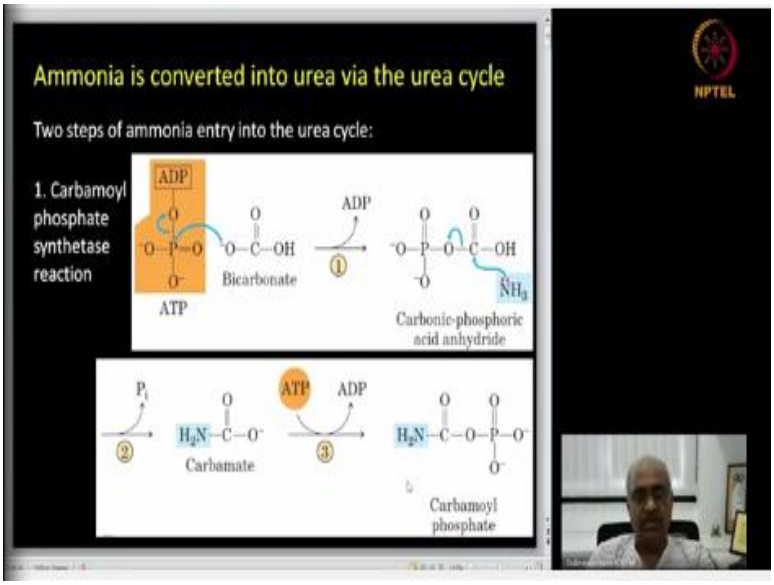
Introduction to Biomolecules
Prof. K. Subramaniam
Department of Biotechnology
International Institute of Technology-Madras

Lecture-28
Urea Cycle and Fatty Acid Catabolism (Part-1/2)

(Refer Slide Time: 00:14)



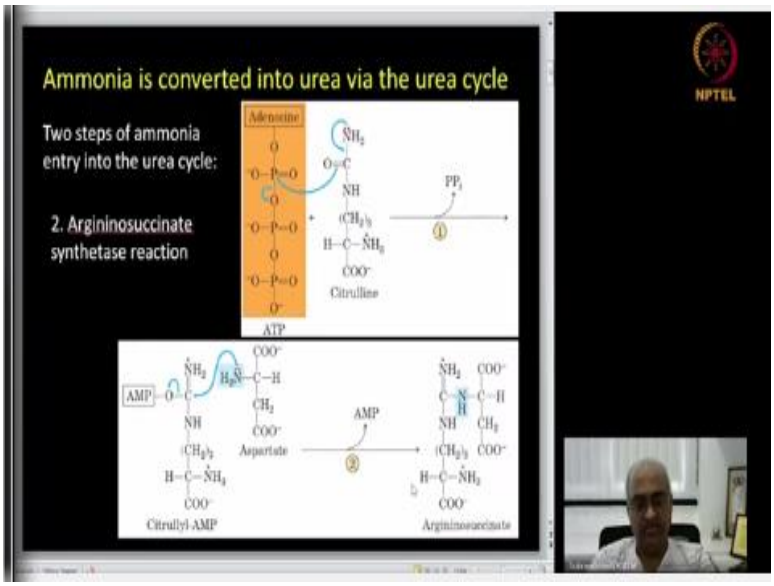
So, today what we are going to do is we are going to look at how this ammonia is excreted? So, as we have already discussed ammonia is toxic and it needs to be immediately excreted. And the process by which it is excreted in mammals like us is via urea cycle and the urea cycle is the only way of nitrogen excretion that we are going to learn in this course. So, there are 2 steps by which ammonia enters into this cycle.

The first one is formation of this molecule called carbamate**,** carbamic acid and this requires an activation of this bicarbonate in the form of this carbonic phosphoric acid anhydride**.** So, this bicarbonate is with the buffer system and therefore it is abundant in our body. And so this is first phosphorylated by hydrolysis of an ATP and this phosphorylated version then it is hydrolyzed, releasing the inorganic phosphate and then you have the carbamic acid.

Then this carbamic acid undergoes the second step of activation by hydrolysis of another ATP, where it gets phosphorylated to form carbamoyl phosphate. So, the enzyme that catalyzes all

these 3 steps together is the carbamoyl phosphate synthetase and so this is how one root of ammonia entry works.

(Refer Slide Time: 01:56)



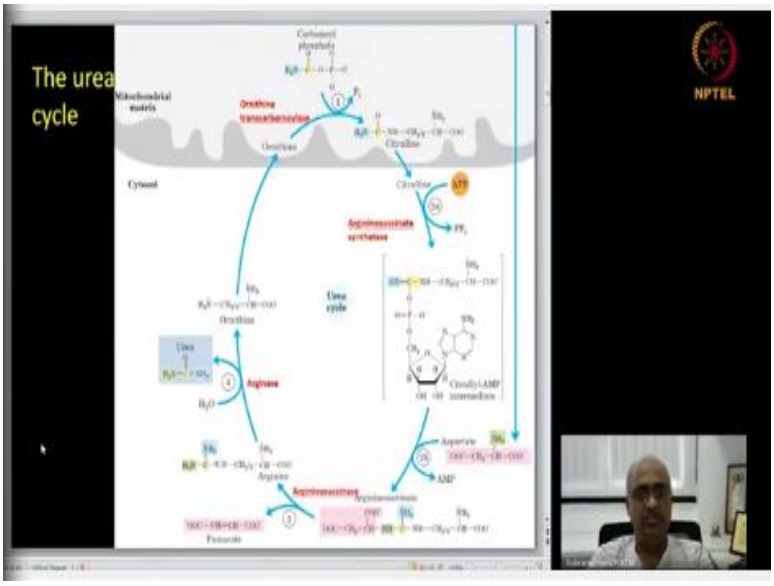
And the second one actually happens is another step in urea cycle, which is entry in the form of aspartic acid**,** as you see here, aspartic acid. So, aspartic acid is the amino group of the aspartic acid is combined with the citrulline. And for that the citrulline we will see in a minute as part of the urea cycle. So, here we have seeing that reaction as an independent reaction.

So, this again is activated by combining with the AMP adenosine monophosphate through this sort of a reaction catalyzed by what is called argininosuccinate synthetase, it is because the product is arginine + succinic acid combined version, so therefore it is called argininosuccinic acid. So, the arginine group primarily comes from this structure of the citrulline and the succinic acid group comes from aspartate minus the amino group.

So, this argininosuccinate reaction combines citrulline and aspartate to form argininosuccinate and the intermediate activation step is what is this citrullyl AMP formation**.** And so the second step is simply removal of the AMP by the combination of this amide bond formation. So, once this is hydrolyzed, this is going to be an alcohol group and then amino group, so it is an amide linkage that is going to form, so this is argininosuccinate.

So, the nitrogen in aspartic acid, now is part of the argininosuccinate, so that is why this is colour. So, this is one entry and here again the colour the ammonia group itself, so this is the other edge.

(Refer Slide Time: 04:03)



So, now let us look at the urea cycle itself. So, here this shaded portion is mitochondria, so this reaction happens inside the mitochondria and these steps in the cytoplasm**.** So, the first step is the carbamoyl phosphate we just saw, the very first set of 3 reactions that we saw where the carbonic acid is activated and forms carbamoyl phosphate by combining with free ammonia, so that is this molecule.

So, this molecule combines with ornithine**,** so we will see the ornithine when we come through the cycle. This is equivalent to the oxaloacetate in TCA cycle, the first molecule with which the entry point like joints. And that produces a molecule called citrulline**.** And the citrulline combining with aspartate to argininosuccinate is what we just saw**.** So, this 1 and 2 are actually this 2a and 2b reactions.

This citrullyl AMP, so this looks big simply because the AMP structure is drawn out, otherwise this is the adenine ribose and the phosphate, so this is the citrullyl moiety. And so from argininosuccinate, so this is called argininosuccinate because this group is a guanidino group.

And guanidino group attached to alpha carbon with amino group and carboxylic acid group is the arginine structure.

So, from this succinic acid is cleaved off by this enzyme argininosuccinase producing fumarate. So, if you look at the TCA cycle there again from succinate we form fumarate, then fumarate to malate to oxaloacetate. So, the same succinic acid when you remove it out, it becomes fumarate. And this fumarate we will follow it again later when we are going to combine urea cycle and TCA cycle.

So, the remaining portion is actually arginine and so essentially what we have done is we have taken aspartate in kept the amino group and removed the carbon skeleton in the form of fumarate**.** So, the amino group remains, this green shaded thing which was combined with the citrulline and that remains as part of the arginine. Now arginase enzyme is going to hydrolyze this portion the 2 amino group with this carbon converted into ketone.

So, this is urea NH 2 C double bond O NH 2, this is what is urea. And the remaining part of this is ornithine**;** this is actually an amino acid. So, you can see it is just that it is not part of our proteins; it is not encoded by any of the triplet codons. So, this it is somewhat like lysine, just 1 carbon less otherwise it is actually lysine. So, then the ornithine again combines with carbamoyl phosphate and the reaction goes on.

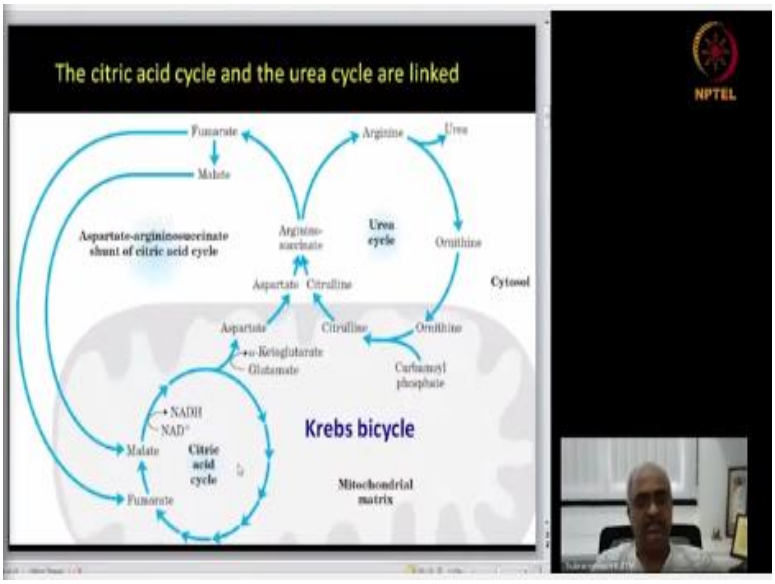
So, you have one reaction, this carbamoyl phosphate formation and then this ornithine transcarbamylase which transfers this carbamoyl group to ornithine that alone happens in mitochondria. The rest of it, it is a very small cycle, so you only have 3 steps other than this, if you include this 4 steps, suppose to 10 step glycolysis or 8 step TCA cycle. So, this is what is urea cycle**,** so these are the key enzymes I have marked them in red and follow the shaded groups as well, that tells you what enters and what leaves.

So, you have 1 amino ammonia coming in the form of carbamoyl phosphate another coming in the form of aspartate. So, how this aspartate formation and all those complexity that is there as part of this mitochondrial thing we need not worry for an introductory course. And that is why I

have not included that here; I have clipped off that, so if urea cycle that is good enough. So, you can easily understand aspartate probably comes from hydrolysis of proteins and the free amino acid aspartate comes or oxaloacetate through transamination form the aspartate one you know how aspartate can come.

So, this is the other amino group; one here and the other here 2 and the 2 are preserved even when we remove this aspartate's carbon skeleton as fumarate. And then both of this is removed in the form of urea**.** So, in operation of one round of the cycle, you get it up 2 ammonia groups in the form of urea, so this is the urea cycle.

(Refer Slide Time: 09:34)



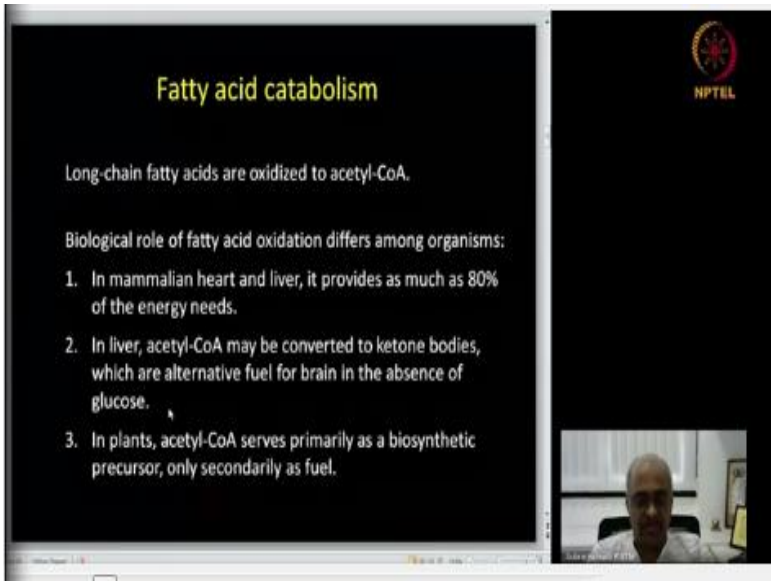
And this via aspartate argininosuccinate shunt links with the citric acid cycle. Since Hans Krebs discovered both these cycles and the connection, this often called Krebs bicycle**.** So, let us see the connection here which is not that difficult. So, we know aspartate enters into urea cycle by combining with citrulline, so that is one entry point and what is the connection to TCA cycle? In TCA cycle oxaloacetate through transamination can form aspartate**,** from glutamate the amino group can be transferred to oxaloacetate forming aspartate.

And the glutamate now the remaining carbon skeleton of that will be alpha-ketoglutarate and that aspartate can enter here. And when the carbon skeleton of aspartate is cleaved off as fumarate, this fumarate can be converted into malate by the same type of reaction that happens in TCA

cycle which is in the mitochondria but here it is going to be in the cytosol. And similar isoenzyme present inside of cytosol does that and both of this actually can interrupt 2 different places in TCA cycle. And that is how via this aspartate argininosuccinate shunt, shunt meaning **a** a side track of the main path.

So, this is the main cycle here and here this is the main cycle and the oxaloacetate getting out of this main path is what we called as the aspartate shunt. So, similarly argininosuccinate it goes this way but then this portion is a shunt in a side track where it becomes malate and enters into TCA cycle. So, this is how TCA cycle and urea cycle are linked, but the linkage requires a dynamic flux between cytoplasm and mitochondria. So, it is all regulated based on the concentrations of individual molecules that you see mentioned here.

(Refer Slide Time: 11:59)



So, that is how the amino acid catabolism we are going to see. So, here what we have actually focused is how nitrogen enters into the biological systems through this nitrogenous complex and formation of glutamine and glutamate only that portion we saw. Then we skipped all the biosynthesis of amino acids and also we have skipped the degradation that is the catabolism of amino acids as well.

So, we only focused on how the amino group is removed out from the amino acids and how that is excreted. So, we saw the amino group the nitrogen entry and nitrogen exit alone we saw and

there again the exit we focused only on what happens in mammals, the urea cycle around we saw. So, there is the complex molecular reactions, biochemical reactions leading to the formation of the 20 amino acids.

And the degradation of the 20 amino acids, that portion we have not ventured into it. Keeping in mind that this is an introductory course where our focus is on the fundamental concepts and a reaction or a mechanism that is actually representative of a large group of reactions, so that is what we focus primarily. And so individual specific details we have kind of ignored, so remember that and if you are curious you can go ahead and learn them from the book, the book has everything.

So, but you need to keep in mind to give you parity with what happens in a good standard that is expected out of undergraduate level education, Lehninger is an introductory biochemistry textbook**.** So, elsewhere a student is expected to learn everything that is given in Lehninger as part of an introductory biochemistry course. This is just a for information sake, so therefore you can map yourself with rest of the world.

So, here for our purposes and our exam point of view we are staying away from those details of amino acid synthesis and degradation. So, now let us turn our attention to fatty acids**.** So, we have dealt with carbohydrates in some detail because glucose becomes the central molecule in all of metabolism in terms of the biosynthesis, supply of carbon skeleton to the formation of other molecules as well as energy production.

In all of that glucose is the central molecule and therefore we spent a lot of time learning about carbohydrate metabolism. And as I mentioned during the discussion on glycolysis much of biochemistry has been learnt and many concepts and enzyme mechanisms have been worked out by focusing on glycolysis and TCA cycles. So, that is why we spent a lot of time on that part. So, nitrogen I already told you about amino acids, then the fourth one nucleic acids we are not even going to touch and the last lift here is fatty acids**.**

Fatty acids, has very important role in terms of the energy dynamics in our system. In terms of the energy storage and the production of energy in everything, they have a central role, second only to carbohydrates. And therefore we will turn our attention to fatty acids for the remaining part of this course, which is only a few lectures; we are going to soon finish the course. So, let us focus on fatty acids now. So, here is some important outline that we need to keep in mind when we are goanna learn about fatty acids.

So, the main summary is in this one short sentence, fatty acids are converted into acetyl CoA. Then we know now what happens to acetyl CoA? Acetyl CoA will enter into TCA cycle will become fully oxidized to carbon dioxide. And then in the process we would have produced reducing equivalence in the form of NADH and ATP and using that energy will be made. So, acetyl CoA we very well know, so therefore all that summary about fatty acid catabolism is fatty acids become acetyl CoA.

Like if you take the 16 carbon fatty acid palmitic acid which is one of the most common one. So, it can form 8 acetyl-CoA**,** so gets broken into 2 carbon moieties. Now let us see how that happens today we are not going to see that, we will prepare ourselves for that. First we will have an overall view into fatty acid metabolism, like starting from digestion, storage and mobilization from storage and how it is transported and so on.

So, the first important point here is, this fatty acid oxidation differs among organisms and it even differs in an organism from organ to organ. For example in mammals in heart and liver 80% of the energy comes from fatty acids primarily stored lipids. But you never want more than 30% of energy source coming from fat; a bulk of it coming from carbohydrate is what it is really good for the overall well-being.

Because fatty acid storage and mobilizing, metabolizing can have unnecessary unhealthy consequences**.** But these tissues can handle as much as 80% requirement from fatty acids alone, that is one. Energy here meaning acetyl-CoA getting oxidized to carbon dioxide. In addition, in liver, acetyl CoA can be converted into other things, like in addition meaning in addition to entry into TCA cycle.

So, it will use the acetyl-CoA via TCA cycle, for it is own energy requirement here it is referring to liver. And in addition to meeting it is energy requirement by oxidizing acetyl CoA via TCA cycle, liver can convert acetyl CoA into ketone bodies. So, ketone bodies are acetone, acetoacetate and beta-hydroxybutyrate, we are not going to learn about how they are formed, except that just you need to remember these 3 molecules are produced when as a product of fatty acid metabolism.

In general for a layman perspective when your diet is very poor in carbohydrate and you meet much of your energy requirement by eating non-carbohydrate food, primarily fat and proteins. You may have heard some people following a diet plan called the keto diet or paleo diet, any of you are aware of anybody who follows that kind of a diet, like where they do not eat carbohydrate, but they eat lot of fat and protein.

You are allowed to eat butter as much as you want; meat as much as you want, but you are not allowed to eat rice or wheat**.** So, somebody has heard of that, so this is a new idea that is being tried out by people, but we do not have any scientific evidence to support whether it is good or bad for health. All that we know is for a good homeostasis, meaning your body in complete equilibrium in a healthy state.

No more than 30% of the calories should come from fat and if you take that into consideration this extreme view of completely depending on fat and protein for a calorie requirement may not be a good idea. But nobody has done a clinical trial on a large population to show that the keto diet is bad or good. So, now just let me tell you what is a keto diet, it is primarily because when you do not have carbohydrate available as a source to operate TCA cycle.

And when you depend on fatty acids, the fatty acids in addition to providing acetyl CoA for TCA cycle, in liver they are converted into these ketone bodies. I just told you, they are the acetoacetate and acetone and beta-hydroxybutyrate. These 3 because they have ketone groups in them, they are called ketone bodies. Usually they are present in diabetic patients as well and they have fruity odour to them.

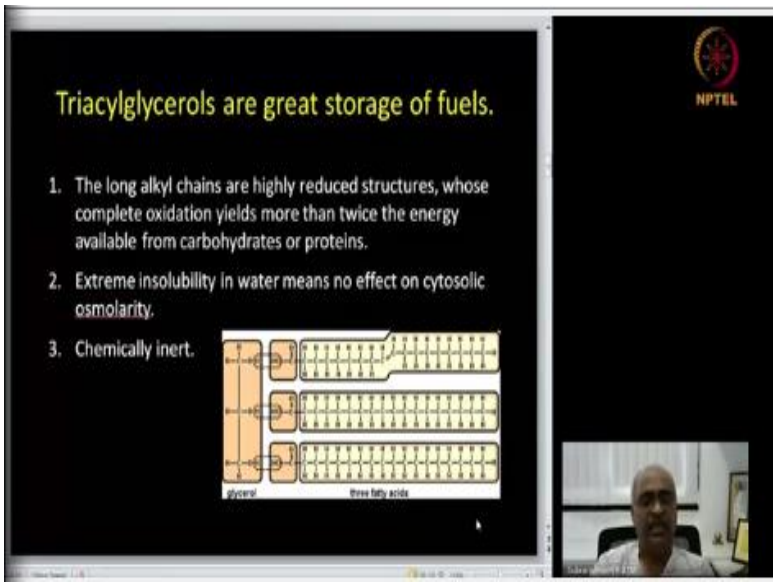
And as a result really seriously diabetic people have this fruity smell next to them, if you go very close to them. That is because they produce a lot of ketone body and that is exhaled through breathing as well. And why these ketone bodies are produced is because if you remember brain normally depends on glucose as the source of energy, because only glucose crosses the blood brain barrier, not fatty acids.

So, in the options of glucose when you are just not eating carbohydrate, then liver produces ketone bodies which also can cross the blood brain barrier and become alternative fuel for the brain and that is the reason liver will produce ketone bodies. And a diet that is completely dependent on this metabolic side path that is fatty acid as the sole source of energy. Because they produce ketone bodies, they are called a keto diet**.**

And someone who alternates, for some time they eat fatty food and no carbohydrate and then later they switch to normal diet and then again go back to fatty diet and that is called keto cycling**.** So, we do not know the benefits of that and there is not any large-scale clinical trial establishing the useful benefit or the unhealthy consequence and therefore we are not going to discuss anymore on that topic.

This is just to connect you to what you may be hearing in everyday life, where in biochemistry that connects? So, that is about liver. And in plants this is primarily a precursor for biosynthesis of other molecules plants never use acetyl CoA as a primary source of energy because they can make carbohydrates as they wish. So, this is the general outline.

(Refer Slide Time: 24:50)



And now we move on to the overall picture of digestion and transport of fat. So, the primary storage form is triacylglycerol, like for example when you eat butter it is all triacylglycerol, it looks like what is drawn here. And this structure is familiar to you, we have learnt at the very beginning itself when we are learning about the molecules of life. So, in the digestive tract, so in amino acids we saw the proteins need to be hydrolyzed and we had the proteases coming in the form of zymogens produced by pancreas.

As well as in the stomach also, you have this parietal cell, sieve cell, sieve cell producing pepsinogen and parietal cell producing HCL, we saw that. So, there the proteases should not lies and digest the glandular cells themselves, therefore we saw them being produced in the form of zymogens, that is inactive form. And then once they come into the stomach or intestine there they are proteolytically cleaved to become active things, so that is what we saw there.

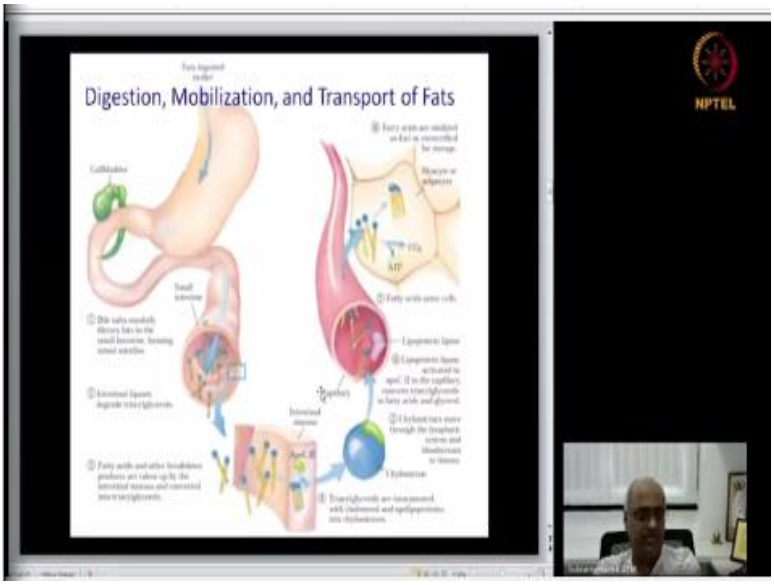
Here a similar peculiarity is that lipids are not water soluble**,** they are hydrophobic and the protein enzymes that are going to work on these lipids, they are hydrophilic. So, as a result you need to emulsify the triacylglycerol and that is the unique concept about a fat digestion that we are going to learn**.** So, there it was zymogen becoming active to work on proteins, here the water insolubility requires emulsification.

And before emulsifying the triacylglycerol, let us look at it is unique usefulness**,** it is unique chemistry that makes it useful in the biological system. So, these are highly reduced structures**,** per unit weight they produce double the amount of energy than carbohydrates or proteins, this I told you earlier, so that is one thing. And second because they are insoluble, they do not contribute to osmolarity**.**

They are presence in the cytoplasm is not going to make water enter into the cell and swell up the cells. That is what is happening, you have glucose or amino acids present in the cytoplasm. So, that problem is not there when you have a triacylglycerol way of storing. And third, they are chemically inert they are not going to produce free radicals or destroy anything; they are going to just happily be there.

So, these are the 3 main aspects of triacylglycerol one, they are highly reduced therefore more energy per unit weight and no contribution to osmolarity, because they are not soluble in water, and they are chemically inert.

(Refer Slide Time: 28:22)



So, this outlines the digestion, absorption of fat. So, the first thing I told you is the emulsification, so the letters are small I will try to. So, bile salts, so these bile salts are produced by liver from cholesterol and they are stored in gall bladder which is attached to the small

intestine right after to the stomach. And when a fatty meal comes in this gallbladder releases these bile salts into the intestine where they emulsify.

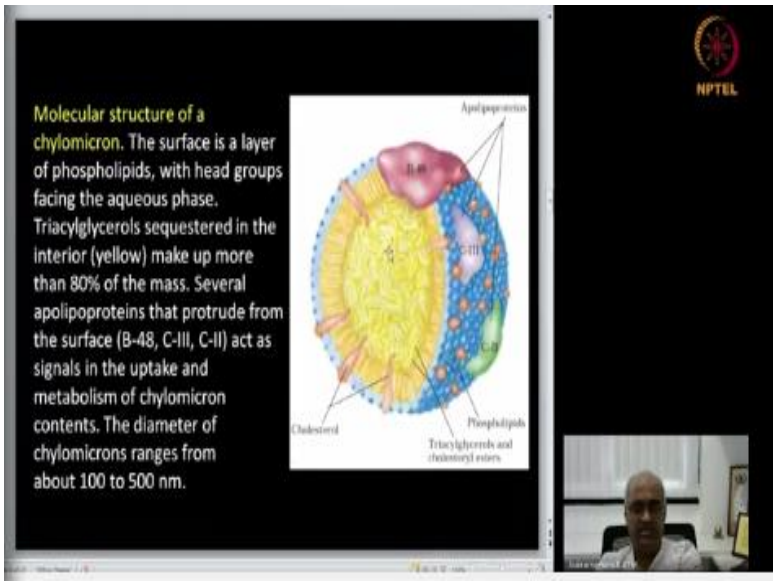
So, they are essentially detergent and the emulsification meaning the large fat aggregates are converted into smaller micelles**,** micelles meaning lipids distributed in water in small particles or droplet like thing. So, that increases the surface area of the fat for the action of intestinal lipases**.** The first step is emulsification and second step is intestinal lipases, so these are going to hydrolyze the triacylglycerol into glycerol and the free fatty acids**.**

So, this is the carboxylic acid and that the hydrophobic change, shown here the blue and yellow. So, and these fatty acids are then taken up by the intestinal mucosa cells where we see this is not showing of the microvilli structure but that is present**.** So, this is the surface of the intestinal mucosa and there they are again converted back to triacylglycerol. And these triacylglycerols combined with a set of proteins called apolipoproteins.

Here shown apoC II, so this is one of the lipoproteins. So, the lipoproteins are actually proteins that associate with lipids and such an association is required because this hydrophobic fat cannot be freely carried to other organs via aqueous blood. So, association of these lipoproteins make them possible to be transported in the bloodstream. And when they associate with triacylglycerol and also phospholipids, you get a structure called chylomicron**,** remember this is an important structure.

So, we will look at it is structure in detail in the next slide. So, this hydrophobic triacylglycerol are made transportable in the body fluids like the lymphatic system and bloodstream by the formation of chylomicrons and what the chylomicrons are I will show you in the next slide. So, we will go to that and then come back that will be easier.

(Refer Slide Time: 31:33)



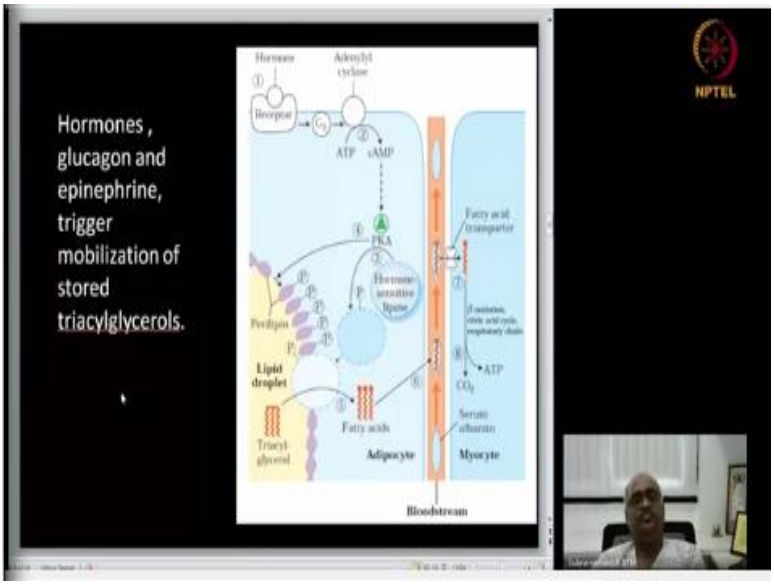
So, this is the chylomicron structure, so inside you have this triacylglycerol**,** so in the intestine triacylglycerols were digested by intestinal lipase forming free fatty acids and glycerol. And the free fatty acids taken up by the intestinal mucosal cells are again converted into triacylglycerol. And that triacylglycerol forms the inner core, surrounded by one layer of phospholipids, so that the outside is hydrophilic.

And then you have this apolipoproteins embedded in them. So, these spherical structures are studded with these lipoproteins. And these lipoproteins are essential they act as signals for the uptake and transport and metabolism of the fat. And these chylomicrons are then transported through the body fluids primarily blood and when they go through the small blood capillaries this apoC II the green structure in this cartoon that activates a lipoprotein lipase in the serum that is part of our blood.

And that hydrolyzes the triacylglycerols into free fatty acids and glycerol. So, you see here free fatty acid 1 carboxy group on the tail. And these are then transported into the cells like either muscle cells myocyte or adipocyte where fat is going to be stored. And there either they are going to be oxidized by via beta oxidation which we will discuss tomorrow. To carbon dioxide and ATP or again re-esterified into triacylglycerol and stored as a storage fat.

So, when you become obese, this is what is happening, this is getting stored and these adipocytes become big and big and that is how it is stored. So, this is the way the fat is digested taken up and transported either used for energy or stored**.** So, we have already seen what is a chylomicron, so this is an important structure for taking care of the lipid transport because lipids have hydrophobic.

(Refer Slide Time: 34:10)



So, now I have a lot of fat stored in my adipocytes and I am starving. So, how are these lipid molecules mobilized from that storage and used for energy production and how is that regulated? So, that is what is explained in this cartoon. So, when blood glucose level goes down meaning you do not have enough source of calorie and you need to mobilize the resource. First of course the glycogen stored in liver will be used up and that is not a large storage.

Like if you start for 24 hours already the glycogen will be fully gone and you will be dependent on fat mobilization. If you go for a jog even a very moderate jog within first 15 minutes your glycogen is gone and then lipid storage gets mobilized. And that mobilization is achieved by these hormones glucagon and epinephrine**.** Glucagon is produced by the alpha cells of pancreatic islets and epinephrine you already know adrenal medulla, so we have already encountered this hormone earlier.

And these 2 hormones sense the level of, so the rather they are produced when the glucose level goes down. So, they are activated in response to sensing the glucose level and when they were produced they go and bind to their respective receptors on the adipocytes, that is a fat cell. And there they activate this adenylyl cyclase, an enzyme this is a G protein coupled receptor and so this is the G protein is here.

So, you will learn about this in cell biology in cell-cell communication, so I am not going to get into the details of this, but this is an important way of signal transduction. G protein coupled receptors and activation of adenylyl cyclase many other hormones do too. So, this adenylyl cyclase is formed cyclic AMP. So, what cyclic AMP means is since we have a AMP structure here in our today's thing.

So, you have this adenosine 3-phosphates and when these 2 phosphates are removed then it is AMP, adenosine monophosphate. But you see here you are going to have 1, 2 two acid groups inorganic acid groups available that could form ester with another hydroxyl group in the ribose of adenosine. So, here you have ribose, so I will just blow it up, so you can have a close look at it.

So, you have imagine that this is not attached to this, so this is a acid group, this an acid group and they can form another ester linkage with this hydroxyl group. So, in cyclic AMP you actually have 1 phosphate linking these 2 hydroxyl groups in the ribose, this of course remains and that is what is cyclic AMP? And this cyclic AMP binds and activates it is sort of allosteric activation of protein kinase A.

And once this protein kinase is activated such as protein kinase becomes active, it goes and phosphorylates a lipase or it is called hormone sensitive lipase simply because only when the hormone is there, it is going to become active. And this is going to bind to this lipid droplet; this is how the lipid is stored in adipose tissues. So, essentially it is triacylglycerol with one layer of phospholipid therefore the surface is hydrophilic.

And in addition to that it has this protein perilipin a very important protein which is present on the surface of the lipid droplets. So, this is how the lipid is stored in adipocytes. In the adipocyte cytoplasm the triacylglycerols all aggregate and form a droplet and on the surface you have a phospholipid. In addition in that outer layer you have this protein perilipin. And this perilipin is the one that prevents access for this lipase access to the triacylglycerol. So, as long as this perilipin is present it is not going to allow this hormone sensitive lipase to act on the triacylglycerols and instead they will remain in the stored form.

But now when the hormone signal comes and this protein kinase A is active, I told you protein kinase phosphorylates this lipase and the phosphorylated lipase is active version. And another substrate for this protein kinase A is this perilipin itself and when this perilipin is phosphorylated, now it allows access. It is confirmation changes such that it no longer inhibits this lipase from acting on this.

So, the experimental observations show that although it is activity increases upon phosphorylation but the major effect in lipid mobilization is actually the phosphorylation of these perilipins. And perilipins when they are defective for this phosphorylation the lipid mobilization does not happen at all. Fat accumulation remains accumulated and it never gets mobilized, even when you have very low glucose and plenty of glucagon trying to signal through this.

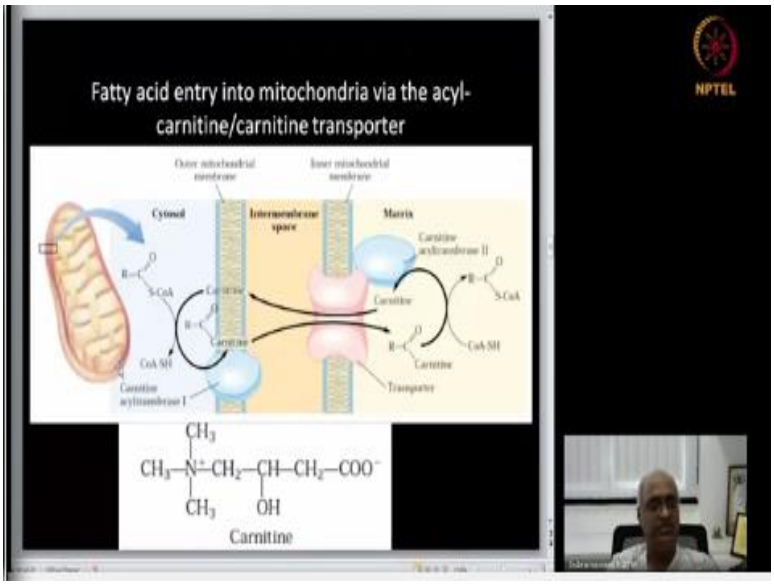
So, this perilipin is an important molecule and it is phosphorylation it is really key in mobilizing triacylglycerol. And once this lipase acts on it and becomes fatty acids then the free fatty acids are secreted into this bloodstream in the blood capillaries where they non covalently bind to serum albumin. So, serum albumin is a very important protein that is the most abundant protein in our body.

And the serum albumin bound free fatty acids are taken up by muscle cells which is requiring the energy via fatty acid transporters into the muscle cells. There it is going to undergo beta oxidation which we will discuss in the next class. At the end of which you will get acetyl CoA, and that acetyl CoA via citric acid cycle will produce the reducing equivalence NADH. And also FADH when the succinate dehydrogenase works and the ATP substrate level phosphorylation

also is there. And these reducing equivalence via respiratory chain are going to produce ATP and carbon dioxide.

So, this is a lipid stored in the adipose tissue becomes carbon dioxide and the energy when you have physical activity like vigorous physical activity like very fast walking or jogging or other household manual labour work when you do, this is what happens. So, this is the overall physiological detail and the endocrine connection of the lipid metabolism. So, the biochemistry of it like how what is beta oxidation, how the long chain fatty acids are converted into acetyl-CoA? So, that portion we will discuss in good length in tomorrow's class.

(Refer Slide Time: 42:45)



So, there is one little detail before we go for today, that is this fatty acid when it comes into the cytoplasm it needs to enter into mitochondria. And as you know mitochondria has outer membrane and inner membrane and the matrix is where TCA cycle happens, beta oxidation also happens matrix. So, the fatty acid has to cross these 2 membranes of mitochondria and that happens via this carnitine cycle.

So, what is carnitine cycle? So, this is the carnitine molecule**,** trimethylammonium group attached to F4 carbon group. It is basically hydroxy, so this is alpha is beta hydroxy butyrate it actually and that you have the trimethyl group and this carnitine is a key molecule. So, there are

three steps as part of this, the first step is the free fatty acid needs to be activated by acyl CoA synthetases, that is not mentioned here, the end product is this.

So, the carboxyl group is now in a acyl group with this thioester. So, we saw this when we were learning about high energy compounds as part of bioenergetics right after ATP, we learnt about this thioesters. They cannot have resonance stabilization; therefore this has high free energy. So, this formation of this carboxylic acid with the sulfhydryl group of coenzyme A. We have learnt about coenzyme A also when we learnt about pyruvate dehydrogenase complex.

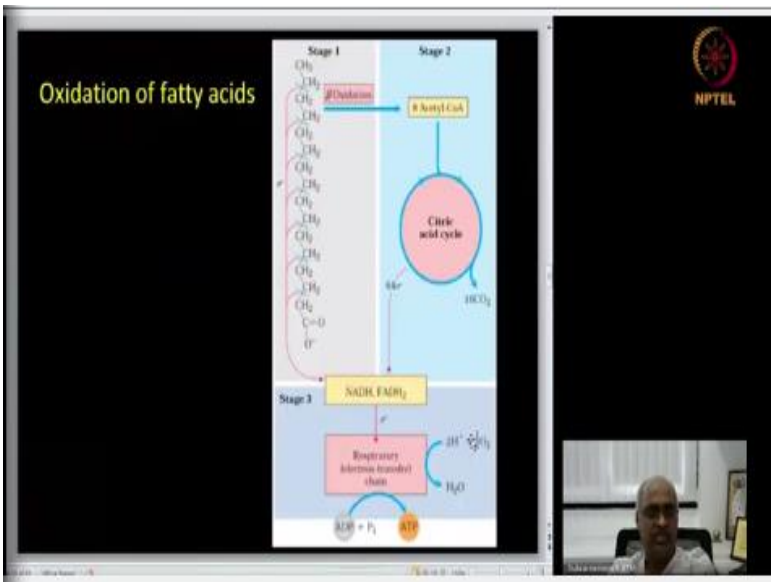
There the hydroxyethyl group, the acetyl group taken from pyruvate is in the form of ester with the coenzyme A there, acetyl CoA we saw. So, similar esterification, thioester formation is catalyzed by acyl CoA synthetases, so this is a acyl moiety, coenzyme is , so production of this is done by those things are simply called ACS, that is first step. Then carnitine acyl transferase I which is present in the outer membrane forms this carnitine acyl group or acyl carnitine**.**

This acyl carnitine crosses the inter membrane space and gets transported via this molecule which is a acyl carnitine, carnitine transporter, in one direction it is acyl carnitine that goes in. Then you have the second inner part of the inner membrane, meaning the matrix side via this carnitine acyl transferase II which then esterifies again with CoA and forming the acyl CoA once more.

And the free carnitine is transported by the same transporter that is why it is called acyl carnitine, carnitine transporter, it transports both these molecules. So, that is how this carnitine cycle operates, the bold black arrows show the cycle. So, here coenzyme A remains here it has it is own function in fatty acid synthesis in the cytosol. Here the coenzyme A again remains here itself, where it is required for connecting glycolysis and TCA cycle.

So, these are 2 different pools of coenzyme A's, but they help via this true transesterification step in transporting free fatty acids from cytosol to mitochondria and mitochondria is where beta oxidation is going to happen, so, that we will discuss tomorrow.

(Refer Slide Time: 46:49)



So, I will stop here and if you have any questions feel free to ask now, what do you say, where are the cycling AMP found? They are in our cytoplasm in different cells, they are kind of ubiquitous, they are there in all our cells, but they are produced only in response to signal transduction, they quickly become AMP and made inactive. So, only as long as you have signalling molecules binding to the receptor adenyl cyclase will produce cyclic AMP.

And since for example in this case the hormone glucagon or epinephrine is like the first messenger. And their arrival leads to the production of cyclic AMP in the cytoplasm and that carries the message therefore it is called second messenger. Like IP 3 that we learnt earlier, so cyclic AMP is there in the cytoplasm of almost all cells and it is level is regulated strictly by the signalling pathway.