فريق طوفان الأقصى

METABOLISM

Modified N. 6

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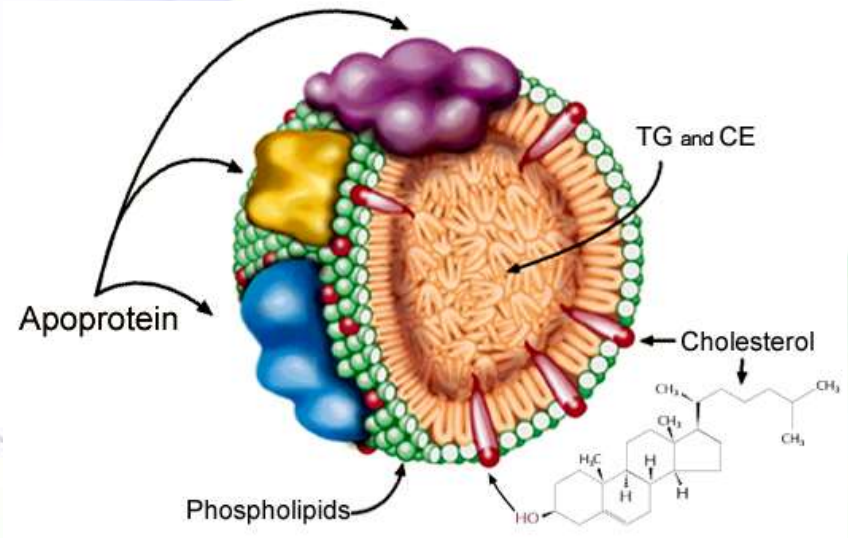
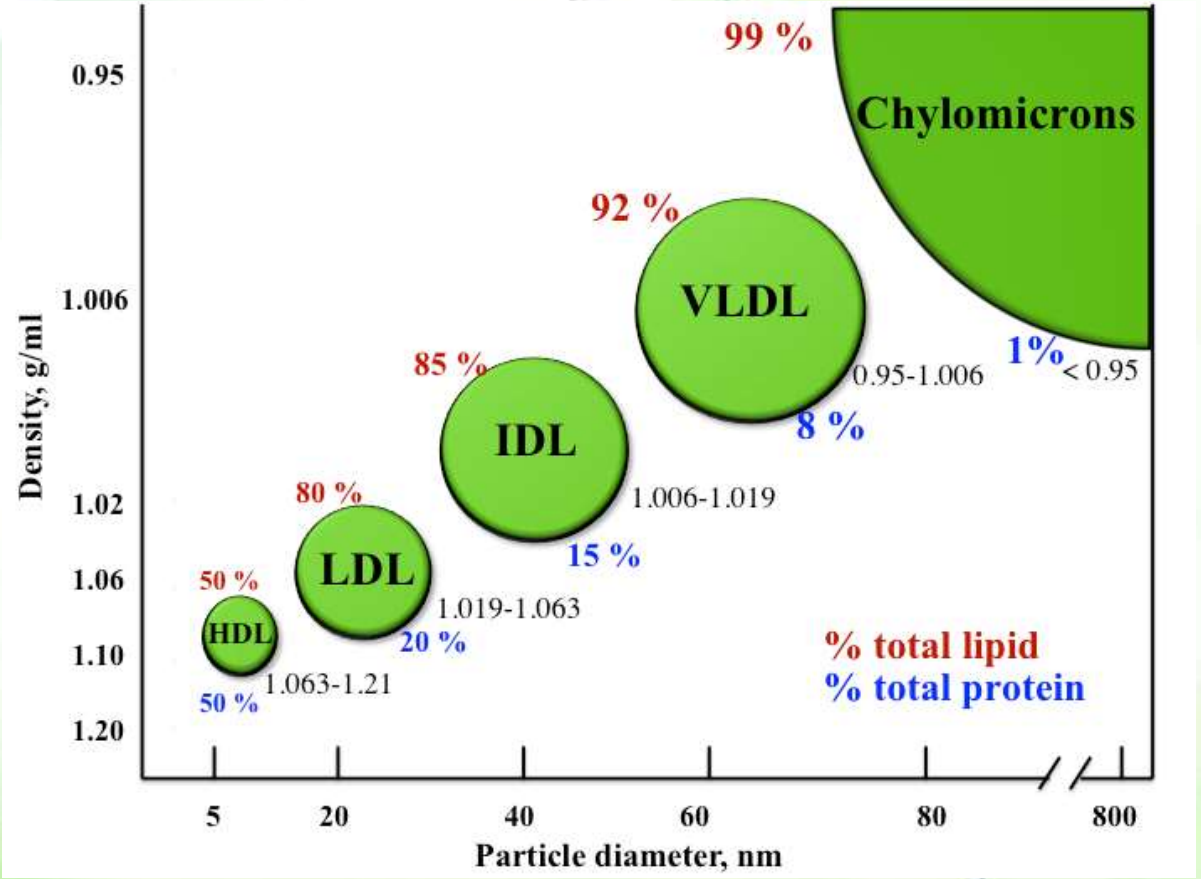
Lipoproteins

Lipids



Function: transport of lipids (cholesterol, cholesterol esters, phospholipids & triacylglycerols) in blood plasma.



As lipid content increases, the density decreases

- Lipoproteins look like micelles, they're composed of lipid molecules mainly phospholipids as well as proteins (known as 'apolipoproteins')
- Lipoproteins transport lipids (found inside the micelle), such as TAGs, cholesterol esters, lipid soluble vitamins, etc
- Lipoprotein's density depends on P(protein):L(lipid)ratio, high ratio means high density.

- VLDL= Very low density lipoprotein
- IDL = Intermediate density lipoprotein
- LDL = Low density lipoprotein
- HDL = High density lipoprotein

- Chylomicrons are the largest , HDLs are the smallest

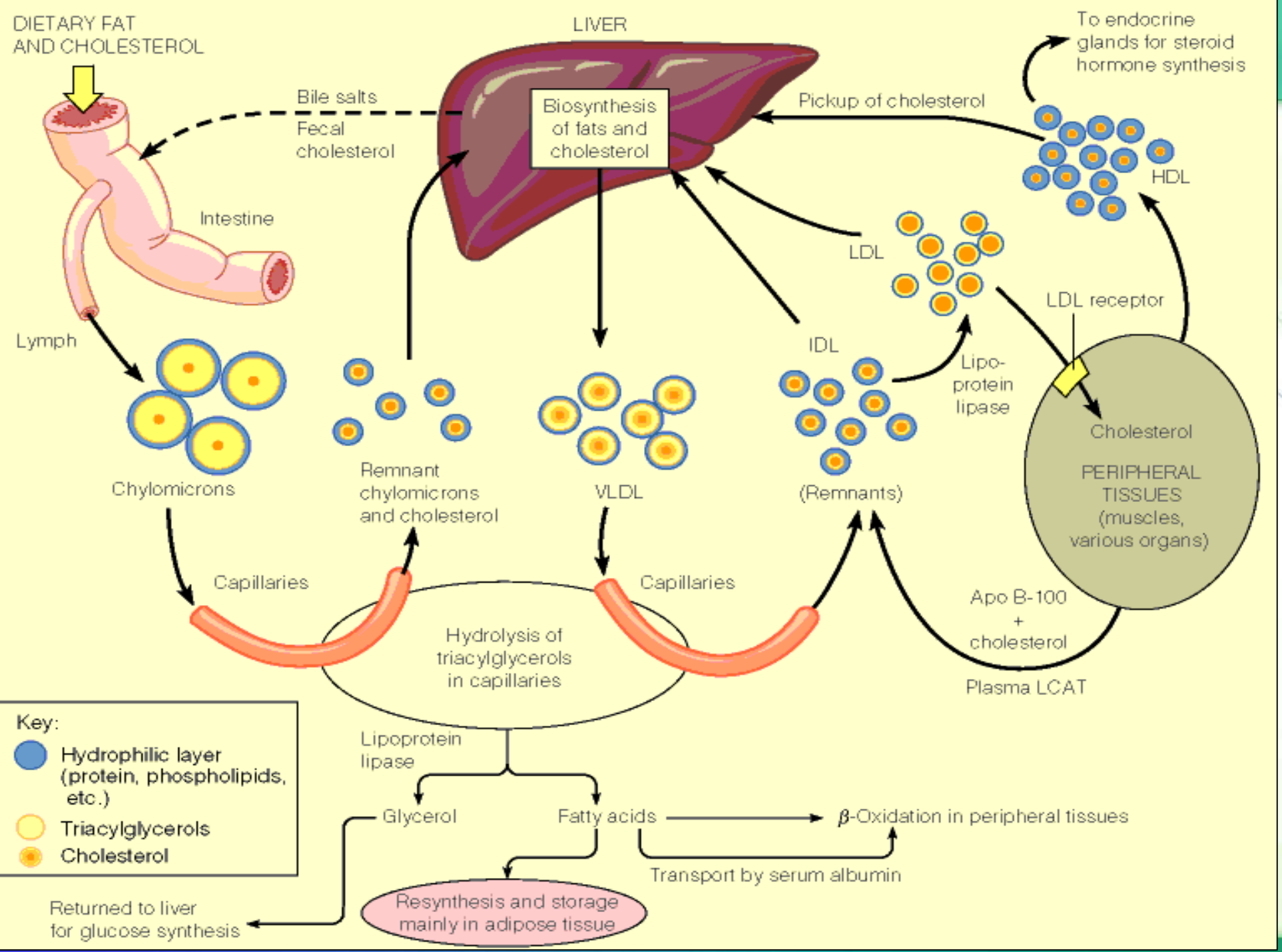
- Chylomicrons are the least dense, HDLs have the highest density
- Chylomicrons contain a lot of TAGs and some cholesterol esters, HDL + LDL contain a lot of cholesterol esters, VLDLs contain a lot of TAGs, but less than Chylomicrons

Composition of lipoproteins

Numbers are NOT for memorization,BUT you have to know which one has the highest density,lowest density and so on. The rows which are in red boxes are required ☺

	Chylomicrons	VLDL	LDL	HDL
Density (g/ml)	< 0.94	0.94-1.006	1.006-1.063	1.063-1.210
Diameter (Å)	2000-6000	600	250	70-120
Site of synthesis	Intestine	Liver	Liver	Liver, intestine
Total lipid (wt%)	99	92	85	50
Triacylglycerols	85	55 Liver	10	6
Cholesterol esters	3	18	50 (bad)	40 (good)
Apolipoproteins	A, C, E, **B48**	C, **B100**, E	B100	A, C, E
Function	Transport of <u>dietary</u> TG to the liver	Transport of TG from the liver to peripheral tissues	Transport of cholesterol from the liver to peripheral tissues	Transport of cholesterol from peripheral tissues back to the liver (cholesterol scavengers)

Indicates the molecular weight of the protein

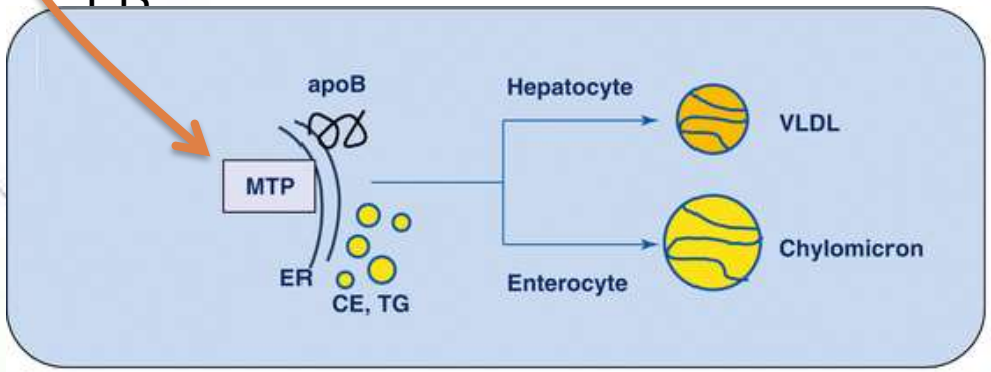


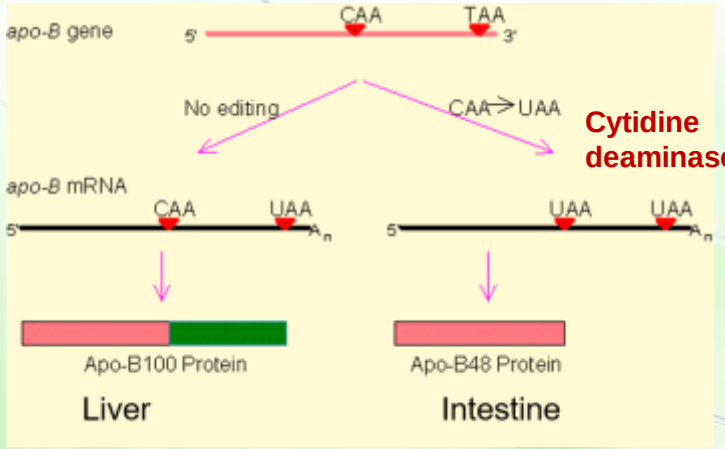
Lipid transport

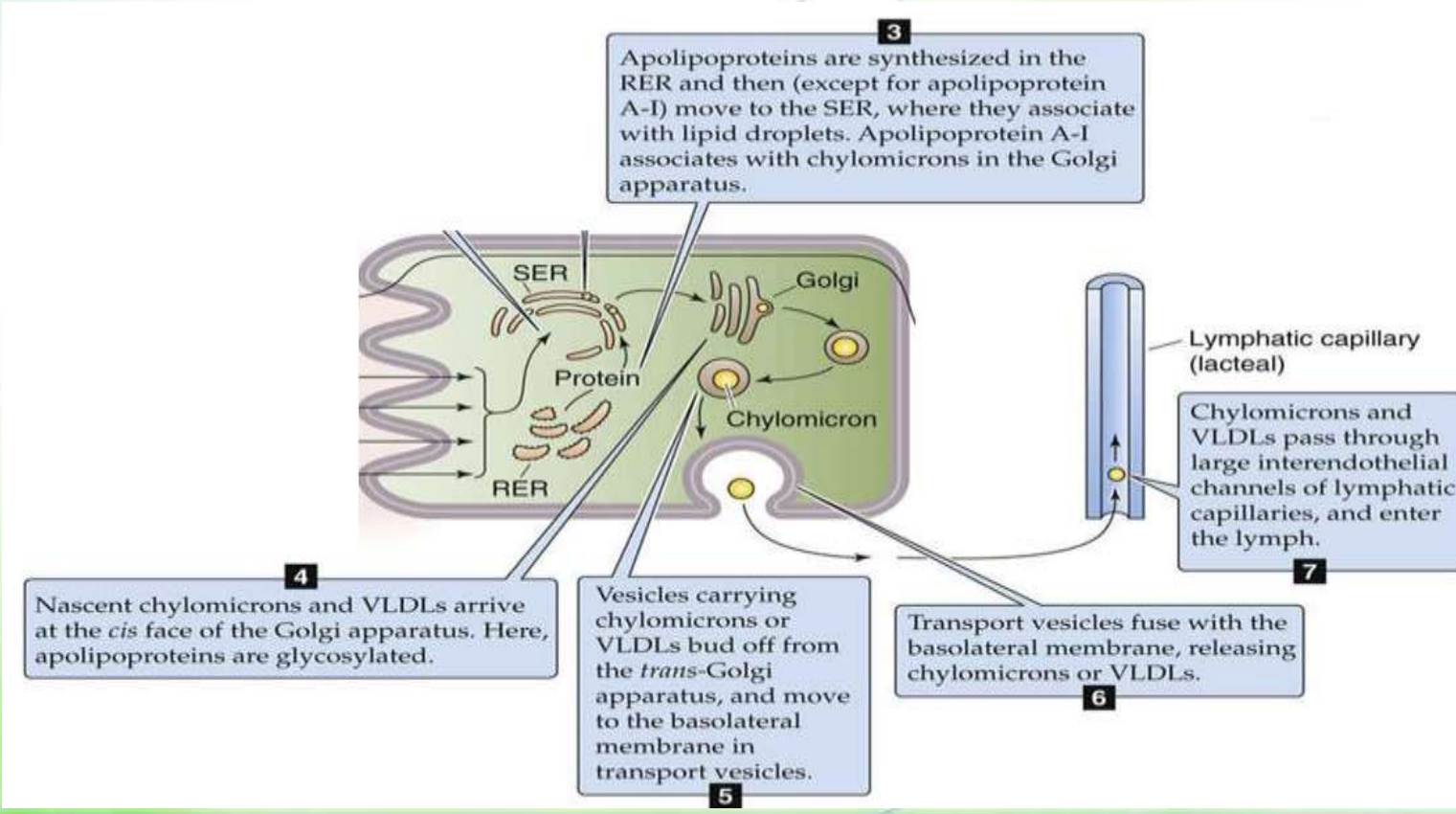
- Chylomicrons carry dietary lipids (TAGs and cholesterol esters) from the intestines and all the way to the liver through the lymphatic system. Fatty acids that are attached to TAGs are released from glycerol and they migrate to tissues. Eventually, TAG's content in chylomicrons decreases and Chylomicrons are thus called ' Chylomicrons remnant ' which are small in size, contain a lot of proteins and cholesterol esters.TAGs undergo simplification, due to the release of fatty acids. Now once these remnants reach the liver, the liver will produce VLDL which are smaller than the original Chylomicrons but larger than HDLs and LDLs. These VLDLs (which carry a lot of TAGs and cholesterol esters as well) leave the liver and travel in the capillaries, now fatty acids are released from TAGs and they move into tissue. Eventually, VLDLs are converted into IDLs, then IDLs will be converted into LDL. Notice that LDLs carry a lot of cholesterol esters which are transported into peripheral tissues. So, the main function of LDL is to carry cholesterol esters to peripheral tissues and this is one reason why LDL is known as 'bad cholesterol', increase FA content in peripheral tissues. Also, LDLs tend to aggregate and cluster in blood vessels if present in high amounts in blood vessels resulting in ATHEROSCLEROSIS. HDLs, on the other hand, carry cholesterol esters from peripheral tissues back to the liver (removes excess cholesterol from the body), that's why it's known as'Good Cholesterol'

Formation and release of chylomicrons

- TAG and cholesteryl esters are packaged in chylomicrons made of phospholipids, nonesterified cholesterol, and apolipoprotein B-48.
- Microsomal triglyceride transfer protein (MTP) is essential for the assembly of all TAG-rich apoB-containing particles in the ER.

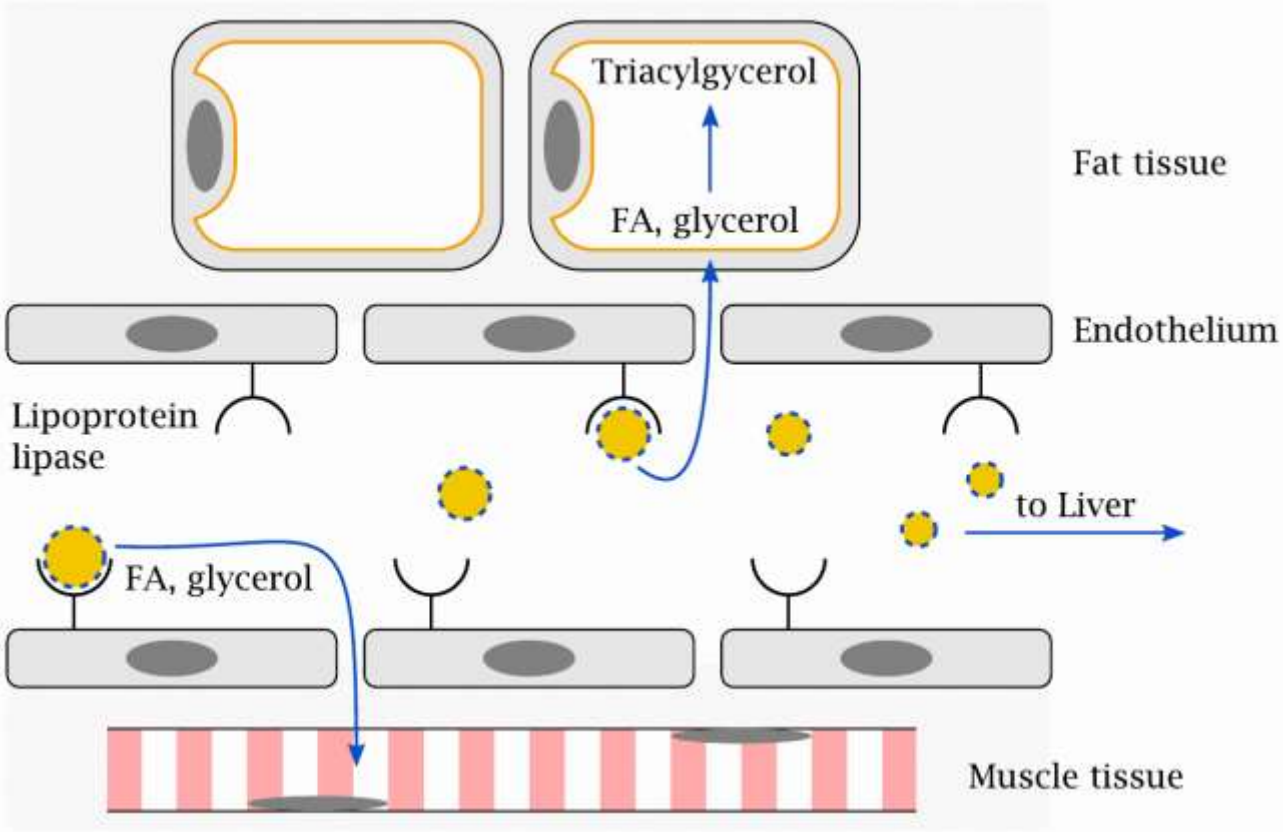






- Chylomicrons formation occurs in enterocytes.
- Apo B48 is structural protein which is involved in the formation of Chylomicrons .
- MTP is responsible for the assembly, it attaches B48 with lipid molecules into Chylomicrons.
- MTP is also found in hepatoocytes, associated with the formation of VLDLs
- apoB48 (in chylomicrons) and apoB100 (in VLDL) are structural proteins and they are produced from the same gene but they have different polypeptide chains ,how so? In entrocytes, RNA editing happens , the gene is transcribed into mRNA, this mRNA is modified in intestines by cytidine deaminase (removes an amino group), it converts Cytosine (amino group) into Uracil (keto group) , leaving the mRNA with a UAA (a stop codon), so the enterocyte’s copy is shorter than the liver's.

Fates of TAGs in chylomicrons



- TAGs in chylomicrons are hydrolyzed in the bloodstream by lipoprotein lipases that are anchored into the surface of endothelial cells.

- The resulting fatty acids have two possible fates:

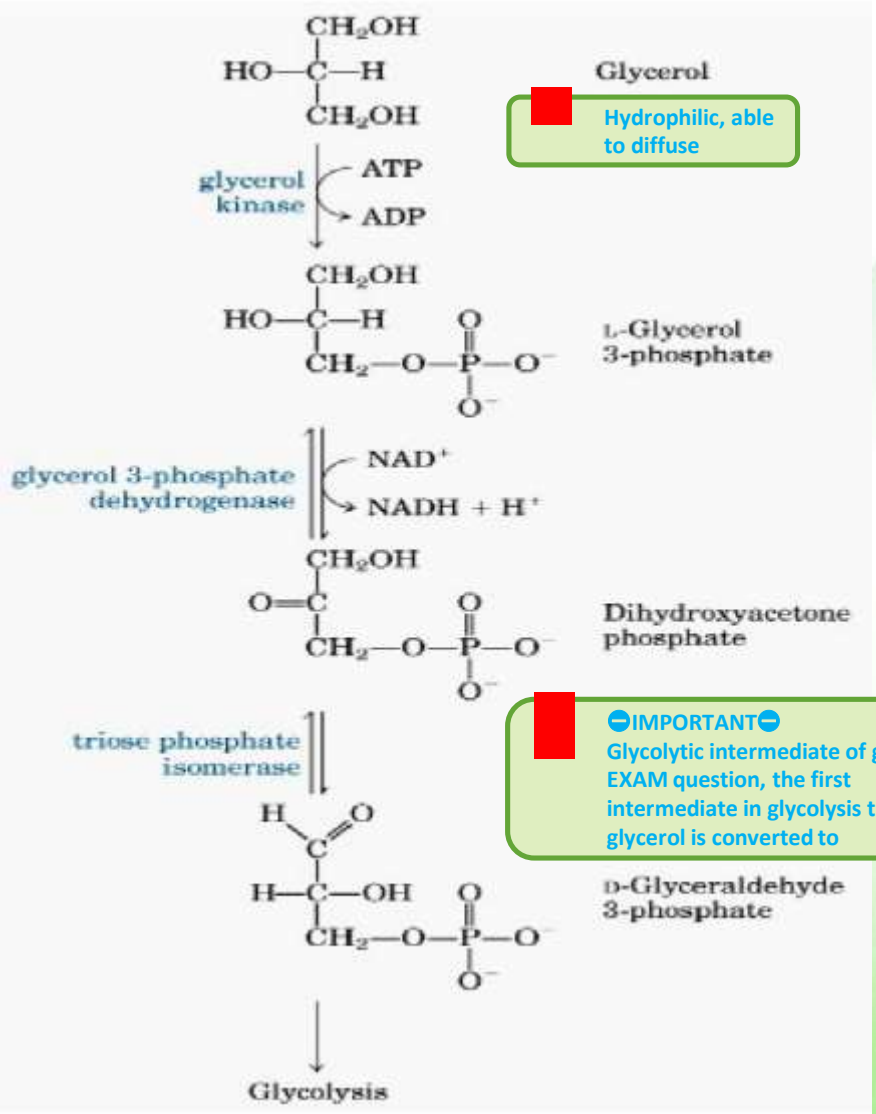
(1) When energy is in good supply, they are converted back to TAGs for storage in adipose tissues.

(2) When cells need energy, the fatty acids are oxidized into acetyl-CoA.

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

- **There are four types of lipsae: lipoprotein lipase, lingual, gastritic, pancreatic.**
- **Lipoprotein lipase detaches fatty acids that are associated with TAG in chylomicrons, releases fatty acids into tissues.**
- **To be active, it must interact with ApoC that is present in Chylomicrons (and VLDLs) with lipoprotein lipase that's present on the cell surface.**
- **In *Familial (hereditary) chylomicronemia (type I hyperlipoproteinemia) , LPL will be nonfunctional due to either a mutation in LPL or apoC , thus they will have high levels of chylomicrons and VLDL.***

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. (Depending on the energy status)

Metabolism of lipids II:

Synthesis of fatty acids

Prof. Mamoun Ahram

Resources

- This lecture
- Lippincott's Biochemistry, Ch. 16

Overview of fatty acid synthesis

Major sites: liver and adipose tissues

- The fatty acids are synthesized by:
  1. Production of malonyl CoA — From Acetyl CoA
  2. Binding of acetyl CoA and malonyl CoA to the fatty acid synthase
  3. Condensation of acetyl CoA and malonyl CoA — Forming a 4 carbon molecule
  4. Elongation of the acyl CoA by 2 carbons per round
     - Reduction, dehydration, reduction
  5. Binding of malonyl CoA
  6. *Repeat steps 3 (acyl CoA), 4, and 5* — Those steps will repeat (increasing 2 carbons at a time) until reaching 16 carbon molecule "palmitoyl CoA, saturated fatty acid" which is released in the form of palmitate
  7. Release of the hydrocarbon chain by a thioesterase (TE)

(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.

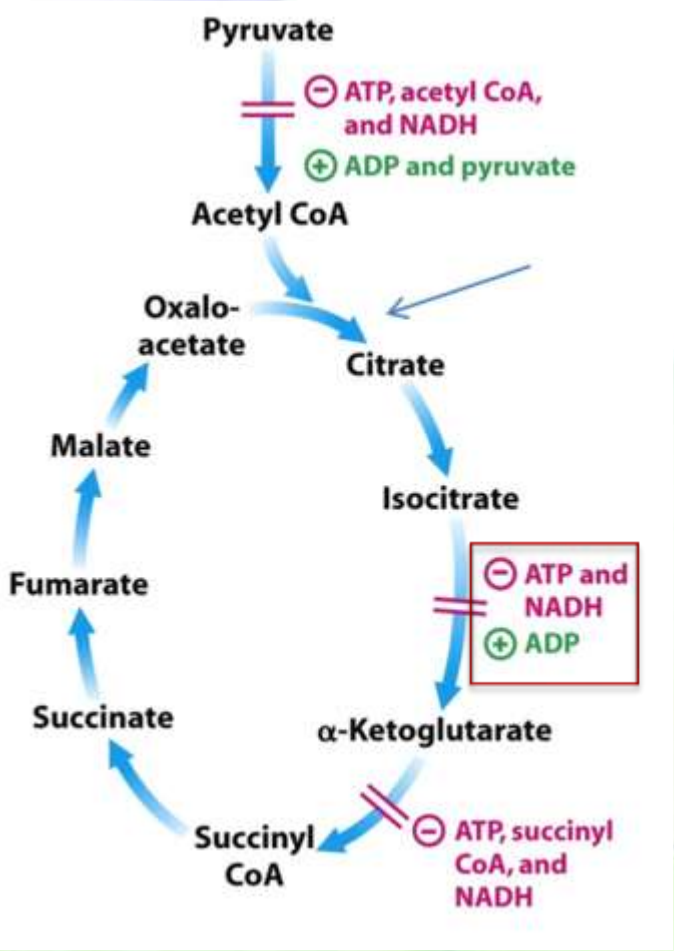
4 .Elongation of acyl CoA by 2 carbons per round (3 reactions) (reduction, dehydration, reduction) will be explained later

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

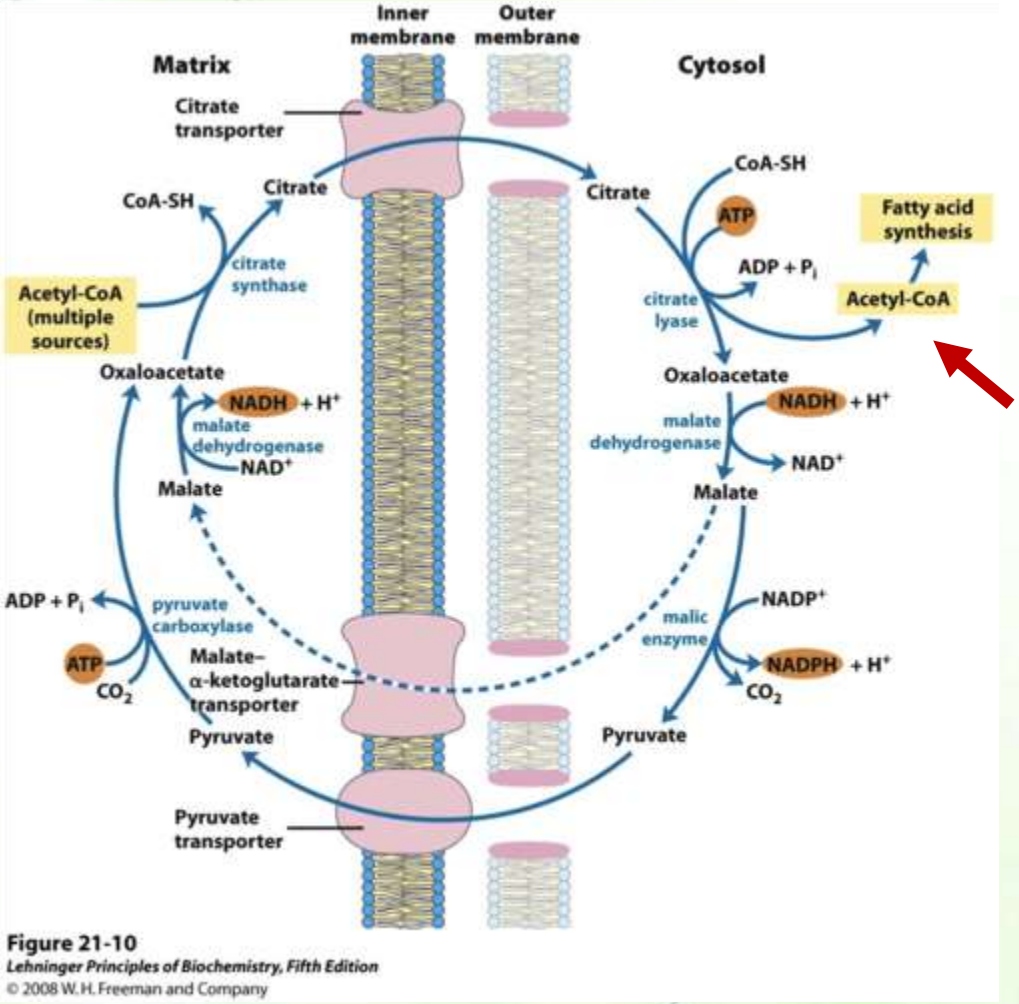


Figure 21-10
Lehninger Principles of Biochemistry, Fifth Edition

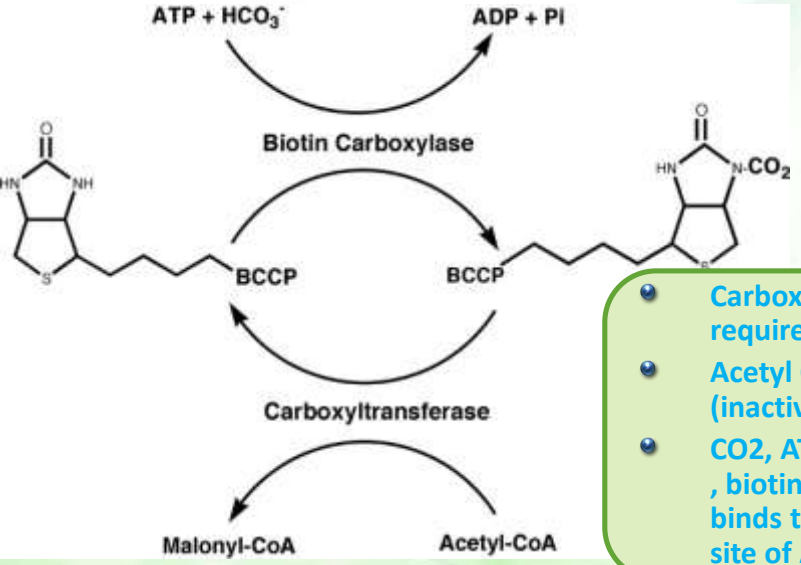
Glucose can be converted to fat, but fat cannot be converted to glucose.

- Let’s trace the process. As we have learned about the TCA cycle, Isocitrate is converted into alpha ketoglutarate, the enzyme responsible is Isocitrate dehydrogenasewhich is highly regulated by the level of energy. If ATP,NADH are high, this means that we have enough energy so there is no need for the TCA cycle and the reaction is inhibited. On contrast, when ADP is high, that indicates the lack of energy so it will be activated. When inhibited, Isocitrate accumulates and thus will be converted back to Citrate (in the mitochondrial matrix), Citrate will leave the mitochondria through a citrate transporter into the cytosol. It will be degraded into oxaloacetate and acetyl CoA (so the mode of transport of acetyl CoA from the mitochondrial matrix into the cytosol is Citrate). Fatty acid synthesis occurs in the cytosol, on contrast to the breakdown which happens in the mitochondrial matrix and this is one of the mechanisms that the cell uses for regulating metabolism. That’s why glucose can be converted to fatty acids but the opposite in not true (Glucose—> Acetyl CoA—> Fatty acids)

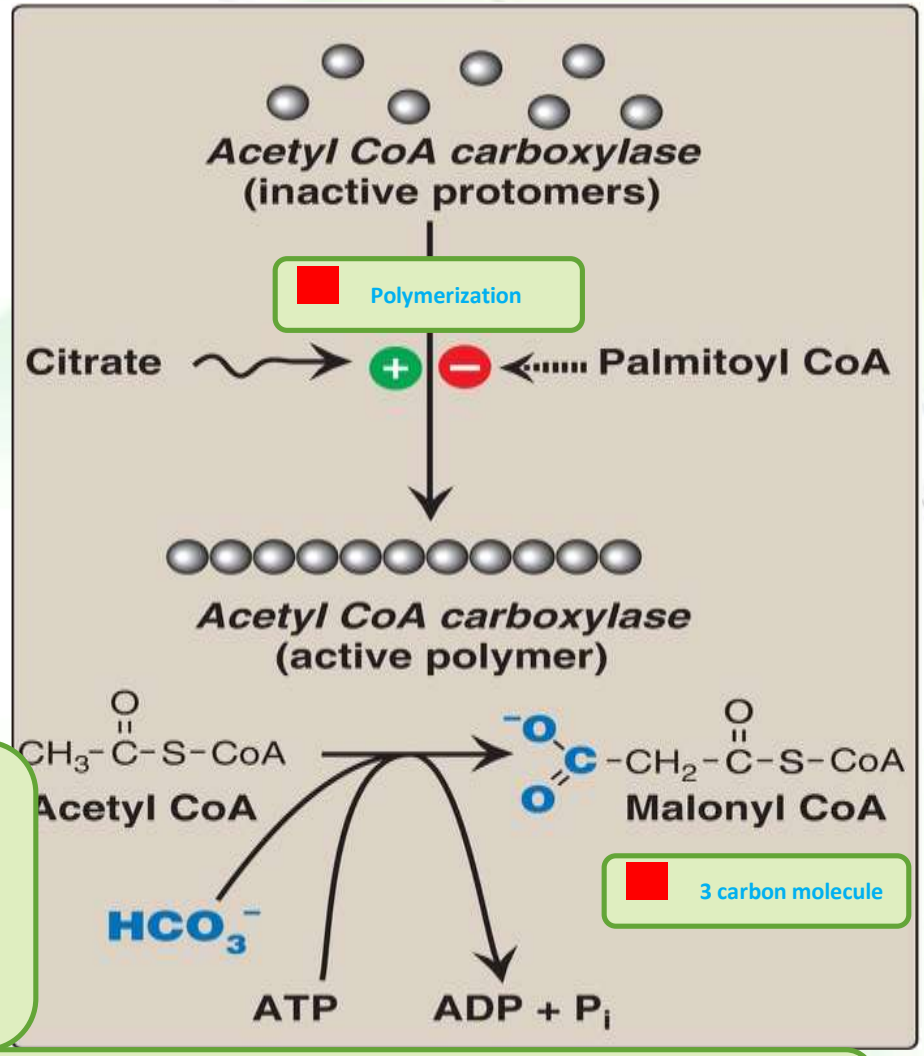
Synthesis of malonyl-CoA

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 takes CO2 and gives back to substrate .

- ATP is needed.
- The reaction is a rate-limiting reaction.

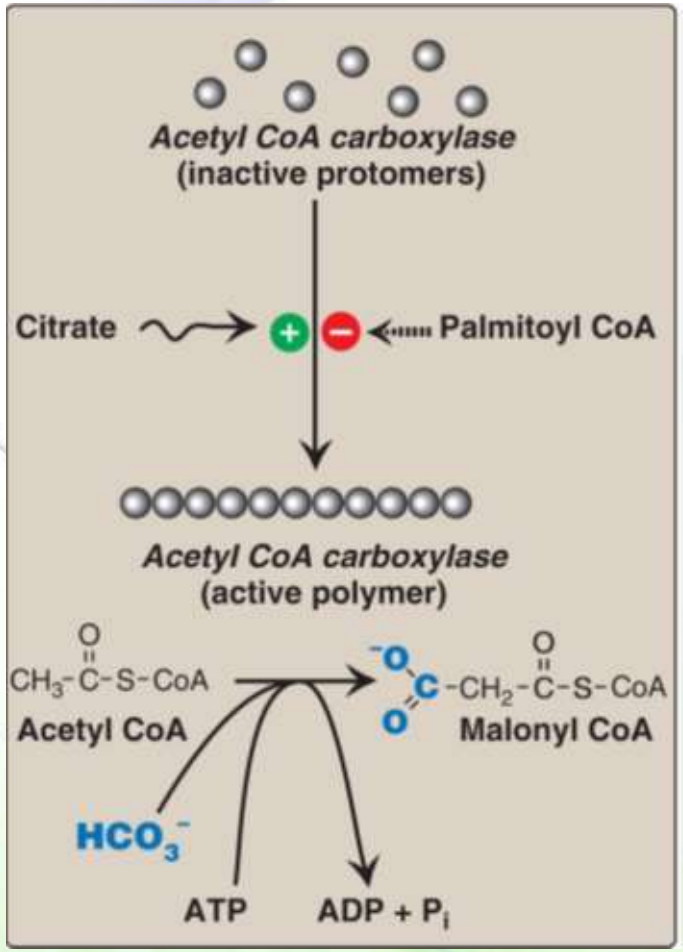


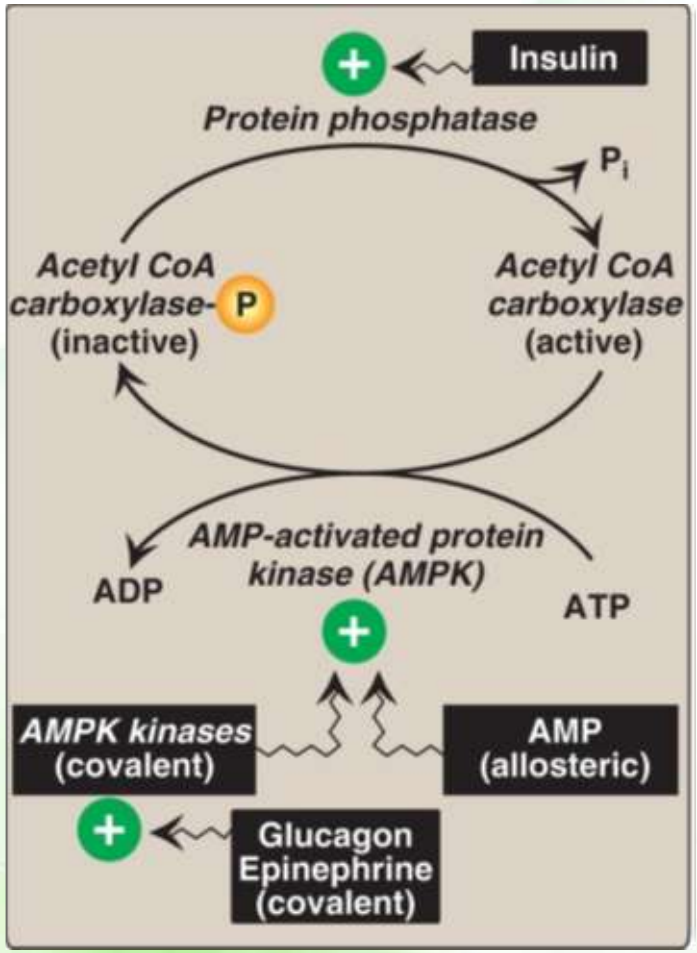
- Carboxylation reaction requires:
- Acetyl CoA carboxylase (inactive protomers)
- CO2, ATP (for condensation) , biotin as a cofactor (that binds to lysine in the active site of ACC to be activated).



- ACC is highly regulated by different steps and mechanisms , for example, it exists as separate polypeptide chains and when the level of Citrate is high (high energy), these polypeptides polymerize and the enzyme becomes active so Anabolism can start.
- But the end product which is Palmitoyl CoA inhibits the enzyme (enough making more of me, do something else with the acetyl coA)

Regulation of ACC





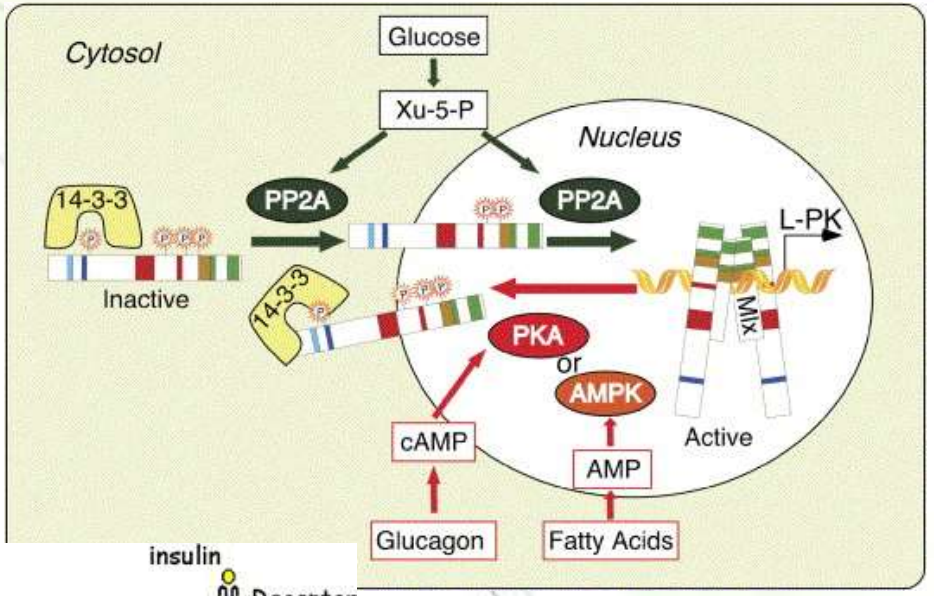
- ACC is inactivated by:
  - Palmitoyl-CoA
  - Phosphorylation by AMPK, which is activated by glucagon and epinephrine.

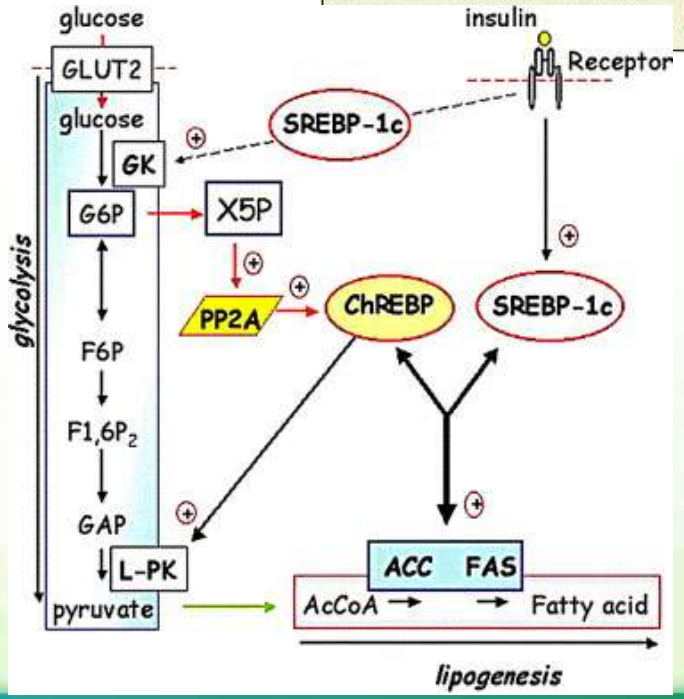
- **The complement in this slide:** Hormonal regulation of Acetyl CoA carboxylase:
- When the levels of Glucagon or Epinephrine elevate due to starvation or lack of energy, then it's an indication of the need to produce energy and to stop the synthesis of fatty acids . Glucagon and epinephrine bind to the receptor and activate PKA which phosphorylates AMP kinase thus activating it. AMP kinase is critical in the metabolic process that it phosphorylates ACC and inhibits its action.
- Another situation, when Insulin's levels elevate (due to the abundance of glucose,high energy state), it binds to the receptor and activates phosphatase enzyme which removes a phosphate group from the inactive phosphorylated ACC , thereby activating it and synthesis of fatty acids go on.

- **Additional information:** This is a mean of short-term regulation

Regulation of ACC synthesis

- ACC synthesis is regulated transcription factors:
  - The carbohydrate response element–binding protein (ChREBP)
    - ChREBP is inactivated by phosphorylation by PKA and AMPK preventing its nuclear localization.
    - It is dephosphorylated by excess glucose.
  - The sterol regulatory element–binding protein-1c (SREBP-1c)
    - SREBP-1 is activated by Insulin.
- Fatty acid synthase, glucokinase, ATP citrate lyase and liver pyruvate kinase are similarly regulated.

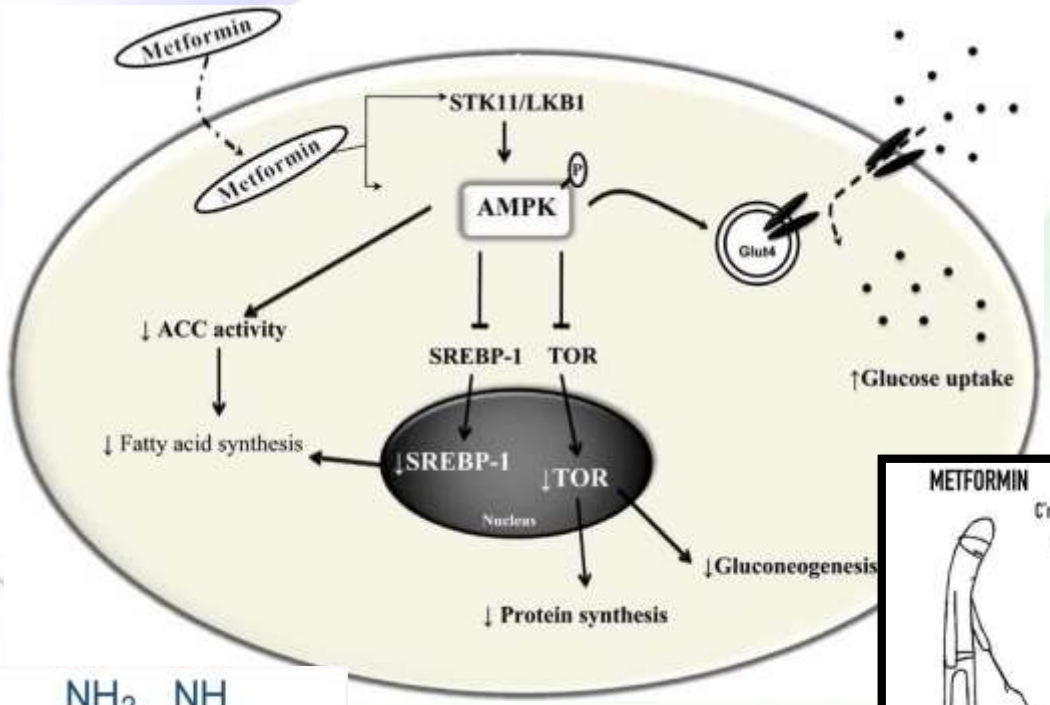




- **The complement in this slide:** The regulatory mechanisms mentioned previously (eg: allosteric regulation, phosphorylation..etc) are quick. On the other hand, a slow mechanism of regulation also takes place which is GENE EXPRESSION. Now let's trace the process…
- Transcription includes the binding of RNA polymerase to the promoter region, RNA polymerase is regulated through the action of transcription factors which bind to the promoter region on the DNA and regulate the RNA polymerase either to activate or inhibit its action. Regions in the promoter which transcription factors bind to are called "Elements".
- We have 2 transcription factors here, ChREBP and SREBP. ChREBP is in the cytosol, AMP kinase phosphorylates it (Remember that AMP kinase gets activated when levels of AMP are high and AMP is an indication of a low energy state so fatty acid synthesis cannot proceed), the phosphorylated ChREBP cannot get into the nucleus and therefore no transcription of Acetyl CoA carboxylase. This is opposed by the binding of Insulin which removes the previously added phosphate group through the action of phosphatase, so ChREBP can now get into the nucleus and bind to the element which will eventually lead to the expression of Acetyl CoA carboxylase and fatty acid synthesis .
- These elements exist also in other promoter regions of different genes, so ChREBP doesn't merely activate ACC, other anabolic enzymes that are utilized in different pathways will be activated too, like glycogenesis. So many genes are activated by ChREBP also many are inhibited when its stuck in the cytosol. **Notice the HARMONY !**سبحان الله

- **Additional information:** This is a mean of long-term regulation.

Metformin

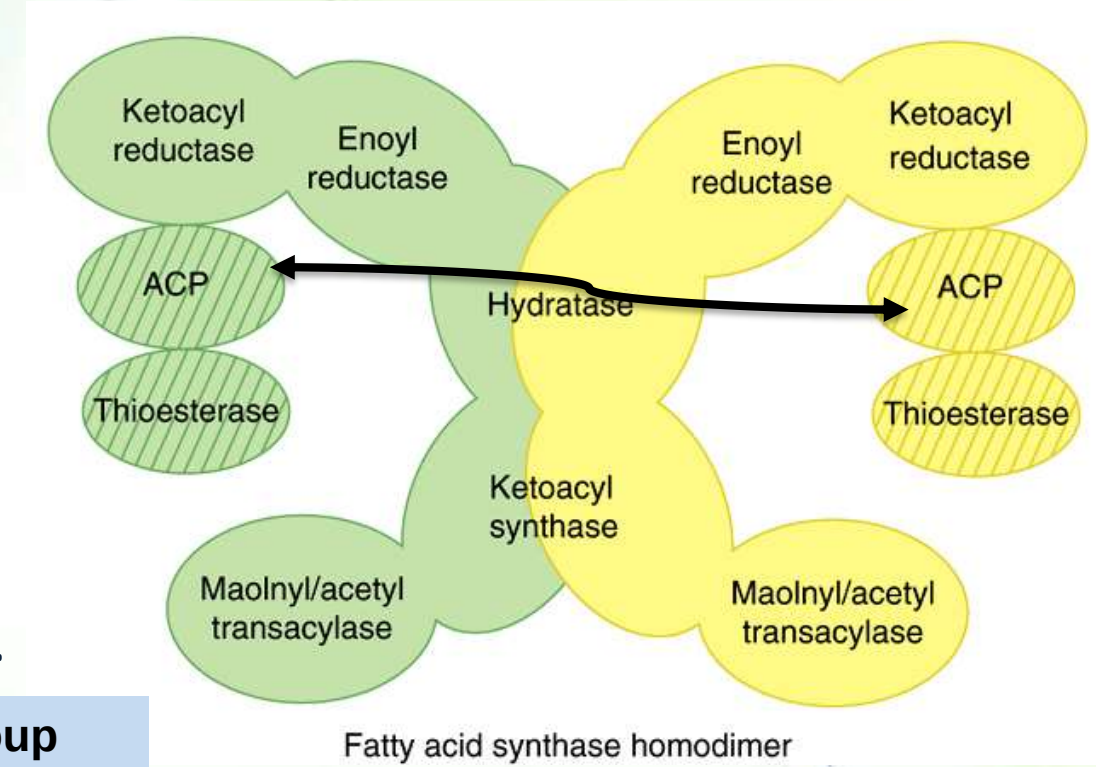


- Metformin lowers plasma TAG through
  - Activation of AMPK, resulting in inhibition of ACC activity (by phosphorylation) and inhibition of ACC and fatty acid synthase expression (by decreasing ChREBP and SREBP-1c).
- It lowers blood glucose by increasing AMPK-mediated glucose uptake by muscle.

NOTE: **Metformin (trade name : Glucophage) is given to pre-diabetic individuals which are susceptible of having diabetes. Its action is by increasing the uptake of glucose into cells and oxidizing it to get energy.Also uptakes TAG, increases the breakdown of fatty acids in the body and inhibits FA synthesis thereby decreasing lipid and glucose amounts in the body. That's how Metformin protects against diabetes!**

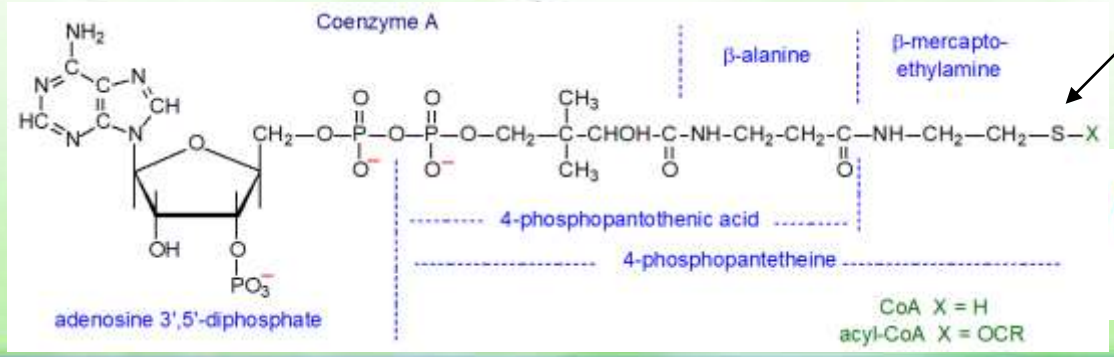
Fatty acid synthase (FAS)

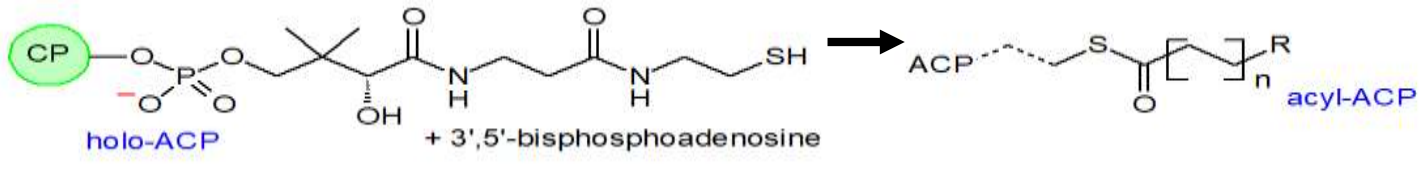
- A multifunctional, homodimeric enzyme
- Each FAS monomer is multicatalytic with six enzymic domains and a domain for binding a phosphopantetheine-containing acyl carrier protein (ACP) domain.
- Phosphopantetheine, a derivative of pantothenic acid (vitamin B5), carries acyl units on its terminal thiol (–SH) group and presents them to the catalytic domains of FAS.
- It also is a component of CoA.



Fatty acid synthase homodimer

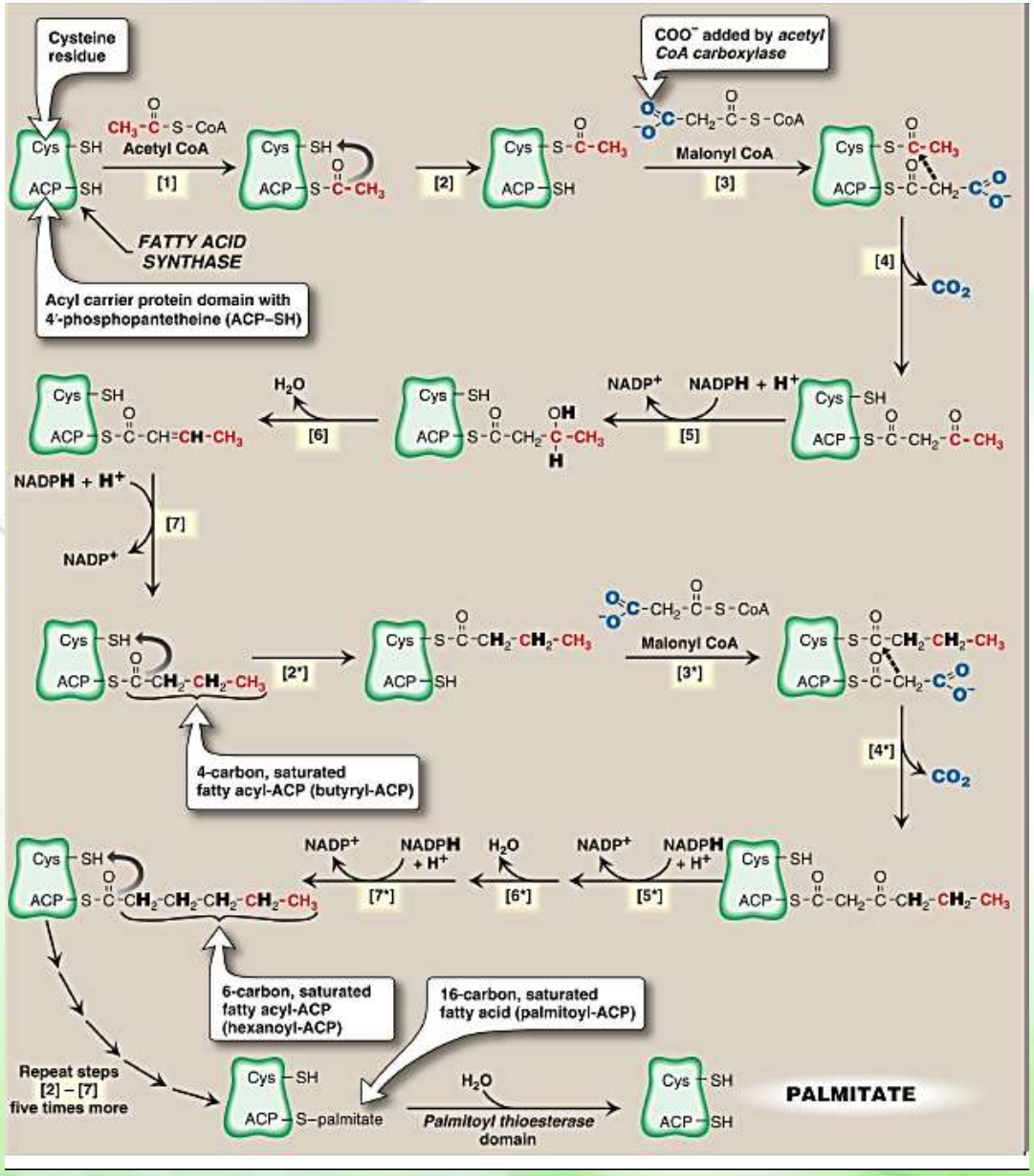
Thiol group (the functional group)

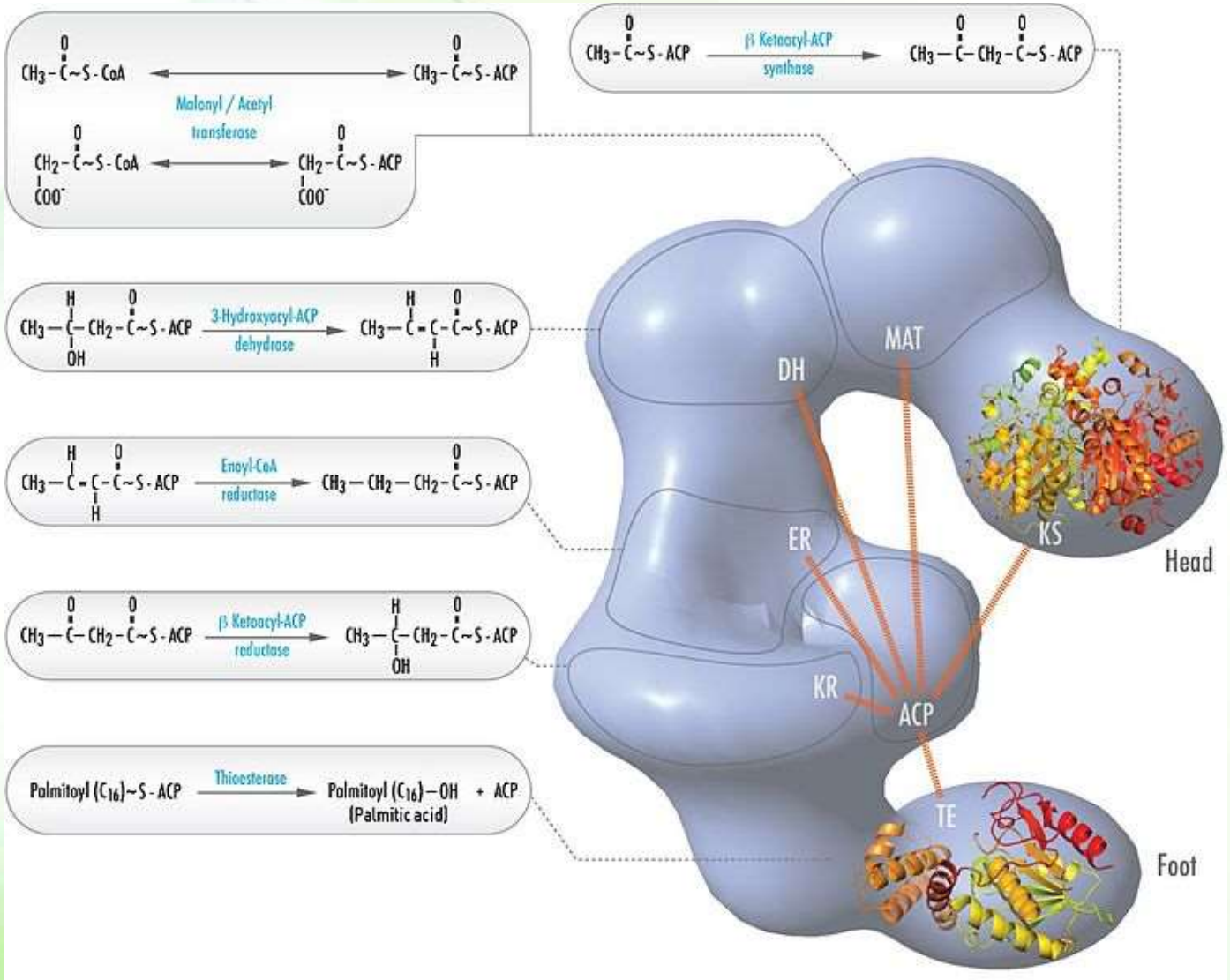




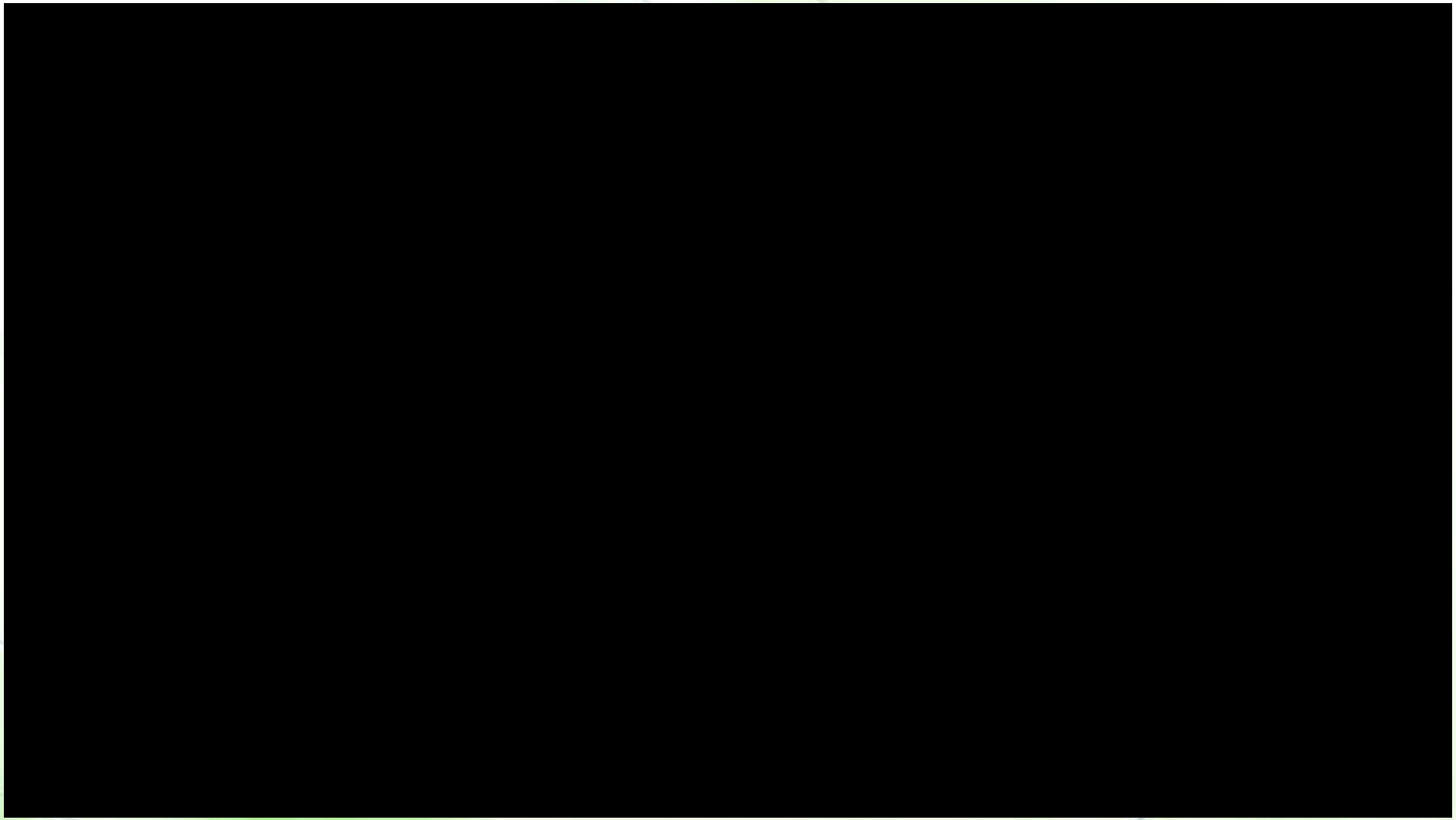
- **Additional information:** The remaining series of reactions of fatty acid synthesis is catalyzed by the multifunctional, homodimeric enzyme fatty acid synthase (FAS).

- **The complement in this slide:** Now Fatty acid synthase: It's a huge enzyme, composed of 2 identical polypeptide chains, each has 6 different enzymic domains and a 7^{th} domain which is an Acyl Carrier Protein (binds to an acyl molecule) . This acyl is part of Phosphopantetheine (a derivative of Vitamin B5).


Cysteine residue
Acetyl CoA
[1]
COO⁻ added by acetyl CoA carboxylase
Malonyl CoA
[3]
[2]
FATTY ACID SYNTHASE
Acyl carrier protein domain with 4′-phosphopantetheine (ACP–SH)
[4]
CO2
NADPH + H+
NADP+
[5]
H2O
[6]
[7]
[2*]
[3*]
[4*]
4-carbon, saturated fatty acyl-ACP (butyryl-ACP)
[5*]
[6*]
[7*]
6-carbon, saturated fatty acyl-ACP (hexanoyl-ACP)
16-carbon, saturated fatty acid (palmitoyl-ACP)
Repeat steps [2] – [7] five times more
Palmitoyl thioesterase domain
PALMITATE


Malonyl / Acetyl transferase
β Ketoacyl-ACP synthase
3-Hydroxyacyl-ACP dehydrase
Enoyl-CoA reductase
β Ketoacyl-ACP reductase
Thioesterase
Palmitoyl (C16)–S-ACP
Palmitoyl (C16)–OH (Palmitic acid)
+ ACP
MAT
DH
KS
ER
KR
ACP
TE
Head
Foot

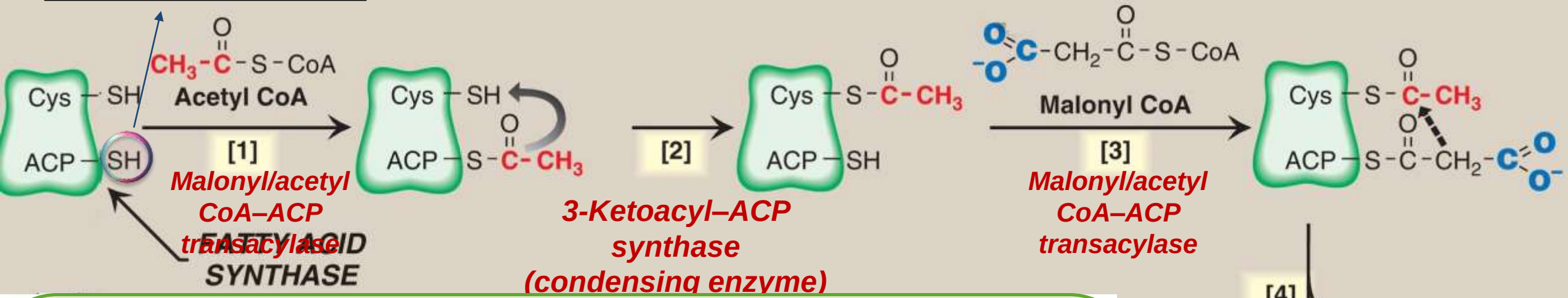
Ketoacyl synthase (KS)
Malonyl/acetyltransferase (AT)
Dehydrase (DH)
Enoyl reductase (ER)
Ketoacyl reductase (KR)
Thioesterase (TE)
Acyl carrier protein (ACP)



po

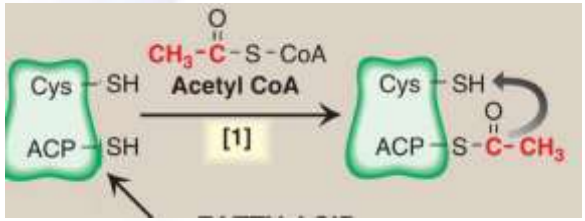
SH resembles Phosphopantetheine

- Question: The energy that is responsible for condensing the acyl molecule with malonyl CoA comes from :
- Ans: Decarboxylation

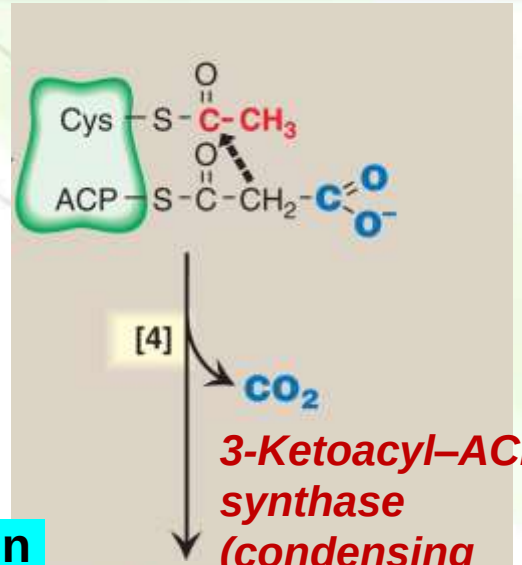


- **NOTE:** Let's dive into the rxn. Look at the enzyme, it has a cysteine residue that its R group ends with a thiol group "كتير منيح"
- At first, Acetyl group of Acetyl CoA detaches and binds with the thiol group of ACP. Then acetyl is transferred to the thiol group of the cysteine and binds to it and the ACP is empty now.
- Now malonyl CoA enters and binds to the ACP and the active site now is bound to an acetyl group plus a malonyl group.
- One of the strategies that enzymes do is to proximate substrates in the right orientation. Malonyl can now attack acetyl which results in decarboxylation (removal of CO2 which is the source of energy)

Condensation, reduction, dehydration, reduction



- Reduction = We need a source of electrons [NADPH]
- NADPH is used in anabolic reactions, NADH is used in oxidation/breakdown

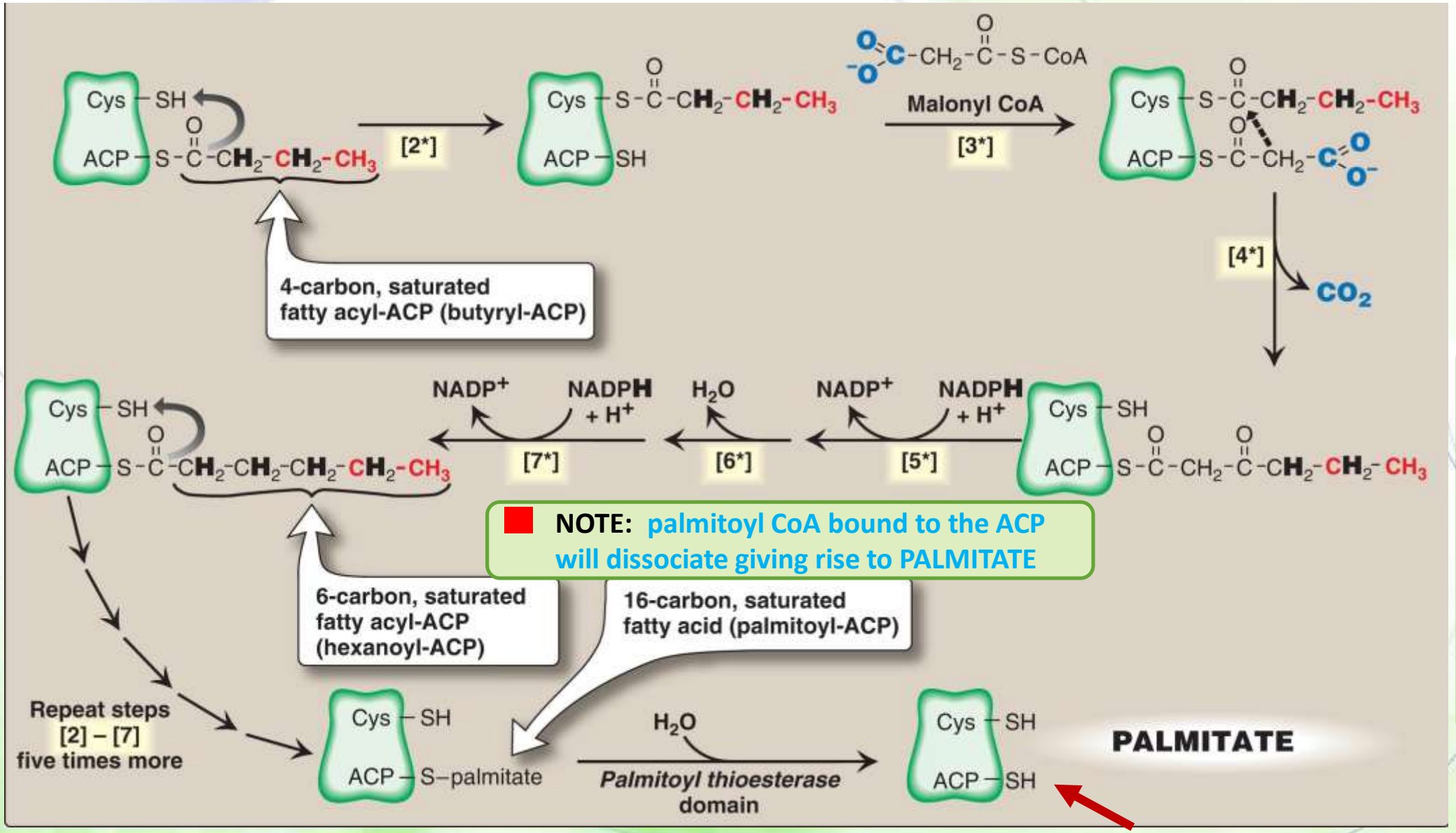


Compare to the first reaction in the previous slide

- The same thing except that here we have 4 carbons but previously we had 2

- **NOTE:** At rxn [5] we have a keto group (C=O), by reduction, the ketone will transform into a secondary alcohol.
- At rxn [6] dehydration will occur, which results in the formation of a double bond between the 2 carbons
- At rxn [7] the double bond is reduced.
- At the end we have 4 carbons molecule that will be transferred into the cysteine residue [recall rxn 1].

NOTE: The process keeps looping 🔁 until we have the desired number of carbons



NOTE: palmitoyl CoA bound to the ACP will dissociate giving rise to PALMITATE

The lactating mammary gland terminates lengthening the chain EARLY.

NOTE:
Fatty Acids produced in lactating mammary glands will have short-medium chains.

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 + $7P_i$

- **NOTE:** Palmitate (16C) = 7*Malonyl CoA (2C each) + Acetyl CoA (2C)
- 14 NADPH : each cycle needs 2 NADPH
- 7 CO2 — comes from 7 Malonyl CoA
- Each malonyl CoA synthesized by ACC needs 1 Acetyl CoA, 1 CO2 and 1 ATP