Biology’s Gasoline: Oxidation of Fatty Acids

Fats: our unpopular best friend- Poor fat! It gets a bad rap at many levels of science and society. But the truth is, it is not only completely vital to our cellular existence, but also a great fuel in its own right. This section will show you some of the basic features of this abundant (and sometimes too abundant) source of energy that we all use, all the time, from birth until death.

We have heard a lot about glucose in the past readings. We have learned about what glucose is, how universal a role it plays in life, some of the ways we break it down, and how the resulting products can be oxidized all the way to CO$_2$, producing useful energy stored as ATP with an efficiency that puts the internal combustion engine to shame. It is the fuel of choice, and stores of glucose, mostly in the form of glucogen in the muscles and liver, will determine how far we can run, or row, or ride. Glucose is indeed a main play-ah (I know, I am about as urban as an Arkansas barn) but it is definitely not the only fuel. In terms of sheer amounts, glucose is in minor supply compared to fats. And that means all and any of us. Even people with very low fat stores, say an elite runner with 5% body fat, still has enough fuel to run many marathons. This source of fuel is not as rapidly accessed as glucose, nor as rapidly implemented by ingestion. But stored fat is an incredibly important source of fuel that we use all the time, need to be operating, and works in all people, large or small, fat or thin. So lets learn about the biochemistry, and then some aspects of the bigger picture for this key “other” fuel.

Acid-uous study of the key structures- A key molecule to think about first is a simple one called a fatty acid. The basic structure of a fatty acid is just this: a hydrocarbon chain and a carboxyl group at the end of the chain. The picture shows a 16 carbon fatty acid, one of the most common in biology, called palmitic acid. Lipids have some pretty crazy names, which often are totally historical and in no way tell you what the structure is from the name. Palmitic acid is an example: it is a major component of palm oil. Who knew!? Fortunately, the key points are separate from the odd names. Anyway, the basic layout of a fatty acid is a hydrocarbon chain with a carboxyl group (hence, the acid) at the end. I have included the rigorous “all atoms” version (bottom) and the much more common “vertex and end are saturated carbons unless otherwise specified” version. The carboyl group is ionized in aqueous solutions buffered at pH7, such as cytosol, or plasma, so usually it is drawn as the negatively charged –CO$_2^-$ version like in the next picture below.
**Acid-uous study of the key structures** - You don’t need to be an Exxon Mobil chemist to see that much of a fatty acid like palmitate (the name of the ionized form of palmitic acid… as my undergraduate chem professor Dr. Wilcox taught me that “the –ic acid gives the –ate salt”) is a hydrocarbon. All that -CH₂-CH₂- is exactly the same energy rich arrangement of atoms found in gasoline, or jet fuel, or propane. And that is why fatty acids provide abundant energy. We can combust those hydrocarbons. But just like with glucose, it is done little by little, to get the energy out a little at a time. The key to fatty acid oxidation lies in the acetyl group, which we have already encountered. Remember pyruvate, how it was harnessed for oxidation after glycolysis? It goes through the PDH complex, where it is oxidized and decarboxylated to yield… acetyl-CoA. That is, an acetyl group (CH₃-CO-) attached to the CoA-SH carrier. This handy version of acetate is then fed into our old pal the Krebs cycle to give a substantial amount of energy. The oxidation of fatty acids proceeds in a conceptually similar manner: the long hydrocarbon chain is broken into acetyl groups, in the form of acetyl-CoA, and then these acetyl groups are oxidized by the Krebs cycle. The AcCoAs are formed one at a time, meaning the fatty acid chain is whittled down two carbons at a time. The nice thing in terms of studying fatty acid oxidation (that is what it is called) is that the same reaction cycle is used each time an acetyl-CoA is made, so really the oxidation of this big long molecule is just a few reactions that happen in repeating cycles until the fatty acid is all converted into useful acetyl groups carried in AcCoA. This is called an “iterative” reaction, an iteration being an operation that is repeated multiple times to complete a process. An iteration is one repeat of such a process. Let’s do it!

Fatty acid oxidation starts with attachment to the CoA carrier. I hope you are getting the (correct) idea that CoA is a universal carrier of carboxylic acids. All the way from one carbon formic acid (which we don’t talk about), through the very frequently encountered two carbon acetyl-CoA, up to large fatty acids including linear and fancier ones.

Although there is much in metabolism that is “unpatterened” and thus a) requires memorization and b) drives poor overburdened students bonkers, it is also true that some things are so consistent that they can be relied on, and that includes CoA being a carrier of carboxylic acids.

**When it’s fatty acid’s turn to burn** - Lets go through the process by which fatty acids are oxidized, starting with a molecule that drifts across the cell membrane from the bloodstream. There is a whole set of things that could have happened before this to
produce that blood-born fatty acid. These could include release from distant cellular stores such as adipocytes that are in the business of storing fat, or release from the large soluble storage devices (sort of lipid spaceships circulating in the blood) called lipoproteins... for now we will focus on the cellular biochemistry of oxidation. But be aware that a lipoprotein biochemist (there are many such critters) would be horrified. You can’t please everyone...

**Activation of a free fatty acid** - When a free FA (fatty acid; standard abbreviation) enters a cell, it is first attached to CoA-SH. This is catalyzed by the enzyme acyl CoA synthetase. Because the thioester bond that is formed requires energy, ATP is hydrolyzed to make the net reaction spontaneous. If you look at the net reaction you will notice an interesting feature: the “parts” left over from the ATP are AMP and PO_4^2−, called adenonsine monophosphate and pyrophosphate ion. What this means is that unlike the more typical case where the “last” phosphate bond is hydrolyzed to produce energy, in this case the “first” P-P bond is broken, thus giving the indicated products. The truth is that all both P-P bonds of ATP have strong exergonic energies of hydrolysis and the choice of which one is broken for energy depends on which enzyme is using the ATP to drive a reaction. What actually happens is that an AMP leaving group is added to the carboxyl group of the fatty acid, allowing the –SH of CoA-SH to perform an efficient and spontaneous nucleophilic attack, thus creating the acyl-CoA, and the AMP we see. Now we have a fatty acyl CoA, often referred to as an acyl CoA, trapped in the cell and activated for easy transfer and biochemical oxidation.

*(Carni)teens today!* – The actual oxidation of fatty acids occur inside the mitochondrion, which makes a lot of sense because acetyl-CoA generated there can be directly metabolized on site by the Krebs cycle. Also, as you will see, the other products of fatty acid oxidation are capable of producing energy by cellular respiration, which as you will learn also occurs in the mitochondrion. So this is fine place for productive conversion of fatty acids into oxidative energy. However, the acyl-CoA we just made from free FA is in the cytosol, while the oxidative action is going to be taking place in the tightly contained mitochondrial matrix, which is the part contained by the mitochondrial inner membrane. As may know or will find out, the inner membrane of the mitochondrion is very impermeable—it needs to be for ATP production by respiration—so we have to find a way to get the newly trapped acyl group across the inner mitochondrial membrane. For this to
occur, the fatty acyl group is transferred to another carrier called carnitine. Unlike CoA, carnitine has a much more specialized function in the cell; it appears to be only involved in fatty acid oxidation. So right after the acyl-CoA is made, the activated acyl group is transferred to the –OH group of carnitine to make acyl-carnitine. (in the pictures, recipient OH is marked with a dotted red circle). This occurs on the outer membrane of the mitochondrion, and the acyl-carnitine adduct can be moved across the normally impermeable inner mitochondrial membrane by the action of a specific transporter. Now here’s the crazy thing: as soon as the acyl-carnitine is moved across the membrane, the acyl group is transferred back to a matrix-located CoA-SH to regenerate acyl-CoA, and free carnitine. So carnitine picks up acyl groups from acyl-CoA made in the cytosol, carries the acyl group across the inner membrane to the mitochondrial matrix, and then immediately transfers it back onto CoA once it arrives in the matrix. The net effect of this elaborate biochemical process is that acyl-CoA is moved from the cytosol to the mitochondrial matrix (from Wikimedia). The enzymes that do this are generally called carnitine acyl transferases (the one in the picture are labeled CPT for carnitine palmitoyl transferase). One of these transferases lives on the outer mitochondrial membrane facing the cytosol, and one lives on the inner membrane facing the matrix, to catalyze the two reciprocal transfers, which are shown in the picture to the left. Now you might ask, “what the heck (or stronger) do you go and make acyl-CoA, only to convert it to acyl-carnitine, only to convert it back to acyl-CoA once inside the mitochondrion? Good question… short answer… don’t know. Perhaps it's a molecular echo of bacteria’s ancient free living lifestyle, or perhaps it provides a greater level of control of use of acyl groups in cell metabolism, or perhaps it is just impossible to evolve a transporter to ship acyl-CoA across the inner mitochondrial membrane. Or some combination of those, or something else entirely. But whatever the reason, this is not some trivial fact to drive young biomedical science students crazy. In
fact, the carnitine-based transport of acyl groups is the rate-determining process for fatty acid oxidation in eukaryotic cell. Meaning, the rate of fatty acid oxidation by mitochondria is determined by the rate of carnitine-based delivery to the matrix. That is why this step is down-regulated by fatty acid synthesis (more on this later) and why you can buy carnitine at Whole Foods (yuppies), Trader Joe’s (hippies), or CVS (sensible millenials). Since carnitine-mediated transport is the key bottleneck in fatty acid oxidation, the thought is that carnitine taken as a supplement would serve as a “fat burner”, and boost utilization and metabolism of fatty acids. The actual studies are not terribly conclusive: it may help in some cases, and may improve the performance of some athletes in some cases, but not do much in other circumstances. Worse yet, more recent studies pose a whole new problem: that too much dietary carnitine can be converted by gut microbes into a compound that is bad for your arteries (Koeth et. al (2013), Nature Medicine 19:576-85)! Nevertheless, I take it for my running just in case. In summary, the carnitine part of the fatty acid oxidation goes: 1) cytosolic acyl-CoA transfers an acyl group to carnitine on the surface of the mitochondrial outer membrane; 2) acyl carnitine crosses the inner mitochondrial membrane by way of an acyl-carnitine transporter, 3) acyl-carnitine transfers its acyl group to CoA-SH in the matrix to regenerate acyl-CoA, now deep inside the mitochondrion where it can be oxidized by matrix enzymes. The picture above tells the same story with thousands of fewer words.

The downward spiral of oxidation- Let the fun begin! The acyl-CoA now within the mitochondrial matrix undergoes a set of 4 reactions that generate an acetyl-CoA and a new acyl-CoA that is 2 carbons shorter. This generation and use of acetyl-CoA is a prevailing theme in fatty acid metabolism. You will see that fatty acids are also built up two carbons at a time, using a set of reactions that are at least chemically the exact opposite of cyclic fatty acid oxidation. But I get ahead of myself. Back to fatty acid oxidation. The first thing that happens to the acyl-CoA is… oxidation. Specifically, our old friend FAD oxidizes the acyl group by removing two hydrogen atoms, becoming FADH$_2$, to produce a double bond between the two and the three carbons, that is, an enoyl-CoA. Here it is useful to do a teeny bit of nomenclature: in fatty acids, the carbon next to the carboxyl group is called the “alpha” or $\alpha$ carbon, and the carbon two away from the carboxyl carbon is called the “beta” or $\beta$ carbon. The poor carboxyl carbon itself
doesn’t get a letter. The IUPAC number system that you learn in O chem is much more reasonable. The carboxyl carbon is 1, the next is 2, etc. The reason I go over this Greek-ish naming is that it is historical and often used. For instance, a very common name for this pathway of fatty acid oxidation is… beta oxidation, because the β carbon is the main site of oxidation, as you will see soon. The pictures will show the chemical process as they occur. The first enzyme is called a “dehydrogenase” for good reason: we have removed a hydrogen molecule (although it is in the form of two H⁺ added to FAD).

Anyway, next a water molecule is added to the double bond to form a β-OH-fatty acid. See… β! This is not an oxidation or a reduction. But it allows the next oxidation to occur. Make sure you are comfortable with why the product of this water addition step is called a β-OH-acyl-CoA. The enzyme that catalyzes this step is called enoyl-CoA hydratase; hydratases generally add (or remove depending on reaction direction) water to a double bond.

Next the βOH-acyl-CoA is again oxidized, this time by another old friend, NAD⁺ to give NADH and a β-keto-acyl-CoA. Look at the picture of this last intermediate: you can see that it looks like a shorter fatty acid with an acetyl group getting a piggy back ride, sort of. This is a β-keto acid attached to CoA, so it is a β-keto-acyl-CoA.

It turns out that this β-keto-acyl-CoA is easily “broken” by addition of CoA-SH to yield an acetyl-CoA, and a new acyl-CoA that is, like we said above, two carbons shorter. That step is shown. Voila! One oxidative cycle is completed. The total products we end up from all of these reactions are: an acetyl-CoA split off from the acyl chain, a two-carbons-shorter acyl-CoA (both products of the last reaction show in this paragraph), along with newly-reduced FADH₂, and NADH made in the earlier reactions. Not bad from a “fuel” standpoint. The NADH and the FADH₂ can be directly used in mitochondrial respiration by the ETC, and the acetyl-CoA can be fed
into the Krebs cycle. Since both of these processes (ETC and Krebs cycle) are right there in the mitochondrial matrix as well, this is a fine place for \( \beta \)-oxidation to be occurring. Now here’s the cool, or at least the easy, thing. Since we ended up with a new acyl-CoA (two carbons shorter than the original one), we can do the same thing again to this smaller substrate with the exact same reactions. This happens again and again until the original fatty acid is “whittled down” to a final acetyl-CoA which is also metabolized by the Krebs cycle. etc. etc (which is double-apt because etc means “etcetera, but also could mean the electron transport chain… cute). So here’s a little question? How many reaction cycles have to happen to sixteen carbon palmitate to convert it all into AcCoAs?

**An exercise I strongly suggest** (hence the red)- The set of reactions that take you from a mitochondrial acyl-CoA to a two-carbon shorter acyl-CoA and a new acetyl-CoA, along with reduced products, are written one by one above. You will notice that the product of each earlier reaction is the substrate of the next. These are the reactions of an iterative metabolic pathway called \( \beta \)-oxidation. So here is my suggestion. Write out the pathway by combining these reactions. You know, like we do A -> B -> C -> D like that. This will help you see the whole train of events. Do it, and you will own it. Trust me.

**The odds are good…** When a long fatty acid molecule is oxidized by the above-described \( \beta \)-oxidation, or simply fatty acid oxidation, you can see that the acyl chain is shortened two carbons at a time. Usually this results in the wholesale conversion of the entire fatty acid into acetyl-CoA molecules and other goodies like FADH\(_2\) and NADH. That is because most fatty acids have even numbers of carbon molecules. This won’t be surprising when you learn about fatty acid synthesis, which, you may have guessed, is built from 2C acetyl-CoAs, and so even numbers of carbons on fatty acids is very common. But there are cases in nature in which fatty acids are made with an odd number of carbon atoms. What happens then? Well, the final product is a three carbon acyl-CoA called “propionyl-CoA” as in propionic acid, which you will recall refers to the three carbon “propane” unit that in this case has one of the carbons dedicated to being a carboxyl group. There is a well-studied pathway that allows us to metabolize this “odd” molecule. It involves adding a CO\(_2\) to it to make 4 carbon methylmalonyl-CoA, and then rearranging the product in two steps to make our old 4-carbon friend succinyl-CoA which we know how to deal with. This happens because there is no major pathway for propionyl-CoA, yet there are multiple dietary sources of odd-numbered FAs. We won’t dwell on this, but it is a very interesting topic we might discuss in the classroom once you have these basics down.
Learning to the point of saturation- Another type of fatty acid that is commonly encountered are called “unsaturated fatty acids”. They have double bonds in various places, introduced either by the dietary organisms who make them, or by us (or our gut microbes). All of the bonds that are biologically introduced during synthesis or processing of fatty acids are “cis” double bonds, like the one in the picture of oleoyl-CoA. Although trans double bonds appear in transient metabolic intermediates (including you will notice, in β oxidation), and are found in low abundance in some dairy products, the only source of abundant trans fatty acids with trans double bonds is from the food industry, where poly unsaturated fatty acids are partially hydrogenated to improve properties for food making. There is evidence that they are not as good for us as cis unsaturated fats, and that is why we see so many labels decrying “no trans fats” which is the nickname for these unnatural fatty acids found in overprocessed foods (which I am quite prone to eat, I am sorry to confess). So what happens when the beta-oxidation process is chugging along and it encounters a natural cis double bond? Well, the basic strategy, which is something we see over and over again, is to try and convert a molecule or an intermediate that is not part of the central pathway into one that is. Often a separate enzyme will help the process, allowing a new molecule to also enter an old pathway. We saw this in glycolysis of several sugars that are not glucose; the strategy there is to modify that sugar into either glucose or something else that is part of mainstream glycolysis. When it comes to metabolic pathways, nature would rather use what is already present rather than invent something totally new.

¡Ole! an example- Lets look at the β-oxidation of oleic acid, an 18 carbon fatty acid with one cis double bond at the 9 position. This is a very common and very important unsaturated fatty acid that we make it from saturate C18 steric acid all the time. The picture shows what happens when β-oxidation encounters this structure. It all starts out normally: the β-oxidation enzymes are pretty uncaring about that is far downstream of the CoA linkage site. In fact, the original hypothesis for the two-at-a-time β oxidation mechanism came about by feeding dogs some very fancy fatty acids with easy to detect functional groups at the ends of the molecule. But eventually, as β-oxidation proceeds, after 3 acetyl-CoAs (CH₃-CO-SCoA) are clipped off one at a time, we end up with the double bond dilemma shown. This particular structure, as simple a variation as it may look, is not a substrate of the next enzyme on the pathway, the one that adds water to the double bond to make a β-OH acyl CoA product. What is there left to do? Well, you will notice that if only the cis-double bond between the 3 and 4
carbon were instead a trans double bond between the 2 and 3 carbon, that would be the correct structure of the water-adding enzyme, and boring β oxidation of a fully saturated fatty acid could proceed. We’re in luck! It turns out there is an enzyme, called enoyl-CoA isomerase, that… isomerizes… this non-substrate into the correct pathway substrate and so allows continued oxidation of this step. This corrective process is shown in the picture above.

**Doubling down**- The same idea applies to the commonly encountered pair of unsaturations that are found in some of the most important fatty acids in physiology. That pair of next door cis double bonds are very common in biology, and shown in the picture above. Let’s suppose that this motif were in the 9 and 12 positions of a fatty acid. It would be called a C18:2 unsaturated fatty acid. Now what happens. Again, β-oxidation is chugging along (represented by red squiggly arrow in pic), until it meets the first obstacle, and the same solution as above occurs: enoyl-CoA isomerase to put the double bond in the correct place, addition of water, oxidation and removal of an acetyl-CoA. But now, the next round stops at an even odder collection of double bonds, a diene, with two double bonds, 2,3 in trans, and 4,5 in cis. This is called a 2,4 diene, and we definitely need some enzymatic help to wriggle out of this covalent mess. Simple isomerization will not help and here we need to spend an NADPH to reduce this non-substrate into something that can be metabolized. In fact, there is an enzyme for this, called (not surprisingly) 2,4 dienoyl-CoA reductase, that uses NADPH to reduce this diene to a mono unsaturated acyl group with the bond between 3,4 which is similar to what we had to deal with in the simpler case of oxidizing oleic acid above. And again, enoyl-CoA isomerase converts this to the pathway substrate with a trans double bond at the 2,3 position for further β oxidation.
Pfeww. That was a lot of work… but remember, you are getting all the acetyl CoA that arrive downstream of this molecular obstacle, so it is, from an energy yield and evolutionary standpoint, worth it. Also, it is worth understanding that unsaturated fatty acids are very common. Even the most “saturated” fat sources have significant fractions (something like 20 percent) of fatty acids with double bonds that need to be metabolized like we said above. We will talk more about unsaturated fatty acids and their incredibly important roles in inflammation and physiology later. Soonish later…

OMG (O-may-ga)! an unsaturated structural interlude- There is, as you can tell, a fairly complex and arcane naming system for fatty acids, due to their long history and consideration in multiple fields of inquiry. It is important to get a few structural ideas down because they are discussed at some length. In the lipid anabolism section we will talk about some of the key ideas of where the various fatty acids come from. But for now, lets just learn enough about unsaturated fatty acids to discuss them easily. An “unsaturation” is simply the presence of a double bond along the alkane or hydrocarbon chain of a fatty acid. Like all double bonds with additions more complex than just hydrogen, they can be cis- double bonds, in which the two largest non-hydrogen groups are on the same side of the double bond when you lay it flat (they are planar, so this makes some sense) and trans- double bonds, in which the two largest non-hydrogen groups are on opposite sides of the double bond. In O Chem, we often see both, and the designation is important in fully defining a molecule with a double bonded carbon. In biochemistry, all naturally made fatty acids are the –cis form. There are trans structures that form in the course of biochemical reactions, but these intermediates, while important during the progress of a metabolic pathway, do not build up and are not nearly as abundant from natural dietary sources. The most common unsaturations are the “cis” bonds and are found in fatty acids with just one such cis double bond, or those with more than one, called monounsaturated or polyunsaturated fatty acids, respectively. The polyunsaturated fatty acids have a characteristic repeat of cis double bonds that is shown in the picture above, which I will repeat here because I hate having to flip back to look at a picture. These polyunsaturated fatty acids include the now very popular “omega 3” fatty acids. “Omega?” you say? WTF!? (What’s that formulation!?). Well, omega is the last letter of the Greek alphabet, and “omega 3” means “third from the last”. Like “You are my alpha and omega”, which is a very nice thing to say to a loved one. But be careful: it is also the kind of thing evil or Hell-dwelling villains will say in big budget movies: “BEHOLD, I am the ALPHA and the OMEGA”. So use this phrase with caution. Probably not a good ice-breaker in your first Tinder message. Anyway, this is a common way to label fatty acids. Omega 3 fatty acids actually are a family of polyunsaturated
fatty acids that have good health benefits, and many physicians and doctors recommend ingesting them on a daily basis. They comprise a number of structures, with varying numbers of double bonds, but always the bond nearest to the end starts on the third-to-the-last carbon of a fatty acid. Similarly, there are omega 6 fatty acids what have multiple cis double bonds with the furthest down from the carboxyl group being 6 from the last carbon. The picture shows “arachidonic acid” one of the most important fatty acids in physiology, and also an omega 6 fatty acid. Convince yourself of this. You will note that the linoleic acid we oxidized on the page above is also an omega 6 fatty acid. We will talk more about these key and in some cases essential fatty acids in later sections on lipid metabolism.

The RER: computing the ATP bang for my O₂ buck?- The two main fuels we use to regenerate ATP are carbohydrates and lipids. Since you now know how each is employed to this noble end, we can start contrasting and comparing them. One of the questions people who aren’t “science-y”, and even those who are, but are not “metabol-y”, will ask you, “which is a better fuel, sugar or fat?”. Fair enough and a reasonable question. The answer (or an answer) is that we have LOTS of fat stores (even someone with low body fat) and much lower stores of sugar or carbohydrates (these terms are used interchangeably in this context), but fat is more challenging to get power from. Recall (if you have had physics) that power is work per unit time, or energy per unit time, and for this carbohydrates are better. So low power tasks like sitting and … being, or TV watching, or inner tubing down a river with a beverage and a dream, are all managed mostly by use of fatty acid oxidation. Glucose and its polymer glycogen are better fuel sources when more power is needed. Beyond this, the first phase of glucose utilization, glycolysis, delivers more power (energy per unit time) than full oxidation of glucose, but is not very efficient at ATP generation. But that is a bit of a hyperlink… for now lets compare full oxidation of glucose to CO₂, and full oxidation of a fatty acid to CO₂. These two modes of fuel consumption are often our two alternatives for longer endurance activities like a bike trip, a strenuous hike, running a long race, and so they are useful to compare.

The RQ or respiratory quotient- A reasonable, and measurable, way to think about fuel usage is to calculate the ratio of CO₂ eliminated to O₂ consumed. This is called the respiratory quotient, or RQ. Since fat and sugar are usually both converted into CO₂ at low to medium intensity exercise, this type of calculation makes sense. If glucose is being used for ATP production, then the balanced equation for one molecule of glucose being fully oxidized results in a ratio of CO2 eliminated to O2 consumed is 1. For the palmitate,
which is the very common 16
carbon unsaturated fatty acid
the RQ is different is about
0.7. This is shown in the
picture. Why do we care about this? The cool thing about the RQ or the similarly used
Respiratory Exchange Ratio, or RER, which is the RQ as measured by the amounts of
these gasses expelled and consumed by a subject as measured by breathing, an is fairly
easy to measure. It isn’t something you can do at home (unless you have some fancy
telemetry-based portable equipment) but it is quite commonly available in a typical
physiology lab or sport science facility.

Get up and breathe! – So from the little calculations above, you can see that when we
are burning only fat, the RER is different from the RER if we are only burning
carbohydrate. This difference, 0.7 for pure fatty acids, and 1.0 for glucose, is easy to
discern with modern measurement techniques. So by measuring this quantity, we can
learn about which fuel is being used in various situations. You know how when you step
on a fancy treadmill like at your health club or in a hotel, you often are presented with a
chart that tells you where the “fat burning” region of exercise is, based on weight and age
and effort. Well, these charts are addressing (in probably too simple a way) the idea that
as exercise intensity changes, the ratio of fat to sugar used changes. And this is reflected
in the RER. So for instance, when people are sitting around, just hanging out, doing little
or nothing (one of my favorite activities), the RER is something like 0.8. By considering
the caloric content of fat vs. carbohydrates, this resting ratio comes to about 70 percent
fat, and 30 percent carbohydrate at rest. If activity increases, the ratio of glucose
increases with increased power-demand, and the mix of glucose to fat will shift, and that
will be reflected in the RER. So there is some sense to the “fat burning” charts on
treadmills. The lowest activity level is the best purely in terms of the fat to carbohydrate
ratio, but the intensity is so low that it is an ineffective way time-wise to burn the desired
calories. But too intense an exercise is both not sustainable for long periods of time, shifts
towards carbohydrate usage as intensity increases. Or at least, that is the thinking. Like a
lot of human physiology, it is definitely not one size (or pace) fits all. The effects of
training, diet, genetics, epigenetics, and now clearly guy microbiome are all important
and complex. Still, long slow distance is a good strategy to remove extra weight, to the
tune of about 100 calories per aerobic mile.

A second fate for acetate: ketone bodies- When we generate all that acetyl-CoA from
fatty acids, abundant energy can be obtained from it by further oxidation in the Krebs
cycle. We just learned about this, and so hopefully it seems natural to imagine that the
acetyl groups produced during mitochondrial oxidation of FAs to acetyl groups can
proceed rapidly and easily. But this requires that there is sufficient Krebs cycle activity to
accommodate the extra acetate. Recall that the first step in the Krebs cycle is the one that introduces a new acetyl group into the cycle: citrate synthase causes the acetyl group of acetyl-CoA to be added to oxaloacetate (OAA) to form citrate, and free CoA-SH. Recall? The citrate synthase reaction is shown to jog your weary memories…

So it is critical to have sufficient OAA to allow the acetyl groups from fatty acid breakdown to enter the Krebs cycle and yield all that useful chemical energy. Well, what happens if OAA levels are low? What happens if there is an overabundance of FA breakdown products acetyl-CoA compared to levels of OAA? It turns out that in these circumstances, something else can happen. It ONLY happens in the liver, but the liver is one of the most important “integrating organs” of metabolism. It is selfless in a number of ways, and this is the first one we will encounter.

**Where the wild acetates go-** In the liver, when acetyl-CoA levels are high and there is insufficient activity of the Krebs cycle to accommodate these high levels, the acetyl-CoA is converted by a simple pathway into a pair of molecules that have the HORRIBLE name of “ketone bodies”. It is horrible because the name sounds like some kind of tissue, or organ, or organelle, but it is none of these things. “Ketone bodies” refers to three related molecules, two of which are important in terms of metabolism. The three molecules are acetoacetate, β-OH-butyrate, and acetone. Yes, acetone, the commonly used solvent in nail polish and superglue remover solutions. A picture is shown of these three molecules. They are not bodies. They are metabolites, and yet we are stuck with the awful name ketone bodies. The basic idea is that the acetyl groups available from excess AcCoA in the liver can be condensed into two related water soluble molecules that are released from the liver for use in other tissues. Let's look at the reactions.

**Bodybuilding: making ketone bodies**—The first reaction condenses two acetyl groups together to from acetoacetyl-CoA and free CoA-SH. This reaction may look familiar because it is exactly the same reaction used at the end of β oxidation, but run backwards.
And it is catalyzed by the same enzyme, thiolase. Now we have a four carbon molecule, acetoacetate, being carried by CoA-SH. In this case, the blue CH$_3$- group displaces the CoA-SH in the black molecule. This is not shocking, because the CH$_3$- group of acetyl CoA is a feisty nucleophile when it gets together with the correct enzyme.

Next, the acetoacetyl-CoA is condensed with a third acetyl group to from HMG-CoA, which has the thorny name β-hydroxy-β-methyl-glutaryl-CoA, or 3-OH-3-methyl-glutaryl-CoA. Look at the reaction, and take advantage of the color-coded acetyl carbons to follow what happens. The acetate attacks the black carbonyl group to form a new bond. Two things about this reaction make it much easier to understand than you may think. First easyfying (my word) feature: because you read and know The Name Game, you now know the HMG is a derivative of the 5 carbon dicarboxylic acid glutarate, which is connected to CoA. In fact, you can tell from the name that this is glutaryl-CoA with both a methyl group and an OH group occupying the 3 or β carbon of the glutary group. Piece of Name Cake! Take a minute or two to convince yourself of this and you will OWN glutarate, HMG, and all of its derivatives forever. Second easyfying feature: The chemistry of this condensation, in which an acetyl group is transferred from acetyl-CoA to a carbonyl group (on the acetoactyl-CoA substrate) is exactly the same chemistry as the formation of citrate. The CH$_3$- group of acetyl-CoA attacks a carbonyl to make a new molecule with two more carbons. In this case, the enzyme is HMG-CoA synthase, and in the case of the Krebs cycle, the enzyme is citrate synthase. Again, convince yourself of this similarity and you will OWN acetyl groups being added to carbonyl carbons.

The third reaction actually produces our first ketone body. The HMG-CoA is broken into free acetoacetate and… acetyl-CoA by use of a CoA-SH substrate. And voila!, we have our first ketone body, acetoacetic acid, sometimes called AcAc. Now you may notice that
the last two reactions look like the hard way to make this free AcAc molecule. After all, we had acetoacetyl-CoA a couple steps ago, but the was not simply hydrolyzed to CoA-SH and free AcAc, which is totally reasonable chemistry. Instead, to generate the free acetoacetate, we first added an acetyl group to one end of acetyl-CoA, using an acetyl-CoA to make HMG-CoA, and then removed an different acetyl group from that HMG-CoA, producing a new acetyl-CoA and free AcAc. Crazy! Why such a roundabout route to make acetoacetate? Why not just hydrolyze the acetoacetyl-CoA molecule to free AcAc and CoA-SH? Not clear. Evolution is smarter than we are.

The next two molecules that comprise the family of ketone bodies occur to this free acetoacetate. A small amount of the AcAc made breaks down into acetone, losing a CO₂ and it is not clear if an enzyme catalyzes this reaction or not (!). This acetone has no apparent metabolic use, and escapes the body through the breath since it is volatile and fully water soluble. Acetone in the breath is a useful clinical indicator of situations in which ketone body formation is extreme, such as diabetes or starvation.

More importantly, some of the AcAc is reduced by NADH to make βOH-butyrate (βHB), which you can picture from your O Chem nomenclature, but I have drawn here to show the reaction. Both the AcAc and the βOH-butyrate are metabolically important ketone bodies that are made in the liver when acetyl-CoA levels are too high to be accommodated by the liver Krebs machinery. But why…?

Take 2: An exercise I strongly suggest (again, written in red)- The set of reactions that take you from acetyl-CoA to the ketone bodies are written one by one above. You will notice that the product of each earlier reaction is the substrate of the next. So just like before with fatty acid oxidation earlier, here is my suggestion. Write out the whole ketone body pathway by combining these reactions. Note, the pathway has a branch, because two things can happen to AcAc once it has been made. As above in the lipid oxidation reaction, doing this will help you see the whole train of events. Do it, and you will own it. Trust me.
What happens to these things? – Why would the liver go to the trouble of making these soluble products of AcCoA? Well, it turns out that they are released into the bloodstream and can be used as an energy source, as fuel, by a variety of other tissues, including brain, heart and muscle. Here is the cool thing: liver can not use AcAc or βHB as a fuel; it can only produce these things for consumption elsewhere. When either βHB or AcAc arrives at another tissue, a couple reactions re-convert that carbon into AcCoA, and those “freshly delivered” acetyl groups are used in to produce energy by oxidation in the Krebs cycles of those recipient tissues. Lets look at those reactions.

Any βHB that arrives at a tissue is oxidized back to AcAc, using the same enzyme that produced it back in the liver. It is the reverse reaction, but the same enzyme.

Now we have to get the AcAc into metabolism to use it. This is done by converting the AcAc to its CoA adduct. This is the one enzyme that is unique to the ketone body utilization reactions, called β-ketoacyl-CoA transferase. The liver does not have this enzyme, and this is why it can only make, and not use these molecules. This simply swaps the CoA from our old friend succinyl CoA (found at about 5 o’clock on the Krebs cycle) to make make acetoacetyl-CoA

Finally, in the last step, the acetoactyl-CoA is converted into two acetyl-CoA by the thiolase reaction we saw in the synthesis of these, and in β-oxidation.

A convenient feature of this whole process is that two of the same enzymes that produce KBs in the liver are also involved in utilizing them in the recipient tissues. You will recognize the names and the reactions The βHB that arrives in a recipient tissue is converted to AcAc by the same enzyme that produced it in liver. And the AcAc-CoA is broken into two AcCoA by the same enzyme, thiolase, that originally condensed two AcCoA into AcAcCoA in the liver. Pretty simple.
What’s the point? But why make it on one tissue only to break it down in another? The way to view ketone bodies is as a “fuel delivery system” that provides tissues an alternate energy source. It turns out (we will learn this later) that the conditions where glucose is low in the bloodstream are precisely the biochemical conditions where the liver makes ketone bodies. So when glucose is low, the liver makes an alternative fuel that allows tissues that need glucose, such as the brain, another option for maintaining essential levels of cellular energy. Once again, it is Liver the Giver.