Overview and Integration of Cellular Metabolism

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Week 06

Lecture 29: Biosynthesis of triacylglycerol, phosphoglycerides and sphingolipids

Hello everyone, welcome back we are continuing with lipid metabolism and in today's class will be discussing the biosynthesis of triacylglycerol, phosphoglycerides and sphingolipids. So, these are the concepts that will be covering will be covering the entire pathway where triacylglycerol biosynthesis is done, how the entire process is regulated and we will also be looking at the overview of phospholipids synthesis, plasma allogen synthesis as well as sphingolipid synthesis ok. So, to start with what is triacylglycerol? Now this is a structure of glycerol, what is glycerol? It is 1, 2, 3 propane triol ok this is glycerol. Now we can attach fatty acyl fatty acid molecule to each and every we can replace this OH group I mean simply replace this hydrogen and we can replace it with fatty acid. Then we will get this molecule where each and every fatty acid molecule is referred to as acyl group a c y l. So, one acyl group if this whole thing remains and one OCR1 COOR1, R1 is replaced it is known as MAG or monoacylglycerol.

When this molecule or this H is replaced by another OCR2 another acyl group it becomes diacylglycerol. Simply when all the three this is as you all know from your chemistry lessons organic chemistry this is all known as SN1 position, this is SN2 and this is SN3 right SN. So, when all the three positions are replaced by acyl groups it is triacylglycerol. So, this is the structure of triacylglycerol which is also abbreviated as TAG all right.

So, let us look how the whole molecule is synthesized. Now you have some basic idea right we discussed during fructose metabolism as well as during glycolysis that these intermediates can be channeled towards synthesis of monoclonal multiple lipids. So, this is the area where we are continuing from carboided metabolism to lipid metabolism. Now see the beauty of it the precursors being glycerol, 3 phosphate and fatty acyl coenzyme A. How do we get fatty acyl coenzyme A? Simple a fatty acid when it enters the I mean fatty acid can get attached to the coenzyme A and it becomes fatty acyl coenzyme A.

For example, when a 16 carbon palmitic acid is synthesized it also can be represented as palmitoyl coenzyme A ok. The main intermediate that is or precursor that is required is glycerol 3 phosphate. Now glycerol 3 phosphate can be obtained from dihydroxyacetone phosphate or DHAP by the action of an enzyme glycerol 3 phosphate dehydrogenase. So, glycerol 3 phosphate dehydrogenase is acting upon DHAP to form glycerol 3 phosphate. Who is helping or who is the reducing equivalent over here NADH.

So, NADH is required to donate 1 H plus into the reaction. Then what happens the dihydroxyacetone phosphate this keto group is reduced and it is replaced by an OH group and this is thus becomes glycerol phosphate ok. So, simple glycerol ok this one glycerol this OH has been replaced by one phosphate group. So, this is glycerol phosphate and since it is third position this is 1 2 3 this is glycerol 3 phosphate ok. Why it is capital L? It is because of the configuration of this carbon the second carbon where if H is located on the right side it is capital L and if H is located on the left side it is capital D or in the opposite side if OH is located on the left side this is L and if OH would have been located in the right side or this side it was capital D.

So, this is the type of isomerism we are not going to details of chemistry right over here, but this is why it is thus capital L glycerol 3 phosphate. Now this glycerol 3 phosphate can also be achieved from one intermediate or from one compound that is glycerol very simple. We can attach one phosphate group directly to glycerol who attaches phosphate group kinase group of enzyme glucose, glucose 6 phosphate fructose, fructose 1 phosphate. So, glucokinase, fructose kinase. So, here glycerol kinase.

So, glycerol kinase by acting on glycerol can lead to formation of glycerol 3 phosphate. Now what happens with this glycerol 3 phosphate? To this glycerol 3 phosphate this H hydrogen atoms of this OH group will be simply replaced by acyl group from fatty acid. What is the enzyme? Simple acyl transferase and if you wonder how fatty acyl co are being synthesized from fatty acid by the action of acyl coenzyme a synthetase. We all know that acyl coenzyme a with the I mean this is an ATP dependent mechanism which uses 2 ATP that is known as activation of fatty acid we all read that during beta oxidation of fatty acid. So, that concept is already clear by now.

So, using that concept we can extrapolate our knowledge to find out that 2 acyl transferase are required where the 2 hydrogen atoms will be replaced. Now what is this? This is diacylglycerol there are 2 acyl molecules R 1 and R 2 diacylglycerol and in the third place 1 phosphate is as also this is diacylglycerol 3 phosphate which is also commonly known as phosphatidic acid or phosphatidate all right. So, up to here if it is clear we can now go to the next slide. Now bit of information about phosphatidic acid this phosphatidic acid is actually place I mean present in a very small amount inside the

cell, but it is the key molecule it plays a sheet anchor role in all type of lipid biosynthesis it is the key intermediate that can be converted to any type of triacylglycerol or glycerophospholipid ok any type of glycerophospholipidol dot PAG. Now let us see how it happens.

So, phosphatidic acid so we need to get rid of this phosphate group somehow. How can we do that? We add phosphate with the help of kinase glucose to glucose 6 phosphate hexokinase. If we were to do the reverse we need phosphatase enzyme same concept also applies over here. So, phosphatidic acid is acted upon by a phosphatase enzyme that get rid of this phosphate group it is gone now we are left with 1, 2 diacylglycerol simple because our goal is to reach to triacylglycerol and as long as the phosphate group is in a third position we cannot attach a acyl group. So, first we need to get rid of this phosphate group it is done with the help of this enzyme phosphatase the diacylglycerol is now ready to accept another acyl group in the third position and with the help of another acyl transverse reaction we are now getting the final desired product that is triacylglycerol which has got an acyl 3 different acyl groups R 1, R 2, R 3 it can be same are acyl same type of acyl group may be present, but the in whole thing generally it is known as triacylglycerol for different side chains may be present in the acyl groups right.

So, how this whole thing is regulated you know triacylglycerol synthesis is an anabolic process all anabolic processes are helped by you got it right insulin. So, whenever we are having increased amount of insulin that is we are having process where there is more and more fatty acid synthesis that also helps in ultimately that helps in synthesis of triacylglycerol because triacylglycerol need fatty acid to start with fatty acyl CoA if it is more and more triacylglycerol will also be more and more who helps in synthesis of fatty acid increased amount of acetyl coenzyme A right and we all know that whenever we are talking about an anabolic reaction insulin is stimulating both the synthesis of acetyl coenzyme A as well as synthesis of fatty acid in turn it becomes fatty acyl CoA and in turn it becomes triacylglycerol. Now when will this process will be hampered now you should know that where from we are getting acetyl CoA we can get acetyl CoA from multiple sources ok we can get it from our diet glucose pyruvate to acetyl CoA as well as from multiple non carbohydrate sources that can be converted via glucogenic amino acid to acetyl coenzyme A that is the process of gluconeogenesis. Now this whole thing is jeopardized in diabetes mellitus where insulin is absolutely low or insulin is not acting in that case what happens this acetyl coenzyme A cannot be directed towards fatty acid synthesis it cannot be utilized and ultimately everything is diverted towards ketone body synthesis that we all know. So this is how TEG synthesis regulated that is by action of insulin and this whole process is jeopardized in case of uncontrolled diabetes mellitus where the phenomena of ketosis is happening and we have already discussed in detail how what are the features of ketosis.

So basically this is the triacylglycerol cycle where upon requirement the triacylglycerol can be broken down to simpler compound like glycerol and fatty acid ok and this fatty acid is acting as a fuel it goes outside the tissue again it can be acted upon by glycerol 3 phosphate when the body is in anabolic mode and again triacylglycerol will be synthesized again it can by the action of lipoprotein lipase glycerol can be broken down. So this whole thing occurs in tandem where the triacylglycerol molecules are broken down and re synthesized during starvation. So during starvation when the body needs more and more sources of energy the molecule is broken down it is utilized and upon refeeding the whole thing can be resumed right. So this was all about triacylglycerol synthesis and the regulation of triacylglycerol synthesis. So remind it in the triacylglycerol cycle the adipose tissue blood and the liver everything is involved.

Now we are moving on to phospholipids. So what are phospholipids? Phospholipids are actually as you have guessed combination of I mean lipid molecule with a phosphate group. What is so special about it? It is a amphipathic substance mean it has got both hydrophilic as well as a hydrophobic region phospholipids are an integral part of membrane ok you already know that. But one thing to remember is the phospholipids synthesis and few example of phospholipids are phosphatidylserine, phosphatidyl ethcholine, ethanalamine and sphingomyelin. Now where the phospholipid biosynthesis is happening it is happening mainly in the smooth endoplasmic reticulum that is SCR and the mitochondrial inner membrane ok inside the cell in eukaryotes.

Basically these are the two areas where phospholipids synthesis is happening. Now again phospholipid synthesis I told you when we need phospholipid or triacylglycerol the key or the central molecule is phosphatidic acid. So how phosphatidic acid what is phosphatidic acid? 1, 2 diacylglycerol 3 phosphate. How phosphatidic acid can be synthesized? Just before a couple of slide we saw it can be synthesized from glycerol 3 phosphate where there is already a presence of phosphate group and simply the two OH molecules are replaced by two acyl group by the action of acyltransferase. Well we also saw a reaction where phosphatidic acid was acted upon by a phosphatase to form diacylglycerol or 1, 2 diacylglycerol.

Well whenever there is a phosphatase enzyme somewhere depending on the metabolic requirement this reaction can be proceeded in opposite reaction with the help of a kinase that is what exactly happens here. So there is another route by which depending on the requirement phosphatidic acid can be synthesized from diacylglycerol with the help of the enzyme diacylglycerol kinase. Whenever there is a kinase enzyme we need someone to donate the phosphate group and in this case it is adenosine triphosphate. So ATP is donating the phosphate group on ATP is being hydrolyzed over it is getting converted to

ADP and with the help of kinase the third in the third place there is formation and entry of a phosphate group by replacing this hydrogen. So 1, 2 diacylglycerol is converted to phosphatidic acid.

So either we are replacing the OH group and phosphate group is already present we are replacing with acyl group or acyl groups are present when we are inserting the phosphate group in any way we have to get that is phosphatidic acid. So this phosphatidic acid or this phosphate group is actually the hydrophilic part ok and the hydrophobic part is actually attached to it ok how this is known as head group attachment ok. Now see this hydrophilic head these are the hydrophobic part the acyl groups the arms ok. So these are the ones that are represented here. So now we need attachment of the head group that is the hydrophilic head.

How it happens? It happens by a phenomena where the phosphoric acid when it condenses it eliminates two molecule of water. So the phospholipid head group is attached to a diacylglycerol by a phosphodiester bond. What is a phosphodiester bond? It is the bond which is formed this is the phosphodiester bond OPO from both side when a phosphoric acid condenses with two alcohol. So over here there is one OH group that is attached to the head group and there is an alcohol that is attached to the phosphatidic acid that is an OH group. When a phosphoric acid condenses over here two water molecules from here HOH will be eliminated from here and one HOH will be eliminated from here ultimately this will form a phosphodiester bond and this is the basic structure of a glycerophospholipid ok.

Now when we look at the synthesis of a phospholipid we will find that in most cases the head group is donated with the help of in CDP form that is cytidine nucleotide. What is a nucleotide? It is a nitrogenous base pentosugar and the phosphate group ok ATP, ADP, CDP these are UDP these are all nucleotides where nitrogenous base that is cytidine pentosugar and the phosphate group is attached. When the phosphate group is not attached it is known as nucleoside we will be discussing them later when we are discussing about nucleotide metabolism but this is the basic chemistry that we should be aware about nucleotides and nucleosides. So Eugene Kennedy found out that whenever phospholipids synthesis are taking place the head groups are donated in the form of the head group formation is taking place whenever there is a attachment of CDP nucleoside nucleotides. So you can see over here whenever there is phospholipid biosynthesis the CDP supplies the phosphate group ok.

So here the phosphate group can be supplied via two ways number one as I told you CDP means cytosine nitrogenous base pentosugar in the presence of in the form of ribose and phosphate group. So CDP can be in the form of diacylglycerol so the

phosphate group can come from the glycerol side or it can be in the form of a CDP alcohol. So over here glycerol so this the CDP diacylglycerol is getting attached to another alcohol right and over here a CDP alcohol is getting attached to 1, 2 diacylglycerol. Anyway in either of the mechanism one molecule of CMP will be lost I mean will be gone and ultimately we are getting the formation of the basic glycerophospholipid structure right. This thing is theoretical whenever a phosphoric acid condenses into the two molecule of water this was thought earlier, but it is Eugene Kennedy who discovered this is not how it happens it actually happens when alcohol specifically during head group donation is in the CDP form and specially during condensation of the phosphate group.

So CDP phosphate either with glycerol or CDP phosphate with alcohol. So these are the two strategies now it has been found out that eukaryotic can use both the strategies to form the glycerophosphate whereas, the bacteria can only use the first pathway right. So addition of polar group diacylglycerol phosphate with CDP or polar group that is activated by CDP alcohol. So now when so now basically we can use the same concept where this phosphatidate right or phosphatidic acid can undergo combination with cytidine triphosphate it undergoes hydrolysis and it forms CDP diacylglycerol because CDP diacylglycerol can participate in all downstream reactions. So over here what is happening phosphatidic acid is being converted to CDP diacylglycerol this CDP diacylglycerol to this now any head group can be attached using this principle.

So the head group can be serine whether you see one CMP molecule is lost so one head group is coming in one CMP molecule is lost and we are getting phosphatidyl serine this structure can vary, but the basic this head group can vary, but the basic structure will remain same in this case what is getting attached a glycerol 3 phosphate molecule right. So this is getting attached with the help of PG 3 phosphate synthase phosphatidyl 3 glycerol phosphate synthase. Now if we continue this with this molecule that is phosphatidyl serine it can be I mean reacted upon various enzymes to form various forms of phospholipids you see if it gets decarboxylated it forms phosphatidyl ethanolamine if it is decarboxylated it if it is methylated by the methyl donor that is S adenosyl methionine it is also known as ADOMET where by the action of methyl transfers it is converted to the form of S adenosyl homocysteine it is also known as ADOHKY these are the abbreviation that means S adenosyl methionine and S adenosyl homocysteine. So one addition of a methyl group I mean multiple methyl transferase it leads to formation of phosphatidyl choline. Now this phosphatidyl glycerol 3 phosphate it can be acted upon by a phosphatase enzyme where this phosphate group is first lost it becomes an alcohol to this alcohol another phosphatidyl glycerol 3 phosphate molecule can be added.

So this whole big structure is actually a mirror image of each other and this is known as cardiolipin it is a special form of phospholipid that is present in the heart it has got multiple function we are not discussing the functional aspect of phospholipids over here, but we are discussing the biosynthetic pathway, but know this cardiolipin is formed in this way. So this is the I mean summary the basic concept is same all alcohol can either be can undergo a reaction with CTP to form the CDP counterpart and thereafter they can act with diacylglycerol to form in this case ethanolamine is forming phosphatidyl ethanolamine choline can form CDP choline it can form phosphatidyl choline we also saw the inter conversion of phosphatidyl choline to phosphatidyl I mean phosphatidyl ethanolamine to phosphatidyl choline by the action of methyl transferase. Now these can also be inter converted by the action of phosphatidyl serine synthase so phosphatidyl serine synthase can convert phosphatidyl ethanolamine to phosphatidyl serine I mean serine will come and ethanolamine will be gone this is a simple replacing of a head group and this case also phosphatidyl choline and phosphatidyl serine can be inter converted by the help of I mean inter conversion of groups. Now as I told you bacteria can only utilize the first way so it can only undergo condensation with CDP alcohol where a head group will come and it will form phosphatidyl serine in this case alright. So this is the summary depending on what are the two mechanisms by which phosphate can be donated using CDP where multiple forms of phospholipids can be formed and inter converted among each other.

Next we will discuss plasmalogen this is a complex structure right this might appear to be very intimidating, but we will dissect the whole thing very slowly. So plasmalogens are a subclass of membrane these are the phospholipids that typically include so basically the structure is the same it is a phospholipid where there will be a head group there will be a phosphate group there will be two acyl groups, but these acyl groups vary in character right. The first one is an vinyl ether bond so there is an unsaturation and there is an ether bond CO C right. So this is the first position and there polyunsaturated fatty acid is present in the second position where the plasmalogens are used they are highly abundant in neuronal immune and cardiovascular cell membranes ok. So let us see how plasmalogens are formed the get your concept very right the only goal over here is to displace the esterified fatty acyl group with a long chain alcohol right.

If we do that we can see how the further process happens. So initial process acyl transfers everything is same right so what happens how can we get the long chain alcohol. So what happens over here you know fatty acyl and dihydroxyacetone phosphate condenses with the loss of a coenzyme A and the first intermeals that is formed is one acyl dihydroxyacetone phosphate ok. So one with one acyl dihydroxyacetone phosphate over here this basic very basic reaction where basically the firstcarbon or the first hydrogen of dihydroxyacetone phosphate has been acylated right

this we all understand. Now in the second case the second acyl group it is treated in a different way what happens in the second acyl group it is reduced with the help of NADPH when we are reducing acyl co it is becoming a saturated alcohol because the coenzyme A is lost.

Now this whole one the one acyl dihydroxyacetone phosphate and one saturated alcohol is acted together it is condensed the first R Co acyl group is lost and we are left with one alkyldihydroxyacetone phosphate basically what we have done we have replaced the acyl group with one saturated or saturated alcohol ok. One alkyl group has replaced the first OH group of dihydroxyacetone phosphate. So from one acyl dihydroxyacetone phosphate it is one alkyldihydroxyacetone phosphate. So if we reduce it what will happen this dihydroxyacetone phosphate I already told you this ketone group will be reduced and now we are getting the structure of glycerol right. So when this is reduced this C double bond O will become CH OH.

So now what is the final structure and we can also replace the acyl group we already told you we can easily get rid of this phosphate group by the help of one phosphatase enzyme and we can acylate one with the help of acyltransferase. So ultimately this leads to one alkyl two acyl glycerol three phosphate in this case whatever what is happening we are not actually getting rid of the phosphate group we are simply first replacing one acyl group in the second position this is R3 right. So this is one alkyl two acyl glycerol three phosphate ok. So this part is clear. In the next part what will happen there will be head group attachment ok.

So head group attachment the concept is absolutely same so this is basically in the form of CDP ethanolamine. So in this case if you are adding ethanolamine CDP ethanolamine will be coming and one CMP will be lost and since this is a phosphate group condensation will happen just like glycerophospholipid was being formed and there will be formation of phosphodiester bond. So there will be one structure where there is an saturated alcohol in the first one polyunsaturated fatty acid in the second and there is a head group in the third position. We are not there yet what we need to do we need to replace the saturated alcohol with an unsaturation we need to induce double bonds who induces this double bonds they are done by mixed function oxidase enzyme. So one NADH is utilized one oxygen molecule is incorporated two molecules of water are lost NAD is generated and there is an unsaturation.

So this is the final structure of plasmalogen ok. And lastly we are left with sphingolipids. So what are sphingolipids? Sphingolipids are a class of lipids that contain a backbone of sphingoid bases where the aliphatic amino alcohols is basically sphingosine ok. They were discovered in the brain extracts in the late 19th century and

were named after the mythological structure Sphinx ok. That is why we are they are known as sphingolipids this is the basic structure of sphingolipids where the R can be an hydrogen group then it will be known as ceramide the R can be a phosphate group then it will become ceramide one phosphate the R can be a phosphocholine it will be sphingomyelin R can be sugar it can be glycospingolipid irrespective of the structure.

So we will not be going to the structural detail we will be just seeing the overview of the important enzymes and the inhibitors that are involved in sphingolipid biosynthesis. For MCQ purpose the most important is the starting material that is L serine and permittoyl coenzyme A where we get permittoyl coenzyme A from permittric acid is synthesized one can undergo one coenzyme A transformation where one coenzyme A will be coming and one SH group will be lost and that will lead to formation of permittoyl coe. So permittoyl coe and serine reacts to form 3 keto sphinganine right that 3 keto sphinganine is then reduced to form sphinganine ok the enzyme being 3 keto reductase. The most important enzyme that comes later I mean after this step this known as sphingosine N acyltransferase also known as ceramide synthase it leads to formation of dihydroceramide mind it when we are talking about ceramide it means the R group is simply a hydrogen molecule. So from the basic structure we can derive multiple structure so if we replace the H we can get multiple complicated version of sphingolipid.

So this dihydroceramide is then desaturated to form ceramide and then subsequently with the help of multiple enzyme sphingomylin synthase we can get sphingomylin where this H will be replaced by phosphocholine alright we can get galactosylceramide for galactosyl will be replaced we can replace it with the help of multiple sphingosine alcohol that will I mean sphingolipids then that will be known as sphingosine. Next very important amount I mean a glucose molecule can replace the H that will be known as glucosylceramide. Now what is most important? In this whole slide most important are the inhibitors because they are the they are the ones that are targeted in case of multiple disease therapy they are the molecules that are our molecules of interest in multiple research grade enzyme for research grade enzyme inhibition they being myriosine that inhibits the enzyme serine, palmitoyl, transferase and fuminesin B1 or FB1 they inhibit the enzyme ceramide synthetase and a very big name PDMP that is 1-phenol, 2-decanol, amino, 3-morpholine, 1-propanol you may not remember this whole big name, but this PDMP inhibits synthesis of glucosylceramide. Now one thing to note is the first few steps before the addition of head groups for all these takes place in the endoplasmic reticulum whereas, the final steps that is the head group attachment takes place in the Golgi complex ok. So, this was about the biosynthesis of triacylglycerol, phospholipid, plasmalogen as well as sphingolipid biosynthesis.

This is the these are the reference slides and I thank you for your patient hearing.