PROFESSOR: We're now at storyboard 23. Let's look at panel A. Our last metabolism topic under the general umbrella of catabolism is ketone bodies. Ketone bodies are only covered in two pages in the book, but they're medically very important. The medical relevance of ketone bodies stems from their role in starvation and their role in diabetes.

Let's look at panel A. This panel shows various sources of and uses of acetyl CoA. It shows that ketone bodies are made from Acetyl coenzyme A. So let's start this part of the lecture with a discussion of where acetyl CoA comes from, and what its various states are including the formation of ketone bodies.

We just finished talking about fatty acid catabolism. So let's start on the right of this figure. We see that a fatty acid can be broken down to form acetyl CoA by beta oxidation, usually with the objective of generating energy. If we follow the acetyl CoA from beta oxidation down into the TCA cycle, we see that it will be fully oxidized to carbon dioxide with the generation of a lot of energy that can be used for mechanical work, biosynthesis, and other things. A second source of Acetyl CoA can be seen to the left, where we see glycogen breakdown to glucose or glucose can be directly imported into a cell. In either case, the glucose that we take in or liberate from its storage depot, glycogen, can be converted into acetyl coenzyme A.

Glycerol can be generated from the backbone of a metabolized triacylglyceride. It enters glycolysis as dihydroxyacetone phosphate and then can progress to acetyl CoA by way of glycolysis. Alanine can transiminate into pyruvate, which is converted subsequently to acetyl CoA by pyruvate dehydrogenase. This diagram shows us that many pathways converge to generate acetyl coenzyme A. Carbohydrates, amino acids, fatty acids, all can act as a source of this important precursor to energy. Aside from being processed by the TCA cycle, on the left, we see a broken line pathway involving fatty acid biosynthesis. This is going to be the next topic we come to after this discussion of ketone body formation. The pathway on the right with the broken lines is the pathway leading to ketone bodies which is called ketogenesis.

In the box at the top of panel A is a cartoon reminding me to tell you that acetyl CoA cannot

escape from the cell. Moreover, it cannot even easily escape from the mitochondrion. In order for acetyl CoA to leave the cell and be transported from one organ to another, it needs to be converted into ketone bodies. Another way to look at ketone bodies is that these are mobile or portable forms of Acetyl CoA that can go from a source organ, which is usually the liver, to a target organ, which may need them in order to generate energy by way of the TCA cycle. The target organ for example could be brain under conditions of starvation, or it could be skeletal muscle if you have to run away from something.

Let's look at panel B. There are five key facts that we need to know about ketone bodies. First, Ketogenesis mainly occurs in the liver. The liver manufactures ketone bodies and then exports them to other organs for use. These reactions typically happen when the levels of oxaloacetate become limiting in their mitochondrion. And I'll give you an example of why this is the case when I talk about starvation a little later in this lecture. The second important fact is that these are the primary metabolic fuels of the heart and skeletal muscle under normal conditions. The third fact is that ketone bodies become the major metabolic fuel of all cells under conditions of starvation, even cells of the brain after a few days of starvation will convert from glucose being the preferred metabolic fuel to accepting ketone bodies as their major source of energy. The fourth fact I want to talk about with regard to ketone bodies is that they are produced in excess in diabetes. I'll talk a little bit more about that later in the lecture. Finally, ketogenesis occurs in the mitochondrion, primarily the mitochondrion of the liver. So these are mitochondrial reactions.

Panel C shows the three classical ketone bodies, acetoacetate, beta hydroxybutyrate, and acetone. From the standpoint of chemical accuracy, it's obvious that beta hydroxybutyrate is an alcohol and not a ketone. Nevertheless, it's lumped in with the ketone bodies for historical reasons. Acetoacetate and beta hydroxybutyrate are what I'll call quote unquote "useful" ketone bodies from the standpoint of serving as precursors to metabolic energy. Acetone by contrast, is not useful by this criterion. Acetone however, is a useful biomarker, because sometimes its presence can help diagnose diabetes.

On a personal note, I come from a long line of diabetics. I remember when I was a little kid, my dad before he was diagnosed, would come home from work at the end of the day. He had very poor circulation. So my two sisters and I would try to rub his legs to give him a kind of massage to make his circulation a little bit better. I remember very clearly my older sister saying, "gee, dad smells like mom's nail polish remover," and that's because he was a diabetic

producing acetone, which was used at the time at least as nail polish remover. We had no idea at the time what was going on. A few months later, my dad was diagnosed as a type 2 diabetic. I now know that the fruity odor we smelled on his breath was acetone. As I said, acetone is a biomarker of this disease.

One last point with regard to the story board. Note that the acetoacetate and beta hydroxybutyrate molecules are acids. In diabetics, these acids can be produced as we'll see later in sufficiently high concentrations to lower the pH of the blood quite substantially. Keep in mind that lowering the pH is the same thing as increasing the concentration of protons in the blood. These concentrated protons will have physiological relevance that I'll discuss later. When a diabetic enters the phase where the pH of their blood is dangerously low, that's called diabetic acidosis.

Let's now turn to Panel D. At this point I want to describe the detailed biochemical reactions that give rise to ketone bodies. To make a ketone body we're going to need three molecules of acetyl coenzyme A. One of these molecules is going to be catalytic. That is, it's going to be restored at the overall end of the process of ketogenesis. Let's start by imagining a scenario in the mitochondrion of a liver cell where oxaloacetate becomes limiting. I'll talk about physiological states under which oxaloacetate becomes limiting or sparse a little bit later.

Acetyl CoA cannot enter the TCA cycle, because citrate synthase lacks oxaloacetate as a reaction partner. The concentration of acetyl CoA starts to accumulate. Then the beta ketothiolase reaction that is the last step in fatty acid bio oxidation reverses owing to the high concentration of product acetyl CoA. So two acetyl CoAs come together in order to form acetoacetyl coenzyme A. Note that I put markers on each of the carbons of the acetoacetyl coenzyme A. A third acetoacetyl coenzyme A is then added to the gamma carbon of the acetoacetyl coenzyme A. That's the carbon that has the filled-in square. The enzyme that catalyzed this last reaction is HMG Coenzyme A reductase, where HMG stands for hydroxymethylglutaryl.

HMG CoA is a six-carbon branch chain molecule. In the present situation, we're going to look at HMG CoA as the source of ketone bodies in the mitochondrion, but I want you to keep in mind that if this reaction were to occur not in the mitochondrion but in the cytoplasm, the resulting HMG CoA could be used for other pathways. For example, HMG CoA in the cytosol is the precursor to cholesterol. With that in mind, let's return our attention to the mitochondrion and ketogenesis. The mitochondrial enzyme HMG CoA lyase will split the HMG CoA, knocking

off an acetyl CoA in liberating as the final product acetoacetate, which is our first of three ketone bodies. Acetoacetate is a beta keto acid and hence, prone to spontaneous decarboxylation. Non enzymatically, this will happen at some slow rate in order to liberate CO_2 and produce acetone, which is our second ketone body. This acetone gives the fruity smell to the breath of a diabetic whose disease is out of control. Acetone is not going to be biochemically useful to us, for example it's not going to be metabolized to generate energy.

The second chemical fate of the acetoacetate is its reduction by NADH using the enzyme beta hydroxybutyrate dehydrogenase. This reduction forms our third ketone body beta hydroxybutyrate, which is a biochemically useful molecule in that it serves as a good metabolic fuel. Acetoacetate and beta hydroxybutyrate do not need any kind of special transporter to get out of the cell into the blood. They diffuse through the mitochondrial membrane and later through the cell membrane. They are then transported by the circulatory system from the liver to organs that need them for energy. As you mentioned above, ketone bodies are portable forms of acetyl CoA. In a real sense, the liver by making these ketone bodies is acting as a food caterer where ketone bodies represent food that's delivered to other organs.

At this point let's look at storyboard 24 panel A. Now let's take a look what happens when the ketone bodies travel by the blood and are taken up by another organ such as muscle. Acetoacetate, which I'll refer to as ketone body one is good to go and is ready to enter the main stream of metabolism. So I'm going to come back to it in a minute. The beta hydroxybutyrate, by contrast, has to be processed in order for it to be useful to the target organ skeletal muscle in this case. In step two, the muscle form of beta hydroxybutyrate dehydrogenase will use NAD plus to oxidize the beta hydroxybutyrate into acetoacetate, which joins the pool of acetoacetate that came in directly from the blood. We now have to put a thioester group on the acetoacetate, and that comes from an unusual source.

In step three you'll see a succinyl coenzyme A from the TCA cycle giving its coenzyme A residue to acetoacetate, which results in the formation of acetoacetyl coenzyme A. This reaction happens in the mitochondrion of the cell. At step four acetyl coenzyme A is converted by beta ketothiolase into two molecules of acetyl coenzyme A. And again, we're going to need another coenzyme A group to come in at this point as part of the beta ketothiolase reaction.

Remember that beta ketothiolase is the last enzyme that's operative in beta oxidation of fatty acids. Here it's doing the same chemistry that it does in beta oxidation. It splits acetoacetyl CoA into two acetyl CoA molecules. And in steps five and six, those molecules integrate into

the TCA cycle. In the TCA cycle they're oxidized to carbon dioxide with the generation of energy. Let me review for a minute before going into a physiological scenario. Way over to the left at step one, the liver has made acetyl CoA and packaged it into two ketone bodies, acetoacetate and beta hydroxybutyrate. They travel in the blood to target tissues, for example, the muscle, or heart, or the brain. In these target tissues these ketone bodies are internalized, converted to acetoacetate, and then to acetoacetyl coenzyme A and then ultimately to several molecules of acetyl coenzyme A. The acetyl coenzyme A that started in the liver, ends up in the target tissue and then can be used to generate energy. This is a particularly important reaction under conditions of starvation and diabetes.

Let's now look at panel b of storyboard 24. As you know, I like to look at physiological scenarios because at least to me, they helped make biochemistry real. The scenario I want to look at is that of diabetes. In Type 2 diabetes, which is the type that I have, my cells have become resistant to taking up glucose. My cells are insulin insensitive. After a meal I have very, very high concentrations of glucose in my blood, because the cells of my tissues are not capable of taking it in. Hence, if I do not take my anti-diabetic medication the sugar concentration in my blood stays high, which leads to some of the medical complications of diabetes. More on that later. Given that there's a lot of glucose in my blood, but it's not getting into my cells, my cells are actually in a technical state of starvation.

Take a look at the pathway I've drawn in panel b. Glucose on the left is not getting into the cell. I've used broken lines for the pathway from glucose to pyruvate and then from pyruvate in the cytoplasm into the mitochondrial matrix. These broken lines are meant to indicate that the pathways involved are just not very active. The sparse activity of these pathways means that acetyl CoA levels are becoming somewhat limiting in the mitochondrion. Because pyruvate is also limiting, the enzyme pyruvate carboxylase doesn't have sufficient pyruvate in order to maintain the oxaloacetate concentration within the mitochondrial matrix. Once again, oxaloacetate is the TCA cycle intermediate that that's at the lowest, that is micromolar concentration.

I'm focusing here on the liver, although I should add at this point that all tissues are similarly limited in the pathways indicated by the broken lines. The liver's response to sensing this limitation in carbohydrate processing is to either take in lipid or to break it down from internal stores, for example, triacylglycerides in order to produce acetyl CoA. But because oxaloacetate is limiting, the step at beta ketothiolase backs up, producing a large amount of

ketone bodies. The ketone bodies are produced in excess, so you can see them escaping into the mitochondrion and later out of the cell, and they go off into the blood. Consequently, the liver of diabetics produces a lot of ketone bodies, because it senses that the body is starving,

To the right of panel B, I have some blood chemistry values that are of relevance to diabetics. In a non-diabetic person, blood sugar concentrations, that is blood glucose is maintained at about 100 milligrams of glucose per 100 milliliters of blood. When I was diagnosed with diabetes, my blood sugar was over 300 milligrams per 100 milliliters. As I recall the symptoms were disorientation. I couldn't walk very easily, I was thirsty, and I urinated a lot. Normal ketone body concentrations are less than 0.2 nanomolar. In a severe diabetic situation, your ketone body concentrations could be 15 to 25 millimolar and the pH of your blood could drop from the mid 7 range down to about 6.8. The kidney responds to the high concentration of glucose and the high concentration of protons, that is the low pH, by increasing urine volume output in order to try to urinate out the glucose and protons. The results are that the diabetic becomes excessively thirsty which again is one of the biomarkers or symptoms of the disease.

The classic historical treatment of diabetes is to give insulin, which will push more glucose into the cell and thus offset the biochemical defect that leads ultimately to ketone bodies and to the high concentration of glucose in the blood. Aside from giving insulin by injection there are other medications that will result in a sort of reactivation of the beta cells in the pancreas in order to produce more insulin naturally. Alternatively, there are medications that will block gluconeogenesis and thus stop the ability of the liver and other gluconeogenic organs from producing glucose. So by blocking gluconeogenesis, one can lower the glucose concentration of the blood.

As you see there are many, many ways to treat this disease. Let me add that it can be a very debilitating disease, leading to blindness, amputation, and cardiovascular difficulties. It's a good idea to try to avoid the risk factors for this disease. It's not fully preventable at least in people who come from families in which nearly everybody gets it, for example, my situation. But by avoiding risk factors you can push off the date of onset by many years.