[SQUEAKING]

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MATTHEW VANDER HEIDEN: OK, hello. So last time, we discussed the pentose phosphate pathway, which can serve as this shunt from glycolysis where glucose can be converted to five-carbon ribulose 1, 5-bisphosphate generating NADPH and ribose.

And this shunt from glycolysis can work with the non-oxidative pathway, where those five-carbon units can then be used by the non-oxidative pathway to re-enter glycolysis, giving cells and ability to generate NADPH.

Now, we described that this non-oxidative pathway can also operate in the reverse direction, such that cells can take products of glycolysis and instead use them to generate ribose for nucleotides and avoid the production of NADPH, really giving cells the flexibility to either make NADPH when they need it, make ribose when they need it, with the ability to either operate in this direction as a shunt and allow NADPH to be produced and material to re-enter glycolysis for further oxidation, or to just generate ribose if they have enough NADPH and they simply need that for nucleotide synthesis.

And we've spent some time discussing that NADPH is a key molecule that allows cells to do reductive reactions. This includes the biosynthesis of reduced carbon, which is, of course, the way that nature stores energy for later use. We saw this used as a way to make reduce carbon as carbohydrates and photosynthesis. And of course, it can also be used to generate fatty acids, which is the most reduced form of carbon that all organisms can have to store energy for later.

And the topic of today is really to go through the pathway that all organisms use to produce those fatty acids and lipids as a way to store this energy as reduced carbon for later. Now, you'll notice I drew up there, as an introduction for the reminder of what we talked about last time with the pentose phosphate pathways, really layering it on top of many of the other pathways in central carbon metabolism that we've already discussed.

And I drew it that way because I want to show that you already know quite a bit. If you think back to the very beginning where we had this complex metabolic pathway chart, well, we've already been able to build quite a complex network of how metabolism works. And really, at this point, you know the basics.

So today's lectures and the three lectures after it, so the four remaining lectures that we have in this course, we're going to cover an awful lot of material. Because, of course, we have to discuss how all of the remaining classes of biomolecules-- lipids, nucleic acids, proteins-- link into the rest of metabolism, which is an awful lot to cover. But what you will see is that, in understanding the basics and the complexity of what we've already described, you actually know already most of what you need to know to understand these remaining pathways.

That is nature continues to repurpose the same relatively simple reactions over and over and over again to really build this complex network that is metabolism, including this diversity of macromolecules. Now, of course, there's a few bits of chemistry that we still have to discuss. We haven't discussed much in terms of nitrogen metabolism. But most of what you need to know you've already covered in understanding glycolysis, the TCA cycle, pentose phosphate pathway, et cetera.

OK. So now onto the topic of the day, which is really lipids and fatty acid synthesis. And so, again, I want to reiterate that organisms store energy as reduced carbon. Fat is the most reduced form of carbon to store. And so if we're going to generate fat, we need a source of electrons.

Because if we're going to reduce carbon, something else has to be oxidized. That is those electrons have to come from somewhere. And as hopefully as clear to you, those electrons will come from NADPH because it forms this useful electron donor for cells.

Now, it should also hopefully be clear at this point that, if oxidation of carbon releases energy, reduction of carbon, therefore, it needs energy input. And so we're also going to need a bunch of ATP if we're going to make fat. And so ADP and NADPH are really the energetic drivers of how we're going to take more oxidized carbon and reduce it to build fatty acids.

Now, all organisms use similar pathways to do this. But, of course, the sources of where the NADPH the ATP come from can be different depending on the organism. And so we described photosynthesis is this process used by photosynthetic organisms, where they can use the light reactions of photosynthesis to make both ATP and NADPH. And we described how that ATP and NADPH could be used to drive synthesis of glucose or other carbohydrates.

And effectively, you can also imagine that that same ATP and NADPH from the light reactions could also be used to form the electron donors and the ATP needed to synthesize fats and lipids. But of course, as animals, we also know that, if we eat too much, we also have the ability to store excess energy as fat. And, therefore, we must also have sources of NADPH and ATP that we can use that are non-photosynthetic.

And those, of course, are the reactions that we've already talked about with glycolysis and the TCA cycle, oxidative phosphorylation as a way to make ATP, as well as NADPH from reactions like the oxidative pentose phosphate pathway as a source of NADPH. Now, eukaryotes will make fatty acids in the cytosol.

And if you think about why that is, it's because mitochondria, remember, is where we did fatty acid oxidation. And so fatty acid oxidation is breaking down fatty acids. Fatty acid synthesis-- building fatty acids.

One set of reactions is in the cytosol, synthesis. One set is in the mitochondria, breakdown, makes sense. Remember, compartmentalized metabolism gives you this ability to favor different pathways.

Mitochondria is better at oxidative reactions. Cytosol is going to be better at reductive reactions. And also, this compartmentalization will keep catabolism and anabolism separate, so another example of a point that we've been making over and over again throughout this course.

Now, if we look up here at our diagram, we're going to make fatty acids from two carbon acetyl-CoA units, OK? And so, remember, when we broke down fatty acids, most fatty acids were even in number. That's allowed us to break them down into acetyl-CoA units. Well, we're also going to build them with two carbon acetyl-CoA units. And so this also contributes to why most fatty acids in nature have even numbers of carbons.

Now, you'll also recall from our past lectures that fatty acids are often esterified to alcohols. Those can be things such as glycerol based alcohols, which allow us to form lipids, either triacylglyerides, those neutral lipids for energy storage, or phospholipids to form membranes. And that, of course, comes from a branch of-- glycerol, of course, comes from dihydroxyacetone phosphate, a molecule in glycolysis.

Now, of course, I'm not going to draw acetyl-CoA now. I'll draw it later. But remember, it is not reduced. And so that's where we're going to need the electrons, NADPH as well as ATP for the energetic requirements in order to take those two carbon units, build the acyl chain, and ultimate reduce them to make fat.

Now, when we do this, you're going to see that carbon dioxide, as well as biotin-- OK, so you might be thinking, oh, what's biotin used for? It's a carboxylation reaction. And so CO2 in a carboxylation reaction involving biotin is going to be required for fatty acid synthesis.

However, you will also see that that CO2 carbon is not incorporated into the fatty acyl chain. And so you'll see that the CO2 is required, but ultimately is added and released, which is exactly the same analogy to what we saw when we did gluconeogenesis. Remember, the pyruvate carboxylase PEPCK reaction in gluconeogenesis, if you look back, a CO2 was added to pyruvate to make oxaloacetate and then release later to generate PEP. That helped drive the energetics of that reaction, no net CO2 incorporation, very similar thing happening in fatty acid synthesis.

Now, in animals, mammals, much of fatty acid synthesis is catalyzed by a giant single polypeptide enzyme called FASN or Fatty Acid Synthase Complex, which is, unlike other complexes we talked about where you might have different polypeptide subunits coming together to form a complex, this is one gigantic polypeptide that is able to catalyze many of the enzymatic steps of fatty acid synthesis.

Now, a bit of trivia is that this is different from plants and bacteria, which will carry out the exact same enzymatic reactions. However, it, rather than using a giant fatty acid synthase complex, will have all the different enzymatic activities broken up and catalyzed by different proteins, separately encoded polypeptides.

Now, all organisms will do this using a protein called acyl carrier protein. And so acyl carrier protein, you'll see, is really analogous to acetyl-CoA. So it's a way to add a thioester, mark a separate pool, and will carry the growing fatty acyl chain as we synthesize it, OK?

So acyl carrier protein is part of FASN, the big polypeptide FASN in mammals that catalyze this thing. The acyl carrier protein is not separate. It's actually built in to the sequence of FASN, whereas acyl carrier protein in plants and bacteria is a small 9 kilodalton protein, all right?

So in mammals, acyl carrier protein-- part of the fatty acids synthase polypeptide in plants and bacteria. Acyl carrier protein is a small 9 kilodalton protein.

And so what acyl carrier protein looks like is that as, part of this 9 kilodalton protein or sequence within FASN, there is a serine residue. Remember, serine has an alcohol on its side chain. And that alcohol on its side chain has a phosphodiester bond to a phosphopantothenate group just like we saw with coenzyme A.

So if you look back in your notes when I drew out coenzyme A, the business end of the molecule was right here with the sulfur on the end there being the one that form the phosphodiester bond. This one, rather than being linked to a nucleotide, in this case is linked to a serine as part of a peptide. And so this is why it's very, very similar.

So this here is same as end of coenzyme A. And so really you can think of ACP, Acyl Carrier Protein, and coenzyme A, in some ways analogous to how we talked about NAD and NADPH-- same functionality, in this case, providing a sulfur to make these thioester bonds as a way to activate the acid on the end of the fatty acid.

One used for synthesis, one use for breakdown-- acyl carrier protein used for synthesis. Coenzyme A, acetyl-CoA is used for oxidation, whereas in, remember, NAD in NADP, one was used to create an NAD, NADH ratio that favored oxidative reactions, the other used to create an NADP, NADPH ratio that favored reductive reactions even though the functionality of the electron donor, in the case of NAD and NADP or, in this case, the carrier function with the thioester bond in ACP and coenzyme A are analogous, really allows you to mark different pools to carry out, in this case, anabolic and catabolic reactions in cells.

So often, we will abbreviate this just as we abbreviate CoA as sort of CoASH or SCoA. In this case, we're going to use ACPSH as an abbreviation for Acyl Carrier Protein. However, remember, just like coenzyme A was this giant molecule, it kind of is a little misleading to write it this way.

Acyl carrier protein is an even bigger molecule, a 9 kilodalton peptide in plants and bacteria. And so, again, misleading to write it this way because it's really this giant group linked to and carrying the acyl chain. OK.

Now, the first step in fatty acid synthesis is carboxylation of acetyl-CoA. This is a very important step of the process. And it is not catalyzed by the fatty acid synthase complex, the fatty acid synthase protein in mammals.

And so, in all organisms, it is catalyzed by a separate enzyme abbreviated ACC, which stands for Acetyl-CoA Carboxylase. So ACC is a pretty famous enzyme. It's argued by many to be the rate limiting step in fatty acid synthesis.

And it makes sense because you're going to see this as we're doing a carboxylation reaction, big energetics here. And that is, obviously, as we learned before, the steps that you regulate are the ones with the biggest change in free energy.

So here's our old friend, acetyl-CoA. And what ACC carries out is it carries out the as biotin found in the active site, which, of course, can contain a CO_2. Just as a quick reminder, how do we put CO_2 on biotin for carboxylic reactions?

And so, remember, CO_2 is in equilibrium with bicarbonate. That bicarbonate can be phosphorylated by ATP to give this phosphobicarbonate. And then that phosphate can be released to add the CO_2 onto the active biotin part of the enzyme.

That then carries that CO2 and can be used to transfer the CO2 to carboxylate, in this example, acetyl-CoA to generate this carboxylated acetyl-CoA three-carbon molecule, which is referred to as malonyl CoA, all right?

So this is exactly the same mechanism that we described before for pyruvate carboxylase in gluconeogenesis, all right. So I drew a part of it here. Remember, if we draw acetyl-CoA, in the enol form of the molecule-- here we have our biotin, CO2.

If you look back at your notes, we drew a mechanism like that. And that allowed us to, in that case, take pyruvate and turn it into oxaloacetate and gluconeogenesis by pyruvate carboxylase. In this case-- identical reaction, but this time you're carboxylating acetyl-CoA to make malonyl-CoA, right?

And it's malonyl-CoA that ends up being the substrate for fatty acid synthase, either the single fatty acid synthase protein in mammals or the same sets of individually encoded activities on different polypeptide in plants and bacteria. All right, so let's draw this out. So here's fatty acid synthase.

And so fatty acid synthase has on it two different acyl carrier protein sites, so two different acyl carrier protein encoded within the fatty acid synthase polypeptide, each of which have attached to them this phosphopantothenate group to make it an acyl carrier protein. Or there would be two separate acyl carrier proteins in plants and bacteria as part of the complex that synthesizes fatty acids.

And each of them can pick up a acyl-CoA. So here's a malonyl-CoA and an acetyl-CoA. Each of those can basically, onto the acyl carrier protein, exchange the thioester bond with the CoA to be a thioester bond with the acyl carrier protein.

And so to get this started, on one site you'll end up going an acetyl-CoA, exchange the thioester bond, such that you release the CoA. And now, you have an acetyl ACP. And on the other side-- a malonyl-CoA exchange that thioester bond releasing the CoA. So you have a malonyl ACP, all right?

So what does that look like? Well, it'll look like this.

OK, so here we're going to have basically-- I'll give you some color, so you can see what's going on.

But here you have, on an upper acyl carrier protein site, the malonyl group, so malonyl ACP. And on this lower site, I put on an acetyl ACP, OK? So what happens is you now release the CO2 that was added by acetyl-CoA carboxylase.

And that allows forming carbon bond from here to here, releasing the ACP on the lower site and generating this longer four-carbon chain on the upper acyl carrier protein site.

OK. Once you have this, now, four-carbon chain, obviously, if we're going to make a fatty acid, we have to reduce this carbon. And so that's exactly what happens. And so how do we reduce it?

We've now seen this many, many times. And so if we use NADPH as an electron donor, oxidizing it to NADP+, that generates this hydride ion to electron carrier.

And now, we net reduce this ketone to this alcohol, OK? Next step is we're going to remove water across that bond, just a dehydration.

All right. Now, we can further reduce that carbon-carbon bond. The electrons, again, come from NADPH as it's oxidized to NADP+.

And that leaves us with a reduced four-carbon fatty acid, a four zero fatty acid here on this, esterified by this thioester to ACP, on the upper site of fatty acid synthase. OK.

All right, so what happens next? Well, we can repeat this cycle. So, now, we can have basically this S-ACP on the lower site pick up a malonyl-CoA, release the CoA.

And then we have, down here, another malonyl-CoA.

Now, they're esterified to the ACP on the lower side. All right, now, we can run this exact same series of reactions again.

OK. That releases CO2, also releases a free S-ACP on the upper site. And now, we are left with this.

This now-- six-carbon molecule with a thioester now on the ACP on the lower side. Hopefully, you're getting the point. I can now take this through the same cycles I took before. But first, we're going to use NADPH to reduce this ketone to the alcohol, the dehydration, use NADPH again to reduce the carbon-carbon double bond.

And we are left with now this S-APP on the lower site.

I can now run this cycle five times. We'll add another malonyl-CoA, this time to the upper site, combine them. Now, you have this growing chain on the upper site, reduction, another malonyl-CoA to the lower site, blah, blah, blah.

Run it five times. Eventually, I'm going to end up with a 16, 0, fatty acyl ACP. And then I can take that, exchange it back for a CoA.

And I end up with a 16 carbon fatty acyl-CoA or palmitoyl-CoA CoA molecule, such that all of the carbons added came from acetyl-CoA except for one. All of that acetyl-CoA was added by, first, adding a CO2 via acetyl-CoA carboxylase, generating a malonyl-CoA that's spending ATP to put the carboxyl group on. Because, of course, we need it to spend an ATP in the acetyl-CoA reaction.

And so seven cycles were needed to generate this 16, 0 fatty acyl-CoA. And so those seven cycles were seven ATP molecules. I also needed 2 times 7, or 14, NADPH molecules. And that allowed me to run this cycle 17 times and make a fully reduced 16-carbon fatty acid.

Now, it turns out that all of this activity stops on the fatty acid synthase molecule when it gets to a 16-carbon palmitoyl-CoA. But of course, as we described before, organisms have longer fatty acids than 16 carbons. And so 18-carbon and longer fatty acids are made in exactly the same chemistry, same reactions that we just described.

The only difference is that they are not made on the fatty acid synthase complex. So they're made in a different location. And so they're made at the ER membrane in eukaryotes, all right, so the ER in eukaryotes.

Even though the enzymes themselves carry out the same reaction, the enzymes that carry out this are encoded by different polypeptides, so-called fatty acid elongase enzymes. Although the chemistry is exactly what we already described. But those enzymes act on the thioester with the CoA, not the thioester with the acyl carrier protein.

But it's still the same. There's still one ATP per acetylcholine for two carbon unit added because that is needed to carboxylate it to make the malonyl-CoA because that's what's driving the addition and two NADPHs per two-carbon unit added to carry out the reduction to take that carbonyl from the acetyl CoA or the malonyl-CoA that's added as a two-carbon unit and reduce it to the fully reduced carbon.

OK. And so that is how you make saturated fatty acids. So what about unsaturated fatty acids? Well, it is not that you just stop and don't make it fully saturated. Nature, first, makes a fully saturated fatty acid. And then it comes back and reoxidizes the fully saturated fatty acid to introduce double bonds in the right positions.

And so for unsaturated, so you start with a saturated fatty acid of the desired length. So you make that first.

And then you use so-called desaturase enzymes to introduce double bonds at the desired location, all right?

So it's a little counterintuitive, but this is effectively why you end up with double bonds at the stereotyped places. So, remember, the delta 9 position is the first place, when we describe the nomenclature of fatty acids, where we always put the first double bond. Why does nature do it this way?

Well, it's very hard to say that, but you can imagine evolution of how you would get these enzyme activities. It's hard to evolve a fatty acid synthase complex that would stop doing the reduction reaction only at specific locations. And so you probably first generate these fatty acids fully saturated because that's the way an enzyme could do it and then, later, have a different enzyme that can pick out a location on a saturated fatty acid to introduce a double bond.

All right, so how can we introduce a double bond? Well, that's an oxidation reaction. And so if we're going to do an oxidation reaction, we need a place for the electrons that we move.

So if we oxidize the fatty acid, that's removing electrons. Those electrons have to go somewhere. You might imagine, if we're oxidizing a carbon-carbon bond, we've seen that reaction before when we did succinate to fumarate.

Succinate dehydrogenase, that was an oxidation reaction. We used FAD. We saw it with the fatty acid breakdown when we first put the double bond in.

We used FAD. And so, indeed, FAD is used in this reaction, but it does not work like other FAD reactions to carry out this oxidation. For whatever reason, the way desaturases work is different.

OK. So these desaturase reactions use oxygen as a final electron acceptor. And they work via a mini-electron transport chain in the ER in eukaryotes OK. So it's a little bit of a different weird mechanism.

But if we think here's our carbon-carbon bond that we're going to oxidize to introduce a double bond and desaturate our lipid, so what is that? Well, it's basically this reaction. OK. So this is, rather than just remove this as a hydride ion, it turns out that those electrons go to oxygen, which, of course, will generate one water molecule.

But there's two oxygen there, so you need to generate a second water molecule. Those electrons have to come from somewhere else. And those electrons come from this little mini-electron transport chain.

So if we oxidize iron, we can get electrons from that reaction. And so this ends up generating a double bond in the fatty acid with two of the electrons going to oxygen and the other two electrons coming from this mini-electron transport chain in this desaturase complex in the endoplasmic reticulum, all right? And so, of course, those electrons have to come from somewhere. So we have to re-reduce. This iron we oxidize to get the electrons from.

And so this works via an electron transport chain similar to what we saw before, where it's this whole series of oxidation and reduction reactions that does happen to involve FAD and FADH2. But ultimately, the electrons are coming from NADH. So oxidation of NADH ultimately is providing electrons that together with oxidation here lead to the reduction of oxygen to water.

And you can, of course, read more about this if you're interested in it, but it's important to point out that this process works by a slightly different mechanism than you might predict from sort of the general principles of the way most double bonds are introduced in carbon-carbon molecules in metabolism. Why it works this way is something we can only speculate about.

OK. Now, mammals only use a disaccharase enzyme that, in a fully saturated fatty acid, can only introduce a double bond at the delta 9 position.

That is between carbons 9 and 10 of a fully saturated fatty acid. And that's because they only have an enzyme complex that does that. So that is they can take an 18, 0 newly synthesized fatty acid and they can make an 18, 1-- or we can make an 18, 1 delta 9 fatty acid, OK?

That's the only desaturase enzyme we have to put into a saturated fatty acid. That is to make completely a de novo unsaturated fatty acid, which is one of the reasons why we don't have a lot of polyunsaturated fatty acids.

Now, we do have enzyme complexes that work similarly to these that can add double bonds at other locations, but they have to start having a double bond already present in those locations. Many of those lipids have to come from the diet. And that's that concept of essential lipids, something that we have to eat from a plant or bacteria that already has a double bond there in order to add double bonds to make some of our other polyunsaturated fatty acids.

So that's how fatty acids are made. But as I alluded to earlier, all of this is taking place in the cytosol. And if we're doing all of this work in the cytosol, maybe you notice that we actually have another problem that nature has to solve.

And that is, because we're doing all this from acetyl-CoA, and so we have to have acetyl-CoA in the cytosol to have this work. But acetyl-CoA, all the ways we've talked or most of the ways we've talked about producing it, happen in the mitochondria. Remember, acetyl-CoA can't get across the mitochondrial membrane.

So we need a way to get acetyl-CoA out of the mitochondria into the cytosol for this whole thing to work. Put another way-- so if we draw here, here's glucose to pyruvate. That's glycolysis. Remember to turn that pyruvate into acetyl-CoA.

We had to move the pyruvate into the mitochondria. That's where the PDH complex was to generate the acetyl-CoA. But if we need acetyl-CoA in the cytosol for fatty acid synthase to make fatty acids, we basically need a way to get that acetyl-CoA back out of the mitochondria in order to generate fatty acids.

Other big source of, remember, was we start with a fatty acid in the mitochondria, run fatty acid oxidation. Again, fatty acid oxidation in the mitochondria generates acetyl-CoA in the mitochondria. We're going to use it to rebuild a fatty acid, need to get it out of the mitochondria into the cytosol.

Coenzyme A is not membrane permeable. Remember, we had shuttles to get fatty acids in the mitochondria. We did the pyruvate dehydrogenase reaction here to begin with. And so it's a problem to get this giant CoA, the CoA from the acetyl-CoA, out of the mitochondria into the cytosol.

OK. So one solution to this, to getting acetyl CoA in the cytosol, is simply to start with acetate, OK? That is take the CoA group off and just have an acetate molecule. And of course, acetate or acetic acid, well, that's food. That's vinegar. All right, the salad you ate has acetate in it, acetyl-CoA.

It can be in the cytosol. We can make acetate if you look back at your notes about how we metabolized alcohol. So alcohol gets metabolized to acetate.

And so that acetate in the cytosol, we can just add a CoA group to it in the cytosol. And how do we do that? Well, we saw the reaction to do this already.

OK. And this is basically when we added any fatty acid to make a fatty acyl-CoA. Remember, the free fatty acid turned into a fatty acyl-CoA. We add this reaction where we used ATP.

We added the AMP to it. And then that [INAUDIBLE] pyrophosphate can drive it forward with the two pyrophosphate molecules and take the AMP off and adds a CoA group. If you look back in your notes, it's the identical reaction to how we made a fatty acyl-CoA.

Well, there's also an enzyme that can act on acetate and do this to make acetyl-CoA. And that's great. It's a way to make acetyl-CoA in the cytosol if you start with acetate.

However, this is not the way it works if you already have an acetyl-CoA in the mitochondria. And so if in the mitochondria you already have an acetyl-CoA-- so this can come from pyruvate via the PDH reaction.

It can come from fatty acid oxidation, this acetyl-CoA in the mitochondria, all right? Well, what do we talk about doing with it in the TCA cycle? Well, the TCA cycle, we can use citrate synthase to generate this citrate molecule.

And it turns out citrate itself can be used as a carrier to export the acetyl-CoA from the mitochondria to the side cytosol, such that now, when you have citrate in the side cytosol, that citrate can now be used where you basically carry out the opposite of the citrate synthase reaction to regenerate acetyl-CoA and oxaloacetate.

Well, if one direction is favorable, the other direction is going to be not favorable. And so you need energy input in one of the directions. And so this costs you ATP. And this is carried out by an enzyme called ATP citrate lyase, often abbreviated ACLY.

So ATP citrate lyase allows you to basically reverse the citrate synthase reaction, such that you can shuttle acetyl-CoA out of the mitochondria to the cytosol using citrate as a molecule. And then, of course, that oxaloacetate can be shuttled back into the mitochondria via something like the malate-aspartate shuttle, which we describe several lectures ago, as a way to make this a complete cycle.

I want to spend a little bit of time talking about this because, here, a lot of metabolism begins to come together, all right? And so when do you want to produce fatty acids? We want to produce fatty acids when you have a lot of ATP.

And so if you have a lot of ATP, this is a situation where the TCA cycle isn't going to want to run. And so that favors citrate export from the TCA cycle into the cytosol. Remember, citrate was this important regulator of glycolysis.

We talked about you have a lot of citrate. Let's slow down glycolysis. Well, that citrate in the side cytosol also now provides a source of acetyl-CoA to take all that extra carbon and turn it into fatty acids, all right?

We also, in the process of moving this out, we generate oxaloacetate. Remember, oxaloacetate, of course, is part of the malate-aspartate shuttle. We'll come back to that in a minute.

But oxaloacetate in the cytosol, that was the product of the pyruvate carboxylase reaction to get oxaloacetate in the cytosol so PEPCK can make PEP and do gluconeogenesis. And so the same thing is also taking oxaloacetate and putting that in this cytosol where it's a good thing to do gluconeogenesis, which is something else that you want to do if you have a lot of excess ATP to store carbon.

And so this is really set up in a way that, now, you have your citrate and oxaloacetate in the cytosol, which are the starting points to generate either fat or carbohydrate as a way to store excess energy if you have plenty of ATP around. Now, it turns out that oxaloacetate in the cytosol is also beneficial in another way because it can be part of a series of enzyme reactions that also benefits fatty acid synthesis.

And that's because it's a substrate for an enzyme. It can create a substrate. It can create malate that's a substrate for an enzyme called malic enzyme, which is another way to generate NADPH.

All right, so let's go through this. So remember, oxaloacetate differs from malate by an oxidation reduction reaction. So remember, we described malate dehydrogenase in the TCA cycle to turn malate into oxaloacetate in the malate-aspartate shuttle.

We pointed out that this enzyme can run in reverse, and so can be used to regenerate NAD in the cytosol. And this oxaloacetate back to malate as part of the malate-aspartate shuttle was a way to regenerate NAD to help keep glycolysis going as an alternative to fermentation, get those electrons into the mitochondria, so we give them to oxygen.

Well, this malate that's made is the substrate not just for the malate-aspartate shuttle to bring electrons into the mitochondria, but is also a substrate for an enzyme called malic enzyme. And malic enzyme is a way to make NADPH. Well, how does this work?

OK. So here's malate. All right, so if we reoxidize this alcohol to bring malate back to oxaloacetate, so that generates hydride ion, which can, of course, be given to a nicotinamide group, the malic enzyme. The nicotinamide group is NAD+ to generate NADPH.

NAPDH is, of course, useful for reducing power to make fatty acids. So what does this generate? Well, this generates-- again, all I've done is regenerate oxaloacetate.

OK. This is oxaloacetate. It turns out this oxaloacetate is retained on the enzyme. And remember, oxaloacetate is a beta-keto acid, so acid group, alpha, beta, beta-keto acid.

And beta carboxylation is favorable. You've now seen this many, many times. This is going to generate enolpyruvate. Enolpyruvate will want to rearrange to pyruvate.

And so, effectively, I can turn malate into pyruvate and generate NADPH. And that's what malic enzyme does. And if you look back at the malate-aspartate shuttle, we used oxaloacetate to make malate. And it was malate that was sent back to the mitochondria.

Well, here, we can also use it to make pyruvate. And then the pyruvate can go back to the mitochondria and use pyruvate carboxylase to generate acetate via pyruvate carboxylase as a way to do anaplerosis for this mini-cycle if you want. A lot of moving parts here, let me be explicit about what's going on and show you how I can build a series of reactions here that is very useful if I want to generate that.

OK. So here's glycolysis. Remember to run glycolysis. I need a source of NAD+ plus for the GAPDH reaction.

If I'm not going to ferment the pyruvate, I need to deal with that NAD+. Well, if I send now that pyruvate here into the mitochondria, that pyruvate can go through the pyruvate dehydrogenase reaction, make acetyl-CoA. Acetyl-CoA combine with oxaloacetate to make citrate.

OK. Export that citrate from the mitochondria to the cytosol. Run the ATP citrate lyase reaction.

Now, I have acetyl-CoA in the cytosol and can use that to generate fatty acids. And of course, that requires NADPH as reducing power to make those fatty acids, OK?

So all series of reactions that you've seen many, many times, this is glycolysis, pyruvate dehydrogenase, citrate synthase, ATP citrate lyase that we just described and, of course, this over here acetyl-CoA carboxylase, of course, and fatty acid synthase to do that.

All right, now, that's great, but we have this NAD+ to deal with. And we need sources of NADPH to balance all the electrons to make this work. Let's show how we can incorporate malate dehydrogenase and malic enzyme as a way to make all this balanced.

So if we take oxaloacetate and make malate, this is our malate dehydrogenase reaction. I've now regenerated the NAD I need in the cytosol to keep that carbon flowing in from glucose to make acetylcholine, right?

Now, I have malate. I can use malic enzyme to turn malate into pyruvate. That's going to serve as a source of NADPH that I can use, again, NADPH in the cytosol to drive fatty acid synthase in the cytosol.

Obviously, you need more than one NADPH to do this, but at least it generates NADPH's reducing power to make the fatty acids. And of course, take that pyruvate, bring it back in the mitochondria. I can run the pyruvate carboxylase reaction.

And I'll regenerate oxaloacetate in the mitochondria. And now, I have a balanced cycle where I can run this cycle to turn glucose carbon into acetyl-CoA for fat and, in the process, also make some NADPH's reducing power to support my fatty acid synthase reaction. This is just one way that I can take all these reactions we talked about and build a pathway that's balanced, at least for the NAD-NADH oxidation piece, and gives us something useful.

Well, it gives us ATP from glycolysis and also gives NADPH from the malic enzyme reaction to do this. And what does it cost me? Well, it costs me a bunch of ATP, right? It cost me ATP here. It costs me ATP there, of course a lot of ATP up here.

But you're doing this when there's energy excess. You have plenty of ATP around. And it's a way that nature then can use this high-energy, high-ATP state as a way to store carbon, ultimately, reduce carbon is fatty acids that can be used later when times are not so good.

All right, so let's spend a couple of minutes talking about the regulation of fatty acid synthesis. It's very straightforward, not really a whole lot to talk about. You really only want to make fat if you have high ATP-ADP ratio.

This is a situation excess citrate in the cytosol. And so high citrate, which, remember, inhibits glycolysis-- well, high citrate is going to activate fatty acid synthase. OK. The big step, though, is acetyl-CoA carboxylase.

That's what makes the malonyl-CoA. That's the big change in delta g. By all the reasons we talked about before, that's a step you want to regulate. And so it turns out that high levels of palmitoyl-CoA-- that is the 16, 0 fatty acid, the product of the fatty acid synthase reaction.

This is going to inhibit acetyl-CoA carboxylase. It makes sense-- have a lot of product around. Stop making it. Stop the step that costs the most, that's hardest to go back to generate it. OK?

What's the other thing? Well, you only want to do this if cells have enough energy to do it. What's their energy charge?

ATP-AMP ratio-- if AMP is high, you also want to inhibit ACC. Low energy-- don't try to make fat. That's basically what you need to know about the regulation of fatty acid synthase.

Now, of course, that's making fatty acids. But of course, most fatty acids are not floating around free in nature. They're stored as lipids. And so just to remind you, here is an image from an earlier lecture.

These were the image of a fat cell, as well as a plant cell here. They both have these lipid droplets that are filled with these neutral lipids, these triacylglycerides. And so you want to store the fatty acid for energy. You put it into a triacylglyceride, pop it into a lipid droplet. And now, you have this efficient way to store all this reduced carbon without having to carry around the weight of the water.

Or maybe you want to use this to generate membranes, right? Phospholipids, you need to generate phospholipids. Both triacylglycerides, phospholipids are built on this glycerol backbone. And I just want to mention briefly the pathway that cells really use or a pathway the cells use to make these glycerol based lipids.

And so as we talked about before, here's our old friend dihydroxyacetone phosphate from glycolysis. Remember, if we reduce this ketone to the alcohol, that's how we made glycerol. It turns out, first, before doing this, the way nature does it is it first adds the fatty acid.

So it takes the fatty acyl-CoA, releases the CoA. And you end up with this intermediate, OK? It's this molecule that then you reduce.

Of course, you can reduce it within NADH. You can also reduce it with NADPH. And that gives us this phospho-monoacylglycerol, OK? So this molecule, with that being the alcohol instead of the ketone-- phospho-monoacylglycerol. Now, come on, add another fatty acyl-CoA.

Releasing the CoA, that gives us a phospho-diacylglycerol, so, now, a fatty acid esterified to that middle carbon. And then this phospho-diacylglycerol, we can remove the phosphate to get just a diacylglycerol and then generate a triacylglycerol by putting on a third fatty acid from a fatty acyl-CoA, releasing the CoA.

And now, we have this triacylglyceride, this neutral lipid that can be packed into the lipid droplet here and store energy as reduced carbon for later. That's great. But what if we want to make a phospholipid?

Well, I'll show you briefly how this works. Don't worry about the details of this. I just want you to have a flavor for how this happens because it illustrates another way that nature repurposes the same reactions over and over again.

And so as one releases this phosphate from the diacylglycerol, it now picks up head group on there. And so one that can be picked up is ethanolamine. And the ethanolamine comes from a molecule called CDP ethanolamine.

If you're interested in the structure of ethanolamine, be reminded of that, you can, of course, look it up. But basically, it's ethanolamine attached to a CDP group releasing a CMP group, which makes a phospho-ethanolamine.

And then that phospho-ethanolamine can be turned into phosphatidylethanolamine. A phospholipid phosphatidylethanolamine can be turned into phosphatidylcholine. And if you look up the difference between choline and ethanolamine, it's adding three methyl groups. And we'll talk about how to do that in one of the upcoming lectures, all right?

And so here's two of the major phospholipids, phosphatidylcholine, phosphatidylethanolamine. They are added to a diacylglyceride by basically taking the phosphate off and adding a phosphate from CDP ethanolamine releasing CMP. What's CDP ethanolamine?

Well, it's very similar to how we already talked about with UDP glucose in glycogen metabolism. And so if you start with the amino alcohol ethanolamine, which itself is made from serine, but we don't have time to talk about how, this can basically be phosphorylated by ATP to put a phosphate on the alcohol to make phosphoethanolamine.

So you have a phosphoethanolamine. And then that phosphoethanolamine can react with a CTP, releasing a pyrophosphate such that the phospho from the ATP is replaced by the CDP. You end up getting a CDP ethanolamine with two pyrophosphate coming off by a series of reactions that, if you look back in your notes, will look identical to how we made UDP glucose in making glycogen. And then the CDP ethanolamine transfers the phosphoethanolamine onto the diacylglyceride to give you the phosphatidylethanolmine.

Why do I mention this? Just because I want you to have a flavor of how phospholipids are made, realize that here is a repurposing of a same series and type of reactions as we saw for glycogen metabolism, but now to make phospholipids, and also to point out that it's really expensive to make a phospholipid, all right? You need three ATPs just to add this ethanolamine group to there. So that's actually fairly expensive. And so a lot of energy goes into cells building these phospholipids.

Now, for the last bit of time today, I want to return to a brief topic of another lipid. And that's this guy here, cholesterol. And so you'll remember that cholesterol is this complex ring structure, lots of reduced carbon there.

It's a molecule that mammals use, if you recall, to keep their membranes fluid. Now, mammals, of course, can make cholesterol. And you probably have heard about cholesterol because high levels of cholesterol have been linked to heart disease. And so a lot of people talk about what their cholesterol levels are, something doctors check a lot.

And this has led the recognition that cholesterol can be associated with vascular disease, has led to the development of a class of drugs called statins. And statins are one of the most commonly prescribed drugs out there. They're a drug that blocks an enzyme in cholesterol synthesis and have been very effective at lowering risk of heart attacks and strokes.

They've also been a huge windfall, a huge moneymaker for lots of pharmaceutical companies. And so if you care about medicine, they're important to medicine. If you care about biotech, they also were very important in supporting the pharmaceutical industry.

All right, now, we don't have time to talk fully about how cholesterol synthesis works. It's a long pathway. You can look it up if you're interested. You have all the tools that you need to understand all of the steps to make cholesterol.

You'll see you build it from acyl-CoA. You need a bunch of NADPH. I don't have time to go through all those steps.

But I will discuss the initial steps, so you can understand how statins work. Because that's important in medicine, and some of you, I know, want to go to medical school. But apart from that, these early steps affect other aspects of biology, making lipid tails for signaling proteins, also introduces a discussion of ketones, which are another important metabolic fuel that we need to talk about as well.

Now, the way that one makes these things is basically you start with three acetyl-CoA molecules. OK. So here's two acetyl-CoA molecules. We can combine those two together, releasing a CoA. And this is really the identical reaction that we just saw for the early steps in fatty acid synthase.

OK. That generates this molecule called acetoacetyl-CoA. And then this can combine with yet another acetyl-CoA to make this molecule, which is called Hydroxy-Methyl-Glutaryl, or HMG, CoA, all right?

I don't need to describe any of these reactions to you. As I said, this is just what we showed earlier for fatty acid synthesis. And this reaction is the identical reaction to citrate synthase in the TCA cycle and takes these three, 1, 2, 3 acetyl-CoAs and makes this molecule, HMG-CoA.

HMG-CoA is the substrate for an enzyme called HMG-CoA reductase. HMG-CoA reductase is the famous target of statins to block cholesterol synthesis.

What HMG-CoA reductase does is it releases that CoA group and uses two NADPH oxidizing it to two NAD+. That effectively is, when you release this CoA, what are you're left with? The acid, you reduce it twice. And you end up with the alcohol.

And that gives you this molecule, which is called mevalonate.

And mevalonate is what you use to build cholesterol. And so statins, by blocking HMG-CoA reductase, basically block the reactions that are necessary to make the precursor mevalonate, which is necessary to make cholesterol. And that's why statins stop cholesterol synthesis.

Now, mevalonate, it turns out, is also used for other things in cells. It's not just used to make cholesterol. It's also used to make a class of molecules called isoprenoids.

Isoprenoids are important. And you'll encounter them if you study signaling or anything like that because these make things like the farnesylation lipid modifications that are oftentimes added to the membrane associated proteins, like signaling proteins. And so statins will also block the production of these molecules that are important for these various signaling proteins.

And so you should have some understanding of what mevalonate is, where it comes from, that it's involved in cholesterol and isoprenoid synthesis, and that it's the target of statins. And of course, you didn't need a whole lot. We already have all the tools to understand that pathway. And if you look up isoprenoid synthesis, cholesterol synthesis, you'll see you'll have the tools to understand that as well.

The last thing I want to talk about is taking the acetoacetyl-CoA. And if I take the CoA group off, what I'm left with is a molecule called acetoacetate, OK? This is basically two acetyl-CoAs brought together losing CoAs times 2.

OK. So this here is acetoacetate. And acetoacetate is a canonical ketone body. What is a ketone body?

Well, it turns out ketones are an alternative fuel that the body can use to glucose, primarily the brain. And so for reasons that no one really understands, a quirk of human and mammalian physiology, is that the brain prefers to use glucose as its fuel. If glucose is not available, it doesn't use fatty acids. It uses ketone bodies instead.

And so the ketone is acetate is the canonical ketone body. This underlies the keto diet, which has become very popular. And so what is the keto diet?

Well, the keto diet is you don't eat any carbohydrates. And so if you don't eat carbohydrates, you don't have a source of glucose. The liver, of course, can do gluconeogenesis. But if you run out of things to make and turn into glucose, now the liver gets into trouble.

Remember, we talked about with anaplerosis of the TCA cycle. There's no way to turn two-carbon units that are made from fat, acetyl-CoA from fat-- mammals cannot turn that back into glucose because we don't have the glyoxylate cycle. And so we can't turn fat into glucose.

And so when the liver can no longer make glucose, it will take acetyl-CoA from the breakdown of fat and make ketones instead. And the ketone is this acetoacetate. People do the keto diet because ketones, like acetoacetate, will suppress appetite. And that's at least how one way it's thought to work.

But I want to talk about what these ketones are and how they fit into metabolism. So here's acetoacetate. If you notice, acetoacetate is a beta-keto acid. Alpha, beta, the ketone is beta to the acid group.

So a beta-keto acid, as I've said now many, many, many times, beta-keto acids can undergo decarboxylation that generates the enol, which can be rearranged to the keto group. And in that case, what's the keto group? Well, the keto group would be this molecule, which is acetone, fingernail polish remover.

If you go to medical school, you'll learn that, if type 1 diabetics have a state of a physiology called ketoacidosis, we don't have time to go into what drives that state, that physiological state. But it's basically a state with very high ketones. They smell like acetone.

The reason is because they have very high levels of acetate, the ketone body. And some of that spontaneously carboxylates to make acetone, all right? The body does this, though. It's making the acetoacetate as a way to give food to the brain.

But because acetoacetate is unfavorable, that's not the form that is put into the blood. Instead, it undergoes an oxidation reduction reaction where this ketone is reduced to the alcohol or interconverted between the ketone and the alcohol. Something gets oxidized. Something else gets reduced. NAD, NADH is the donor. And that leads to this molecule, which is called beta-hydroxybutyrate.

And beta-hydroxybutyrate is the canonical ketone that circulates in your blood. And measurement of beta-hydroxybutyrate levels really tells you if you're really doing the ketone diet properly. So basically, your liver, if it doesn't have enough glucose, will have to break down fat.

It can't turn the acetyl-CoA from fat into glucose. So instead, it'll turn it into acetoacetate, which will convert to the more stable beta-hydroxybutyrate. And then the more stable beta-hydroxybutyrate can be oxidized by peripheral tissues, predominantly the brain, as an alternative source of fuel to glucose when the liver can't maintain glucose.

To why this happens this way, no one really knows why it uses ketones instead of that. But it fits with all the physiology, and it also fits the metabolism that we've already learned about to see why it works this way. I just want to point out, for those of you who are doing the keto diet, to do it properly.

Yes, you have to limit carbohydrates. That's the most popular way to do it. But to really make this work, you also have to eliminate other sources of carbon that can be used for gluconeogenesis, mostly amino acid, so lowering protein.

So a true ketogenic diet is really all fat because that is what basically makes acetyl-CoA really the only carbon source that the liver can use. And then it has to only generate ketones, beta-hydroxybutyrate, which then circulates as an alternative oxidizable carbon source for the brain. OK. Thanks so much.