Overview and Integration of Cellular Metabolism

Prof. Arindam Ghosh

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

Indian Institute of Technology Kharagpur

Week 06

Lecture 27: Metabolism of Ketone Bodies

Hello everyone, glad to see you again in your lecture series on Overview and Integration of Cellular Metabolism. We are continuing with lipid metabolism, we are in week 6 and today's lecture revolves around metabolism of ketone bodies. The concepts that we will be covering in this class is how ketone bodies are generated that is ketogenesis, how ketone bodies are utilized, how the whole thing is regulated, what are the consequences of ketogenesis, how can we diagnose the ketogenesis and ketoacidosis and how can we treat the ketogenesis using principles of biochemistry. Now, last time we discussed in diabetes mellitus in type 1 diabetes mellitus, I showed you what the intra tissue organ I mean in relation how ketone body was generated and I mentioned we will be discussing them detail today is that day ok. Now, we will be extrapolating our concepts that we gathered regarding type 1 and type 2 diabetes mellitus, we will be focusing mainly on what is the pathogenicity of type 1 diabetes mellitus specially in this class. So, basic thing that we need to understand from our knowledge in carbohydrate metabolism and lipid metabolism now that we have covered both carbohydrate metabolism as well as fatty acid oxidation prior to this class is the concept of how fats are burned under flame of carbohydrate.

This is a justification type of question that we often find in exams right specially in medical exams. So, what does it mean? So, when we are cooking something under flame of gas this is the basic thing that we are doing. It means the heat that is being produced by the LPG is being used to cook the food. Similarly, the when we are planning a diet which has got fats and carbohydrate and we preferentially want to break down the fats to produce energy that is the principle of all keto diets that many health conscious or diet nutritionists are promoting nowadays.

The basic principle is in order to digest fats we need some amount of carbohydrate or specifically when the acetyl coenzyme A that is produced by breaking down of fatty acids via beta oxidation when we need that acetyl coenzyme A inside the TCA cycle in order to produce energy the TCA cycle has to be working right. However, that thing

when that thing is jeopardized that is when a specific intermediate of TCA cycle that is OAA or oxaloacetate is not there. Why it is not there? We will be discussing it again. So, oxaloacetate actually it is shunted towards something known as gluconeogenesis. You already know what is gluconeogenesis since the body in any case where the body has less glucose body will try to make more glucose either by degrading the stored carbohydrate that is glycogen or by accumulating other compounds in order to form glucose.

So, oxaloacetate is one such compound that is shunted towards production of glucose by gluconeogenesis and that actually jeopardizes the TCA cycle. What happens if TCA cycle is jeopardized? The acetyl coenzyme A which was originally meant to produce energy by going into the TCA cycle and ultimately electron transport chain does not happen cannot happen because the TCA cycle itself no longer remains a cycle. In order for a TCA cycle to continue as a cycle the intermediates have to be replenished. This has already been discussed in the concept of anaplerosis and anaplerotic reaction. So, in this case when oxaloacetate it is already diverted towards gluconeogenesis TCA cycle gets jeopardized and the acetyl coenzyme A from fatty acid cannot be used fatty acid oxidation.

This now leads or forces the acetyl coenzyme A to get diverted into an alternate pathway. This alternate pathway is nothing, but ketone body production ok. So, I will again we will be discussing the condition that actually leads to this sort of metabolic alteration is diabetes and untreated I mean fasting and untreated diabetes ok. In starvation and untreated diabetes mellitus these are the condition when this is prolonged fasting ok. Just by having some ritual if you are overnight fasting ketone bodies would not rise much ok, but in case of prolonged fasting in starvation and in untreated diabetes special in type 1 or insulin dependent diabetes the older make an older name or type 1 diabetes autoimmune nature where insulin is deficient this pathway kicks in and leads to production of a huge amount of ketone bodies right.

So, let us discuss some things about ketone bodies. What are the ketone bodies? What are the compounds? The ketone bodies are mainly 3 if you are asked what are the example of ketone bodies they are acetone, acetoacetate and beta hydroxy butyrate. If examiner goes one step further and asks you what do you mean by primary ketone body, what do you mean by secondary ketone body? Then you have to keep in mind the primary ketone body is acetoacetate and the secondary ketone bodies are beta hydroxy butyrate and acetone. Why? Because of their metabolic chain of production I mean the consequence how they are produced in the metabolic cycle or pathway. What you need to keep in mind again for MCQ purpose that this ketogenesis or production of ketone body happens in two specific area.

One is inside the liver the organ and where inside the liver that is hepatocytes the liver cell that is inside the mitochondria. So, to be very specific ketone body production happens in liver mitochondria. You can have many type of questions or combination type of question match the following all except using these combination. Suppose ketone bodies are produced in brain mitochondria, brain cytosol, liver mitochondria. So, be very careful examiner can mix and match and you need to select the correct option right.

So, let us see what are the steps by which ketone bodies are produced. I already told you the starting compound is acetyl coenzyme A. Where it is coming from excess acetyl coenzyme A is now in the system by breakdown of fatty acid ok beta oxidation. So, two molecules of acetyl coenzyme A in the first step that is the condensation reaction they combine two carbon compound combines to form acetoacetyl coenzyme A ok. So, actually if you just look at the skeleton there are two acetyl groups and this forms a acetoacetyl co one coenzyme A is lost in the reaction right.

The enzyme is thiolase I told you whenever you are getting the word thio it means involvement of a sulphur molecule. In this case who has got the sulphur the coenzyme ok the co SH is actually having a disulphide group. So, the enzyme thiolase is actually catalyzing the condensation reaction. So, two molecules of acetyl coenzyme A combines to form acetoacetyl coenzyme A right. In the next step what happens this acetoacetyl coenzyme A combines with another acetyl coenzyme A and a water molecule by the enzyme HMG coenzyme A synthase or HMG co A synthase what is the full form of HMG beta hydroxy beta methyl glutaryl coenzyme A.

You know glutaric acid is a 4 carbon having COH in both ends. So, this is the alpha carbon and this is the this is the alpha carbon sorry and this is the beta carbon this is the alpha and this is the beta is the functional group ok. So, beta hydroxy beta methyl glutaryl coenzyme A. Now, you will find this name HMG co A in other areas ok, in other metabolic cycle in one very close upcoming cycle that is cholesterol metabolism the same name HMG co A will be found. But we should note that the HMG co A the cholesterol metabolism actually happens in cytoplasm or cytosol.

So, the HMG co A that is found in cytosol is actually used for cholesterol production, but here we are discussing about mitochondria right. The mitochondrial HMG co A is directed towards synthesis of ketone body right. So, HMG co A is broken down by the enzyme HMG co A lyase. This is the most important enzyme of ketone body synthesis. Why this is most important? This is the reason why ketone body synthesis can take place only in the liver because this enzyme is present only in the hepatocyte mitochondria.

So, HMG co A in the next step is broken down to form acetoacetate and this is our primary ketone body ok with a CO group. So, acetoacetate is formed this is the primary ketone body and in the next step what happens from this acetoacetate two secondary ketone bodies are formed. One is via reduction ok the enzyme beta hydroxy butyrate dehydrogenase ok. This is the enzyme that is forming beta hydroxy butyrate and this reaction is actually reversible. The enzyme dehydrogenase actually also leads to the conversion of beta hydroxy butyrate back to acetoacetate ok.

Then actually the nomenclature if you are getting confused about the name just remember the enzyme beta hydroxy butyrate dehydrogenase acts on beta hydroxy butyrate. Dehydrogenase means it takes up H plus ion. So, this OH HOH from here will be gone and the ketone body will be formed, but now we are discussing about the synthesis of the buty hydroxy butyrate the same enzyme is actually reversible. So, when it is forming beta hydroxy butyrate one molecule of NADH is used and basically this acetoacetate is reduced. So, this is a type of reduction reaction.

So, upon reduction acetoacetate produces beta hydroxy butyrate and how acetone is formed? Acetone is basically formed by spontaneous decarboxylation. One molecule if we keep acetoacetate in an environment it will spontaneously break down it will lose carbon dioxide by the action of the enzyme acetoacetate decarboxylase and it happens I mean acetone is formed ok. So, remember this is the primary or often it is denoted by the symbol 1 degree and these are the secondary ketone bodies acetone and beta hydroxy butyrate. So, this is the in a nutshell the whole pathway of ketone body synthesis. So, first again let us recapitulate two molecules of acetyl coA condensing by the enzyme thiolase to form acetoacetyl coA.

Again HMG coA synthase acts on acetoacetyl coA and another molecule of acetoacetyl coA, acetyl coA combines to form HMG coA this is in mitochondria. In mitochondria what happens? HMG coA lies this is the key enzyme of ketone body synthesis only present in mitochondria one acetyl coenzyme A is lost. This coA is actually lost to acetyl coenzyme A and acetoacetate is formed this is the primary ketone body and via reduction this secondary ketone body and via spontaneous decarboxylation acetone and beta hydroxy butyrate are formed ok. So, this is all about ketone body formation. Next we need to discuss about ketone body utilization or oxidation of ketone bodies.

So, what does it happen? Just now we discussed ketone bodies are produced exclusively in the liver only and only in the liver. Liver cannot use ketone bodies ok. So, who can use ketone body other than liver extra hepatic tissue. So, extra hepatic tissues means now let us see who are the ones that loves to use ketone body and who are the ones who may not love to use ketone body, but if offered with ketone bodies under special circumstance

can choose to use them ok. So, cardiac muscle and kidney means heart and kidney the especially the cortex of the kidney and heart muscle or cardiac muscle prefers to use ketone body rather than glucose.

So, if given a choice whether you want to utilize glucose for your fuel or ketone body they choose ketone body ok. So, this is very important that is why ketone body production is normal in our body. It is the over production that is problematic right. So, ketone body is normally produced. Next in situations where glucose is not available.

So, that is one situation where nature has I mean we are seeing that when glucose is absent excess ketone bodies are produced in diabetes or in starvation right. So, nature I mean our body has adopted in such a way that even when glucose is absent the tissues like brain and skeletal muscle can still function with ketone body as backup energy sources right. So, basically with the exception of liver and RBC almost all types can use ketone body as fuel in emergency situation. So, what is the reaction that is actually happening? So, we so how basically they are using ketone bodies. We need energy production from ketone bodies right.

So, beta hydroxy butyrate is now being the reverse direction we are going. So, by the same dehydrogenase now NADH is produced on NAD is utilized to form acetoacetate. In the next step acetoacetate utilizes succinyl coenzyme A ok. Succinyl coenzyme A combines with acetoacetate with the enzyme thiophores. This thiophores is also known as beta ketoacetyl co A transferase ok.

So, this with the help of this thiophores enzyme acetoacetate forms acetoacetyl coenzyme A ok. One coenzyme A is attached and in the next step what will happen? Another coenzyme A will be utilized with the help of thiolase. So, this is the condensation reaction I told you whenever we are seeing thio coenzyme A I mean sulphur group in the form of I mean disulphide group here in the form of coenzyme is coming into action and another coenzyme A is being incorporated. So, that two molecules of acetyl coenzyme A are produced. Now, this acetyl coenzyme A can now be directed towards energy utilization by various methods ok.

So, this is how ketone bodies are used up in the periphery. Now, you see considering the normal scenario what happens in usual cases liver very slowly produce ketone bodies in their own pace right. And so, that extra hepatic tissue whatever amount that is present can easily metabolize them. How and that is why the amount of ketone body in blood is actually very very low it is less than 1 milligram per deciliter and there is almost negligible or no excretion of ketone bodies in urine right. But when this process is messed up means whenever there is excess production of ketone body I mean excess

production to that of utilization ratio then only the amount of ketone body will grow up.

So, when the production outpaces the tissues capacity to utilize them now ketone bodies are getting built up and what happens this results in increased ketone body in blood that is known as ketonemia. Ketone bodies being volatile I mean acetone is a volatile compound right. If you keep a bowl of acetone it will evaporate and it will lead to a very sweet smell of acetone in the room. For those who have for those who know how acetone smells like almost it smells like nail polish remover ok. I am for that construct one common household material I mean item where acetone can be found anyway.

So, acetone number 1 it rises in body, rises in blood that is known as ketonemia being volatile the breath will smell of acetone ok. So, that is known as acetone breath sweet smell fruity smell is found in breath and it is highly soluble in water. So, acetone is also excreted in urine. So, combination of these three ketonemia acetone like breath and ketoneuria that is excretion of ketone bodies in urine these three conditions together are known as ketosis or state of ketosis means all three are present. So, now let us look into the cause ofketosis in detail I might be repeating myself because already I have discussed a bit.

Now let us see in detail what happens the nutritional supply of glucose is reduced during starvation. So, starvation is one condition when there will be ketosis right. So, let us see what happens. So, in starvation for prolonged amount of time there is no glucose we already discussed during regulation of blood glucose what happens during absorptive phase post absorptive phase etcetera that first our body glucose will be utilized thereafter the glycogen stored glycogen will broken down to produce glucose thereafter proteins will be fats will be broken down. So, that multiple sources of energy via beta oxidation will be utilized in the form of acetyl coenzyme A and lastly the glucogenic amino acids or proteins will also be broken down.

So, that ultimately the glucogenic substance will produce glucose and that will be utilized by the common pathway of glucose utilization that is TCA cycle, but if this is hampered what happens TCA cycle is hampered why again I told you oxaloacetate if this is less why oxaloacetate will be less oxaloacetate will be less because of it being a substrate for gluconeogenesis body will be trying to produce more and more glucose as long as glucose is available from any sources body will try to produce glucose. This leads to a situation where there is no glucose excess glucose excess acetyl coenzyme A is formed from breakdown of fatty acid now this acetyl coenzyme A has got nowhere to go and this leads to production of ketone bodies alright. There is also another hormone that promotes ketogenesis that is high level of glucagon do you know when glucagon level is high glucagon level is high when the body needs to synthesize more glucose or when the

body is a hypoglycemic state. So, the body is already in a hypoglycemic state. So, the metabolic pathway is triggered in such a way.

So, that the ketone bodies are only directed towards I mean the acetyl co is only directed towards ketone body synthesis and that is further triggered by presence of high level of glucagon. Anyway initially we do not need to bother as I told you because the brain the mains requirement of glucose in our body is to make sure the brain is functioning the brain can get 75 percent of its energy from ketone body. Now since we are discussing starvation one thing has to be kept in mind that apart from prolonged starvation that is a situation where someone is intentionally starving or he or she might be trapped in a situation where there is no food to eat. The one condition that may closely remake this metabolic state is known as hyperemesis gravidarum or excess vomiting during pregnancy which in which a pregnant mother specially in the early stage of pregnancy cannot eat and there is hypoglycemia and then ketosis might develop in those cases right. So, this was about starvation right again common pathology you see the whole thing again acetyl coenzyme A coming and getting diverted here because it cannot be utilized by the oxaloacetate deficiency in the TCA cycle TCA cycle is jeopardized right.

So, what happens in diabetes mellitus believe it or not the pathology is exactly the same. In diabetes mellitus there is deficiency of insulin. Insulin is an anabolic hormone what it does it helps in synthesis of lipids or storage of lipid whenever there is less insulin or absent insulin glucagon takes over and glucagon promotes lipo acid promotes breakdown of fatty acid because we are dealing with a scenario where we need more and more glucose whatever energy sources is there we need to break it down. So, there is already an excess production of acetyl co excess breakdown of fatty acid, but again TCA cycle is hampered why TCA cycle is hampered because oxaloacetate is shunted towards production of glucose. Why even if there is high amount of glucose for example, why we need gluconeogenesis in type 2 diabetes mellitus.

See in type 1 diabetes mellitus there is absolutely no insulin and this is the condition where mainly ketone bodies are formed right. Even in type 2 diabetes mellitus when there is some amount of glucose, but insulin is not acting basically due to lack of insulin action and insulin sensitivity the cell senses a scenario where glucose cannot be uptaken. So, basically the intracellular mechanism mimics that of starvation right. So, in diabetes also the intracellular mechanism is same as starvation mind it in diabetes mellitus there is excess amount of glucose already otherwise the condition would not have been diabetes right. So, even if excess amount of glucose is present in blood in plasma it cannot get inside the cell glucose uptake is hampered that is why inside the cell the substrate for gluconeogenesis are being used to produce glucose.

And that is why oxaloacetate again it is diverted and again we are trapped in a scenario where there is no oxaloacetate and TCA cycle is hampered and that is why the excess acetyl coenzyme A is diverted towards production of ketone bodies right. So, in a nutshell whenever there is a high glucose to insulin ratio there will be that leads to ketogenesis. And ultimately the level of glucagon rises with starvation diabetes mellitus that we already know and what inhibiting I mean what excess glucagon and low insulin will do it will help in triggering gluconeogenesis. So, when there is excess glucagon is oxaloacetate is hampered. So, TCA cycle is jeopardized and when there is excess lipolysis more and more TCA cycle will be generated and that leads to scenario where we have no TCA cycle, but more and more acetyl coenzyme and ultimately there will be production of ketone bodies.

So, let us see how this ketone body formation whether it can be controlled or not. So, now, if we know the stages of ketone body formation we can easily regulate its production. So, number one the precursors of ketone body production that is free fatty acids ok that is free fatty acids when it is broken down via the action of glucagon or lipolysis it actually if lipolysis is controlled then the ketone body production can be controlled. So, who does that it is actually done by a balance between insulin and glucagon. So, now, we are actually looking at a situation how body controls this ketogenesis.

So, we already discussed what are the condition in which ketogenesis can be increased. Now, we are looking at a ketone body homeostasis. So, glucagon promotes lipolysis where insulin inhibits. So, the body is in perfect balance. Now, the next stage is the fatty acids has to go inside the mitochondria right.

So, suppose this is the mitochondria the fatty acid has to go inside the mitochondria in order to undergo beta oxidation right the fatty acids cannot be utilized in the cytosol. So, who does it we already read in beta oxidation that is carnitine pathway. So, carnitine acyl transferase this is the cat one transferase is there and it is actually regulated by malonyl CoA. We will be discussing malonyl CoA in fatty acid in this more and more, but malonyl CoA is a compound which actually regulates. Now, what happens increased glucagon is increased glucagon causes a reduction in malonyl CoA.

So, glucagon what it does it reduces malonyl CoA. Now, what happen the inhibitory effect of malonyl CoA will be gone and more and more fatty acid will go inside the mitochondria it will lead to more and more beta oxidation and more and more production of acetyl coenzyme A right. So, the last step what is what happens the last level. So, this is one level of regulation again insulin and glucagon. So, increase glucagon cause a reduction in condition like diabetes and starvation.

So, in normal cases it does not happen. So, in the last step what happens when excess acetyl CoA is created. So, excess acetyl CoA is created when the first two stages are increased. Now, we are seeing in a case of starvation or diabetes what happens oxaloacetate is routed to gluconeogenesis resulting in reduction of oxaloacetate availability. So, TCA cycle is not able to utilize the oxaloacetate properly and ultimately when citric acid cycle cannot function properly the excess acetyl CoA goes into the ketogenic pathway. So, basically at three levels it is controlled number one lipolysis by glucagon.

So, when we are seeing excess production of keto anisis what happens in starvation diabetes there is excess fat breakdown lead into excess fatty acid in plasma that excess fatty acid goes inside the mitochondria and undergoes the beta oxidation. So, there is a level of control at the entry of fatty acid if that is entry is controlled there will be less and less production of acetyl CoA and ultimately when these two are uncontrolled even inside the mitochondria there is availability of oxaloacetate is a factor. So, if there is increased gluconeogenesis and oxaloacetate is decreased TCA cycle is hampered then it leads to production of ketone bodies. So, now let us see what are the features of ketogenesis I mean what are the manifestations when there is excess ketone body in body number one these are acid beta hydroxy butyric acid right this actually decreases the pH of the body and this leads to a condition known as metabolic acidosis right. So, acetoacetate and beta hydroxy butyric acid acid beta hydroxy butyric acid both have got their acidic component and there is a acidic in nature which reduces the pH not only reduces the pH it also utilize the capacity of all the body buffers that is bicarbonate buffer it is one of the first line buffers that is also utilized.

So, all the bicarbonate is also used up resulting in decreased pH mind it whenever there is an unbalanced acid first the buffer system will try to trigger to compensate the acidostic imbalance. So, there is increased acidosis and I mean there is decreased pH and there is acidosis because of depletion of buffer. Next there is acidotic breath this is known as kusmals breathing typically the patient tries to hyperventilate when the acid has developed the body tries to get rid of excess acid by washing of all the carbon dioxide this is phenomena was hyperventilation this is a phenomena of compensatory hyperventilation right to wash of the carbon dioxide therefore, this pattern of breathing is noted in metabolic acidosis and known as kusmals respiration. I already told you why sweet smell of acetone is found in breath because acetone is volatile in nature when it comes in breath. Next these ketone bodies are osmotically active they attract water molecule inside the cell and that is why there is excess loss of water that leads to diarrhea and dehydration along with water what happens sodium is also lost and this dehydration as well as sodium loss electrolytic imbalance can lead to coma there is loss

of sensation.

So, how can we diagnose ketosis since acetone is very soluble in water ketone body comes out in urine and ketone body in urine is diagnosed by a test that is Rothera's test I am not going into the detail of how Rothera's test is performed, but when Rothera's test is positive of pink ring or purple colored ring will be formed in the test tube at the junction of two liquid. Now since Rothera this ketosis happens in both starvation and diabetes can be differentiate by just undergoing a urine test absolutely yes because in case of diabetic benedict test along with Rothera's test will be positive that is it will give a brick rate precipitate whereas in case of starvation benedict test will be negative only Rothera's will be positive and lastly how can we manage or treat this condition mind it to treat diabetic ketoacidosis we need to inject glucose and insulin glucose to do I mean replenish the hypoglycemic state and insulin so that all the metabolic phases are reversed. Now this insulin has got a very complicated or very vital role by which it actually triggers entry of potassium into the cell so potassium becomes very much trapped inside the cell there is hypokalemia. So we need to be clinician need to be very crucial regarding the level of electrolyte and as I already told you the level of bicarbonate is depleted in ketone body ketosis because buffer systems are used up we also need to re-administer bicarbonate and preserve the fluid and electrolyte balance. So this is the conclusion the we have covered the concept of ketone analysis how ketone bodies are synthesized how they are used up and how they are regulated what are the metabolic features and symptoms how they are diagnosis diagnosed and how they are managed.

So these are my references for this class and I thank you for your patient hearing I will see in the next class. You