◉Sheet ○Slides

Subject :	β-oxidation pathway
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Number :	20

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

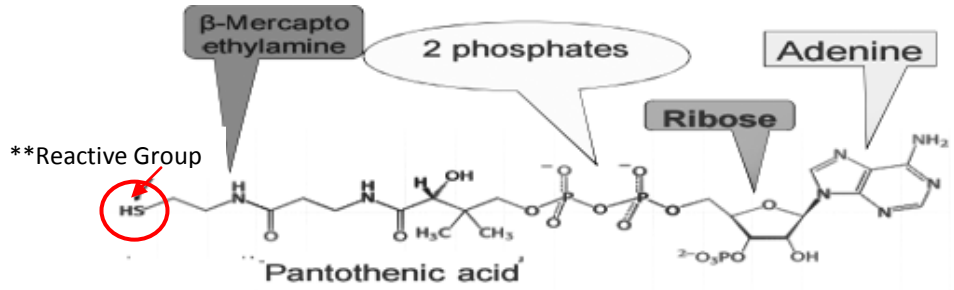
Fatty acids are transported to tissues bound to albumin, why?

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) [oxidation at β carbon followed by cleavage of two carbon units].

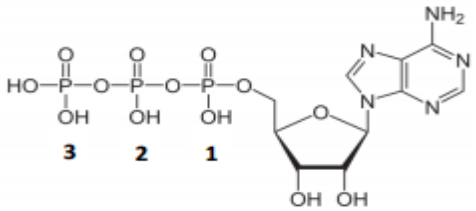
1- Activation of the fatty acid:

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 found in limited amount and is formed from adenine, Ribose, 2 Phosphate groups (Like ADP until now), Pantothenic acid (B5 vitamin) and β-Mercaptoethylamine.



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. (We cleaved a high energy bond and formed another high energy bond).

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 **PP_i** 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:

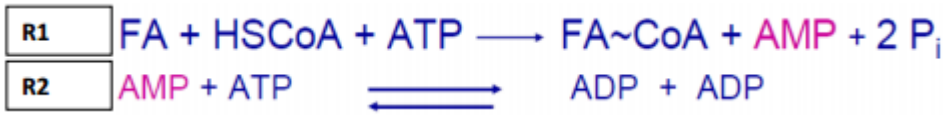
$$FA + HSCoA + ATP \longrightarrow FA\sim CoA + AMP + 2\ P_i$$

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 favoring 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).



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

Using one ATP and converting it to AMP + 2Pi is equivalent to the hydrolysis of 2ATP into 2ADP.
<u>How many ATP molecules in fatty acid activation</u>? → 2 molecules
<u>How many</u> <u>ATP molecules are used by the enzyme itself</u>? → Only one

- 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.

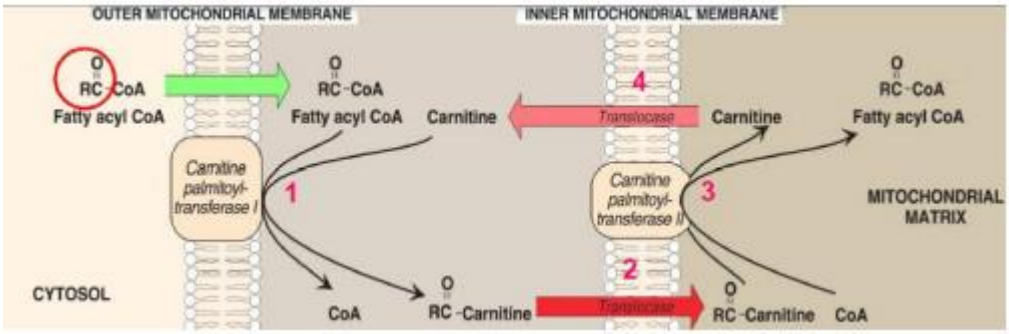
However: if we're dealing with a medium-chain or a short-chain fatty acid (4-10), then these two can be activated by Thiokinase <u>in the mitochondrial matrix itself</u>, and enter the β-oxidation pathway there.

Although the long-chain fatty acid is activated at the outer mitochondrial membrane, it still should get into the mitochondrial matrix in order to be oxidized because the enzymes exist there.
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.

Carnitine Shuttle:



1) The Fatty Acyl-CoA enters the outer mitochondrial membrane with no problems, because the inner membrane is the impermeable one.

2) A small carrier **Carnitine**, in the intermembrane space, accepts the Acyl group from the Acyl-CoA in the 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: it is named it as Palmitoyl, because the most common fatty acid is the palmitic acid.

So now we have **Acyl-Carnitine**.

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

4) The Acyl group from Acyl-Carnitine is transferred to CoA to give Acyl-CoA once again by an enzyme, similar to the first mentioned, called the "**Carnitine Palmitoyl-transferase II**".

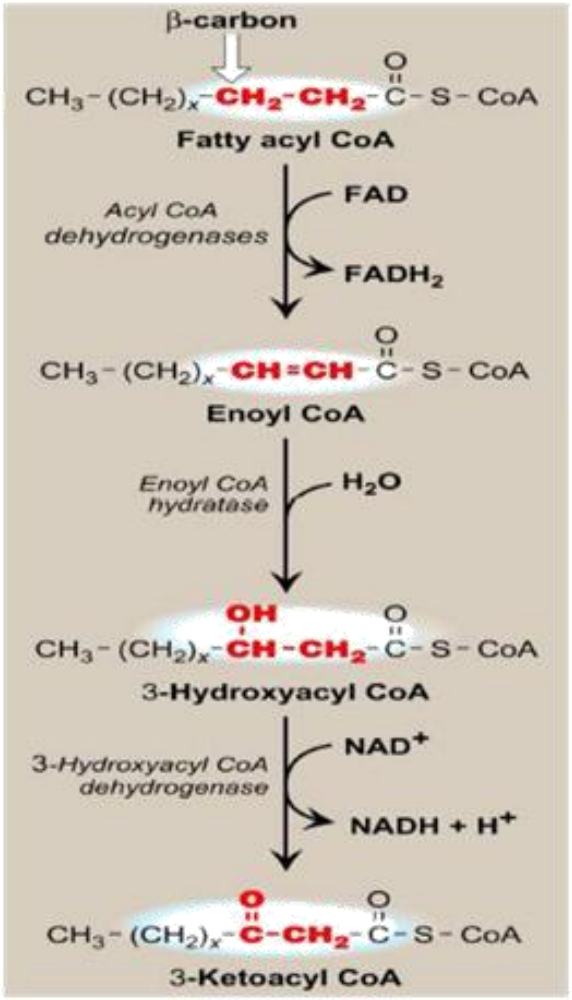
*Note: CoA doesn't pass the inner membrane.

5) The freed Carnitine can go back to the intermembrane space to repeat its function.

So you can somehow think of Carnitine a transport vehicle that carries the Acyl group across this barrier (it is a shuttle).

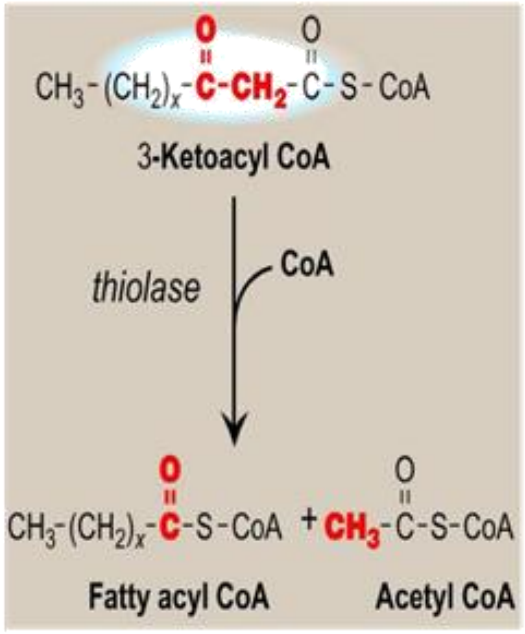
Now that we have the Fatty Acyl CoA inside, we can continue with the *next step:*

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 forming a hydroxyl group on the β carbon specifically to form **3-Hydroxyacyl CoA**, which is a secondary alcohol.
- Now we oxidize the secondary alcohol, by reducing NAD^+, and that will 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. The difference between "hydratase" and the "hydroxylase" is that 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**".

- The enzyme "Thiolase" as a smart enzyme because it gave us Fatty acyl CoA saving two molecules of ATP. How? 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 say it's a smart enzyme.

- 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 **"**two carbons less**". It is called β Oxidation cycle**; but of course, this cycle has to end.

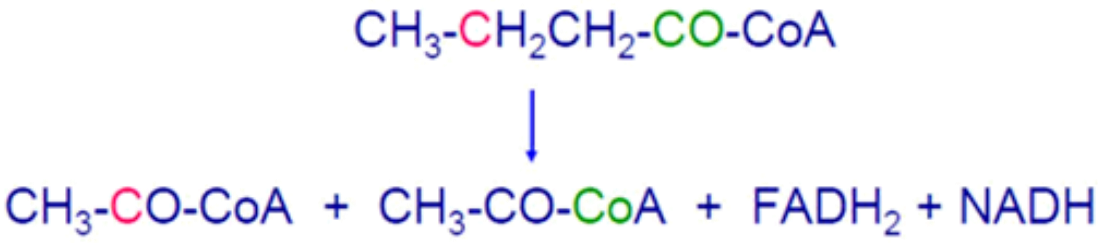
- So building up on that we should be able to understand this example:

  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 will be formed?

    Each β oxidation will give us **one** $FADH_2$ and **one** NADH. So six of them will give **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 the **4** carbon Fatty acyl CoA goes through another β oxidation reaction.



- 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 molecules. And of course $FADH_2$ plus NADH.

- 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 molecules.

- 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**.

❖ 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:

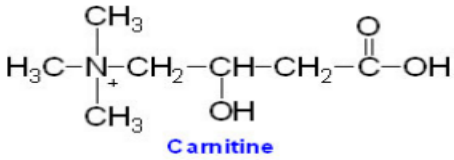
2. We compare them per gram:

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 emphasizes 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: *(ine = Amine)*



Carnitine

•Carnitine transports the Fatty acyl CoA across the inner mitochondrial membrane

•Muscles have the highest activity for Carnitine because they consume fatty acides as source of energy during exercises.

•Sources of Carnitine: 1) dietary 2) they get synthesised in the liver and the kidney from the essential amino acid Lysine. 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 an essential *amino acids* which are needed for the synthesis of our proteins.

• Carnitine has other functions, such as:
1- Export of branched chain acyl groups from mitochondria.
2- Excretion of acyl groups that cannot be metabolized in the body.

Carnitine deficiency: 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 (Rare).

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.

❖ Now what do you think the person with Carnitine deficiency will suffer from?

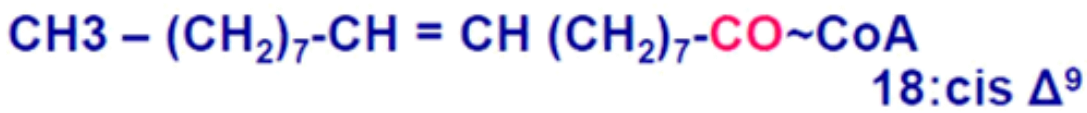
- Impaired ability to oxidize fatty acid as source of energy, which leads to weakness of the muscle, and muscle pain after exercise.
- Using glucose as source of energy, this leads to hypoglycaemia. Because the glucose is supposed to be saved for the brain, so if the muscle uses glucose as the source 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 solve the problem (body builders use it). 💪

❖ We can just avoid long chain and take short and medium chain fatty acids because they don't need a shuttle (short chain fatty acid exists in the butter and milk).

Oxidation of Unsaturated Fatty Acids:

❖ An example of an unsaturated fatty acid is the Oleic Acid, which has a double bond at carbon number **9**, in the figure below.



❖ **18:cis** ^{9} means that it's composed of **18** carbons, and at the carbon number **9** there's a double bond with a **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 cycles, 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).

- If that this fatty acid was saturated, then the next step yould 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.

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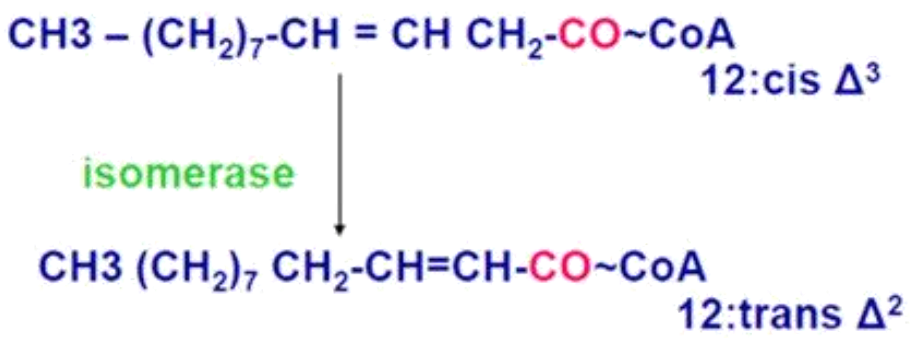


- So the problem is solved by the enzyme "Isomerase" as seen above, which shifts the double bond to be between the carbon 2 and 3.

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

Acyl CoA dehydrogenase also produces trans double so we need this trans double bond to be produced by this isomerase also.

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

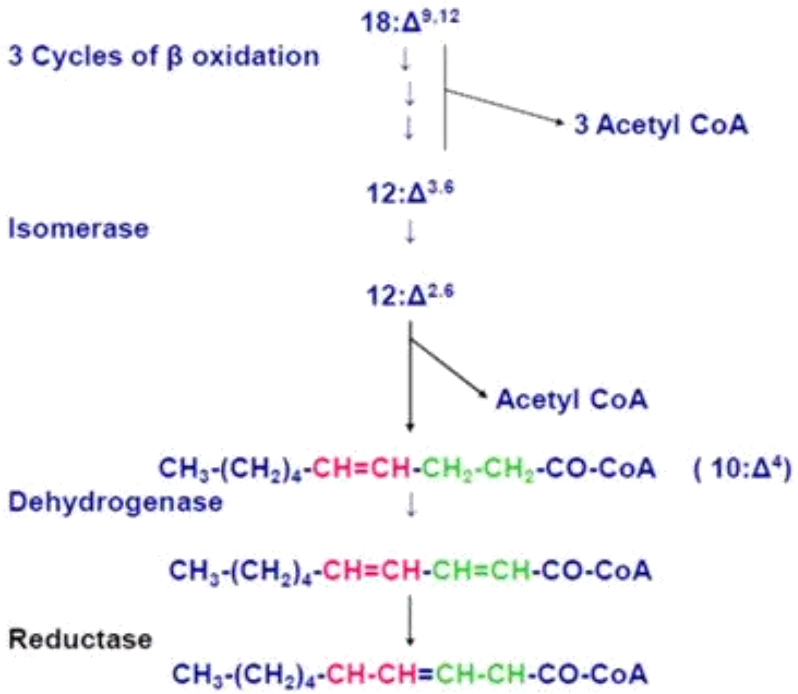
1) We start with Linoleic Acid which has two double bonds (**18: ^{9,12}**).
2) After 3 β oxidation cycles we'll have (**12: ^{3,6}**) .
3) "Isomerase" will make it (**12:^{2,6}**).

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.

4) It'll undergo one more cycle of β oxidation to get (**10: ^{4}**)

**we can't just move to carbon number 2 because isomerase only moves the double bond to the adjacent carbon.

5) "Dehydrogenase" enzyme will introduce a new double bond between carbon 2 and 3.
So the new molecule is (**10: ^{2,4}**).



6) "Reductase" enzyme adds two hydrogens, one at carbon **2** and one at carbon **5** and a new double bond is formed between carbons number **2** and **3** (2 double bonds are reduced to one).

After that we'll get **10:** ^{3}. Then "Isomerase" will do its work again.

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Good Luck anyway :")