

Glycolysis

1 Glucose enters a cell and is quickly phosphorylated on the 6th carbon by ATP. This “traps” the glucose in the cell as the charged phosphate group changes the way glucose fits in a glucose transport protein (GLUT). Glucose with a phosphate attached is too large and polar to escape by passive diffusion through a bi-lipid membrane layer.

2 If the enzyme “glycogen synthase” is available, and the cell has enough energy that it does not necessarily need the glucose to make ATP, then this newly phosphorylated glucose may be attached to a chain of glucose molecules called **glycogen**. This is handy because later, when blood sugar begins to drop, glucose will be cleaved from glycogen and made available to go through glycolysis. Also, the phosphate may be removed and the glucose will be put back in the blood to bolster blood sugar levels. These processes called **glycogenesis** (glycogen synthesis) and **glycogenolysis** (glycogen break down) occur in muscle cells to a small extent and in liver cells to a large extent.

3 A phosphorylated glucose that does not become part of the stored glycogen will undergo a conformational change and become fructose. The fructose molecule has another phosphate attached to it from ATP. This double phosphorylated 6 carbon fructose is now primed to be divided into 2 - 3 carbon sugars – each with one phosphate attached. The remaining glycolytic reactions will now happen twice because there are 2 – 3 carbon molecules called Glyceraldehyde-3-phosphate.

4 The energy available in the glucose molecule is found in the form of “chemical energy”. This energy exists in the C - H bonds – or more specifically within the electrons that constitute the carbon – hydrogen bonds. The dehydrogenase enzyme in step 4 will remove two hydrogens (2 protons and 2 electrons) from Glyceraldehyde-3-phosphate. Oxidized **Nicotinamide Adenine Dinucleotide (NAD⁺)** accepts and bonds with one of the protons and both of the electrons. The other proton does not bond with the NAD⁺ but will be found nearby. This may be written as $\text{NAD}^+ + \text{H}^+ + \text{H}^+ + 2\text{e}^- \rightarrow \text{NADH} + \text{H}^+$.

Because NAD⁺ acquires 2 new electrons, we say that NAD⁺ is reduced. The 3 carbon molecule that the protons and electrons were removed from is oxidized. This is an example of a redox reaction. For simplification, the reduced form of NAD⁺ will be referred to as NADH (instead of NADH + H⁺).

Think of NAD⁺ as an electron carrier. It is like an empty taxi cab. It comes in and parks near the “dehydrogenase” enzyme and as the reaction occurs, NAD⁺ acquires 2 high energy electrons and a proton as passengers. This “taxi” is now occupied and will be referred to as NADH. Later, we will see that these new “passengers” will need to be dropped off for other metabolic reactions to proceed. When NADH unloads its “passengers” NAD⁺ is reconstituted and becomes available to go back and participate in reactions again. Without NAD⁺ involvement, the

dehydrogenase enzyme would not be able to complete the reaction and glycolysis would stop at this point. Notice that if glycolysis stopped at this point, ATP would not be generated in glycolysis because the ATP generation steps are yet to come. It is important to have enough NAD^+ around to keep the reactions going.

Another important effect of the dehydrogenase reaction in step 4 is that an inorganic phosphate (P_i) ends up being bonded to the 3 carbon molecule from step 3. We now have two 3 carbon molecules called 1,3 bisphosphoglycerate.

5 In step 5 there are several biochemical reactions that ultimately accomplish one very important outcome – **Substrate-Level Phosphorylation**. In glycolysis, Substrate-Level Phosphorylation is the transfer of a phosphate group from a 3 carbon organic molecule to ADP. This reconstitutes ATP which can be used in other important energy consuming processes of the cell. Substrate-Level Phosphorylation is different from Oxidative Phosphorylation which will be discussed in Step 12.

Notice that because there are 2 - 3 carbon molecules to donate phosphate groups, 4 ATP molecules will be generated. Notice that for every glucose molecule in glycolysis, 4 ATP are made. However, 2 ATP are required at the beginning steps of glycolysis, so the net production of ATP in glycolysis is 2 New ATP for every glucose molecule.

6 The 2 - 3 carbon molecules left after Substrate-Level Phosphorylation are called pyruvate. **Pyruvate** is the end product of glycolysis. The fate of pyruvate will depend on whether there is enough oxygen available to the cell or not.

If a hypoxic (meaning that oxygen is deficient) condition exists, then a dehydrogenase enzyme will perform a reaction that is actually the reverse of what we saw in step 4. A hydrogen ion and 2 electrons will be removed from NADH and put onto pyruvate. This causes pyruvate to become **lactate**. You have probably heard of lactic acid before. Lactic acid is acid form of the conjugate base lactate. It has often mistakenly been referred to as a negative thing – a waste product or a product that makes muscles fatigue or maybe even a product that causes muscle soreness.

While it is true that lactate is produced when muscles work very hard (because the body cannot deliver oxygen fast enough), it is **not** true that lactate causes pain or fatigue. In fact, lactate is easily absorbed and converted back to pyruvate by other cells of the body. Lactate does not last long in the blood and it is **not** something that courses through us like a poison causing all kinds of trouble.

You might be asking why this conversion of pyruvate to lactate is even necessary. Remember that the reactions of step 4 are not possible without NAD^+ . If we continually made NADH and had no way to reconstitute or recycle back NAD^+ , then we would soon have to stop glycolysis and wait until more NAD^+ became available. Since none of the ATP producing steps of glycolysis can happen until NAD^+ arrives, we would not be making ATP which could kill the cell.

Making lactate is a quick way to free up NAD^+ to go back to step 4 and allow the Substrate-Level Phosphorylation reactions to take place. This is called **Anaerobic Metabolism**. Anaerobic metabolism is very fast, but not very efficient (not a lot of ATP per glucose molecule). It is good for sudden bursts of intense activity but cannot sustain activity for very long.

7 If Oxygen is available then pyruvate is transported to the mitochondria. Pyruvate moves across the two mitochondrial membranes and a whole new sequence of metabolic steps proceed in the mitochondrial matrix. The culmination of all the metabolic reactions in the cytoplasm and the matrix of the mitochondria are called **Aerobic Metabolism**. It is called aerobic because oxygen is used in step 11. Aerobic Metabolism results in many more ATP than were produced by glycolysis alone.

Citric Acid Cycle (Krebs Cycle)

8 Steps 8-12 complete the story of aerobic metabolism of glucose. After pyruvate is transported into the mitochondria, another dehydrogenase enzyme (actually a very large enzyme complex) will accomplish several things. It will remove 2 protons and 2 electrons from pyruvate. This creates NADH (actually 2 NADHs because there are 2 pyruvates). Also, the reaction results in the loss of a carbon and two oxygen atoms (released as CO_2). Finally, the remaining 2 carbon molecule is attached to Coenzyme A.

Coenzyme A (often referred to as simply CoA) is derived from pantothenic acid (Vitamin B5). **Acetyl CoA** is the name used for the product of the reaction in step 8. The "Acetyl" prefix specifically refers to the 2 carbon group that is being transported by the CoA. Black dots in the summary figure help us keep track of the carbons that originated from the glucose molecule way back at the beginning of glycolysis. Notice that ultimately all the black dots are released as CO_2 so that the metabolism of glucose leaves us with no accumulation of carbons in the cell. Acetyl CoA will enter and participate with the reactions of the Citric Acid Cycle.

9 Step 9 represents the activities of the **Citric Acid (or Krebs) Cycle**. The Citric Acid Cycle involves a lot of steps. The intent for our level of understanding will not involve details of all the reactions.

10 The important things to remember about the Citric Acid Cycle are...

- A 4 carbon molecule called oxaloacetate combines with the acetyl (2 carbon) group of Acetyl CoA (which came from glucose or fatty acids or possibly even some of the amino acids). This will yield a 6 carbon molecule called citric acid. Citric acid will be changed and manipulated as this 6 carbon molecule ends up recycled back to oxaloacetate – thus the term "Citric Acid Cycle".

- During the reactions of the citric acid cycle, CO_2 will be lost twice. This means that if you are counting, you will realize that every carbon of the original Glucose or Fat is ultimately lost as CO_2 . This is the reason we have to breath continuously as we do.
- Hydrogens with electrons are transferred to NAD^+ . This creates 6 NADH molecules.
- FAD is reduced to FADH_2 . This yields 2 FADH_2 molecules.
- Substrate-Level Phosphorylation will yield an ATP for each turn of the Citric Acid Cycle (or 2 total for each glucose).

Electron Transport Chain

11 NADH is carrying a proton and 2 high energy electrons that need to be “dropped off”. FADH_2 is also carrying high energy electrons and a couple of protons. These electron “carriers” are able to donate these electrons to an enzyme complex found in the inner mitochondrial membrane. Think of the “**electron transport chain**” as being like a bucket brigade. A series of proteins pass 2 electrons from one to another. Sometimes when the electrons are passed, a little bit of the energy from the electrons is used to induce a conformational change in some of the protein structures. This conformational change results in the transport of protons from the inside of the mitochondria to the intermembranous space (the space between the inner and outer mitochondrial membranes). Also, some of the energy released as the electrons move through the electron transport chain is given off as heat. NADH donates the electrons to the electron transport chain at complex I and FADH_2 donates electrons at complex II. There are more protons pumped from electrons moving down the transport chain from complex I than from complex II. For this reason, NADH yield more ATP ultimately than FADH_2 . Whether from NADH or FADH_2 , any donated electrons will move down the transport chain to the last protein acceptor and cannot go back to previous components of the chain.

Oxygen, accepts the electrons from the last protein complex (complex IV) of the chain. As oxygen accepts the electrons, the oxygen becomes reactive and capable of forming a covalent bond with two protons and water is formed (H_2O). If you have ever wondered what oxygen does and why it is so important to breath into our bodies, now you know. Oxygen is the final electron acceptor.

Notice that NADH becomes NAD^+ at the beginning of the electron transport chain. Also, FADH_2 becomes FAD. This recycles these electron carriers such that they can be used again in earlier metabolic reactions. This has been mentioned, but it is worth mentioning again. Without NAD^+ , reactions that use NAD^+ cannot occur.

Think about this question: *What if there was no oxygen available?* Predict what would happen to the metabolic pathways discussed...

Answer: If there was no oxygen, then the high energy electrons would sit on the protein complexes in the electron transport chain. They can't go back and they can't move forward

because there is no FINAL electron acceptor. Complex I would have electrons that couldn't move as well, so NADH could not "drop off" any more electrons. Since NADH could not become NAD^+ , it would not be very long before a severe shortage of NAD^+ stopped the metabolic reactions from occurring. This would basically grind the Citric Acid Cycle to a stop. Also, pyruvate could not become Acetyl CoA. Basically, none of the mitochondrial metabolic processes could occur. Aerobic Metabolism would not be possible. However, Glycolysis would still be possible. You might recall the possibility to regenerate NAD^+ in the cytoplasm if Lactic acid is made (Anaerobic Metabolism).

Hopefully, now you can see why oxygen is so important.

12 In step 11, we learned that as high energy electrons passed down the chain of protein acceptors, energy was used to move H^+ ions into the intermembranous space. This generates a proton gradient. This means that there will be a higher concentration of protons in the intermembranous space than there is inside the mitochondria matrix. This proton gradient represents "potential energy" because the protons will try to flow down their gradient if a passageway opens up and allows such movement.

Step 12 represents the idea sometimes referred to as **chemiosmosis**. This is a term that refers to the fact that protons tend to flow down their gradient through a selective protein channel. This protein channel, called ATP-Synthase is a very intricate and specialized molecular machine. This protein literally turns as the protons come through it and this kinetic energy is used to bring ADP and inorganic phosphate together so that ATP is created. The synthesis of ATP through chemiosmosis is referred to as **Oxidative Phosphorylation**.

While, glycolysis gives us 2 ATP per glucose molecule, the electron transport chain gives us approximately 34 ATP per glucose molecule. We say "approximately" because it is difficult to say exactly how many ATP we get. This is because some ATP is used to shuttle molecules in and out of the mitochondria and there is likely some "leaking" that occurs when protons from the intermembranous space accidentally escape by some other way than through the ATP synthase enzyme complex. However, it is generally accepted that aerobic metabolism yield between 18 and 19 times more ATP than anaerobic metabolism. It is a good thing that our cells have mitochondria!

Although aerobic metabolism gives us much more ATP, it takes longer to do it. You might think of aerobic metabolism being useful for endurance activities but less useful for activities that require both high speed and high intensity work.

13 Fat can also be used to make ATP. The metabolism of fat is called Beta Oxidation. Triglycerides are dismantled into glycerol and fatty acids. The glycerol can actually be converted into Glyceraldehyde-3-phosphate and then it completes the glycolytic reactions. The fatty acids are transported into the Mitochondria. Once in the Mitochondrial matrix, fatty acids are dismantled 2 carbons at a time and each 2 carbon piece is converted to Acetyl CoA. Acetyl CoA enters the Citric Acid Cycle. A single triglyceride molecule can ultimately yield a lot more Acetyl

CoA than a glucose molecule. So, we say that fat is much more energy dense than sugar because we get more ATP per gram of fat than we do with sugar. However, notice that the end product of Beta oxidation is Acetyl CoA (not pyruvate). So, anaerobic metabolism is not possible with fat metabolism. We can only burn fat when there is enough oxygen available to the cell.