BOGDAN FEDELES: Greetings, and welcome to 5.07 Biochemistry online. I'm Dr. Bogden Fedeles.

Let's metabolize some problems. I have a good problem for you today. This is problem one, from problem set nine. It is a problem in which we're going to calculate how much energy we get from metabolizing a molecule of fat, more specifically, a molecule of triacylglycerol.

Here is a structural representation of the triacylglycerol. Recognize the glycerol molecule in the middle here, that it's holding together three fatty acids. Now notice, I picked a short-chain fatty acid, a long-chain fatty acid, and a fatty acid that actually has a double bond.

Now when this molecule gets metabolized, it's going to be acted upon by an enzyme called the lipase. It's going to hydrolyze the molecule into its constituents.

Obviously the lipase is going to use water molecules, and it's going to break it down into glycerol, shown here. Glycerol. Then, this fatty acid that has two, four, six, C6 fatty acid. This fatty acid has two, four, six, eight, 10, 12, 14, 16, C16 fatty acid. And this one, it's an unsaturated fatty acid, has a double bond, and if you count the carbons it should add up to 15 carbons. So not only it has a double bond, but also it's an odd-numbered fatty acid.

In order to figure out how much energy we can get from this one molecule of fat, we will look at how much energy we get from each one of these constituents-- namely, the glycerol and the three fatty acids-- and calculate what is the maximum amount of ATP we can generate when we metabolize each one of these molecules completely to CO2 and water.

In order to keep track of how much energy we get from each of the molecules, let's make a handy table right here. So we're going to put glycerol and the C6 fatty acid, and the C16 fatty acid, and C15 fatty acid.

All right. And each one of these, we're going to follow the metabolism, and see how much ATP we're going to need to put in or generate. Also, the redox cofactors in ADH or FADH2. Also, for fatty acids, we're going to be dealing with beta-oxidation. And pretty much every single molecule, when it's going to be born completely, it's going to generate first acetyl-CoA. All right. And here, we're going to tally up the total amount of ATP for each one of them. And then we're going to tally up for the entire molecule.

So let's start with the glycerol molecule. Now, if you have watched the problem set seven, you might recognize the following pathway. As we just discussed, triacylglyceride can be hydrolyzed to form glycerol. And the glycerol, then, is first phosphorylated by glycerol kinase, and oxidized by glycerol phosphate dehydrogenase, to generate dihydro acetyl phosphate, which can then enter the glycolysis, and follow glycolysis all the way to pyruvate, and then acetyl-CoA. From then, acetyl-CoA can go into the TCA cycle.

So let's tally up how much energy we can get from one molecule of glycerol. Let's take a look specifically at the steps where we are generating ATP, or generating redox cofactors, such as NADH or FADH2.

First, we need to put in ATP. Neglect glycerol kinase. But we're going to get back one ATP in the phosphoglycerate kinase step, and one ATP in the pyruvate kinase step. So the net ATP formation is one. Now in the glycolysis, we're also going to generate one NADH, in the pyruvate dehydrogenase another NADH, and the glycerol-3-phosphate dehydrogenase will generate also an NADH.

Now, keep in mind, this NADH is going to be outside the mitochondria, so we're going to have to use a shuttle to bring it in. But we're considering that we're using an efficient shuttle, that gives us the full amount of energy for this NADH. So, once again, the total, it's going to be three molecules of NADH.

So, going back to our table, we said the glycerol is going to give us a net one molecule of ATP, three molecules of NADH. There's going to be no FADH2, no beta-oxidation, and we're going to get one molecule of acetyl-CoA.

As you've seen by this point many times, acetyl-CoA will enter the TCA cycle, where it's going to be completely oxidized to two CO2 molecules, and in the process is going to generate the equivalent of 12 ATPs. Let's take a look at where those are coming from.

Here is a schematic of the TCA cycle. And one acetyl-CoA molecule comes in, and it's going to generate one, two, three molecules of NADH, one molecule of FADH2, and one molecule of

GTP. Now, if we keep in mind that for every FADH2 we generate about two ATPs, and for every NADH we generate three ATPs, that's a total of, 3 times 3 is 9, plus 2 is 11, plus a GDP is equivalent to an ATP. That's about 12 molecules of ATP per molecule of acetyl-CoA that enters the TCA cycle.

So now let's tally up how much energy we can get from one molecule of glycerol. So we know we get one ATP, three NADHs, now each NADH is going to give us three molecules of ATP. Now FADH2, we know these give two molecules of ATP. We don't have any beta-oxidation-- we're going to be talking more about fatty acids-- and acetyl-CoA we just talked about, we get 12 molecules of ATP per acetyl-CoA. So, the total here is 12 plus 9, plus 1, that's going to be 22 ATPs from one molecule of glycerol.

Now let's talk about the fatty acids. In order to metabolize them, first we need to activate them into thioesters. These are going to be thioesters formed with coenzyme A, or CoA.

Here's an overview of the activation process by which fatty acids become fatty acid thioesters. Of course, this is written for the C6 fatty acid, but would occur for any other fatty acid, regardless of the chain length. Now this process is catalyzed by acetyl-CoA synthetase, that uses ATP to first generate this mixed anhydride, with AMP. This process is called adenylation. So this activates the acid, which then reacts with coenzyme A shown here, HS-CoA, which will generate the thioester of the fatty acid.

Now, here we are using one molecule of ATP, but we're breaking it into alpha-phosphate to generate pyrophosphate, which is then broken down into two inorganic phosphates. And the energy in this reaction, catalyzed by inorganic pyrophosphatase, drives the reaction towards the right. Now, since we're generating AMP in the second step, we need a second molecule of ATP to convert this AMP back to ADP.

So, overall, this process requires two molecules of ATP to generate one molecule of the fatty acid thioester.

Once a fatty acid is activated into a thioester with coenzyme A, it can now undergo beta-oxidation. This is a set of reactions in which the fatty acid is broken down into a shorter fatty acid and one molecule of acetyl-CoA. The process then can repeat over and over, until the entire fatty acid is broken down into acetyl-CoA molecules.

So let's take a look at how beta-oxidation works.

Here is an overview of beta-oxidation pathway. We're starting with a fatty acyl-CoA thioester that has "n" carbons, and by the end of the process, we're going to get a thioester that has "n" minus 2 carbons, and the remaining two carbons are going to be in the form of acetyl-CoA.

Now, this beta-oxidation involves four steps. In the first step, we're going to use a dehydrogenase to oxidize this single bond between the alpha and beta carbons, and make a trans double bond. So this is the alpha carbon, this is the beta carbon. So these fatty acyl-CoA dehydrogenases, there are actually several of them, and they have different specificities for short, medium, long, and very long chain fatty acyl-CoAs. But regardless, there will be some dehydrogenase to act on any length fatty acyl-CoA, and introduce this trans double bond.

In the next step, we add one water molecule to generate a beta-hydroxyacyl-CoA, which is subsequently oxidized to generate a beta-ketoacyl-CoA. In this oxidation, we're going to use NAD, generating one molecule NADH.

Finally, the thiolase, or beta-ketoacyl thiolase, is going to break down this bond between the alpha and beta carbons, in a reverse Claisen reaction, to generate one molecule of acetyl-CoA. And the remainder of the fatty acid is another thioester.

So one round of beta-oxidation is going to generate one molecule of FADH2, and one molecule of NADH, as well as one molecule of acetyl-CoA.

Now let's update our table with the information we just learned. So, as we just said, for every fatty acid we need to expend two molecules of ATP, to transform them into the thioesters. That's why I put here minus 2 for each one of the three fatty acids. We also learned that in beta-oxidation, we generate one molecule of FADH2, and one molecule of NADH. So, for every beta-oxidation, we generate the equivalent of five ATP.

Now we're ready to calculate how much energy we can get from each of the three fatty acids in our problem. Now, for the C6 fatty acid that's what's represented here, we discussed, we're going to activate it, we're going to need to use two ATP molecules and coenzyme A. We're going to form the thioester. And then we're going to do beta-oxidation

Now, for a fatty acid that has six carbons, we're going to do the beta-oxidation, and the molecule is going to cleave there, and we're going to do it one more time, the molecule is going to be cleaved there. So we're going to do two rounds of beta-oxidation. And each one of these two carbons is going to become an acetyl-CoA. So we're going to generate three acetyl-

CoAs.

Now, similarly, for the C16 fatty acid-- two, four, six, eight, 10, 12, 14, 16. All right, it's going to first activate two molecules of ATP and coenzyme A to form the thioester, with 16 carbons. And this will undergo beta-oxidation. And we're going to do it one, two, three, four, five, six, seven times. OK, so seven rounds of beta-oxidation is going to generate eight molecules of acetyl-CoA.

Now let's go back and put in all this information into our table.

So for the C6 fatty acid, we expended two molecules of ATP to activate it, and then we did two rounds of beta-oxidation, and we generated three molecules of acetyl-CoA.

For the C16 fatty acid, again, we activated then we did seven rounds beta-oxidation, and we generated eight molecules of acetyl-CoA.

So for the C6 fatty acid, we have a grand total of, 3 times 12 is 36, plus 2 times 5, 10, is 46, minus 2, is 44 molecules of ATP. For the C16 fatty acid, well, 8 times 12 is 96, plus 35, minus 2, that's 129 molecules of ATP.

Now the C15 fatty acid is going to be a little bit more complicated, because on one hand, it has a double bond, so we need to figure out how to deal with that. On the other hand, it's an odd chain fatty acid, and as you imagine, the beta-oxidation breaks off two carbons at a time. So the last time we do beta-oxidation, we're going to be left with three carbons. That's called propionyl-CoA, and we'll have to figure out what to do with that.

Just as with the other fatty acids, the C15 fatty acid is going to need to be activated into a thioester with CoA. So this is going to cost two ATP molecules, and, of course, we need to add the CoA. And now, this is the thioester of our C15 fatty acid.

Now, since the double bond is pretty far away from the business-end of the molecule, we can do a number of rounds of beta-oxidation. In fact, we can do beta-oxidation once, twice. So two rounds of beta-oxidation is going to give us this molecule. Two rounds beta-oxidation.

In each one of these rounds we're going to generate one molecule of acetyl-CoA, so two acetyl-CoA. So we get this fatty acid taiyo thioester, which contains a beta-gamma double bond.

Now, it turns out there is an enzyme that can isomerize this double bond into an alpha-beta double bond. So this is what is going to happen next. The double bond moves from the beta-gamma to the alpha-beta. Now, this looks a lot like an intermediate in the beta-oxidation. Once again, this reaction is catalyzed by am isomerase.

And this alpha-beta unsaturated thioester can continue in a manner similar to beta-oxidation. So, first it's going to add water to form a hydroxyl here at the beta position. Then that hydroxyl is getting oxidized to form a keto group. And the thiolase is going to generate acetyl-CoA, and another thioester shown here.

So, from here, we're going to generate one molecule of NADH, and one molecule of acetyl-CoA. All right, now this is a thioester of a completely saturated fatty acid. Now of course, it's still odd chained, so we have one, six, seven, eight, nine carbons. So we can do beta-oxidation actually three times.

So, three rounds of beta-oxidation. And it's going to take us to three molecules of acetyl-CoA. And the last portion of the molecule is going to be this molecule, which we call propionyl-CoA, is a three carbon thioester.

So far we have generated two, three, and another three here, six molecule of acetyl-CoA. And we've done, two, another three, five rounds of beta-oxidation. And we also generated an additional NADH molecule.

So now let's update our table with this information, and then we're going to figure out what happens to propionyl-CoA.

As we just discussed, the C15 fatty acid is going to get activated, so we need to ATPs there. Then it's going to undergo five rounds of beta-oxidation. And we generated a total of six molecules of acetyl-CoA, and one additional molecule of NADH.

Let's now take a look at propionyl-CoA, and see how we metabolize it, and how much energy we can generate from it.

It turns out the first step is to expand from a three carbon molecule to a four carbon molecule. This happens by adding one CO2. Of course, this process will require the expense of an ATP molecule. We generate this methylmalonyl-CoA, which is a branched four carbon chain molecule. And another enzyme, racemase, is going to interconvert this stereocenter from the S-configuaration to the R-configuration.

Finally, this methylmalonyl-CoA is going to undergo a rearrangement of the groups to generate a linear molecule, succinyl-CoA. Now, this is one of the most fascinating transformations in the whole biochemistry, and involves an enzyme called methylmalonyl-CoA mutase, which requires cobalamin, or the coenzyme derived from vitamin B12.

This unusual transformation catalyzed by the methylmalonyl-CoA mutase, the enzyme that requires vitamin B12 cofactor, it's fascinating because it involves a carbon skeletal rearrangement of the molecule. And this reaction occurs via a radical mechanism. The radical is obtained by breaking a carbon metal bond. In this case, it's a carbon-cobalt bond.

Now succinyl-CoA is a familiar molecule, you've encountered it in the TCA cycle. However, we cannot use the TCA cycle directly to completely metabolize succinyl-CoA, as all the intermediates in the TCA cycle are in fact in catalytic amounts. So, we're going to use just part of the TCA cycle, to generate a molecule, malate, which then can be converted into pyruvate. And pyruvate can then generate acetyl-CoA to re-enter the TCA cycle and be completely metabolized.

Here is the TCA cycle, to refresh your memory. And this is succinyl-CoA that we can generate from the methylmalonyl-CoA mutase. Now, as we said, succinyl-CoA is going to be converted to malate. Malate can then escape the mitochondria and continue its transformation towards pyruvate. So, in this process, succinyl-CoA is going to generate one molecule of GTP to form succinate. And succinate to fumartate is going to give us one more molecule of FADH2. Then malate will escape the mitochondria.

So to summarize what happens in the TCA cycle, succinyl-CoA is going to give us a molecule of GTP, and then one more molecule of FADH2. And it's going to make it to malate, and then malate is going to be converted to pyruvate using the malic enzyme.

Now, this is an oxidation and decarboxylation that happens in one step. For the oxidation, we're going to need NADP, instead of the usual NAD. So we're going to generate one molecule of NADPH. Now for the purpose of this problem, we're going to treat NADPH as equivalent to NADH.

So malate, via the malic enzyme, is going to form pyruvate. And then pyruvate, in the pyruvate dehydrogenase, is going to lose one CO2 and form acetyl-CoA. In the process we'll also generate one more NADH, and now acetyl-CoA can re-enter the TCA cycle and be completely metabolized, generating in the process about 12 ATP equivalents.

So now let's go back, and update our table with all this information that we found out about propionyl-CoA.

So as we said, propionyl-CoA required first the loss of another ATP to activate it, to form the methylmalonyl-CoA. But then methylmalonyl-CoA converted to succinyl-CoA. Succinyl-CoA generated one molecule of GTP, so we're going to put plus 1 back here. Then we're going to get one molecule of FADH2 the generating succinate, from going from succinate to fumarate. And then, we're going to generate two more molecules of NADH, one at the malate enzyme step, and one at the pyruvate dehydrogenase step, so we have plus 2 here. And, of course, in the end, we get one more molecule of acetyl-CoA as well.

So now we have a total of seven molecules of acetyl-CoA, five rounds of beta-oxidation, one FADH2, three NADHs, and at a loss of two ATPs. So this totals up to 118 ATPs for the C15 fatty acid.

Now we can tally the entire ATP yield of a molecule of fat, of this triacylglyceride, and that's going to be 313 molecules of ATP. Now that's a lot of energy from one single molecule of fat.

Now, this problem has an additional question, and it asked us to contrast how much energy we get from a six carbon fatty acid and compare that to how much energy would get from one molecule of glucose, which also has six carbons.

Let's take a look at our table here. The six carbon fatty acid generates about 44 molecules of ATP when completely oxidized. By contrast, one molecule of glucose would generate only about 34 to 36 molecules of ATP.

If you want to follow the same analysis that we did here-- remember glucose, well, we need to spend two molecules of ATP to activate it, but then we're going to generate four molecules of ATP going to pyruvate. And then there's going to be two molecules of NADH generated, one at the GAPDH step, one at the pyruvate dehydrogenase step, and finally, we're going to generate two molecules of acetyl-CoA. So the total is going to be, 2 times 12 is 24, plus 4 times 3 is 12, is 36, plus 2, is 38.

So as you guys can see, the C6 fatty acid generates actually more ATP than one molecule of glucose. And that's probably reasonable, because the C6 fatty acids have a lot more C-H bonds. In other words, the carbons are more reduced. In glucose, we have a lot of hydroxyls, so the carbons are in a slightly higher oxidation state. Therefore, there's less energy generated total.

Of course, one molecule of glucose pales in comparison with one molecule of fat, which has hundreds of ATP generated, as we thought in part A of this problem.

Well, that sums up this problem. I hope it helped you realize why fats, or triacylglycerides, are so much more energy dense than other nutrients, such as sugars or amino acids.