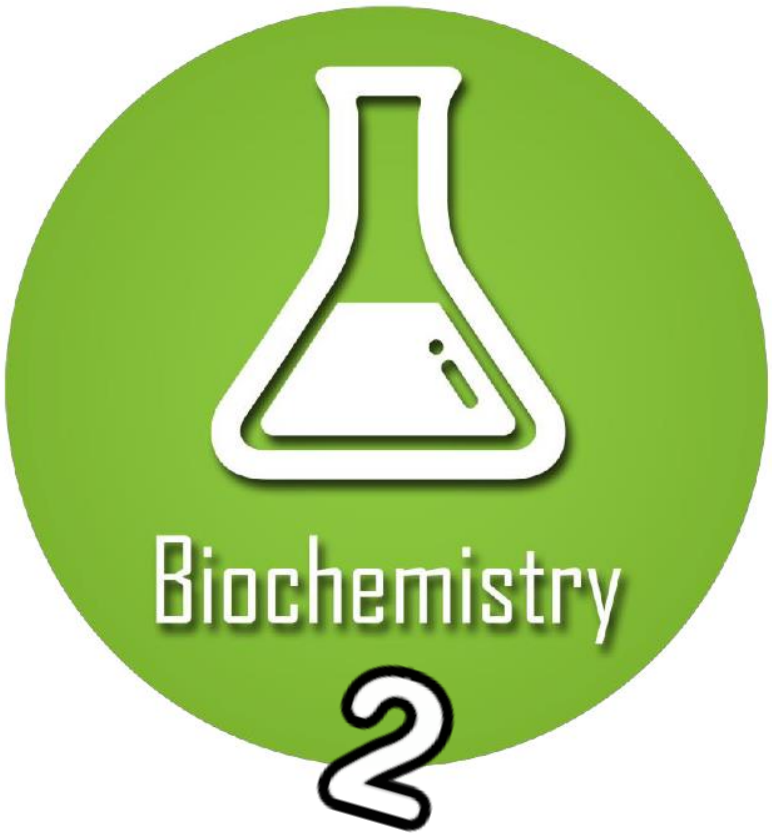
2014 2020
Medical Committee
The University Of Jordan



◉Sheet ○Slides

Number: 13

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Subject: Fatty acid oxidation

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In this lecture we're going to talk about the β-oxidation pathway in detail.

Fatty acids are transported to tissues bound to albumin, why?

Because they're insoluble in water, so they can't be found free in the plasma, which is mainly water.

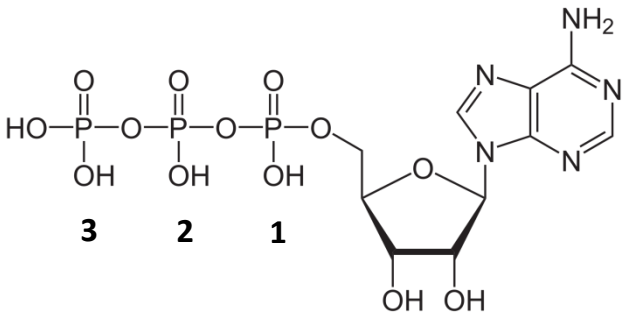
In the tissues they are degraded by a three step pathway called "beta oxidation" (β-oxidation).

1- Activation of the fatty acid:

In order for fatty acids to be a source of energy they have to be activated first, and this activation happens by joining the fatty acid to "Coenzyme A", CoA is added to the fatty acid side chain forming a thioester bond and the resulting compound is called "acyl-CoA". Now this bond is a high energy bond, so forming it would require some high energy as well; and we get that energy by cleaving ATP to form AMP and pyrophosphate.

> Side note: The "ester" bond is formed between a carboxyl group and a hydroxyl group (-OH). But if a bond is formed between a carboxyl group and –SH group (rather than –OH) then we call a Thioester Bond.

Notice that we are cleaving a high energy bond (the bond between no.**1** and no.**2** in the figure, which resembles the structure of ATP), but we're making another high energy bond. So the energy change is very minimal that it's close to zero, which means that this reaction is reversible.



So, by now we have this reaction:

$$FA + HSCoA + ATP \rightleftharpoons FA\sim CoA + AMP + PP_i$$

But we don't want the reaction to be reversible, so how can we make it irreversible?

We make it irreversible by contentiously removing one of the products as soon as they are formed. And that's exactly what happens when pyrophosphate is rapidly hydrolysed into two phosphate groups by an enzyme called Pyrophosphatase, pushing the reaction in the forward direction.

$$PP_i + H_2O \longrightarrow 2\,P_i$$

So by the summation of those previous two reactions, we end up with this final one:

$$FA + HSCoA + ATP \longrightarrow FA\sim CoA + AMP + 2\,P_i$$

By all of that we can conclude that we cleaved two high energy bonds, we formed another high energy bond, and made the reaction irreversible by favouring the forward direction.

You saw above that we used only one ATP, and we converted it into AMP. But this is actually equivalent to using **two** ATP molecules. **So how come?**

We know that ATP and ADP are continuously recycled, meaning that the ADP that is formed has to be converted back to ATP. So how can we convert AMP to ATP?

Well, the first step is converting AMP to ADP. And we do so by transferring a phosphate group from an ATP to that AMP, so we end up with two ADPs, (R2).

R1 $FA + HSCoA + ATP \longrightarrow FA\sim CoA + AMP + 2\ P_i$

R2 $AMP + ATP \rightleftharpoons ADP + ADP$

Now if you combine R1 (which is the same one as mentioned before) with R2, you'll notice that the two AMPs will cancel out, and the ATP will be added up resulting in **two** ATP molecules

So R1 + R2 will give us:

FA + HSCoA + <u>2ATP</u> → FA-CoA + 2ADP + 2Pi

Conclusion: using one ATP and converting it to AMP + 2Pi is equivalent to the hydrolysis of 2ATP into 2ADP.

- The enzyme that catalyses the reaction, from a fatty acid to Acyl-CoA, we call it ***Thiokinase.*** We also call it *Acyl-CoA Synthetase.*
  Note: the difference between "Synthetase" and "Synthase" is that the "Synthetase" requires ATP while the other one doesn't.

- The location of the reaction is in the "Outer Mitochondrial Membrane" at the cytosolic side of the mitochondria.
  However: if we're dealing with a medium-chain or a short-chain fatty acid, then these two can be activated by Thiokinase **IN** the mitochondrial matrix itself, and enter the β-oxidation pathway there.

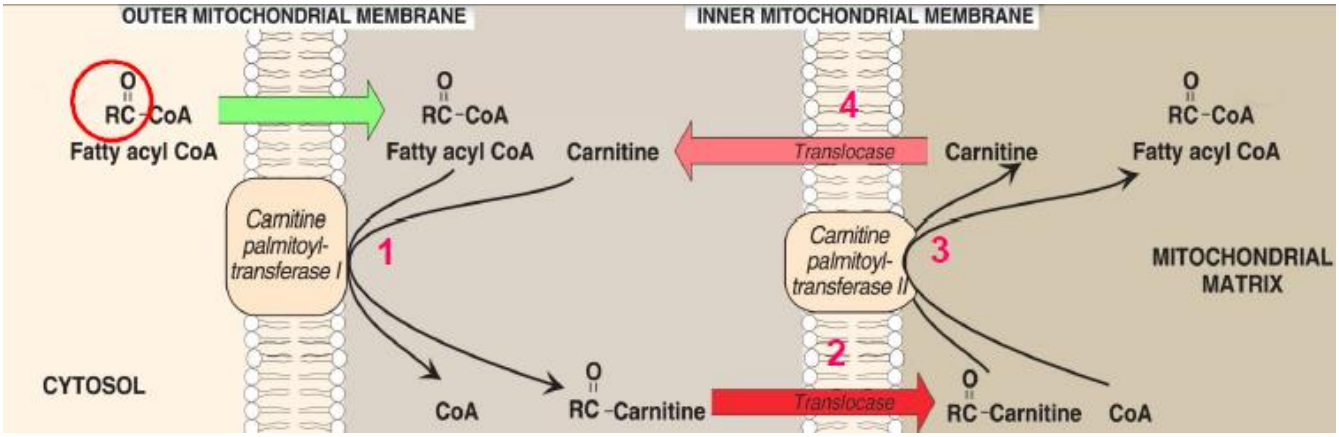
- Although the long-chain fatty acid has been activated at the outer mitochondrial membrane, it still should get into the mitochondrial matrix in order to be oxidized.

- So we have to transport Acyl-CoA, which resulted from the activation of the long-chain fatty acid, across the inner mitochondrial membrane to the matrix. But the problem here is that the inner mitochondrial membrane is impermeable to Acyl-CoA, because it's relatively considered a large molecule with a lot of negative charges.

- So to solve the problem we must have a carrier system, and it's known by the "Carnitine Shuttle". The Carnitine Shuttle helps to introduce the Acyl group into the mitochondrial matrix.

- The Carnitine Shuttle consists of: a carrier molecule, two enzymes, and a membrane transport protein.

So now let's discuss in detail how the Carnitine Shuttle works:



- Notice that the Fatty Acyl-CoA entered the outer mitochondrial membrane with no problems, because remember: we said that the inner membrane is the impermeable one.

- Also notice the presence of Carnitine, in the intermembrane space, which is a small carrier molecule that accepts the Acyl group from the Acyl-CoA in this reaction (numbered as "**1**" in the figure).

- The enzyme that transfer the Acyl group from Acyl-CoA to the Carnitine is known as "Carnitine Palmitoyl-transferase I" or "Carnitine Acyl-transferase"
  Note: they named it as Palmitoyl, because the most common fatty acid is the palmitic acid.

- So now we have Acyl-Carnitine.

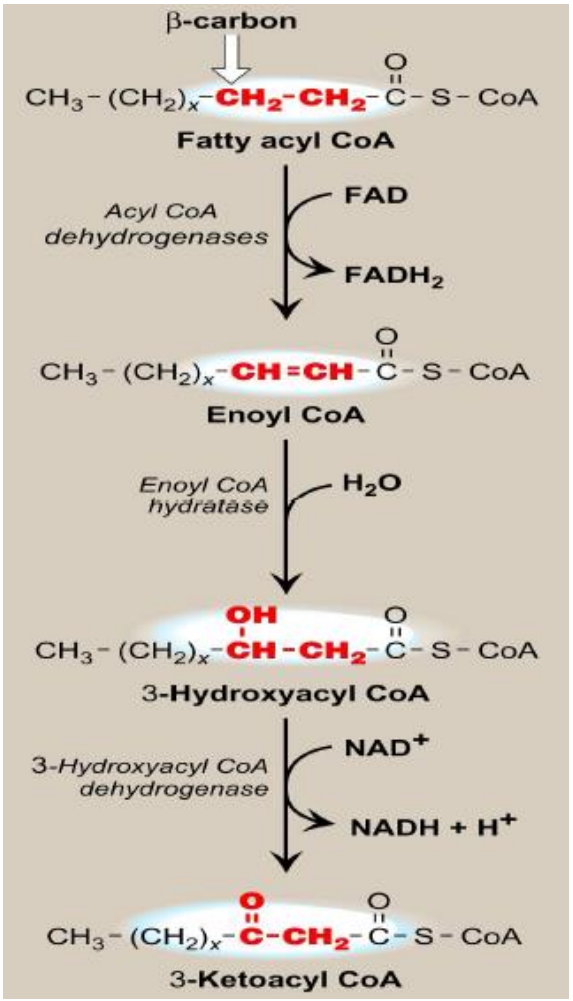
- The Acyl-Carnitine can cross through the inner mitochondrial membrane into the matrix with the help of a membrane transport protein called Translocase.

- And by an enzyme, similar to the first mentioned, the "Carnitine Palmitoyl-transferase II" transfers the Acyl group form the Acyl-Carnitine to CoA to give Acyl-CoA once again. And the freed Carnitine can go back to the intermembrane space to repeat its function.

- So you can somehow think of Carnitine as a car, or a transport vehicle that carries the Acyl group across this barrier.

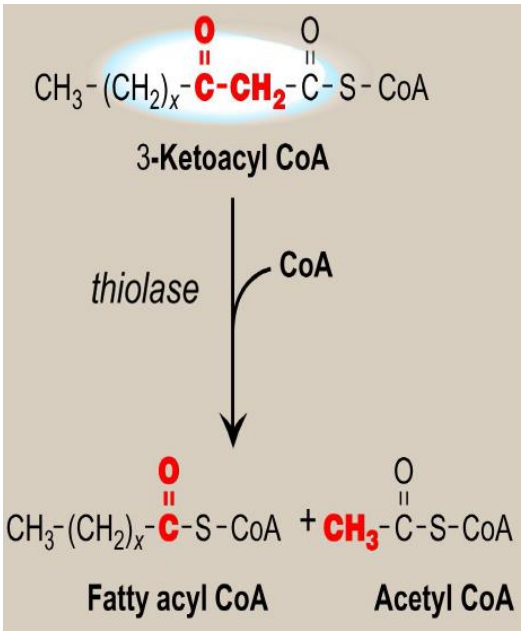
- Okay, now that we have the Fatty Acyl CoA inside we can continue with the *next step:*

2- Oxidation of the beta carbon:



- So we want to oxidize the beta carbon, as you can see in the figure to the right.
- First, there is a reduction of FAD into $FADH_2$ by accepting the hydrogens from carbon α and β.
- Because of that we introduce a double bond between those two carbons. So now it's an alkene group. And the molecule is called Enoyl CoA.
- The next step is adding water to that alkene resulting in a hydroxyl group on the β carbon to form 3-Hydroxyacyl CoA, which is a secondary alcohol.
- Now we oxidize the secondary alcohol, by reducing NAD^+, and that will of course give us a ketone. So the product is named 3-Ketoacyl CoA.
- Conclusion: by those three reactions, the β carbon got oxidized from CH_2 into C=O,
- Note: you should be smart enough to notice that those three reactions are not very new to us, since we have encountered similar reactions before in the Citric Acid Cycle. Where Succinate nearly looks like Fatty Acyl-CoA (has the same CH_2=CH_2 in the middle) which gets oxidized to form Fumarate, then we add water to it forming Malate, and finally we oxidize Malate to form Oxaloacetate.
- We haven't talked about the enzymes yet, but all their names make sense if you understood the reaction they catalyse. So let's go through them in brief:
  - The first enzyme is called "Acyl CoA dehydrogenase", which oxidizes Acyl CoA and reduces FAD. You should know that whenever we remove two hydrogens from two adjacent carbons we always use FAD as the hydrogen acceptor.
  - The second enzyme is "Enoyl CoA hydratase". It adds water to its substrate. And you should know the difference between "hydratase" and the "hydroxylase"; the former adds water, while the latter adds only a hydroxyl group.
  - The third enzyme is known by "3-Hydroxyacyl CoA dehydrogenase", which removes two hydrogens from its substrate, but not from two adjacent carbons. So that's why it reduces NAD^+ rather than FAD.

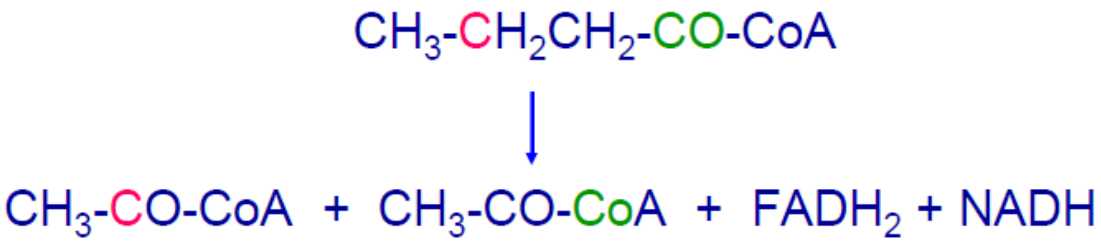
3- Cleavage of Ketoacyl CoA



- So now that we have "3-Ketoacyl CoA" the next will be the cleavage of the bond between carbon α and β.
- Now imagine that we cleaved that bond by adding of water, so what do you think the products will be? In case of adding water, the reaction will be "hydrolysis" and the products are Fatty Acid and Acetyl CoA.
- But that's not the case here. Notice that we added "CoA" and not water, therefore the reaction will be "thiolysis".
  Note: the –SH of the CoA attacked the carbonyl carbon (C=O) so we ended up with Fatty acyl CoA, and Acetyl CoA.
- Now that we understood the reaction that happened, it'll be easy to name the enzyme, which is called "Thiolase".
- We can think of the enzyme "Thiolase" as a smart enzyme, but why? Because it gave us Fatty acyl CoA. But if water did the cleavage then the product will be a Fatty Acid, so we'll *again* need to activate that Fatty Acid to give us Fatty acyl CoA, which costs two ATP. So the enzyme "Thiolase" saved us energy here by adding CoA instead of water, and that's why we said it's smart.
- Notice that in the last page we started the β oxidation with Fatty acyl CoA, and after four reactions we ended up with another Fatty acyl CoA, but what's the difference between them? Well, the last Fatty acyl CoA is actually **"<u>two carbons less</u>"**.
- So building up on that we should be able to understand this example:
  - Consider that we started the β oxidation with a **16** carbon Fatty acid (palmitic acid).
  - Then we did the β oxidation process "**6**" times.
  - How many $FADH_2$ and NADH do you think will be formed?
    Simple and easy talk: each β oxidation will give us **one** $FADH_2$ and **one** NADH. So six of them will give us **6** $FADH_2$ and **6** NADH.
  - And how many Acetyl CoA? Again, it'll be also **6**.
  - We also said that after each β oxidation we'll get a Fatty acyl CoA that is 2 carbons less. So after 6 β oxidations we'll end up with a **4** carbon Fatty acyl CoA.

Because remember that we started with **16**.

- Now let's enter that **4** carbon Fatty acyl CoA into another β oxidation reaction, what do you think the result will be?



- As you see in the figure above, we entered the **4** carbon Fatty acyl CoA into an additional β oxidation, so we ended up with **<u>two</u>** Acetyl CoA. And of course $FADH_2$ plus NADH.

- So, again, to sum up the example as a whole: we started up with a **16** carbon fatty acid, we activated it, and then we did the β oxidation **7** times to yield:
  - **7** $FADH_2$
  - **7** NADH.
  - **8** Acetyl CoA.

- Okay now what if we want to calculate how many ATPs we're getting out of this?

- The doctor in this example assumed that each $FADH_2$ gives us **2 ATP**, and each NADH gives us **3 ATP**. SO:
  - **7** $FADH_2$ will give us: 7 × 2 = 14 ATP
  - **7** NADH will give us: 7 × 3 = 21 ATP
  - **8** Acetyl CoA will give us: 8 × 12 = 96 ATP (assuming they went through the TCA cycle).

- So the total ATP is: **14 + 21 + 96 = 131.**

- But we used two ATP for activation of the fatty acid at the first step. So: **131 – 2 = 129 ATP.** And that's what we call **the net ATP moles per mole of 16 carbon fatty acid**.
- The doctor said that he'll bring a question about these calculations.

- Well you can see that this is a lot of energy. Now how about we compare this amount of energy with the energy produced by the oxidation of glucose? See there are two ways to do that:
  1. We compare the same amount of carbons; 3 molecules of glucose with 18 carbon fatty acid. And by that we're comparing 18 carbons of glucose with 18 carbons of fatty acid. *<u>NOTE</u>: this isn't really a great way of comparing, the better way is mentioned next:*

2. We compare them per gram:
   We divide **129** over the **Molecular weight** of the 16 carbon fatty acid. By that we get the moles of ATP produced per one gram of C16 FA. **(129/MW of C16 FA = n. ATP produced per gram)**.

   Now we do the same with the glucose to get the number of ATP per gram of glucose. So by comparing those two numbers you'll be surprised that **the number of ATP produced by one gram of "16 carbon fatty acid" is almost twice (or more) as the number produced by one gram of glucose.**
   That emphasises that the amount of energy produced by the oxidation of fatty acids is almost twice as much as ,or more, the energy produced by the oxidation of glucose.

Carnitine:

- We said that the Carnitine transports the Fatty acyl CoA across the inner mitochondrial membrane.

- Why do we care about Carnitine? Because we can have Carnitine deficiency.

- The sources of Carnitine are either dietary or they get synthesised in the liver and the kidney. You can find it in meat, because meats are muscles, and muscles mainly get ATP by the oxidation of fatty acids.

- If our dietary source of Carnitine is inadequate, then we can synthesise Carnitine BUT at the expense of essential *amino acids* which are needed for the synthesis of our proteins.

- We have other functions of Carnitine other than it being a shuttle, such as:
  1- export of branched chain acyl groups from mitochondria.
  2- excretion of acyl groups that cannot be metabolized in the body.

- So as we said we have Carnitine deficiency, well it's not very common, but it can be classified as: **secondary deficiency**, or **congenital deficiency**.

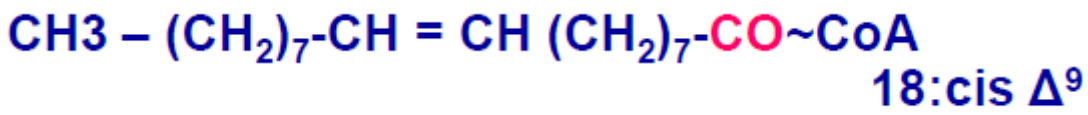
- Congenital deficiency is inherited and it means that someone is born with an inability to produce adequate amount of Carnitine.
  EX: **1-** decrease in enzyme that is involved in Carnitine synthesis.
  2- decrease in tubular reabsorption in the kidney, so such person will lose Carnitine the urine, which leads to congenital Carnitine deficiency.

- Secondary deficiency could be due to decrease in the secretion of Carnitine because of a liver disease, or malnutrition, as well as increased requirements…

- Now what do you think the person with Carnitine deficiency will suffer from?
    - Impaired ability to oxidize fatty acid as source of energy, which leads to weakness of the muscle, and muscle pain after exercise.
    - Using glucose as source of energy, this leads to hypoglycaemia. Because the glucose is supposed to be saved for the brain, so if the muscle uses glucose as the source of energy we'll suffer from hypoglycaemia.
    - Accumulation of fatty acids and branched acyl groups in cells.
- Carnitine is sold in pharmacies, which most people use for the build-up of their muscles, but it's not scientifically proved that they could benefit from it. And some take it as a food supplement.

Now let's talk about the **Oxidation of Unsaturated Fatty Acids**:

- An example of it is the Oleic Acid, which has a double bond at carbon number **9**, in the figure below.



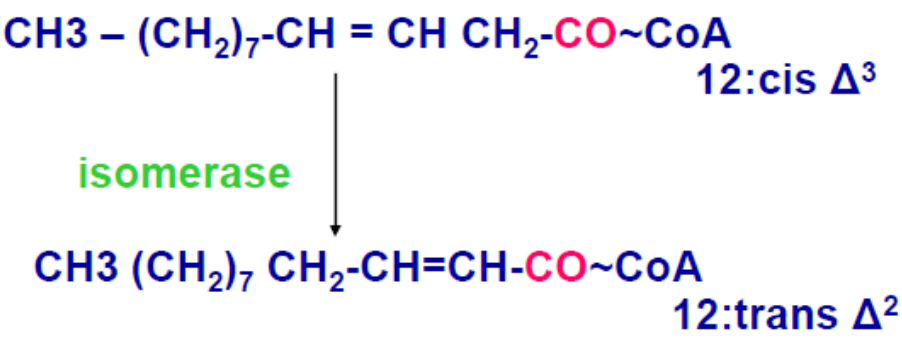
- **18:cis Δ^9** means that it's composed of **18** carbons, and at the carbon number **9** there's a double bond with the **cis** configuration.
  Note: the double bonds present normally in the fatty acid are of **cis** configuration, but the **trans** configuration happens during the process of hydrogenation.
- The oxidation of **unsaturated** fatty acids is similar to the **saturated**, but it's modified. So we have to modify the β oxidation pathway to deal with this double bond.
- Let's see how this is oxidized:
    - After **3** β oxidation pathways, of the above Fatty acyl CoA, what do you think the result will be?
    - Well of course we'll have **3** $FADH_2$, **3** NADH, and **3** Acetyl CoA. And a fatty acyl CoA with **6** carbons less:

    - So accordingly the double bond we'll be at carbon number **3**. (Compare the two figures).

- At this very point the doctor said he'll bring a question about the location of the double bond and it'll be either in the final exam or in the makeup.

- Okay, imagine that this fatty acid is saturated, then the next step will be introducing a double bond between carbon 2 and 3.

- But you see in the last figure, we already have a double bond between carbon 3 and 4. So this double bond will prevent the formation of a new double bond between carbon 2 and 3.

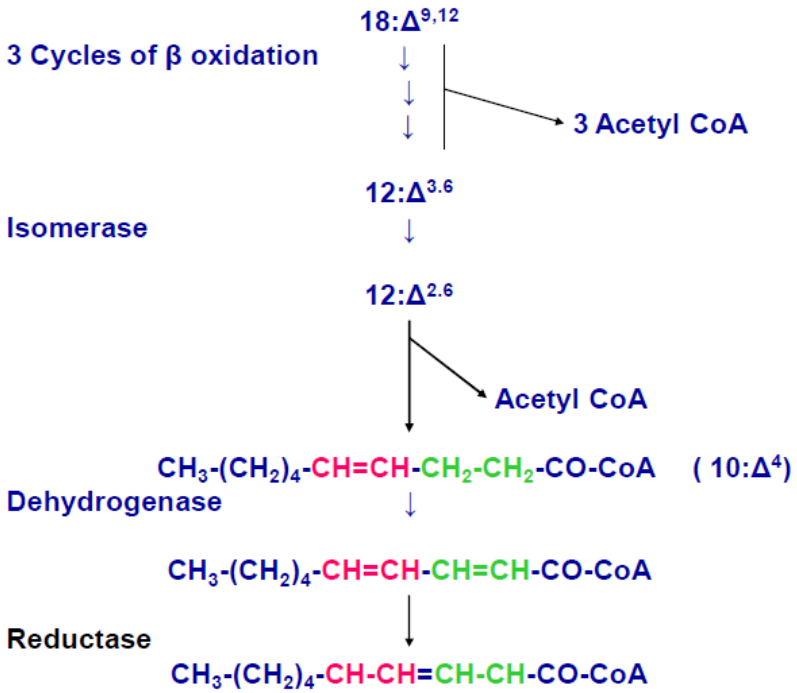


- So the problem is solved by the enzyme "Isomerase" as seen above, which shifts the double bond to be between the carbon 2 and 3.

- Notice also that the **cis** bond became **trans** as the result of the hydrogenation reaction.

What if the unsaturated fatty acid has **two double bonds?**

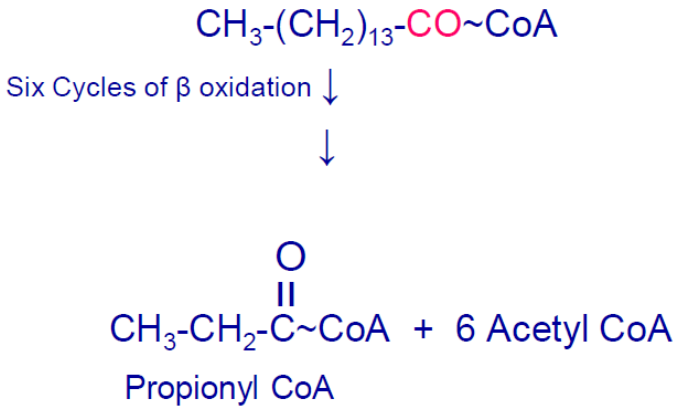
- We start with Linoleic Acid which has two double bonds (**18: Δ^{9,12}**).
- So after 3 β oxidation cycles we'll have **12: Δ^{3,6}** .
- And with "Isomerase" we'll get to this **12: Δ^{2,6}** .
- So this will undergo one more cycle of β oxidation to get **10: Δ^{4}** .
- Notice that we used "Isomerase" to shift the double bond to carbon **2**, and then to get rid of it. So that's why we ended up with only **one** double bond.
- And now by a "dehydrogenase" enzyme we introduce a new double bond between carbon 2 and 3.
- So the new molecule is **10: Δ^{2,4}.**
- Now the "Reductase" enzyme adds two hydrogens, one at carbon **2** and one at carbon **5**.



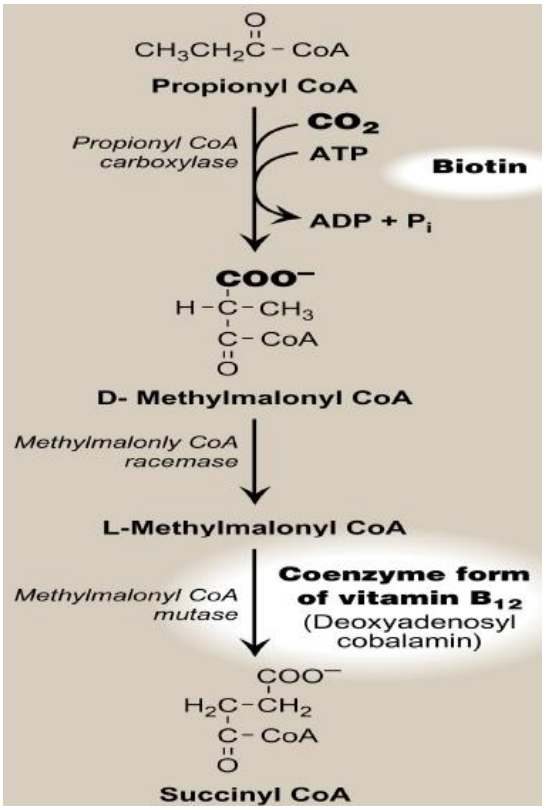
- After that we'll get **10: Δ^3**. And now what? The "Isomerase" will do his work again.
- The doctor said that he'll **not** ask us about the energy yield **in the <u>unsaturated</u> fatty acids.**

The oxidation of fatty acids with <u>odd number of carbons</u>:

- Most of the fatty acids as you learned contain an **even** number of carbons. But if you consumed animal fat, they contain odd number of fatty acids.
- Look at this example with fatty acid with 15 carbons.
- **Six cycles** of β oxidation will proceed as usual.
- The end product will be **6** acetyl CoA and a carboxylic acid with three carbons called "**Propionyl CoA"**.



- Now how can we deal with Propionyl CoA?
- The first step of utilizing Propionyl CoA as source of energy is adding a carboxyl group as CO_2. And this process requires ATP.
- So the product is a malonic acid called D-Methylmalonyl CoA.
- Obviously the enzyme that catalyzes this reaction is "Propionyl CoA carboxylase"
- And of course since the enzyme is carboxylase then it needs Biotin as cofactor.
- Now we switch the "D" form of Methylmalonyl CoA to the "L".
- What happens now that we transfer the carboxyl group to the terminal carbon to result in Succinyl CoA, which is an intermediate of the TCA cycle. And this reaction requires **Vitamin B_{12}**.
  NOTE: there are only two reactions in humans that require Vitamin B_{12}, and this reaction is one of them.



Nowadays you can measure the level of vitamin B_{12} in the plasma; we take a blood sample and give it to the laboratory.

But this technique was not available like 30 years ago. So how did we measure it back then? We used to measure the levels of Methylmalonyl CoA, if it's elevated then we have a vitamin B_{12} deficiency.

The Sheet is Finally Over