4. Power: Pathways that make ATP

4.1 The human body has a duel power system

In hybrid cars, such as a Prius™, power is supplied by two systems. For long-term travel, gasoline is used to move the pistons, which then causes the wheels to move. This process uses O₂ and an equation of the reaction of gasoline with oxygen, O₂, is the same as what occurs in metabolism:

\[ \text{C,H (gasoline in cars, food in people) + O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{energy} \]

The above reaction is said to be aerobic, meaning that it uses O₂.

The hybrid car has another power system; this system is electrical, powered by batteries that are charged when the car is moving. When the car is getting its energy from the electrical system, it is working anaerobically, meaning it is not using O₂.

The human body is much more complicated than a car, in part because we eat so many kinds of foods that are used as fuel. It is also much more efficient than a car; much of the power produced by a car is lost to friction. In this chapter we are going to tell how energy is produced in the cells of humans. We can think of this as how we produce power – the “power” being ATP, instead of the pistons that the car used.

Like a hybrid car, the human body has duel power systems. There is an anerobic system by which ATP is produced without using O₂. The anerobic pathway is called glycolysis. “Lysis” means “to break” and this pathway breaks sugars (6 C compounds) into 3 C compounds. The enzymes for this power system are located in the cytoplasm of the cell. Glycolysis is important in anerobic muscles. When you sprint or lift weights, you use this pathway. Glycolysis is also used when excess sugar is eaten. In this case sugar goes to 3 C, which then gets converted into fat.

The body, in addition, has an aerobic system. This power system resides in the mitochondria, and metabolic pathways in the mitochondria produce CO₂ and H₂O when the fuel reacts with O₂. This power system takes two C compounds and converts them to CO₂ in one pathway (called the citric acid cycle). It forms H₂O in a pathway called oxidative phosphorylation. The title of this pathway indicates that it requires O₂. The aerobic system produces most of the body’s ATP. Without O₂ being supplied, death will occur rapidly, because most of tissues of the body rely on mitochondria to continually supply ATP.

4.2 Fuel for the engine: Starting materials for metabolism

Carbohydrates, fat and proteins are broken down into smaller components in the gastrointestinal tract.

When we think of sugar we usual think of table sugar. Table sugar molecules are composed of two different monosaccharides, fructose and glucose. Lactose is a sugar found in milk; it is composed of two sugars, galactose and glucose. Maltose is a disaccharide of two glucose molecules hooked together. Complex carbohydrates, such as flour and starch, get broken down into monosaccharides in the stomach and intestine.
Table 4.1 Disaccharides

<table>
<thead>
<tr>
<th>Common disaccharide</th>
<th>Where we find it</th>
<th>Monosaccharide components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>Table sugar</td>
<td>Fructose and glucose</td>
</tr>
<tr>
<td>Lactose</td>
<td>Milk, dairy</td>
<td>Galactose and glucose</td>
</tr>
<tr>
<td>Maltose</td>
<td>Barley and product of fermentation</td>
<td>Glucose and glucose</td>
</tr>
</tbody>
</table>

Monosaccharides sugar molecules enter the blood plasma from the intestinal tract. Some people have the inability to break down some of these compounds as their digestive systems lack a particular enzyme. In lactose intolerance, the enzyme to break lactose into glucose and galactose is missing or is defective. These people are advised to avoid milk products.

From the intestinal tract, the monosaccharide enters the portal vein system and gets transported to the liver.

Figure 4.1

Carbohydrates and amino acids from proteins are transported from the stomach and intestine via the portal vein. Blood exits the liver by the vena cava, which goes to the heart. From the heart, the blood enters the general circulation. The blood circulation insures that sugars and amino acids from digestion are first circulated through the liver. The liver can change these before they are put into circulation for use by all other tissues.

Just as carbohydrates are broken down to small units, proteins get digested to amino acids in the intestinal tract and are put into the portal vein. This circulation ensures that the first organ that sees ingested carbohydrates and amino acids is the liver. The liver can and does modify the molecules before putting the molecules into the blood of the vena cava, from where blood goes to the heart, and then is pumped throughout the body.

The digestion of fat and introduction of fat into the blood stream is different from carbohydrates and proteins and will be discussed in Chapter 7. The starting material for fat metabolism in the cell is a breakdown product of fat: it is fatty acid.

4.3 Overview of pathways that produce ATP

To summarize what we learned before, fats, proteins and carbohydrates are converted to CO$_2$ and H$_2$O and in this process ATP is formed. The word “pathway” is used to describe the sequence in the break-down of food. The word pathway is used to indicate that the compounds are not broken down willy-nilly, but are broken in a definite sequence, by enzymes. Enzymes are specialized proteins that are catalysts. A catalyst is a substance that makes a reaction go
fast. Enzymes catalyze all chemical reactions in every pathway of the body. A pathway always involves the action of many enzymes.

Let’s briefly go over what each pathway does. **Glycolysis** is the first pathway to metabolize sugar. This pathway, found in the cytoplasm breaks sugar into 3 C compounds, pyruvate and lactate. ATP is formed. In the mitochondria, the **pyruvate dehydrogenase** enzyme changes the 3 C compound is changed into 2 C compound (acetate in the form of acetyl CoA) and CO$_2$ is released. Extra H’s are also formed, and temporarily stored in a compound called NADH.

Fatty acids are changed into 2 C compound, acetyl CoA, and H’s are transiently stored in NADH and FADH. (NADH and FADH are talked about in the next section). This pathway is called **β-oxidation**.

In metabolism food is converted into CO$_2$ and H$_2$O. The pathway that produces CO$_2$ is called the **citric acid cycle**. This pathway also transiently stores H in NADH and FADH. The pathway called **oxidative phosphorylation** takes H from NADH and FADH and adds it to O$_2$ that we get from breathing. This produces H$_2$O. In this pathway, many ATP are formed.

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**Figure 4.2**

In this figure, follow the C’s. In the cytoplasm, 6C sugar is broken to 3 C compounds in glycolysis, and ATP is formed.

In the mitochondria fatty acids are made into 2 C compound (acetyl CoA), and 3 C pyruvate from glucose is made into 2 C acetyl CoA.
Figure 4.3

In this figure, look at the H’s. H is gotten from fatty acids in β-oxidation, from 3C in pyruvate dehydrogenase, and from 2C in the citric acid cycle. The molecules that transiently store H’s are NADH and FADH.

The H’s indirectly react with O₂ to form H₂O in oxidative phosphorylation. Many ATP molecules are formed.

4.4 Four new compounds: NAD, NADH, FAD and FADH and importance of vitamins in metabolism

Before we describe how fuel makes ATP, we need to introduce a new concept in metabolism – the importance of vitamins. Two vitamins are described that required for cells to produce ATP. These vitamins are niacin (vitamin B₃) and flavin. We will describe why they are essential to metabolize food.

In the previous chapter we introduced ATP and ADP. These two molecules cycle between each other. When energy is required ATP goes to ADP. Then metabolism of food transforms ADP back to ATP. The molecule pairs, NAD and NADH, and FAD and FADH, like ATP and ADP, cycle between each other. Like ATP, they are inside of the cell, not in the blood plasma.

Remember that the overall equation of metabolism is:

(C, H, O from food) + O₂ → H₂O + CO₂ + energy (as ATP)

NAD and FAD are two compounds that, in effect, take H from food, and put it onto O₂ to make H₂O. NAD and FAD are made from vitamins, niacin and flavin, respectively. When NAD and FAD take H from fuel, they become NADH and FADH, the H in their name indicating that they now have an extra hydrogen, H. They indirectly add this H to O₂, and in so doing they return to being NAD and FAD, respectively.

Here is NAD (full name is nicotinamide adenine dinucleotide) and NADH (reduced nicotinamide adenine dinucleotide):
NAD and NADH are large molecules. The tail contains two ribose molecules (ribose is a 5C sugar), two phosphate groups (phosphate plus oxygen) and adenine. The adenine, ribose and two phosphates are identical to ADP, and this is an example of cellular economy. One molecule part is used for another. This tail helps to bind these molecules to specific enzymes. The business end is the nicotinamide end. This end gains hydrogen from food and loses hydrogen (H) to O\textsubscript{2} to form H\textsubscript{2}O during metabolism.

Flavin adenine dinucleotide (FAD and FADH) is similar to NAD and NADH in that this pair also has a big tail. In the Figure below “R” represents the big tail.

The head part of the molecule is called flavin and it is shown above. It is where the action occurs – what changes during the chemical reaction. The big tail of FAD, like the tail of NAD, helps to hold these molecules in the proper location in the enzymes that use them.

Our bodies have enzymes that allow it to make ATP from smaller molecules, but we cannot synthesize (make) flavin or nicotinic acid; they are vitamins. A vitamin is a compound that is essential for our metabolism but which we cannot make from simple foods. From a biochemical point of view plants are more sophisticated than we are. Plants can make these compounds. We get them from the plants that we eat.

Part of NAD and NADH is nicotinamide, formed from nicotinic acid. “Nicotinic” sounds like something coming from cigarettes, but smoking does not give it to us. Grains have a high content of nicotinic acid. We also get it from meat, but the animals we eat in turn got it from plants. Another names for it is niacin and vitamin B\textsubscript{3}. With a deficiency of the vitamin niacin in the diet, a disease called pellagra results. Symptoms of pellagra are skin lesions and general problems with metabolism. Nicotinic acid is used as part of NADH and NAD. It is also used in the synthesis of various compounds, seen in the next chapter. So, when vitamin B\textsubscript{3} is lacking, the enzymes that use NADH or NAD do not function and many organs are affected.

Niacin deficiency is very rare, as nicotinic acid is found in many foods. However, niacin deficiency is sometimes seen in alcoholics. These patients get most of their calories from alcohol and so their diets may be deficient in niacin. Alcoholism may also impair the absorption
of niacin in the gut. Pellagra occurred in the American south during the first part of the 20th century. This was due to the prevalence of corn in the diet, lacking in niacin.

Flavin is also gotten from our foods and it is a vitamin. Flavin with a ribose sugar attached to it is called riboflavin. It is found in green plants, tomatoes and also in meat, eggs and milk. Riboflavin is used in many processes in the body, and deficiency in flavin, like a deficiency of niacin, causes general problems, including anemia. Children with riboflavin deficiency do not grow well.

Nicotinic acid and flavin are not stored in the body, and everyday they are excreted from the body in urine. Because they are lost each day, it is good to include fruits and vegetables in your diet every day! As a side point, flavin is fluorescent, and if you are taking vitamin supplements, you might notice that your urine has a fluorescent yellow-green color. Excess flavin is removed by the kidneys and gives a yellow-green color to urine.

4.5 Metabolism to make ATP

With this background, we now take a deep breath and go over the metabolic pathways that give energy. The pathways are summarized at the end of the chapter. Glucose breaks down to 3 carbon compounds. The 3 carbon compound then goes to 2 C, acetate (in the form of acetylCoA. Fatty acids break down to 2 C acetate also in the form of acetyl CoA. So the ending pathways are the same for glucose and fatty acids – both form acetylCoA. The acetate is broken to CO₂ and H₂O, forming ATP. Therefore, both glucose and fatty acids ultimately form CO₂ and H₂O.

4.5.1 Pathway 1. Glycolysis splits glucose to form two 3 C compounds; ATP formed

Glycolysis is the pathway that metabolizes sugars. Some common sugars are glucose, fructose and galactose. The figure represents a cell. All human cells have the glycolysis pathway in their cytoplasm. This pathway does not use O₂.
The initial step is to get glucose from blood into the cell. Various cells have different transporter molecules to take glucose from the blood. Glucose transporters in some tissues are sensitive to some hormones.

The hormone that has a major role in glucose transporters is insulin. Insulin is secreted by pancreas when glucose levels get high. When insulin is present, the transporters of fat and muscle become active and glucose goes inside these cells. The transporters in liver and brain cells are not sensitive to insulin. These tissues take in glucose whether insulin is present or not.

When glucose gets into the cell it gets phosphorylated. A phosphate gets put on to the 6th carbon position. The enzymes that do this are called glucokinase (in liver and pancreas) and hexokinase in other tissues. When two different enzymes catalyze the same reaction they are called isoenzymes. The picture of glucokinase was shown in Chapter 3, so you are already familiar with it. Glucokinase puts a phosphate group on glucose, to form glucose-6-phosphate:

![Glucose-6-phosphate structure](image)

Once the glucose has a phosphate on it, it is trapped inside the cell and the destiny of this molecule is to be changed somehow within the cell. Metabolites with phosphates on them do not circulate in the blood plasma. We already saw that with ATP, NADH and FADH.

Several things can happen to glucose-6-phosphate. It can be converted to the storage form of carbohydrates, glycogen. Alternatively, it can be used for synthesis of other molecules. Or, it can be broken to 3C compounds in reactions where ATP is made. This last pathway is glycolysis and is what we are interested in here.

In glycolysis, a series of enzyme-catalyzed reactions occur. All the intermediates have phosphate bound to them, and ultimately these phosphates get transferred to ADP to form ATP. Two ATP's are used to start the pathway, and 4 ATP's are formed. In the red blood cell, these extra two ATP's molecules are used to maintain the ion gradients in the cell.

The final step of glycolysis is the removal of phosphate from the intermediates to form lactate and pyruvate. Without phosphate, these compounds are free to leave the cell. This is what happens in the red blood cell, which has no mitochondria. Cells that have mitochondria can convert lactate and pyruvate to CO₂ and acetylCoA.

The products of glycolysis, **pyruvate** and **lactate**, have three C’s. This is pyruvate:

![Pyruvate structure](image)

The other product of glycolysis is lactate.

![Lactate structure](image)

The difference between propionate and lactate is in the number of H's.
Here is where NADH comes into play. NADH adds H’s to pyruvate; in losing H’s it becomes NAD.

The glycolysis pathway is absolutely essential for human life. But only under some times, it becomes the major energy supply. During vigorous exercise, the supply of O\textsubscript{2} to muscle is not sufficient to supply the mitochondria so that mitochondria can keep up with production of ATP. Then glycolysis produces most of the ATP for the energy required for muscle contraction. This is called “anaerobic” exercise. (On an exercise bicycle, anaerobic exercise is when the muscles are rapidly contracting. Aerobic is a slower motion, that allows sufficient O\textsubscript{2} to be delivered to the mitochondria.)

In some cancer patients when the cancer is very advanced, high lactate in the blood indicates that glycolysis is producing much of the body’s ATP. Glycolysis does not require O\textsubscript{2}, and in large tumors, O\textsubscript{2} is not delivered to the center of the tumor, so glycolysis becomes predominant. But, like a hybrid car that cannot go for very long on the anaerobic electrical system, the human body cannot last very long solely on anaerobic metabolism of glycolysis. More ATP is required than what is supplied by glycolysis.

To summarize glycolysis: the final products of glycolysis are lactate/pyruvate and ATP. For each glucose molecule that is metabolized to two pyruvate or lactate molecules, two ATP molecules are formed.

4.5.2 Pathway 2. Pyruvate dehydrogenase splits three C into two C plus CO\textsubscript{2}; NADH formed

Pyruvate has 3C’s and it comes from glucose, via glycolysis and it is also a product of some amino acids. First, pyruvate enters the mitochondria. Then the pyruvate dehydrogenase\textsuperscript{1} enzyme chops pyruvate into acetate and CO\textsubscript{2}. In doing this, it converts an ADP molecule into ATP.

This is the first time where we see the production of CO\textsubscript{2}, the final product of metabolism. CO\textsubscript{2} goes into the blood stream and it leaves the body in the lungs. The other two products of the pyruvate dehydrogenase reaction are acetate and NADH. Pyruvate dehydrogenase ties up acetic acid with coenzyme A, abbreviated CoA. This is acetyl CoA:

\[
\text{H}_3\text{C}-\text{CO} \quad \text{O} \quad \text{H} \quad \text{CoA}
\]

The CoA tail prevents acetate from leaving the mitochondrion, just as phosphate prevents glucose-6-phosphate and other intermediates of glycolysis from leaving cells. The final products of pyruvate dehydrogenase are ATP, NADH, acetylCoA and CO\textsubscript{2}. You notice that we have gotten energy, ATP, from the action of the pyruvate dehydrogenase enzyme.

4.5.3. Pathway 3. β-oxidation breaks fatty acid to 2 C; FADH and NADH formed

The break-down of fat into CO\textsubscript{2}, yields many calories in the form of ATP. The starting material in fat metabolism is a fatty acid, shown here:

\textsuperscript{1} The names of many enzymes end in “-ase”. In contrast, note on Table 4.1 that sugar molecules end in “-ose.”
First, a CoA is added by an enzyme to keep the fatty acid inside the cell:

Then an O atom is placed at the third C. In chemistry the first C from the carboxy end is called the $\alpha$ (alpha) C and the second one is called the $\beta$ (beta) C. Hence, this pathway is called $\beta$ oxidation.

In putting the O onto the fatty acid, NAD goes to NADH and FAD (flavin) goes to FADH.

Next, the fatty acid splits at the place indicated by the blue arrow. The two products are acyl CoA and acetylCoA. The two products have similar sounding names! Acetyl CoA means a two C unit; it is a derivative of acetate. Acyl is a generic name for any fatty acid group, irrespective of the number of C's.

You see that you have gotten acetylCoA and a fatty acid CoA that is two C’s shorter than the starting acid. Then the process starts over. The 16 C fatty acid is chopped to become 14 C plus acetyl CoA, each time producing NADH and FADH. Then again and again, to make 12 C, then 10 C, then 8 C and so on until finally all the fatty acid is chopped down to acetyl CoA.

The final products of $\beta$-oxidation are acetyl CoA, NADH and FADH.

4.5.4 Pathway 4. Citric acid cycle takes two C (acetyl CoA) to CO$_2$; NADH and FADH formed

Acetyl-CoA is formed from the breakdown of fat (via $\beta$-oxidation) and of sugar (via glycolysis and pyruvate dehydrogenase). Acetyl-CoA is oxidized by O$_2$ to CO$_2$ in a series of steps in the mitochondria. This pathway is called the citric acid cycle. It is called a cycle because the molecules that are involved in the pathway regenerate themselves, and the word citric refers to one of compounds in the cycle. We will also mention the citric acid cycle later when protein use as food is discussed. Acetyl-CoA combines with a 4 C compound. Then, in a series of steps, those two C’s that came from acetate get chopped off to form CO$_2$, and the 4 C compound is regenerated.
Every acetyl group gets converted to two CO$_2$ molecules. Once acetyl gets into the pathway, it is the end of it in the body. The CO$_2$ produced goes into blood, the blood transports it to the lungs, and from the lungs it is exhaled to the atmosphere. To convert acetyl to CO$_2$, three NAD’s and one FAD are reduced to NADH and FADH, respectively.

The final products of the citric acid cycle are CO$_2$, NADH and FADH.

4.5.5 Pathway 5. Oxidative phosphorylation uses O$_2$ to make H$_2$O; NADH and FADH transformed back to NAD and FAD with the formation of ATP

Oxidative phosphorylation is the powerhouse ATP producing pathway of the body. Oxidative phosphorylation is the pathway where FADH and NADH from pyruvate dehydrogenase, β-oxidation and the citric acid cycle ultimately react with O$_2$ to produce H$_2$O, the other final product of metabolism. In so doing, FADH and NADH are converted back to FAD and NAD, respectively. While this is happening, ADP is phosphorylated to form ATP.

The proteins that catalyze oxidative phosphorylation are embedded in the inner membrane of the mitochondria. Many of the proteins that carry out these reactions are colored like hemoglobin, the protein that makes blood red.
Electrons are transferred between these proteins in the membrane of mitochondria. NADH goes back to NAD and O\textsubscript{2} goes to H\textsubscript{2}O. During these processes, ATP is made from ADP. For each NADH converted back to NAD, 3 ATP’s are formed. For FADH going to FAD, two ATP’s are formed. You can see why the mitochondria are the cell’s power house.

The pathways used at a given time depend whether we are using fat or sugar for energy. There is one pathway, glycolysis, and one single step, pyruvate dehydrogenase, for sugar metabolism. There is another pathway, β-oxidation, for fat. These pathways converge since the C product of both sugar and fat is acetate, a 2C compound. Acetate is bound in a compound called acetyl CoA. Acetate is degraded to CO\textsubscript{2} in the citric acid cycle (pathway 4). The citric acid cycle produces NADH, which is described below. NADH reacts with O\textsubscript{2} in a series of reactions to form ATP.

### 4.6 Road map of pathways

Figures 4.4 and 4.5 summarize the pathways for glucose (sugar) and fat metabolism. Note that CO\textsubscript{2} and H\textsubscript{2}O are the final products of metabolism of both fat and carbohydrates. The metabolism of both leads to ATP production.

Proteins also give us energy. Proteins are broken down to amino acids in the intestine. Like fat and carbohydrates, amino acids are metabolized to form CO\textsubscript{2} and H\textsubscript{2}O, thereby producing ATP. Some amino acids are metabolized using the pathways for glucose; some are metabolized like fatty acids.

![Sugar, Fat, and Protein Metabolism](image-url)
4.7 When do these pathways operate? How is the production of ATP regulated?

The pathways are complex, but in a way they are quite simple. The simple version is that six C sugars go to three C molecules, which then gets converted to two C’s. Fatty acids get converted to two C’s. The two C molecule, coming either from glucose or fat, goes to CO$_2$. The side products of these reactions, FADH and NADH, put an H on O$_2$ to produce H$_2$O, this process coincides with making ATP.

At a deeper level the metabolism to produce ATP is quite complicated. Why is that? Probably, it is because all of the reactions must be regulated so that balance is maintained.

The major regulating molecule is ADP. The pathways that produce ATP are stimulated when the cell needs ATP. In a resting cell, the levels of ATP are high. When the cell uses energy, ATP goes to ADP, because ATP is used for energy.

$$\text{ATP} \rightarrow \text{ADP} + \text{P}$$

At high ATP, oxidation by O$_2$ is inhibited: the reactions of oxidative phosphorylation are not going on. On the other hand, when ADP is high, then mitochondria suddenly use O$_2$ and they oxidize NADH to NAD and FADH to FAD. When NAD is available, then the citric acid cycle goes into gear and acetylCoA goes to CO$_2$. When NAD is high, β-oxidation can occur and fatty acids get broken down. When NAD is high, pyruvate dehydrogenase is activated, so that propionylCoA goes to acetylCoA and CO$_2$.

So these three pathways all go fast when ADP is high, indicating the cell needs ATP. Oxidative phosphorylation, citric acid cycle, β-oxidation and pyruvate dehydrogenase pathways slow down when ATP is high. All of these mitochondrial pathways depend upon O$_2$ to keep NAD and FAD ready to accept H atoms.

4.8 Hormones regulate metabolism

When you look at the pathways, you can see another question. You notice that acetylCoA can come from fat or from glucose. How does the cell “know” which food source to use? The
answer is easy for the brain. The blood brain barrier does not allow fat to go into the brain. So the brain uses glucose under usual conditions (and keto-acids coming from fat during starvation). For other tissues the major regulation is by the hormone insulin. When blood sugar is high, the pancreas produces insulin. Insulin prevents the breakdown of fat to fatty acid, and therefore β-oxidation does not occur. Insulin stimulates glycolysis and pyruvate dehydrogenase. Therefore the acetylCoA used in the citric acid cycle will be obtained from glucose. Excess acetylCoA from glucose is made into fat (Chapter 6). Insulin also stimulates the production of fat from glucose.

When a person does not have sugar in the blood, insulin is low. Low insulin stimulates the release of fatty acids from adipose.

We will discuss how insulin works on important enzymes of metabolism in Chapter 11.

Glycolysis is hormonally regulated indirectly by insulin, but it also sensitive to low energy levels, i.e., low ATP, in the cell. Unlike oxidative phosphorylation, which is stimulated by ADP, glycolysis is stimulated by AMP, which stands for adenosine monophosphate. AMP is made from ADP, catalyzed by an enzyme called myokinase. The reaction is:

$$2\text{ADP} \rightarrow \text{ATP} + \text{AMP}$$

When ADP is high, this reaction takes a phosphate from one ADP on another one and makes ATP, which can then be used for energy by the cell. The remainder AMP stimulates glycolysis, and by this means more ATP is supplied.

### 4.9 Insulin regulates the fuel sources

The metabolism of carbohydrates and fat gives us ATP. The figure below illustrates how the pathways are regulated by one hormone, insulin, and by the energy requirement of the cell.

**Figure 4.4.**

Oxidative phosphorylation is stimulated by ADP. When oxidative phosphorylation is occurring, NADH becomes NAD. NAD is supplied to pyruvate dehydrogenase, β-oxidation and citric acid cycle pathways. Glycolysis is stimulated by AMP and high insulin. Low insulin is required for fat release from adipose.

### 4.10 Long term regulation: lactate threshold
In exercise, the point where lactate is produced is called the lactate threshold. This occurs where O\textsubscript{2} supply does not keep up with the requirement for ATP, and is unable to remove the lactate produced from glycolysis. A trained athlete will be able exercise for a longer period compared to a “couch potato” before lactate builds up. The training program of an athlete increases the number of mitochondria in the muscle, the amount of capillaries and the power of the heart, so that O\textsubscript{2} can be delivered faster. Changing the level of the enzymes is a long-term regulation of metabolism.

Thyroid hormone also controls metabolism. People who have low thyroid have low metabolism, meaning that these pathways are going slowly. With excess thyroid, these pathways run in full gear. But, thyroid hormone does not directly act on the enzymes of mitochondria or glycolysis. It uses another form of regulation that is not fully understood.

4.11 Cases

4.11.1 Diseases where ATP levels are too low in red blood cells

Here are descriptions of two diseases that involves glycolysis and affects red blood cells (RBCs). These diseases are both described as being hemolytic anemia. Hemolytic means that the RBC’s break down too soon. (“Hemo” refers to blood and “lytic” comes from the word lysis, to break apart). A normal RBC lasts in the blood for about 120 days. If the RBC is defective for some reason, the RBC is taken out of circulation sooner than that, and the patient will have fewer RBCs. The patient will have anemia – not enough RBCs. In the following cases patients have similar symptoms, but the reasons for the hemolytic anemia are different.


A group of 8 patients were recovering in the hospital following gastrointestinal surgery. They were in the hospital for 3 to 4 weeks, during which time they were given nutrition through a catheter in the subclavian vein. The nutrition included amino acids, glucose and vitamins.

The hematocrit and hemoglobin was low (8-10 g/dl; normal 12-15 g/dl) for these patients. These two values showed the doctors that the patient had lower than normal amount of red blood cells (RBC’s) The researchers took blood from the patients and examined the red blood cells. They lyzed the cells to release the enzymes contained in the cells and to examine the levels of metabolites. They found that the enzymes in the RBC’s were normal, but the level of ATP was low. They also found that phosphate levels were low.

**Diagnosis, treatment and discussion:** In this case, the patients suffered from a nutritional lack of phosphate. With a low level of phosphate, the conversion of ADP to ATP was slow. If ATP is not regenerated fast enough, ATP levels will decrease. ATP is constantly be used to maintain the salt levels in RBC’s. Without the proper salt levels, RBC’s will be unstable.

These patients were treated by including phosphate in their intravenous (IV) nutrition. This kind of hemolytic anemia caused by phosphate deficiency is no longer seen in hospital patients with long-term IV nutrition because, after this paper was published, phosphate is included in the intravenous fluid.

**Case 2. Hemolytic anemia in a boy.** (Br. J. Hematol. 132, 523-9, 2008, Noel N., Flanagan, JM et al)
This patient was the only son of a healthy couple from Barcelona. Pregnancy was normal, but after delivery the baby showed severe anemia (Hb 7.3 g/dl; normal 10-12) and jaundice. Jaundice occurs because there is an elevated amount of bilirubin, a break-down product of hemoglobin. The anemia recovered without blood transfusion. Red blood cell morphology appeared normal under a microscopy. At 2 years of age, the patient was hospitalized due to severe anemia (Hb 6.6 g/dl) and jaundice for which he received exchange transfusion and antibiotics. The diagnosis of a deficiency in an enzyme of glycolysis was established at the age of 3 years during a hemolytic crisis. Blood sample was taken from the boy and tests for enzyme activities was undertaken. After examining the enzymes in the RBC’s, diagnosis was made. This patient has phosphoglycerate kinase deficiency.

**Diagnosis, treatment and discussion:** This patient has a defect in an enzyme that takes a phosphate from an intermediate of glycolysis and puts it on ATP. Because this enzyme was defective, the ATP levels in the RBC’s are low, because glycolysis cannot go fast enough to keep the ATP high. Blood transfusion will alleviate the anemia. Since glycolysis is required for all cells, and nerve cells use glucose, not fat, for metabolism, they are also affected. The boy continued to be ill. At 7 years of age the hemolytic crises were associated with a progressive neurological impairment leading to mental retardation.

The above two cases illustrate two examples of metabolic diseases. The first is a nutritional disease. The second is a genetic disease that runs in families. Both affect glycolysis; in both cases glycolysis becomes slower than it should be to keep ATP levels high to maintain the salt levels. In both cases the hematocrit (number of red blood cells in the blood) and hemoglobin is low because the RBC’s are degrading too fast. These diseases particularly affect RBC’s because RBC’s do not have mitochondria, which supply most cells with most of their ATP. Note that at longer times, the brain of the boy was affected. Glycolysis is needed for brain function.

### 4.11.2 Case where mitochondria do not produce enough ATP

The case below is a disturbed metabolism in mitochondria.

**Case 3: Coma in a family on a holiday trip** (Adapted from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5402a2.htm)

Family of four (husband, wife and two children) went on an annual Christmas vacation to their cabin in the north woods. An oil furnace heated the cabin. After unwrapping their presents on Christmas eve, the family went to bed.

The husband awoke at 2:30 AM feeling nauseous and confused. He was unable to wake his wife or his children. He called 911.

**Diagnosis, treatment and discussion:** The emergency response team gave the family members oxygen and opened the windows of the cabin. All family members responded by becoming alert. They had no long-term effects. The diagnosis was CO (carbon monoxide) intoxication, due to faulty ventilation of the oil furnace. CO is deadly because CO replaces O₂ in hemoglobin, and consequently there is not enough O₂ to maintain oxidative phosphorylation. CO also inhibits the last enzyme in oxidative phosphorylation. For these two reasons, oxidative phosphorylation is slowed. Not enough ATP is produced by the mitochondria to maintain functions of the cell. Without a constant supply of ATP in the brain, death occurs.

### 4.12 “Cheat sheet” of pathways
The pathways that produce ATP and the locations of the enzymes of these pathways are listed below in Tables and diagrams below. The Table and figures are given as a “cheat sheet” to help you study.

Table 4.2. Pathways for energy production

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Location</th>
<th>Starting compound and its fate</th>
<th>Ending compound</th>
<th>Energy compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Glycolysis</td>
<td>Cytoplasm</td>
<td>Glucose (6 C’s) gets converted to 3 C (pyruvate and lactate).</td>
<td>Pyruvate, lactate (3C)</td>
<td>Net 2 ATP’s formed</td>
</tr>
<tr>
<td>2 Pyruvate dehydrogenase</td>
<td>mitochondria</td>
<td>Pyruvate (3 C’s) gets converted to 2C’s (acetyl CoA) and CO₂.</td>
<td>Acetate (2C as acetyl CoA)</td>
<td>NADH produced from NAD; One ATP molecule formed</td>
</tr>
<tr>
<td>3 β-oxidation of fatty acids</td>
<td>mitochondria</td>
<td>Fatty acids broken to 2 C units (acetyl CoA)</td>
<td>Acetate (2C as acetyl CoA)</td>
<td>NADH produced from NAD; FADH produced from FAD</td>
</tr>
<tr>
<td>4 Citric acid cycle</td>
<td>mitochondria</td>
<td>Acetyl CoA gets converted to CO₂</td>
<td>CO₂</td>
<td>NADH produced from NAD; FADH produced from FAD</td>
</tr>
<tr>
<td>5 Oxidative phosphorylation</td>
<td>mitochondria</td>
<td>NADH goes to NAD; FADH goes to FAD; O₂ goes to H₂O.</td>
<td>H₂O</td>
<td>NADH to NAD, 3 ATP formed. FADH to FAD, 2 ATP formed.</td>
</tr>
</tbody>
</table>

*Powerhouse*
5. Storage and retrieval from glycogen: Metabolism during short term fasting

5.1 Storage form of carbohydrates is glycogen; Glycogen is fuel for quick energy

The whole of metabolism is this:

\[ C_{6}H_{12}O_{6} \text{(food)} + O_{2} \rightarrow CO_{2} + H_{2}O + \text{energy} \]

This is the overall reaction that gives us ATP, as we outlined in the previous chapter. We emphasized that this reaction must occur at all times. We get \( O_{2} \) from breathing, and we must breathe at all times. The other part of metabolism is \( C, H \) and \( O \), which represent the major elements in food.

Luckily for us, our bodies store food, so we do not have to be eating all the time. Without our bodies storing food, civilization would cease because we would have no time for education, music, art, work or hanging out with friends.

We already know that the three forms of food are carbohydrates, proteins and fat – and these forms of food are also stored for fuel. But we do not store the same molecules that we eat. They must be processed to be suitable for storage. And there must be a way to retrieve the stored molecules when the body needs energy from the stored molecules.

The various types of stored fuel are retrieved in different ways and at different times. Figure 1 shows what metabolites are supplying to GB after eating, and then during fasting and starvation.

**Figure 5.1**

After eating, glucose in blood comes from food. Some of this glucose is removed from blood by being metabolized to \( CO_{2} \). Excess is made into glycogen and fat. After several hours, glucose is released from liver glycogen (this chapter). When liver glycogen is used up the liver makes glucose from amino acids of protein (Chapter 6). Some tissues, such as heart, use fatty acids for fuel at all times. After long term fasting, keto acids are used for fuel. They are made from fatty acids in the liver (Chapter 7).
This chapter deals with the storage and retrieval of fuel from carbohydrates. The next two chapters discuss protein and fat, respectively.

Carbohydrate is stored as glycogen. We are starting with glycogen because we are thinking of our patient, GB, and what happens after he eats. Glycogen is mobilized for energy after a short term fast. Glycogen is made from glucose and in the liver it breaks back down into glucose. This glucose goes into the blood stream and supplies fuel for the brain. In other tissues, glycogen breaks down into glucose-1-phosphate, and the particular tissue uses this for its own fuel. The two organs that have large glycogen stores are liver and muscle.

### 5.2 Glycogen in liver and muscle

All tissues store glycogen. But because so much of our overall metabolism occurs in liver and muscle, these tissues are emphasized. Here is the overview of the storage and release of fuel from these two tissues:

- **Liver** is the unselfish “good-guy” of metabolism. Liver takes sugar from the blood stream and makes it into glycogen after eating. When glucose in the blood gets low, glycogen stored in the liver is broken down to glucose. This glucose goes out from liver into the blood stream. The brain (and other tissues) picks up this glucose from the blood and uses it for energy. The source of fuel from liver glycogen for the brain is especially important for short term fasting. Short-term is, say, less than about 12 hours after eating.

- **Muscle** uses both fat and glucose for energy, but fast, white muscles, used for sudden motion, primarily use mostly glycogen as a fuel. Muscle stores glycogen, and muscle’s stored glycogen gets broken down into glucose-6-phosphate. The glucose that comes from muscle glycogen never leaves the muscle cells, and instead, glucose 6-phosphate gets converted to 3 C compound (pyruvate and lactate). Consequently, the muscle uses its glycogen for itself. Muscle is “selfish”– it does not directly share glucose from its stored glycogen with other organs.

### 5.3 Hormones regulate the use of glycogen

The use of glycogen is different in the liver and in muscle. Glycogen storage and release in both tissues are hormonally controlled.

One player is **insulin**, which was introduced in the last chapter. Three other hormonal players are **epinephrine**, **glucagon** and **cortisol**. Insulin stimulates the storage of fuel; Epinephrine, glucagon and cortisol stimulate the release of fuel from storage in the tissue. “Glucagon” sounds a lot like “glycogen” but we will remember that:

- **glucagon** is a hormone and
- **glycogen** is the storage form of sugar

Both glucagon and insulin are made in the pancreas. The pancreas is an organ that lies behind the stomach and under the liver, and it makes both endocrine and exocrine hormones and enzymes. Glucagon and insulin are **endocrine** hormones; the pancreas secretes them into blood plasma, not the digestive tract. Pancreas also excretes enzymes that are used to break down food in the digestive tract; these enzymes are considered exocrine enzymes.
Glucagon and insulin are both made of amino acids, so they are classified as polypeptides (in effect they are mini-proteins). The pancreas contains a specialized substructure, picturesquely called “Islets of Langerhans”. One type of cell within the islets is the α (alpha) cell. α cells secrete glucagon. Another type of cell is the β (beta) cell. β cells secrete insulin. At “normal” glucose levels, a low level of insulin is secreted but after a high carbohydrate and protein meal glucose and amino acid levels in the blood go up. The β cells respond to high glucose by secreting insulin into the blood. Insulin acts on many tissues, and its action is to reduce glucose levels in the blood. Without eating, glucose levels decrease more and more in the blood. Then the hormone glucagon comes into play. Its function is to stimulate the release of glucose from stored glycogen, thereby increasing glucose levels in the blood.  

Two other hormones act to increase blood glucose levels. Both are made in the adrenal cortex, a gland that is above the kidneys. Epinephrine is derived from an amino acid, and it is a hormone that elicits a short-term release of glucose from stores. Cortisol is the third hormone; it is produced in the cortex of the kidneys. Cortisol is derived from cholesterol. It acts to stimulate the making of glucose from protein. We will discuss the role of cortisol in maintaining glucose levels the next chapter.

5.4 Storage of carbohydrates after eating

5.4.1 Liver

In the previous chapter we showed what happens to glucose when ATP is required: glucose gets transformed to a 3 C compound with the production of ATP by glycolysis. Then it gets chopped to 2 C, which goes to CO₂ in the mitochondria. In doing this, mitochondria consume O₂ and produce ATP.

Now we look at what happens when the liver cell has more glucose than needed to make ATP for its energy needs. After a high carbohydrate meal, glucose in the blood plasma is high. High glucose stimulates the pancreatic β-cell to secrete insulin. Insulin stimulates glycogen synthesis. Glycogen does not accumulate indefinitely, however. Unlike fat where we can store more and more (getting fatter and fatter), when the glycogen store is filled, no more goes in. When liver is “full” of glycogen, glycogen accounts for about 10 % of the weight of the liver. After the glycogen store is filled, excess sugar gets transformed to 3 C’s (lactate and pyruvate), then to 2 C’s (acetyl group in acetylCoA) and then made into fat.

After eating, when glucose in the blood is high, insulin is secreted. So insulin in blood goes high while glucagon secretion goes down. Consequently, after eating carbohydrate, the hormone

2 There are two other types of cells in the Islets of Langerhans. The d cells secrete somatostatin and the PP cells secrete pancreatic polypeptide. These hormones serve to regulate insulin and glucagon. So we have hormones regulating other hormones! But for the level of discussion here, we will not consider the action of somatostatin and pancreatic polypeptide.

3 Another name for epinephrine is adrenaline.

4 Epinephrine controls blood flow and makes the heart beat faster. There is a related compound called norepinephrine that also play a role. We are interested in metabolism here, but only point out that the effects of hormones are more complex than what we describe.

5 You might have noticed that in the morning your abdomen feels flatter than at night. This is because the stored glycogen in the liver has been used up overnight.
ratio of insulin to glucagon (I/G) is high. This hormone set stimulates the storage of glycogen in the liver and the transformation of excess glucose into fat. Figure 1 diagrams this.

**Figure 5.2**
Storage of fuel in the liver after eating carbohydrates

High insulin/glucagon (I/G) stimulates glycogen synthesis. When glycogen stores are filled, high I/G stimulates fat synthesis.

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**5.4.2 Muscle**

Glucose is also taken into the muscle after a high carbohydrate meal, when I/G is high. Glucose is also taken into the muscle during exercise, even when insulin is low. These processes are diagramed in Figure 5.3.

**Figure 5.3**
Storage of fuel in muscle after eating carbohydrates.

High I/G promotes glucose uptake into muscle. Glucose is also taken into muscle during exercise. High I/G promotes glycogen synthesis.

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**5.5 How glucose is released from glycogen**

**5.5.1 Liver – the good guy**

We learned in Chapter 4 that fat gets broken down into acetyl CoA, which gets oxidized to CO₂ in the citric acid cycle. Two C’s are in the acetyl group and two CO₂ molecules are formed. So fatty acids are not converted into glucose – they get broken to CO₂, CO₂ is lost in breathing. The brain needs glucose for metabolism. The brain is selective in what it takes from the blood. The
brain is protected by what is called the “blood-brain barrier”. The capillaries in the brain form a barrier that prevents the brain from taking fatty acids in the blood.

So, there needs to be a source for glucose and the source of glucose for the brain is the liver. Under “short-term” fasting conditions, glycogen in the liver gets degraded back to glucose. Hormones control the breakdown of glycogen to form glucose-6-phosphate. When glucose is low, insulin goes down, and glucagon goes up. This is the trigger for the enzymes that break down glycogen to act.

Figure 5.4
Pathways in the liver after short-term fasting.

An enzyme called “glucose-6-phosphate phosphatase” (a long name!) catalyzes the final step in getting glucose from glucose-6-phosphate. “Phosphatase” refers to an enzyme that breaks off phosphate, and the name of the enzyme means that it breaks off phosphate from glucose-6-phosphate.

This enzyme is found in the liver. Other tissues do not have this enzyme, and so once glucose has a phosphate on it, in other tissues this glucose stays within the cells. (There is an exception: a small amount of this enzyme is in the kidney, and the kidney supplies some glucose in long term fasting. We will discuss this in the next chapter). Consequently, other tissues use glycogen as an energy store only for themselves. Since they do have the enzyme glucose-6-phosphate phosphatase they cannot release glucose into the blood stream for the use of other tissues.

So this is why we call the liver “the good guy”. It releases glucose from its glycogen stores into the blood for the brain to use.

The enzymes that release glucose from glycogen are also triggered to act in an emergency by the hormone epinephrine. We mentioned before that GB is walking in the woods and a bear jumps out. The hormone epinephrine (aka adrenaline) comes into play. Epinephrine stimulates the break-down of glycogen in both the liver and muscle. The glycogen from the muscle gets metabolized to lactate in the anaerobic, fast muscles. Glucose goes to CO₂ in the aerobic muscles. Both muscle types will be used when GB runs away. GB is saved!

5.5.2 Why and when muscle uses its stored glycogen

6 Remember that the names of many enzymes end in “-ase”.
Muscles attached to our skeleton enable us to move, search for food, escape danger and do work. Muscles consume much of our bodies' energy. A person who is rigorously skiing, running or swimming consumes up to 6000 kcal/day. When this person is sedentary, the amount of calories consumed is about 1500 kcal/day or even less. From this it is clear that muscles consume a lot of ATP when we are active – and they must be ready for sudden action at all times.

Muscles are biological engineering marvels. Muscle cells have many fibers and the fibers are composed mainly of two proteins, called actin and myosin; both of these form filaments. When muscles are relaxed, the filament composed of actin molecules and the filament composed of myosin molecules do not interact with each other. When muscle contracts, a portion of the myosin binds to actin. To bind, energy is required, and ATP is used. As the muscle contracts, the portion of myosin “walks” along the actin, much like a caterpillar walking. This makes the muscle shorter, and the muscle pulls upon a bone causing it to move.

![Figure 5.5. How muscles work.](image)

When muscle is relaxed, actin and myosin filaments are non-interacting.

When muscle contracts, actin and myosin interact. The molecules of myosin bind to actin. For this to occur, ATP must be used.

What makes muscle contract? Muscle contracts in response to nerve stimulation. Nerves cause the release of calcium (Ca) from specialized organelles in the muscle cell. (These membranes are the endoplasmic reticulum organelles, see Figure 1 in Chapter 3). In response to the presence of Ca, ATP is hydrolyzed by myosin, to form ADP and P, and the muscle contracts. When the muscle is no longer stimulated, Ca gets pumped back into the membranous organelle, and the muscle relaxes. It is again extended.

The function of the amazing muscles is related to metabolism. There are two basic types of skeletal muscles: fast and slow. Without resorting to autopsy of humans, we can illustrate these two muscle types during our Thanksgiving dinner. The wild turkey, which is the progenitor of our domestic turkey, has a mixture of red and white muscle on its breast. In the domestic turkey, there is a selection for white muscle, so that we can have white meat for dinner. White muscles are called “fast muscle”. The wild turkey survives by flying rapidly to cover when a predator is near-by. It accomplishes this using the fast muscle on its breast for flight. The white muscles use glycolysis to make ATP. On the other hand, when the turkey is picking up corn from the ground, it needs to stand for a long time. It uses the “slow muscles”. The leg of turkey has predominantly red muscles, they contain many mitochondria, and a red protein, called myoglobin that makes the muscle red and serves to transport O₂ in the cells. Fat is the main fuel of red muscles and oxidative phosphorylation is used to make ATP in the slow muscles.
Fast muscles use glycolysis for energy; and they work in quick response to stimuli. Fast muscles are full of glycogen. Under stimulation, glycogen breaks down to glucose, which gets transformed into lactate with the formation of ATP via the glycolysis pathway.

Now comes the neat thing. Nerve stimulation causes the release of Ca from the endoplasmic reticulum, and the Ca causes myosin and actin to interact and the muscle contracts. This very same Ca stimulates the break-down of glycogen to form glucose-6-phosphate. If you are texting while you are reading this, the white muscle fibers in your figures are being stimulated to contract by Ca, which also stimulates a little squirt of glucose-6-phosphate to come from glycogen, and then this glucose is used to produce ATP in the fiber. As we noted above, epinephrine also stimulates the breakdown of glycogen in muscle. This also quickly produces glucose-6-phosphate in the white muscle cells, for a quick response for movement.

Figure 5.6 White and red muscles
White, fast anaerobic muscles, use mainly glycogen for energy
Red, slow aerobic muscles, use fat and glucose for energy. Aerobic muscle fibers have many mitochondria.

Figure 5.7 gives summary of the metabolism in muscle during exercise. After anaerobic exercise lactate that builds up in the muscle, gradually leaks out.

Figure 5.7 Glycogen break-down in muscle
During exercise, glycogen is broken down to glucose-6-phosphate, stimulated by Ca ion. Glucose provides ATP by glycolysis. In aerobic muscles, the pyruvate gets oxidized to CO₂ in the mitochondria, via citric acid cycle and oxidative phosphorylation. In anaerobic muscle, lactate is produced. The lactate goes into the blood stream, and the liver metabolizes it.

In humans, the aerobic and the glycolytic fibers are mixed together in each muscle type. Sprinters and weight lifters tend to have high content of glycolytic muscle fibers, which allow
them to exert a burst of work for a short period of time. People who specialize in sports that require long sustained effort have muscles with high content of aerobic muscles that use fat as the primary fuel source. Humans can outrun most animals and if we train, we are able to run long distances for long periods of time. Tarahumara people from Mexico can run over 400 miles up and down canyons in a period of two days. We would expect that their muscles would be rich in mitochondria, and that during running the major source of energy would be coming from fat, not the glycogen stores.

5.6. Summary: Hormones regulate the storage and retrieval of glycogen

Two major concepts are introduced in this chapter. The first concept in this chapter is that hormones regulate storage and retrieval of food molecules. The second major concept explains why hormone regulation is so important. The important concept is that the storage and release of a fuel depends upon the organ. We saw that glycogen is used differently in liver and in muscle.

High level of insulin stimulates the enzymes that contribute to the storage of glucose in the form of glycogen. Low levels of insulin and high level of glucagon stimulates the enzymes that catalyze glycogen breakdown back to glucose. The breakdown is further stimulated by adrenaline in both liver and muscle and by Ca\textsuperscript{2+} in muscle.

In this book we are emphasizing basic concepts. A theme is that there are key enzymes that when either stimulated or inhibited control which pathway is working. In case of glycogen, the substances that stimulate synthesis, inhibit break-down, and vice versa. If you take an advanced course in Biochemistry, you will be given greater detail about how hormones affect particular enzymes. To whet your appetite for further study, at the end of the chapter is a summary of the hormonal regulation by insulin of glycogen synthesis and glycolysis.

5.7. Clinical case

This case illustrates what happens when glucose cannot be stored as glycogen, or retrieved from storage.


ST is a 43 year-old male patient, who in the past few years has had episodes of aching fingers, stiffness of legs and arms and suffers from general weakness. He noticed that the muscles in his hand show atrophy – i.e., they are wasting away. Indeed, the physician estimated about a 30 – 60% decrease in overall muscle strength.

His blood chemistry showed some deviations from normal. After an 8 hour fast the blood glucose was 3 to 3.5 mM (normal control: 4 to 4.5 mM). In the hospital after another 8 hour fast, keto-acids were detected in the urine. (Keto-acids are made from fatty acids and they are normally made under long-term starvation conditions. The presence of keto-acids indicates that fat is being used as fuel. This will be described in Chapter 7).

In order to study why ST had a low amount of glucose in blood after not eating for 8 hours, the physicians ordered a glucose tolerance test. In a glucose tolerance test,
the patient first fasts overnight, and then drinks a drink containing 100 grams of glucose. Following the drink, samples of his blood are taken and analyzed for glucose. Here is the result of the glucose tolerance test.

![Glucose tolerance test](image)

**Figure 5.8 Glucose tolerance test**

The glucose levels in blood after ingesting glucose is shown in the solid line for normal control person. The glucose level in the patient is shown in the dotted lines.

**Diagnosis and Comments:** This patient, ST, was found to be deficient in an enzyme required for the breakdown of glycogen. The enzyme that was deficient is the same in liver and muscle. So both the liver and muscles are affected.

So we now ask how the impaired enzyme is affecting ST. The weakness and wasting of muscle is consistent with low ATP. In this patient, ATP is low during anaerobic exercise. He cannot release glucose from glycogen, and therefore glycolysis is impaired, because glucose-6-phosphate is not being made from glycogen. The patient noticed wasting of muscles in his hands. The muscles in the hands are rich in white muscle fiber, and these muscles rely on glycolysis to make ATP.

In a normal person after eating an excess of glucose, glucose concentration in the blood increases. Insulin is released and insulin stimulates the removal of glucose. As glucose levels drops, the level of the hormone glucagon increases. Glucagon stimulates the breakdown of glycogen from the liver. The glucose released from liver glycogon goes into the blood, and blood glucose levels remain constant.

In ST, giving glucose causes an increase in glucose levels in the blood that is larger than seen in the controls. The reason for this, glucose is not being made into glycogen because the glycogen levels are already full (because the enzyme to breakdown glycogen is missing). At long times after taking glucose, the level of glucose in ST’s blood is below that seen in the control patient. That is because glycogen in the liver cannot be broken down to glucose.

A question for you to think about: what would you do for this patient?

5.8 For further study

Here is the pathway showing the enzymes that are the major points of regulation.
Glycogen synthetase: It is indirectly stimulated by insulin. Insulin inhibits the formation of cyclic AMP. At low cyclic AMP, synthetase is active. (High cyclic AMP, the enzyme that breaks down glycogen is activated).

Phosphofructokinase: AMP stimulates this enzyme; AMP is high when ATP is low, so AMP serves to “tell” this enzyme that more ATP is needed. Phosphofructokinase is also stimulated by 2,6 frutobisphosphate, a compound that is high at when insulin levels are high and glucagon levels are low (I/G is high).

Pyruvate kinase: Pyruvate kinase is inhibited by having a phosphate put on the enzyme. At high I/G proteins called protein phosphorylases are stimulated. These proteins remove phosphate from pyruvate kinase, and pyruvate kinase is stimulated.
6. Sugar is made from proteins and small molecules; toxic N is removed

6.1 Our bodies keep blood sugar levels from dropping during fasting by making glucose from other molecules

In the past chapter, we saw how liver glycogen maintained glucose levels. But, even after glycogen is depleted, blood glucose does not drop to zero. If we were to follow the blood sugar of GB, we would find that his blood sugar only gradually declines, even when most of liver glycogen is gone.

When glycogen is gone, where does glucose come from? It turns out that liver and, to a lesser amount kidney, makes glucose from amino acids of proteins and other small molecules. Making glucose is called **gluconeogenesis** (genesis means “to make” and “neo” means new, so this word means making new glucose). Gluconeogenesis in GB occurs when glucose from diet and glucose from glycogen is depleted, as in an overnight and longer fasting.

Table 2 lists the main small molecules that are made into glucose.

<table>
<thead>
<tr>
<th>Gluconeogenic compounds</th>
<th>Structure</th>
<th>Source and use</th>
</tr>
</thead>
<tbody>
<tr>
<td>alanine</td>
<td><img src="image" alt="alanine" /></td>
<td>From protein. Liver uses mainly alanine for gluconeogenesis</td>
</tr>
<tr>
<td>Glutamate/glutamine</td>
<td><img src="image" alt="glutamate" /></td>
<td>From protein. Liver and kidney use for glutamate and glutamine for gluconeogenesis</td>
</tr>
<tr>
<td>Pyruvate/ lactate</td>
<td><img src="image" alt="pyruvate" /></td>
<td>Lactate is formed from glycogen by anaerobic muscle during exercise. Lactate and pyruvate is formed from glucose via glycolysis in red blood cells (RBC’s).</td>
</tr>
</tbody>
</table>
glycerol

<table>
<thead>
<tr>
<th>Blood glucose, mmol/l</th>
<th>Hormone response</th>
<th>Metabolic pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 8</td>
<td>Exceeds renal threshold, and glucose is lost in the urine, along with water, and electrolytes (Na⁺ and K⁺). Insulin high</td>
<td>Glycolysis, glycogen synthesis, fat synthesis, protein synthesis</td>
</tr>
<tr>
<td>5.5</td>
<td>Insulin secretion increases as sugar levels increase</td>
<td>Glucagon stimulates glycogen breakdown in liver; Epinephrine stimulates glycogen breakdown in liver and muscle</td>
</tr>
<tr>
<td>4.6</td>
<td>Insulin secretion decreases, but does not go to zero</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>Increased secretion of glucagon and epinephrine</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>Cortisol secretion</td>
<td>Muscle protein break-down; gluconeogenesis</td>
</tr>
</tbody>
</table>

The other part of this chapter is how to deal with a by-product of protein metabolism. Our proteins are always being broken down and remade. Proteins are made from amino acids and they are composed of C, H, O and N. The N released when our proteins are broken down produces ammonia (NH₃). Excess protein from diet also makes NH₃ and bacteria in the intestine make NH₃. Ammonia is very toxic. Therefore, in the discussion on protein metabolism, we must also talk about how N is removed in the body.

There are two ways the body gets rid of ammonia. The most important way is that it is transformed into urea (NH₃CONH₂) in the liver. Urea produced by the liver goes into the bloodstream, and the kidney excretes it into the urine. Under long-term starvation, there is another way to get rid of excess N. The kidney produces ammonia from glutamate and glutamine, and ammonia is excreted into the urine. During this time, the C’s of glutamate and glutamine are converted into glucose by the kidney.
6.2 Overview of glucose production in liver and kidney during fasting

This part of the book may be the most shocking – we make glucose from protein! So before we compare metabolism in liver and kidney, let us get an overview of when and how much glucose the two organs produce. As glycogen gets depleted in the liver, liver steps up its production of new glucose. The production of glucose, in kidney, in contrast is increased during long-term fasting, i.e. starvation. The time-course and source of C’s for glucose production are shown in Figures 6.2 and 6.3.

Figure 6.2
Glucose released from liver after fasting.
Figure is adapted from this web site:
http://www.medbio.info/Horn/PDF%20files/homeostasis1.pdf

Figure 6.3
Glucose released from kidney after fasting.
Note that the y-axis is expanded relative to Figure 6.2. Kidney makes far less glucose than liver.

Here are some things to notice about these graphs:

1. When glucose from glycogen goes down, liver steps up the production of glucose from amino acids. It turns out that the amino acid alanine is the major source of C’s to make glucose.

2. The production of glucose from amino acids in the liver declines after several days of fasting. In the next chapter we will learn why. (Hint: In long-term fasting, ketoacids are made from fatty acids, and the brain uses this as fuel. Glucose is no longer the major fuel for brain and the break-down of protein is reduced.)

3. The kidney steps up production of glucose after long-term fasting. The different time course of making glucose tells us that the liver and kidney make glucose for different reasons. As will be explained below, the major sources of C’s for making glucose by the kidney are glutamine and glutamate. These amino acids release NH$_3$, and this serves to adjust the pH of urine during starvation.
4. Glycerol serves to make glucose in both liver and kidney. Glycerol comes from triglyceride, fat.

5. Lactate and pyruvate are made into glucose in both organs. These substances are produced by glycolysis in red blood cells (RBC’s). Since all lactate and pyruvate is made back into glucose, RBC’s in effect do not use up glucose nor contribute to net glucose levels in blood.

Figure 6.2 does not show what is happening during exercise by anaerobic muscle. In anaerobic exercise, glycogen is broken down to glucose-1-phosphate, which gets converted to lactate, via glycolysis. Lactate goes into the blood, and the liver uses this lactate to make glucose.

6. Under short-term fasting conditions, the amount of glucose produced by the kidney is less than 20% of the total, but during long-term starvation, the kidney production of glucose can equal that of the liver.

### 6.3 Sugar from glycerol

We will describe the use of glycerol and pyruvate/lactate to make glucose first, and then discuss the more significant use of protein as a precursor for making glucose.

A small amount of the C’s in sugar made by gluconeogenesis is obtained from fat. This is a fat molecule, also known as triglyceride:

![Image of triglyceride molecule]

When fat is broken down, it splits into fatty acids and glycerol. Fatty acids go to 2 C units and are not made into glucose. But glycerol, which is 3 C is also part of fat. When fat is being made, glycerol, a side product of glycolysis, is used. This glycerol part is used when fat is being broken down to make glucose.

![Figure 6.4](image)

Fat is broken down into fatty acids and glycerol. Glycerol is made into glucose in the liver, and from there glucose goes into the blood stream.
Figure 6.5 shows the pathway for making glucose from glycerol. In the cytoplasm the 3 C molecule combines with another 3 C molecule to produce 6 C molecule, glucose 6-phosphate. The final step is that glucose-6-phosphate loses its phosphate and glucose is formed. This enzyme is only found in the liver and kidney, and so these are only organs that make glucose.

Figure 6.5
Glycerol, 3 C’s is converted into glucose, 6 C’s, in liver.

Since fat is always being used, the amount of glycerol made into glucose is relatively constant over the period of starvation (Figures 6.2 and 6.3). But during many days of fasting, the overall production of glucose from the liver decreases (Figure 6.2). Then the percentage of glucose obtained from glycerol becomes more significant.

6.4 Sugar from lactate and pyruvate

Lactate and pyruvate (both 3 C’s) are made from glucose. Lactate is formed from glycogen in anaerobic muscles during strenuous exercise. Lactate and pyruvate are always being made from glucose in RBC’s by glycolysis since glycolysis provides ATP for RBC’s. Without ATP the cells would break.

Lactate from muscle and RBC’s goes into the blood circulation, and is taken in by the liver, which can make it into glucose. This glucose goes into the blood circulation and can be used by other tissues, including the brain, or can be recycled back to the tissues that originally produced lactate.
Figure 6.6
Glucose is used in RBC’s to make ATP by glycolysis. The products are 3 C compounds, pyruvate and lactate. The liver makes glucose from pyruvate and lactate, but the amount of glucose made is equal to the amount broken down in RBC. So this process recycles glucose but makes no extra.

For RBC’s, there is no net glucose gained or lost by this process, so RBC’s do not supply lactate to make glucose for the brain. In muscle, the possible amount of lactate available to make glucose is limited by the amount of glycogen.

Here is the pathway for making glucose from lactate and pyruvate:

Figure 6.7
Lactate is made into glucose in the liver. First, lactate is converted to pyruvate. A C is added to make a 4 C compound. This compound exits the mitochondrion, and the C is removed and a phosphate is added. The 3 C compound adds to another 3 C compound and glucose-6-phosphate is eventually made. The phosphate is removed to make glucose.

6.5 Roles of proteins in the body

Now we are getting to that important idea that proteins can be made into glucose. “Everyone” knows that fat accumulates when we eat too much, and fat is diminished when we eat less. Athletes know the importance of glycogen in athletic efforts. Marathon runners attempt to increase their glycogen stores by eating carbohydrates the night before a race. But, how many people realize that protein is converted into sugar, and that this conversion occurs normally during every day?

Unlike glycogen and fat, which are storage forms of food, there is no storage form of protein that just sits there and does nothing except wait to be metabolized. All protein in our bodies has uses. Yet, proteins play an important role in nutrition and are used to supply energy. Proteins that we eat, whether the protein comes from plants or animals, are broken down to amino acids in the intestine, and these amino acids are used to make the proteins of our body. When
excess protein is eaten, the amino acids are transformed into fat and glucose in the form of glycogen. During fasting, protein of our body is used as fuel for energy. We have emphasized the importance of glucose in brain metabolism. During fasting, the same proteins that enable us to move, transport ions and many other things are converted into glucose by the liver. The kidney also makes glucose from amino acids, but the regulation of gluconeogenesis is different in liver and kidney.

6.6 Amino acids are involved in gluconeogenesis

Because new proteins are always being made we absolutely need to eat proteins in order to survive. Proteins contain 20 different amino acids. The sequence of amino acids determines the function of the protein. The proteins within all cells that have mitochondria are made within the cell from amino acids from the blood.

The metabolism of each amino acid is unique, but nearly all amino acids can be made into sugar. All organs and cells contain proteins. Muscle tissue has the largest amount of proteins in the body. Muscle proteins are always being made and being degraded. A typical lifetime for a muscle protein is about 10 days. That means that about 7% of muscle proteins are broken down and remade in a day. During fasting more protein is broken down than is remade. Although all amino acids are released when protein is broken down, enzymes in muscle convert them so that the major amino acids released into the blood are alanine, glutamate and glutamine.

Alanine has 3 C’s and it is a derivative of propionate and is related to pyruvate:

![Alanine structure](image)

Alanine is termed a “non-essential” amino acid. This means that it can be made in the body from other components. For instance, alanine can be made from pyruvate, using the N from another amino acid. This reaction can also be reversed: alanine can be made into pyruvate by giving the amino group to another molecule. This is the reaction:

![Alanine reaction](image)

In this equation R stands for any amino acid. This equation says that another amino acid can give its amino group to pyruvate; in so doing alanine is formed. The double arrow also indicates that alanine can be used to form amino acids too. The amino group of alanine can be put on the “R”- acid, to get an amino acid and pyruvate. Since all 20 amino acids are needed to make proteins, and each protein molecule is made of a definite sequence of amino acids, this swapping of amino groups helps to have the right ratio of amino acids available for making proteins.

Glutamate is another amino acid that is made in the body and hence it is also classified as a non-essential amino acid. Glutamate has five C’s and two carboxyl groups.
In the citric acid cycle (Chapter 5), we indicated some compounds with 5 C’s. When glutamate loses its amino group it becomes one of these 5 C compounds. You may be familiar with glutamate as a food additive – monosodium glutamate. Glutamate is a common amino acid, found in most proteins.

The final non-essential amino acid that we discuss is glutamine. This is glutamine:

Glutamine is like glutamate except that it has an amino group where glutamate has OH. Notice that glutamine has two N’s. This is important because glutamine helps to get rid of excess N.

Alanine, glutamate and glutamine are used to transport N from the various tissues to the liver, where the excretion product of N, urea, is made. Their carbons can be made into glucose. Glutamine also brings N to the kidney, where NH₃ is formed.

6.7 Amino acids made into sugar

We have seen the fate of fatty acid metabolism: formation of the 2 C unit, acetylCoA, which gets metabolized in the mitochondria to CO₂ to form ATP. Fatty acids are not converted into glucose. 2 C units are not converted into 3C units, and 3 C units are required to make glucose, which is a 6 C compound.

Figure 6.8 shows the sources for C for glucose when GB does not eat.

The amino acids are converted into glucose by the scheme in Figure 6.9. Alanine gets converted into pyruvate. During eating conditions, the enzyme that breaks alanine into two C’s is activated. During fasting, cortisol is high, glucagon is high and insulin is low. These conditions stimulate an enzyme that puts 1 C on pyruvate to make a 4 C compound. This compound exits the mitochondria. The enzyme that converts the 4 C into 3 C is stimulated by cortisol, as is the other enzymes that make glucose.
Vanderkooi

6.6 ATP, glycogen, protein

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Figure 6.9  Making glucose from alanine, pyruvate.

The figure also indicates that cortisol stimulates the enzymes of gluconeogenesis in the liver.

Figure 6.10

Glutamate and glutamine are the other amino acids involved in gluconeogenesis. The metabolism of glutamate and glutamine uses some of the reactions of the citric acid cycle (Chapter 4). The pathway is shown in the figure below.

Glutamine, which is C 5, first loses one N group to form glutamate, and then it loses the other N to form a 5 C compound in the mitochondria. This compound loses CO$_2$, and forms NADH and FADH in a series of chemical reactions. Remember that NADH and FADH are used in oxidative phosphorylation to make ATP. The 4 C compounds undergo reaction and one of them comes out of the mitochondria. In the cytoplasm, the 4 C compound loses CO$_2$, and becomes 3C. From then on, gluconeogenesis in the cytoplasm takes over.

6.8 Hormones regulate the production of glucose from amino acids

The hormone cortisol stimulates the making of enzymes that catalyzes reactions in the cytoplasm that changes 4C to 3C. In this way, cortisol stimulates the breakdown of some
proteins to produce the starting materials for gluconeogenesis (alanine, glutamine and glutamate) and the synthesis of other proteins that aid in making these amino acids into glucose.

When glucose is broken down to the 3 C compounds pyruvate and lactate in glycolysis, ATP is formed. When 3 C compound is used to make glucose, energy, ATP, is required. This ATP can be coming from the metabolism of amino acids themselves. We noticed above that the metabolism of glutamine produces NADH and FADH, which can be used to make ATP. But during gluconeogenesis ATP mainly comes from the oxidation of fatty acids. They are broken down to acetylCoA, and enter the mitochondria, and are oxidized to CO$_2$, forming ATP. So, although fatty acids do not give C’s to make glucose, their oxidation provides the energy for glucose formation by giving ATP.

6.9 What to do with waste N: Liver

At all times, in sickness or health and under feeding or starvation, proteins are being made and degraded. Some proteins last for a long time – up to our lifetime; the protein molecules in the lens of the eye are as old as our age. Some proteins survive a short time, only hours or even minutes. For example, PEP carboxykinase, the main enzyme that regulates making glucose, is synthesized when cortisol is high, but it is constantly being degraded. When feeding occurs, the levels of PEP carboxykinase drop rapidly, because cortisol levels fall and the enzyme is no longer being made.

Because proteins are being broken down continually, at all times we need to be making new protein. The amino acids from one protein are used to make another proteins. Since the amino acids generated when a certain protein is degraded may not exactly contain the amino acids needed to synthesize a different protein, at all times in our lives we need to eat some protein. The N’s from the excess amino acids need to be removed.

The liver is once again the “good guy” in that it removes N’s. Ammonia (NH$_3$) is generated from glutamine and glutamate and then this ammonia reacts with CO$_2$ and ATP to form a compound called carbamyl-phosphate.

$$\text{NH}_3 + \text{CO}_2 + 2 \text{ATP} \rightarrow \text{carbamyl phosphate} + 2 \text{ADP}$$

This is carbamyl phosphate:

![Carbamyl phosphate](image)

Carbamyl phosphate has one N and 1 C.

Carbamyl phosphate picks up a nitrogen atom from another amino acid in a series of reactions, called the urea cycle. Most of these reactions occur in the mitochondria.

Finally urea is formed. This is urea:

![Urea](image)

Urea goes into the blood. Kidneys take the urea from the blood, and the urea is excreted in urine.
The urea cycle is operating at all times, because protein degradation and synthesis is occurring at all times. If your diet is high in protein, more N is removed by the urea cycle, because the excess N from the amino acids must be disposed of.

### 6.10 N is released when glutamate and glutamine are metabolized in kidney

<table>
<thead>
<tr>
<th>Figure 6.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney uses glutamine and glutamate to produce glucose during long-term starvation.</td>
</tr>
</tbody>
</table>

During prolonged starvation fat is gradually used up, and then there is severe protein breakdown. Eventually, liver cannot make all extra N into urea. This is when gluconeogenesis in the kidney becomes important.

The kidney takes in glutamine and converts it to glutamate, releasing $\text{NH}_3$. This is the reaction:

Then the amino group of glutamate is also removed to yield $\text{NH}_3$ and a 5 C compound. This compound is made into glucose as in Figure 6.11. $\text{NH}_3$ is released into the urine. We will see that during long starvation, acids are formed, and some of these leak into the urine. $\text{NH}_3$ helps to keep the pH of the urine to the proper value.

### 6.11 Protein breakdown in disease

Protein breakdown occurs during many diseases. Viral infection stimulates protein breakdown from muscle. You may have felt weak after flu (caused by a virus), even after the infection is gone. A patient with HIV (also a virus disease) often suffers from wasting of muscle. Presumably, protein breakdown serves to give amino acids, which are used to form antibodies that fight the infection. Also, mucus – the scourge of sinus infections – is largely made of carbohydrate. If you are not eating during a cold, this carbohydrate is made from protein.

There are brain-body interactions regarding protein metabolism too. Cortisol is higher in stress. Long-term high level of cortisol leads to general loss of strength, as muscle is broken down. Many people complain that they tend to get colds or other infections during stressful times.
Long-term high levels of cortisol can ultimately reduce the levels of antibodies, proteins made in white blood cells that help ward off infection.

### 6.12 Hormonal regulation of protein breakdown and gluconeogenesis

Life is not simple. The production of cortisol is regulated by a series of other hormones. When glycogen stores in the muscle are close to depletion, the hypothalamus in the brain secretes a hormone called CRF (Corticotropin-Releasing Factor). CRF stimulates the pituitary gland to release ACTH (adrenocorticotropic hormone). ACTH, as its name implies, stimulates the adrenal gland to produce cortisol.

![Figure 6.5](image)

Cortisol production is stimulated by other hormones.

Cortisol and adrenaline are both produced in the adrenal glands, but in different locations in the gland. Cortisol is produced in the cortex and epinephrine in the medulla. Epinephrine is produced due to innervation and hence it is rapidly made, as nerve from brain stimulates it production. The production of cortisol is slower. Cortisol’s effect on metabolism is slower too. Cortisol stimulates the synthesis of key enzymes involved in glucogeneogenesis. It takes time to make a protein.

### 6.13 Summary

GB did what Mom said not to do: GB skipped breakfast. Yet, blood glucose did not go low, and GB passed a Calculus exam. The liver made glucose from amino acids. Cortisol stimulated the break-down of some proteins mainly in muscle. It also stimulated the synthesis of enzymes that catalyze the reactions that make glucose in the liver.

### 6.14 Case study

Health care people say that they “practice medicine.” This is a good phrase because it means that they recognize that they do not know everything and that they should learn from their patients. The group of patients described in this case helps to emphasize the importance of gluconeogenesis.

A group of children who have hypoglycemia (low blood sugar) with high keto-acids in the morning
Physicians observed that some children have low blood sugar and high keto-acids when they wake up after sleeping. In the paper cited above, 8 of these children were studied and compared with children whose blood sugar remains normal. The children ranged in age from 30 to 56 months. The affected children were all small (growth in the 20th percentile or lower) for their age. Most of the children suffered from seizures, and several had experienced coma.

The children were given glucose tolerance tests. This test is described in Case ??. The glucose levels during this test were the same within error for the patients and the control group. The glucose tolerance tests shows that the insulin response was normal. Glucose goes into glycogen during this test, and therefore the children are able to make glycogen. In both patient and control children, glucose in the blood went up, indicating that glycogen from the liver was broken down.

Next the investigators gave alanine by intravenous infusion. The glucose levels in the children went up, but not as high as normal.

The final test was to give the children cortisol. In this case, alanine restored glucose levels to normal.

The diagnosis was that in these children, cortisol was low. When cortisol was present, the children's level of alanine went up because cortisone stimulates the break-down of muscle, thereby releasing alanine. The glucose levels went up because cortisone also stimulates the enzymes of gluconeogenesis and alanine was made into glucose.

The children usually "out-grow" this condition, and by age 5 or 6 they produce appropriate levels of cortisone so that gluconeogenesis occurs during short-time fasting.

6.15 Appendix for additional study

The pathways to make something are always different from the pathways to break something down. We see that in glycogen synthesis. The enzymes that make glycogen are different from the enzymes that break glycogen down.

Glycolysis (break down of glucose) and gluconeogenesis (the making of glucose) pathways are unique in that both pathways share some enzymes. But some important enzymes are stimulated or inhibited and this is what ensures that the proper pathway is operating. Figure 6.12 shows the key enzymes that are activated or inhibited.

Starting from alanine, muscle breakdown is stimulated by cortisol. Alanine is transformed into pyruvate by transamination (it gives it amino group to another compound).

**Pyruvate kinase** is inhibited. High glucagon stimulates the formation of a compound called cyclic AMP, this stimulates an enzyme called protein kinase. Protein kinase puts a phosphate onto pyruvate kinase, and the enzyme becomes inhibited.

Pyruvate goes into the mitochondria where a C is added to it by **pyruvate carboxylase**. This enzyme is stimulated by acetyl CoA. Acetyl CoA is high when fatty acids are being used for fuel. Fatty acids are used for fuel in I/G is low. Fatty acid oxidation supplies ATP, needed for gluconeogenesis.

**PEP carboxykinase** converts 4 C to 3 C. The synthesis of this enzyme is stimulated by cortisol. Without cortisol present, there is little PEP carboxykinase.

**Fructose phosphatase** is stimulated by low I/G. It is not directly stimulated, but indirectly; an activator fructose 2,6-bisphosphate. Fructose phosphatase is inhibited by AMP. AMP is high when ATP is low; high AMP, on the other hand, stimulates phosphofructokinase, an enzyme of glycolysis.
Glucose-6-phosphatase converts glucose-6-phosphate to glucose. This enzyme is in the endoplasmic reticulum. Its synthesis is stimulated by cortisol.

Figure 6.13
Alanine gets converted into glucose.