Overview and Integration of Cellular Metabolism

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Week 06

Lecture 28: Biosynthesis of Fatty acid and its regulation

Hello everyone, welcome back to your lecture series on Overview and Integration of Cellular Metabolism. We are in week 6, this is lecture number 28 and we will be dealing with a very important topic of biosynthesis of fatty acid and its regulation. So, we are in lipid metabolism and the concepts that we will be covering today are about the fatty acid synthase multi-enzyme complex and how it helps in de novo synthesis of fatty acid. We will be looking at the citrate transport system and how finally the whole thing is regulated. So, it is very important. So, buckle up and let us move forward.

Now, looking at the fatty acid synthesis from a historical perspective it was discovered by the very famous scientist Fyodor Leinen, who was awarded Nobel Prize for his discoveries in this fatty acid metabolism along with Conrad Bloch in the year 1964, he was a German scientist right. So, you see de novo synthesis of fatty acid means it is synthesized newly de novo inside ok, inside our body from scratch. So, this all means the de novo synthesis of fatty acid. Now, the primary fatty acid that is the main fatty acid that will be looking in our class today's synthesis of palmitic acid.

Palmitic acid is a saturated 16 carbon fatty acid right. So, what are the organs or in which fatty acid synthesis majorly takes place? They are the liver, the fat cells that is adipose tissue and lactating mammary gland ok. These are the organs in which fatty acid synthesis takes place much more compared to all other organs. So, the mainly more than 90 percent of the fat synthesis of the body that is lipid synthesis takes place in these three organs right. Now, looking at the enzyme, we all know when we are dealing with we all know now in metabolism whenever we are dealing with any pathway the tools by which the path reactions are catalyzed are enzymes.

Now, a very interesting thing about this whole fatty acid synthesis pathway is it is catalyzed by something known as fatty acid synthase multi enzyme complex. You have heard about this concept in pi-v-5 dehydrogenase reaction there was PDH complex alpha ketoglutarate dehydrogenase complex this is a bigger version of that. Here the multi

enzyme complex consists of seven different enzyme it is a large polypeptide which has got seven different active site. Not only that this seven different active site large polypeptide is acting in pair. So, it is present as a dimer.

So, these are the constituent names of the fatty acid synthesis multi enzyme complex or it is often abbreviated as FAS complex. So, what are the names ketoacyl synthase often abbreviated as KS malonyl or acetyl transacylase it is often abbreviated as MAT. In some textbooks you may find they are denoting it separately MT and AT, but this is basically one single subunit that catalyzes two different reactions depending on the case scenario. Next one is dehydratase or DH next enoyl reductase ER next ketoacyl reductase or KR ACP is acyl carrier protein and last one is thioesterase again it is abbreviated as TE. So, why I am saying all these abbreviations because from the next slide onwards we will be using these abbreviations, but if you are getting confused you need to roll back to this slide and then look at the names in detail what are the abbreviations that stand for right.

So, these this is all about the structure of the multi enzyme complex now what is the this is actually the 3D structure how in three dimension the polypeptide chains are associated with each other in real time right. So, what is the advantage of having this multi enzyme complex instead of having seven different enzymes why what is the utility of all these enzymes being together in large polypeptide as you can see these are the advantages that have been laid down that is since fatty acid synthesis is occurring in cytosol and it is a vast area. So, the intermediates can go here and there, but the beauty of the multi enzyme complex is the all the enzymes are together. So, the intermediates can immediately react once it has been acted upon by the previous enzyme. So, this is the I mean reactionary feasibility which is mass I mean hugely increased due to presence of all the enzymes nearby otherwise if one enzyme is present here another one enzyme is present there it delays the reaction progress right and one more big advantage at genetic level is one gene that codes for all the enzymes.

So, basically if we can up or down regulate that enzyme or I mean gene all the proteins that will be coded I mean the single polypeptide will effectively increase the enzymes in equimolar concentration. So, all this actually increases the efficiency of fatty acid synthesis reaction. So, the ultimate word is increasing the efficiency of fatty acid synthesis or lipogenesis that is why nature has chosen all of these enzymes to be club together as a multi enzyme complex. Now, the process of fatty acid synthesis is basically cyclical we have discussed that we will be analyzing how a 16 carbon palmitic acid synthesis takes place. The whole 16 carbon is not added together it is repeated in cycle.

So, in each cycle the chain is starting from 2 carbon that is acetyl coenzyme A and 2 carbons are being added sequentially. So, in all 4 carbons are added in each cycle we will

be seeing it very soon. Now, why acetyl coenzyme A from where did you hear the name acetyl coenzyme A very common we have heard it in carbohydrate metabolism during TCA cycle and very lately you have heard it in beta oxidation where we are discussing an opposite pathway discussing a catabolic pathway where the large fatty acid was broken down into small molecule of 2 carbon acetyl coenzyme A. Well this fatty acid synthesis is not the simple reversal, but the starting material is the same that is acetyl coenzyme A. So, acetyl coenzyme A is the starting material and it is converted to malonyl coenzyme A by the enzyme acetyl coenzyme A carboxylase and this is the rate limiting regulating key reaction in the whole fatty acid synthesis.

Now, you might ask or you might wonder this name acetyl coenzyme A carboxylase is not in this abbreviation and you are very right because acetyl coenzyme A carboxylase or acetyl co A carboxylase is a separate entity it is an separate enzyme which is not a part of fatty acid synthase complex ok. So, first the action of acetyl co A carboxylase converts acetyl coenzyme A to malonyl coenzyme A, acetyl coenzyme A is a 2 carbon compound it uses 1 carbon is added from bicarbonate and it results in formation of a 3 carbon malonyl co A, but this is the main key reaction and the cofactor of this acetyl coenzyme A carboxylase enzyme is biotin or vitamin H. Now, this acetyl coenzyme A carboxylase in itself is a bit complex enzyme. So, let us look how the acetyl co A carboxylase enzyme acts ok. So, it is very interesting you just follow along first we will discuss about the I mean the cofactor biotin plays a very important role.

It in between the reaction it becomes carboxy biotin. So, at first the biotin moiety is carboxylated and thereafter the carbon molecule shifts from shifts to acetyl co A to from malonyl co A. So, just look at here the first step of this reaction is basically transfer of the carboxyl group to biotin. So, this whole acetyl acetyl co A carboxylase enzyme functions as 3 separate enzymes in itself the first function is biotin carboxylase ok. So, the first the with the help of ATP this this is energy dependent process this HCO 3 minus the carbon from HCO 3 minus is transferred to biotin all right and hence biotin becomes carboxylated.

So, this is known as carboxy biotin right. Now this carboxy biotin undergoes an internal conformational change where the carboxyl group is just flip flopped from the biotin carboxylase domain to the trans carboxylase domain. So, basically transfer of the activated CO 2 this is the activated carboxyl group from biotin carboxylase region to trans carboxylase active site ok. As you all know from regulation of enzyme activity a substrate always mimics the enzymes active site. So, the this acetyl co A carboxylase enzyme has got 2 active sites in itself which can bind to the carboxyl group of biotin internally.

So, a transformation occurs and this is the trans carboxylase activity. And in the final phase then only with the help of this trans carboxylase activity the carboxyl group is transferred to acetyl co enzyme A. So, a 2 carbon acetyl coenzyme A becomes a 3 carbon malonyl co A and the biotin that is the coenzyme is again regenerated. So, in the whole thing basically what happens acetyl coenzyme A along with biotin a carbon molecule from bicarbonate comes to from carboxy biotin and in the next step the carboxyl group goes to acetyl co and biotin is regenerated to form malonyl coenzyme A. So, now we have got malonyl coenzyme A which is actually the form by which the acetyl coenzyme A molecule enters into the fatty acid synthase complex right.

Now the very starting molecule is of course, acetyl coenzyme A. So, in the first step one acetyl coenzyme A will bind to fatty acid synthase complex and in the next step a malonyl co A will bind to fatty acid synthase complex. So, it is all about binding. Now where does it bind those fat acetyl coenzyme A and malonyl coenzyme A have got affinity towards the sulfhydryl group where this sulfhydryl or SH group is located it is located at the enzyme ketoacyl synthase or KS and who helps in transfer that is the enzyme a MAT acyl transacylase or malonyl transacylase and with the help of acyl carrier protein. So, now just see what happens this is the basic concept where acetyl co and malonyl co A first attaches to the SH group of the condensing enzyme.

So, the first step in the first step what happens acetyl group from acetyl co A is transferred to the SH group by the enzyme malonyl or acetyl transacylase. I told you the same enzyme codes for transfer of acyl group from acetyl co A as well as from malonyl acetyl group I mean so it is transferred to the enzyme ketoacyl synthase you see over here what is happening one molecule of acetyl coenzyme A is coming and the coenzyme A part is lost the acetyl group is being attached to the ketoacyl synthase domain KS with the help of acyl carrier protein or ACP ok ACP actually helps in everything you will see. So, this is the first step where the coenzyme A part is lost and the acetyl group CH3CO is transferred to the ketoacyl synthase KS. So, if this step is clear in the next few steps it will be very easy for you to understand. So, please make sure you are understanding this whole thing acetyl group comes ok ACP has got a sulfhydryl group it helps in attachment.

So, ACP actually bends over it helps in attachment of the acetyl coenzyme A acetyl group to the ketoacyl synthase domain. In the next step similar reaction happens what happens a malonyl group is also transferred to the ACP SH group by malonyl transacylase. So, the acetyl group was transferred to ketoacyl synthase and the malonyl group is transferred to the ACP fine. So, both has got disulfide linkages I mean sulfhydryl groups acetyl group is attached to KS and malonyl group is attached to ACP. Now these two reacts very closely ok.

So, the ACP is actually lying. So, the malonyl group on the acyl carrier protein is lying very closely is located very closely in three dimensional structure to the acetyl group of ketoacyl synthase fine. Now now the enzyme is actually primed for all the reactions. So, in order for the enzyme to function it needs to receive the initial group first it started with an acetyl group that was allotted to ketoacyl synthase domain and one malonyl group that was allotted to the acyl carrier protein. Now these two react these two condense together with the help of the enzyme ketoacyl synthase ok.

So, what happens you see the acetyl group from the ketoacyl synthase is actually transferred to the malonyl group. Now a bonding has been formed and it has led to the formation of acetoacetyl ACP and one carbon dioxide is lost fine. So, acetoacetyl ACP where malonyl coevas three carbon acetyl coevas two carbon however one carbon dioxide is lost. So, now it has become a four carbon acetoacetyl group and it is always attached to acyl carrier protein or ACP. So, acetoacetyl group is attached to acyl carrier protein.

Mind it when you are learning beta oxidation we learned everything was in coenzyme A form. In this case fatty acid synthase all the intermediates are in ACP form it is bound to this ACP right. So, in the next step what happens you see acetoacetyl ACP now a reduction happens, reduction with the help of NADPH very important. So, when an aldehyde or keto group is reduced it leads to the formation of an OH group or alcohol. So, this is beta hydroxy butyryl ACP ok.

So, this keto group has been reduced to alcohol who has donated the hydrogen who is the reducing equivalent here NADPH very important it is not NADH. We discussed what are the roles of NADPH it was helping in fatty acid synthesis during our HMP shunt lectures. So, now, you see this is the step where NADPH is I mean exerting its role as a reducing equivalent as a hydrogen donor there are more. So, in the next step what happens there is dehydratase enzyme action. So, a water molecule simply goes out when a water molecule goes out if you can I mean if you just remove an HOH from here what will happen there will be a introduction of a unsaturation or a double bond this is exactly what happens here and the resultant compound is known as B delta 2 trans enoyl or delta 2 butanoal ACP delta 2 butanoal acyl carrier protein and the enzyme that is acting is beta hydroxy acyl dehydratase or simply DH right.

So, now, when the unsaturation has been reached the next part is again a reduction using NADPH the name of the enzyme is enoyl reductase what will happen the unsaturation will be gone. So, the whole purpose was to get rid of this OH group you see. So, in order to get rid of this OH group we introduced an unsaturation with the help of dehydratase

enzyme and again we have reduced with the help of NADPH and this is now butyryl ACP or a 4 carbon acyl carrier protein. So, a 2 carbon has now been converted to a 4 carbon which is attached to the acyl carrier protein. So, now, what will happen you remember initially acyl coenzyme A was bounded to acyl carrier protein and it was translocated to ketoacyl synthase.

So, that it could receive another molecule of malonyl CoA well the same thing is going to happen over here. So, now, translocation of butyryl group to the ketoacyl synthase domain happens and finally, the acyl carrier group will become free to receive another molecule of malonyl coenzyme A and the second round of at this whole thing will be repeated. So, first acetyl group of acetyl coenzyme A got attached to KS then malonyl a 3 carbon was attached to ACP and ultimately those 2 reacted and they after a series of 4 reaction they formed a 4 carbon this 4 carbon is again transferred to KS and now again when another malonyl CoA will be attached to ACP the same series of reaction will happen 1 molecular carbon dioxide will be lost and ultimately the chain will increase by another 2 carbon. So, this process is repeated for 7 cycles why 7 cycles it started with 2 acetyl coenzyme A 2 carbon 7 more cycle 14 additional carbon and ultimately we get the 16 carbon palmitic acid. So, when the desired length is achieved then only the 16 carbon fatty acid will be detached from acyl carrier protein by the enzyme TE or thioester is mind it during all this internal cycle the action of TE is not required.

TE is only required when the desired length of fatty acid has been reached and thus we get the 16 carbon palmitic acid with all unsaturated CH3 at the end CH2 CH2 CH2 in between and COO at the end or COOH right this is the palmitic acid and palmitic acid is COOH. So, if we look at the stoichiometry or the calculation. So, just as I told you it starts with 1 acetyl coenzyme A and finally, 7 malonyl coenzyme A is added to it, but ultimate as we saw each step in each step 1 carbon dioxide is lost right and where from this 7 malonyl coenzyme A came this was actually by the first reaction where carbon dioxide this is also known as CO2 fixation reaction where carbon dioxide in the form of bicarbonate actually converts acetyl coate to malonyl coenzyme A. So, if we look at the entire thing this looks like 8 molecules of acetyl coenzyme A along with 7 molecule of ATP 14 molecule of NADPH because NADPH is required in 2 steps. So, 7 cycles 2 steps 14 NADPH it gives rise to 1 palmitate or palmitic acid 14 molecule of NADP plus that is the oxidized form of NADP and ultimately 6 8 acetyl co molecule and you know the others if you just look at the cycle you will get easily get the stoichiometry what are the initial and the final compound.

So, ATP hydrolysis happens this is an energy dependent process. Now, this whole thing fatty acid synthesis is exclusively happening in the cytosol I told you why in the cytosol mainly because the presence of high amount of reducing equivalent what is the reducing

equivalent NADPH. So, in cytosol the NADPH is much more compared to NADP plus, but I told you this NADPH is not present in all the cells and all the organs it is only present in those organs where this fatty acid synthesis is happening more and more it is happening in excess. So, what are the 3 organs I told you in hepatocyte, lactic memory glands adipose tissue these are the organs where there is excess production of NADPH by HMP shunt these are the cells where maximum amount of HMP shunt is happening these are the cells where maximum amount of fatty acid synthesis is happening. And I also told you that apart from production of NADPH by the enzyme glucose 6 phosphate dehydrogenase this is the main source of NADPH production malic enzyme also plays an important role right.

Now one question you should be having in your mind right now that we need acetyl coenzyme A for fatty acid synthesis, but this acetyl coenzyme A is produced from pyruvate and this pyruvate dehydrogenase reaction happens in the mitochondria right. So, where from we are getting acetyl coenzyme or how acetyl coenzyme is coming out from the mitochondria to the cytosol. It is actually not directly coming out because the mitochondrial membrane is not permeable towards to acetyl coenzyme A. So, it actually gets transferred in the form of citrate across from the mitochondria to the cytosol ok. So, this is the basic cycle we can look at it in a elaborate fashion this is the same thing that we have elaborated in this figure.

So, you see inside the mitochondria acetyl coenzyme A is formed from pyruvate. It now condenses I mean it is converted to citrate with the help of citrate synthase enzyme citrate synthase enzyme is a part of that is for you to answer right. So, in the form of citrate it can come outside into the cytosol there it is I mean actually it is acted upon by the enzyme citrate lies very important citrate lies ok. Here one now with the help of this enzyme the acetyl coenzyme A is generated back and it is also an ATP dependent process and this acetyl coenzyme A is used for fatty acid synthesis and the citrate gets converted to oxaloacetate. Now in the next step oxaloacetate is acted upon by the enzyme malate dehydrogenase is cytosolic malate dehydrogenase there is also another mitochondrial malate dehydrogenase that also you already know by now.

So, malate dehydrogenase now acts up is acted upon by malic enzyme to form pyruvate all right. This malate or pyruvate are actually permeable to the mitochondrial wall or inner membrane preferably pyruvate is much more permeable. So, pyruvate again goes inside it gets transformed to oxaloacetate and the whole cycle is completed whereas, malate can also be I mean transferred in a small amount and also malate will be acted upon by oxaloacetate to form I mean by malate dehydrogenase to form oxaloacetate and you know what the cycle is going on over here. So, you can see this all health thing looks like a spiral due to this I mean layout the fatty acid synthesis complex is also I

mean the whole thing the citrate transport along with fatty acid synthesis complex which was discovered by Fyodor Linen is known as Linen's spiral. Now as I told you the fatty acid synthesis reaction is not a simple reversal of fatty acid oxidation.

So, there are differences the very first difference that you are already guessed by now that is the location beta oxidation takes place in mitochondria fatty acid synthesis takes place in cytoplasm right. The intermediates are present as coenzyme A whereas, the intermediates in fatty acid synthesis are covalently linked to the sulfide group of acyl carrier proteins. The all the enzymes of beta oxidation are isolated they are independent enzymes whereas, it is present as a multi enzyme complex. Two carbon units are added in case of beta oxidation or two carbon units are broken down at a time in case of beta oxidation whereas, two carbon units are added, but as three carbon malonyl coen right. Next the trans the coenzymes over here are NAD and FAD whereas, you already saw it was the reducing equivalent is NAD pH right.

The transporter of course, carnitine path is very important for to form acyl carnitine this is the pathway by which fatty acids get inside the mitochondria whereas, acetyl coenzyme A comes out of mitochondria as with the help of citrate transporter. And in the regulation of course, you know by now this is anabolic so, it will be helped by insulin and it will be suppressed by glucagon and the counter this is a catabolic pathway beta oxidation. So, insulin inhibits it and glucagon promotes it right. Now, let us look at the regulation of fatty acid synthesis as I told you the main enzyme is acetyl coenzyme A carboxylase. So, if you control the activity of acetyl coenzyme A carboxylase we can control the activity of fatty acid synthesis.

So, who how it is controlled first end product so, permittoyl coen, permittate is formed in excess we do not need the formation of more permittate. So, permittate actually inhibits the acetyl coenzyme A carboxylase enzyme. We all know by the covalent modification glucagon, epinephrine that is all the catabolic hormone will prevent this anabolic process right, but there is one allosteric stimulator who is the allosteric stimulator that is citrate ok. If citrate is present in excess it will help its own breakdown by the help of the enzyme citrate lies and more and more acetyl coenzyme A will be formed and more and more acetyl coenzyme A will now be utilized by the acetyl coenzyme A carboxylase and insulin acts as a trigger for activation. As we all know insulin being an anabolic hormone will help in the fatty acid synthesis.

Not only that it has been found that the dephosphorylated form of the enzyme is active of course, if insulin is activating it is dephosphorylated and in dephosphorylated form the enzyme undergoes it and polymerizes to form a long filament that this is the electron microscopic structure of the acetyl coenzyme A carboxylase enzyme. So, looking at the

regulation in term of short term and long term regulation the amount of acetyl coenzyme A and the amount of ATP if it is more if it is in excess. So, what it will do it will inhibit isocitrate dehydrogenase isocitrate dehydrogenase is an enzyme of TCA cycle. It means the body has got more energy we do not need any more energy we do not need any more ATP we need to now store things. So, lipogenesis will be favoured and this actually increases the amount of citrate since the enzyme isocitrate dehydrogenase was utilizing citrate if it is inhibited citrate will the concentration of citrate will rise and this will help in up regulation of acetyl co A carboxylase as we saw in the previous slide.

And of the acetyl this citrate is acting as a carrier of the acetyl coenzyme A that we just discussed right. So, more and more citrate means more and more fatty acid synthesis. We also discussed about covalent modification basically glucagon we all know it how it is acts by the cascade increased glucagon means increased amount of cyclic AMP it will activate protein kinase and it will happen phosphorylation of acetyl co A carboxylase and phosphorylated form is inactive it will be switched off. So, more glucagon means we do not need more fatty acid now glucagon means catabolic fatty acid will be broken down in the form of beta oxidation. I told you palmitoyl co A in is inhibiting how it is inhibiting it number one it inhibits acetyl coenzyme A carboxylase it also inhibits translocation of citrate from ito quantity to cytosol right also it inhibit G 6 p d.

So, G 6 p d inhibited there will be no NADPH again there will be no deducing covalent the fatty acid synthesis will be hampered. And lastly this is the long term regulation where diet actually plays a very important role and this is very I mean you can guess it from your common sense since the body has got if the diet is of low fat and high carbohydrate the body needs more fat then the activity of acetyl coenzyme A carboxylase and fatty acid synthesis will be increased. So, there will be more and more lipogenesis and if we are fasting and having a high fat diet then the body will try to break down all the fatty acid then the activity of acetyl coenzyme A carboxylase will be decreased. And what actually happens in high fat low carbohydrate diet we all saw that it is it leads to especially Atkins diet it leads to production of more and more ketone bodies because oxal acetate has been shunted towards gluconeogenesis there is no TCA cycle acetyl coenzyme A cannot be broken down even beta oxidation is happening and ultimately ketogenesis is formed. So, to conclude this lecture has covered the concept of the fatty acid synthesis multi enzyme complex and how it helps in all the reaction of de novo synthesis what are the citrate transport system acetyl coenzyme is getting out from mitochondrial cytosol and how the whole fatty acid synthesis is regulated.

So, these are my references and thank you for your patient hearing.