Overview and Integration of Cellular Metabolism

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Lecture 44: Branched chain amino acid metabolism and their disorders

Hello everyone, how you all doing? Today we will be discussing the metabolism of branched chain amino acid and their disorder. This is class number 44 and the concepts that we will be covering are how the branched chain amino acids look like, what is the chemistry, what is the common metabolic fate, how they are catabolized in the body and whether there are any disorders of this branched chain amino acid metabolism that are abbreviated as BCAA's we will be discussing it all, alright. So first of all branched chain amino acid whenever we are referring BCAA's there are three valine, isoleucine and leucine, mind it is different from lysine that is not this leucine, valine, leucine and isoleucine they are all essential amino acid means body cannot synthesize whenever we are dealing with an essential amino acid metabolism we do not need to know how they are synthesized in body because we need to consume them from diet. Whenever the rule is we are discussing a non-essential we also need to know what are their sources, ok. So the metabolic fate of all these amino acids right at the beginning let me discuss for MCQ purpose. Valine is a glucogenic amino acid, leucine is a major ketogenic amino acid most important ketoionic amino acid that leads to formation of ketone bodies.

Isoleucine has a fit on both the boards, right. It is both ketogenic and glucogenic amino acid, right and these amino acids actually since they can produce either glucose or ketone body they serve as a very important alternate source of fuel whenever there is condition that leads to deficiency of glucogen, glycogen that is starvation, right. So there then the metabolism of these amino acids become very vital, ok. So if we look at the catabolism since these are essential amino acid there is no synthesis so we will only see how they are broken down in the system.

They the for there it involves multiple steps, ok. Each amino acids undergo their own way has their got their own way of being broken down, but the first three metabolic reactions actually are common, alright. The first three type of metabolic reaction are common and in some cases the enzymes are also common. So the first step is transamination, the second step is oxidative decarboxylation and the third step is a

dehydrogenation. So all the three amino acids undergoes these reaction to start with, then they will be diverted into their individual metabolic fate.

So when we are discussing the first step we already know transamination takes place by a transaminase enzyme. Where the transaminase enzyme is acting on? It is acting on the branched chain amino acid. So the enzyme is named as branched chain amino acid amino transferase. Any amino transferase or transaminase enzyme requires a cofactor, vitamin B6 pyridoxine in the form of pyridoxal phosphate. So these fundamentals should be absolutely clear by now.

Now the this has actually three isoforms. So this transaminase for branched chain amino acid has got three iso enzymes acting depending on their site of action and one is actually very specific for leucine. Both I mean this enzyme is present both in the cytosol and the mitochondria also the activity is very high in the muscles right. And who increases the activity of these enzymes in muscle naturally starvation, when we need to break down more and more branched chain right. When muscle is been degraded to produce energy then this enzyme is reduced and the process of catabolism of branched chain amino acid will starts right.

So this is the transaminase enzyme mind it I have the slide has been designed in such a way. So that each and every amino acid will be I mean branched chain amino acid and their corresponding product will be shown in different color right. So the black corresponds to the products of valine, blue for leucine and green for iso leucine for your easy understanding. So the first step what happens they are converted to their respective keto acid very fundamental rule any transamination amino acid will be converted to a keto acid. The name of those keto acid alpha keto iso valerate for valine, alpha keto iso caproate for leucine, alpha keto beta methyl valerate for iso leucine alright.

If you are finding it difficult to remember the names you can choose to omit I mean you can choose not to remember these specific names because the most important thing to remember in this branched chain amino acid metabolism are the enzymes ok. So after the first of transamination the second common step was oxidative decarboxylation right. It takes place by the help of an enzyme dehydrogenase it is acting on branched chain keto acid because keto acids are produced in the earlier step. So this is known as branched chain keto acid dehydrogenase. If this enzyme is present in the inner mitochondrial membrane and in mechanism it is very similar to pyruvate dehydrogenase complex which was taught in carbohydrate metabolism which was converting pyruvate to acetyl coenzyme A.

Similar enzyme alpha ketoglutarate dehydrogenase complex right. So those enzymes

were having actually 3 enzymes and 5 coenzymes same. This has also got dihydro lipol transacetylase dihydro lipol dehydrogenase along with 5 coenzymes TPP, lipoamide, FAD coenzyme A and NAD. So multiple vitamins in their active form are playing a role in this step. So what happens alpha keto acid dehydrogenase this is branched chain alpha keto acid dehydrogenase converts their keto acid to respective acyl coathio esters ok.

This is a regulatory enzyme and its activities increase by dephosphorylation mind it since the mechanism is same PDH was activated by insulin dephosphorylation alpha ketoglutarate dehydrogenase again by dephosphorylation. Similarly this enzyme is also activated by dephosphorylation very important although it is a catabolic enzyme. And more active form occurs in the liver in well fed state and muscle during starvation muscle during starvation that we already know right. But in liver it is in well fed state where the activation occurs. So what happens actually the keto acids are being converted to their respective thio esters what are the names isobutyrile coenzyme A, isovaleryl coenzyme A and alpha methyl butyrile coenzyme A ok.

This reaction as I discussed requires 5 coenzymes but remember NAD and coenzyme A must ok. Also TPP is required thiamine pyrophosphate vitamin B1 right. This enzyme is very important because it is lacking in maple syrup urine disease we will be discussing it very soon, but remember the name of this enzyme branched chain alpha keto acid dehydrogenase ok. Next dehydrogenation another dehydrogenation ok. So what happens oxidation of these thio esters are formed formed in the oxidative decarboxylation step leads to double bond formation or alpha beta unsaturation right.

It is analogous to the FADLing dehydrogenation the beta oxidation that was taught in the fatty acid degradation. You know whenever we are discussing about dehydrogenation we should know a concept dehydrogenation does what it introduces double bond it takes up hydrogen molecule. So this will become this alright. If this is an NAD dependent dehydrogenase what happens the NAD forms NADH2 the hydrogen is removed from the same carbon. Whenever it is an NAD dependent dehydrogenase the result will be the same double bond will be formed, but the same carbon atom will lose two hydrogen.

Whereas if it is FAD dependent dehydrogenase what will happen the hydrogen will be lost from adjacent carbon atoms ok. This is the reaction mechanism that happens ok. In this case these are moved from adjacent carbon atoms and this leads to an alpha beta unsaturated acyl-CoA derivative. What are the names of those derivatives? They are methyl acryl-CoA, beta methyl crotonyl-CoA and tig ly-CoA. So this is actually the third or common reaction that is taking place via the FAD dependent dehydrogenase.

Mind it in fact individual enzyme can be named just like this. So for example if you are

degrading isoleucine or leucine it will be isovaleryl-CoA dehydrogenase that will lead to formation of beta methyl crotonyl-CoA. So as you know enzyme can be named after a substrate on which it acts. So dehydrogenase was acting on that compound so that dehydrogenase forms with the methyl crotonyl-CoA. Why I am saying this? It is important we will see in the disease section.

Now what happens after these three common reactions these individual amino acids go to their own path H and individual amino acid suffers a different metabolic fate. In case of valine it undergoes conversion to beta hydroxyisobutyrate which generally which again undergoes a dehydrogenation to form propionyl-CoA and we all know when propionyl-CoA is found how it can be converted to methyl manly-CoA and then succinyl-CoA and ultimately to glucose via the new glucogenesis and carbohydrate metabolism pathway. Next we move to leucine. Leucine has got a different fate where it undergoes a carboxylation to form beta methyl glutamyl-CoA and ultimately it forms HMG-CoA or hydroxymethyl glutaryl-CoA. You already know the name HMG-CoA you have read it in cholesterol synthesis, ketone body synthesis where you have been taught how HMG-CoA lies enzyme.

As we will see in the next slide and in case of third pathway what happens tiglyyl-CoA is again converted to alpha methyl beta hydroxybutyryl-CoA then it is converted to alpha methyl acetoacetyl-CoA by dehydrogenation ok. So, I hope this slide is clear again let me tell you you do not need to remember these individual steps ok. In the grand scheme of things when you are revising for the entire curriculum this is not so much important compared to the disease section because disease section is a must know area in this branch in amino acid metabolism. This is nice to know if you can answer it you will get more marks in viva or you may answer a very tough question, but if you miss the disease section that will be discussing later it will be problem because that is a must know area. So, after this step what happens the HMG-CoA is acted upon by HMG-CoA lies this we already know how via three steps carboxylase, a mutate, racemase and the mutate it is converted to succinyl-CoA.

You already know that here beta ketothiolase it breaks down this alpha methyl acetoacetyl-CoA to form both acetyl coenzyme A and propionyl coenzyme A ok. This is a recap of last class what we discussed how propionyl-CoA is the fate of propionyl-CoA how propionyl-CoA is converted to succinyl-CoA which was also taught in beta oxidation of othcine fatty acid right. Anyway so, if we look at the fate valine enters exclusively in the glucogenic pathway leucine exclusively enters the ketogenic pathway because acetoacetate and acetyl-CoA both are precursor of ketone body they form ketone bodies right. And this enters glucogenic pathway by propionyl-CoA as well as ketogenic pathway by acetyl coenzyme A.

So, very interesting. So, considering the interrelationship of amino acid this diagram was taught in last class while we discussed how methionine and threonine was being converted to succinyl-CoA. And I told there will be new players that are joining well those two players are valine and isoleucine because both are converted to succinyl-CoA ok. So, if we just magnify it a bit valine multiple steps 7 steps to be precise is converted to propionyl-CoA and isoleucine by a one step less it converts to acetyl coenzyme A and also propionyl coenzyme A by the beta keto thiolase enzyme and we all know how propionyl-CoA leads to production of succinyl coenzyme A. So, looking at the metabolic fate where our branch in amino acid metabolism or branch in amino acid are lying you see isoleucine and leucine are forming acetyl coenzyme A leucine is exclusively forming acetyl coenzyme A whereas, isoleucine is also forming succinyl coenzyme A along with valine alright. So, we have discussed how branch in amino acid enter into the TCA cycle or they enter or they have a ketogenic fate alright.

So, this is the final common pathway of carbon skeleton where our today's amino acid have also been addressed in this piece of puzzle. Now, we move on to the various disorders of branch chain amino acid metabolism. The first disorder which is the important of metabolism very important is maple syrup urine disease MSUD right why it is called? It is first of all because the urine of the affected individual smells like maple syrup or burnt sugar alright. You see if you are an I mean maple syrup may not be available in our country although it is available now because more westernized food are being available in shopping malls etcetera right, but burnt sugar smells very similar right we do not have maple tree in India right. Anyway and the enzyme that is deficient I told you that is branch in alpha keto acid dehydrogenase which was converting the keto acid into their thio esters this enzyme is deficient.

So, what happens I already told you this is the blockage leads to hamper of conversion of these keto acids into their respective thio esters. So, what will happen both the branch in amino acid and their corresponding keto acid will be elevated. The first step was amino acids all being transaminated to keto acid in the next step these keto acids were being converted to the esters if the esterification does not happen both the two previous steps the intermediates substrates will be high. So, that is the reason why plasma and branched chain amino acid and their keto acids are highly elevated ok. And since this keto acids are excreted in urine specially the branch chain keto acid they are also known as branch chain ketoneuria.

Now, this disease that is classic maple syrup urine disease is actually referred to the term where there is the maximum absence of the enzyme activity of this enzyme branched chain alpha keto acid dehydrogenase ok. The incidence is 1 per 1 lakh bar and

in fact, it has been found that less than 2 percent of normal enzyme activity is present that actually leads to a problem where these branched chain amino acid cannot be metabolized in any way and it cannot metabolize their intermediates will be accumulated in the blood alright. So, what are the symptoms of classic maple syrup urine disease which is also abbreviated as MSUD. So, accumulation of branched chain now the symptoms are very common in any in border of amino acid metabolism whenever something is or amino acids are cannot be metabolized they will be accumulated they will saturate the transfer they will help in a brain development, they will be mental retardation, intellectual disability, abnormal movement all these things that we already read in previous amino acid metabolism disorders will fit into this disorder as well ok. So, what happens impairment in transport and function of other amino acid because of accumulation of excess amount of branched chain amino acid.

When other amino acids are less available protein biosynthesis will be reduced not only that this branched chain amino acid actually competitively inhibit the very important amino acid glutamate dehydrogenase. Remember GDH was an important enzyme which was helping in transamination followed by deamination that is trans deamination removal of nitrogen group. If it is hampered there will be ammonia tocetia as well because ammonia cannot be removed from the system. So, anyway after, but since these are in border this is an in border of metabolism the deficiency of this enzyme or the problem in the BCKD gene is already present to start with and the baby will exhibit subtle symptom to start with. What are the subtle symptoms in the first 1 or 2 days that is poor feeding either breastfeeding or bottle feeding the baby will appear to suckle very poor there will be lethargic and irritable it will cry it will failure to thrive will happen.

There will be abnormal movement if this is continued and the disease is not detected what will happen it leads to focal neurological signs. What are those focal neurological signs? What are those focal neurological signs? These are abnormal movement for example, athetosis, hypertonia, spasticity muscles will be very spastic there will be increased tone. Opisthotonous, opisthotonous means the posture that is found in tetanus where if this is the head of the baby I am drawing a schematic diagram and this is the table the baby will curve like this on the table the baby will appear this will be the hand of the baby and mind you the eyes are looking up this is the baby. So baby will be curved like a bow this is also known as opisthotonous it is found in one of the key features of tetanus, but this is also found in classic mapel sera furin disease if there is excess accumulation of brancheda and amino acid and there keto acid in the system and ultimately it can lead to convulsion and coma. And if MSUD is left untreated the central neurological function will hamper and ultimately lead to respiratory failure followed by death ok.

So this is a very serious disorder which needs to be detected very early and it needs to be promptly treated right. So how we can diagnose? Diagnosis actually very easy if you know the biochemistry and metabolism of it now diagnosis of every disorder is very easy urine contains branched chain keto acids and also those branch and amino acid like valine, leucine and isoleucine alright. Rothera's test is positive when does Rothera's test become positive in case of ketoneuria mind it right. You know in classic Rothera's test when we boil and cool the urine the acetone and acetoacetate may get evaporated and that might not give a positive Rothera's test when, but these branch and keto acids are not heat labile. So even if we boil and cool the urine still Rothera's test will be positive ok.

This is one applied importance that might help in practical exam. So Rothera's test we got one answer from phenylketonuria also mapelsirapurin these are the two disorders of protein or amino acid metabolism in which the Rothera's test is positive for ketone bodies alright. Now the diagnosis depends on enzyme analysis I told you the level of the enzyme in classic MSUD it is less than 2 percent right. And very important the diagnosis should be done prior to or one week after birth right. Means if we let the baby undiagnosed this symptoms are continuing and the baby if it is detected prenatally it is very good, if it is not detected prenatally and if we cannot detect even if one week has passed by then the brain development is so much hampered that that will lead to permanent mental retardation and it can also even lead to death.

What is the treatment? Well the culprit since those are essential amino acids and their metabolism is hampered and they are not synthesized in the body we can restrict their availability by low by designing a diet in which there are low very low amount of branch chain amino acids. Mind it every amino acid is essential for protein synthesis right, but in when they are not needed this branch chain amino acids or every amino acid is broken down in their own catabolic pathway. So, very low amount must be given because it is essential for brain development, but not in excess right. So, a very important balance needs to be struck. There are other variants of Mappel-Sireh purine disease that are not severe.

Intermediate Mappel-Sireh purine disease is one such in which the same enzyme is deficient ok. All Mappel-Sireh purine disease are due deficiency or inactivity of this enzyme or less activity of this enzyme and branch in alpha keto acid dehydrogenase. In this case 3 to 8 percent of normal enzyme activity is present, in classic it was less than 2 percent all right. So, what happens since it has got greater level than residual enzyme activity in classic MSU, the symptoms are generally late to appear all right. In that case classic it was appearing within first 24 to 48 hours and if you do not treat by 7 days the B might die.

Here 5 months to 7 years because still then the whatever residual level of a branch and keto acid dehydrogenase activity is there, it will help to normalize the brain function. Mind it whenever there is more enzyme activity the diagnosis will be much later because there will be less problem whenever there are severe enzyme deficiency the diagnosis will be earlier right. So, it depends on the amount of enzyme that is deficient right. The symptoms are actually same as that of classic MSUD, but generally milder and they appear late in case of intermediate MSUD. Further lighter variant is known as intermittent Mappel-Sireh purine disease.

It is also known as intermittent branch and ketoneuria it is a milder variety because 8 to 15 percent of enzyme activity are present ok. So, what happens contrary to classic and intermediate MSUD all right these individuals have got normal growth and normal intellectual development why because the enzyme activity with 15 percent enzyme activity generally there is no problem all right. So, it will not be diagnosed unless there is a crisis situation. When there will be muscle degradation and when there is increased catabolism of branch in amino acids only then this will lead to a problem right. This is in case of excess stress, in case of starvation or in case of infection when there is an increased catabolic state when excess muscles are being degraded then only when excess branch in amino acids will be degraded then only the feel of the absence is I mean the absence is felt all right.

So, metabolic crisis leading to seizures coma branded is still a possibility it may happen, but if the disease diagnosed and careful diet is planned. So, that after diagnosis suppose in case of a crisis the symptoms happen all right, but after the crisis is over if a dietician gives specific foods which are less in these branched chain amino acid or special formula feed then this disease will be taken care of. There is a very minor variant of my pelsida purine disease that is also known as thiamine dependent ok because I mean that is similar to intermediate right. It is because the even if the enzyme is not active right somehow it the function can be improved by over I mean excess dose of vitamin B 1 or thiamine why because the enzyme branched chain keto acid dehydrogenase needs thiamine as a cofactor all right. So, large dose of thiamine will is the activity generally if the thiamine response MSUD is present newborns will do not present as a I mean this does not present as a disorder.

So, newborns rarely present with symptoms, but whenever the baby is growing up for example, 5 to 8 months or even 5 years where intermediate MSUD was being diagnosed first we can actually check the by dosage excess dose of thiamine and that actually helps in treating the disorder. After MSUD there is isovaleric acidemia which is nothing, but specific inborner of leucine metabolism. The first three reactions are common and the

first was transamination, second was dehydrogenation and third one was FAD dependent dehydrogenation right. The problem lies in the reaction 3 of leucine metabolism. So, what was the enzyme in the reaction to isovaleryl CoA was produced right and this isovaleryl CoA cannot be further metabolized.

So, isovaleryl CoA dehydrogenase is deficient it was converting it to 3 or beta methyl protonyl CoA this conversion cannot happen. So, what happens isovaleryl CoA in the form of isovaleric acid is accumulated in the body and it is I mean the I mean it is level is very high in sweat and it is whenever that is common theme whenever an intermediate is accumulated its level will rise in sweat and urine. But a characteristic how we can diagnose it is this acid is secreted in the body fluid and it imparts a cheesy odor to breath and body fluid ok. And the symptoms include acidosis, lethargy and mental retardation that is the same to all amino acid intermediates if they get accumulated that is leucine further can be accumulated it will the concentration rise in brain and it will lead to hampering of other amino acid metabolism leading to mental retardation by I mean those keto acids that were being accumulated in MSUD from leucine is also getting accumulated in isovaleric acidemia right.

So, the symptoms are more or less the same. And lastly hypervalinemia this is a disorder again an inbound of metabolism what happens the transamination of valin is selectively impaired the first step impaired again very common what will happen high plasma and valin level, but since it is selective valin transamination defect leucine and isoleucine will remain normal. But again the clinical feature its same similar growth failure, hyperkinasia, abnormal movement, vomiting, nystagmus means rapid eye movement to and fro eye movement it can be up down it can be horizontal there is a nervous CNS sign and inability to circle that is irritability to breast feed. So, this are the disorders of branched chain amino acid metabolism to conclude this lecture session has covered the chemistry of branched chain amino acids their metabolic fate. We showed we learnt what are the steps of catabolism of branched chain amino acid, we learnt about the disorder, maple syrup urine disease most important, we learnt about the variation of maple syrup urine disease the symptoms the diagnosis the treatment also we learnt about isovaleric acidemia and also hypervalinemia which are selective defects in the metabolism of leucine and isoleucine and valine. So, these are my references for today's class I thank you all for your patient hearing. Bye.