Overview and Integration of Cellular Metabolism

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Lecture 56: Integration of Metabolism III (Metabolic Control Analysis)

Welcome back. We are starting our lecture on Overview and Integration. So, we are going to discuss integration of cellular metabolism. We were in the very important part of this lecture that is integration of metabolism. In this class, we are going to discuss the Metabolic Control Analysis. Now, before that let us discuss something about the changes or metabolic changes related to some specific conditions like diabetes mellitus.

Now, why diabetes mellitus we are discussing here? In the last class, we have discussed star fit cycle where cells under if undergo starvation, it is manifested as different metabolic adaptations. Now, diabetes mellitus is one such condition which is very much similar to the starvation phase of metabolism. Why? Because even if there is adequate supply of glucose, there is insulin deficiency or the insulin resistance is there. So, functional inability of insulin is actually causing or hampering glucose uptake in cell.

So, that is similar pattern to starvation. So, but are the effects of insulin those will not be imparted in diabetes mellitus. Like insulin is important for uptake of glucose inside cell, glycogen synthesis for storage inside cell, storage of lipid in the form of triacyl glycerol because it forms VLDL in cell, then it inhibits neoglucogenesis or glycogenolysis. So, in diabetes just the opposite we will see like there will be no glucose uptake, glycogen synthesis will not be there rather because the cells are deprived from the availability of glucose glycogen stored glycogen will be degraded. So, enhanced glycogenolysis will be there also neoglucogenesis will be exaggerated because cells are not having glucose or some sort of energy should be there.

So, that is also neoglucogenesis amino acids supplied those will be synthesizing glucose. Then what is happening to adipose tissue? In adipose tissue insulin is basically causing the storage function in the form of triacyl glycerol. Also the fatty acids which are generated by hydrolysis of triacyl glycerol with the help of the enzyme lipoprotein lipase those fatty acids will be uptake in inside the adipose tissue and those fatty acids are will be synthesizing triacyl glycerol. So, basically there is increase of triacyl glycerol

and it inhibits the lipolysis the stored triacyl glycerol will not be degraded. In case of diabetes mellitus functions of insulin is not there.

So, what will happen the stored triacyl glycerol will basically be degraded and will be will come to the circulation. So, there is hyperlipidemia the mobilization of fat is increased in diabetes mellitus. Then normal function of insulin in skeletal muscle is uptake of glucose supplies energy storage of glucose in the form of glycogen amino acid sorry amino acids are utilized for synthesis of protein all these things will be hampered because thus and there is no insulin. So, what will happen the stored glycogen will be depleted proteins which are forming the skeletal muscle those will be catabolized as a new glucogenetic material. So, these are the manifestations which occurs via this metabolic chart you can easily calculate or easily extrapolate what will be the metabolic changes in diabetes mellitus.

So, here you can see there is degradation of protein increased in muscle. So, there will be muscle wasting diabetes patients of diabetes mellitus they appears very there is a catechic appearance of the patients because those proteins are actually utilized for new glucogenesis also glycogenolysis is are there. So, those are apart from the available glucose in the circulation which are not able to be uptaken inside the cell they are also supplying glucose. So, there is hyperglycemia then there is lipolysis free fatty acids are available which are forming more acetyl coenzyme A those acetyl coenzyme A forms ketone bodies causing ketoacidosis also lipoprotein lipase which were actually important for uptake of triacylglycerol in the cells those like that lipoprotein lipase is inactive or rather cannot be activated. So, circulating chylomicron and VLDL will be very high causing hypertriglyceridemia.

So, these are the manifestations of diabetes mellitus the metabolic changes. Then we are moving on to metabolic changes in trauma or critical illness where the stress level is very high majority of the manifestation in trauma and critical illness are based on the release of stress hormones because there is there is stress hormone release from HPO hypothalamo pituitary adrenal axis. What are the stress hormones cortisol epinephrine norepinephrine glucagon growth hormones they are also released along with in the later stages cytokines are also released from the tissues. So, what happens this stress hormones along with cytokines adipokines they are basically causing they are basically imparting an environment of uncontrolled catabolism as well as the anabolic signals they are also blocked. So, definitely the energy expenditure the body composition all are actually altered in critical illness or any trauma due to this stress hormone or inflammatory signals.

Now, let us see one by one what happens that in trauma based on the severity of the

trauma the oxygen requirement the energy requirement are proportionately increased in the body. So, there is major trauma causing excess release of catabolic hormone also it decreases the anabolic signal releases inflammatory cytokines also catecholamines sympathomimetic response they are all causing the metabolic disorganization. Now, in trauma the major source of energy is basically free fatty acid because what happens for carbohydrate or glucose there is a in there is an environment which is mimicking insulin resistance. Now, those free fatty acids are mostly coming from triglycerides along with that energy is obtained from proteolysis as well. Now, the phases of trauma divided in 3 phases actually now what happens in the very initial phase within 24 to 48 hours it is a phase of shock where body tries to preserves it energy for the future repair.

So, what it does it decreases the total body energy expenditure as well as urinary nitrogen excretion and in these case the stress hormones are increased. In the second phase which occurs in 2 to 7 days that is basically the time when there is flow the phase of flow when the body starts to adjust with what has happened. So, basically there is catabolism at this phase the energy requirement is high. So, oxygen consumption is high metabolic rate is high utilization of glucose is reduced as I already told there is a environment of insulin resistance. So, what will happen the energy will come from triglyceride derived free fatty acid as well as proteins from muscles proteolysis.

So, there will be muscle wasting. In the third phase now body is at its recovery phase. So, there is a there is an anabolic state which is occurring in 3 to 8 days after an uncomplicated surgery their body starts to restore whatever has disorganized. So, there will be positive nitrogen balance because there is protein synthesis is increased as well as weight which have been reduced the restoration of body weight and muscles will be there. So, these are the 3 phases of trauma.

So, these are the metabolic changes I have already discussed in the carbohydrate metabolism what happened because there is a phase of insulin resistance first the glucose will try to come from hepatic glycogen. So, the glycogen stores will be depleted after that and not only after that simultaneously there is energy provision from free fatty acid oxidation. So, there is lipolysis and also proteolysis causing negative nitrogen balance. So, the mode of treatment in trauma is basically provide glucose as energy. So, that muscle wasting protein catabolism lipolysis can be prevented as well as there is a there is an environment of insulin resistance I told.

So, exogenous supply of insulin should be there. So, that the cell can uptake glucose for their civil. Next we are coming to the very important topic metabolic control analysis. Now, before that I want to highlight few terms like rate and flux what is the difference between rate and flux we say metabolic rate we say metabolic flux why differently. Now,

remember rate is basically it is concentrated over one single enzyme.

So, we are when we are talking about individual component individual enzyme there we say the rate of the reaction whereas, flux is about the whole reaction we are talking a whole pathway we are talking about all the enzymes all the metabolic metabolites in the pathway. So, the rate of all the reactions finally, forming the metabolic pathway they are we utilize the term flux. Then we are coming to regulation and control. Now, control is a very very basic term when we want to control something means either we can allow it to go as it is or we can stop it or we can increase it or we can decrease it. So, that is a control over the output of the system whereas, regulation talks about homeostasis.

So, even if there are different fluctuation in the external environment when there is an attempt to maintain homeostasis attempt to maintain the internal condition temperature concentration of the enzymes concentration of the substrates when they are tried to be kept in a homeostasis condition that is known as regulation. So, remember once again regulation and control are not the same thing. Then we are talking about metabolic control now metabolic control is something we are talking about quantitative estimation. So, till now there is qualitative estimation that if we block this enzyme this pathway will be inhibited, but we do not know how much the pathway will be inhibited. So, till now majority of the metabolism related concepts are actually based on the qualitative discussions.

Now, just imagine a map which gives all the roads with all the signals of a city does it indicate at all how the traffic will go unless a real time traffic is shown how much the flux how much the run how many cars are running how in how many speed how they are control if we do not discuss these points the map is of no use if we want to discuss the flux or the traffic of the city. So, this is just metabolic control analysis is even if we have all the metabolic pathways we know what is the rate limiting enzyme what is the regulatory enzyme unless we discuss or quantitate how much the external signal can affect the enzyme or the metabolite it is not clear. So, metabolic control talks about quantitation of such. Now, the conventional concept is based on single rate determining step hypothesis what is that we say in cholesterol synthesis HNG coenzyme A reductase is the rate limiting step as if this is the only enzyme based on that the whole cholesterol synthesis is actually dependent and the whole reaction is based on the enzyme only. Now, the enzyme as told that is the rate limiting step or the single rate limit determining step is the slowest and if we control that enzyme the whole metabolic pathway can be regulated, but actually it is not various experiments have explained that metabolic pathway is basically the effort of all the enzyme related to that pathway.

So, it is the control of the flux to the metabolic pathway is basically distributed among

all the enzymes located in that pathway and that control also that control there is not only on the enzymes, but also on the substrate supply how much the substrate is there how much the oxygen is there what are the intermediates what are the effect of those metabolites or intermediates over the enzymes or over other metabolites what are the external effect like hormones hormones are the external compounds which is not a substrate which is not an metabolite. So, how they will control based on these things all the enzymes and all these factors actually determines the flux of a complete metabolic pathway that is actually discussed in metabolic control analysis. Now, why metabolic control analysis is important? Now, remember down I mean now assume we want to produce something in a pathway we want to over produce something. So, we need to identify which enzyme to be induced how much to be induced and other factors how they will affect this induction for that we need to know metabolic control analysis. Also if we want to understand the action of some drugs or hormones even we want to develop some drugs which are targeted to specific enzymes basically this is the commonest thing whenever we want to block some pathway we want we actually target to some enzyme which has major hold over that pathway.

So, how they are affecting what will be the metabolic effect of those can be studied by metabolic control analysis. Similarly pathogenesis of different metabolic defects also can be studied from metabolic control analysis. Now, metabolic control analysis are studied via different experiments for long time and the role of each enzyme in a pathway can be elaborated by experiment. Now, here I am going to discuss one such experiment concentrating the pathway glycolysis. So, these are the enzymes of glycolysis you all know hexokinase 4 or glucokinase phosphoructokinase 1 phosphor hexo isomerase these are the enzymes of glycolysis.

Now, how those are isolated from rat liver. So, the liver of rat is actually isolated and homogenized in those in that homogenized sample of liver all the soluble enzymes are there glycolytic conversion of glucose can be done through this homogenate. Now, while conducting the glycolysis what additionally is done here you can see exogenously apart from that homogenate which contains all the glycolytic enzymes exogenously purified hexokinase 4 additionally is added and you can see there is a burst of increase in the flux. Similarly, additionally purified phosphorructokinase is added there is increased in the flux, but not as much as done by hexokinase. Next is addition of purified phosphor hexo isomerase with has which has no effect.

So, this is one very important pathway to describe what is regulation and what is control and what is rate limiting step. Why we often says what are the regulatory enzymes hexokinase in glycolysis hexokinase phosphorructokinase and pyruvate kinase, but remember flux as you can see is increased by hexokinase because more glucose comes

more glucose comes more glucose is converted to glucose 6 phosphate via hexokinase. Whereas, phosphorructokinase is basically trying to maintain the homeostasis by regulation how whenever there is increase glucose 6 phosphate availability increase glucose availability causing increase glucose 6 phosphate or somehow glucose 6 phosphate is very much high in cell. So, what will happen there will be very high glycolysis no phosphorructokinase will decide how much glucose 6 phosphate is to be converted to fructose 1 6 bisphosphate. So, it regulates and maintain the homeostasis.

So, that is the difference between regulation control and flux control. Next we are moving on to discussing some coefficients related to this metabolic control analysis one such is flux control coefficient which is denoted by C. Now, flux control coefficient is basically the quantitative expression of relative contribution of each enzyme and relative contribution of each enzyme where when the enzyme is changed the change of enzyme concentration it affects the metabolic flow how much is actually discussed by flux control coefficient. So, flux control coefficient C can be of different value 0 means changing the enzyme has no impact actually. So, the coefficient is 0 to the highest where if you change the enzyme the flux is very much regulated this is the determining enzyme of this enzymatic pathway.

So, basically flux control coefficient can be 0 to 1 similarly it can be negative also. Now, where flux control coefficient is negative where you have a branched reaction suppose this is one reaction where A is B is formed from A and then C is formed from B. Now, if there is a branch where B can form D. So, here is the enzyme X which has negative flux control coefficient over this pathway because what is happening because of this enzyme X B is actually siphoned off to the other pathway. So, the production of C will be very low.

So, basically there is a negative flux control coefficient. So, flux control coefficient can be positive as well as negative. Now, remember one very important thing flux control coefficient is not the property of the enzyme rather it is the property of the system because all the metabolites all the environments are actually affecting the enzymes role over the pathway and it also depends on the availability of the substrate and additional external effectors like allosteric regulators or other cofactors like that. Then there is metabolite effect metabolites also tend to counteract the changes in the amount of the enzyme and several enzyme in combination actually control the total flux. So, what happen suppose you see this reaction where X is forming Y by X S enzyme forming Z by Y DH enzyme and Z is forming X 1 by Z S enzyme.

Now, suppose this Y DH enzyme activity is increased what will happen Z will be formed more now because there is. So, Y DH will be depleted very soon actually if Y

DH is increased. So, there substrate availability for the Y DH will be actually low. So, the function of Y DH will be low, but because product availability of Z is high.

So, Z S function will be high. So, what will happen Y DH finally, the Y DH will be low, but the sorry, but the Z S will be high. Similarly, because Y is depleted product inhibition over X S will be reduced. So, again X S function will be high. So, this is how the all the metabolites can affect the enzymes function and control the flux. But then if that is a systems property what is enzymes property? Enzymes property is basically quantitated and described by elasticity coefficient.

Now, what is the elasticity of the enzyme? Elasticity is basically the enzymes intrinsic kinetic property how it is actually controlling the flux based on the availability of the metabolite. So, it is the quantitative expression of the responsiveness of a single enzyme to the metabolite changes. So, if the metabolites change how the enzyme will react that is elasticity coefficient. Now, just concentrate on this is the very classical pattern of all the enzymes which is following Michaelis maintenance hypothesis we do not have this is not an enzymes class. So, I am not discussing the Michaelis maintenance hypothesis you need to go back to the enzyme ology part for that.

But here for all the Michaelis maintenance protocol based enzyme you can see this is the substrate concentration which when increased how the enzymes rate are affected it is described in this graph or plot. Now, initially you can see in a very mild increase of substrate there is proportionate increase of the enzymes activity. So, at the very initial phase the elasticity of the enzyme is very high based on the substrate concentration. But at the end when there the substrate concentration is increased the enzymes rate is not that that much increase. So, the elasticity is low because the enzymes intrinsic properties change why because now the enzyme is totally or completely saturated by the substrate.

So, you can see based on the intrinsic property of the enzymes the elasticity or it is responsiveness towards the metabolite change is actually regulated. Now, elasticity can be positive because metabolites are actually stimulating the rate of reaction by like substrates more substrate more activity activators which activate the enzyme function. Just the opposite occurs with inhibitors or due to product inhibition there is negative elasticity. Now, remember most of the enzymes have more than one substrate.

So, there is more than one product. So, one product can affect the other reaction as well. So, and also based on that specific substrate or product enzymes are having different types different elasticity coefficient. So, elasticity coefficient is enzymes intrinsic property. Flux coefficient is the systems property. Now, what is response coefficient? Now, all these flux control coefficient or elasticity coefficient I am talking about the

metabolites and the enzymes.

Response coefficients talks about the role of external effectors external molecules which is not a metabolite which is not a substrate which is not an enzyme as well. So, like one important one is hormone or allosteric regulators. So, how these external effectors are affecting the flux via the enzymes action is actually quantitated by response coefficient. Now, what is then response coefficient? It is the quantitative expression for the relative impact of an outside factor on the flux of that metabolite. Now, responsiveness depends on two things flux control coefficient and elasticity coefficient how? Now, in a pathway what is the role of that enzyme how much the enzyme is actually controlling the pathway that decides the responsiveness.

This is the reaction and this is our enzymes. Now, X is the main enzyme which is affected by external regulator suppose hormone. So, this pathway will be highly affected because by the external effector we are actually regulating the main enzyme here. So, this is actually we are talking about flux control coefficient how much important the enzyme is or how sensitivity of the pathway towards that enzyme. Likewise if we affect Y with the hormone Y is not the major flux controlling enzyme here. So, the enzyme so, the pathway will not be affected the response coefficient will be low.

So, basically flux control coefficient determines how much responsiveness will be shown by the pathway towards the external effector. Similarly also we are talking about the elasticity of the enzyme the externals effector which is denoted as P how much impact it is imparting over the enzymes activity that is the internal intrinsic role of the enzyme elasticity of the enzyme. So, basically the strength of the outside effector how much it affect the enzyme is decided by elasticity and that decides the responsiveness. So, both the flux control coefficient as well as the elasticity coefficient they decide the responsiveness of the pathway.

So, it regulates the response coefficient. So, metabolic control analysis it actually clears multiple concepts. So, this is basically this is clearing the doubts which has been proposed by the traditional approaches of single rate limiting enzyme instead the role of the whole flux control is actually distributed among multiple enzyme and that is quantitated by the flux control coefficients different flux control coefficient. Also it has discussed the different roles of different enzymes in a metabolic pathway also it has cleared what can be a what can be the regulatory role of enzyme versus what can be the rebalancing role of enzyme. Also metabolic control analysis predicts via different experiments that flux towards a specific product is mostly increased by the individual enzymes concentration. So, as a whole in a pathway if the individual enzyme concentration is increased via over production or external provision in that case the flux

of that pathway can be increased without hampering the other metabolic pathway in the body.

So, this is how metabolic control analysis not only discuss how it discuss how much it is the quantitative representation of metabolism. So, these are the concepts we have discussed today the different metabolic changes which are occurring in diabetes mellitus as well as acute illness critical illness and trauma and also the metabolic control analysis along with its different coefficients these are my references and. Thank you all see you in the next class.