# 23

# Biochemistry 2

Doctor 2018 | Medicine | JU

Sheet ○ Slides


**Done By**

Abdullah Firas

**Contributed in the Scientific Correction**

Anas Zayad

**Contributed in the Grammatical Correction**

Anas Zaqut

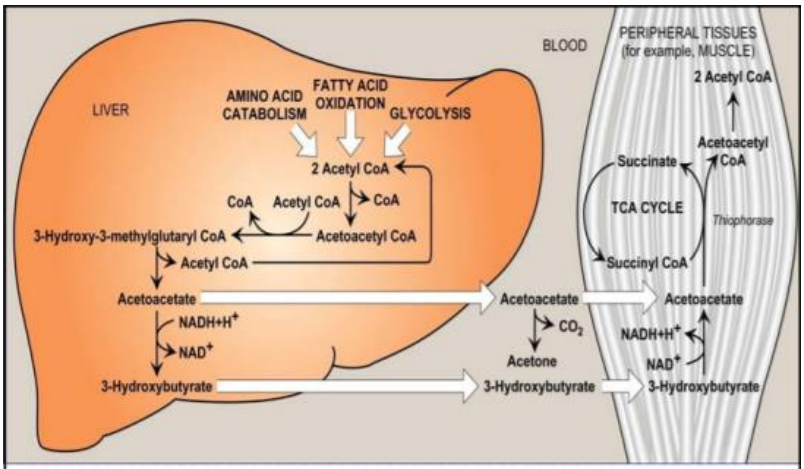
**Doctor**

Faisal Al-Khatib

# Ketone body synthesis

The goal of ketone body synthesis is to regenerate CoA to keep Beta-oxidation going on. The same idea is repeated in glycolysis when NADH accumulates as a byproduct of pyruvate production, as a result, the pyruvate is then reduced into lactate by LDH in order to regenerate $NAD^+$ to keep glycolysis going.

Ketone bodies are used by the skeletal muscles, cardiac muscles, and the brain. All of which cannot utilize fatty acids to their benefit, ketone bodies can be thought of as a processed form of fatty acids that the brain can manage, like a bird feeding its little ones.



**Use of Ketone Bodies by Peripheral Tissues: Skeletal Muscle, Cardiac Muscle and In Brain During Prolonged Fasting (Starvation)**

As you all may know, the brain utilizes carbohydrates as its main source of energy. However, in cases of prolonged starvation or carbohydrate deprivation, the brain can utilize ketone bodies as an alternative source of energy. This gives the body a chance to regenerate glucose through gluconeogenesis. It also ensures that amino acids will not be used as the alternative source of energy since it has multiple bodily functions including involvement in gluconeogenesis.

Despite the liver producing the ketone bodies, it **cannot** utilize it as a source of energy up due to the depletion of Oxaloacetate in the liver for gluconeogenesis. One important detail is that Acetoacetate and 3-Hydroxybutyrate are **interchangeable**, meaning, you can get one from the other through reduction ⇌ oxidation, respectively.

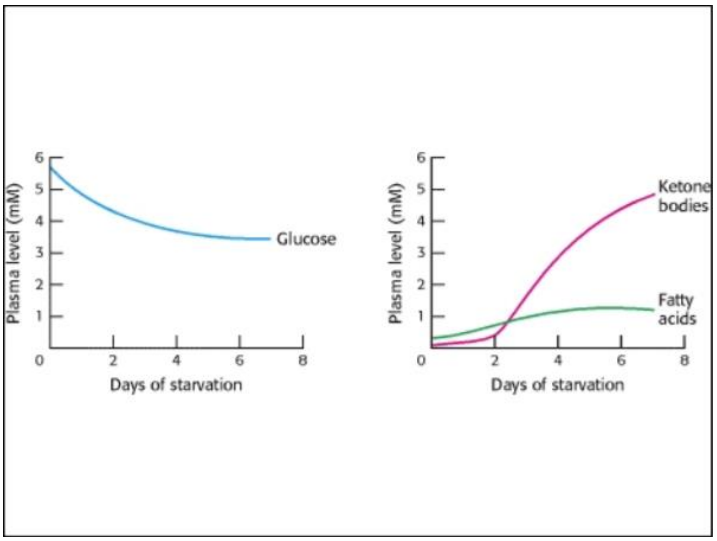
The production of ketone bodies increases during prolonged fasting because they are needed for energy production in the peripheral tissues.

In order to be used up, **acetoacetate** must be activated by joining it to **CoA** which is taken from Succinyl CoA (part of the TCA Cycle). The product formed during this process is **acetoacetyl CoA** (the last intermediate in Beta-oxidation), which can be broken down by thiolase into Acetyl CoA, which then goes into the TCA cycle.

During fasting, blood glucose levels are 5.5 mM and decrease with time, but as the fasting becomes prolonged, blood glucose levels never drop to zero. Instead it remains at a constant level of 3.5 mM. It is maintained by both gluconeogenesis and glycogenolysis.

Fatty acid usage is increased within the first two days.

The usage of ketone bodies, increases substantially two days after prolonged starvation from near zero levels to nearly 4.5mM.




## Fuel metabolism in starvation

The idea is to decrease dependence of the brain on the glucose →decreasing gluconeogenesis →decreases protein degradation later on. This allows individuals to survive for longer periods of time (weeks) and use the stored triglycerides.

**Fuel metabolism in starvation**

| Fuel exchanges and consumption | Amount formed or consumed in 24 hours (grams) 3rd day | 40th day |
|---|---|---|
| Fuel use by the brain | | |
| Glucose | 100 | 40 |
| Ketone bodies | 50 | 100 |
| All other use of glucose | 50 | 40 |
| Fuel mobilization | | |
| Adipose-tissue lipolysis | 180 | 180 |
| Muscle-protein degradation | 75 | 20 |
| Fuel output of the liver | | |
| Glucose | 150 | 80 |
| Ketone bodies | 150 | 150 |

Note: We don't need to know the numbers and specific details of this table, what we do need to know is what increases or decreases during starvation or prolonged fasting.

11:50

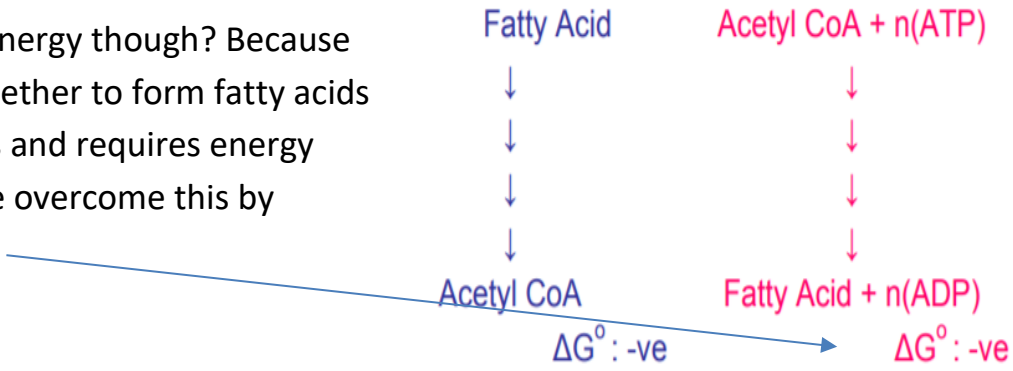
# Fatty Acid Synthesis (Slide #3)

Fatty acid synthesis occurs mainly in the liver due to excess carbohydrates. Excess carbohydrates can be converted to fatty acids, the opposite however is not true, as we cannot produce pyruvate from acetyl-CoA.

**In fatty acid synthesis:**

1. The source of carbon is Acetyl CoA.
2. Reducing power comes NADPH's reducing abilities as fatty acids are more reduced than Acetyl CoA.
3. The source of energy is ATP.

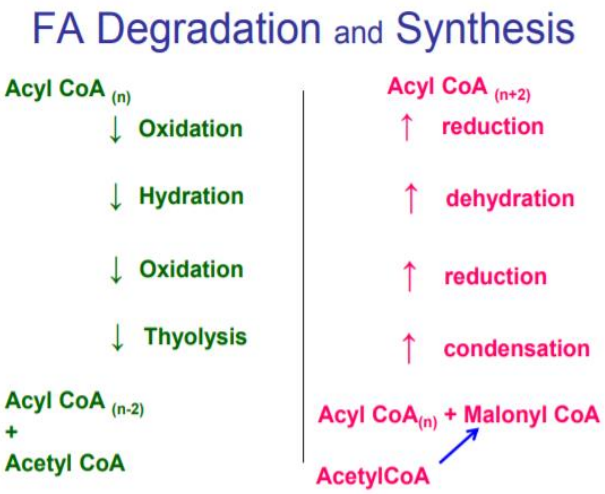
Why do we need energy though? Because linking carbons together to form fatty acids is not spontaneous and requires energy ($\Delta \mathbf{G}^{\circ}$=+ve). So, we overcome this by coupling with ATP.




Fatty acid synthesis and oxidation are opposites of each other. Despite their similarities.

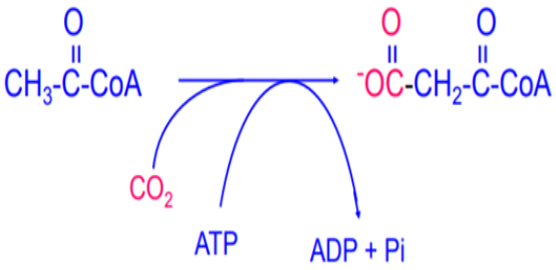
**BUT** there are key differences in:

1. The location of each reactions: FA synthesis occurs in cytoplasm while FA oxidation occurs in mitochondira.
2. The enzymes that catalyze each reaction.



**Malonyl CoA (a dicarboxylic acid):**

Malonyl CoA can be produced by the carboxylation of Acetyl CoA by Acetyl CoA carboxylase (rate limiting step).

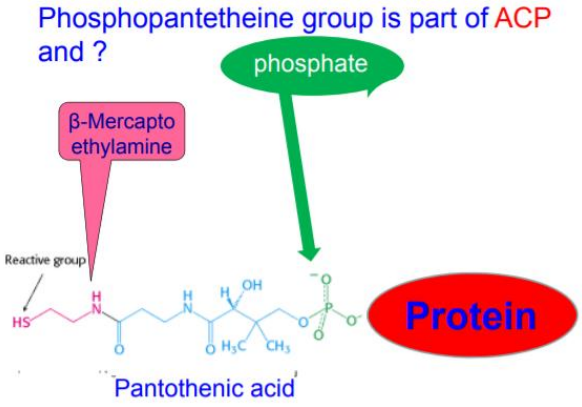



ACC requires: (ATP,Biotin, and source of $CO_2$).

the source of $Co_2$ is $HCO_3^-$

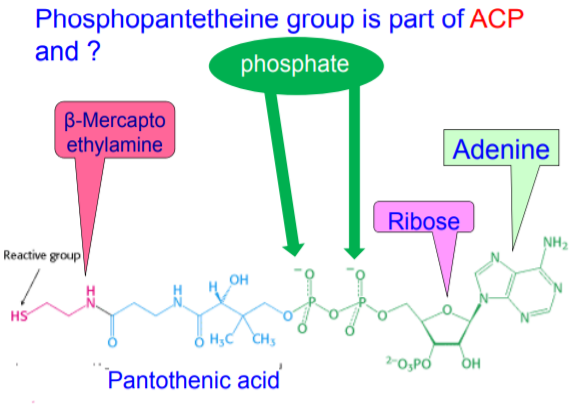
## ***Fatty Acid Synthase***

- This enzyme catalyzes the remaining steps (multifunctional enzyme complex).
- It is Dimer made up of two chains (if only one chain is present, the complex is nonfunctional).
- Each has seven catalytic activities:
    - ACP (acyl carrier protein domain): this domain carries the acyl unit on its terminal (-SH) group and present them to the catalytic domain of fatty acid synthase enzyme.
    - CE (condensing enzyme domain): catalyze the condensation reaction between the building block of fatty acid.
    - All subunits (domains) have **(-SH)** to bind with building fragments.
    - Remember -SH group is part of cysteine amino acid.
- All enzymes exist in one in one complex in order to increase the efficiency.
- One domain is known as Acyl Carrier Protein (ACP)
    - It carries intermediates (Acyl/Acetyl/Malonyl groups) during catalysis so the intermediates don't leave the enzyme complex.
    - ACP is joined to a Phosphopantheine group and a Reactive SH group.



- ACP (acyl carrier protein) and CoA share a phosphate group, pantothenic acid, and β-Mercapto ethylamine. They differ in the lack of a second phosphate group, ribose, and Adenine in the ACP.

Enzymes DO NOT leave the complex, and the reactions take place within the cytoplasm.

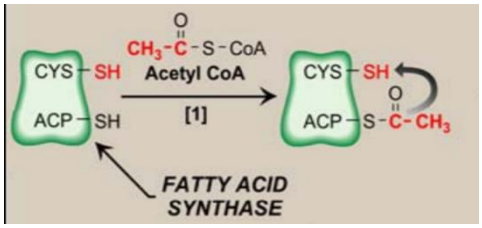



## Mechanism of fatty acid synthesis (20:40)

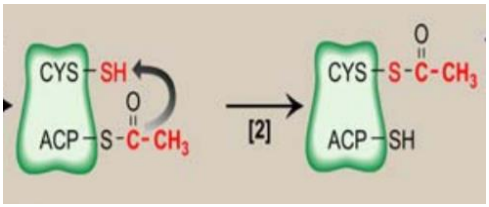
**Before Reading: this method is only effective in producing fatty acids that are up to 18 carbons in length, any fatty acids longer than that are produced in the method discussed in the next sheet.**

In fatty acid synthesis:

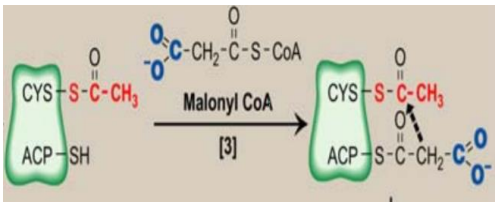
1. the acetyl group or (Acyl group) is transferred from acetyl-CoA or (Acyl-CoA) to the -SH group of the ACP domain.

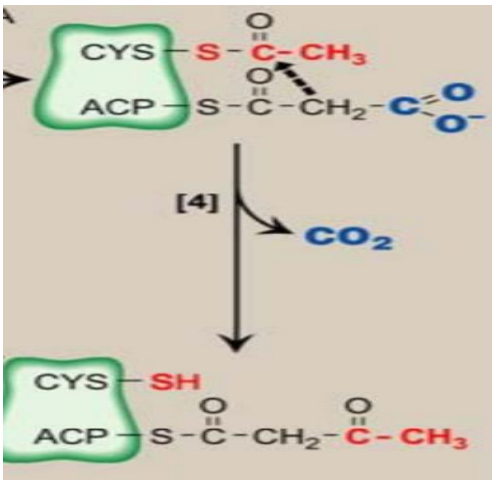



2. The two-carbon fragment is transferred temporarily to -SH group of a cysteine residue on the condensing enzyme domain.

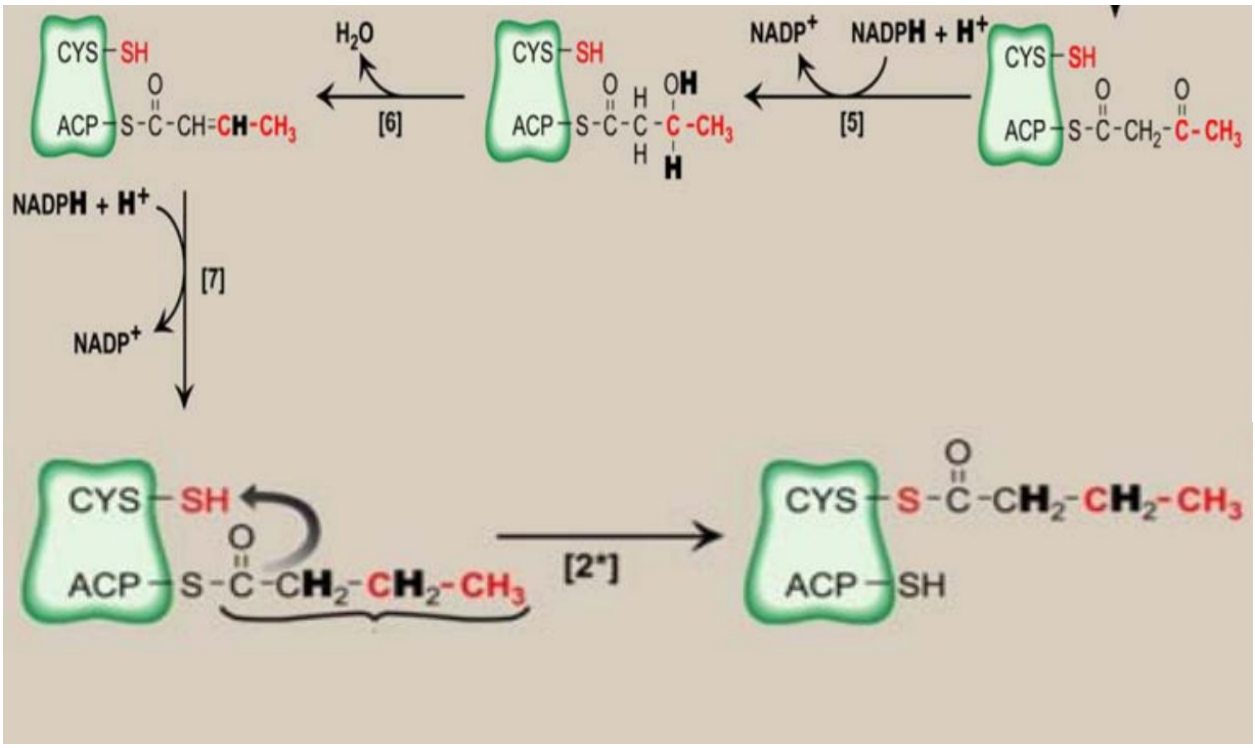



3. ACP accepts a three-carbon malonyl group from malonyl CoA.
4. The acetyl group on the cysteine residue condenses with the malonyl group on ACP as the CO2 originally added by acetyl CoA carboxylase is released. The result is a four-carbon compound called ketoacyl APC.
    - The release of $Co_2$ drives the reaction to forward direction.
    - This reaction catalyzes by condensing enzyme.
    - So, we add $Co_2$ by ACC enzyme to elevate the internal energy for acetyl-CoA, enable it for condensate with the two-carbon fragment molecule. On the other words $Co_2$ need for catalytic amount (catalytic purpose).

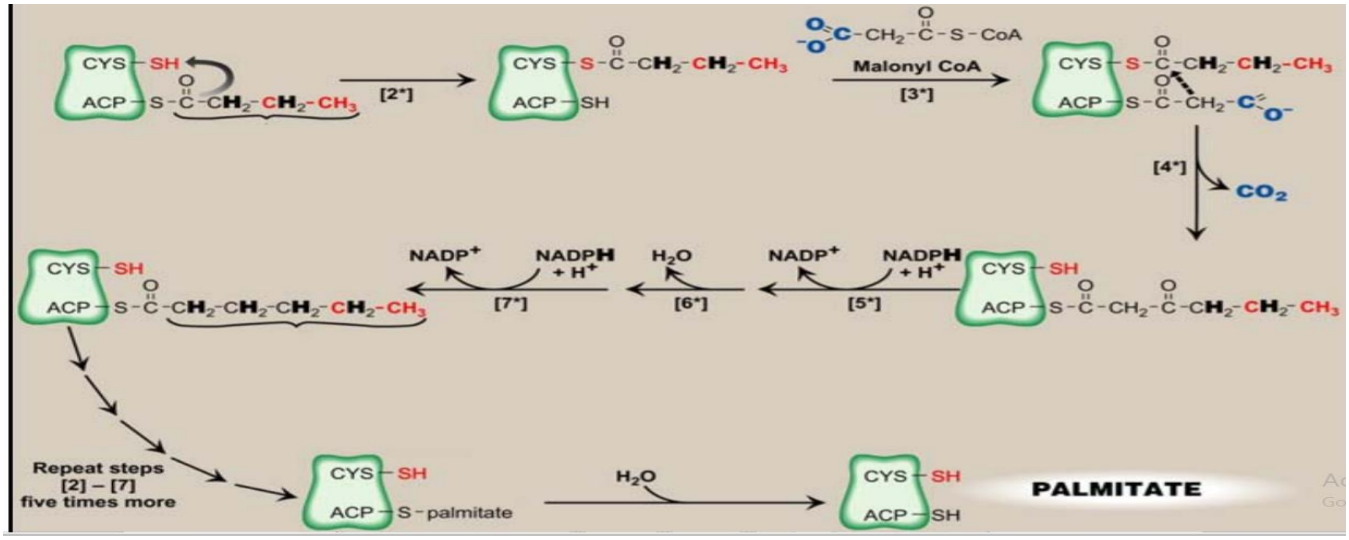


5. The next three reactions (5+6+7) involve converting of ketoacyl-ACP to saturated Acyl-ACP by:
    1) using reducing power of NADPH to convert the ketone group to alcohol OH.
    2) dehydration reaction, this reaction generates double bond.
    3) The double bond is then reduced using another NADPH forming Acyl-ACP.




6. The reactions throughout 2-7 (the synthesis cycle) are repeated many times, depending on the number of carbons in fatty acid.
palmitate (16:0) in this example the cycle will repeat six times not including the first cycle(in total 7 cycles).



**EXTRA:**

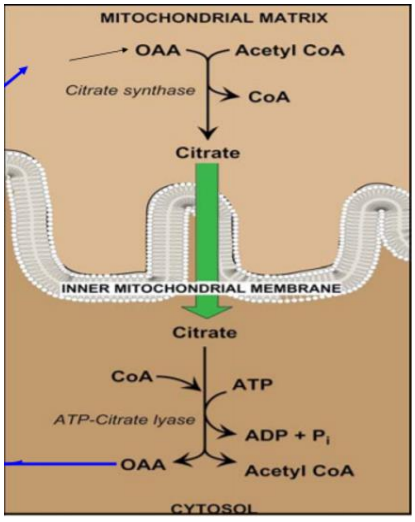
Palmitate is released from ACP by the Palmitoyl thioesterase domain (via hydrolysis).

## The net reaction of palmitate synthesis:

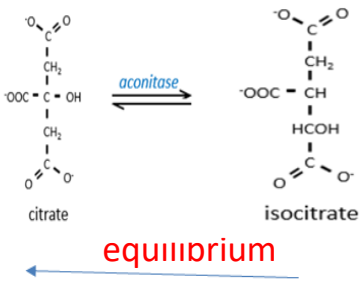
- 7 cycles of synthesis are needed.
- 7 malonyl-CoA are needed (1per cycle).
- Only one acetyl-CoA (in the first reaction only).
- 14 NADPH (each cycle needs 2 NADPH).

You must be familiar with calculating the net of the reaction.

## Production of Acetyl CoA in the Cytosol




- Acetyl-CoA synthesis occurs inside the mitochondria by the oxidation of the pyruvate through PDH enzyme.
- Because Fatty acid synthesis occurs in the cytosol, we need to transport the acetyl-CoA to the cytosol, because the inner membrane of the mitochondria, however, is impermeable to Acetyl-CoA.
- The first step that takes place is the condensation of Acetyl-CoA with Oxaloacetate which results in Citrate formation, this reaction is catalyzed by Citrate Synthase.
- normally this citrate will continue in the Krebs cycle to give iso-citrate and so on, however, the isocitrate dehydrogenase enzyme is inhibited by the high concentration of ATP present in the cell (ATP is an allosteric inhibitor of isocitrate synthase) the increasing isocitrate concentration result in the shift of the equilibrium toward citrate formation (citrate reflects a high energy state).
- when high concentrations of citrate are present within the cell it will leave the mitochondrial matrix and move into the

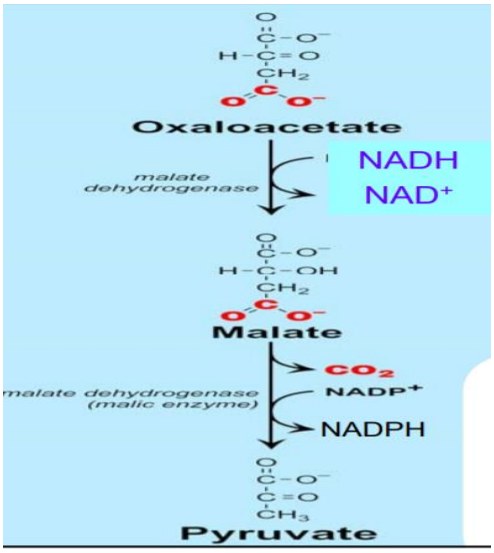


cytoplasm by a citrate carrier protein present in the inner mitochondrial membrane.

- Now that citrate is in the cytoplasm it is cleaved back to acetyl CoA and oxaloacetate by ATP-Citrate lyase enzyme.

Note: This reaction is not the reversible unlike the condensation of OOA and Acetyl-CoA forming, because ATP is required to form a high energy thioester bond between acetyl group and CoA, now acetyl-CoA can be used in fatty acid synthesis.

- Oxaloacetate cannot pass the inner mitochondrial membrane to get back to the matrix, so it is reduced to malate through the oxidation of NADH. This is the last reaction of Krebs' Cycle; however, it occurs in the opposite direction.
- Malate undergoes oxidative decarboxylation by, which is catalyzed by an enzyme known as Malate Dehydrogenase (also known as Malic enzyme), producing a pyruvate. The enzyme used is NADP+ dependent. Notice that we produced NADPH in this reaction, the NADPH produced is used in FA synthesis.

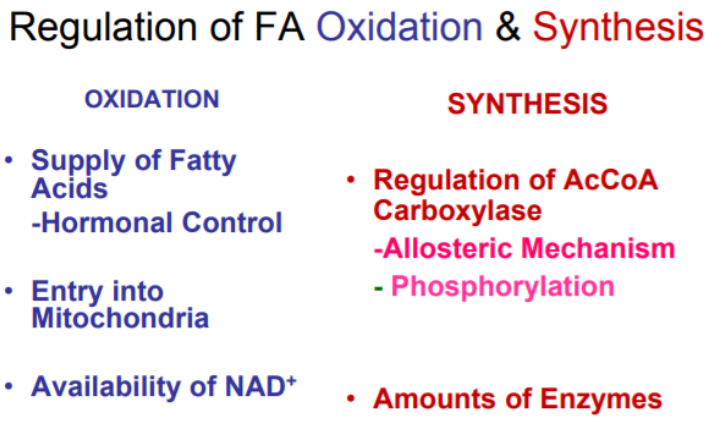



- We produce eight out of the fourteen NADPH needed for the synthesis of palmitate (one for each Acetyl CoA) the remaining six NADPH are produced by the Pentose Phosphate Pathway.

## *Regulation of fatty acid oxidation and synthesis:*

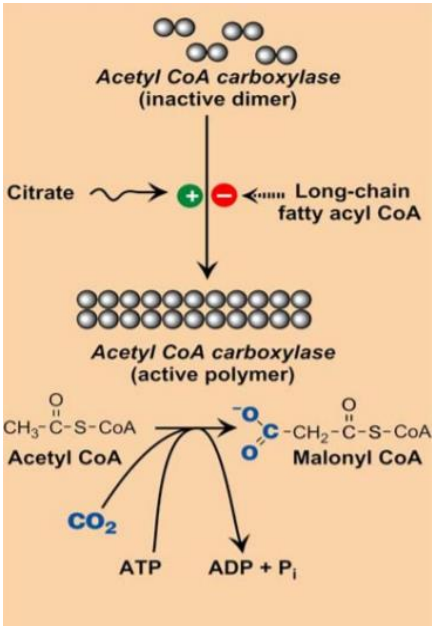
Synthesis and oxidation do not occur at the same time because it would be a waste of energy and time, that means that when one process is highly active, the other one is inhibited.

This figure compares the requirements of regulation in both the oxidation and synthesis fatty acids.




1. **Regulation of synthesis:**
   A. **Allosteric regulation of acetyl CoA carboxylase:**

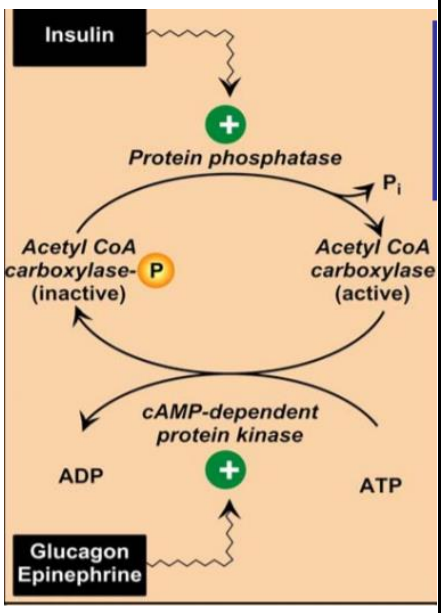
We know that this enzyme catalyzes the carboxylation of acetyl-CoA to malonyl-CoA, this reaction is both the rate limiting and committed step in fatty acid synthesis.




The ACC enzyme is allosterically activated by citrate, converting the enzyme from an inactive dimer to the active polymer which promotes fatty acid synthesis, in other words citrate reflects the high energy state of the cell.

The ACC enzyme is also allosterically inhibited by long chain fatty acyl-CoA (palmitoyl-CoA), which is the end product of the pathway (product inhibition), this converts the enzyme into the inactive form, promoting fatty acid degradation.

**B. Hormone-mediated, covalent regulation of ACC:**




This pathway regulates the enzyme through phosphorylation, the phosphorylation of ACC is induced by glucagon, which reflects a low energy state in the body, so we need to save our glucose by preventing of fatty acid synthesis because it requires glucose to converted into acetyl-CoA, so the phosphorylation of ACC inhibited the ACC enzyme by activating of cAMP-dependent protein kinase.

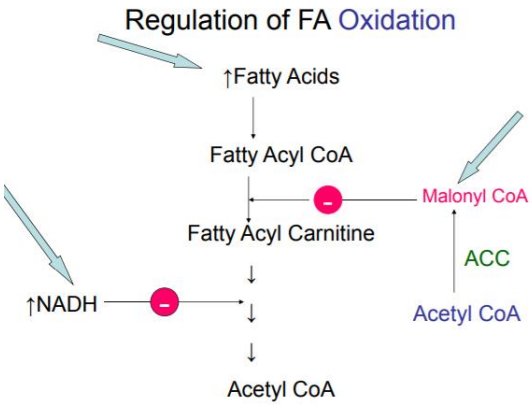
On the other hands, dephosphorylation is activated by insulin which reflect the high energy state, so we need to store the energy in the form of fatty acids, insulin activates protein phosphatase, these proteins activate the ACC enzyme by the removal of the phosphate group (dephosphorylation).

2. **Regulation of fatty acids oxidation:**

The availability of $NAD^+$ regulates the fatty acids synthesis because the fatty acid oxidation needs $NAD^+$, so if there is a depletion in $NAD^+$ the oxidation will stop, therefore the level of $NAD^+$ inside the mitochondria is always high enough to maintain the fatty acids oxidation.

Oxidation is also controlled through hormonal control and through its entry into mitochondria.

This figure summarizes the regulation of fatty acid oxidation:




1. Fatty acids availability: high level of fatty acids will increase the level of oxidation.
2. High level of NADH will inhibit the oxidation.
3. High level of malonyl-CoA will inhibit the fatty acid oxidation because the malonyl-CoA indicates that fatty acids synthesis is activated, so malonyl-CoA inhibits the entrance of fatty acids into the mitochondria for their oxidation.