Enzyme Science and Technology
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Module - VI
Enzyme Catalyzed Biochemical Reactions
Lecture - 29
Lipid Metabolism

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT, Guwahati. And what we were discussing? We were discussing about the different properties of the enzyme in the course, Enzyme Science and Technology. And so far, what we have discussed? We have discussed about the enzyme classification and nomenclature followed by; we have also discussed about the enzyme structures.

So, we have discussed about the primary structure, secondary structure, tertiary structures and the quaternary structures. And in the previous couple of modules, we have also discussed how you can be able to produce the enzyme in the bulk quantities. So, that you can be able to use them for studying its properties or you can use them for other kinds of applications.

So, in this context, in this particular module, we are discussing about the important reactions where the enzymes are playing crucial role. And in this context, if you recall in our previous lecture, we have discussed about the carbohydrate metabolisms and when we were discussing about the carbohydrate metabolism, we have said that we have we have discussed about the glycolysis and the kreb cycle.

And we discussed how the different enzymes are playing the, you know, conversion of the one substrate into the product and how that is actually facilitating the production of energy through the production of NADH and as well as the ATP. And then in this particular lecture today, we are going to continue our discussion about the reactions where the enzymes are involved in producing the energy for the cell.

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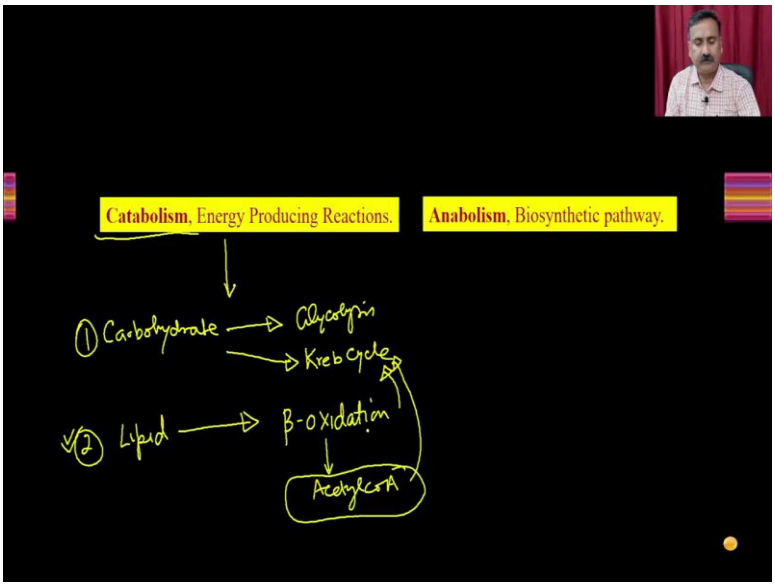

So, in this context, what we have said that all the reactions which are actually producing the energy are coming under the under the broad category that is the catabolism. And catabolisms are actually mainly being done for the two biomolecules; one is carbohydrate and within the carbohydrate, we have discussed about the glycolysis and we have also discussed about the kreb cycle.

And apart from carbohydrate, we also have the another biomolecule which is mainly being used for the energy production and that is the lipids. And the lipids are undergoing for the energy production in a series of reactions collectively been considered under the beta oxidations. And beta oxidation is also been linked to the kreb cycle.

So, the end product of the beta oxidation is that the acetyl CoA and the acetyl CoA is actually going to be a going to be get feeded into the kreb cycle and that is how the all these two, these two all these 3 pathways are interrelated to each other. So, let us discuss about the lipid metabolism and how the beta oxidation is producing the energy and what are the different enzymes are involved in this particular type of metabolic pathway.

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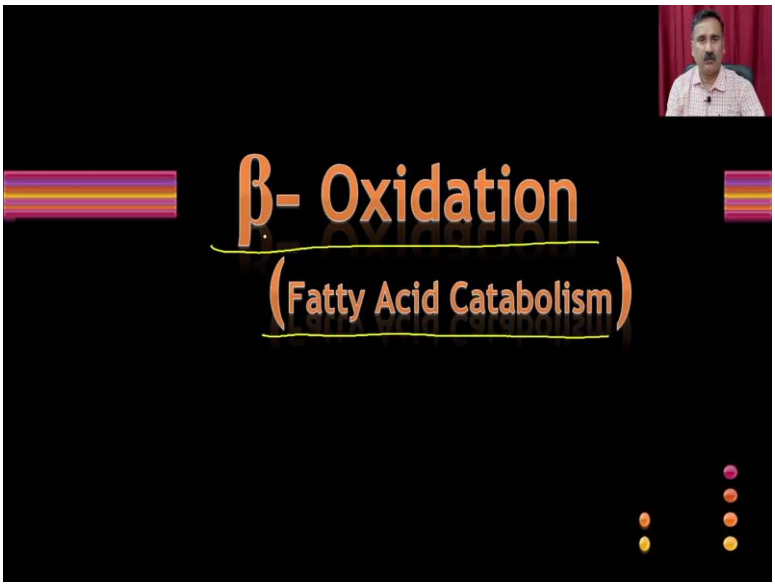

So, beta oxidation or the fatty acid catabolism. So, beta oxidation is being started when there will be a entry of lipid molecules inside the cell. But that does not start from that point of view, it starts when you are actually taking the lipid rich diet in your food. So, if you go back how the lipid catabolism started.

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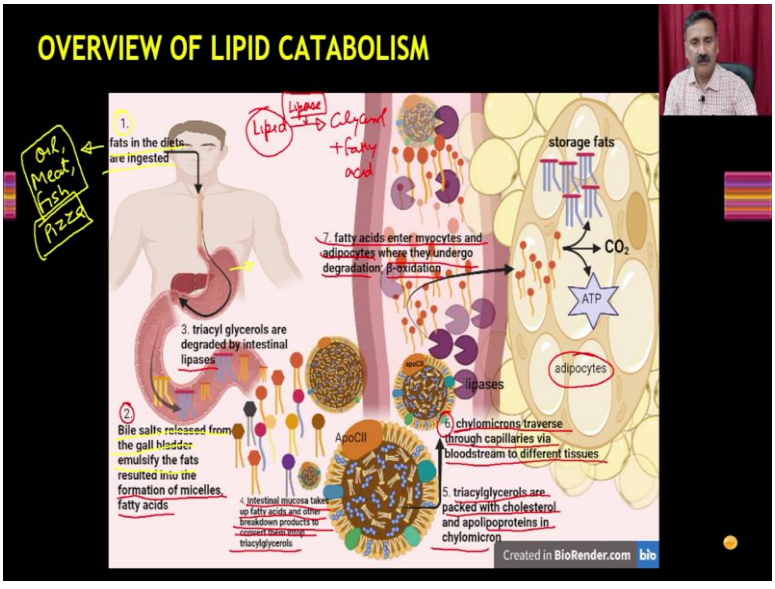

The lipid catabolism started with the fat in the you know in the diet which are being ingested. So, you have the fat rich diet like the oil, you can have the fatty, you know the meat and you can also have the fish.

So, when you take these including the pizza also right. So, when you take these lipid rich molecules they will be going to be ingested into the system and I am sure we have many of the enzymes which are involved. So, they will enter into the stomach. So, the first step is that the fats are going to be ingested through in into the diet ok and then will it will enter into the stomach right.

So, there is no digestion of the fat into the stomach and then the when it enters into the small intestine the bile salts are going to be released from the gallbladder and that is actually going to emulsify the fat resulted into the formation of the micelles and resulting into the formation of micelles and the fatty acids. And in the these emulsified fat is actually going to be entered into the small intestine and then it is actually going to be degraded by the enzyme which is called as lipases.

So, lipases are actually going to get convert into the lipid into the glycerol and it is actually going to generate the fatty acid. The fatty acid could be the saturated fatty acids or unsaturated fatty acids, so that anyway we are not taking care into this, right. So, it is actually going to be digested by the lipases. So, you have the pancreatic lipases which are actually going to act on the lipid molecules and that is how it is actually going to produce the fatty acids.

These fatty acid molecules are then been taken up by the intestinal mucosa. So, intestinal mucosa will take the fatty acid and other breakdown product to the convert them into the triglycerides. And you see this this is the chylomicron, but you see and the chylomicron is going to be taken up by the is the way in which the fatty acids are going to be transported within the blood right. So, the triglycerides are then going to be packed with the cholesterol and apolipoprotein in a in the form of a chylomicron.

So, chylomicrons are going to be packed where you have the lipid bilayers and within the lipid bilayer you have the cholesterol, triglycerides and all these. So, these are like a liposomes and where you have the several types of apolipoproteins and you also have the cavity in which the lipid molecules are going to be filled. So, you have the triglycerides and the cholesterol and then in the step number 6 the chylomicrons are transverse through the capillaries via blood stream to the different issues.

So, chylomicron will actually transport into the blood and that its going to be present in the blood and they will actually going to go to the different organisms ok. Then in the

step number 7 the fatty acid which are enter going to be enter into the myocytes and adipocytes where they will undergo the oxidations that is the beta oxidations.

And apart from this if there is a no requirement of energy there is a you know low metabolic pathways, then the fatty acids are actually going to be again getting converted into the lipids and that is how it is actually going to be stored into the adipocytes.

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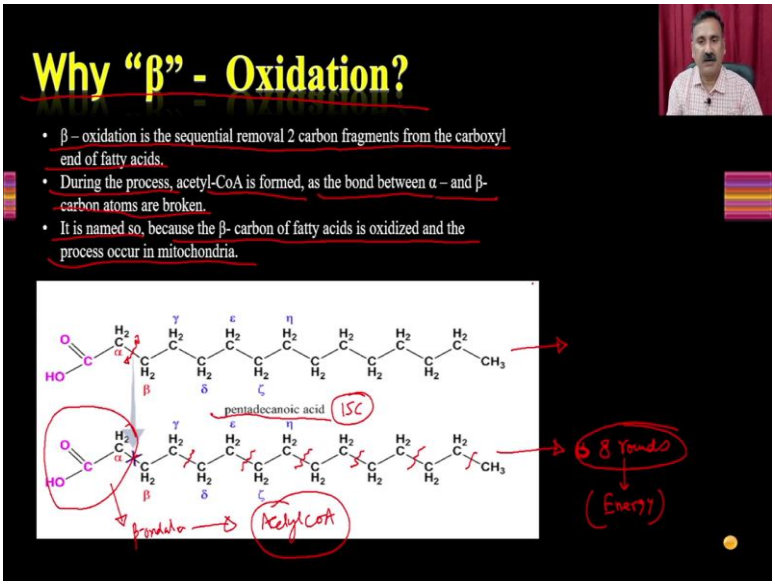

Now, what is the beta oxidation? So and why it is called as the beta oxidations? So, beta oxidation is a sequential removal of 2 carbon fragment from the carboxyl end of the fatty acids. During this process the acetyl CoA is actually going to be formed and as the bond between the alpha and the beta carbon are going to be broken down.

It is named as so because the beta carbon of the fatty acid is oxidized and the process occurs into the mitochondria. So, this is what you are going to see. This is actually a lipid molecules or the fatty acids which is called as the pentadecanoic acid. Pentadecanoic acid means it is actually going to have the 15 carbon fatty acids and the bond between you see the this is the bond between the alpha carbon and the beta carbon.

So, this bond is actually going to be broken down and that is how this particular moiety is actually going to be released and that will undergo into the beta oxidation to produce the acetyl CoA ok.

So, every 2 carbon from the this particular fatty acid is actually going to be released and that is how it is actually going to produce the acetyl CoA. So, you can imagine that if this fatty acid has to go through the beta oxidation it will actually go for the beta oxidation for the 8 rounds ok.

So, in the 8 rounds only then it is actually going to get converted. For example, in the second round when the they will this bond is going to be broken down it is actually going to broken down from here, then it is actually going to be broken down here then its going to be broken down here like that ok

And ultimately it will going to have the single you know the acetyl CoA which is going to be left and that is how it is actually going to be fully oxidized and it will actually going to produce the large quantity of energy right. So, beta oxidation is a multi-step process; one of the major crucial step is that you take these particular you know degradation product and it will transported that fatty acid into the mitochondria because beta oxidation occurs inside the mitochondria.

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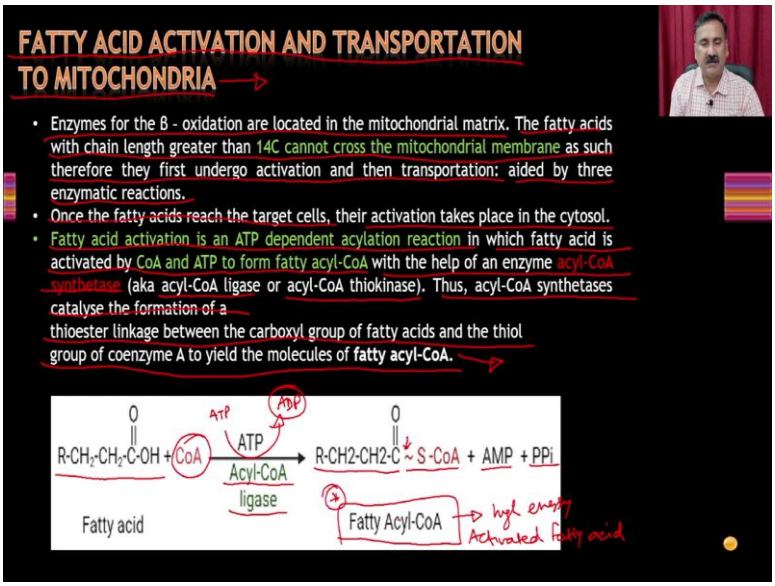

So, the fatty acid first step is the fatty acid activation and the transportation into the mitochondria. So, if you recall when we are talking about the carbohydrate metabolism we said that the carbo in the carbohydrate metabolism also a glucose molecule which is going to be you know which is going to be taken up by which is going to be taken up into the cell is actually going to be phosphorylate and then only the molecule is going to

be in activated state and that is how it is actually going to be committed for the glycolysis.

Same is true in the case of beta oxidations. You have to activate the fatty, so that it is actually going to be committed for the energy production and then it will actually going to be transported into the mitochondria. So, the enzymes for the beta oxidations are located into the mitochondrial matrix. The fatty acid with the chain length greater and than 14 carbons cannot cross the mitochondrial membrane as such therefore they undergo the activation and then transportion added by the three enzymatic reactions.

Once the fatty acid reach the target cell their activation takes place in the cytosol. Fatty acid activation is a ATP dependent a acylation reaction in which the fatty acid is activated by the coenzyme A and the ATP to form the fatty acyl-CoA with the help of an enzyme which is called as acetyl CoA synthetase, which also going to be called as the acyl CoA ligase or the acyl CoA thiokinase.

Thus, acyl CoA synthetase catalyzes the formation of the thioester linkage between the carboxyl side of the fatty acid and the thiol group on the coenzyme A to develop the fatty acyl CoA ok. So, this is the reaction what it is going to catalyze, so you or the end this is the fatty acid and it is actually going to react with the coenzyme A in the process of the ATP.

So, the one molecule of ATP is actually going to be consumed and it is going to be get converted into ATP and the reaction is going to be catalyzed by the acyl CoA synthetase or the acyl-CoA ligase both are the same name for this these are the two different names for the same enzyme

And then it is actually going to form a fatty acid with acyl-CoA ok. So, this is actually a very high energy bond which is actually going to be formed between the fatty acid and the coenzyme A and it is going to form the AMP and PPI and that is what it is called as fatty acyl-CoA.

This is a very high energy molecule because its it going to be contains the thioester linkage and that thioester linkage is very very you know energetically very active and that is how this molecule is going to be in a activated this is called as the activated fatty acids.

So, basically you now what you have done is you have put the tag on this particular fatty acid and saying that this fatty acid will go inside the mitochondria and it is actually going to be undergo the (Refer Time: 14:25)

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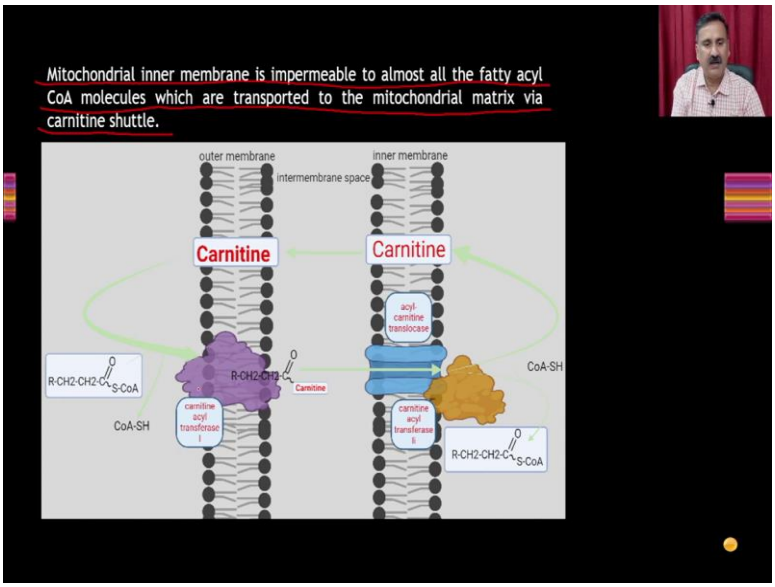

Then mitochondrial inner membrane is impermeable to almost all the acyl CoA molecule which are transported to the mitochondrial matrix via the carnitine shuttle. So, there is a carnitine shuttle through which the fatty acyl co enzyme molecule are going to be transported.

So, where you have the carnitine proteins and you also have the carnitine acyl transferase and all these two molecules are actually going to be participate into a shuttling processes and that is how it is actually going to take the you know the fatty acyl CoA from the cytosolic site and it will actually transport that into the mitochondrial site.

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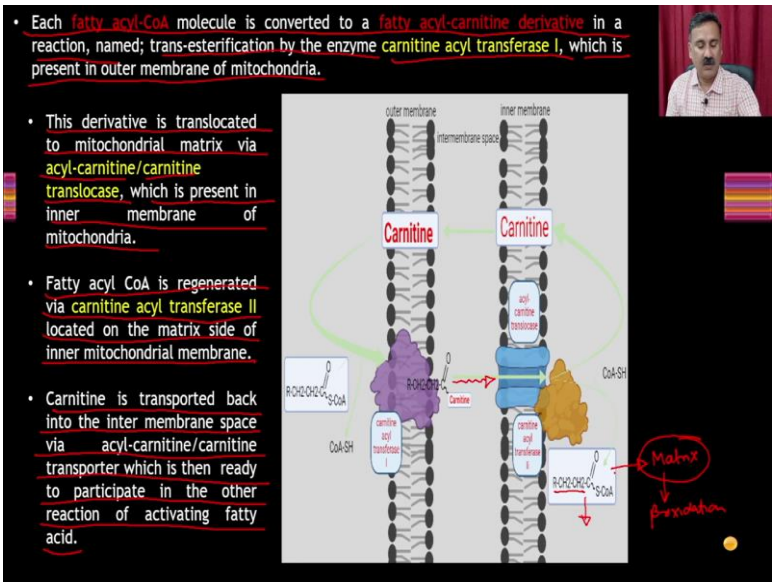

How that happens? It happens that the each fatty acyl CoA molecule is converted into the first the fatty acyl carnitine derivatives in reaction named the trans esterification and it is this reaction is going to be catalyzed by the carnitine acyl transferase I, which is present in the outer membrane of the mitochondria.

So, this is what this enzyme is actually going to take up the fatty acyl CoA the target and it is actually going to generate the fatty acyl carnitine derivatives. So, the carnitine protein is going to be tagged and then the this derivative is translocated to the mitochondrial matrix via the acyl carnitine or the carnitine translocase which is present in the inner membrane of the mitochondria. So, this is the acyl carnitine translocase.

So, first in the first step the they will take the fatty acid and the fatty acid is going to be tagged with the carnitine protein. And once this happens it is actually going to get a particular type of you know token and this with the help of this token it is actually going to be get inside the inner mitochondria right.

And the fatty acyl CoA is regenerated while the carnitine acyl transferase II. So, once it enters into the mitochondrial matrix the carnitine acyl transferase II which is the this enzyme is actually going to reverse the reaction.

So, in the first step the carnitine is been tagged in this step the carnitine is actually going to be removed from the fatty acyl CoA and that is how the fatty acyl CoA is actually

going to be released into the matrix. So, the carnitine acyl transferase II which is located onto the matrix side of the inner mitochondria. Carnitine is then transported back into the inner mitochondrial space by the acyl- carnitine transporter which is then ready to participate in the other reaction of the activating fatty acid.

So, then this carnitine what is going to be released it is actually going to be recycled back into the outer membrane and that is how this carnitine will again participate into the conjugation reaction. And that is how the fatty acyl CoA is going whatever the fatty acyl CoA is going to be generated into the cytosol part will actually going to be get into the mitochondria.

Now, once the fatty acyl CoA will enter into the mitochondrial matrix it is actually going to be participate into the beta oxidation.

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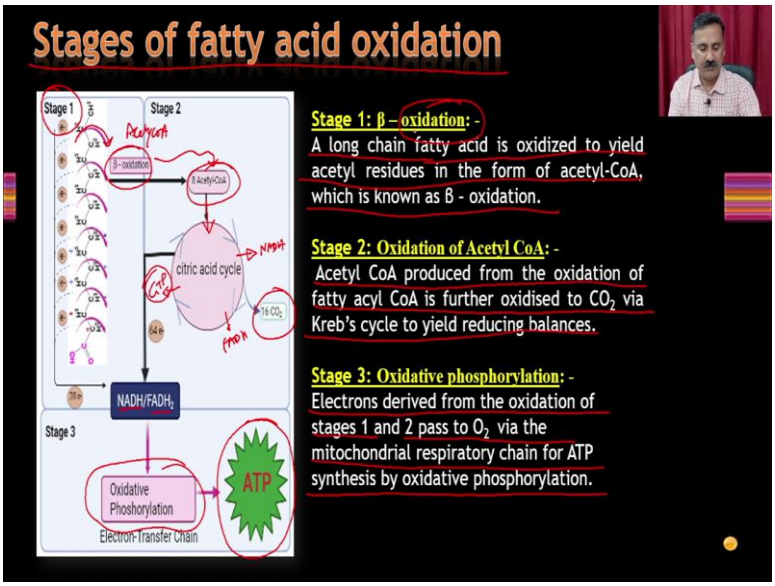

So, the stages of the fatty acid oxidations. So, in the beta oxidation you have the 3 stages. So, 1st step is the oxidation step. So, a long chain fatty acid is oxidized to yield the acetyl residues in the form of acetyl CoA which is known as the beta oxidations. Then in the stage 2 you are going to have the oxidation of acetyl CoA.

So, acetyl CoA produced from the oxidation of the fatty acyl CoA will further oxidize to the carbon dioxide while the kreb cycle to yield the reducing equivalents. And in the stage 3 you are going to have the oxidative phosphorylations. So, electron derived from

the oxidation of the stage 1 and stage 2 passes through the oxygen via the mitochondrial respiratory chain for ATP synthesis by the oxidative phosphorylation.

So, what will happen is that in the stage 1 you are going to have the beta oxidation which means after every second carbon the fatty acid is actually going to be broken down and that is actually going to generate the acetyl CoA. This acetyl CoA is actually going to enter into the mitochondria right.

So, it is actually going to be produced into the mitochondria right. And that acetyl CoA is actually going to enter into the kreb cycle. Remember that the pyruvate is actually going to be converted also into the acetyl CoA and that is why acetyl CoA will enter into the kreb cycle and will form citric acid and that is how it is actually going to produce the carbon dioxide right.

And then in this acetyl CoA this kreb cycle also it is actually going to produce the NADH. It is actually going to produce the FADH and its also going to produce the GDP right. All these molecule will then enter into the oxidative phosphorylation. So, all the NADH, FADH2 and all that will enter into the electron transport chain and that is how they will actually going to produce the large quantity of ATP. So, 1st step is the oxidation.

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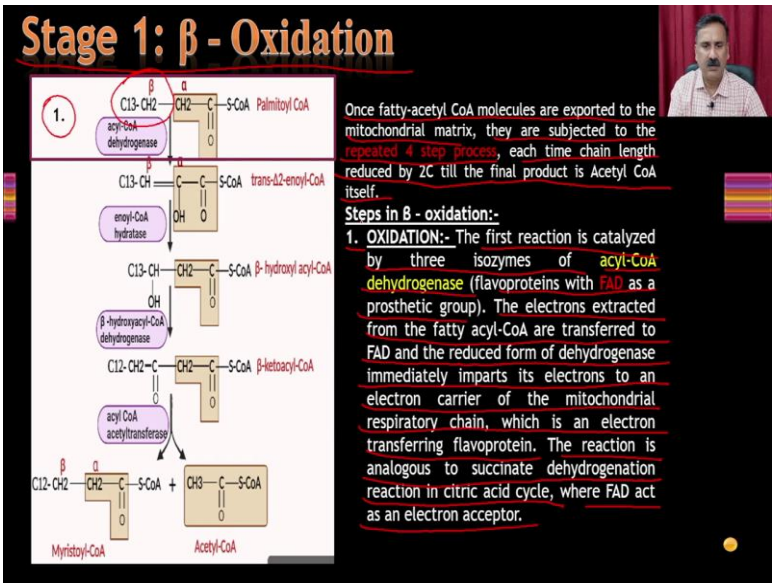

So, in the stage 1 we have the beta oxidations. So, once the fatty acid molecules are exported to the mitochondrial matrix, they are subjected to the repeated four step process. Each chain length reduced by the 2 carbon till the final product is acetyl CoA itself.

So, what are the steps in the beta oxidations? So, 1st step is the oxidation. So, this is what the 1st step; the 1st step is the oxidation. The 1st reaction is going to be catalyzed by the three isoline of the acyl CoA dehydrogenase. Flavoprotein with FAD as a prosthetic group,

The electron extracted from the fatty acid acetyl CoA, acyl CoA are transported to the fatty FAD and the reduced form of dehydrogenase immediately imparts its electron to an electron carrier of the mitochondrial respiratory chain which is an electron transferring flavoprotein. The reaction is analogous to the succinate dehydrogenase reaction in the citric acid cycle where FAD act as an electron acceptor.

So, the first is that acetyl CoA dehydrogenase is going to take up this beta 2 carbon molecules right and then it is actually going to you know reduce the, it is going to use that and it is going to produce the 1 molecule of FADH2.

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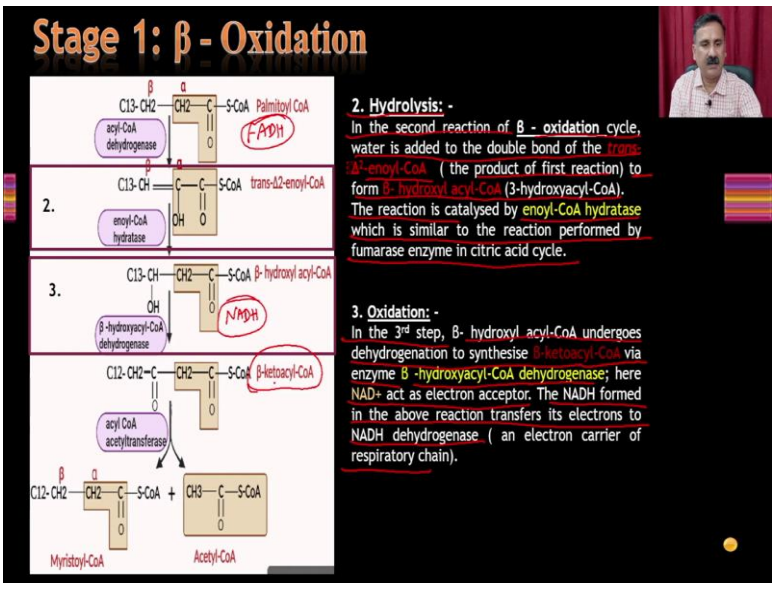

Now, in the step 2 it is actually going to have the hydrolysis. So, in the second reaction the you know beta oxidation cycle water is added to the double bond of the trans enoyl

CoA the product of the first reaction to form the beta hydroxy acyl CoA or the 3 hydroxy acyl CoA. The reaction is going to be catalyzed by an enzyme which is called as the enoyl CoA hydratase.

So, this is the first second reaction where these molecule which are going to be released from the acyl CoA dehydrogenase will enter into the second reaction where its going to go through the hydrolysis process and this hydrolysis process it is actually going to produce 3 hydroxy acyl CoA and which is.

So, this reaction is catalyzed by an enzyme which is called enoyl CoA hydratase which is similar to the reaction performed by the fumarase enzyme in the citric acid cycle. Then we have the second round of oxidations. So, in the 3rd step beta hydroxyl acyl CoA undergoes the dehydrogenation to synthesize the beta keto acyl CoA via enzyme is beta hydroxy acyl dehydrogenase.

Here the NAD plus act as a electron acceptor the NADH is going to be formed in the process and its electron to the NADH dehydrogenase. And so, in the 3rd step you are going to have the production of another of another molecule of NADH right. So, in this molecule this step you have produced the FADH, in this molecule this step you have produced the NADH and it is also going to produce the beta keto acyl CoA right and the enzyme name is the beta hydroxy CoA dehydrogenase, right.

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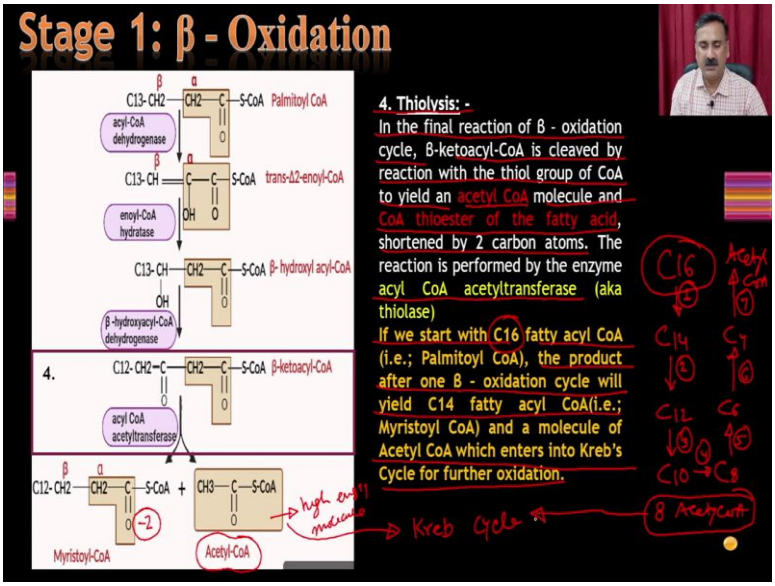

So, beta acyl beta keto acyl CoA will undergo the second the next step and next step is called as the thio thiolysis. So, in the final reaction of beta oxidation cycle the thioacyl CoA is cleaved by the reaction with the thiol group of CoA to yield an acetyl CoA.

So, far it is been done right, so at this stage there will be thiolysis and because of this thiolysis it is actually going to form the acetyl CoA and this is the remaining molecule of the fatty acids. So, this reaction is going to be catalyzed by the enzyme which is called as acyl CoA acyl transferase and this it is going to give you acetyl CoA molecule and the CoA thioester of the fatty acid which is shortened by the two carbon units.

So, this is actually going to be shortened by the two carbon right. Whereas these two carbon are now going to be present along with the coenzyme A. So, that is how it is going to form the acetyl CoA. And acetyl CoA is a high energy molecule right, so its going to be high energy molecule and which you will enter into the kreb cycle for further oxidations.

The reaction is going to be catalyzed by the enzyme which is called as acetyl acyl CoA acyl transferase or it will also going to be called as acyl CoA acetyl thiolase. For example, if we start with the C16 fatty acid CoA like the palmitoyl CoA the product after one beta oxidation will yield the C14 beta oxidation and so on and a molecule of acetyl CoA which is inter into the kreb cycle for further oxidation.

Which means if we started with C16 it will go through after the first round of beta oxidation it is actually going to form the C14 after that it is going to form the C12 that is going to called as like 10 like that its going to be like C8, then it is going to be C6 C4 and then ultimately it is going to form the acetyl CoA right this is going to be C2 right.

So, you can see that how many how many beta oxidation are going to happen 1, 2, 3, 4, 5, 6 and 7 ok. So, after the 8, 7 rounds of beta oxidation it is actually going to fully oxidize this fatty acid chain ok which is only containing the C16 molecules and in this process, it is actually going to generate the 8 molecules of acetyl CoA this means 8 molecule by all these 8 molecules of acetyl CoA, then enters into the kreb cycle.

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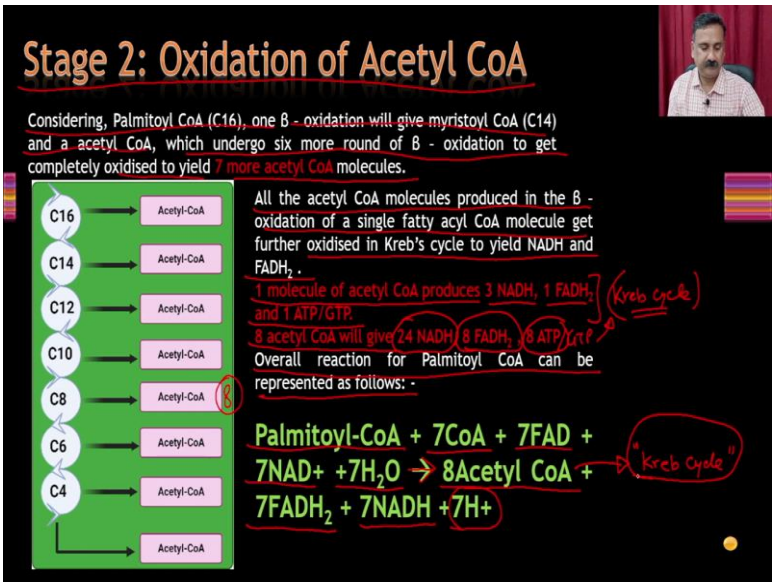

Then the step 2 is the oxidation of the acetyl CoA. Considering that palmitoyl will generate the another acetyl CoA which undergoes the 6 round of beta oxidation to get the completely oxidized to produce the 7 more acetyl CoA molecule all the acetyl CoA molecule produced in the beta oxidation of a single fatty acid molecule get further oxidized in kreb cycle to produce T NADH and FADH 2 and you can see that from C16 will C14, C12, C10 like that.

So, it is actually going to be it has gone 2, 3, 4, 5, 6, 7 and 8 molecules of acetyl CoA. So, if you see 1 and 1 molecule of acetyl CoA produces the 3 molecules of NADH 1 molecule of FADH 2 and the 1 molecule of GTP in the kreb cycle. So, this is the yield of the kreb cycle remember that in the previous lecture we discussed about how much is the energy which is going to be produced when we are discussing about the kreb cycle.

So, 8 acetyl CoA will give 24 molecules of FADH NADH 2, 8 molecules of the FADH 2 and 8 molecules of ATP or GTP only from the kreb cycle. So, overall reaction; so overall reaction for palmitoyl CoA can be represented as palmitoyl CoA plus 7 molecules of coenzyme A plus 7 molecules of FAD plus 7 molecules of NAD plus 7 molecules of water molecules and that is actually going to give you the 8 molecules of acetyl CoA 7 molecules of FADH 2 and 7 molecules of NADH and 7 molecules of H plus.

This means all these 8 molecules are further going to give you the more amount of the NADH and the FADH 2 in the kreb cycle.

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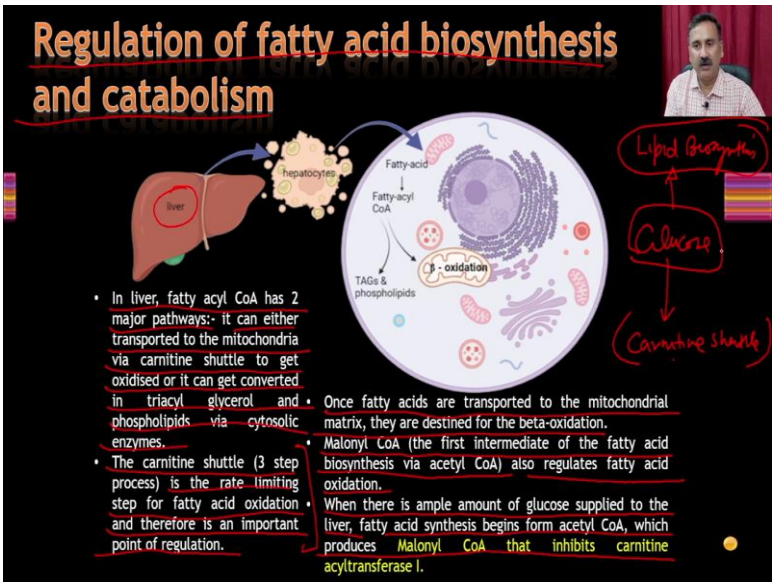

Now, how we can be able to regulate the fatty acid biosynthesis and as well as the catabolism? So, you have the 2 molecules, 2 major organs which are or single major organ that is the liver which is actually going to regulate the fatty acid biosynthesis and catabolism.

So, in the liver the fatty acyl CoA has 2 major pathway; it can either transported to the mitochondria via the carnitine shuttle to get oxidized or it can convert it into triacyl glycerides and the phospholipids via the cytosolic enzymes. The carnitine shuttle that is the 3 step process that we have already discussed right is the rate limiting step for the fatty acid oxidation.

And therefore, it is an important point of regulations which means as soon as the fatty acid will enter inside the mitochondria it will actually going to be get oxidized right. So, the carnitine shuttle which where you are actually utilizing the 2 different types of enzymes and the carnitine protein is the rate limiting step. So, that so the more and more amount the fatty acid which is going to be delivered inside the mitochondria it is actually going to be get oxidized eventually.

So, once the fatty acids are transported to the mitochondrial matrix they are destined for the beta oxidations for example, the malonyl CoA the first intermediate of the fatty acid bio synthesis via the acetyl CoA also regulate the fatty acid oxidation. When there is ample amount of glucose supplied to the liver fatty acid synthesis begins from the acetyl

CoA which is reduced malonyl CoA that inhibits the carnitine transferase, which means if there is a enough amount of glucose it is actually going to block the carnitine shuttle right which means if you are.

So, if there is a enough amount of glucose that will actually going to stop the carnitine shuttle on one side and it is actually going to induce the lipid biosynthesis which means it is actually going to ask the cell to accumulate the extra amount of lipids.

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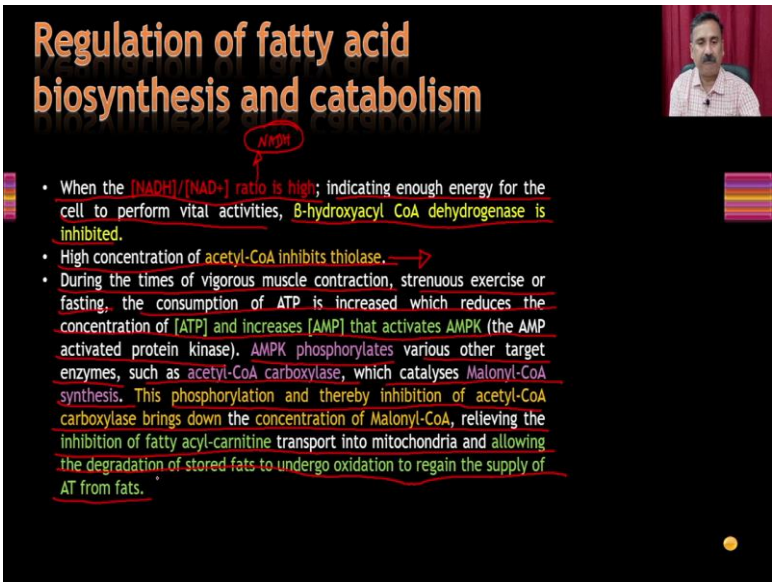

So, other parameters that also regulate the fatty acid biosynthesis and the catabolism is when the NADH and the NAD plus ratio is very high this means the cell has sufficient quantity of the NADH indicating the enough energy for the cell to perform the vital activity. So, beta hydroxyacyl CoA dehydrogenase is inhibited.

So, under these conditions the beta hydroxyacyl CoA dehydrogenase is been oxidation is been inhibited. A high concentration of acetyl CoA inhibits the thiolase inhibits thiolase. Then the 3rd is during the time of vigorous muscle contractions the strenuous exercise or fasting the consumption of ATP is increased which reduces the concentration of ATP and increases the AMP that activates the AMPK.

The amp activity protein kinase and AMPK phosphorylates the various other targets enzymes such as acetyl CoA carboxylase which catalyses the malonyl CoA synthesis.

This phosphorylation thereby inhibition of the acetyl CoA carboxylase bring down the concentration of malonyl CoA relieving the inhibition of fatty acyl carnitine transported into mitochondria and align the degradation of the stored fat to undergoes the oxidation to regain supply of ATP from the fats.

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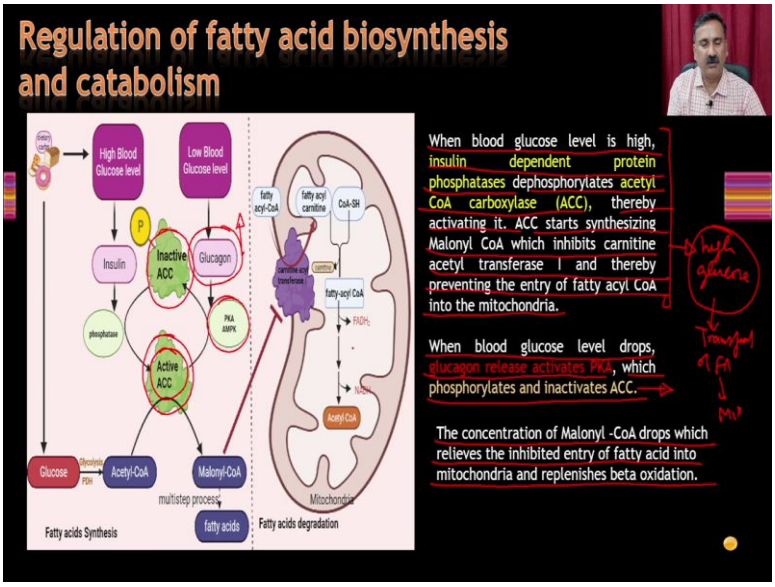

Then we have the another bacteria also when the blood glucose is very high the insulin dependent phosphorylate protein phosphatase dephosphorylates the acetyl CoA carboxylase thereby activating it. The ACC starts synthesizing the malonyl CoA which inhibits the carnitine acetyl transferase I and thereby preventing the entry of fatty acyl CoA into the mitochondria this is very very important to understand that when you have very high glucose.

Then the high glucose is inducing the insulin dependent protein phosphatase activity and as a result it is actually going to inactivate the carnitine acyl transferase I the enzyme which is actually coupling the carnitine to the fatty acid acyl CoA complex and that is how it is actually going to inhibit the transport of the transport of the fatty acid into the mitochondria.

So, when the glucose levels drops the glucagon releases activates the protein kinase A which phosphorylates and inactivates the ACC. So, this is and the concentration of the malonyl CoA drop which release the inhibits the activity of fatty acids into the mitochondria and replenishes the beta oxidation.

So, this is all about the what will be the condition when you have the high glucose versus the light glucose. So, when you have a high blood glucose it is actually going to induce the insulin dependent phosphatase activity and that actually is going to convert a inactive ACC into the active ACC and as a result of this it is actually going to inhibit the activity of the fatty acid transport into the mitochondria and that is how it is actually going to block the beta oxidations

Whereas when you have the low blood glucose that is actually going to induce the glucagon mediated phosphorylation reaction and that is actually going to reverse the activity this means it is actually going to inactivate the ACC and as a result it is actually going to induce the production or the transportation of the fatty acids into the mitochondria for the fatty acid oxidations.

So, this is all about the catabolic reactions what is mean responsible for the energy productions and you might have seen that we have discussed about the role of different types of enzymes in you know starting from the catalyzing the different types of reactions phosphorylation reactions.

When you are discussing about the carbohydrate metabolism. And then when we were discussing about the fatty acid metabolism, we have also discussed the role of enzyme in facilitating the transport of the fatty acid from the cytosolic side into the mitochondrial side with the help of the carnitine shuttle.

So, enzymes are actually very very crucial for running the catabolic reactions to for the energy productions. So, in this is all about the what we have discussed for the today's lecture in our subsequent lecture we are going to discuss about the role of enzyme in the anabolic reactions and we are going to take up some of the biosynthetic pathways and we will discuss about the role of enzyme in those pathways. So, with this I would like to conclude my lecture here.

Thank you.