Overview and Integration of Cellular Metabolism

Prof. Aritri Bir

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Week 05

Lecture 24: Fatty acid catabolism (Oxidation of Fatty acids) - I

Hello everyone, welcome back to the lecture series session of our lecture series on Overview and Integration of Cellular Metabolism. Today we are going to start Fatty Acid Catabolism mainly the oxidation of fatty acids. So, in this sessions in the discussions of fatty acid oxidation what we are going to discuss are how mobilization of the stored fat occurs finally, that stored fat is actually utilized for different types of oxidation. So, we are going to discuss different types of fatty acid oxidation then obviously, we are going to highlight beta oxidation of fatty acid where we will discuss how activation of fatty acids occurs, how those are transported and finally, how the main reaction series of beta oxidation occurs in case of even chain fatty acid as well as odd chain fatty acids. Then we will have a comparative discussion of beta oxidation between saturated and unsaturated fatty acid. We will discuss different I mean the bioenergetics of beta oxidation then the metabolic regulation and finally, diseases associated with beta oxidation.

And then we will proceed to different other types of fatty acid oxidations like alpha oxidation, omega oxidation, peroxisomal oxidation of fatty acids like that. So, mobilization of stored fat occurs from the storage depot of lipids and neutral lipids are stored in adipocytes. Adipocytes of the fat storing organs like adrenal cortex the steroid synthesizing cells of adrenal cortex as well as the sex steroid sex hormone which are steroid hormone actually which are produced from ovary and testis these are the tissues or organs where neutral lipids are stored in the form of adiposomes. Now, what is adiposome? Definitely adiposomes are lipid droplets actually.

So, lipid droplets where in the central core there are lipids more hydrophobic lipids like steroid sterol esters triacylglycerol these are forming the cores. And these core is surrounded by a monolayer of amphipathic lipid that is phospholipid. Now this core lipid droplet is surrounded by a surface protein which is known as perilypin. Now perilypin has one very important role in mobilization of stored fat. Now, this mobilization from the storage tissues mostly occurs through some hormonal signals.

Signals when there is depletion of the readymade energy supplier that is glucose or carbohydrate products when those stores are depleted that gives a signal. Basically low blood glucose actually gives a signal via hormones like epinephrine and glucagon. So, these are the hormones used in mobilization of storage of stored lipids and they come out from these adiposomes. So, this mobilization basically occurs as I told that it is the signal comes from epinephrine and glucagon hormones. Now what is epinephrine and glucagon do? They actually activate the enzyme adenyl cyclase.

Now this adenyl cyclase what it does? It converts ATP to cyclic AMP via G protein coupled receptor. Now the cyclic AMP if you remember from the previous classes that the cyclic AMP activates cyclic AMP dependent protein kinase A. Now this protein kinase A is important for phosphorylation of different proteins enzymes like that. So, one such important protein here is peri lipine which was forming the surface of adiposomes. Now this peri lipines via protein kinase A is phosphorylated in different regions.

So, there is phosphorylation of peri lipine. Now there is another hormone that is hormone sensitive lipase. Now hormone sensitive lipase definitely lipase enzyme is actually breaking down triacylglycerol to glycerol and fatty acids. Now hormone sensitive lipase here is also phosphorylated and activated by protein kinase A. So, once hormone sensitive lipase is phosphorylated it is definitely is definitely is targeting for targeted for breaking down the stored triacylglycerol in the adiposome.

Now important thing is peri lipines are the proteins which actually separate the adiposomes or rather it prevents the access to the core triacylglycerols in conditions when the breakdown is not required. Now when peri lipine is phosphorylated it basically attracts the hormone sensitive lipase and it causes the movement of hormone sensitive lipase over the or towards the adiposomes. Now basically hormone sensitive lipase is attached to the membranes of adiposome where the enzyme is activated and actually breaking down the triacyl core of adiposomes. Now remember for mobilization of fat or rather breakdown of stored triacylglycerol both required one is peri lipine phosphorylation another is activation of hormone sensitive lipase by phosphorylation. But remember only activation of hormone sensitive lipase will not help peri lipine phosphorylation is mandatory and required.

Now what happens when this hormone sensitive lipase is activated there is release of fatty acid and triacylglycerol. Now these fatty acid from adipocytes enters blood and circulated in albumin bound form. So, remember free fatty acid is circulated in through albumin bound form. Now these fatty acids enters oxidation and produces energy. What

is the fate of glycerol? Now glycerol in the stored triacylglycerol, glycerol is the least important part actually they glycerol takes very low plays very low important role in producing energy it is only 5 percent of energy producing biomolecule.

So, what is the fate of this glycerol? Glycerol is basically phosphorylated to form glycerol 3 phosphate. Now these glycerol 3 phosphate enters glycolysis. How it is basically once again with the help of dehydrogenase it forms dihydroxyacetone phosphate remember that is one product important in glycolysis. Now dihydroxyacetone phosphate is treated with another glycolytic enzyme that is triose phosphate isomerase to produce glycerol dihydes 3 phosphate and you if you remember the glycolysis class that this glycerol dihydes 3 phosphate further enters glycolysis. So, glycerol can enter glycolysis and produces and follows further reactionary steps.

Now let us move on to the oxidation of fatty acids. Now fatty acid oxidation can be of different types based on the which carbon is actually oxidized. Now fatty acids are carboxylic acids containing long aliphatic chains. Now this is a carboxylic acid having carboxyl group as acid group. Now the naming of the carbon it is the part of chemistry you should know or you should remember actually that the first carbon adjacent to the carboxyl group in fatty acid is known as alpha carbon.

The next one is known as beta carbon then it goes on alpha beta gamma delta likewise. Now when alpha carbon is oxidation in alpha carbon occurs that is alpha oxidation. Similarly, if there is oxidation of beta carbon that is beta oxidation. Now what is omega carbon? Omega carbon is the farthest carbon from the carboxyl group. So, this is omega carbon.

So, in case of omega oxidation these carbon is oxidized. So, based on which carbon is oxidized there is naming of the types of fatty acid oxidation beta alpha omega like that. Now the commonest one in our body is beta oxidation of fatty acid. Now what is beta oxidation? So, beta oxidation of fatty acid is basically oxidation of the carbon which is positioned in beta region. So, there is oxidation of beta carbon as well as so, there is oxidative removal remember beta oxidation is breaking down of fatty acid.

So, there is oxidative removal of successive 2 carbon units. So, in each cycle there is removal of 2 carbon units in the form of acetyl coenzyme A and that starts from the carboxyl end. So, from the carboxyl end means from the beta position and this process is known as beta oxidation as I told you already because there is oxidation of at the beta carbon position and there the splitting occurs. So, this is our beta oxidation. Now for fatty acid oxidation one very important step is activation of fatty acid.

Remember we discussed about activation of glucose by addition of UDP. Basically activation of fatty acid occurs via addition of coenzyme A molecule. Coenzyme A molecule basically is a complex of pantothenic acid and beta mercaptoethanolamine. Now when this coenzyme A is added to fatty acid forms fatty acyl coenzyme A this is the activated form of fatty acid and it occurs in the cytoplasm. So, what happens once again there is mobilization of stored fat triacylglycerol breakdown from adipocyte free fatty acid bound to albumin are circulated through the circulation enter cell enters in the cytoplasm.

In cytoplasm there is activation of fatty acid. Now the important enzyme for activation of fatty acid is fatty acyl coenzyme A synthetase. Now remember synthetase and synthetase there are two types of enzymes in case of synthetase there is utilization of ATP. So, here you can see there is utilization of ATP. So, there is utilization of ATP.

Now fatty acyl coenzyme A synthetase based on the chain length of fatty acid there are different isomer like short chain fatty acid for short chain fatty acid there is short chain fatty acyl coenzyme A synthetase. Similarly medium chain fatty acid there is medium chain fatty acyl coenzyme A synthetase and long chain fatty acids as well. Now this enzyme is present on the outer surface of the mitochondrial membrane remember outer mitochondrial membrane this enzyme is attached to outer mitochondrial membrane. Now this is a two step reaction activation is a two step reaction where an intermediate that is fatty acyl adenylate is formed and ATP hydrolysis occurs it on ATP hydrolysis there is formation of AMP and pyrophosphate and that pyrophosphate is immediately hydrolyzed to produce inorganic phosphate. So, basically there are two high energy phosphate bonds are utilized.

So, basically formation of AMP from ATP not ADP it is adenosyl monophosphate fine. So, now important thing is sometimes small chain fatty acid can be activated by thiophores enzyme as well using succinyl coenzyme A as the coenzyme A molecule donor. For succinyl coenzyme A for small chain fatty acid there can be succinyl coenzyme A otherwise for other short chain medium chain long chain fatty acid fatty acyl coenzyme A synthetase enzyme is required. Now after activation main beta oxidation occurs and the main beta oxidation related enzymes are located inside mitochondrial matrix, but remember activation has occurred in outside mitochondria in cytosol. Now what will happen because fatty acids are not freely permeable to mitochondrial membrane.

So, definitely what we need is a transporter a transporter which is based on the protein or the molecule carnitine. Carnitine is beta hydroxy gamma trimethyl ammonium butyrate. Now carnitine is here acting as a transporter for long chain fatty acids.

Remember those fatty acids which are the chain length is 12 carbon or shorter they can they can diffuse to the mitochondrial membrane and enter mitochondrial matrix, but for long chain fatty acids they are not freely permeable. So, they need carnitine transporter and that transport mechanism is known as carnitine shuttle.

Now let us see what how this carnitine shuttles actually occur. Now before that carnitine regarding carnitine few points you need to remember that is it is synthesized from the amino acid lysine and methionine very important for the MCQs synthesized mostly in liver and also in kidney huge amount abundantly present in muscle. There is a variation of serum or tissue level during growth or pregnancy when high amount of carnitine is required. So, the circulatory carnitine is also high or in case of ageing process where the synthesizing capability of cell is low. So, there is low serum level of carnitine.

Remember whenever there is carnitine deficiency bones are the most important part which are affected during physiological low carnitine level. So, there is osteoporosis in case of elderly subject when there is carnitine deficiency. So, this is our carnitine shuttle. Carnitine shuttle is important for remember for carrying or transporting long chain fatty acid from outside mitochondria to inside mitochondria. Now the first step is formation of acyl carnitine.

So, fatty acid activated fatty acid and carnitine forms a complex that is acyl carnitine with the help of the enzyme CAT 1. Carnitine acyl transferase 1. So, you can see here the fatty acid and coenzyme A forms the activated fatty acid which joins with carnitine to form this acyl carnitine complex. Now it is formed in the outer mitochondrial membrane. From outer mitochondrial membrane it can this acyl carnitine complex can diffuse through intermembranous space and comes outside the inner mitochondrial membrane.

Now inner mitochondrial membrane is not permeable. So, what is required? One transporter that is known as translocase. Now this translocase this is our translocase this is our translocase fine. Now this translocase what it does? It transfers the acyl carnitine complex from intermembrane space to the mitochondrial matrix. So, there is transfer of acyl carnitine inside mitochondria.

Now the next step is definitely reformation of fatty acyl coenzyme A. So, there is fatty acyl coenzyme A reformed by another enzyme that is carnitine acyl transferase 2 CAT 2. And what happens to the carnitine? Carnitine goes back to the cytosolic surface via the translocase enzyme. So, once again what we can see that there is formation of acyl carnitine with the help of the enzyme CAT 1. This acyl carnitine is transported inside mitochondrial matrix via translocase there is reformation of acyl coenzyme A and

carnitine transfers back to the mitochondrial through the mitochondrial intermembrane space to outer mitochondrial membrane.

So, now, activated fatty acid is inside the mitochondria. Now what happens? There are the main beta oxidation pathway consist of 4 enzyme catalyzed reaction. Those are dehydrogenation, hydration, again dehydrogenation and finally, there is thiolitic cleavage or splitting and that split compound is released as 2 carbon product acetyl coenzyme A. The other product is a another fatty acyl coenzyme A which is 2 carbon less than the previous one because 2 carbon compound is released as acetyl coenzyme A. That substrate once again follow these 4 steps of reaction and this cycle goes on till there is till the final product is either 2 or 3 carbon compound.

So, this is how beta oxidation occurs. Now, let us see step by step. So, as I told you there is dehydrogenation, hydration, again dehydrogenation and finally, thiolitic cleavage and these are the important enzyme. These are the enzymes related to the step that is FAD link dehydrogenase, hydratase then another dehydrogenase which is NAD link dehydrogenase and finally, there is thiolase for cleavage. Now, let us discuss one by one. FAD link dehydrogenase is basically what it is what it doing? It is causing dehydrogenation and introduction of one double bond between the alpha and beta carbon.

So, there is introduction of one double bond and remember these double bond is of trans variety. So, there is production of trans delta 2 NOA coenzyme A the product is having trans bond. Remember all the fatty acids which are the endogenous fatty acids or dietary fatty acids those are mostly cis configuration, but this product is trans configuration and that has some importance we will discuss later. Now, this enzyme acyl coenzyme A dehydrogenase it is one FAD linked enzyme and has multiple isoform based on the chain length. For very long chain fatty acid that is very long chain acyl coenzyme A dehydrogenase VLCAD similarly MCAD medium chain acyl dehydrogenase and short chain for short chain fatty acid short chain acyl dehydrogenase.

Now, the electron dehydrogenase is causing electron transfer electron is taken up by the FAD of the in attached enzyme. So, basically the what is formed is a reduced enzyme and that reduced enzyme which is attached to now after dehydrogenation which is attached to FADH 2 are is immediately oxidized via transferring this electron to electron transport chain of mitochondria and the electron carrier is electron transport factor or ETF. So, this is one type of electron carrier which is entering which is carrying electron to the electron transport chain of mitochondria. Next is hydration by hydratase now this enzyme hydratase is specific for the trans bond. So, trans bond is important for specification of hydratase.

Now what is formed after the addition of one molecule of water that is beta hydroxy acyl coenzyme A which is L-stirio isomer and now L-stirio isomer is acted upon by this enzyme NAD dependent dehydrogenase. So, once again NAD dependent dehydrogenase actually specific for the L isomer of beta hydroxy acyl coenzyme A. So, finally, what is formed is beta keto acyl coenzyme A. So, another electron is transfer to NAD and forms NADH once again this NADH enters electron transport chain of mitochondria. And the last one is thiolitic cleavage with the enzyme acyl transferase acyl coenzyme A acyl transferase here it is acetyl transferase also known as thiolase.

What is formed is a 2 carbon short fatty acyl coenzyme A then the previous one. So, we started with palmitic acid which is a 16 carbon long compound and here what is the product C 14 that is myristoyl coenzyme A and released product is acetyl coenzyme A. So, the 2 carbon unit which is released is acetyl coenzyme A via thiolitic cleavage. So, these are the 4 sequential reaction which constantly occurs still all the products are either acetyl coenzyme A and or some or one 3 carbon compound that is propionyl coenzyme A. Now, remember based on the chain length of fatty acids the last 3 reaction can be done by 2 different sets of enzyme.

How in those fatty acids which are having 12 or longer carbon chain they are treated by multi enzyme complex which contains these 3 enzyme. Now that multi enzyme complex is known as trifunctional protein TFP trifunctional protein. Now trifunctional protein is a hetero octamer which is having 8 subunit amongst them 4 are alpha 4 are beta. So, alpha 4 beta 4 subunit. Now each alpha subunit contains these 2 enzyme activity hydratase and NAD dependent dehydrogenase and the beta subunit contains thiolase activity.

Now what is the importance of having multi enzyme complex? Now in case of all the multi enzyme complex remember when all the enzymes are stuck together or complex together what happens once product is will be utilized as substrate of another enzyme. Now there will be very less diffusion while transferring those substrate from one enzyme to another. So, there will be efficient substrate channeling when there is a multi enzyme complex. Now while getting treated by this TFP multi enzyme complex the chain lengths get shorter when it is shorter than 12 carbon compound it is treated by another set of enzyme that is soluble enzyme complex which consists all the 4 enzyme. So, this is the importance of having multi enzyme complex in beta oxidation of fatty acid.

So, remember what has been the product for fatty first cycle of beta oxidation that is fatty acyl coenzyme A which is having a chain length 2 carbon shorter than the substrate and one acetyl coenzyme A is released. Now what happens this substrate once again enters dehydrogenation, hydration again dehydrogenation again thiolatic cleavage. So,

there is release of another molecule of 2 carbon acetyl coenzyme A and again the substrate enters this cycle till all the products are broken down to either 2 carbon or 3 carbon compound. So, that is called beta oxidation cycle. So, let us discuss palmitic acid for case of for in case of palmitic acid what will happen palmitic acid is a 16 carbon compound.

So, what will happen after one cycle there will be release of acetyl one molecule of coen, acetyl coenzyme A forming C 14 which is known as myristoyl coenzyme A 14 carbon long. And second cycle there will be release of another molecule of acetyl coenzyme A 12 carbon long lorile coenzyme A is formed. Similarly there is third cycle C 10, fourth circle C 8, fifth cycle C 6, sixth cycle C 4 and finally, this is our seventh cycle where actually 2 acetyl coenzyme A from C 4, 2 acetyl coenzyme A are released. So, what will be there? So, there will be 7 cycles of beta oxidation in case of palmitic acid and 8 molecules of acetyl coenzyme A production. So, what we have learned from this session these are the key points that the stored triacyl glycerol is mobilized on requirement via enzyme hormone sensitive lipase from adipose tissue.

The mobilized fat is activated by fatty acyl coenzyme A mobilized fat in the form of free fatty acid is activated by fatty acyl coenzyme A synthetase. Short chain and medium chain fatty acid can enter inside mitochondrial matrix freely whereas, for long chain fatty acid transporter is needed and that transporter is carnitine shuttle. Similarly there is beta oxidation beta oxidation follows 4 sets of sequential reaction of dehydrogenation hydration again dehydrogenation and thiolite splitting which gives of sequential release of acetyl 2 carbon acetyl coenzyme A. So, these are the take home messages these are my references and see you in the next class of oxidation of fatty acid. Thank you.