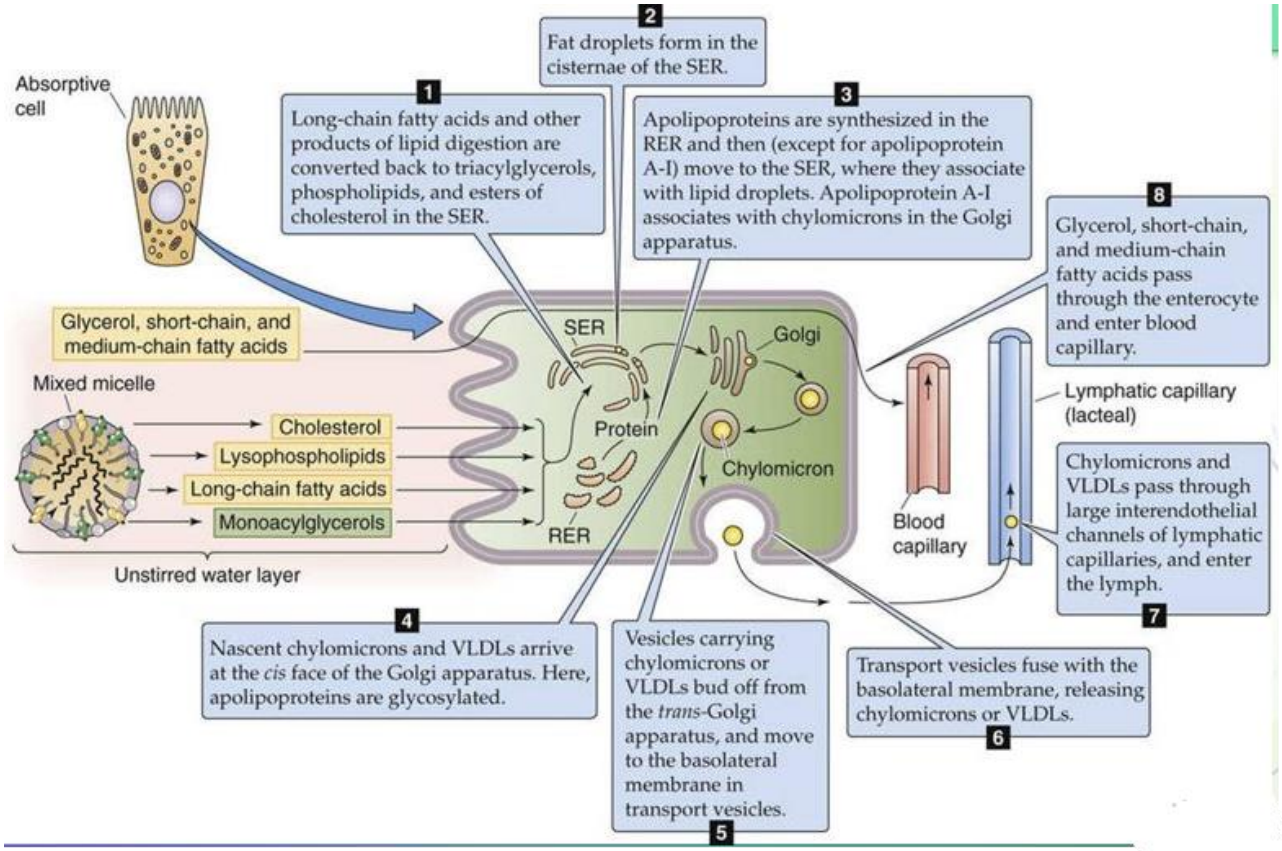
- **Now chylomicrons remnants will reach the liver with a apolipoprotein E which comes from HDL, so Apo E and Apo C get transferred from HDL to chylomicron remnants and it will bind to Apo E receptors on the liver.**
- **When TAGs are removed, chylomicrons remnants would contain cholesteryl esters, phospholipids, apolipoproteins, fat-soluble vitamins, and a small amount of TAG).**
- **Chylomicron remnants bind to apoE receptors on the liver via their apoE and are endocytosed.**
- **The intracellular remnants are hydrolyzed to their component parts.**




✓ **Type III hyperlipoproteinemia: mutations in apoE gene leading to decreased clearance of chylomicron remnants.**

**A better view: -**

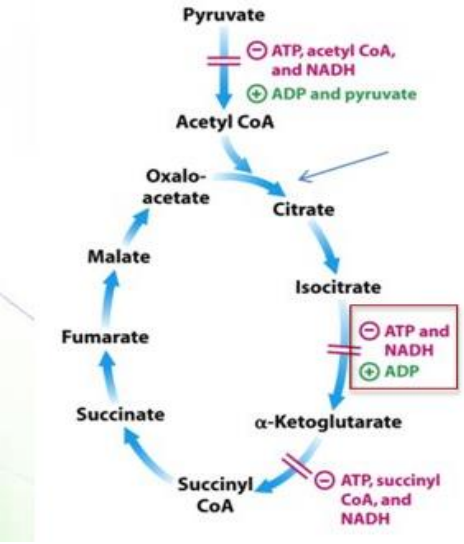


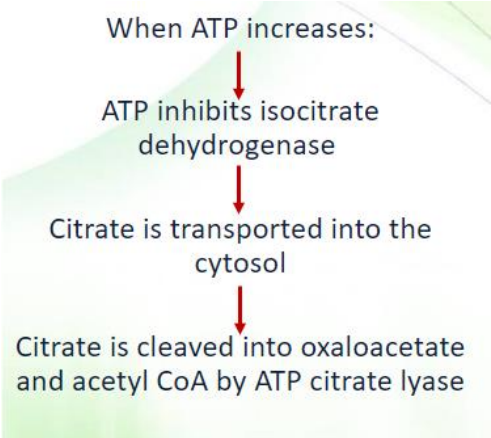

**A new topic: -**

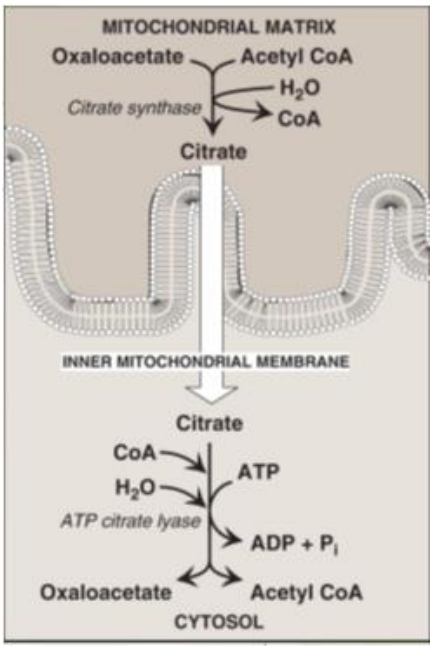
# Synthesis of fatty acids

## Mitochondria to cytosol transport of Acetyl-CoA

- **Synthesis of fatty acids occurs when there are a lot of ATP and Glucose, now increasing glucose , increases pyruvate , increases Acetyl-CoA which joins the oxaloacetate and forms citrate then it get isomerized into isocitrate then via isocitrate dehydrogenase it will be converted to Alpha ketoglutarate , isocitrate dehydrogenase is inhabited by ATP and NADH , the isocitrate and citrate will accumulate in the mitochondria and citrate get transported to cytosol and in the cytosol citrate is cleaved into oxaloacetate and Acetyl-CoA, so by this proses we transported Acetyl-CoA from mitochondria to the cytosol where it will be used for fatty acid synthesis.**



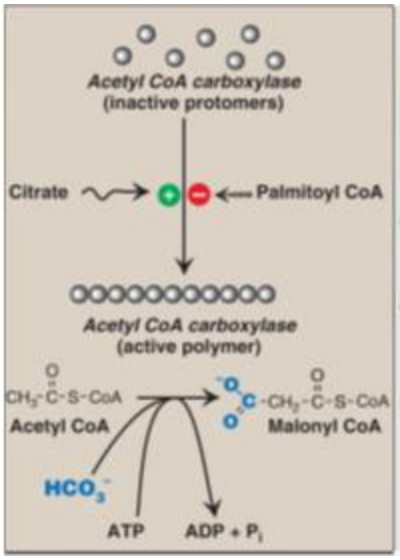



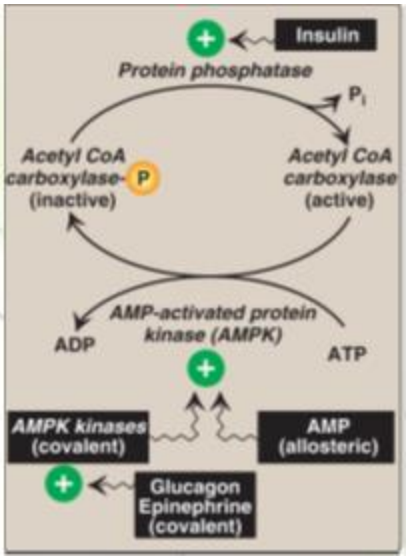




# Synthesis of malonyl-CoA

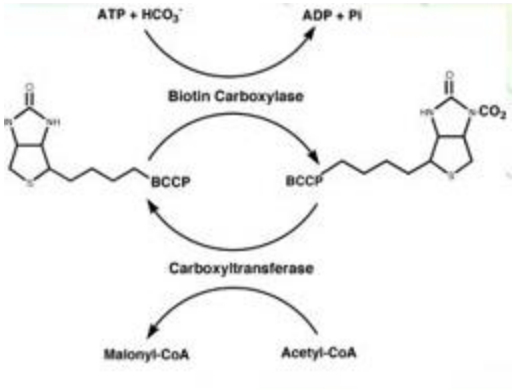
- There are 2 important enzymes for fatty acid synthesis: ACC and FAS

- **Acetyl CoA carboxylase (ACC) transfers a carbon from $CO_2$ (as a bicarbonate) via biotin (vitamin B7), which is covalently bound to a lysyl residue of the ACC and results in a formation of malonyl-CoA (3 carbon molecule).**
  - **ATP is needed.**
  - **The reaction is a rate-limiting reaction**
  - **ACC is an allosteric polymeric enzyme**




- **ACC is inactivated by:**
  - **1-Depolymerization by palmitoyl-CoA.**
  - Palmitate is the end product of fatty acid synthesis
  - **2-Phosphorylation by AMPK (AMP Kinase)**
  - **AMP kinase is an energy sensor so if we have low energy the enzyme gets activated and vice versa.**
  - **AMP kinase is also hormonally regulated so if we have Glucagon or Epinephrine it will get activated.**



- Now remember AMP Kinase inhabit the ACC thus inhabits the fatty acid synthesis and that make sense because we are under low energy state.
- How AMPK inactive ACC??
- By phosphorylation

  - **ACC synthesis increases by:**

  - 1-Insulin activates protein phosphates which remove the phosphate from ACC and active it.
  - 2-Excess calories via the transcription factor carbohydrate response element–binding protein (ChREBP)
  - 3-Insulin via the transcription factor sterol regulatory element–binding protein-1c (SREBP-1c).

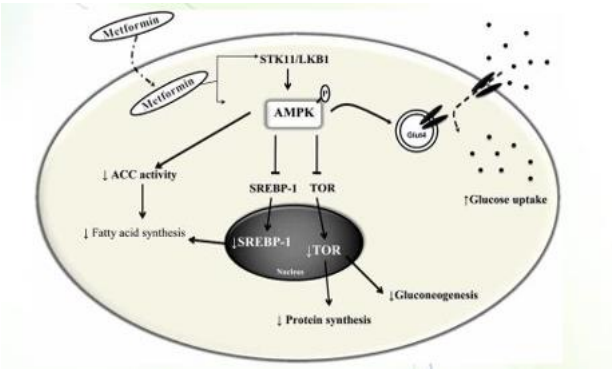
- **Notes:**
- **A low-calorie or a high-fat, low-carbohydrate is inhibitory of ACC synthesis.**
- ***ACC* synthesis is also upregulated by carbohydrate Fatty acid synthase is similarly regulated.**
- **Also the second enzyme FAS is regulated in the same manner of ACC regulation**

صلّ على النبي

## Metformin

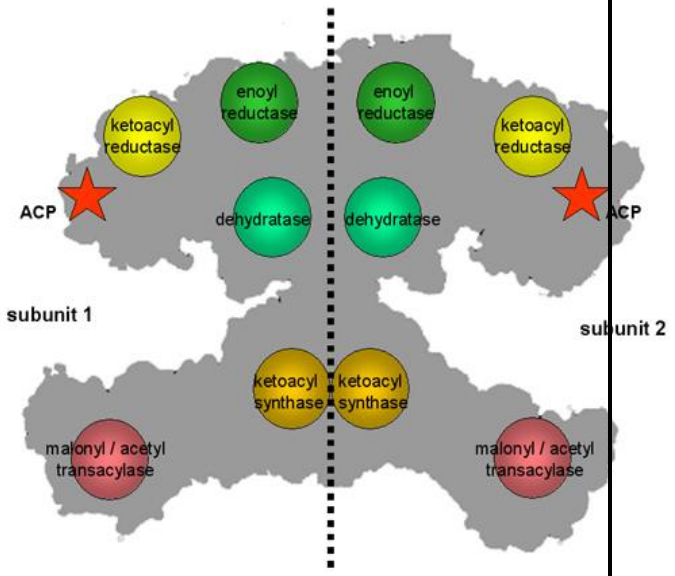
**Drug that regulate the glucose level of the blood**

- **Metformin lowers plasma TAG through :**
- **1-Activation of AMPK, resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and fatty acid synthase expression (by decreasing SREBP-1c).**
- **2-Lowering blood glucose by increasing AMPK-mediated glucose uptake by muscle.**




**The second enzyme that is important for fatty acid synthesis is *Fatty Acid Synthase(FAS):***

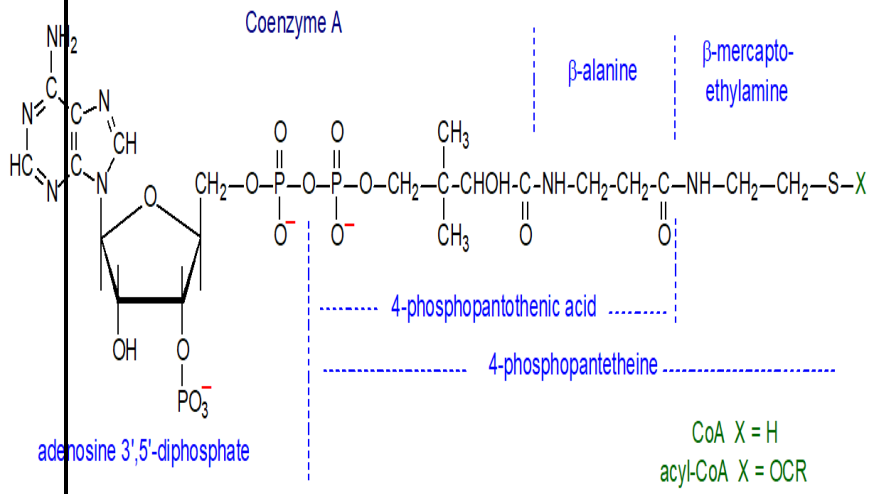
- **A multifunctional,homodimeric enzyme(each subunit contains a multienzymatic structure,see next)**

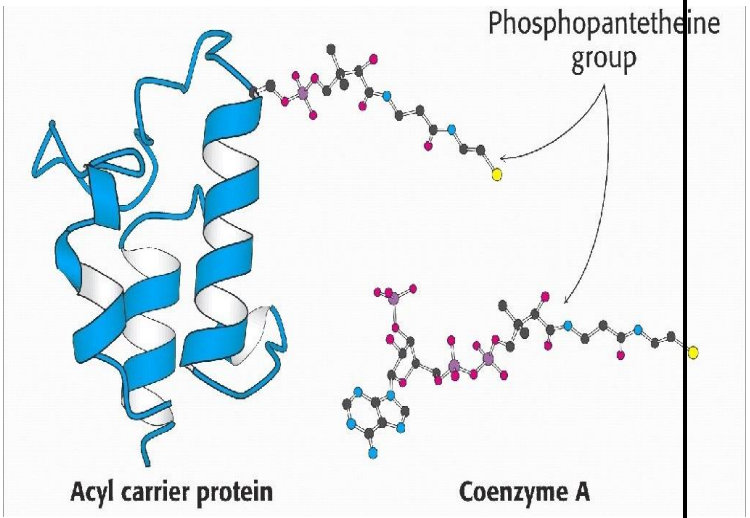



- **Each FAS monomer is multicatalytic with six different enzymes domains(similar to the principle of pyruvate dehydrogenase,a complex that consists of three different catalytic enzymes)**

- **It is associated with two molecules,the first one is phosphopantethiene-containing acyl carrier protein (ACP) domain ( a protein associated with phosphopantethiene- a derivative of vitamin B5(pantothenic acid),which is used to synthesize CoA)**

- **Phosphopanthethiene carries acyl units on its terminal thiol (-SH) group and presents them to the catalytic domains of FAS (thiol is important being responsible for the activity of FAS as well as the this molecule)**



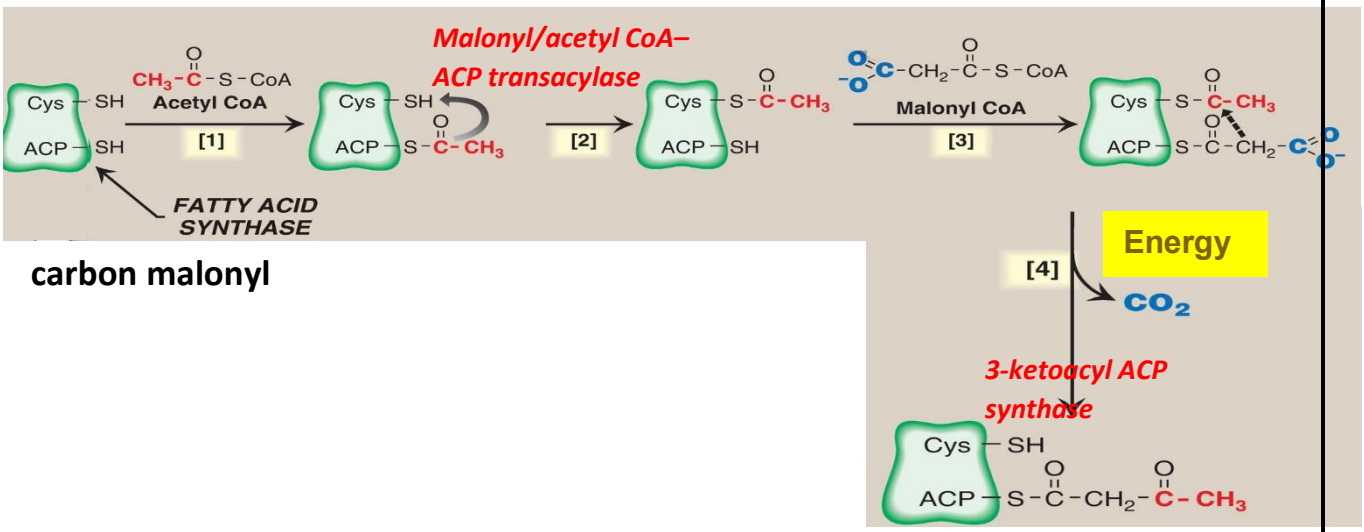



## WHAT ARE THE REACTIONS?

### We'll take them in three stages:

**Stage 1:We have the enzyme,in the active site we have ACP accompanied with a thiol group,close to a *Cysteine* residue which also has a thiol group.The first enzymatic activity is catalyzed by trans acylase,what is does that it connects acetyl CoA(which binds to ACP) transferring it to the Cys residue.Having Cys attached to the acetyl group,malonyl CoA -which we synthesized before by carboxylase- comes in,so now we have two molecules:acetyl CoA and malonyl CoA,attached to the active site.Consequently,a condensation reaction occurs,malonyl CoA attacks acetyl CoA(bound to cys),resulting in the release of $CO_2$.Acetyl CoA has 2 carbons,malonyl has 3,so the outcome is a four-carbon molecule.Here the synthetic activity of FAS manifests.**

- **Remember we're talking about a condensation reaction,so we need energy,which comes from the decarboxylation(release of $CO_2$) of the three-carbon malonyl**

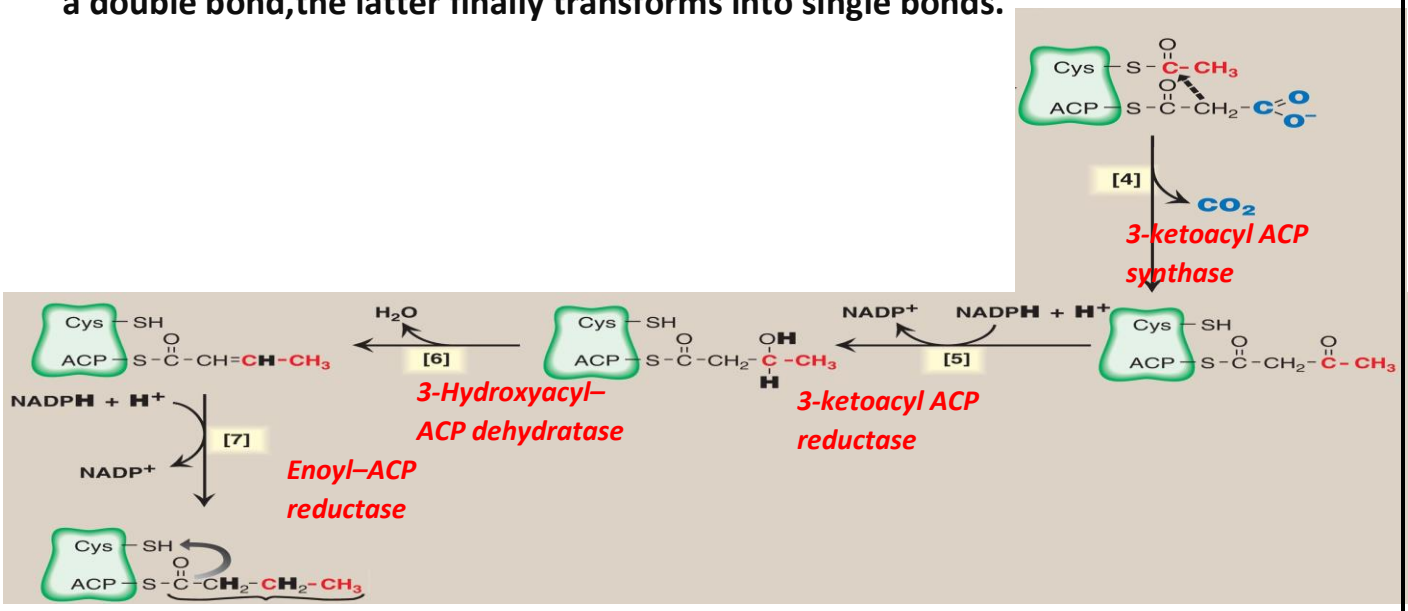



**Stage 2:consists of 4 ordered reactions:condensation(which we've just talked about) reduction,dehydration,reduction that are repreated several times.**

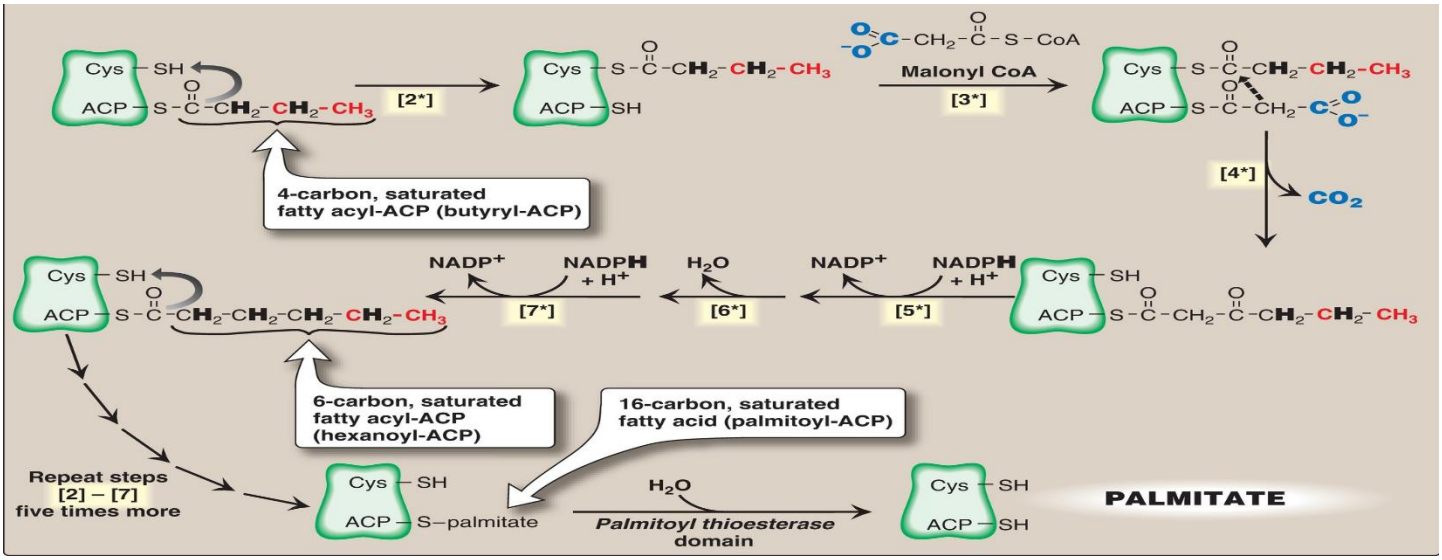
**The two reduction reactions are catalyzed by reductases.In terms of oxidation-reduction,fatty acids get reduce concominant with oxidation of NADPH,ofc 2 NADPH.**

**The resultant molecule is a butyryl,a four-carbon compound.Let's summarize how it forms.**

**After the attack malonyl performs a four-carbon molecule forms(distinctly,it has a ketone group).This molecule gets reduced with the ketone transforming into secondary alcohol,followed by dehydration in which the hydroxyl group becomes a double bond,the latter finally transforms into single bonds.**




**Stage 3:Just like CoA binding to ACP then transferred to the Cys residue,the butyryl already formed binds ACP,then it gets transferred to cysteine.Malonyl CoA comes again,repeating the same reactions:condensation,reduction,dehydration,reduction,with the consumption of 2NADPH moelcules,the result is a six-carbon compound.Subsequently,these reactions keep repeating over and over until we get a 16-carbon molecule,*Palmitate,*which gets released by a thioesterase.**



## STOICHIOMERTY OF PALMITATE SYNTHESIS

- 1 acetyl CoA enters the reaction (2C)
- 7 malonyl CoA (x2=14C)
- Every round,2NADPH are consumed(x7 for each malonyl)
- 8CoA are released (from acetyl&malonyl)
- With 7 malonyl being in the context,they need to be synthesized,and this is done by the carboxylase enzyme previously mentioned.

Together they form palmitate

## SUMMARY:

$$\text{Acetyl CoA} + 7\ \text{malonyl CoA} + 14\ \text{NADPH} + 20\ H^+ \longrightarrow \text{palmitate} + 7\ CO_2 + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ H_2O$$

The equation for the synthesis of the malonyl CoA used in the preceding reaction is:

$$7\ \text{Acetyl CoA} + 7\ CO_2 + 7\ \text{ATP} \longrightarrow 7\ \text{malonyl CoA} + 7\ \text{ADP} + 7\ P_i + 14\ H^+$$

Hence, the overall stoichiometry for the synthesis of palmitate is:

$$8\ \text{Acetyl CoA} + 7\ \text{ATP} + 14\ \text{NADPH} + 6\ H^+ \longrightarrow \text{palmitate} + 14\ \text{NADP}^+ + 8\ \text{CoA} + 6\ H_2O + 7\ \text{ADP} + 7\ P_i$$

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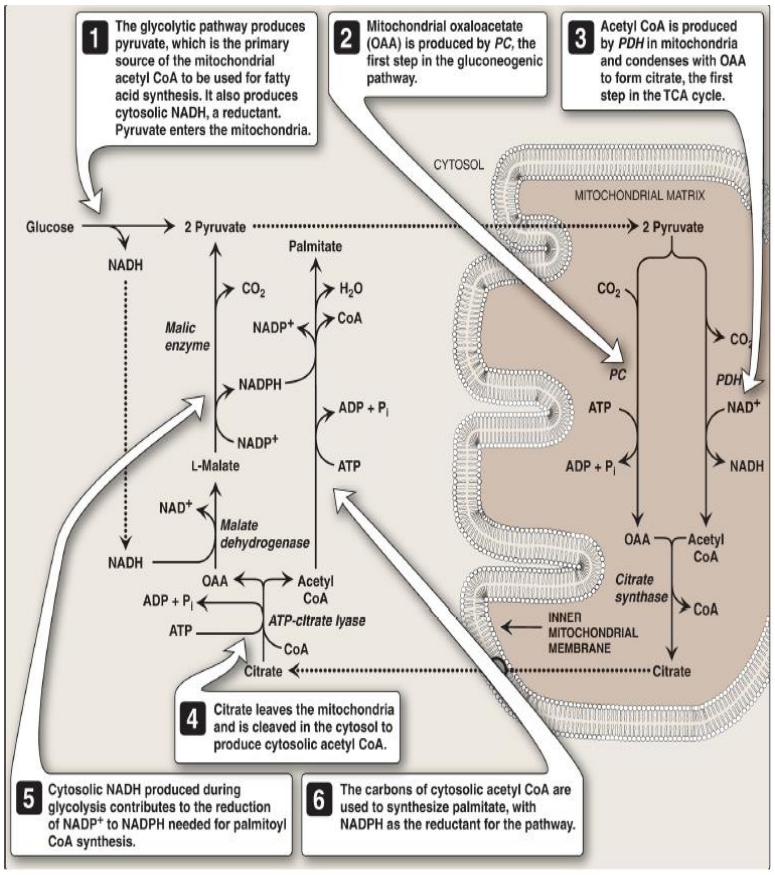
## SOURCES OF MOLECULES




**Zooming in into the mitochondria,pyruvate is converted into *acetyl CoA* as well as *oxaloacetate*,with a condensation reaction they form *citrate,*which exits into the cytosol,and gets cleaved into acetyl CoA&oxaloacetate. Considering NADPH,it comes from 2 sources,the first of which is PPP,which produces 2NADPH per round *in the cytosol,*therefore can be used for fatty acid synthesis.The second source is the conversion of oxaloacetate to pyruvate,oxaloacetate is converted into malate that in turn is converted into pyruvate(malate to pyruvate reaction releases CO2 and produces NADPH utilized in fatty acid synthesis).**

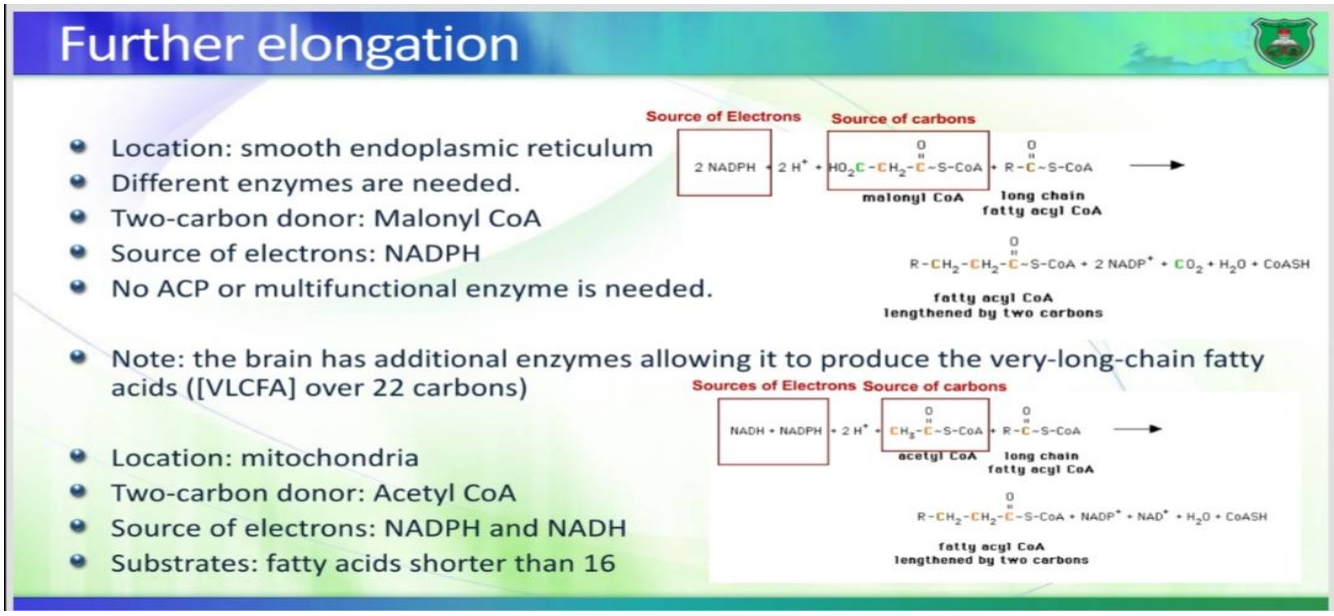
- ☑ **To sum up,acetyl CoA,acetyl CoA carboxylase and fatty acid synthase contribute to the formation of palmitate(a 16-carbon fatty acid)**

**By this,we've made a 16-carbon FA.BUT can we make longer chains?**
**Ofc we can,and this depends on where it takes place,it may occur in the smooth endoplasmic reticulum or mitochondria.**

- **Smooth endoplasmic reticulum:We need a source of carbon(i.e:malonyl CoA) and a source of electrons(i.e:NADPH,because an anabolic reaction is taking place).Palmitate surely should be activated to begin the reaction,two carbons are transferred from malonyl CoA in order to produce an 18-carbon FA.**

  **The enzymes that catalyze this reaction aren't the same as the previous one.Here there is no need for fatty acid synthase or ACP,these are just different enzymes.**

- **Mitochondria:In mitochondria,the substrates are fatty acids shorter than 16C.The source of carbon here is acetyl CoA NOT malonyl CoA,while the source of electrons(each reaction requires $2e^-$) is both NADH and NADPH,different sets of enzymes carry out the process,and the result is**



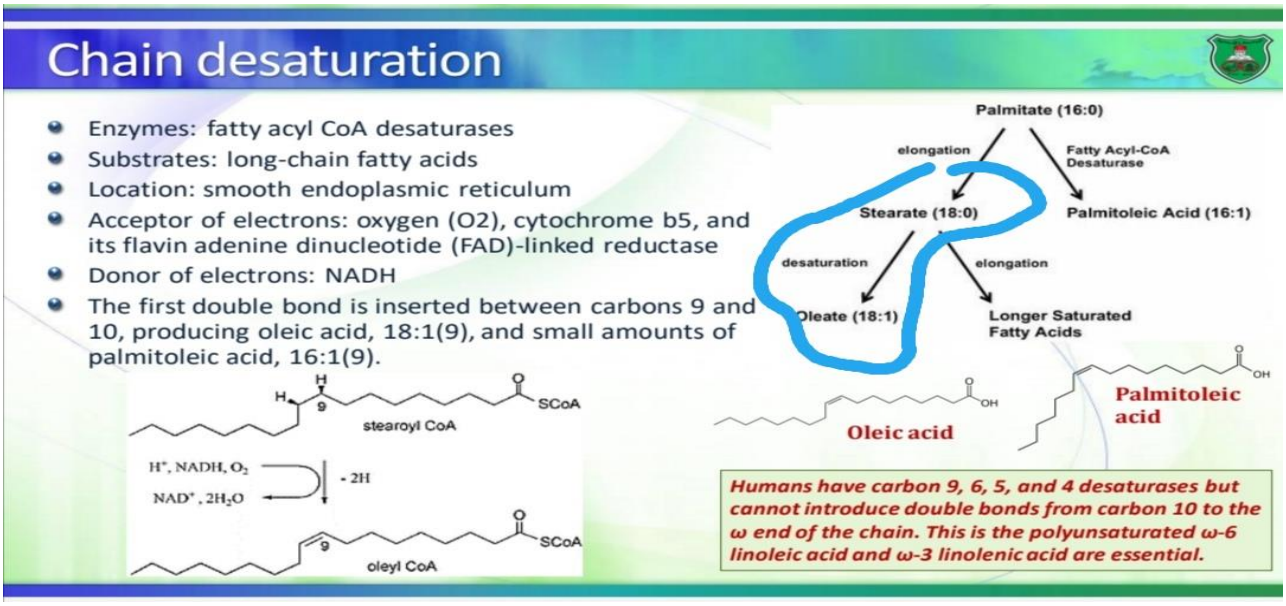

**longer fatty acid chains.**

**Now,don't we have unsaturated fatty acids that contain double bonds?**

**A:Yes we have,but the question is,how are they synthesized?**

**<u>Actually there are two sources,either our cells make them or we get them from diet.</u>**

- **In our cells:we have the enzymes which are called desaturases,they create double bonds by oxidizing fatty acids,also can take stearate(saturated 18-carbon fatty acid) creating double bonds as in oleic acid(olive oil,monounsaturated 18-carbon fatty acid,the double bond is between C9 and C10).**
  - **But wait a minute,if we can produce double bonds into fatty acids,why do we say that linoleic and linolenic acid are essential? A:Because we don't have the enzymes which create double bonds after C10,we only have enzymes introducing double bonds on C4,5,6,7,9,10 but NOT beyond C10.Hence we need these two fatty acids.**




**<u>After synthesizing fatty acids,what's next?</u>**

- **Their fate depends on the tissue,either liver or adipocyte.**
- **Examining triacylglycerols generally,we find that the fatty acid on C1 is typically saturated,while that on C2 is unsaturated,and the third one exists in either.**
- **How to make triacylglycerols? The three steps are:**
  - ☑ **Synthesis of glycerol 3-phosphate(active glycerol backbone)**
  - ☑ **Synthesis and activation of fatty acids**
  - ☑ **Triacylglycerol synthesis**
- **How can fatty acids be activated?**

  **A:Using CoA**
  - ☑ **First ATP is used as a source of energy,releasing two pyrophosphate to become AMP that attaches(conjugates) to the fatty acid.(run by the enzyme thiokinase)**
  - ☑ **The AMP bound gets replaced by CoA producing Acyl CoA**
  - ☑ **This reaction needs energy,provided from the immediate cleavage of pyrophosphate forming two phosphate groups.**
  - ☑ **This reaction keeps proceeding in the forward direction,by playing with delta G .In terms of bioenergetics,delta G can be reduced by lowering products' conc.As a consequence,endergonic reactions turn to be exergonic,so the reaction goes forward.And this is the situation here,when forming the AMP-bound acyl molecule,along with pyrophosphate later converted to phosphates,we are reducing the**

amount of pyrophosphates(the product),thus the reaction keeps moving forward.

- **Now,how is glycerol 3-phosphate synthesized?**

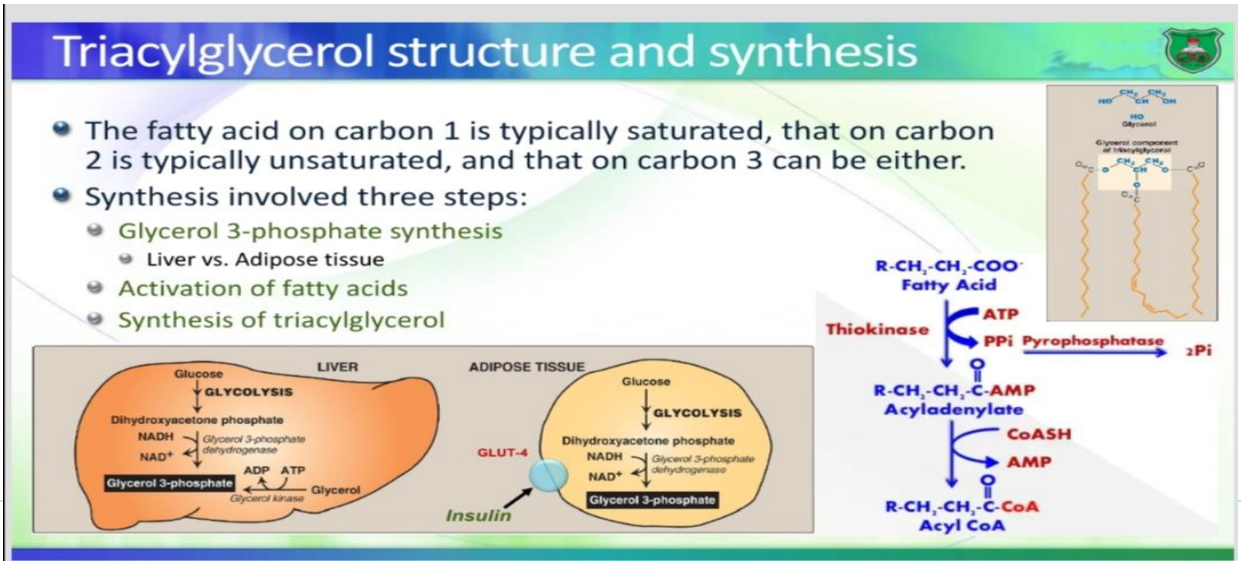
  **A:In fact,it's tissue-dependent process.**

  **In liver and adipose tissue,one mechanism is similar,which is glycerol 3-phosphate synthesis by the pathway(glucose to Dihydroxyacetone phosphate to glycerol 3-phosphate).What forms the precursor of glycerol 3-phsophate is dihydroxyacetone phosphate.**

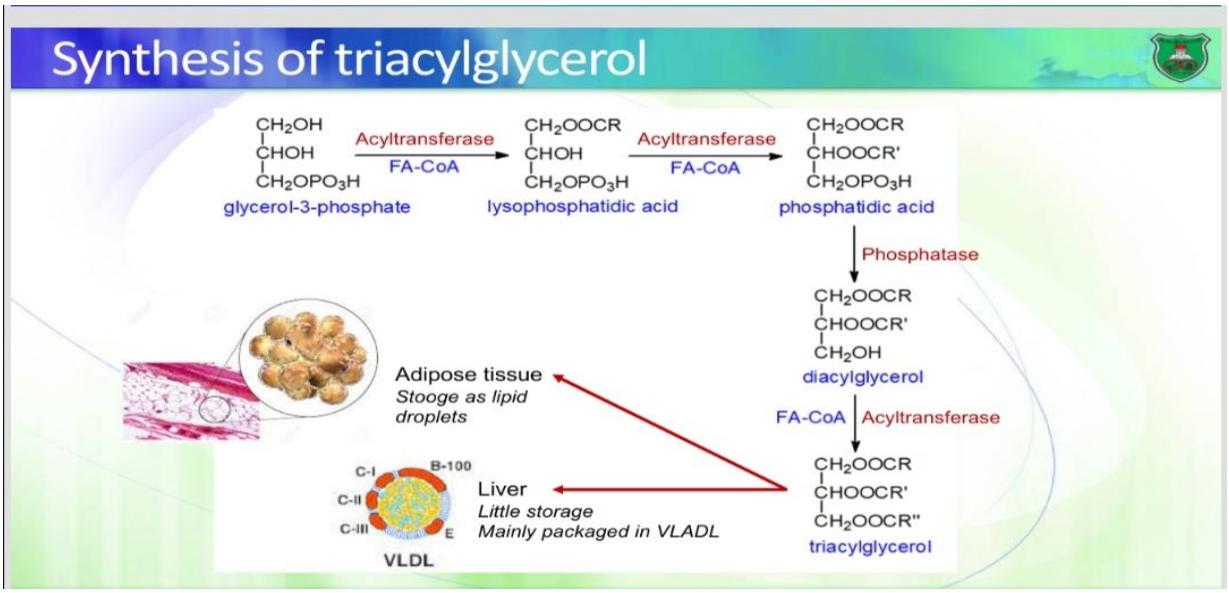
  **Liver has a distinct pathway,in which glycerol 3-phosphate is produced by directly phosphorylation of glycerol,catalyzed by glycerol kinase.**

  **This enzyme isn't present in adipose tissues,and this is of an advantage.The sole pathway to synthesize glycerol 3-phosphate is glucose-dihydroxyacetonephopshate-glycerol 3-phosphate.So in cases of shortage of glucose,fatty acid synthesis is no longer a choice.Regulation of glucose uptake is carried out by insulin,which controls GLUT-4 expression in high-glucose status.High blood glucose stimulates insulin secretion,resulting in subsequent GLUT-4 activation,which increases glucose levels in the cells,finally forming glycerol 3-phosphate (then triacylglycerols) ,provided that glycerol is available.**

  **In cases of scarce of glycerol,no insulin will be released,no GLUT-4 will be expressed,eventually there won't be any glucose entering the cells to synthesize glycerol 3-phosphate.Therefore,the pathway of glycerol 3-phosphate synthesis and triacylglycerols is blocked.That is the benefit of lacking glycerol kinase in adipose tissue,stricting glycerol 3-phosphate synthesis starting with glucose.Lack of glucose indicates no triacylglycerols.**



- **Once glycerol 3-phosphate is synthesized and fatty acids are activated,we're ready to make triacylglycerols.Two fatty acids get sequentially transferred to glycerol 3-phosphate by the action of acyltransferases producing phosphatidic acid,which of a phosphate gets removed(by phosphatase),followed by a third acyltransferase transferring the third fatty acid,finally giving a glycerol attached to three fatty acids(triacylglycerol).Finally,the fate of triacylglycerols depends on the tissue,in adipose tissue triacylglycerols are stored as lipid droplets.In liver,little triacylglycerol is stored yet it gets packaged in VLDL and the latter gets out into peripheral tissues.**




**تم بحمد الله**

**"نهاية مادة اختبار منتصف الفصل"**