Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 17

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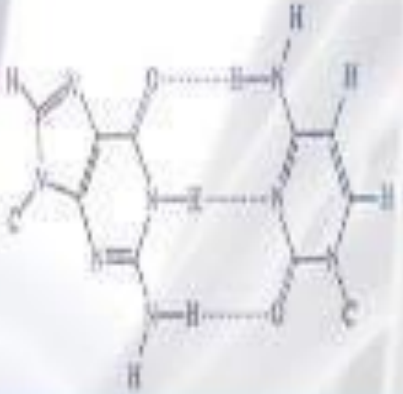
Biochemistry



Majida Al-Fogaraa'

بسم الله الرحمن الرحيم

Fatty Acid Synthesis, Regulation, and Double Bonds

Note: this sheet was done on section 3's record.

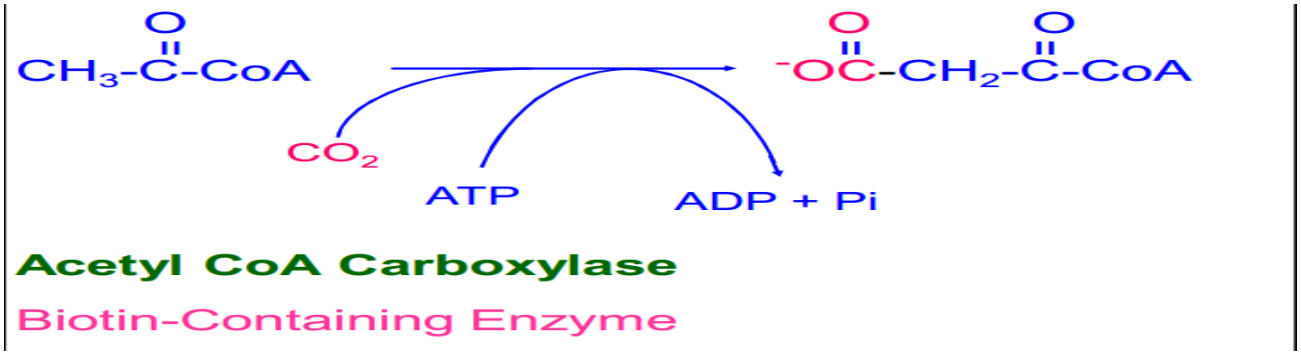
Recap:

Yesterday we talked about fatty acid synthesis.

An Overview of the process of fatty acid synthesis.

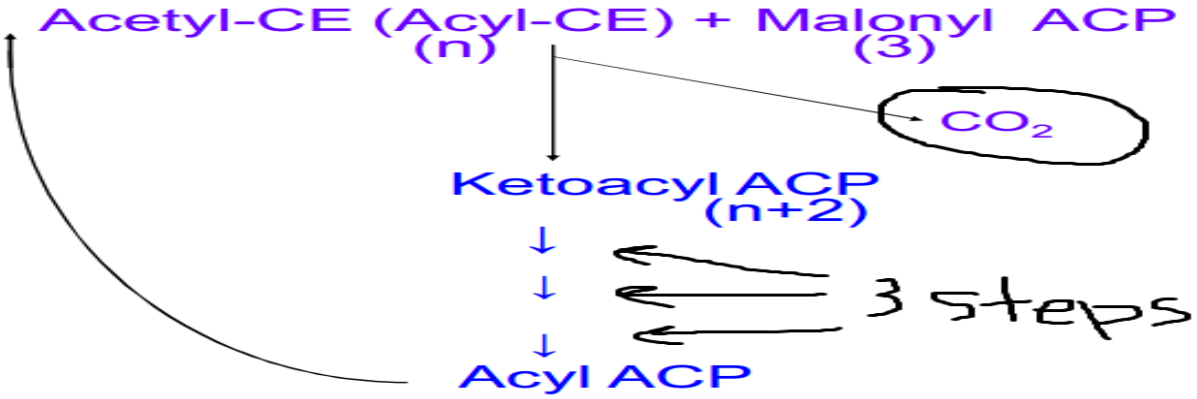
The first step of fatty acid synthesis:

Carboxylation of acetyl CoA to provide malonyl CoA by **acetyl carboxylase**. All other reactions are catalyzed by **fatty acid synthase**, which is a large molecule made from two subunits. The subunits associate together to form an active dimer. The reaction starts by the transfer of an acetyl group to the condensing enzyme and the transfer of a malonyl group to ACP (acyl carrier protein).



The second step of fatty acid synthesis:

Then condensation happens, with the loss of CO2 that drives the reaction in the forward direction. 3 steps then occur, resulting in the reduction of the ketoacyl group on the beta carbon into an acyl group.



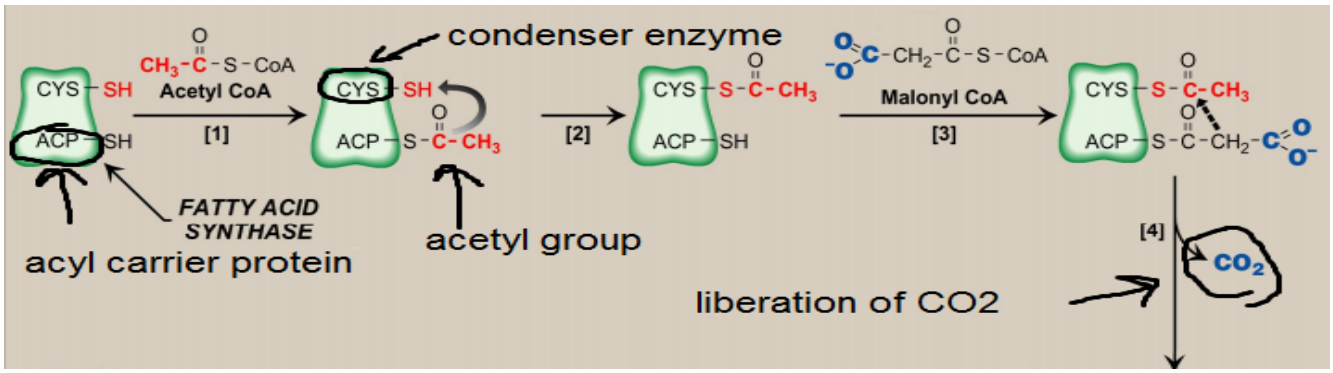
The third step of fatty acid synthesis:

Condensation. Several repetitions of the 3 steps results in the formation of acyl ACP, which is transferred again to the condensing enzyme. The process is repeated again and again.

Lets look at the individual steps and see them in more detail.

Condensation step

The acetyl group is linked to a condensing enzyme. The condensing enzyme contains an -SH group in its active site, which is used to bind to the substrates.
The malonyl group is linked to the ACP portion of the fatty acid synthase. The condensation occurs by the attack of the carboxyl group of the acetyl group to the malonyl, resulting in the liberation (release) of CO2, and now the condensing enzyme is free.



What drives the condensation reaction in the forward direction?

The release of CO2 makes the reaction irreversible and exergonic.
It is as if the CO2 that was added in the first step (carboxylation of Acteyl CoA) is used to activate the acetyl group for condensation, because once condensation occurs, the CO2 is released, which drives the reaction in the forward direction.
Decarboxylation reactions are always exergonic reactions, they drive reactions in the forward direction

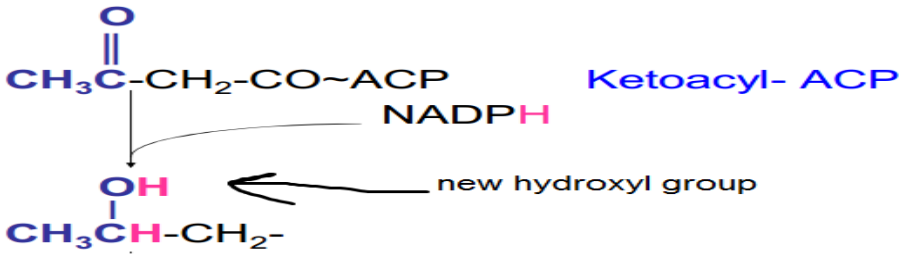
Now we have a ketoacyl ACP. Notice the 2 carbons that come from malonyl (that were originally from acetyl). The newly formed 4 carbon compound now has a double bond (ketone group) at its beta carbon. We are going to reduce this ketoacyl beta double bond into CH2. To achieve this, 3 reactions occur that are almost the reverse/opposite to those that occur in oxidation.

Keto Acyl ACP Reduction

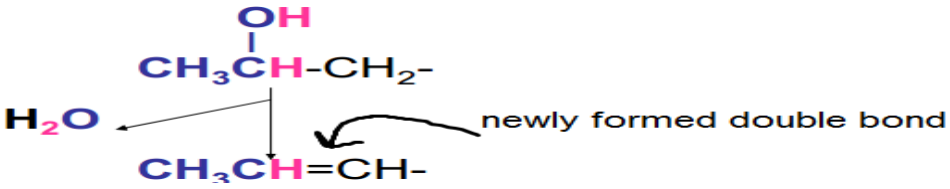
Firstly, the ketone group will be reduced by the reducing agent NADPH.

NADPH is like NADH except it has one more phosphate group. Both have the same reduction potential and both are high energy compounds. But the difference between them is 1 extra phosphate group on NADPH. NADPH is used by the cell for reduction purposes and not for production of energy by electron transport chain like NADH. NADH is oxidized in electron transport chain, and NADPH is not. NADPH is used for synthetic reactions.

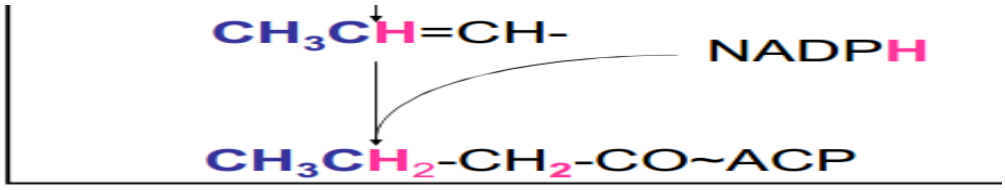
NADPH reduces the ketone group into a hydroxyl group. The doctor didn't write the rest of the compound (for simplicity) because the focus of the reaction is now on the left part of the molecule.



Now the hydroxyl group attached to the beta carbon will undergo **dehydration** (loss of OH from beta and H from the alpha carbon). This is the opposite of what happens in oxidation. This results in the formation of a double bond between the beta and alpha carbon.



Another reduction reaction will reduce the double bond into a single bond by the addition of two hydrogens, facilitated by NADPH.



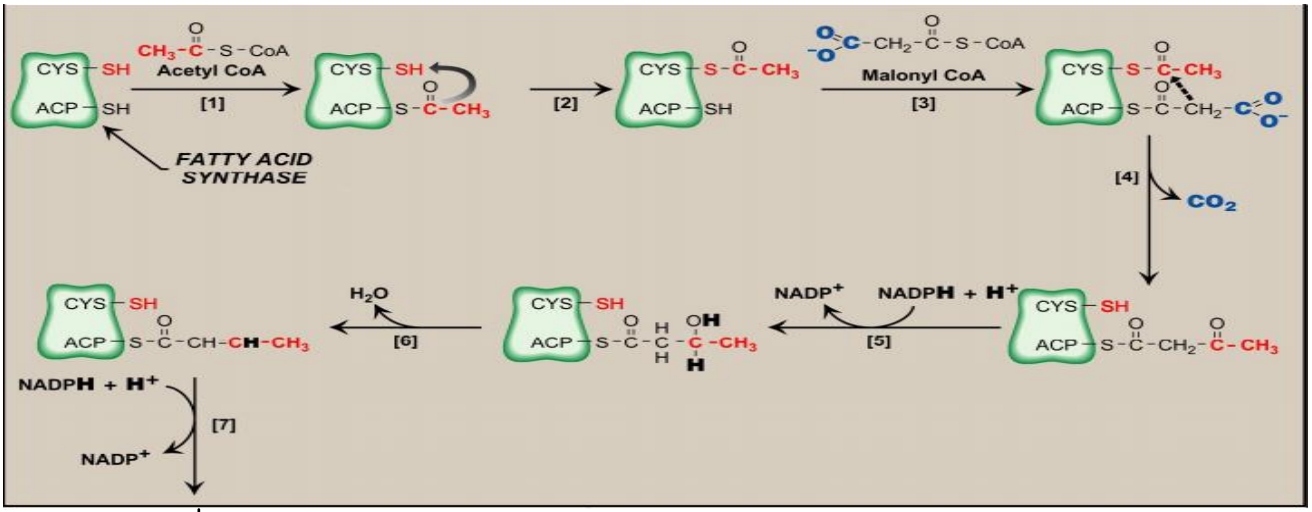
So, by these three steps, the keto group is reduced to a methylene (CH2) group. They convert the whole ketoacyl group into an acyl group. By this, we make a fatty acid with 4 carbons, starting with acetyl CoA and malonyl CoA (which aslan originated from acetyl CoA).

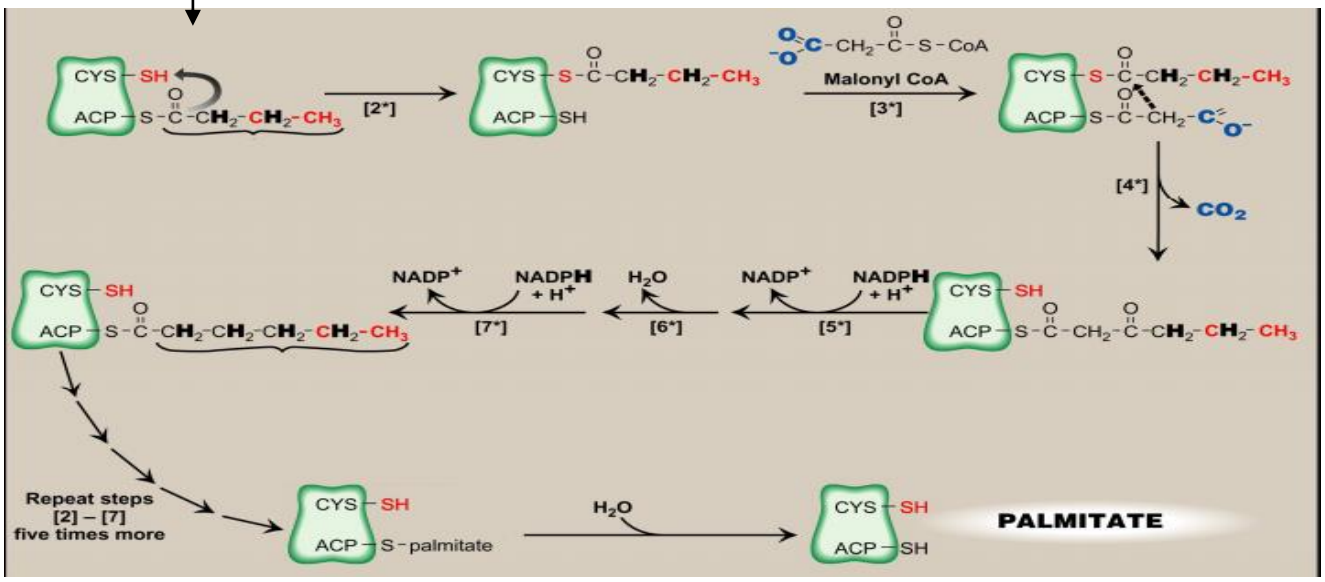
The next step is **carried by ACP**. The Acyl Carrier Protein again transfers its load to the condensing enzyme and the reactions are repeated again and again. The reactions of condensation and reduction (dehydration) are repeated over and over until the fatty acid is 16C (palmitate). Remember this point, the limit is 16C.

From the book, we have a diagram showing how the reaction occurs in details for fatty acid synthase. (recap)

Fatty acid synthase contains the condensing enzyme that has –SH group and the ACP part of the protein that carries the intermediate.

1- Transfer of acetyl group to ACP, so ACP is now a part of the acetyl group.
2- The acetyl group is transferred to the condensing enzyme. ACP is now free.
3- Transfer of a malonyl group into ACP. So now we have an acetyl group and a malonyl group attached to the enzyme.
4- Now condensation occurs.
The first two steps were just to transfer the starting materials/substrates into the enzyme for enzyme activity. Now condensation occurs
5-The ACP now carries the ketoacyl group.
6-Reduction, 7- dehydration, and another 8- reduction gives us the acyl group.
9- This is repeated again and again.
Look at it, you will find that the reactions are repeated over and over.
10- In the end the fatty acid synthase will be bound to palmitate. Palmitate has 16C.
11- Hydrolysis of palmitate then occurs, and palmitate/palmitic acid is released.
For this reason we have 7 enzymatic activities (there are 7 subunits and each subunit has an actions in the process) for attaching the substrates, release of substrate by hydrolysis (which is the last reaction), dehydrations and reductions and etc etc etc.





Fatty acid synthase is a **multifunctional enzyme.**

Why do we have multifunctional enzymes?

What is the advantage of multifunction enzymes?

Catalysis is more efficient. Rather than having several enzymes separated from each other and the product of each enzyme being the reactant of the next enzyme, the same enzyme molecule carries the intermediate molecules from one active site to another, as if it were a machine. So having two polypeptide chains together, they complete the functions of each other. Awwww….. :')

The fatty acid synthesis pathway was thoroughly studied by Dr. Saleh al-Wakeel, who spent most of his life studying the process of fatty acid synthesis in bacteria and mammals.

A student asked:

Does all of this happen without ATP?

YES. ATP was used in the first reaction to convert acetyl CoA into malonyl CoA.

If you understood the reaction well you should be able to answer the following questions:

Considering the synthesis of Palmitate:

Q: *What is the net reaction*?

We can also say: *how many cycles of synthesis/condensation reactions occurred*?

A: 7 cycles are required (so 7 condensation reactions)

Q: *How many malonyl CoA are used*? (as malonyl CoA, not acetyl CoA)
Condensation starts with acetyl CoA and malonyl CoA, so as it continues, we add one malonyl CoA every time. Each condensation reaction needs one acetyl CoA activated in the form of malonyl CoA, so 7.
A: 7

Q: *How many acetyl CoA are used?* (As acetyl CoA, not for malonyl synthesis)
Only ONE acetyl CoA. Only the first 2 carbons from the omega end/terminal (for eg. carbons #15&16 in palmitate) come directly as acetyl CoA, all other carbons will be converted from acetyl to malonyl followed by decarboxylation, and then incorporated into the fatty acid. So one acetyl CoA is used as such by FA synthase While 7 more molecules are converted to malonyl CoA.
A: 1

Q: *How many NADPH are consumed*?
Since we have 7 cycles, and each cycle has 2 reduction steps, then…?
A: 14

Production of cytosolic Acteyl CoA for fatty acid synthesis

What is the source of acetyl CoA for fatty acid synthesis?

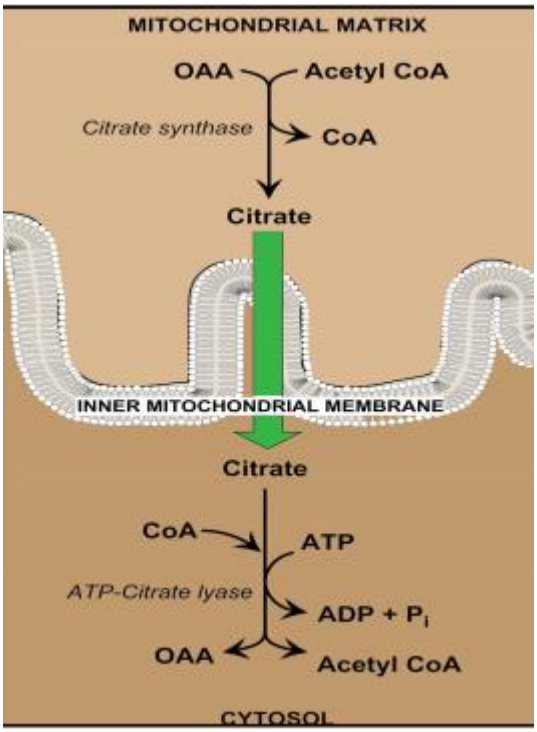
Glycolysis. Pyruvate dehydrogenase is a mitochondrial enzyme. The acetyl CoA is produced in the mitochondria, however the synthesis of fatty acids occurs in the cytosol. So now, we have a problem. The InterMitochondrialMembrane is impermeable to Acteyl CoA, Acteyl CoA produced in the mitochondria cannot cross the IMM as expected, so we need a carrier molecule.
So how is Acteyl CoA transferred?

Look at this reaction:

Acteyl CoA reacted with **O**xalo**A**cet**A**te will give citrate by the action of the enzyme **citrate sythase**. If you remember, this is the first reaction of the citric acid cycle. Here we have two cases. 1) If energy is required by the cell, citrate will continue in the citric acid cycle to give isocitrate and continue to give alpha keto glutarate..etc. However, 2) if the energy level in cell is HIGH (meaning we have adequate ATP), then isocitrate will NOT be oxidized, because the cycle is regulated at the level of isocitrate. (we don't need any more energy)

So *isocitrate dehydrogenase* is inactive. This will lead to an accumulation of isocitrate, which is in equilibrium with citrate.

So, an accumulation of isocitrate will lead to more citrate. The increase in concentration of citrate allows it to pass through a citrate carrier that will take it across the IMM from the mitochondrial matrix into the cytosol (there is a carrier molecule that is specific for citrate).



Citrate in the cytosol is cleaved again into OAA and Acteyl CoA. Notice that this reaction (cleavage of citrate to OAA and Acteyl CoA) is NOT the reverse of the first reaction. Think of it as if the product of the *citrate synthase* enzyme is the substrate of the cytosolic enzyme. They are **NOT** the same reaction occurring in opposite directions. They have different reactants, and different enzymes as well.

In the cytosolic reaction (breakdown of citrate) we use ATP.

***Why is ATP needed here*?**

Look at the products (OAA and Acetyl CoA). **They are high energy compounds** (Acteyl CoA is a high energy compound) **so energy is required to link the acetyl group with CoA**. And that's why the enzymes are different (citrate synthase in the **mitochondria** and ATP-citrate lyase in the **cytosol**)

ATP also helps to push the reaction [citrate → OAA] in the forward direction. The reaction (synthesis of citrate [OAA → citrate]) is normally exergonic, with a –ve delta G -as what we took in TCA cycle-. So the rxn in the opposite direction [citrate → OAA] will be endergonic, with a +ve delta G, so the ATP is used/utilized to push this rxn [citrate → OAA] in the forward direction.

The dr. didn't mention the following, but it's for more illustration.

If we have this rxn: A+B → C+D and this is an exergonic rxn, -ve delta G

So the reverse rxn: C+D → A+B will be an endergonic rxn, with a +ve delta G

* However, remember that the mitochondrial and cytosolic rxns mentioned above are **NOT** the same reaction occurring in opposite directions. They have different reactants, and different enzymes as well.

A student asked, *why isn't ATP a product of the first reaction*?
Answer: because the reactions are not opposite and they have different enzymes.
The rxn [OAA → citrate] has a –ve delta G but not to extent that is adequate for ATP production. The enzyme doesn't produce ATP. If the 1st rxn produces ATP, it must be called a substrate level phosphorylation. And as you know [OAA → citrate] which is the 1st rxn in TCA cycle doesn't produce ATP.

In this reaction, OAA has to come back to the matrix, but it doesn't.
Instead, OAA is reduced to malate, (this is the last reaction of the TCA cycle but in reverse). Here, it is in the opposite direction, a reduction reaction. The cofactor is NADH.
Malate CAN go back. Malate is oxidatively decarboxylated
Malate is a dicarboxylic acid. It loses CO2 in the process of oxidative decarboxylation and is converted to pyruvate, a 3C compound. This reaction is catalysed by malate dehydrogenase (DH).

Malate DH can be found as an enzyme in the TCA cycle , catalyzing malate to OAA. Malate to pyruvate is also catalyzed by cytosolic malate DH, also known as *Malic enzyme*, the enzyme that catalyzes the oxidative decarboxylation of malate to pyruvate.
It is called a **dehydrogenase** (even though it's also a decarboxylase) because it is a **redox** reaction (oxidation-reduction rxn).

What is the other product of this oxidative decarboxylation?
The other product is the reduction of **NADP+ to NADPH**

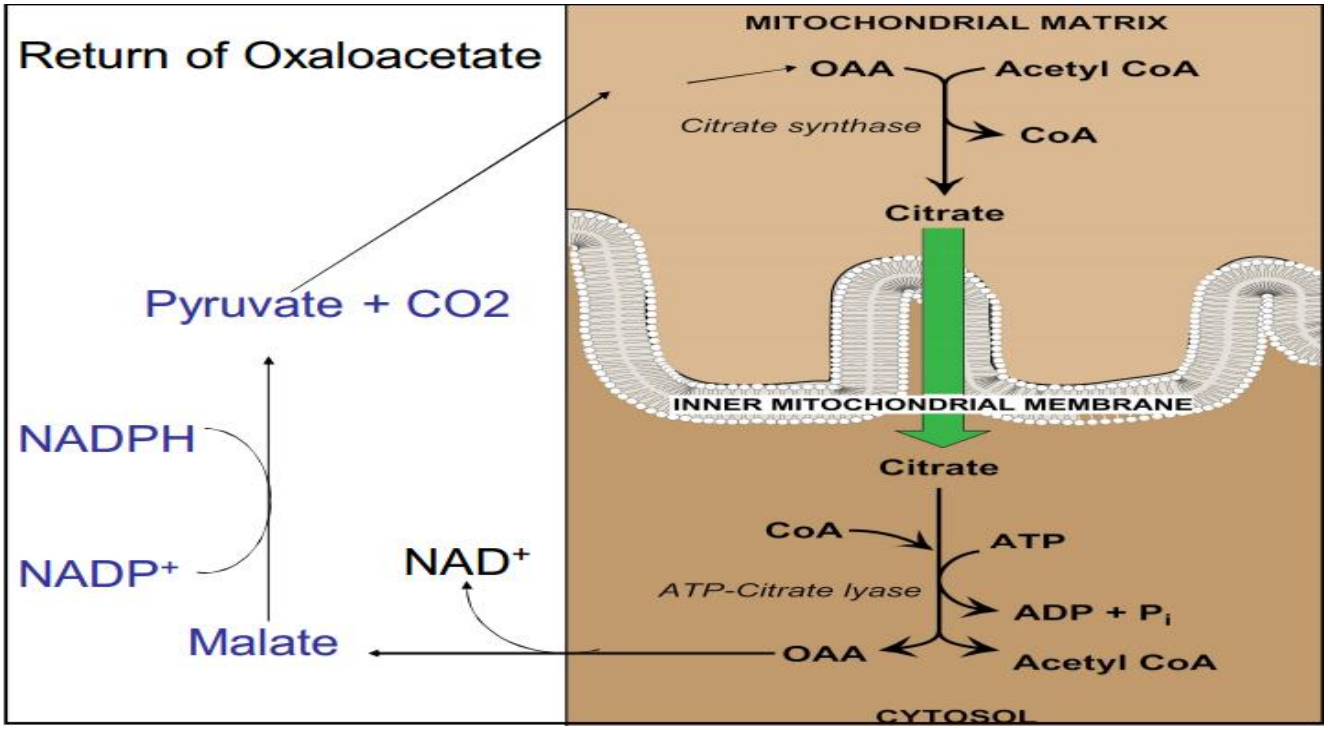
This reduction is advantageous to fatty acid synthesis. Fatty acid synthesis, as mentioned previously, requires NADPH. So NADPH is available here.
This process occurs during fatty acid synthesis, to make the Acteyl CoA and thus NADPH is produced.
NADPH is a product that will be used in fatty acid synthesis. So this is an advantage.

Pyruvate can enter into the mitochondria, and by mitochondrial pyruvate carboxylase, it will be reverted back into OAA.

So we have a **cycle** here. We start with OAA, and together with mitochondrial acetyl CoA, it forms citrate. Citrate, once outside, is cleaved into OAA and acetyl CoA. OAA is made into malate, which turns into pyruvate, and ultimately becomes OAA.

What is the net reaction?

Acetyl CoA is transferred outside the mitochondria. And NADPH is produced. Also, energy is needed in the form of ATP in two reactions. So the net effect is transfer of Acteyl CoA outside the mitochondria, and another advantage is the production of NADPH.



How many NADPH are produced in this manner during the synthesis of Palmitic acid?

A: 8

BUT WHY?

Firstly, we need 8 Acteyl CoA (palmitate is a 16C fatty acid, so if each Acetyl CoA is 2C, then simple math would suggest I need 8 of them)

Synthesis of palmitic acid requires 8 Acteyl CoA, 1 as acetyl CoA and 7 as malonyl CoA

8 Acteyl CoA will be transferred in the manner explained above, producing 8 NADPH (since every 1 acetyl CoA that crosses ultimately produces 1 NADPH).

So more than half of the required NADPH comes from this pathway.

But if you look back, we require 14 NADPH to synthesize one palimitic fatty acid.

Okay, I have 8 from this transport process, what about the other 6 NADPH?

The rest of the 14 NADPH comes from the PentosePhosphatePathway. This is why the PPP is active in the liver during fatty acid synthesis, and when glucose is available/abundant, it is converted into fatty acid (and some will be used for production of NADPH)

Which tissue is most active in fatty acid synthesis?

In males, the liver.

In females (besides the liver), in the mammary glands. Nursing mothers have lactating mammary glands, and this is a place where a lot of NADPH is produced, because the mammary gland produces fatty acids. *Which kinds*? Usually short and medium chain fatty acids.

Regulation of Fatty Acid Oxidation and Synthesis.

Regulation of Synthesis

Oxidation and synthesis should not occur at same time. If they did, it would just be a waste of energy, a futile cycle. (It would be a loss of energy, because synthesis requires ATP directly, and degradation doesn't produce ATP directly)

So if synthesis and degradation occurred at the same time, we would only be wasting energy. This is why they're tightly regulated.

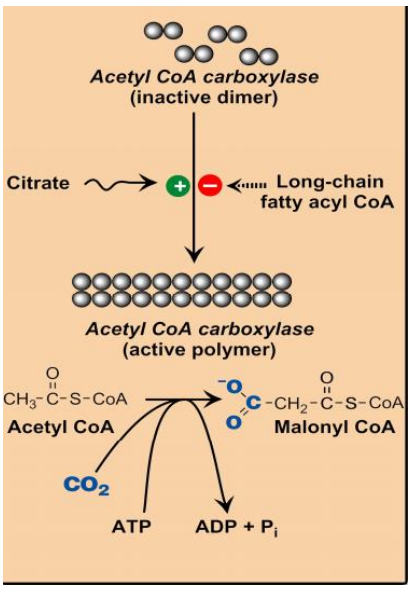
*** Regulation of Acteyl CoA carboxylase can occur by three methods:**

1- Allosteric regulation

2- Phosphorylation

*** The amount of available substrates and enzymes.**

1-Allosteric regulation



The *Acteyl CoA carboxylase* enzyme, can exist as an inactive dimer or an active polymer. It becomes active by **the presence of citrate**. Citrate converts the inactive form into the active form.

Why citrate? High levels of citrate in the cytoplasm indicate that the energy level of the cell is high. More building blocks are available. If the energy level is high, and the building blocks are available, why not make fatty acids? So fatty acid synthesis is active during conditions where citrate building blocks are available and high energy, because citrate gets out of mitochondria, because of high level of ATP.

Long chain fatty acyl CoA is ultimately the end product of fatty acid synthesis, so if it accumulates, this means synthesis should be turned off. This is an example of negative feedback. Notice how the last product of synthesis, the long chain acyl CoA, is the **inhibitor** of the early enzyme, *Acteyl CoA carboxylase*, which is the regulatory enzyme that catalyzes the rate limiting step.

2- Phosphorylation (covalent modifications)

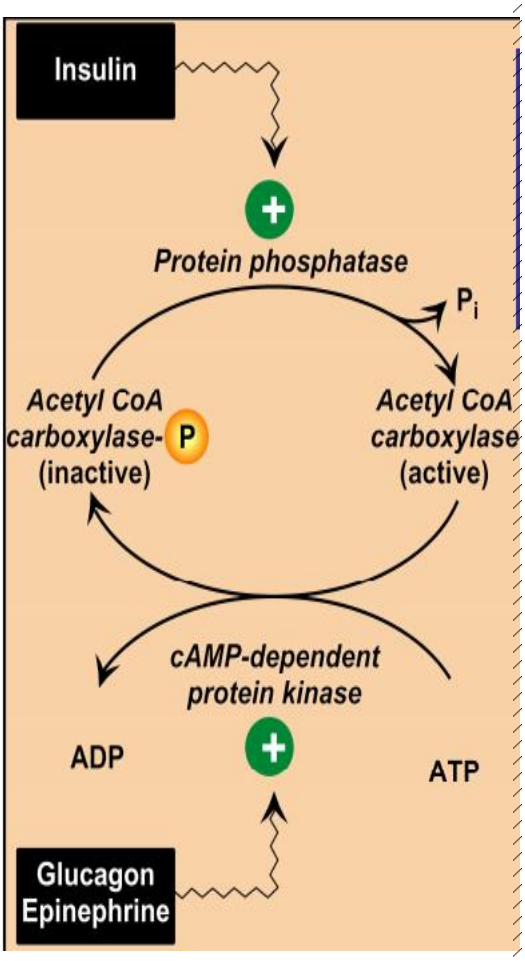
We can add a phosphate group to the end of the enzyme, converting it to its inactive form. Acteyl CoA carboxylase is inactive when **phosphorylated**. The inactivation occurs by **cyclicAMP (cAMP) dependent protein kinase**. It can also be inactivated by **AMP dependent kinase**. We have two kinases, cAMP dpk AMP dpk, AMP kinase is active when AMP levels are high, which means that energy in cell is very very low, so we have no need for fatty acid synthesis at this time.
So when the concentration of AMP is high, it stimulates a kinase called AMP dependent kinase that adds a phosphate group, inactivating acetyl CoA carbocylase, thus inhibiting fatty acid synthesis.

Glucagon and epinephrine also act by a similar mechanism, by activating the cAMP dependent protein kinase. They both lead to inactivation of Acteyl CoA carboxylase.

How does the Acetyl CoA carboxylase go back to its original state? By dephosphorylation. Dephosphorylation is catalyzed by *protein phosphatase*. It will revert it to its active form. Notice that this is dependent on **insulin**. Insulin stimulates protein phosphatase. Protein phosphatase removes the phosphate group from the Acteyl CoA carboxylase, and converts it to active form.

What is the physiological significance of this?
High insulin means high blood glucose. Cells deal with high blood glucose by converting it into fatty acid.

So we have discussed hormonal regulation. Hormonal regulation happens over relatively longer period of time.



Regulation of oxidation:

1-Mainly regulated by supply of fatty acids.

We also have hormonal control, like glucagon and epinephrine, which stimulate hormone sensitive lipases, making free fatty acids available. The presence of free fatty acids will cause oxidation to happen.
So, oxidation is mainly dependant on substrate concentration.

2- entry into mitochondria

3- Availability of NAD+, considering the fact that NAD+ is one of the products.

Regulation of fatty acid oxidation in the diagram

1- when level of fatty acid is HIGH, they enter into the mitochondria and oxidation becomes active.

2-availability of NAD+. Fatty acid oxidation converts NAD+ into NADH. When NAD+ level decreases, NADH levels are high. NAD+ by itself is needed for oxidation. If it is not available, this will inhibit oxidation. It is simply another example of availability of substrate. *They are linked together; fatty acid oxidation does not happen unless NAD+ is available.*

3- Entry of fatty acyl group into mitochondria. Remember that the entrance of fatty acids into the mitochondria requires carnitine shuttle. It requires enzymes that transfer acyl group from acyl CoA to carnitine. This step is inhibited by malonyl CoA.

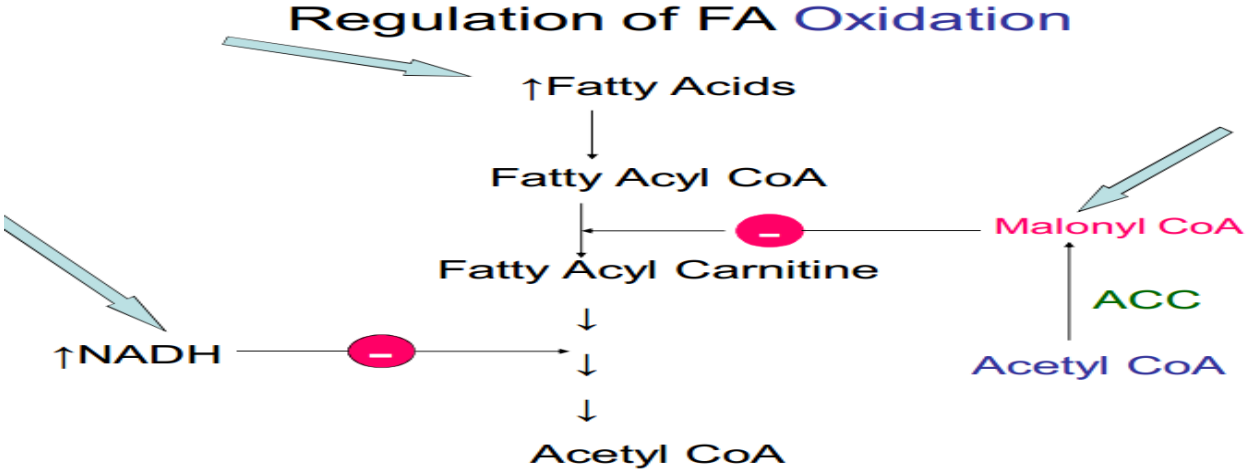
What is the significance of this?

Why is malonoyl CoA an inhibitor of the process?

Shu da5alo?

Malonyl CoA is an intermediate in fatty acid synthesis. It is produced by Acteyl CoA carboxylase and used by fatty acid synthase. So high levels of malonoyl CoA tells the cell not to oxidize fatty acids

So *oxidation* can be inhibited by one of the intermediates of fatty acid *synthesis*.



Why do we need to know this stuff?

This is basic science that can have some real life applications. Consider one of the healthcare problems nowadays. Millions of people suffer from obesity. Obese people have a low rate of fatty acid oxidation, so they have fatty acid accumulation in their adipose tissue.

Most drugs in the pharmacy are enzyme inhibitors, there are no enzyme stimulators because enzymes normally work by high activity. You can only inhibit the activity.

Let's suppose we could inhibit an enzyme for the sake of humanity…

Suppose you want to choose an enzyme to inhibit in order to **increase** fatty acid oxidation, which enzyme do you choose?
In other words, we want to design a drug that inhibits an enzyme in order to increase the rate of fatty acid oxidation, *which enzyme do you choose*?

If you thought of *Acteyl CoA carboxylase*, you're that much closer to becoming a millionaire. If you didn't, don't worry, you can still charge super high kashfiyehs.

We choose this enzyme, Acteyl CoA carboxylase, because it produces malonoyl CoA. Malonoyl CoA inhibits fatty acid oxidation. So if we inhibit the enzyme, we'll have less malonoyl CoA, and therefore, we increase the rate of fatty acid oxidation. So the idea is inhibiting an enzyme that leads to an increase in the rate of fatty acid synthesis.
So this is basic science that can be applied.

Then the doctor proceeded to read an excerpt about a discovery of such an enzyme from Al-Rai newspaper.

If you're interested in this, please check the record of section #3 at this time (00:39:56).

He also says that if you were to find a chemical that could inhibit *Acteyl CoA carboxylase*, you'd be a "multi millionaire".

Elongation of fatty acids

We have seen that fatty acids are synthesized up to 16 C (palmitate). What about 18C fatty acids, 22C, 24C, etc?

These are produced by **elongation**. Elongation occurs in the endoplasmic reticulum. It has a **similar sequence of reactions** but with **different enzymes**.
So same idea, malnoyl CoA with acyl CoA, 16 or more, with two NADPH, same sequence etc..
BUT the reaction occurs in the ER, *not* in the cytosol.
So now, our aim is to elongate fatty acids.

Sometimes elongation happens in **mitochondria** for short chain fatty acids by a sequence of reactions, which are basically the reverse of fatty acid oxidation. They use the same set of enzymes of fatty acid oxidation, except for the first reaction of fatty acid oxidation, which cannot be reversed. So this reaction happens by using different enzymes.

The **purpose** of this elongation in the **mitochondria** is for dietary short chain fatty acids. For example, eating a lot of butter, which is rich in short and medium chained fatty acids [we want to store these fatty acids but our fat can't be like butter], so it will be digested and taken to the mitochondria to be elongated. And elongation can't happen by fatty acid synthase.

Introduction of Double Bonds

Desaturation

Desaturation (converting a saturated fatty acid into an unsaturated fatty acid) is possible in humans. It is possible to introduce double bonds. Therefore, unsaturated fatty acids can be synthesized in humans, starting with saturated fatty acids as the substrate.

We can introduce double bonds, but **not after carbon 9**. Fatty acids with double bonds after carbon 9 **cannot be produced in humans**. However, these fatty acids are important for growth and normal functions, thus they are essential fatty acids. Essential = Important, yet can't be produced in humans.

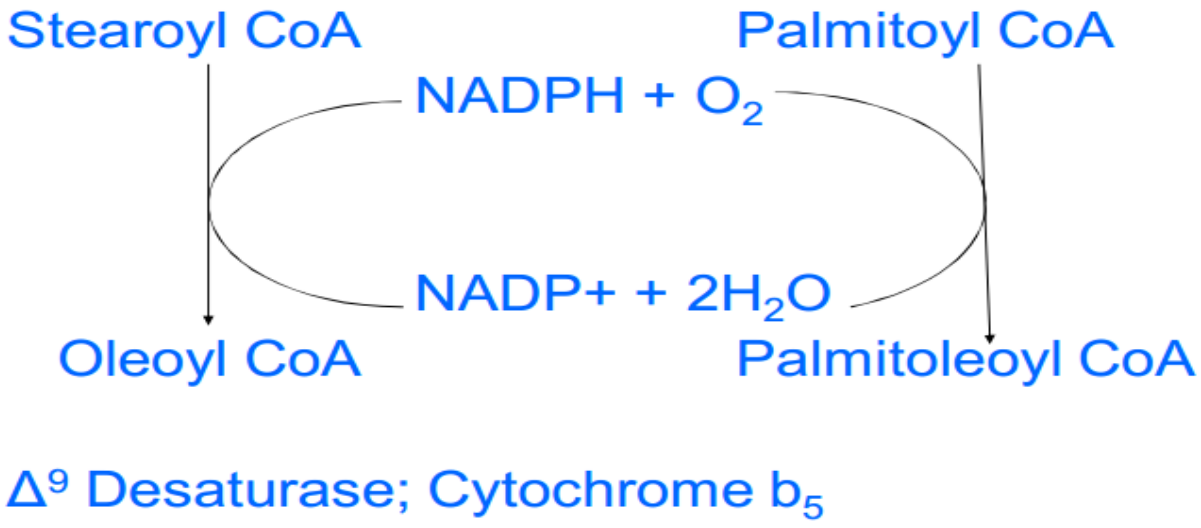
We **CAN**, however, produce fatty acids with double bonds **before carbon 9.** Oleic acid and palmitoleic acid can occur in ER. No double bond can be introduced beyond carbon 9 in humans. So by definition, *is oleic acid an essential fatty acid*? No it is not. But it does have important functions.

Look here at stearoyl CoA. How can we introduce a double bond?

The introduction of a double bond requires an enzyme called ***desaturase***. It is a sequence of enzymes that starts with the introduction of oxygen onto stearic acid at carbon 9 that results in formation of hydroxyl-fatty acid, followed by desaturation (dehydration) with removal of OH & H forming a double bond. Notice that this reaction, O2 + NADPH + stearoyl CoA will be converted into oleoyl CoA by a sequence of steps.

Why is NADPH needed by this reaction?
It is an oxidation reaction, so why is NADPH required?
The mechanism for the reaction is introduction of oxygen atom on the fatty acid to make a hydroxyl group. For this, you need one oxygen atom, which comes from the O2 and for this to happen the other atom has to be reduced by NADPH that is used to activate the oxygen molecule reducing one oxygen to water and allowing one oxygen atom to be added to the fatty acid followed by desaturation (dehydration) reaction which is done by delta 9 desaturase (cytochrome b5).

This is how Oleic acid is produced and in the same manner Palmitoleic acid is produced.



And that concludes this sheet. For more information and to get a better understanding of things, read Lippincott's chapter 16. Good luck :D
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