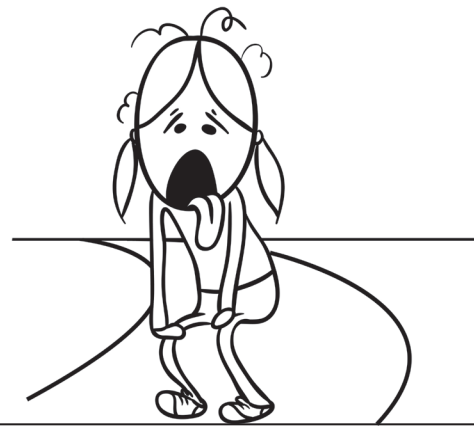


I Hate Running! And Lactate Is to Blame, Right?

by

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Introduction

I hate running! That was Shelby’s first thought upon hearing the announcement in class about an exercise challenge where students in two different courses competed to see which course could log the most miles during a semester. Her second thought was that she was sorry she even came to class this day; her friends were excited about starting the exercise challenge right away and wouldn’t stop talking about it but Shelby was skeptical and not at all enthused. For as long as she could remember running was difficult; she always felt out of breath and her muscles burned painfully whenever she would attempt to run more than a mile. The next day would be even worse due to muscle soreness. Because of this, Shelby never stuck to any sort of training program even though she had heard about the benefits of doing so in several kinesiology courses she had taken in college. For her, it was always much easier to tell someone else what they needed to do to get in shape than having to follow her own advice.

“So, what do you think about this exercise challenge?” Patrick asked after class. Shelby just rolled her eyes and said, “I think you’ll excel like you do with everything, but I’m not interested.” Patrick just giggled. “But what if we exercised together? We could use the time to not only get in shape but also discuss the concepts we’re learning in class. Maybe it would even help our grades a little.” Shelby definitely needed help with the course material and Patrick talked a lot about how he had in-depth experience in many areas of anatomy and physiology. “Ok,” Shelby said, “but don’t expect me to be able to keep up with you. I can tell from looking at you that you exercise a lot.” Patrick just smiled and said, “How about we start first thing tomorrow morning with just a two-mile run? Maybe we could even do a few sprints for fun!”

As Shelby had predicted, the run was physically hard. Her muscles burned, she was short of breath, and she couldn’t wait for it to end. “Wow, that was awful,” Shelby said to Patrick as they sat in the grass, stretching after their run. “I never want to do that again! I’m just not made for running, especially endurance running. I’m sore already thanks to all the lactic acid in my muscles.” Patrick shot her an inquisitive look and said, “I don’t think it’s the lactic acid that’s making you sore, but I could be wrong. I remember hearing something about this in class.” Shelby paused for a moment and then said, “Maybe it’s lactate then? Isn’t that produced in skeletal muscle during exercise? Or maybe lactic acid and lactate are the same thing? My track coach in high school used those terms a lot so I think they might be interchangeable. He even made us do drills that he called ‘lactates’ just to make us sore, so it’s got to be one of them.”

“Well, regardless,” Patrick said, “I know lactic acid and lactate are only produced in muscle when it’s being heavily used and runs out of oxygen. Maybe with training you won’t make those compounds at all and won’t be sore after you run.”

Activity 1

The conversation above highlights several common misconceptions about skeletal muscle cells, the effects of training, and causes of muscle soreness. Can you identify these? Jot down your thoughts below before continuing.

| <i>Shelby’s Misconceptions</i> | <i>Patrick’s Misconceptions</i> |
|--------------------------------|---------------------------------|
| | |

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Part I — Blood, Skeletal Muscle Cells, and Glucose Uptake

To untangle the misconceptions in the conversation between Shelby and Patrick, let's begin by looking at the structures within skeletal muscle cells that are involved in energy production at a submaximal level of exercise in an untrained person. This would be the case for Shelby, who is just beginning an aerobic exercise program that includes mostly endurance training occurring in 20–30 minute bouts. In addition, let's examine some common metrics such as lactate threshold and blood pH to assess what is happening in an untrained person during exercise.

During the first 30 minutes of Shelby and Patrick's run at a submaximal level (40–70% VO_2 max), blood glucose levels increase due to glucose release from the liver (Zinker *et al.*, 1990), providing skeletal muscle cells with a concentration gradient of glucose for uptake and subsequent ATP production. However, glucose is a large polar molecule and cannot simply diffuse unaided into the sarcoplasm through the phospholipid bilayer comprising the sarcolemma of skeletal muscle cells. Instead, to take up blood glucose, skeletal muscle cells must produce and insert glucose transporters, commonly referred to as GLUTs, into their sarcolemma. These GLUTs are a diverse family of large, integral membrane proteins that permit facilitated diffusion of glucose down its concentration gradient. Skeletal muscle cells can express several different forms of GLUTs, but the most common form is GLUT4 (Gaster *et al.*, 2000; Goodwin, 2010; Richter & Hargreaves, 2013). Interestingly, the insertion of GLUT4 into the sarcolemma can either be stimulated by insulin binding to its receptor on skeletal muscle cells that are resting, or by an insulin-independent mechanism during contraction of the skeletal muscle cells (Richter & Hargreaves, 2013).

Activity 2

Using the information above, complete the flow chart (Figure 1) on the following page to get a better understanding of the sequence of events that occurs during exercise. Then label all structures shown in the diagram (Figure 2), which represents a portion of a skeletal muscle cell's sarcolemma during exercise. Also label the extracellular fluid compartment, blood, and sarcoplasm. Indicate where in the body glucose and insulin would have come from and how they are transported to the skeletal muscle cell.

Questions

1. Why does glucose uptake by a skeletal muscle cell require a transporter such as GLUT4? In other words, why isn't simple diffusion possible?
2. What stimulates the insertion of GLUT4s into the sarcolemma?
3. The existence of GLUT4s in the sarcolemma does not guarantee glucose uptake into the cell. Why? What else is required?
4. Exercise helps reduce blood glucose levels in people, even if they are insulin resistant and thus their cells can no longer respond to insulin efficiently. How is this possible?

Figure 1. Exercise flow chart.

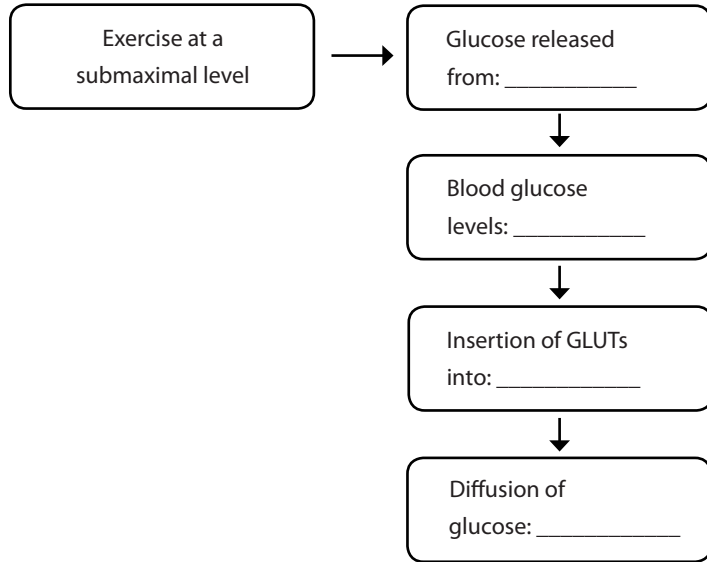
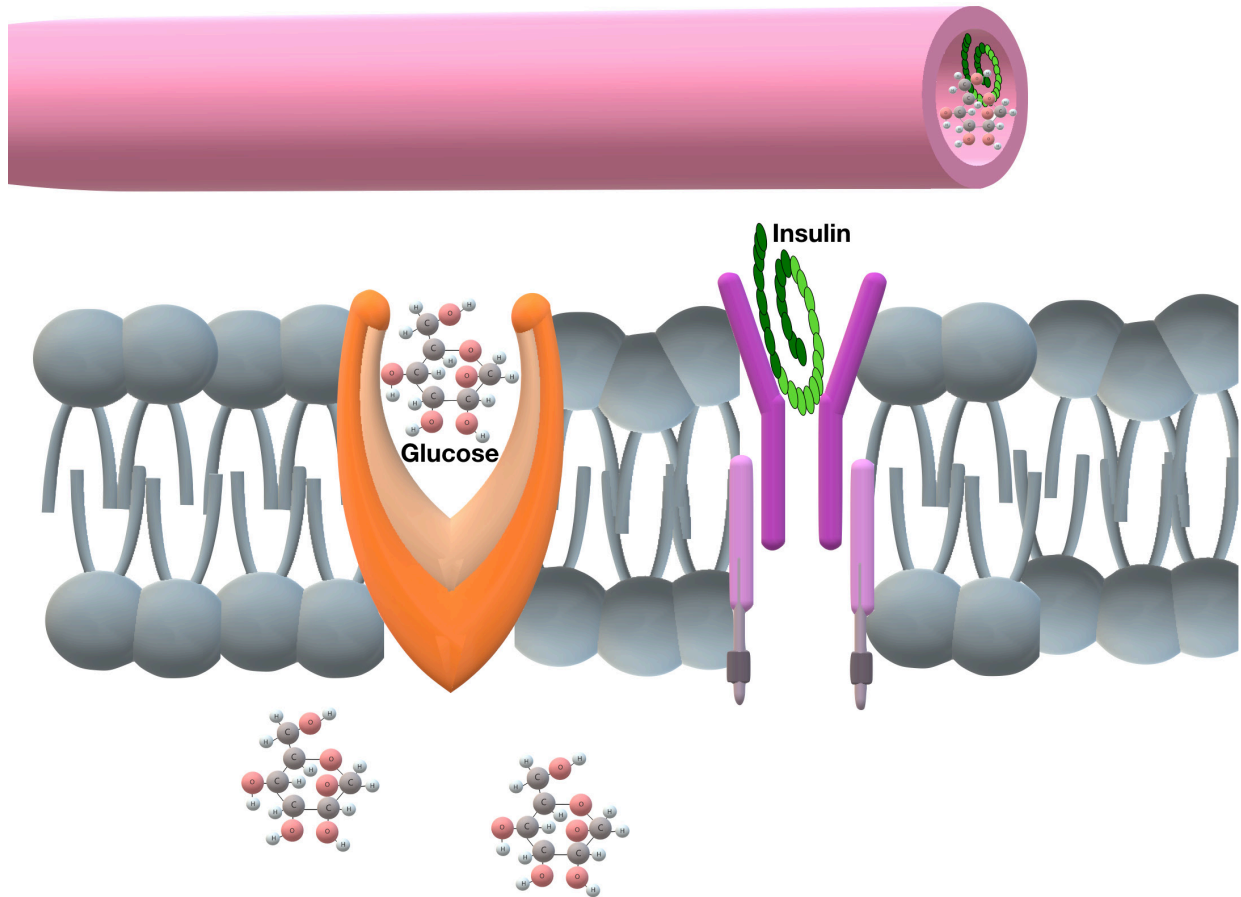


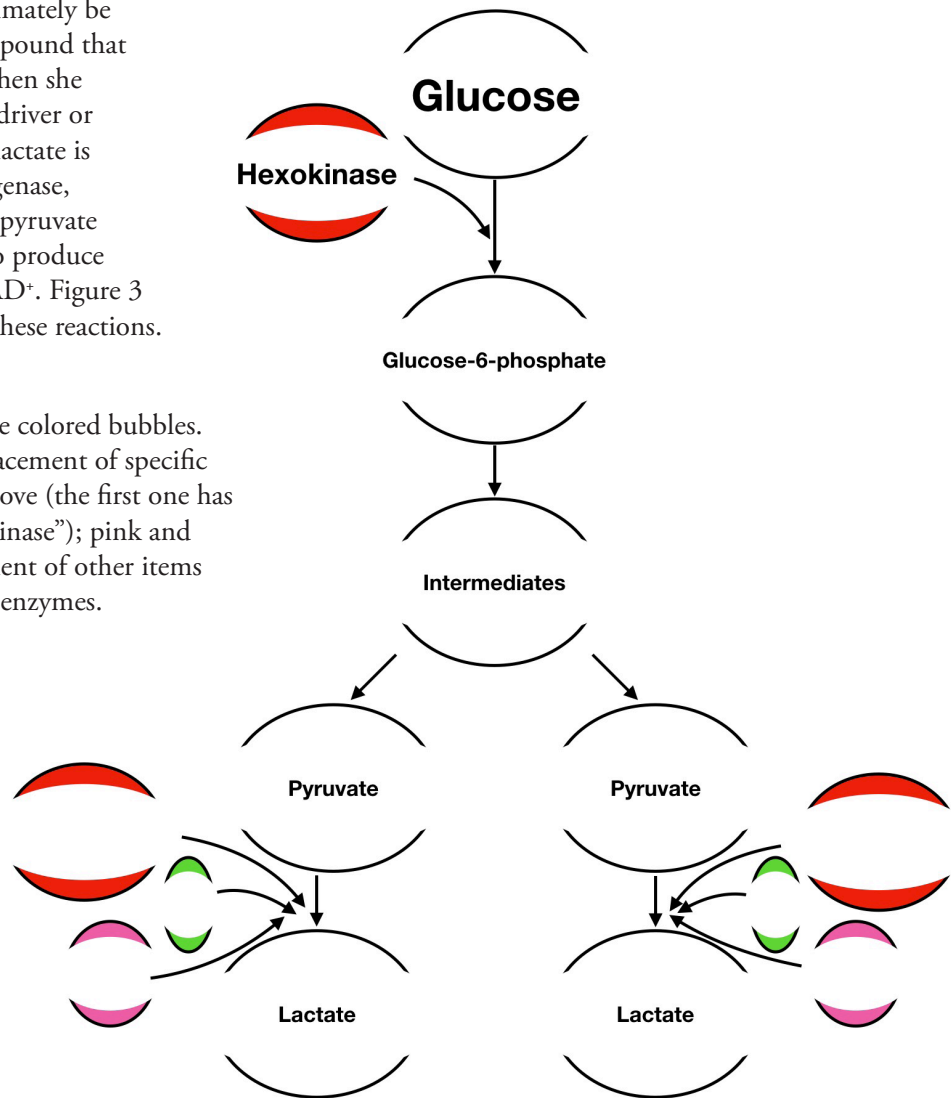
Figure 2. Portion of a skeletal muscle cell's sarcolemma during exercise.



Part II – Glucose Utilization in Skeletal Muscle Cells

After glucose enters an active skeletal muscle cell an enzyme called hexokinase converts it to glucose-6-phosphate by the addition of a phosphate from ATP. Not only does the creation of this new compound kick-start the process of glycolysis (breakdown of glucose), but it also prevents glucose from accumulating in the cell's sarcoplasm, which could slow or even stop subsequent diffusion of additional glucose molecules from the blood and extracellular fluid by reducing the concentration gradient that drives the diffusion of glucose into the cell. As the process of glycolysis continues, another phosphate from an additional ATP molecule is donated resulting in energized, yet unstable intermediates that will eventually yield useful products such as ATP, NADH, and two molecules of pyruvate. Many of the pyruvate molecules will ultimately be converted to lactate, the very compound that Shelby was most worried about when she began her running program. The driver or catalyst converting pyruvate into lactate is an enzyme called lactate dehydrogenase, or simply LDH, which combines pyruvate with NADH and a proton (H^+) to produce lactate while also regenerating NAD^+ . Figure 3 shows a simple representation of these reactions.

Figure 3. From glucose to lactate.



Activity 3

Complete Figure 3 by filling in the colored bubbles. Those marked red indicate the placement of specific enzymes mentioned in the text above (the first one has been filled in for you with “hexokinase”); pink and green bubbles indicate the placement of other items used in the reactions catalyzed by enzymes.

Questions

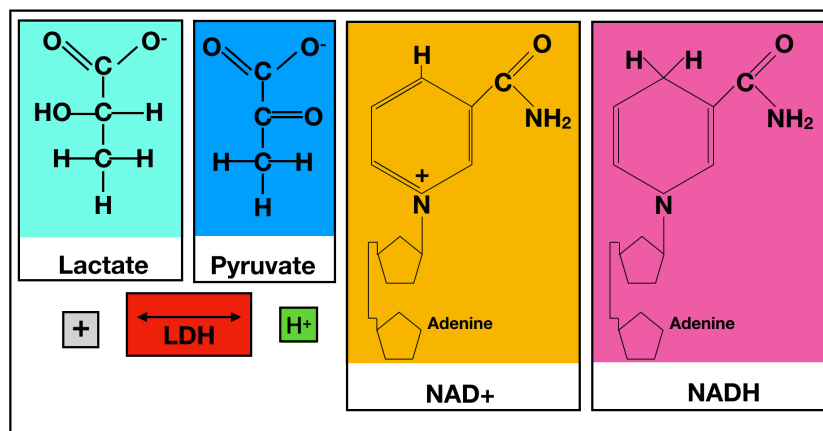
- In an actively contracting skeletal muscle cell, what would cause a decrease in the rate of glucose diffusion into the cell?
- In order to produce lactate from pyruvate, what items or inputs are required?

It is crucial to note that the processes described above occur in the sarcoplasm of muscle cells and do not require oxygen, even if oxygen is present in sufficient amounts. The reason for this is that no carbons are cleaved from glucose or any intermediate during glycolysis, thus no O_2 is required and no CO_2 is produced. Furthermore, lactate is always an end product of glycolysis due to the presence of LDH, even if oxygen is present in sufficient amounts (Rogatzki *et al.*, 2015). In fact, lactate is constantly created in fully oxygenated cells and potentially ten times more lactate than pyruvate is ultimately produced from glycolysis, even when the skeletal muscle cell has adequate access to oxygen and is at rest (Brooks, 2000). At one time, lactate was thought to simply be a cellular waste product or a dead end to anaerobic respiration. We now know this isn't true at all and that lactate serves many functions for the cell in which it was produced as well as in other cells of the body. For example, the production of lactate helps prevent pyruvate from accumulating in the sarcoplasm, which could actually impede the production of pyruvate in the future. Lactate production also involves the regeneration of NAD^+ so that glycolysis can continue and decreases the number of free protons (H^+) that could lower intracellular pH. Furthermore, lactate can be converted back to pyruvate within mitochondria, which can then be used to fuel the Krebs cycle. In fact, even lactate that "spills" out of muscle cells and enters the blood is useful to cells of the heart, kidneys, and liver, which can use lactate from the blood for ATP production! Thus, the production of lactate should not be seen as a waste product, but instead as both a useful product and substrate.

Figure 4. From pyruvate to lactate.

Activity 4

Using the information provided above and the scrambled objects in Figure 4, construct the reaction converting pyruvate into lactate. Include the correct placement of LDH, $NADH$, NAD^+ , protons and even addition signs. Then circle the two items that lactate "accepted" that make its chemical structure different from that of pyruvate.



Correct Equation: _____

Questions

- During glycolysis one molecule of glucose, which has six carbons, is split into two molecules of pyruvate, which have three carbons each (Figure 4). Each pyruvate molecule can then be converted into a molecule of lactate, which also has three carbons. Oxygen is not required for any of these processes to occur. Why?
- After lactate is produced in the sarcoplasm, where might it go and how might it be used?
- How does the production of lactate protect or buffer the cell from acidosis, which is defined as the accumulation of H^+ in a fluid filled compartment?
- Look at the structure of lactate (Figure 4). Do you think it can leave the cell via simple diffusion? Why or why not?

Part III – Lactate Utilization in Skeletal Muscle Cells

If you look closely at the LDH enzyme (Figure 5 below) you will notice it has an arrow pointing in two different directions, meaning it catalyzes a reaction that is reversible or can occur in either direction; however, the characteristics of the LDH enzyme strongly favor the production of lactate from the substrate pyruvate regardless of O₂ levels. As a result, lactate production can outpace lactate use in some cells, for example, untrained skeletal muscle cells used during sub-maximal endurance activities like jogging. In these cells, the production of lactate far exceeds use; consequently, lactate accumulates and diffuses or “spills” out of the cell via a transporter, called MCT, and into the blood. One of the reasons skeletal muscle cells from untrained people cannot efficiently use the large amount of lactate they produce is that they do not yet have sufficient numbers of mitochondria to sustain them during endurance or aerobic activities. Interestingly, it is the mitochondria that ultimately serve as the “sink” for much of the lactate produced from glycolysis. Known as the intracellular lactate shuttle mechanism (Brooks, 1999; Cruz *et al.*, 2012), lactate produced from pyruvate by LDH in the sarcoplasm can enter the mitochondria where another isoform of LDH converts lactate back to pyruvate for use in the Krebs cycle, a process which ultimately results in at least ten times more ATP production from glucose than that produced by glycolysis in the sarcoplasm. Thus, the more lactate produced and the more LDH available both in the sarcoplasm and within the mitochondria, the more ATP can ultimately be produced via aerobic respiration. In other words, lactate is ultimately used to fuel ATP production within the mitochondria aerobically, or in the presence of oxygen.

Activity 5

Label the structures in Figure 5 and then identify three possible fates of lactate in a skeletal muscle cell.

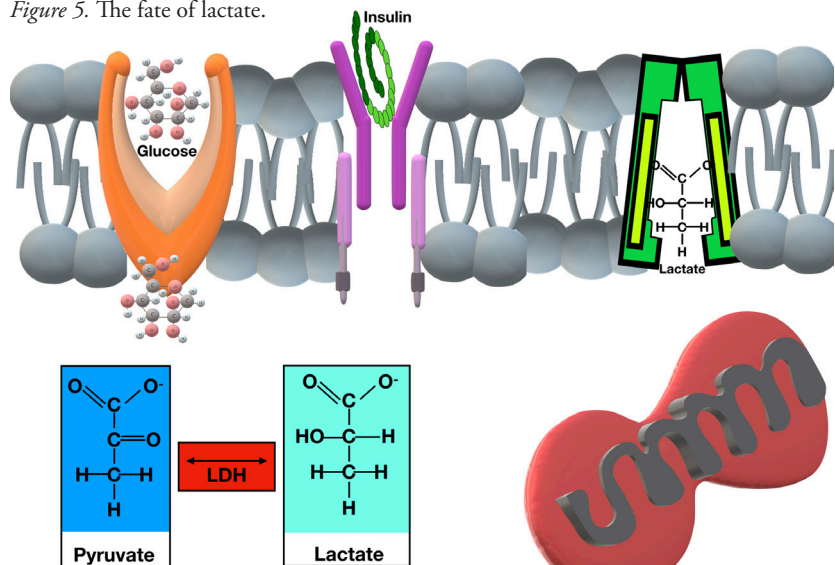
Fates:

1. _____

2. _____

3. _____

Figure 5. The fate of lactate.



Questions

- If lactate is an energy source for the cell in which it is produced, why does it “spill” into the blood; in other words, why do blood lactate levels increase during exercise especially in untrained people?
- LDH is an enzyme and thus a protein. In order for a skeletal muscle cell to increase the amount of LDH within the sarcoplasm, what cellular processes must be completed? What organelles need to be added to Figure 5 in order for LDH to be produced?

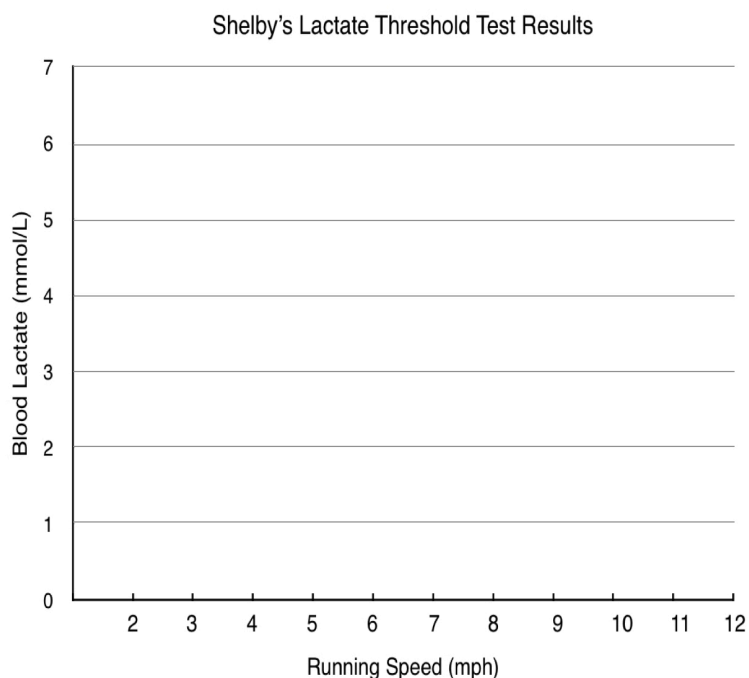
A common way of measuring the point at which lactate spills out of working skeletal muscle cells and into the blood is the lactate threshold test. This test indicates the level of work (speed, for example) or exertion at which the amount of lactate produced in cells exceeds the amount of lactate that can be used either by the cells producing it or by other cells in the body that have the ability to pull lactate from the blood for use as an energy source. As mentioned before, cells of the liver, heart, and kidney can use lactate for ATP production (Robergs *et al.*, 2004). At rest, blood lactate levels remain fairly steady with production matching use. But when an untrained person like Shelby begins jogging, the production of lactate within skeletal muscle cells increases faster than it can be used causing it to spill into the blood, in turn causing blood lactate levels to spike.

Activity 6

Examine the results of a lactate threshold test from an untrained person like Shelby who is just starting an endurance training regimen (Figure 6). Using the information in the table, construct a line graph in the area provided by plotting running speed against blood lactate and then identify the running speed at which the amount of lactate produced by skeletal muscle cells has likely overcome the ability of many of the body's cells to use it, thus causing it to accumulate in the blood.

Figure 6. Lactate threshold test results.

| Running Speed (mph) | Blood Lactate (mmol/L) |
|---------------------|------------------------|
| 2 | 1.8 |
| 3 | 1.9 |
| 4 | 2.0 |
| 5 | 2.1 |
| 6 | 2.2 |
| 7 | 4 |
| 8 | 4.5 |
| 9 | 5 |
| 10 | 5.4 |
| 11 | 5.9 |
| 12 | 6.3 |



Question

13. Through dedicated training, the speed or level of effort coinciding with a person's lactate threshold improves. Within the skeletal muscle cell, what changes might have occurred to permit this improved lactate threshold, resulting in less lactate spilling out into the blood?

Part IV – Extracellular Fluid and Blood Composition Changes with Skeletal Muscle Cell Activity

Recall Shelby's first run with Patrick, a run that she definitely did not enjoy. Her legs burned and she felt as though she couldn't catch her breath, while Patrick, who was in better shape, didn't have those issues despite going the same pace as Shelby. "Patrick! This is every bit as hard as I remember it being back in high school! I feel like my legs are on fire and my chest just burns. Why is this?" asked Shelby. "I don't know," said Patrick, "but I vaguely remember something about this from a previous chemistry course; I think it has something to do with lactate decreasing the pH of muscle cells." Shelby shot him an inquisitive look and said, "Hmm... I've had several chemistry courses and that doesn't sound quite right to me."

The exchange above between Shelby and Patrick captures some common questions and misconceptions people have about the cause of muscle pain during or after exercise. To gain a better understanding of what actually occurs to cause this discomfort, let's take a closer look at the inner workings of muscle cells and how they influence the fluid-filled environments around them.

During exercise, extracellular fluid and blood composition change due to reactions occurring within the body's cells. For example, the amount of lactate diffusing out of skeletal muscle cells and accumulating in the extracellular fluid and blood increases, with lactate efflux and accumulation occurring more rapidly in untrained people like Shelby compared to trained athletes like Patrick. Additionally, the pH of fluid environments can also change during exercise as H^+ accumulates in the sarcoplasm of skeletal muscle cells during extended periods of rapid contractions due to ATP hydrolysis. Eventually, the H^+ ions will spill into the extracellular fluid and blood, causing the pH to decrease in both of these areas if not adequately buffered. The increase of lactate and H^+ in the extracellular fluid and blood occurs simultaneously as lactate produced within skeletal muscle cells is removed by a transporter, specifically the monocarboxylate transporter (MCT), which is embedded in the sarcolemma and was introduced to you earlier in Figure 5. This transporter functions as a symporter, permitting the diffusion of both lactate and H^+ at the same time (Robergs *et al.*, 2004); in fact, both must be available in order for this transporter to change shape or conformation and expel H^+ and lactate.

For a century or more this simultaneous increase in blood lactate and H^+ during exercise has perplexed scientists and it was originally believed that lactate production within skeletal muscle cells was the cause for increasing H^+ concentrations because both lactate and H^+ leave the cell at the same time and accumulate in the blood at the same time. As a result, it was also believed that the sensation of discomfort perceived by Shelby was due to the accumulation of lactate. But as we've just seen, the production of lactate is not at all the source of increased H^+ levels within cells or blood as the production of lactate is alkalinizing (it actually increases rather than decreases the pH). So why has lactate been so misunderstood?

To answer this question, a very brief history is warranted for the terms "lactate" and "lactic acid." In 1780 a Swedish chemist named Carl Wilhelm Scheele first discovered lactic acid in samples of soured milk (thus the adjective "lactic" because it pertained to milk) and isolated the compound using impure conditions. Despite some initial criticism of Scheele's work, other chemists of that period had also verified the presence of lactic acid in additional organic tissues such as fresh milk, meat, and blood (Robergs *et al.*, 2004). While we now know that the presence of lactic acid—and not lactate—in many of these experiments was due to impure samples and bacterial fermentation, the term "lactate" stuck and has been associated ever since with the increased presence of H^+ and resulting acidosis in working human skeletal muscle cells, extracellular fluid, and even in the blood, regardless of the true cause. And once these terms became interchanged, correcting the issue has proven very difficult. In fact, even recent studies have mistakenly interpreted correlations illustrating the simultaneous increases of lactate and H^+ in the blood during exercise as cause and effect (Juel *et al.*, 2004). But according to Robergs *et al.* (2004) no experimental evidence has ever revealed a cause-effect relationship between lactate production and acidosis. In summary, this large and enduring misconception in the field of skeletal muscle physiology is due to improper scientific techniques that occurred centuries ago, continued misinterpretations of correlations depicting simultaneous increases in blood lactate and H^+ levels, and the sustained use of "lactate" and "lactic acid" interchangeably. Precise language in science is important!

Activity 7

Using the information above and your work in Figure 5, complete the flow chart in Figure 7 to better understand the sequence of events during exercise. Then, complete Figure 8 below by identifying the monocarboxylate transporter (MCT) and indicating what items diffuse through this transmembrane protein. Indicate how these items were produced in the cell and what changes would occur in the sarcoplasm, extracellular fluid, and blood as a result of these items leaving the cell through this protein.

Figure 7. Exercise flow chart.

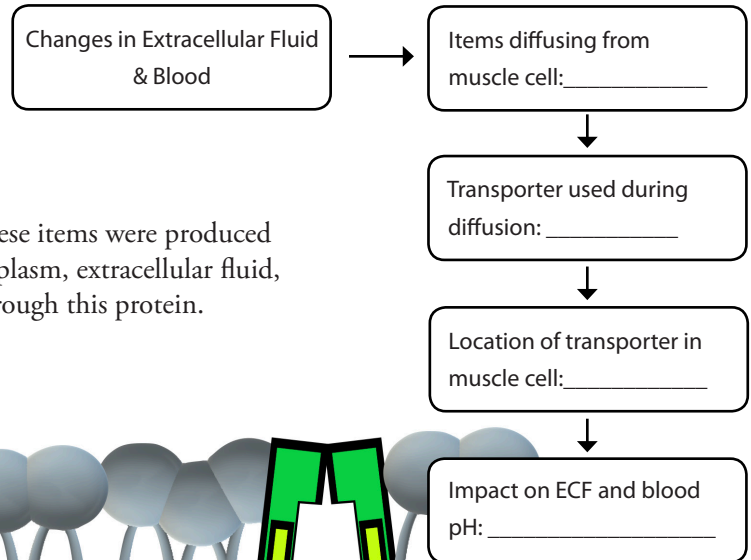


Figure 8. MCT and facilitated diffusion.

CC(O)C(=O)[O-]
 Lactate
Produced from: _____

H^+
 Proton
Produced from: _____

Questions

14. Suppose you are measuring changes in blood composition in real time in an untrained person like Shelby who is jogging at a moderate pace on a treadmill and you notice that as lactate levels increase in her blood, so do H^+ levels. Why is this? Is lactate the original source of these additional H^+ ions?

15. Hypothesize how Shelby's lactate threshold test would change with dedicated aerobic training. What changes would occur within skeletal muscle cells to delay the spilling of lactate into the blood.

Part V – The Real Cause of Muscle Soreness

As you have noticed by now lactic acid and lactate, while often used interchangeably, are not the same. For example, lactic acid is a true acid, meaning it donates a proton (H^+) while lactate is a proton acceptor, meaning it accepts H^+ ions. Furthermore, lactic acid is most commonly produced during times of intense exercise when anaerobic metabolism is the only option for skeletal muscle cells and glycogen must be used as the fuel source. Lactate, however, is constantly produced by the cell during times of rest and times of low to moderate intensity exercise, which is generally fueled by the diffusion of glucose from the blood and burned via aerobic metabolism (Westerblad *et al.*, 2002).

But what about the painful after effects noted by many recreational runners like Shelby and even sometimes by trained athletes like Patrick after a hard training session? Interestingly, muscle soreness is likely caused by the accumulation of H^+ in the extracellular fluid just outside of the sarcolemma. The production of H^+ can result from many different reactions in the skeletal muscle cell and as H^+ flows out of the cell via facilitated diffusion through MCTs in the sarcolemma it accumulates in the extracellular fluid and is thought to activate sensory neurons that convey information about the skeletal muscle cell to the brain (Westerblad *et al.*, 2002). In other words, the accumulation of H^+ in the extracellular fluid may cause extraneous afferent action potentials to be generated and sent to the brain where they are interpreted as pain.

Activity 8

Complete Figure 9 comparing and contrasting lactic acid and lactate in skeletal muscle cells.

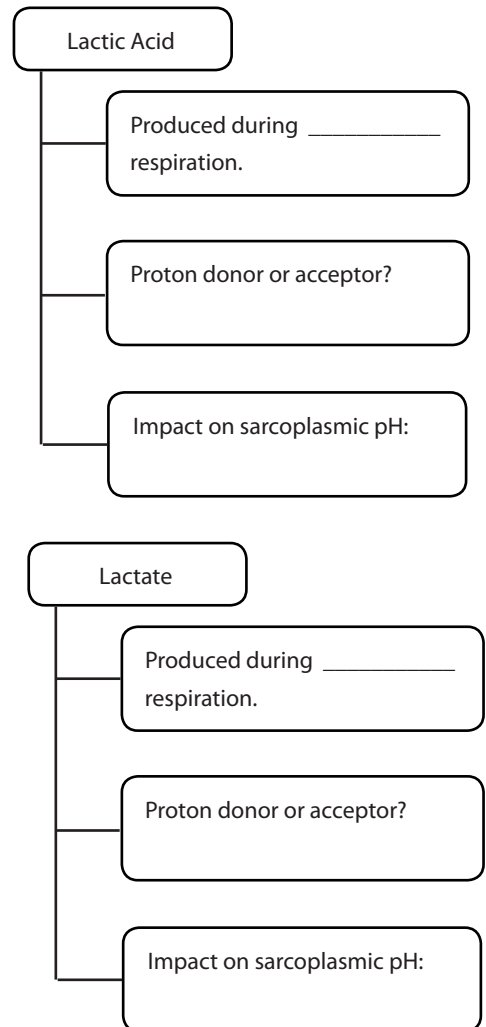
Questions

16. Skim back through this case study and identify the causes of H^+ production and accumulation in the sarcoplasm, extracellular fluid, and blood. Is lactate ever the original source of these ions or just guilty by association? Then, go back to the very first page of this case study and re-examine your thoughts from Activity 1. Can you generate a more detailed list of misconceptions now?

17. What is the relationship between the production of lactate and the burning sensation many people like Shelby describe after they begin a new exercise regime or activity? How is the brain made “aware” of this situation?

18. Hypothesize what changes could occur within skeletal muscle cells that would help prevent the burning sensation after completing an aerobic exercise activity like jogging.

Figure 9. Lactic acid vs. lactate.



In spite of her initial discomfort, Shelby discovered that running actually was enjoyable and kept at it. Having Patrick to encourage her made a big difference as well, and she found herself thinking about metabolism of glucose and production of lactate within her muscles as she ran. As a result, she started getting better grades on exams and quizzes. After a few weeks she noticed that she could run with Patrick for the full two miles at a moderate pace without pain during the run or soreness the day after. Shelby called Patrick the day after they had ramped up their running distance to 3.5 miles, and she couldn't stop herself from excitedly gushing to her friend. "Patrick, this is amazing. I think what's happening is that I've gotten better at using lactate. I'm pretty sure it's because my cells have increased production of the LDH enzymes and have increased mitochondrial density, both of which allow me to use whatever lactate is produced much more efficiently so less spills out into my blood. What do you think?" After a long awkward silence Patrick finally said, "Uh, I think I've created a monster. I was just hoping for a date." Shelby gave a respectable pause and said, "Well thanks, Pat, but you're a little slow for me."



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