24

◉Sheet ○Slides

DONE BY

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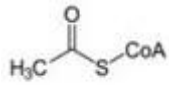
Fatty Acid Synthesis

- Occurs mainly in the Liver (to store excess carbohydrates as triacylglycerols(fat)) and in lactating mammary glands (for the production of milk in the breast) and to a lesser extent, in adipose tissue.

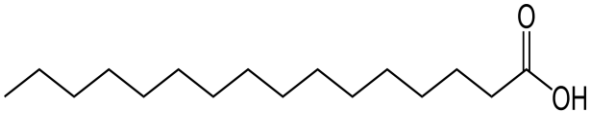
  Note: it occurs mainly in the liver because it receives the blood coming from the small intestines which carries the substances that are absorbed from the intestines (remember that blood carries glucose and other carbohydrates).

- It requires 3 substances:
  1- **Carbon source** → Acetyl CoA *(it is the only source of carbons in FAS)*
  2- **Reducing power** → NADPH.
  We need NADPH as a reducing power in fatty acid synthesis because we are going from a less reduced/more oxidized molecule to a more reduced/less oxidized molecule.
  Remember that in NADPH is used in reductive biosynthetic process.

Note:
Reduction → increasing Hydrogen or decreasing Oxygen.
Oxidation → decreasing Hydrogen or increasing Oxygen.
So, for example if we compare Palmitic acid with Acetyl CoA (starting molecule of fatty acid synthesis) we'd see that Palmitic acid (a 16-carbon fatty acid) has relatively more Hydrogen atoms than Acetyl CoA.



Acetyl CoA



Palmitic acid

  3- **Energy input** → ATP.
  Energy is required in anabolic reactions.

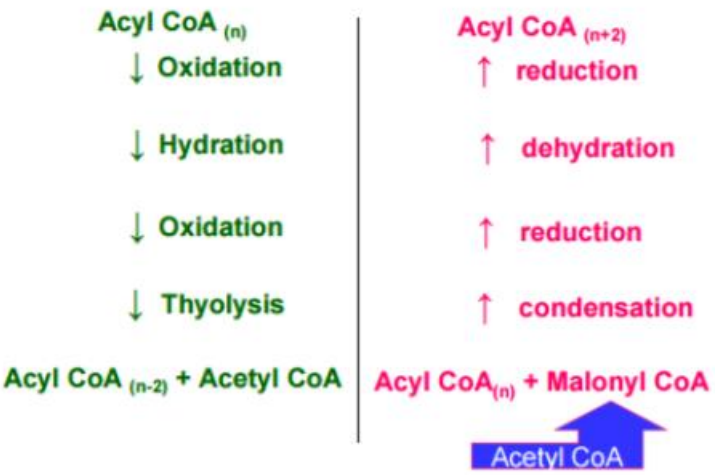
-The degradation of fatty acids by beta oxidation produces NADH, FADH2 and also releases some of the energy in the form of heat. So, overall the △G is negative.

While in the synthesis of fatty acids in which we are going from Acetyl CoA to a newly synthesized fatty acid (opposite from the degradation of fatty acid) △G is positive but by *coupling it with ATP hydrolysis* △G would then be **negative**.

Note: Enzymes reduce the activation energy.

Comparison between FA synthesis and degradation:

If you notice in the picture the two processes are (chemically speaking) the opposite of each other.



In FA degradation Acyl CoA undergoes repeated cycles of beta oxidation.

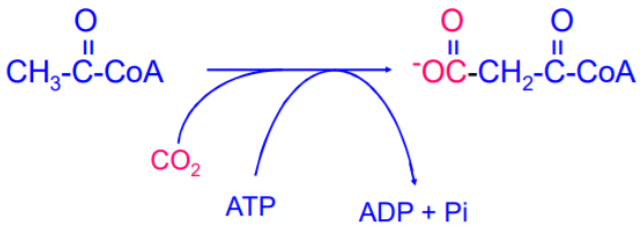
In every cycle the following happens: oxidation of the Acyl CoA followed by hydration then oxidation for the second time and then thyolysis and by that we would have removed 2 carbons from the Acyl CoA in the form of Acetyl CoA.

In FA synthesis the opposite of that happens, Acyl CoA undergoes condensation (opposite of thyolysis) with Malonyl CoA (derived from Acetyl CoA; we'll cover this reaction next) and then the resultant molecule undergoes reduction then dehydration and finally reduction again and by that we would have added 2 carbons to the Acyl CoA.

Note: They use different enzymes and occur at different places in the cell; Oxidation occurs in the Mitochondria whereas Synthesis occurs in the cytoplasm.

Malonyl CoA:

- It is a dicarboxylic acid that comes from the carboxylation of Acetyl CoA.



This reaction is catalyzed by **Acetyl CoA Carboxylase** (this reaction is considered the first step of FA synthesis and is *the rate limiting step*).
Acetyl CoA Carboxylase needs biotin (carrier of the activated CO2) as a coenzyme.

Carboxylation reactions are endergonic while **Decarboxylation** reactions are exergonic. So, we need energy in order for this reaction to proceed in the forward direction; this energy comes from the hydrolysis of ATP.

recall from sheet 14
The addition of CO2 (from HCO3-) to biotin requires ATP.

(with ATP this rxn becomes exergonic because the energy required to add CO2 is less than the energy released from the hydrolysis of ATP)

The remaining 4 steps are catalyzed by the same enzyme complex **Fatty Acid Synthase.**

Fatty Acid Synthase:

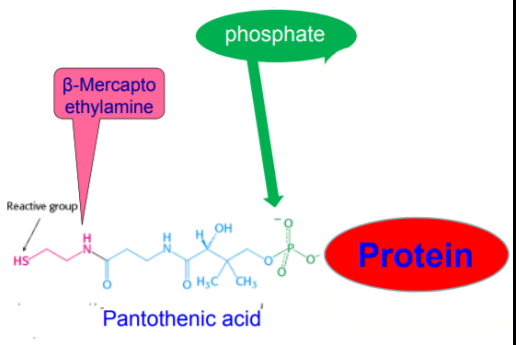
- It is a multifunctional enzyme complex that is responsible for catalyzing 4 reactions in FA synthesis. What do we mean by saying that it's an enzyme complex?
  It is a large enzyme that has several active sites (each catalyzes a specific reaction) in which the product of each reaction is the substrate of the next reaction.
- It is a dimer of two identical polypeptide chains.
  Although the two chains are identical, one polypeptide is not active alone (because part of the reactions occur at one of the polypeptide chains and the other part occurs at the other polypeptide chain), but when they dimerize, they produce an active enzyme (they complete each other).
- Each has seven catalytic sites.
- One active site is the **Condensing Enzyme** usually called **CE** for short (it catalyzes condensation and has a reactive -SH group).
- One domain is **Acyl Carrier Protein** usually called **ACP** for short.

This domain is linked to Phosphopantetheine which:

- has a reactive -SH group in which FA synthesis intermediates bind to.

- made of 1 phosphate + pantothenic acid + β-mercapto ethylamine linked to a protein.

What makes it differ from CoA is the absence of a second phosphate group, and ribose and adenine (replaced by the protein).



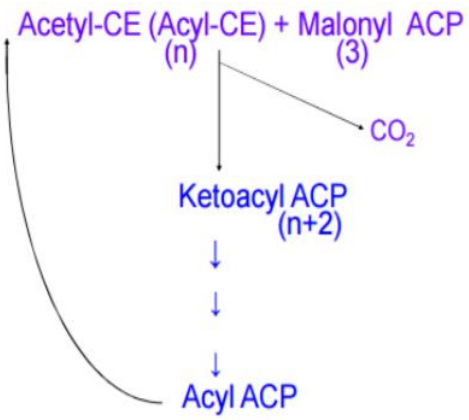
ACP's function is to carry intermediates (Acyl, Acetyl, Malonyl Groups) during catalysis from an active site to another.

-SH group can form a thioester bond with a carboxyl group; it is a high energy bond.

This domain is called Acyl Carrier Protein *it's like a large CoA* (both CoA and ACP carry acyl groups; CoA during oxidation of FA while ACP during FA synthesis).

Overview of FA synthesis:

- We start with an Acyl CoA (or Acetyl CoA initially) and Malonyl CoA.
- They are joined together by the condensing enzyme and CO2 is released forming Ketoacyl CoA which has 2 more carbon atoms than the initial Acyl CoA.
- Ketoacyl is reduced, dehydrated and then reduced forming Acyl CoA.
- Acyl CoA will repeat the cycle (each cycle 2 carbons are added).



Fatty Acid Synthesis (FAS):

- Keep in mind that the carboxylation of Acetyl CoA by Acetyl CoA Carboxylase (ACC) is the first step in FAS and is also the rate limiting step.

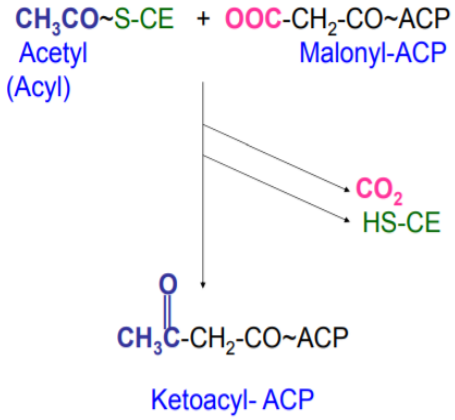
- **Condensation:**

  Firstly, an Acetyl group is transferred from Acetyl CoA to the -SH group (thioester bond) of the **ACP** domain.

  Next, this two-carbon fragment is transferred to a temporary holding site, the -SH group (by another thioester bond) of a cysteine residue on the **condensing enzyme** domain.

  The now-vacant **ACP** accepts a three-carbon malonyl group from malonyl CoA (another thioester bond is formed).

  The acetyl group on the cysteine residue condenses with the malonyl group on ACP as the CO2 originally added by ACC is released. The result is a Keto Acyl ACP which is a four-carbon unit attached to the ACP domain that has a ketone group on the β-carbon.



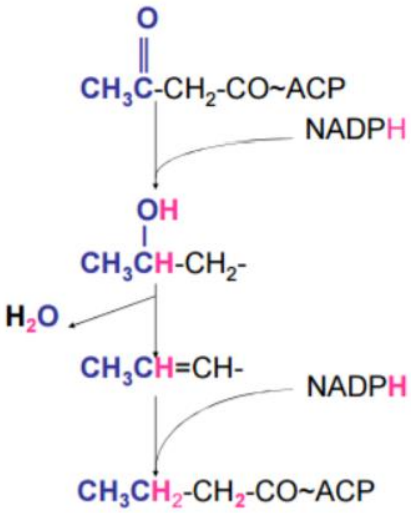
Why do you think the Condensation reaction proceeds in the forward direction?

1- The release of CO2 (Recall that decarboxylation is exergonic).
   This CO2 came from Malonyl which was originally, Acetyl CoA. So you can see that the CO2 was only added to raise the energy level of Acetyl CoA, *this CO2 molecule will not appear in the end product and can be used again in the same step so only catalytic amounts of CO2 is needed.* (it's like what we did to Pyruvate when we transformed it into Oxaloacetate then to PEP).
2- The cleavage of the thioester bond (Which is a high energy bond).

Note: in FA synthesis in humans we only get *even chain fatty acids* while in animals odd chain fatty acids are produced in FA synthesis because they start with Propionyl CoA instead of Acetyl CoA.

- **Reduction → Dehydration → Reduction:**

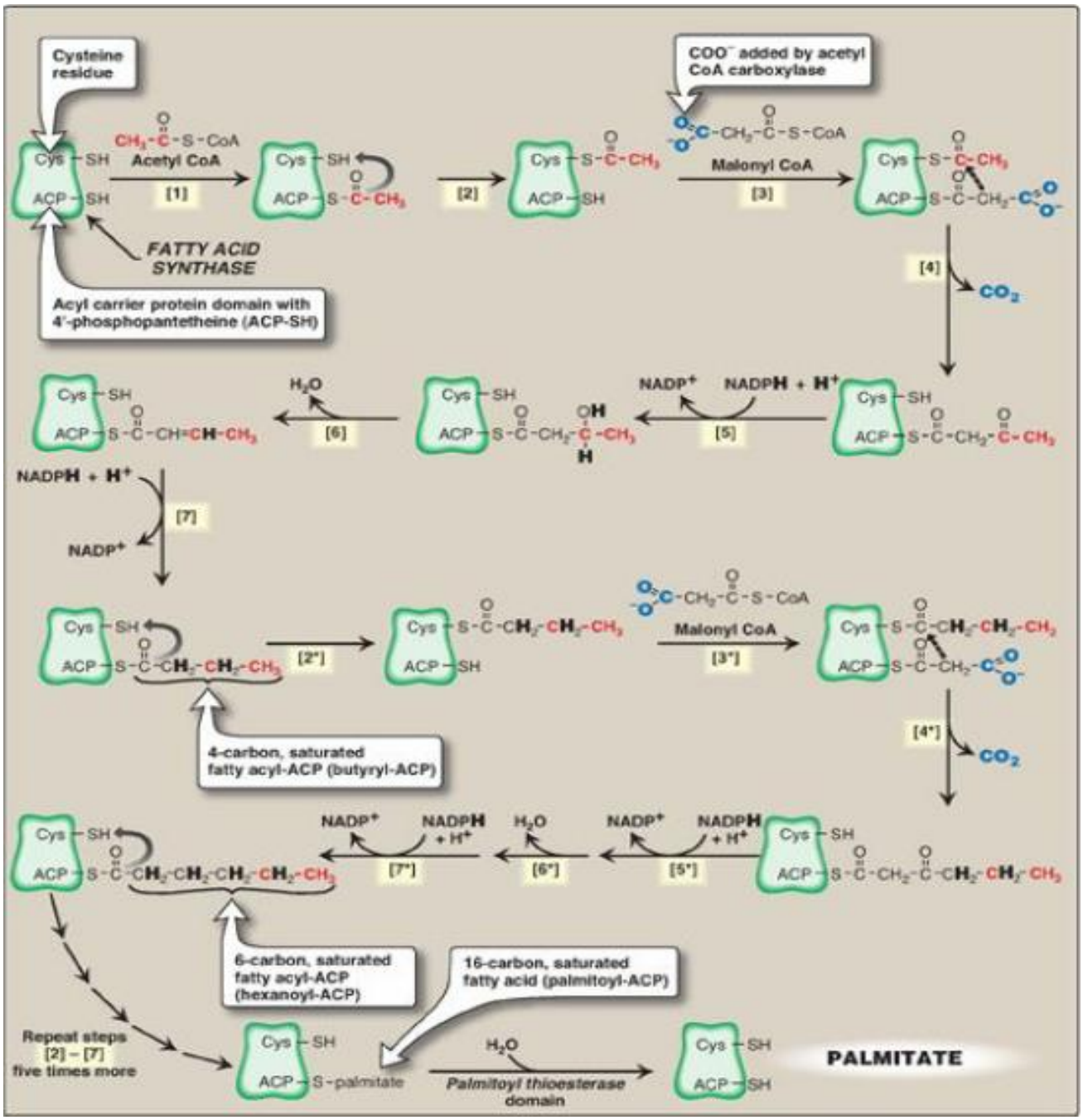
- The objective here is to go from Keto Acyl ACP to Acyl ACP. So, what happens here?



1- Reduction of the ketone group forming a hydroxyl group (at the β-carbon) using NADPH.
2- Dehydration to form a double bond.
3- Reduction of the double bond using another NADPH forming Acyl ACP.
4- Then the acyl group (bound to ACP domain) is transferred to the CE to repeat this cycle.

This cycle is repeated until we get **palmitate** (the primary end product of FAS). Palmitate is released from ACP by the help of Palmitoyl thioesterase domain (hydrolysis happens) to regenerate a free Fatty Acid Synthase.

Look at the picture in the next page thoroughly for better understanding of what just had been said.(notice where was the Acetyl group added in step 4)
or look at the picture from your book (page 185)



Revision of what happened:

The Acyl group is attached to the Condensing Enzyme (CE) by a thioester bond (with the reactive -SH group) and Malonyl CoA loses the CoA and bind to the reactive -SH group of ACP (Also by a thioester bond) then condensation happens by the CE and CO2 is released which would give us Keto Acyl ACP (has a ketone group on the β-carbon) which has 2 more carbons than the previous Acyl group (not 3 carbons Because a CO2 molecule was released during the condensation) then the resulting Keto Acyl ACP undergoes 3 reactions (reduction → dehydration → reduction) and we end up with Acyl ACP Then the cycle is repeated till we get palmitate.

So basically, in order to synthesize palmitate de novo we need:

- 7 cycles of FAS.
- 7 Malonyl CoA (1 per cycle).
- 8 Acetyl CoA.
    - 7 for the creation of Malonyl CoA.
    - 1 for the first FAS cycle as our Acyl CoA in our first cycle is of 2 carbons and is Acetyl CoA (this is the only Acetyl CoA that will act as a substrate for Fatty Acid Synthase enzyme).
      You'll find this Acetyl CoA carbons numbered 15 and 16 of the palmitate fatty acid.
- 14 NADPH (2 per cycle as we have 2 reduction reactions in each cycle).

Q) How many Acetyl CoA (as such -not as Malonyl)?

Only 1.

Remember that in the first condensation reaction we added Malonyl CoA to Acetyl CoA then Malonyl CoA is added to Acyl CoA.

Production of cytosolic Acetyl CoA:

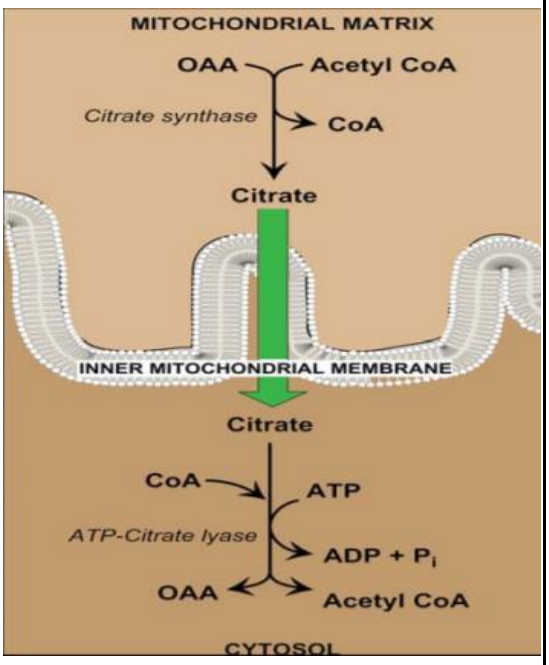
What is the source of Acetyl CoA?

We get Acetyl CoA from pyruvate dehydrogenase, but this Acetyl CoA is produced in the mitochondrial matrix and as we have studied before, the inner mitochondrial membrane is impermeable to Acetyl CoA.

*Note: excess carbohydrates can be converted to fatty acids but excess fatty acids cannot be converted to carbohydrates because **pyruvate to Acetyl-CoA** is an irreversible reaction.*

So, this Acetyl CoA condense with Oxaloacetate to give Citrate (catalyzed by Citrate Synthase) normally this citrate will continue in the Krebs cycle to give iso citrate → α-ketoglutarate and so on…

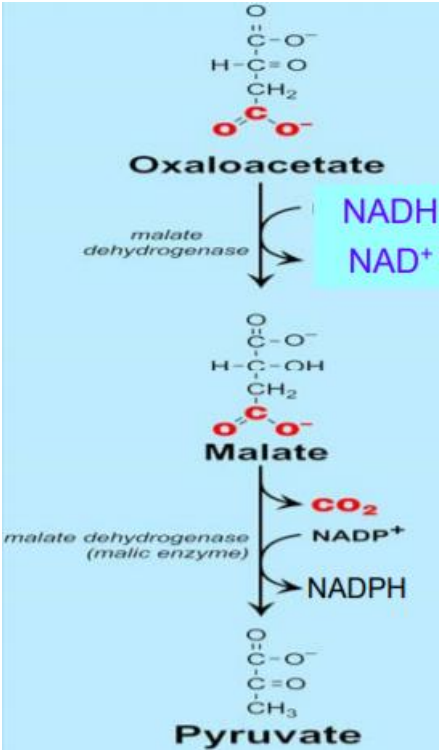
But if isocitrate dehydrogenase is inhibited (Responsible for the conversion of iso citrate to α- ketoglutarate) by the high concentration of ATP present in the cell then isocitrate concentration will be high resulting in a high concentration of citrate. And when high concentration of citrate is present it'll start getting out of the mitochondrial matrix to the cytoplasm by a citrate carrier protein present in the inner mitochondrial membrane.



Now that citrate is in the cytoplasm it is cleaved back to acetyl CoA and oxaloacetate (not by the reverse reaction).

1- OOA + Acetyl CoA → Citrate
   - Enzyme: Citrate synthase
   - No ATP used
2- Citrate → OOA + Acetyl CoA
   - Enzyme: Citrate Lyase
   - ATP is required because one of the products has a high energy bond (a thioester bond with CoA).

Oxaloacetate cannot pass the inner mitochondrial membrane to get back to the matrix.

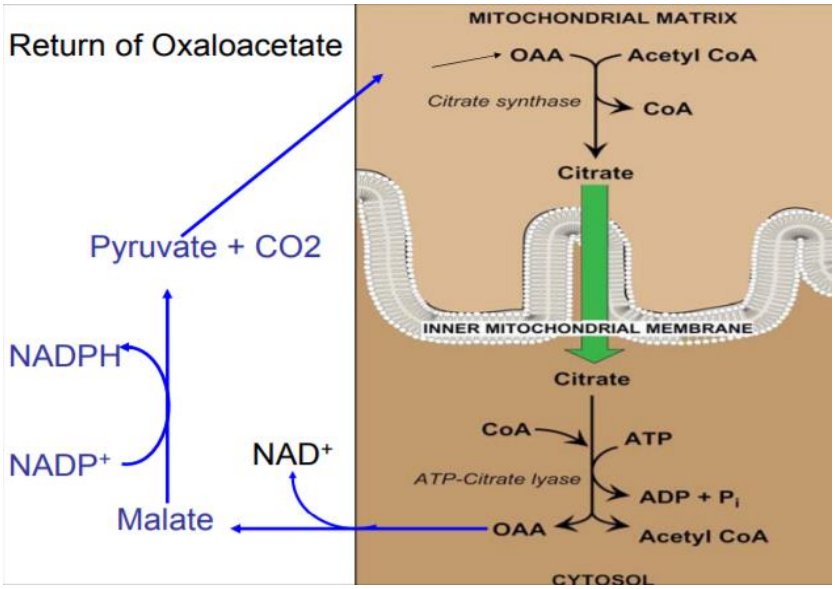


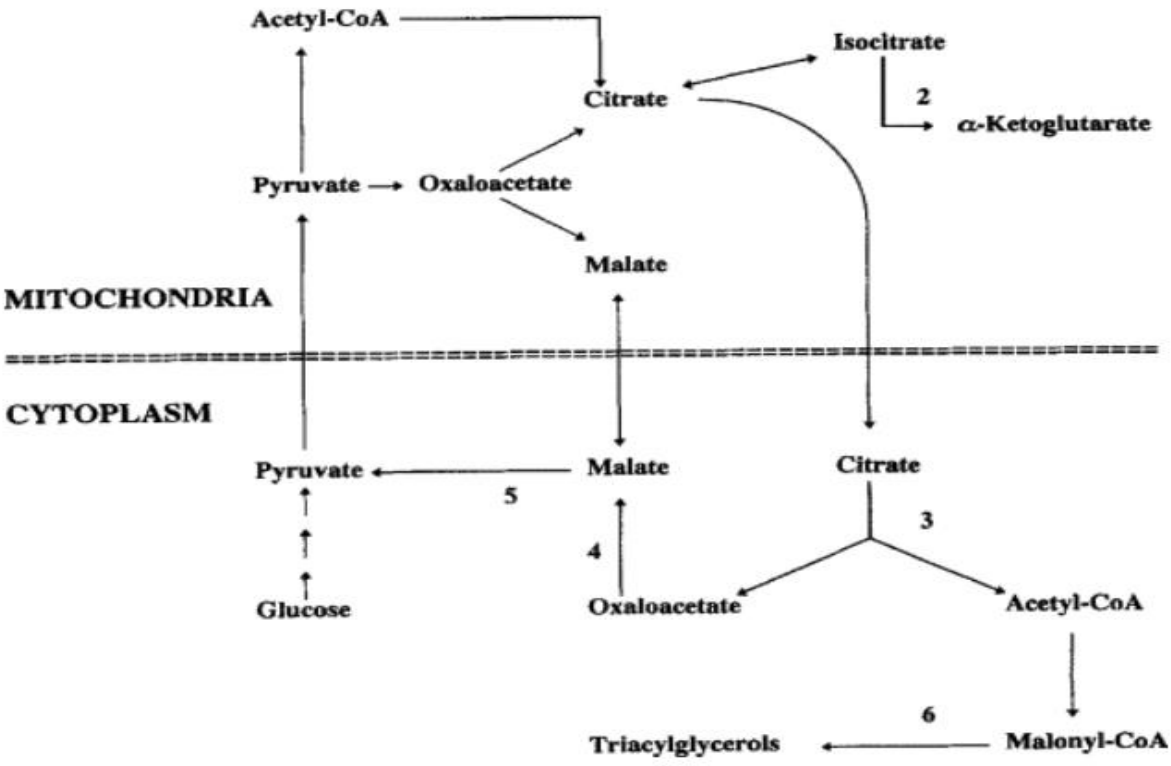
- So now oxaloacetate is reduced to malate by oxidizing NADH, this is the last reaction of the kreb's cycle occurring in the opposite direction. (malate can now enter the mitochondrial matrix by the malate-shuttle but it will not enter that way during fatty acid synthesis)
- Malate undergoes oxidative decarboxylation by an enzyme also called Malate Dehydrogenase (Loss of CO2 and oxidation of a hydroxyl group to a ketone group) producing Pyruvate. The enzyme used is NADP+ dependent malate dehydrogenase (malic enzyme) (sometimes referred to as cytosolic malate dehydrogenase). Notice that we produced NADPH in this reaction, the NADPH produced is used in FA synthesis.

We produce 8 of the 14 needed NADPH for synthesis of palmitate this way (1 for each Acetyl CoA) the remaining 6 NADPH are produced by the Pentose Phosphate Pathway.

Note: Even though the enzymes involved in the two mentioned reactions (OAA → MALATE → pyruvate) are both called malate dehydrogenase (both work on malate) these 2 enzymes are of different complexes from each other.

- Then **Pyruvate** enters the matrix and gets carboxylated to give **Oxaloacetate**





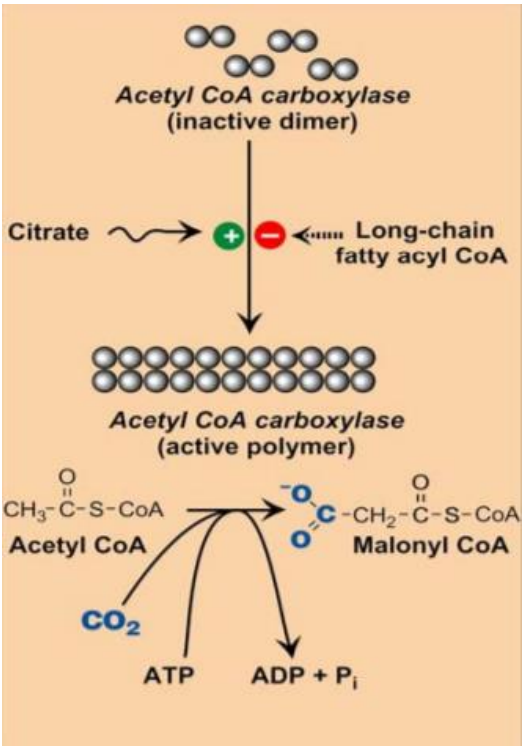
This picture wasn't in the slides; I just thought it'd be helpful ☺

Regulation of fatty acid synthesis and degradation:

- Synthesis and oxidation do not occur at the same time because it would be a waste of energy. These 2 are reciprocally(بالتبادل) regulated (That means that when one process is highly active, the other one is inhibited).
- Oxidation is regulated by:
  - Supply of FA's
  - Entry of FA's into the mitochondria
  - Hormonal regulation
  - Availability of NAD+.
- Synthesis is regulated by:
  - The regulation of Acetyl CoA Carboxylase, because ACC catalyzes the first step of FA synthesis.
    - By either allosteric regulation or phosphorylation
  - Amount of enzymes

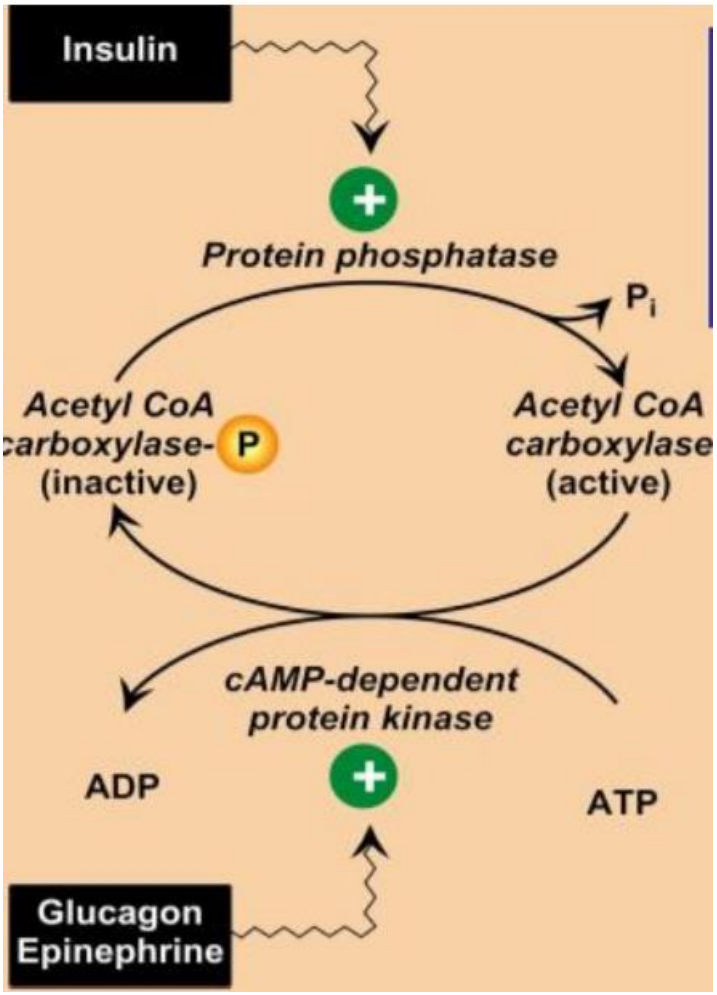
Allosteric regulation:

- ACC can exist in 2 forms
  - Inactive dimer
  - Active polymer
- Citrate stimulates the conversion of the inactive dimer form into the active polymer form.
  - Because citrate indicates high energy level and abundance of building blocks.
- Long chain fatty acyl CoA which is the end product of FAS is an inhibitor (negative feedback) and thus stimulates the conversion of the active polymer to the inactive dimer.



Phosphorylation:

- When Glucagon or Epinephrine level is high it stimulates cAMP dependent kinase which adds a phosphate group to Acetyl CoA Carboxylase transforming it to the inactive form. (Because they indicate low blood Glucose level → we don't want to synthesize FA).
- When insulin level is high it stimulates Protein phosphatase to remove (by hydrolysis) the phosphate transforming it to the active form. (Blood Glucose is high → we want to synthesize FA).
- Notice that adding a phosphate group on an enzyme means to keep or maintain glucose.



The end