Overview and Integration of Cellular Metabolism

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Lecture 35: Urea Cycle – Regulation and Enzyme Deficiency Disorders

Hello everyone, welcome to Protein Metabolism. We are continuing with our lecture series on overview and integration of cellular metabolism. And today's topic is the second part of the continuation of last class that is urea cycle. Over here we will be discussing the regulation of urea cycle and we will be also be learning the enzyme deficiency disorder. So basically we will be covering these concepts the first few concepts are already covered in the last class and today we will be dealing with these concept that is regulation of urea cycle, the various enzyme deficiency disorder, how to diagnose and how to treat the urea cycle deficiency disorders alright. So, we have already learnt how urea cycle is taking place alright.

We have learnt how all the nitrogen's are entering into the mitochondria, how they are getting prepared to participate in the urea cycle ultimately each and every steps we have all learnt. Now, just for recapitulation these are the important enzyme the first enzyme was carbamoyl phosphate synthetase I which was required for prerequisite for urea cycle in this for the synthesis of carbamoyl phosphate. Subsequently the enzymes are ornithine trans carbamoylase, then argininosuccinate synthetase, argininosuccinate lyase and arginase I. We also discussed mnemonics by which you can easily remember all the intermediates and all the enzymes right.

So, this is the sequence of urea cycle which was also discussed in previous class. So, I hope you can remember this slide if you do not it is good time to watch the previous class again and then attain this class according to your own pace. And as I have already told you, you should have by now drawn your own version of urea cycle with all the enzymes. So, now we can actually focus on what are those enzymes and what will happen if each of these enzymes are defective and what will be the problems that will be leading to all the metabolic symptoms right. So, we all know the final reaction was this ammonia carbon dioxide and 3 ATP was participating along with aspartate from TCA cycle it was leading to progression of urea and fumarate which was going into the TCA cycle and ultimately 2 ADP, 1 AMP and 1 BPI.

Now, over there I discussed that will be reading about carbamoyl phosphate synthetase 1 in detail. Well, carbamoyl phosphate synthetase 1 has got an isoform or iso enzyme that is carbamoyl phosphate synthetase 2. You should remember that the first two reaction are occurring in the mitochondria. So, definitely if we compare these isoforms the first enzyme is looking it is taking place is actually so, called in the mitochondria whereas, the isoform is located in the cytosol right. We all know CPS 1 is a product of or is a component of urea cycle, but CPS 2 will be reading in detail very soon when we go into the nucleotide metabolism is a part of pyrimidine biosynthesis.

This point is very important a positive effector of CPS 1 is NAG, N-acetylglutamate very important role it will be playing in the regulation of urea cycle. Next there is no known effector of CPS 2 right. What is the source of nitrogen to act on CPS 1 it is ammonia and for CPS 2 it is glutamine. Is there any inhibitor of N I mean CPS 1 there is no known inhibitor. So, if we have got an acceptor it will increase more if you do not have the effector it will still go on nobody is there to stop urea cycle why because it is very important if urea cycle stops there will be many problems in our body which is the discussion of today's topic and CTP is the inhibitor of caramel phosphate synthetase 2.

So, looking at the nitrogen balance this figure we are now very familiar with or you can easily conceptualize. So, we are getting dietary proteins which are contributing to the amino acid pool from their tissue proteins are either getting synthesized or tissue proteins are breaking down to again contribute to the amino acid pool and this amino acid can be reused to either produce important products or they can be excreted as ammonia and urea alright. So, about 80 percent of acetate nitrogen is the form of urea alright. So, how this urea cycle is actually regulated. So, there are multiple regulatory levels number 1 basically there are 2 one is coarse regulation one is fine regulation.

So, what is coarse regulation broadly basically coarse regulation deals with inducing enzyme of urea cycle basically the carbonyl phosphate synthetase 1 as well as all the 4 enzymes of the cycle whenever there is an increased demand. So, when there will be increased demand in case of high protein diet in case of high protein diet there will be more and more nitrogenous waste that will be produced and there will be more and more urea cycle will be needed to get rid of those waste. Also remember in case of prolonged starvation where all the bodies carbohydrate and lipids have been utilized thereafter the bodies nitrogen or the amino acids and proteins are broken down where the muscle destruction occurs in the last phase of starvation after 72 hours or even after 3 to 4 days right. So, new alkalinity is happening then glutamate dehydrogenase is induced because catabolism of protein is happening and that also increase the demand for urea cycle alright. So, what is the way of negating this whole thing.

So, reverse way I mean reverse regulation will happen whenever animals are well fed with diet that is rich in carbohydrate and fat. So, remember high protein diet is actually inducing urea cycle diet which is devoid of protein will decrease the urea cycle. Also animals with protein free diets if we have diet plan as such that there are no protein in diet then urea cycle will be suppressed because there will be no need of urea cycle. So, this is the course regulation alright. Now, look at the fine regulation fine regulation is done by allosterically affecting the enzyme CPS 1.

I told you carbamoyl phosphate synthetase 1 is the most important or regulatory enzyme of urea cycle. So, who is the actually the allosteric activator or effector it is N-acetyl glutamate. So, if the body or if somehow we have more and more N-acetyl glutamate it means there will be more and more activity of carbamoyl phosphate synthetase 1 and there will be more and more urea cycle. Now there it does not end there. We can actuallysynthesize N-acetyl glutamate in the body by the help of glutamate and acetyl coenzyme A.

You already know by now what is the source of acetyl coenzyme A right from carbohydrate metabolism knowledge and also lipid metabolism and we already also know how glutamate is produced right. Now this enzyme N-acetyl glutamate synthase alright it synthesizes N-acetyl glutamate from glutamate and acetyl coA. This enzyme can again be allosterically activated by the factors like high protein diet, more glutamate, arginine it is a very strong activator of N-acetyl glutamate synthase as well as prolonged starvation. So, all those factors that was discussed in the course regulation along with high glutamate and arginine is actually activating N-acetyl glutamate synthase which is into the production of more N-acetyl glutamate or NAG and this NAG is actually activating the CPS 1 enzyme that is the regulatory enzyme of urea cycle right. This whole process of course and fine regulation helps in regulating the urea cycle whenever there is an increased demand for urea cycle alright.

There is also another level of regulation which is actually biological regulation by the phenomenon of compartmentalization. What is compartmentalization? You are familiar with this figure where I discussed the first two reactions of urea cycle are taking place in the mitochondria right and the rest that is taking part in the cytoplasm. Now how this proteins or how the molecules are transferred across mitochondria cytoplasm well there are dedicated transporter. For example, ornithine is transported via an ornithine transporter, citrulline is transported out by citrulline transporter. Few of these compounds are freely permeable by the mitochondrial membrane through the mitochondrial membrane they do not need transporter right, but for ornithine there is a transporter which will also play a vital role during the urea cycle disorders.

However, how the compartmentalization helps? Since the cycle is designed in such a way that few steps will be in the mitochondria and few steps will be in the cytosol there is one step where fumarate is produced right. Well when fumarate is produced the fumarase enzyme alright actually has got you see the inhibitory effect of fumarate on its own production is minimized because argininosuccinate lies is in the cytoplasm while fumarase is in the mitochondria. So, what happens? This absence of fumarase is actually helping the cycle to continue alright. Otherwise what would have happened the product would have been inhibited it would have been feedbackily inhibited, but it is not inhibit in a feedback manner because the enzyme fumarase is not present in the mitochondria. So, where fumarase is present it is present in the cytosol.

So, when fumarate is coming out it is actually broken down in the cytoplasm. Once again fumarate when it is formed it actually. So, how this basically happening fumarate is actually inhibiting its own formation what do you understand by this means it will it would have inhibited the enzyme argininosuccinate lies. So, argininosuccinate lies actually breaks down argininosuccinate to form arginine and fumarate right. So, if fumarate was present over there fumarate would have inhibited feedback inhibition, but it is going outside why because it needs to replenish aspartate since it is going outside because fumarate is present in cytosol the inhibitory effect of fumarate on its own formation is negated all right.

So, I hope I have explained already explained twice. So, I hope now it is clear if it is not you can pause the video rewind back and rewatch again right. So, looking at the amount of blood urea the normal level is 20 to 40 mg per dl this is a very common indicator of renal function that is always presented I mean prescribed by clinician whenever routine blood investigation is given urea and creatinine well you have read about urea will be reading about creatinine later right. And it is since it is an indicator of renal function when it will increase physiologically it increases with age. So, the level of urea in a new born baby will be much lower compared to the level of urea in a adult person and it will be lower compared to an elderly in elderly patient the amount of urea increase will be high normal range of urea.

And pathologically in a disease process the amount of urea is actually increased with renal failure very important it is not increased in protein intake all right very important. Because whenever there is a balance whenever the excretory system is working no matter how much urea is produced by the urea cycle irrespective in spite of a high protein diet urea will be cleared urea is will be washed out in urine. So, only and only if the excretion system is at fault the outbound system is problematic then the level of urea will rise all right. So, let us return to the deficiency of urea cycle enzymes which is a main topic of

today's discussion. So, this is again an overview all right.

So, one thing to note is the first two steps are happening inside the mitochondria I am repeating myself over and over again. So, that the thing is reinforced into your brain and ornithin is transported into the mitochondria with the help of an ornithin transporter all right. And the enzymes are carbon phosphate synthetase 1 ornithin trans carbamoylase argininosuccinate synthetase argininosuccinate lyase and arginase and these are the gene names that are coding for the this ornithin transporter is ORN1 ok. These are the genetic gene name of the genes that are coding for these enzymes and everything is very important. So, considering the enzymes of urea cycle each enzyme can be deficient individually and there have been cases individual cases that have been studied and well documented where each and every enzyme can be deficient and ultimately they result in ammonia intoxication .

Although the diseases of urea cycle are rare that is 1 in 30000 live births, but still they are they cases are often found because in countries like India where population is in billions suddenly this 1 in 30000 the number does not remain 1 in 30000 increases whenever we are talking about tertiary care center specially the newborn clinic right . You should note for MCQ purpose that all the enzyme of urea cycle diseases are inherited all the diseases of urea cycle are inherited in autosomal decisive manner except I will again discuss later ornithin trans carbamoylase deficiency which is X linked very important MCQ all right. Now, the reaction 1 and 2 what is the reaction 1 and 2 the first reaction carbamoyl phosphate synthetase 1 and ornithine trans carbamoylase these two reaction are more concerned I mean the deficiency of these two enzymes are much more serious because they lead to direct accumulation of ammonia they are much more problematic. Whereas, the later enzymes they help in accumulation of intermediates and they are less toxic compared to direct accumulation of ammonia all right. Now, we will be for the next few slide we will be discussing the few bits and pieces few facts about urea cycle deficiency disorders right.

So, whenever and you newborn is actually I mean you see these enzymes are actually coded or formed in whenever the baby is in fetal stage. So, the deficiency of these enzymes will be manifested as and when the baby is born right. So, this is a reason of congenital hyperammonemia the time hyperammonemia again will be discussing later and all we have also discussed it previously in our earlier classes means an excess of ammonia all right. So, whenever ammonia is acquired when can it be hyperammonemia when can it be acquired whenever the ammonia extracting mechanism is at fault means liver failure it was ok, but suddenly it has gone wrong. So, liver failure and whenever a baby is born with urea cycle enzyme deficiency it is a cause of congenital hyperammonemia .

Now, most of it are partial deficiencies generally total deficiencies are not found and they are very fatal, but still one or more of the several enzymes may be deficient in at least several days of time are given to the treating team by which they can diagnose and treat the disorder otherwise there will be permanent brain damage. I already told you for the first few enzymes the first two specifically they are much more dangerous because ammonia is accumulated where in the other hand the later stages the multiple intermediates are accumulated that can diffuse in from the hepatocyte into the blood and ultimately they are passed into the urine. Still the collective features are mimicking ammonia toxicity. So, in general the deficiency of urea cycle enzyme are termed as hyperammonemia and what is the feature of hyperammonia it is the it is a triad of actually hyperammonemia n k-phallopathium respiratory alkalosis very important now we cannot extend our class in discussion in detail about acid base disorders, but please note that high amount of ammonia stimulates the medullary respiratory center to cause hyper ventilation. Ammonia medullary center stimulation hyperventilation and hyperventilation means rapid breathing leads to wash out of CO2 which leads to respiratory alkalosis alright.

Ultimately ammonia toxicity you already know leads to features like vomiting, irritability, lethargy and severe mental retardation. There was depletion of TCA cycle there are multiple neurotransmitters that are problematic we have already discussed what are the mechanism of ammonia toxicity and if the toxicity happens right from the birth there will be progressive and severe mental retardation alright. It may be may seem normal at birth, but with ongoing days they will become lethargic and activity will be very much compromised. Now regarding the management of treatment it is mainly symptomatic right. So, whatever is the culprit we need to identify and we need to treat right.

For example, we can actually decrease the ammonia or the effect of these deficiencies by letting these enzymes not act. So, these are urea cycle enzymes right when they will be acting more and more whenever the urea cycle is in full force. So, when or how can we decrease the level of urea cycle we told you in regulation low protein diet. So, low protein diet can actually restrict urea cycle. You know urea cycle is also needed for synthesis of arginine.

So, if we supplement arginine the body will sense that ok we do not need to perform urea cycle and therefore, the intermediates of urea cycle if there is some deficiency will not be accumulated alright. So, mainly these and frequent feeding by ultimately protein free diets can help in the ammonia level remaining low and prevent brain damage right. I discussed ornithine transporter deficiency it is a very important deficiency it is not an

enzyme, but is a transporter that is if it is deficient what will happen? Ornithine will be accumulated right. So, ornithine gets accumulated leading to hyper ornithinemia urea cycle will be hampered what happens when urea cycle is hampered it leads to hyperammonemia and inside the mitochondria what happens since ornithine is not available right lysine is carbamylated to form homocytrulline ultimately there is an increase in level of homocytrulline. So, three things are increased hyper ornithinemia, hyperammonemia and hyper homocytrullinemia this is known as HHH syndrome which is a diagnostic feature which is a syndrome named in ornithine transporter deficiency again a very important multiple choice type of question from the urea cycle deficiency disorders.

Now, you all know brain is very sensitive to ammonia. So, what is the regime I told you frequent small feed and low protein diet. Now, regarding the specific treatment there can be some chemical agents that the mechanism of action which will be discussing later slide is to give benzoate intermediate that is benzoate and phenyl acetate ok. So, benzoate and phenyl acetate or phenyl butyrate ultimately gets converted to soluble intermediates like hippuric acid and phenyl acetyl glutamine. We will be seeing this reaction detail later do not bother right now, but know this treatment is treating with some intermediates which will lead to formation of soluble products that will be excreted and thus the subsequent formation of these products will ultimately deplete the nitrogen from the system.

If it is not clear I will be again addressing this slide very later this thing very later right. Next one thing to be noted since citrulline is present in significant quantities in milk right there is a deficiency you can tell it right now when there is citrulline is acting in what reaction citrulline is being converted to next product. If that enzyme is deficient naturally citrulline will be accumulated and that will lead to citrullinemia. So, in case of citrullinemia the breast milk is to be avoided right because already citrulline is increased due to deficiency of urea cycle we do not need extra amount of citrulline from diet right. Next I already told you for very important MCQ question ornithine transcarbamoylase is the only inherited urea cycle disorder that is X linked all are autosomal recessive right.

So, increased levels of ammonia are also accompanied with high glutamine levels in blood why glutamine? Glutamine synthase I already told you glutamine synthase where glutamate is acting upon capturing on ammonia to become glutamine and glutamine is the major carrier of ammonia. So, whenever ammonia is increased glutamine will be increased right. Next argininosuccinate lyase deficiency well if you want to follow these step by step it is very important that you are also following the urea cycle that you have drawn then it will be very easy for you to locate what are the enzymes and the product that is immediately before will get accumulated this is the story of all enzyme deficiency

disorder. So, the reactant on which the enzyme is acting will be accumulated right. So, whenever there is a deficiency of argininosuccinate lyase it acts on argininosuccinate right or argininosuccinic acid.

Argininosuccinate accumulates in the body and ultimately it is leading to metabolic acidosis right. So, argininosuccinic aciduria is a case where argininosuccinate is elevated in the CSF and it is excreted in urine all right. A special clinical feature that happens in argininosuccinate lyase deficiency is trichorexis nodosa it is characterized by friable and tufted hair the hair is very much tufted and it easily breaks. So, this is the microscopic figure in which the breakage of the hair is being shown it is broken internally. So, one gentle pull the hair will be very brittle all right.

The most mild deficiency is actually the last enzyme deficiency arginase mind it whenever the upper enzymes are inhibited or enzyme are deficient the diseases are much more severe the manifestation much more severe as we move along the cycle the symptoms become mild. So, in case of arginase deficiency it appears within 2 to 4 years of age right. So, how to diagnose this urea cycle deficiency disorders? Nowadays and the gold standard of detecting enzyme of urea cycle disorder is by establishing the metabolites using tandem mass spectrometry also two mass spectrometry that are coupled or MS MS ok tandem mass spectrometry. So, this is again one slide which is showing all the disorders right I will be discussing all these disorders very soon in another table, but for now you should remember whenever the enzymes are later enzymes there will be increased accumulation of both arginine and citrulline. So, argininosuccinate synthetase argininosuccinate lyase or arginase they are characterized by increased arginine and citrulline.

In case of arginase deficiency there is more and more accumulation of arginine only. Whereas, the initial enzymes that is carbamoyl phosphate synthetase bond deficiency or ornithine trans carbamoylase or N-acetyl glutamate synthase the enzyme that was synthesizing NAG they are characterized by decreased amount of arginine and citrulline very important. So, later enzymes more arginine citrulline former earlier enzymes less arginine citrulline whenever we are measuring these these amino acids in the plasma alright. And regarding genetic inheritance I told you OTC the ornithine trans carbamoylase is X linked other everyone is autosomal recessive. One important information that is if we measure the organic acid in urine in case of carbamoyl phosphate synthetase deficiency there is normal or decreased urinary orotic acid this is the marker ok.

So, next high urinary orotic acid happens in ornithine trans carbamoylase and normal again normal or decreased urinary orotic acid happens in N-acetyl glutamate synthetase

deficiency. So, if we look at these enzyme deficiencies one by one the first enzyme deficiency that is carbamoyl phosphate synthetase I is termed as hyperammonemia type I alright it is characterized by severe mental retardation and developmental delay. Next N-acetyl glutamate synthase is a variant of hyperammonia type I because it is actually helping in the syntheses helping in the enzyme CPS I and we can actually treat that enzyme deficiency by treatment with N-carbamoyl L-glutamate which actually activates CPS I. Why N-acetyl glutamate synthase the main job this enzyme is to activate CPS I. So, if this is deficient this enzyme is actually lowered, but if with this treatment the whole thing is taken care of.

Hyperammonemia type II ornithine trans carbamoylase deficiency leading to orotic aciduria alright. So, this is what happens here the channeling of the intermediate to pyrimidine synthesis. Next hyperornithinemia when it happens in case of ornithine transport system disorder not only hyperornithinemia hyperammonemia and hyper homocytrelinemia also happens right. Citrulinemia when it will happen the enzyme that acts on citrulline that is arginine synthetase if it is deficient the product the reactant earlier to it will be accumulated right. Next argininosuccinic aciduria when it happens in case of argininosuccinate lye's deficiency.

So, if this is deficient this will not be acted upon there will be more and more accumulation of argininosuccinic acid the clinical feature trichorexis nodosae I have already seen the photon lastly most mild arginase deficiency here what happens argininemia happens because there is more and more accumulation of arginine inside CSF. Now, considering the level of ammonia in blood it is actually very low it is less than 50 micromole per liter the standard being 11 to 30 or 15 to 45 whatever unit it is less than 50 right depending on labs unit of representation may be different. Whenever it is going beyond 100 the patient will start to lose consciousness there will be altered sensorium and if the value goes above 200 there will be coma and convulsion. However, this blood ammonia generally it is whenever a blood ammonia is raised along with respiratory alkalosis in babies that will lead to a clinical suspicion of urecycled disorder, but in adults mainly whenever ammonia is high it is related to acquired or hepatic NK phallopathy and mind it hepatic NK phallopathy the levels of ammonia not well correlated because ammonia estimation may have some errors whenever finch clist is I mean fist is clench or there is application of tourniquet or leading to processing time extension ammonia level may be falsely elevated or lower right. And moreover statistical studies have found out that the sensitivity specificity all those things as well as the correlation of the disease whenever the disease cured even then the ammonia levels appear to be very high.

So, ammonia level is not a good indicator of hepatic NK phallopathy in case of acquired

hyperammonia. So, how can we treat this ureicycle deficiency disorders? Mainly whenever we are restricting the protein because protein free diet or low protein diet we need to make sure the essential amino acids are deficiencies are not hampered. How? We need to provide the corresponding keto acid in diet because amino acids are converted keto acid by trans amination. Maximum calorie have to be given by intravenous glucose and lipids right. Whenever there is block in a argininosuccinate lies we can supplement arginine diet because arginine is actually synthesized with the help of that enzyme.

And in the first two blocks we can actually supplement with benzoyl co or benzoate and phenyl acetate or phenyl butyrate which will give rise to soluble compound that are excreted in urine. See over here whenever there is formation or block in this enzyme arginine synthesis will be defective we need to supplement arginine. And this is how we can actually treat with external compound like benzoate. So, benzoate is actually combining with glutamine is actually combining with all the glycine to form hippurate right. Glycine is the very glycine is a amino acid that is extremely needed for all practical purpose.

Also phenyl butyrate is getting converted in vivo to phenyl acetate and it is finally, getting converted to phenyl acetyl glutamine. So, what happens? So, all the intermediates that is glycine and glutamine are now depleted from the system. Body needs more and more glycine and glutamine in order to excrete ammonia or in to maintain normal metabolism. So, very important line subsequent synthesis of glycine and glutamine to replenish the body pool of this compound actually removes ammonia from the blood stream. So, we are actually indirectly removing ammonia by depleting their source their sources of the molecules which are actually produced for ammonia removed.

So, more and more ammonia will be utilized in order to produce this compound. So, thus hyperammonia will be treated. So, that is it about today's class to conclude the two lecture series on urea have been completed and this lecture has covered the regulation of urea cycle, the various enzyme deficiency disorders, how we can diagnose the enzyme deficiency disorder, what are the symptoms and what is their treatment. So, these are the few references for my today's class and I thank you all for your patient hearing. Bye.