[SQUEAKING]

[RUSTLING]

[CLICKING]

MATTHEW VANDER HEIDEN: So last time, we discussed the TCA cycle. And that allows us to then, you know, say how we can take glucose and completely oxidize those six glucose carbons into CO2. And you know, of course, glycolysis converts glucose into two pyruvate. Pyruvate has two carbons, and then pyruvate dehydrogenase can release the first of those carbons as CO2. It generates an acetyl CoA. That acetyl CoA then enters the TCA cycle, and two other CO2s are released-- one of the isocitrate dehydrogenase step, one of the alpha ketoglutarate dehydrogenase step.

Now, we discussed that the TCA cycle is useful because it allows you to oxidize anything that can be turned into acetyl CoA into CO2, and that includes fatty acids, which we will spend a lot of time talking about today. Now, the TCA cycle, as I mentioned, just by review, is also very useful as a way to make stuff-- lots of useful intermediates. But we discussed last time if we're going to do that-- that is, because it functions as a cycle-- if we remove stuff from the cycle, something has to be added back in-- so-called anaplerosis-- in order to have it continue to function as a cycle.

Now, you'll note-- we weren't explicit about this, but this is oxidation. Remember, carbon oxidation is generally favorable. So delta G of this will be less than 0. However, unlike glycolysis, where we discussed that most of the harnessing of energy-- that is, the favorable oxidation of glucose to pyruvate-- was captured to directly keep an ATP/ADP ratio high in the cell, you can see that most of the energy output of the TCA cycle isn't actually direct synthesis of ATP or GTP. Instead, what we have is we have most of that energy as being captured and charging up a ratio of NADH to NAD, or FADH2 to FAD. And we'll see over the next several lectures how we can harness electron transfer from those molecules to oxygen to make ATP, as well as do some other work.

But before we get to that, I want to first focus a bit on fat, which, of course, is the most reduced carbon biomolecule in the cell. And so that's chains of fully reduced carbon. And of course, this is the most chemically dense way to store energy-- as carbon. Intuitively, you know this, because what's oil and gas? It's chains of reduced hydrocarbons. Of course, those are better fuels than wood, which are, as we saw before, based on carbohydrate alcohol carbons. You also know intuitively that fat has more calories than sugar-- exactly same ideas.

And so I want to start by discussing what biological fat is, and then that will lead into a discussion about how we can oxidize the fat, also, as a way to get energy. Now, most biological fat is packaged into molecules called lipids. And so I want to make clear that lipids are not the same thing as fat. More correctly, a lipid contains fat-- or more precisely, something called a fatty acid.

And so what is a fatty acid? Well, it's this fully saturated hydrocarbon with a carboxylic acid on the end. And so we have a carboxylic acid followed by some chain of fully saturated hydrocarbons. So it differs from oil and gasoline in that it has this carboxylic acid handle, if you will, on the end that basically allows biology to build and break down these fatty acids.

Now, most biological fatty acids have even numbers of carbons. And it turns out that that's a consequence of the fact that it's built and broken down into these two carbon acetyl CoA units. And the most common lengths-- that is, how long these chains are. So in biology, they can vary anywhere from 4 to 36 carbons long. But the most common ones are 12 to 24 carbons, with 16 carbons and 18 carbons being by far the most abundant in cells.

Now, it's worth mentioning what some of the names of these more common ones are, because you'll see them. And so the fully saturated 16 carbon fatty acid-- so 16 carbon total. 14, 15, 16, including the carboxylic acid. This is referred to as palmitate. Or it's drawn in the acid form, palmitic acid. And the systematic name for this is hexadecanoic acid.

The other common one, the 18 carbon version-- so same molecule, but two carbons longer. That's referred to as stearate, or stearic acid, if drawn in the acid form. Or the systematic name would be octadecanoic acid.

Now, oftentimes things like palmitate can also be drawn like this. So there's 16 carbons. Another way to draw palmitate. And you'll note that palmitate as well as stearate here are fully saturated. What do I mean by saturated?

I mean fully saturated by electrons. There's no way to reduce this molecule further, unless, of course, we reduce the carboxylic acid on the end. And so palmitate would be the most saturated 16 carbon fatty acid. Stearate is the most saturated 18 carbon fatty acid.

Now, if I add a double bond to one of these molecules, that's an oxidation. So just like when we added a double bond to succinate to make fumarate in the TCA cycle. I showed that last time. That's an oxidation reaction. And so an unsaturated fatty acid is a fatty acid that is no longer fully saturated with electrons. That means it is not fully reduced.

And so, remember, you store energy as reduced carbon. And so an unsaturated fatty acid stores less energy than a saturated fatty acid because it's more oxidized than the saturated fatty acid. And so just as a couple examples here-- so here's the example of a 16-carbon unsaturated fatty acid. So I introduce a double bond. I'll draw it in the acid form here. And so here you got 5, 6, 7, 8 plus 7 is 15-- 16 carbons total, one double bond. This is all middle oleic acid, more systematically named hexadecene acid. And the 18-carbon version would be as follows.

So I added two extra carbons on this end of the molecule. This is oleic acid, or octadecanoic acid. Now, note I put a double bond in here. You'll remember from organic chemistry that double bonds can exist in a trans or a cis form. And so if it's trans, it would be like this. If it's cis, the double bond would be like that in the carbon chain.

And so just to show you here on the models that this can matter, so here is a double bonded carbon. And so these two red guys are cis to each other. And this purple is trans to that. Now, if I put this onto a fatty acid chain, you can see that there's a big difference there. So if I add the double bond in here and it's continued the chain here on a trans bond, it's a relatively straight molecule, whereas if I go here in a cis bond, I now introduce a kink into the alkyl chain.

So biological fatty acids are always cis. And this is structurally important because it actually creates this kink in fatty acids that are not fully saturated. Now, obviously, as you might guess, the number of double bonds and the locations of the double bonds will change the structure and therefore the properties of the fatty acids. And so we need a nomenclature that allows us to describe what's going on here.

And so the simplest nomenclature is as follows, where we basically have the number of carbons and the fatty acids, say 18, followed by the number of double bonds. So an 18:1 fatty acid, that's oleic acid-- so 18 carbons long, one double bond-- 18:0 would be steric acid-- 18 carbons long, zero double bonds; 16:0, palmitate-- palmitic acid-- 16 carbons long, zero double bonds. So if we have one double bond, oftentimes this is referred to as a MUFA, or monounsaturated fatty acid. And, of course, if I have many double bonds-- more than one-- that's a PUFA, or a polyunsaturated fatty acid-- probably heard those terms.

So what's an example of a polyunsaturated fatty acid? Well, a common one is an 18:2 polyunsaturated fatty acids-- 18 carbons, two double bonds. The common biological one is called linoleic acid-- and CH3-- and so 1, 2 double bonds, 4, 5, 6, 7, 8, 9, 10, 17, 18 carbons total, 18:2 fatty acid, linoleic acid-- formal name is octadecadiene oleic acid. If I make it more unsaturated-- so 18:3-- that is linolenic acid, or octadecatrienoic acid.

And if I painfully draw out the entire thing-- so we've got 1, 2, 3, double bonds, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 17, 18 carbons total-- 18:3 polyunsaturated fatty acid with three double bonds in it. In all of these cases, all of those double bonds are cis double bonds. And you'll also notice that the spacing of them is not entirely random either, at least as I drew it. The first of all is that these are not conjugated double bonds, so you see that there's a carbon in all cases between the double bond.

And also, you'll notice that most of the double bonds are actually started, if you count from this end-- from the acid end-- this would be carbon 9 and 10-- so 1 plus 7 is 8, 9, 10. Each of them-- the first double bond I put in is between carbons 9 and 10 going in this direction. And that's a consequence of the conserved enzymes that introduced these double bonds into the molecule.

Now, obviously this spacing matters. And so we need a way in the nomenclature in order to denote that. That is where exactly the double bonds are located. And there are sort of two ways. We can count we can count from one end. Or, we can count from the other end.

Now, the most systematic way-- correct way now is to count from the carboxylic acid end. So very similar to sugars, we counted carbon 1 of sugars to be the one that was closest to the end of the molecule where the carbonyl was located. So same thing in fatty acid, so this would be carbon 1 count in that direction. And so the nomenclature would be then to put it in a delta 9, would be a double bond between the 9th and 10th carbon counting from the carboxylic acid.

And so the 18:2 fatty acid I drew here, linoleic acid, would more precisely be 18:2, delta 9, delta 12. That's because it's carbon 9, 10, 11, 12-- so carbon 9 and 10 and 12 and 13. Linolenic acid-- 18:3, delta 9 12, 15-- so 9, 10, 11, 12, 13, 14, 15 to say where the double bonds are in the polyunsaturated fatty acids. As you can see, most polyunsaturated fatty acids is a consequence that they're not conjugated. You'll see that the double bonds are placed every three carbons.

Now, this three carbon spacing is maintained even in the-- often in biology, even in the exceptions, where the first one is not between carbon 9 and 10. And so a classic example of that is the polyunsaturated fatty acid arachidonic acid, which is a 20 carbine fatty acid with four double bonds at carbons 5, 8, 11, and 14. So arachidonic acid, you may have heard in the past, is a key signaling fatty acid. It's mobilized and used to generate some inflammatory mediators.

It's the enzyme that acts on this to generate the inflammatory mediators-- is the target of very common drugs, like aspirin or other nonsteroidal anti-inflammatory drugs-- ibuprofen, et cetera. And this molecule, drawing it like this, should fully describe to you what it would look like. Now, there's also an older nomenclature that numbers fatty acids to name them and where the double bonds are located-- not from the carbonyl side, but from the other end of the molecule. And I mentioned this nomenclature because it's still used quite often in popular culture.

And so to do this, it's basically-- refers to this, I guess, as the alpha carbon. So counting in Greek, going this direction, the final carbon in the fatty acid, omega-- the last letter the Greek alphabet. And so if you count from the other end, we could also give this different nomenclature. And so in this case, our 18:3 linolenic acid, 18:3, delta 9, 12, 15, we could also say is 18:3, omega 3, 6, 9. So now we're counting from this end-- 1, 2, 3, first double the bond, 4, 5, 6, next double bond, 7, 8, 9, next double bond.

And so thus, 18:1, by this nomenclature-- 18:1, delta 9, would be the same as 18:1, omega 9 because it's obviously meeting in the middle. But if we did 18:1, delta 12, that would be 18:1, omega 6. 18:1, delta 15 would be 18:1, omega 3. And so I mention this because you'll hear about so-called omega 3 fatty acids. The major omega 3 fatty acid that they're talking about is linolenic acid. And it's an omega 3 fatty acid using the nomenclature counting from the other side. Or, you could also say it's 18:3, delta 9, 12, 15.

Now, I want to mention one of the reasons you hear about omega 3 fatty acids is that, in general, there are fatty acids such as those that humans cannot make. And so we have to get those from the diet. And so this is the concept called essential fatty acids. And so somewhat like vitamins, there's things out there that our physiology uses that we have to get from other organisms making. And therefore, we have to eat them. And so if you hear this term essential fatty acid, it's basically referring to specific fatty acids where we lack the enzymes to place all the double bonds to be in the place where it's useful for some aspect of our biology.

Now, lots of nomenclature here-- the reason I discuss this is because this diversity of chain length and double bonds creates different properties and nature uses these diverse chemical properties of the fatty acids-- that is, different links and different degrees of unsaturation or saturation positions of the double bonds-- to take advantage of those properties to do different things in biology. And so a lot of this has to do-- a lot of why fatty acids are useful is that they are not soluble in water or poorly soluble in water. You know this just from the common experience of making salad dressing. And so you mix the oil in the vinegar in the salad dressing, and so the oil is largely made out of fatty acids, made out of lipids. And those fatty acids are not soluble in the vinegar part, the water part of the molecule.

Now, the fatty acid themselves-- the chain length and the number of double bonds will affect other properties, such as the melting temperature. So in general, the melting temperature of a fatty acid will decrease with shorter chain length and decrease with more unsaturation-- so more double bonds. So the more double bonds I put in and the shorter it is, the lower the melting temperature. And so you guys know this, from just cooking experience, to be the case.

And so animal fatty acids tend to be more saturated. And because they're more saturated, they have a higher melting temperature. And so they're solids at room temperature. So think about it-- animal fat, butter, lard-- these things are solid at room temperature. So plant fatty acids-- they tend to be more unsaturated fatty acids. And they're liquids at room temperature.

And you know this because cooking oil made from plants is typically a liquid at room temperature. Now, you might be aware that olive oil has a lot of monounsaturated fatty acids, a lot of MUFAs. Olive oil, unlike other plant oils, which are more polyunsaturated fatty acids-- if you put those in the refrigerator, the olive oil will tend to form a solid, whereas your canola oil will not. And that's a consequence of the fact that the more unsaturated fatty acids are, the lower the melting temperature. And so the more likely it is to be a liquid or a solid in the fridge or at room temperature across these different fatty acids.

To show here on the slide, here's just something I stole from the textbook. It basically gives the composition of some things you might be aware of-- so olive oil, butter, and beef fat. So you can see as we go down the spectrum here, you have longer chain lengths and a reduction in the number of double bonds. And, of course, beef fat, if you've ever handled it, is a much firmer solid at room temperature than butter, which is of course solid at room temperature, whereas olive oil is not. And that follows this with the chain length and the double bonds really affecting the melting temperature of these different fats.

Just a couple of sides so you can better understand your food-- and so you may have heard about or seen on the side of your food packaging hydrogenated oils. So what's a hydrogenated oil? Well, that's basically taking a plant oil, which has unsaturated fatty acids and hydrogenating it. That is adding hydrogen.

Well, what is that? It's not really the hydrogen that's being added. It's adding electrons so it's taking it from being a unsaturated fatty acid to chemically making it a saturated fatty acid. And it's a way to take plant oils and make it such that it's solid at room temperature. An example of a hydrogenated oil would be margarine-- plant oil that would be liquid, reduce it chemically such that it's fully saturated, and now it's a solid at room temperature.

Sure you've also heard of so-called trans fats. So what's a trans fat other than something cooked up in the devil's kitchen? So trans fats are basically taking animal fat and introducing chemically double bonds into them such that you have this solid that is now a liquid at room temperature. Now, this is done chemically to introduce those double bonds. And so if you're introducing a double bond chemically by oxidizing the fatty acid, you'll get some cis and some trans.

Cis is what biology does because it introduces them with an enzyme. Trans versus cis is not controlled when it's done chemically. And so this leads to these unnatural trans fatty acids, which lead to health issues and are now banned in many cities, including here in Cambridge, Massachusetts. Last aside is-- as all of us have probably experienced our oils or fats going rancid-- so what is that? So that's oxygen oxidizing the fatty acid. And so if you want to protect your oil from going rancid, the thing to do is just keep it sealed, right? Keep oxygen away from it and your oil will last a lot longer.

So that's fatty acids. In biology, most fatty acids aren't sitting around by themselves. They're esterified to an alcohol. And as we mentioned in an earlier lecture, a lipid equals a fatty acid that's esterified to an alcohol.

So we spent some time in the prior lecture talking about doing this to make phospholipids. And that's an example of a lipid-- fatty acids esterified to make this phospholipid, which gave us both a polar and a nonpolar end and allowed us to create membranes. And so lipids have lots of important functions in cells. And so there's the barrier function, which is basically membranes, things like phospholipids we talked about last time. There's also signaling functions of lipids.

So I mentioned arachidonic acid earlier. You'll certainly encounter signaling functions of lipids in other courses. And then the last one, which is really the reason why we talk about it now, is lipids are great for energy storage because it's the most reduced carbons, so the most dense way to store energy as reduced carbon. So the simplest lipid and the one used for energy storage is referred to as a triacylglyceride, often abbreviated as a TAG-- triacylglyceride. And we can see now how we can make a triacylglyceride as well as how it relates to other pathways in metabolism that we've encountered.

And so here's our old friend from glycolysis. Hopefully you recognize this molecule as the phosphorylated triose dihydroxyacetone phosphate, also an intermediate in glycolysis. And so if we reduce this ketone to the alcohol-- so two electrons from NADH. Reduce that ketone, that will oxidize the NADH to NAD+. If we also remove the phosphate, now what do we have? Now we have the alcohol.

And this molecule is glycerol. And so now this glycerol, as we talked about before, we can take three fatty acids, esterify them to each of those alcohols. And now we have a glycerol molecule with three fatty acids esterified to the alcohols on the glycerol. This is a triacylglyceride. Now, of course triacylglycerides are not soluble in water.

And so when we make these in cells for energy storage, they're stored as so-called lipid droplets. And so if you look here at the slide, here's an example. This up here is an adipocyte from an animal. This down here, I think, is a plant cell.

And so in both cases, you have these large droplets that would be basically droplets of triacylglycerides that basically form as a way for long-term energy storage. And so while we think of, at least as people, our adipocytes, our fat cells, as storing our fat, they do. But they're really specialized cell types that have these giant lipid droplets, whereas many cells actually have much smaller lipid droplets as a way to store triacylglycerides as a way to store energy as reduced carbon.

I also want to say that if you look at this, unlike the phospholipids that we described earlier as ways to build membranes, these don't have a charge on them. And so they're sometimes referred to as so-called neutral lipids. And it's really this neutral lipids that allow them to clump together in these oil particles, if you will-- these lipid droplets in cells that are good for energy storage. Now, why specifically are neutral lipids good for energy storage? Well, really chemically dense way to store energy, the most reduced carbon.

And by forming these droplets, not unlike the oil in your salad dressing, another neutral lipid forming droplets within the vinegar, this is a way that you store reduced carbon without having to carry around water. And so if we store energy as carbohydrates, starch or glycogen, these molecules have to exist in water. So you're carrying around the starch and glycogen.

But as an animal, we're also carrying around the water. If we're carrying around reduced carbon as lipids, we can now exclude the water from it. And so it's much more dense. And we can, in a more efficient way, carry around this material without having to carry the water. And so gram for gram, fat is a much more efficient way to pack on calories that we can burn later than storing it as carbohydrate.

Fat's also nice because it forms a nice insulator. And so it makes sense. As animals, we need to survive the winter-- pack on all kinds of calories as fat, don't have to carry around as water. Just have the energy there as reduced carbon, and then we can slowly release it over the course of the winter, as well as use it to keep us warm, and then build up those stores again during the summer months when there's more food available.

Fat, of course, has months worth of energy packed into it, whereas carbohydrates that we carry around-- our glycogen-- has less than a day's worth of energy. So we could live off of our fat for the winter. We can't live off of our glycogen for the winter. Now, the big trade-off here is that it's much slower to mobilize fat. We have to get into these lipid droplets and break off little pieces of it, get them into aqueous water soluble pieces to break it down. Glycogen, of course, is already in water, can break it down much faster.

And so it's much slower to mobilize the fat. But it can be much more efficient in terms of what's stored, whereas glycogen can be mobilized a lot faster. And that's really part of our physiology. You will burn your glycogen first when you exercise before you start burning a lot of fat.

And I think many have heard about that from just reading and thinking about what you know about exercise physiology. Now again, I want to make the point that we all know that fat has more calories than sugar. And the reason for that is, to be clear, is because it's more reduced. And so the energy that's released from burning fat, just like the energy that's released from burning sugar, comes because the transfer of electrons from the reduced hydrocarbon to oxygen is favorable. And that is how energy is released.

And so fat is more reduced than sugar, and so more electrons to transfer, and so more energy released than burning sugar. So now let's go through, in a pathway sense, how does nature, rather than taking gasoline and just igniting and releasing a lot of energy, how does nature stepwise break down fatty acids in a way that energy release can be controlled in the same way we described it for carbohydrates-- glycolysis in the TSA cycle, controlled stepwise energy release that can be captured to do things like make ATP. How does the same thing work for oxidation of fatty acids? Well, if as organisms we store fatty acids in these triacylglycerides or other neutral lipids, the first step is we have to get them out of the neutral lipids.

And so if we start with a triacylglyceride, the first step is to use a lipase molecule. So a lipase just breaks that ester bond. And so you basically now have glycerol plus the three fatty acids. We're going to spend most of our time today discussing how you break down the fatty acids.

However, I want to mention, it should be clear how you're also going to metabolize the glycerol. So glycerol-- we can oxidize the glycerol. So now if we oxidize that alcohol, what are we going to get? We're going to get dihydroxyacetone. And then if we phosphorylate one of the ends of that with ATP, now we get dihydroxyacetone phosphate. And that, of course, can go into glycolysis, generate pyruvate, generate acetyl-CoA, oxidize that acetyl-CoA in the TCA cycle, get energy from the glycerol part of the lipid.

So what about oxidizing the fatty acids? Well, the first thing we have to talk about to oxidize the fatty acids is, where is this going to occur in the cell? And so lipid droplets are floating out there in the cell. And so you mobilize these pieces with the lipase. Well, the glycerol now is sitting there in the cytosol. That can form the hydroxy dihydroxyacetone phosphate and be burned in glycolysis, which is in the cytosol.

But once we generate that pyruvate-- remember that pyruvate had to get into the mitochondria where we turned it into acetyl-CoA. So acetyl-CoA was present in the mitochondrial matrix. So that is here in the matrix of the mitochondria where the TCA cycle happens-- remember, glycolysis in the cytosol, TCA cycle in the matrix. And so that pyruvate eight needed to get into the matrix.

So we could turn it into acetyl-CoA. And then that acetyl-CoA was in the right place to be entered into the TCA cycle and turned into CO2. Well, fatty acid oxidation has the same thing.

And so if those fatty acids are generated out here in the cytosol when they're lipases remove them from the lipid droplet, it turns out we're going to burn the acetyl-CoA we get from the breakdown of the fatty acids. That acetyl-CoA needs to be in the matrix so it has access to the TCA cycle. And so we need to get the fatty acid from the cytosol inside the mitochondria. Now, part of this-- remember, our CoA group that I drew last time is this giant molecule. And so acetyl-CoA is not this little two carbon unit. It's this big, giant molecule.

And so by generating the acetyl-CoA in the mitochondria, we obviate the need to get this giant CoA group across the mitochondrial membranes. But, of course, we do need to get the fatty acid into the right location as well. And so the way that that fatty acid is transported there is actually via a system called the carnitine shuttle. But there's sort of a little bit of a roundabout way to do it.

And that is, we are going to need to do two things-- get the fatty acid inside the mitochondria as well as activate i with this coenzyme A group. And it turns out that the fatty acid is first activated in the cytosol by adding the coenzyme A group to the acid on the end, very much like acetyl-CoA. And so here is some generic chain length fatty acid. And it turns out that there's an enzyme called acyl-CoA synthetase that is going to take ATP and adenylate this acid group on the fatty acid.

And so this is basically an AMP that's been used to adenylate the fatty acid itself. Now, you'll notice by doing this, we generate a pyrophosphate. And that pyrophosphate can be turned into two inorganic phosphates-- same trick we've seen before that basically can pull a reaction that otherwise would be unfavorable forward. In this case, it's adding the CoA group to this fatty acid. And what happens next is that CoA comes in and replaces the AMP such that you generate this fatty acyl-CoA molecule, which is basically acetyl-CoA but with some arbitrary longer a number of reduced hydrocarbons in the chain-- so not a 2-carbon unit, but a fatty acid-- a many carbon unit fatty acid with whatever other properties happen to be on fatty acid, where now you have this fatty acyl-CoA instead of the fatty acid.

So it turns out this fatty acyl-CoA then is subjected to a shuttle called the carnitine shuttle to actually get it into the mitochondria. And so what is carnitine? So carnitine is a small molecule-- looks like this.

So this here is carnitine. And it turns out that this hydroxyl group, basically, is swapped for the CoA on the fatty acyl-CoA. And so if this here is some generic fatty CoA, you end up with this molecule, which is called a fatty acyl carnitine.

So all I've done is take the CoA off and move the fatty acid to make this ester here with the alcohol on the carnitine. And this fatty acyl carnitine can now be transported across the mitochondrial membrane-- so from the cytosol to the mitochondria. Here we have our fatty acyl carnitine. And then that fatty acyl carnitine can exchange a CoA for the carnitine and regenerate the fatty acyl-CoA in the mitochondrial matrix.

This whole process is referred to as the carnitine shuttle and is effectively a complex way to move fatty acyl-CoAs from the cytosol into the mitochondria, where they can be oxidized. Here's another picture of it that is maybe drawn in a different way because it's a little bit confusing as something to describe, but basically you generate this fatty acyl-CoA in the cytosol. And then the fatty acyl-CoA is exchanged for a fatty acyl carnitine. Fatty acyl carnitine goes into the matrix and is used to regenerate the fatty acyl-CoA.

The enzymes that do this is something called CPT, or carnitine palmitoyltransferase. Write that down-- carnitine, which is obviously refers to palmitate as the common fatty acid, although CPT will catalyze many fatty acyl carnitine, fatty acyl-CoA interconversions. And so by doing that interconversion on the cytosolic side and the mitochondrial side, you can use carnitine as a handle to transfer fatty acyl-CoAs from one compartment to another.

Now, once that fatty acyl-CoA is in the mitochondria, it can now be oxidized to acetyl-CoA. So now, let's discuss the series of steps that is referred to as fatty acid oxidation, and abbreviated FAO. And so, of course, we start here with our generic fatty acyl-CoA in the matrix of the mitochondria of some arbitrary chain length.

And so the first thing that we're going to do is oxidize this carbon-carbon bond-- that is, introduce a double bond here. That's an oxidation reaction. It's exactly the same reaction that we saw convert succinate to fumerate in the TCA cycle.

That reaction used FAD as an electron acceptor. So we oxidize that carbon-carbon bond, FAD gets reduced to FADH2. This is carried out by an enzyme called acyl-CoA dehydrogenase and generates that intermediate.

Again, now just like we did in the TCA cycle when we converted fumerate into malate, we added water across this double bond. Do exactly the same thing here. That generates this intermediate. Now we can oxidize this carbon here, this alcohol-- oxidize it to the ketone.

Of course, you know how to do this. We've now seen it a million times. So that generates this hydride ion, which can be transferred to NAD+, reducing it to NADH.

And now what happens next is CoA basically breaks that carbon-carbon bond. And what are we left with? We're left with over here release of a acetyl-CoA group, which can then go down and be further oxidized in the TCA cycle as well as this fatty acyl-CoA that is two carbons shorter than the one we started with. That can then go back up, repeat the cycle again until, if you start with an even number of carbons, the last one leaves you with two acetyl-CoA molecules.

And so per two carbon units that we run through this cycle of fatty acid oxidation, what we get is we get an acetyl-CoA that comes out, we get an FADH2, and we get an NADH. Now, of course, if we start with an unsaturated fatty acid, we don't have to introduce a double bond into it, we don't get the FADH2, we get less energy produced from that molecule. And so if this acetyl-CoA goes on and enters the TCA cycle and we fully oxidize it to CO2, we get three more NADH, we get another FADH2, and we get a GTP. It's written up there as a reminder.

And so with a fully saturated per two carbons out, we basically get four NADHs, two FADH2s, and a GTP. And that's a fair amount of energy, if you will, released from the oxidation of this fatty acid all the way to CO2. We'll come back to that accounting in a second. But first I want to say, what happens if you happen to start with an odd number of carbons.

So I mentioned that most biological fatty acids have even numbers of carbons. But there are odd chain carbon lengths of fatty acids. Some bacteria make these. Sometimes it just happens. Obviously, if you do that, you'll end up at the end with this molecule, which is a 3-carbon acetyl-CoA called propionyl-CoA.

And so cells need a way to deal with this 3-carbon propionyl-CoA. Well, the way they deal with it is they carboxylic it. So if we're going to add a CO2 to a molecule, how do we do that? Well, we need a co-factor.

Remember, we did this with the pyruvate carboxylase reaction. This was the reaction of biotin. So we started with bicarbonate. We phosphorylated the bicarbonate. And then that phosphorylated bicarbonate, there was biotin in the active site of the enzyme. That released the phosphate. I had enzyme biotin with a CO2 on it.

And so if we take this propionyl-CoA here, which is drawn in the keto form, and we redraw it in enol form of the propionyl-CoA, we carry out similar reaction that we saw before. And now we end up with this molecule. So, effectively, adding the CO2 to this carbon to the second carbon in. And that's this molecule, which is called methyl-malonyl-CoA. I'm going to redraw methyl-malonyl-CoA just to show you how cells deal with this.

So this is methyl-malonyl-CoA. I just re-drew it in a way that there's some colors on the different molecules. What I'm going to do is I'm basically going to take this group and this group and swap their positions. And if I do that, I will end up with this molecule, which hopefully you recognize as succinyl-CoA from the TCA cycle.

Now, the mechanism for how that swap happens, I don't have time to go into. But this complex intermolecular rearrangement requires a cofactor. That cofactor comes from vitamin B12. And so here's a picture of vitamin B12. You can see by looking at it why I don't want to draw that out for you.

It's a cobalt-containing cofactor-- sometimes referred to as cobalamin. If you're interested, you can look up the chemistry for how vitamin B12 helps catalyze this intermolecular rearrangement to go from methyl-malonyl-CoA to succinyl-CoA. But the important take home is that if you have an odd chain fatty acid, use a biotin-containing enzyme to carboxylate propionyl-CoA to methyl-malonyl-CoA, and then B12 to rearrange that methyl-malonyl-CoA to succinyl-CoA. And now this can enter the TCA cycle and be oxidized as well.

So now you have the details of how you can start with a fatty acid and oxidize it to CO2. Now, I think it's useful if we compare the output of what we can get from glucose oxidation to CO2-- carbohydrate oxidation to CO2-- to what we can get if we start with a fatty acid and oxidize it to CO2 and sort of illustrate that there are more calories in fat than there are in sugar. And so for this comparison, we'll compare glucose, which, of course, has six carbons in it to a 6:0 fatty acid-- also six carbons.

And what do we get if we take this less reduced versus more reduced molecule and fully oxidize it to CO2? Well, if we start with glucose-- so if we take that glucose and we run glycolysis-- and so take that glucose and turn it into two pyruvate molecules, of course we get from that two ATPs and two NADHs. And then if we take those two pyruvate molecules and turn them into two acetyl-CoAs plus two CO2s-- that's the pyruvate dehydrogenase reaction-- we get two more NADHs. And then if we take those two acetyl-CoAs and turn them into four CO2s, that's the TSA cycle, what do we get out?

We get two GTP molecules. We get two FADH2 molecules from the succinate dehydrogenase step. And we get 2 times 3 equals 6 more NADHs. And so our total yield is 4 ATP equivalents-- remember, the GTPs can be interconverted with ATP-- two FADH2s, and 6, 7, 8, 9, 10 NADHs.

What if we start with our 6:0 zero fatty acid? Well, to metabolize it, first we have to take that fatty acid and we have to make our fatty acyl-CoA. That's going to cost us two ATP. Why two ATP? Because remember when we charge that fatty acetyl-CoA, which is now erased, I think-- yes, it is-- we converted an ATP to an AMP. And so that's two ATP equivalents to charge that fatty acid to a fatty acyl-CoA. Next, we take that fatty acyl-CoA and we turn it into three acetyl-CoA molecules via fatty acid oxidation cycle.

That's two trips around to generate the three acetyl-CoAs. So that will give us two FADH2s and two NADHs. And then once we have those three acetyl-CoA, we can turn them into six CO2s in the TCA cycle. So that's three GTPs, three FADH2s. And 3 times 3 is 9 NADHs for a total yield of 3 minus 2 is 1 ATP equivalent, 5 FADHs, and 11 NADHs.

Now, I went through this exercise because it's not immediately apparent that the yield of fatty acid oxidation gives you more energy from full oxidation than oxidizing the equivalent carbohydrate, at least in terms of ATP. You get four ATPs directly from full oxidation of glucose, whereas you only get one ATP equivalent directly from the complete oxidation of this 6-carbon fatty acyl-CoA. Now, I say this because most of the energy, if you will, that's released from oxidation of fat and sugar isn't actually directly producing ATP. It's actually being used to charge up this NADH, and NAD+, or FADH2/FAD ratios in the cell, which we will see is also a lot of energy, because those electrons can be transferred to oxygen and be used to do other work down the road.

Now, your book will tell you that each of these NADHs or FADH2s are worth on the order of one to three ATP. And I guess this kind of fits intuitively what you might guess based on the thermodynamics of the GAPDH reaction. So if you go back to that, remember our oxidative phosphorylation we described at GAPDH roughly generated an ATP.

And so even if we say one ATP equals an FADH2 or an NADH, which, of course, there's not a direct relationship to that, you can still, even with that, say that our fatty acid oxidation-- if we add up all these numbers, we would get 17, whereas if we add up all these numbers, we get 16. So I guess that says that there's more coming from fatty acid oxidation than from complete glucose oxidation. But you probably also learned in high school or somewhere else that NADH gives you more ATP than FADH2.

That's true. Our goal is to understand why that's the case. And so those numbers will only get better for fatty acid oxidation in terms of ATP equivalents when we can describe how to do those conversions. However, to really appreciate how these electron carriers equal energy and equal biological energy, we really need to go back and revisit some concepts in bioenergetics and thermodynamics to really understand what biological energy is. And that will also help us understand mitochondrial oxidative phosphorylation, which is really the process that allows us to interconvert these electron carriers and their ability to transfer electrons to oxygen as a way to generate favorably synthesized ATP.

So hopefully, you will remember from our previous lectures that for any reaction, any pathway, any process to occur, it has to be thermodynamically favorable-- that is, delta G has to be less than 0. And remember, ATP to ADP was useful because that reaction is very favorable. And so we could couple ATP to ADP conversion to otherwise unfavorable reactions. And that is what allowed us to have ATP be useful.

And it was useful because delta G equals delta G naught prime plus RT times a log of the products of the reaction over the reactants of the reaction. And so if ADP is a product in ATP is a reactant, it was actually that ATP/ADP ratio that was providing the energy, if you will, to drive the reaction. This was also, if you recall, why we described that oxidation of reduced carbon because it was favorable, was able to be coupled to reactions that keep this ATP/ADP ratio high, so that that high ratio could then support otherwise unfavorable reactions.

But now, you hopefully appreciate that in reality, most of the energy from carbon oxidation is not directly captured as ATP. It's being used to charge up these other ratios-- NAD/NADH, FADH2/FAD. And the energetics of doing that follow exactly the same rules as ATP or really any other reaction. And effectively, it's the transfer of electrons that is favorable or not that really is going on here, just like we discussed for ATP to ADP. And so we can couple other reactions to those ratios as a way to make other reactions possible.

Now, ultimately it turns out that these things, these redox ratios, are more useful than ATP because these electron transfers-- favorable electron transfers-- remember, biological energy is all about oxidation and reduction-- can be used to drive ATP. We'll see that when we described how OXPHOS in the mitochondria really works. But it can be used for other things as well. We'll see we can use it to make heat. We can do it other work, like move ions, et cetera.

And so-- we can even make glucose, right? gluconeogenesis. We needed a source of NADH. And so ultimately, all biological energy, of course, has to come from the sun. And photosynthesis also is about capturing solar energy as these oxidation and reduction pairs.

And so if we appreciate this, what we realize is that it's really these transfers of electrons. Remember, there's no free electrons in biology. And so it's really coupling oxidation and reduction reactions that are favorable that ends up being how bioenergetics largely works. Now, I like to be explicit about this because sometimes people get confused by oxidation and reduction reactions and focus on charge.

And I just want to point out oxidation and reduction reactions are really moving electrons. And this is irrelevant of charge. So I add an electron to an uncharged molecule, I get a negatively charged molecule. Add it to a positively charged molecule, get a neutral molecule. Add it to a more positively charged molecule, now have a positively charged molecule.

It's adding these electrons. Each of these are reduction reactions. In that direction, they would be oxidation reactions. And so NAD+ plus 2 electrons going to NADH, FAD plus 2 electrons going to FADH2-- all reductions in this direction, all oxidations in that direction.

Now, because there's not free electrons, these reactions have to happen in pairs. And so if we consider a pair, here's lactate interconversion with pyruvate. So alcohol and lactate to the ketone and pyruvate-- this direction is an oxidation. That means the electrons have to go somewhere-- NAD+ to NADH. This is a reduction.

If we go from pyruvate to lactate, that's a reduction. We can reoxidize NADH back to NAD+. Of course, you'll remember from glycolysis fermentation, this inner conversion is catalyzed by LDH. And effectively, if you're going to use lactate for energy-- so we oxidize the lactate, generate NADH.

If we're going to use it for fermentation, we produce lactate, reoxidize the NADH back to NAD+. How does LDH know which direction to go in? How does any reaction, any pathway know which direction to go in? Its delta G.

Delta G-- well, it's delta G naught prime plus RT times the log of, in this case, the pyruvate lactate ratio times the NADH/NAD+ ratio. And so how oxidized or reduced NAD+ to NADH is will determine how oxidized and reduced the pyruvate lactate ratio is. In other words, this must be true for absolutely any redox pair.

And in general, remember carbon oxidation is favorable. And that's because oxidizing carbon to give those electrons to something downstream, ultimately oxygen, is favorable. That's really what's driving each of these pathways. Now, how favorable any of this is, of course, can be quantified? And if we want to know this for this redox pair or any redox pair, of course, this is related to some equilibrium constant.

And we've already discussed that we can have this term delta G naught prime that is relevant to the equilibrium constant. But it's still, because delta G determines what happens, it's still that equilibrium constant plus the ratios of the reactants and products that will really determine if the reaction happens. However, it turns out it's useful to think about-- when electrons can go to donated or accepted in lots of different reactions, it's useful to come up with a term that helps us know what is the propensity of an individual pair to accept or donate an electron in either direction.

And we have a term for this. It's denoted E naught prime, which is the standard reduction potential that basically describes for a pair of molecules-- NADH+, NADH, pyruvate, and lactate-- in an oxidation or reduction reaction, how likely is it to give up its electrons in one direction or the other. And so the units of this is volts. And the standard reduction potential can be calculated as follows. And of course, it's related to the equilibrium constant of a reaction.

And so the equilibrium constant overreaction, delta G naught prime-- related to the equilibrium constant-- is this formula. N is the number of electrons transferred, F is the Faraday constant, and delta E naught prime is the change in standard reduction potential from electron donation from one pair to the next pair. So if we use our lactate pyruvate example, we have lactate going to pyruvate plus 2 electrons. So that's the electron donor. It's being oxidized.

And then you have NAD+ plus the electrons going to NADH. It's being reduced-- two electrons, of course-- it's being reduced. And so this has a standard reduction potential. This half reaction has a standard reduction potential.

And so the difference between these standard reduction potentials-- that is, who receives the electrons, delta E naught primed 2 minus delta E naught prime 1 gives us this change in standard reduction potential, which I can plug into this formula, which is related to the equilibrium constant and tells me which direction of electron transfer is going to be favored, at least at equilibrium. And so if this number is related to equilibrium, there's a negative here.

And so if this term is positive, delta G naught prime will be negative. And that means that electron transfer will be favored at equilibrium. If this number is negative, that means the reverse electron transfer will be favored at equilibrium. And so it stands to reason then that electron transfer from smaller standard reduction potential to larger standard reduction potentials will be favored. Hopefully that makes sense-- so smaller to larger.

Now, that could be more negative to less negative, negative to positive, positive to more positive. As long as that delta is positive, electron transfer will be favored. Now, of course, the ratios still matter. But this standard reduction potential is useful because it can help us know which direction transfer wants to occur between any redox pairs at equilibrium.

And so recognizing this, you must know that carbon oxidation electron transfer in general is going to be favored to NAD to make NADH. And in general, that NADH electron transfer is going to be favored to oxygen. And it's coupling those favorable electron transfers that ultimately is allowing the system to use oxidation and reduction reactions to drive these various pathways. And that energy release from these electron transfer reactions can be used to make ATP and do other work, as we will talk about in great detail in the next lecture.