THE POTENTIAL OF PH AS A DETERMINANT OF MUSCLE FATIGUE DURING STEADY-STATE EXERCISE

by

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This paper attempts to answer four fundamental questions: what are the causes of muscle fatigue during steady state exercise, how is pH related to muscle fatigue, what technologies exist to measure pH during exercise, and what future steps must be taken to make use of this connection. This paper examines muscle fatigue as a whole, as well as the role pH plays in predicting the onset of muscle fatigue in exercising muscle. Current literature on the physiological and temporal links of pH to lactate threshold and muscle fatigue are examined. This paper makes the assertion that while acidosis may not cause fatigue or even be exactly temporally correlated with muscular fatigue, there is a strong enough correlation between the two for pH measurement to have potential use in preventing muscle fatigue and subject dropout during steady state exercise. Finally, there is a review of current technology and methods for measuring pH *in vivo* in order to determine the most efficient and practical way forward for pH measurement to be used in this manner.

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Introduction

The Exercise and Environmental Physiology Lab at the University of Oregon, directed by Dr. John Halliwill, conducts research into the "hormonal, neural, or metabolic factors that are responsible for changes in the cardiovascular system during exposure to environmental and physical stresses" (Halliwill, 2016). Physical stresses, such as running, encourage the body to adapt so that the stress is easier to handle in the future. Cardiovascular adaptations involve helping bring more oxygen to the exercising muscles, whether through improving heart or lung function, improving muscle efficiency, or by building new blood vessels. Environmental stresses, such as cold or hot weather, or low oxygen situations at high altitude cause many of the same adaptations, albeit through different pathways. With this overarching goal in mind, current research is focusing on the benefits exercise can provide to an aging population, with specific research in signaling pathways for angiogenesis, muscle healing, and inflammatory processes. My research is intended to lead to the use of improved technology and methodology to more accurately and effectively continue this research.

Many experimental protocols that are used within our lab involve making subjects exercise for extended periods of time, often up to an hour. These exercises include dynamic knee extension, cycle ergometry, treadmill running, and other protocols. During such an exercise protocol, if the subject becomes too tired to continue for the entire duration we must often discard the data. Becoming too tired to maintain work at the same level as it was being performed during exercise is termed muscle fatigue. It is thought that this muscle fatigue is related to the body switching to new forms of breaking down glucose into energy. Even if the subject is able to continue

exercising, these new forms of energy production introduce entirely new physiological mechanisms which can interfere with data collection, leading to poor data or even faulty conclusions. It is in our best interest to make sure that our subjects are able to maintain the prescribed work effort for the entirety of the exercise protocol. Current methods of preventing subject dropout involve having the subject perform a maximal effort test to see how hard they are capable of working, and then picking a work level based on a percentage of their maximal work effort, usually around 60% of maximum. While this method has proved fairly effective, it is limited in a couple of ways. First, it is a blanket rule applied to all subjects, which ignores physiological variation. Second, it relies on obtaining an accurate maximum workload for each subject, which can depend on subject motivation. Third, the estimation is based on a guess at where anaerobic threshold will occur, and because it is only an estimation, it may prove wrong in some cases. This paper is intended to determine a more quantitative measurement that can be used to prevent muscle fatigue. Further, it specifically examines the role of pH as a determinant of muscle fatigue.

This paper begins with a review of the terminology and physiological concepts necessary to discuss the role that pH plays in muscle fatigue. It then goes on to examine the possible causes of muscle fatigue, the literature supporting them, and the ability of each to be used as a biomarker to determine the onset of muscle fatigue. Next, there is a discussion of the role of pH in muscle fatigue, beginning with past ideas, examining current literature, and drawing conclusions on its usefulness for our purposes. There is also a more in-depth discussion of the role lactate threshold plays in aligning with anaerobic threshold and acidosis. The next section after that is a review of the

technologies that exist to measure intramuscular pH, and the paper concludes with recommendations for future research. Overall, I argue that while acidosis may not be directly causally or temporally related to the onset of muscle fatigue, there is a close enough correlation to justify exploring the use of near-infrared spectroscopy as a tool to prevent subjects from exercising at a level above their lactate threshold during steady-state exercise protocols.

Review of terminology

Glycolysis

In order to understand many of the physiological concepts that will be discussed, a basic knowledge of the way in which humans produce and use energy is necessary. Energy is mostly stored in the body in the form of ATP, or adenosine triphosphate. This molecule is specifically useful because the three phosphate groups are in a high energy conformation (Hall 2013). It takes a lot of energy to attach a third phosphate to adenosine diphosphate (ADP), and a lot of energy is released when the third phosphate group is let go from ATP. In this manner ATP acts as a sort of battery, holding energy until it is needed. There are three main ways in which muscles obtain ATP; creatine phosphate, anaerobic respiration, and aerobic respiration (Hall 2013).

Carbohydrates which are ingested are broken down into glucose, and either directly turned into ATP or turned into glycogen for storage. Some ATP is attached to creatine in the form of phosphocreatine (PCr). Because this can be immediately broken down, PCr provides energy for the first 5-10 seconds of exercise, and at maximal exercise (Hall 2013). During exercise, your body takes the glucose stored as glycogen in muscles and turns the glucose into ATP (adenosine triphosphate), in addition to using free glucose within the blood stream. The two methods of doing so are anaerobic and aerobic. Both methods begin by turning glucose into pyruvate, which produces a net 2 ATP (Hall 2013). Anaerobic respirati7on turns pyruvate into lactate through a very simple reaction (producing 1-2 H+ in the process), which can then be turned back into glucose in the liver. Because of this, glucose is turned to energy very quickly, albeit in an inefficient manner (one glucose molecule only makes 2 ATP molecules). This is

useful during sprints around 30 seconds in duration (maximal exercise), when your body needs energy quickly.

After this period of time, your body switches to aerobic respiration, which uses pyruvate in a series of reactions termed the Krebs cycle. This cycle begins by breaking down pyruvate into acetyl-CoA, which then goes through a circular series of processes which ultimately produces NADH to be used in the electron transport chain, and carbon dioxide as a byproduct, as well as others (Hall 2013). It is important to note that fats and lipids can also be used as a source of acetyl-CoA, as well as amino acids to an extent. Within the electron transport chain, NADH and FADH2 provide hydrogen ions which, through the creation of a chemical gradient, power a system which synthesizes ATP (Hall 2013). This process takes much longer than turning glucose into lactate, but it is far more efficient (one glucose molecule makes somewhere around 36 ATP molecules). Because of this, exercise which demands a more constant supply of energy (steady state exercise) tends to use aerobic respiration (Hall 2013). This is important because both methods have a different effect on the pH of muscle.

The PCr system helps immediately synthesize ATP with little effect on H+ concentrations. However, after this system is no longer in use, it begins to resynthesize PCr. This is hypothesized to have an alkalization effect on the muscle (Robergs et. al 2004). When the muscle is only using anaerobic respiration, muscular pH was once thought to be lowered due to the production of lactic acid, although this is now known to be false. When the muscle is only using aerobic respiration, hydrogen ions are both produced during the Krebs cycle and used in the ETC (Hall 2013). The overall impacts of both systems on muscular pH are still up for debate. In our research, we often attempt

to isolate one system of glycolysis or the other in order to control for variables. When exercising for longer than a minute at a rate that is too high in energy demand for just aerobic respiration, anaerobic respiration will begin to help supplement the required energy. Because the presence of anaerobic respiration will somehow impact muscular pH, it is thought that the transition into both types of glycolysis is measurable through H+ concentrations.

Acidosis

It has been widely accepted for much of the past century that lactic acid production directly leads to acidosis of the muscle. However, modern research has shown that lactic acid is not produced through anaerobic respiration before dissociating into lactate and a hydrogen ion. Instead, lactate is a direct product of the reaction (Robergs et al. 2004). Whether this lactate is correlated with acidosis is discussed in depth later, as arguments have been made supporting lactate production having both alkalinizing and acidifying effects on the muscle. Instead, as discussed in a paper by Robergs et al., acidosis is thought to stem from a variety of other intramuscular sources. The phosphocreatine system, when breaking down into creatine and ATP, is thought to be “alkalinizing to the cell, as a proton is consumed in this reaction” (Robergs et al. 2004). Additionally, whether blood glucose or glycogen is used as a substrate for glycolysis matters, as “using glycogen as the source of G6P, as opposed to blood glucose, is less acidifying to muscle during intense exercise” (Robergs et al. 2004). Still, both sources have an acidifying effect on the muscle. Another source of acidification is thought to be ATP hydrolysis, an essential component of muscle contraction, due to the production of a hydrogen ion in the reaction (Robergs et al.

2004). Finally, NADH production is thought to produce hydrogen ions as a byproduct, overall having an acidifying effect (Robergs et al. 2004) As with most physiological concepts, debate surrounds the exact effects of each system on acidosis. For instance, Robergs makes the argument that lactate has an alkalizing effect, while Lindinger makes the argument that it has an acidifying effect (Lindinger et. al 2005). The exact implications of this debate on the role of pH on muscle fatigue will be discussed later.

Muscle Physiology

When discussing muscle fatigue, it is important to have an understanding of how muscles contract. There are two important terms that will be used throughout this paper, excitation-contraction coupling and cross-bridge cycling. Excitation-contraction coupling refers to the multiple steps between a neural signal arriving at the muscle and the muscle contracting. The first step of this is acetylcholine being released from the alpha motor neuron attached to the muscle. This ACh release depolarizes the muscle membrane, and this depolarization travels along the membrane into an indent into the muscle called a T-tubule (Hall 2013). The depolarization leads to a ryanodine receptor channel on the sarcoplasmic reticulum to open. The opening of this channel allows calcium release from the SR, where it can then go on to take part in cross-bridge cycling. The SR then reuptakes calcium for later release, and extra calcium is released out of the muscle cell in order to repolarize the cell membrane (Hall 2013). This process is important because the inhibition of this process at any point can lead to muscular fatigue.

Cross-bridge cycling refers to the process in which muscles contract using actin and myosin. Specifically, calcium released from the SR in the previous process attaches

to troponin C, which allows a myosin head to bond to actin (Hall 2013). Upon bonding, the myosin performs a power stroke, pulling on actin. The myosin is then stuck to the actin molecule until a new ATP bonds to it. ATP bonding releases the myosin from the actin, and hydrolysis of the ATP resets the myosin for the next cross-bridge cycle (Hall 2013). Like excitation-contraction coupling, interruption at any point of this cycle can lead to muscle fatigue.

Steady-State Exercise

One term that is used extensively throughout this paper is steady-state exercise. There are multiple types of exercise, between maximal and submaximal, continuous and interval, isometric and isotonic, etc. Steady-state exercise simply refers to exercise at any intensity at which physiological variables—such as heart rate, breathing rate, blood metabolite levels, lactate—remain the same. Steady-state exercise could theoretically occur at any exercise intensity below anaerobic threshold, and for any length of time. Realistically, steady-state exercise tends to be anywhere from about three to five minutes to an hour in length (Hall 2013). While steady state exercise could occur at 5% of maximum effort, for the purposes of this paper, I use steady-state exercise to describe exercise at or near the anaerobic threshold. It is this intensity of exercise that we most often have subjects work, and the purpose of this entire paper is to develop a way of ensuring subjects remain at that physiological steady-state.

Lactate Threshold

In this paper, I use the term lactate threshold to indicate the point at which lactate begins to build up, although there are a number of related concepts which must

be discussed. When a subject is working hard enough for both types of glycolysis to be necessary, lactate begins to build up in the blood plasma, which is termed the lactate threshold (Denadai et al. 2005). It has traditionally been thought that the lactate threshold correlates to the onset of muscle fatigue. In the past, we have used a maximal exercise test to estimate the intensity where a subject's lactate threshold is, and then pick a point below that for steady state exercise. With this research, we hope to use a method of continuous monitoring of pH to not only accurately know intramuscular pH during exercise, but hopefully determine when a subject crosses the lactate threshold by a subsequent sharp decrease in intramuscular pH. This will allow more accurate methods of ensuring subjects remain below lactate threshold during steady state exercise, and will hopefully improve dropout rates during studies which require extended exercise bouts.

Lactate threshold is closely related to the ideas of Maximal Lactate Steady State (MLSS) and Onset of Blood Lactate Accumulation (OBLA). Because of this, it is not uncommon to find researchers using these terms interchangeably. However, there are specific differences in the terms. MLSS describes the maximum level of exercise at which lactate levels remain steady (Denadai et al. 2005). This recognizes the fact that muscles are not only involved in lactate production, but lactate clearance. In truth, some minimal level of anaerobic respiration is occurring at any workload, even below lactate threshold. However, at some point lactate production outpaces lactate clearance. Right before this occurs is termed the MLSS. OBLA is a very similar concept. It would make sense that right after crossing the MLSS, the excess lactate would enter the blood and mark the onset of blood lactate accumulation. Indeed this is often true, especially in

whole body exercise. Contraction of multiple large muscle groups can produce large amounts of lactate, which would then begin to accumulate in the blood. However, with a very small muscle mass such as in a finger, lactate production might outpace lactate clearance in the muscle, while the blood experiences no noticeable rise in lactate levels. This finger would have crossed the MLSS while the body as a whole would not have reached OBLA (Denadai et al. 2005). For simplicity's sake, I avoid both of these terms in favor of the all-encompassing term lactate threshold.

Additionally, I often imply in this paper, as do other researchers, that lactate threshold and anaerobic threshold occur at the exact same time. Again this is not true. Anaerobic threshold refers to the moment when exercise is too great for aerobic respiration alone, and anaerobic respiration must also help. Lactate threshold is not necessarily dependent on anaerobic threshold, as it is simply a measure of the onset of lactate build-up in the muscle. While these two events may not occur at the exact same time, they usually occur in close proximity to each other. Additionally, it is very difficult to tell if a muscle is using anaerobic respiration or not. However, it is relatively simple to measure lactate levels in the muscle. For this reason, in this paper I often use lactate threshold as an approximation of anaerobic threshold due to its simplicity in measurement. There is a more in-depth discussion on the validity of this assumption later.

Muscle Fatigue

Muscle fatigue has been highly debated for many years. The general definition of muscle fatigue is a lowered ability for the muscle to produce force, whether due to clinical conditions or simply exhaustion. There have been a multitude of proposed mechanisms of what causes muscle fatigue, each with their own supporting research. Fatigue can be split into two main categories; central and peripheral fatigue. In a 1997 study by Davis and Bailey, the assertion is made that "the unwillingness to generate and maintain adequate CNS drive to the working muscle is the most likely explanation of fatigue for most people during normal activities" (Davis and Bailey 1997). CNS fatigue has been hypothesized to function through serotonin, dopamine, or acetylcholine deficiencies. According to Davis, "Good evidence suggests that increases and decreases in brain 5-HT activity during prolonged exercise hasten and delay fatigue, respectively" (Davis and Bailey 1997. In addition, "several cytokines have been associated with reduced exercise tolerance", and "ammonia in the blood and brain during exercise could also negatively effect the CNS function and fatigue" (Davis and Bailey 1997). Davis concludes with "clearly fatigue during prolonged exercise is influenced by multiple CNS. . .factors" (Davis and Bailey 1997). Unfortunately, it is not this clear. In 2016, Contessa et al. question whether central fatigue even exists. "Unlike the directly observable and verifiable influence of peripheral factors of muscle fatigue" they state, "direct empirical evidence of central fatigue has yet to be revealed" (Contessa et al. 2015). Contessa et al. continue on to cite multiple research studies on central fatigue, and state that only one study even "attempted to take into account the influence of peripheral factors on central fatigue" (Contessa et al. 2015). They went on to perform an

experiment, which was “able to replicate empirical results from studies of fatiguing contractions. . .without requiring the involvement of central factors”(Contessa et al. 2015). Whether or not central fatigue exists and to what extent is still clearly under debate, and therefore does not provide an easily measurable predictive biomarker of fatigue, which is what we are searching for.

The second main category of fatigue—peripheral fatigue—can further be split into two categories; substrate fatigue and metabolite fatigue. By definition, substrate fatigue is supposedly caused by a lack of enough substrate to produce energy, while metabolite fatigue is supposedly caused by the byproducts of exercise somehow inhibiting further contraction. The three main substrates involved in substrate fatigue are ATP, glycogen, and creatine phosphate. A deficiency in ATP is the most obvious cause of fatigue, as ATP is the body’s form of stored energy. Logically, it would make sense if muscles have a set amount of ATP available to them that when stores run out during exercise, the muscle would no longer be able to contract. Indeed, a lack of ATP would lead to muscle fatigue for this reason. However, there is extensive research showing that upon reaching muscle fatigue, ATP levels are still sufficient to maintain contraction, as running out of ATP would result in rigor mortis (Jennett 2001). This suggests that ATP deficiency is most likely not a primary cause of muscle fatigue in healthy individuals. Glycogen plays a similar role as ATP in substrate fatigue. Only a small amount of ATP is readily available to the muscle in the form of PCr or glucose in the blood. The majority of glucose is stored in the muscle in the form of glycogen, so that during extended exercise, the muscle has a steady supply of glucose. It would again make logical sense that a deficiency in glycogen could interrupt the supply of glucose to

the muscle, leading to fatigue. However, as noted by Ortenblad et al. in 2013, “a direct cause-and-effect relationship between glycogen and muscle function remains to be established”. This study specifically suggests a link between glycogen and SR calcium release during excitation-contraction coupling (Ortenblad et al. 2013). While such a link may indeed exist, muscle glycogen is not currently a practical, easy to measure substance that could allow us to determine onset of muscle fatigue. The final substrate, phosphocreatine, is only used within the first approximately 15 seconds of exercise, or at very high intensities. While a strong connection between PCr level and muscle fatigue may exist, it would only indicate muscle fatigue at maximal exercise. Because we are interested in muscle fatigue during long-term steady state exercise, it is unlikely that PCr will be useful to us as a biomarker of muscle fatigue.

Peripheral metabolic fatigue is the subject of the largest amount of fatigue research. Evidence seems to suggest that at least a portion of muscle fatigue is due to metabolite production, if not the vast majority of it. One important metabolite is hydrogen, which is produced during a wide array of metabolic reactions and serves to create a more acidic environment. However, as pH will be the primary focus of this paper, it will be discussed later.

Chloride is physiologically very important to muscle contraction. Cl- channels are in both the T-tubule and along the membrane of muscle. Cl- influx is involved heavily in depolarization of the membrane, helping to signal calcium release from the SR. Cairns et al. suggest that “normal [Cl-] protects against excessive fatigue in situations in which run-down of the transsarcolemmal K+ gradient occurs”, such as during high stimulation frequencies or tetany (Cairns et al. 2004). These results suggest

that Cl- may play a role in preventing muscle fatigue, and decreased Cl- could allow for additional fatigue to occur. However, the results also seem to apply mostly to situations of maximal contraction, so additional research would be necessary before attempting to use Cl- levels to predict muscle fatigue during steady state exercise.

If Cl- plays an important role in protecting against fatigue in situations where potassium concentrations are diminished, then it makes sense to look at the role K+ plays in muscle fatigue. Even more so than chloride, potassium plays an essential role in maintaining polarization of the sarcolemma and T-tubule. During exercise, muscles experience a decrease in potassium concentration as it leaves the muscle. According to Clausen et al., "this leads to depolarization, loss of excitability and contractile force." (Clausen et al. 2007). While this has been shown before, "little is known about the effects of these physiological increases in extracellular K+. . . on contractile endurance" (Clausen et al. 2007). In this paper, Clausen et al. examine the role that potassium plays in fatigue in rat muscle. They find that "excitation-induced increase in [K+] is an important cause of high-frequency fatigue, and the Na+,K+-pumps are essential for the maintenance of contractile force in the physiological range of [K+]o" (Clausen et al. 2007). While this indicates a strong connection between potassium and muscle fatigue, it is important to note that the correlation has been found between potassium and high-frequency fatigue, such as maximal contraction. While intramuscular potassium deficiency may play an essential role in fatigue at maximal contraction, it may play a lessened role in fatigue during extended steady state exercise.

While ATP provides energy for contracting muscle and has been examined as part of substrate level fatigue, its byproduct ADP plays an important role in metabolic

fatigue. ADP is known to work with ATP as a natural check and balance system, where excessive ATP encourages ATP hydrolysis and excessive ADP prevents critical steps in glycolysis and encourages ATP synthesis. This is directly tied to the idea of inorganic phosphate, as ATP hydrolysis results in ADP and Pi. According to McLester, "Pi release is coupled to the powerstroke of the crossbridge cycle. The accumulation of Pi during exercise would lead to a reversal of its release step, therefore causing a decrement in force production capability" (McLester 1997). He goes on to conclude that "Pi accumulation is probably the largest contributor to the fatigue process in exercise of any duration" (McLester 1997). He continues on to detail that ADP plays a role not only in a "reduced oscillatory power output", but in a "slowing of the rate constants (and therefore a decrease in the maximal velocity of shortening" (McLester 1997). This experiment suggests that at any intensity or duration, both ADP and Pi play an essential role in causing muscle fatigue. If there is a convenient method of measuring either of these metabolites in an accurate and non-invasive manner, they could potentially play an important role in predicting muscle fatigue during steady state exercise.

It is important to note that external factors may play a large role in influencing fatigue. In high altitude environments, muscles may not have access to the amount of oxygen necessary for normal aerobic respiration, which may lead to fatigue (Hall 2013). Dehydration can be a major cause of fatigue, as hydrolysis is an extremely common reaction involved in metabolism (Hall 2013). In addition, dehydration may lead to poor blood flow and impaired cognitive function, making exercise more difficult. Finally, heat may play a major role in fatigue, as it may influence dehydration or reaction speeds (Hall 2013). While these three factors may play a significant role in muscle fatigue,

dehydration and altitude should be controlled for in any experiment. For this reason, heat production seems to be the most influential and potentially useful external cause of fatigue during steady-state exercise within a lab setting. A relationship between muscle heat production and muscle fatigue is an area that should be explored further.

Finally, lactate has often been thought to be a cause of muscle fatigue. As discussed in the section on glycolysis, the function of lactate in the body is as a byproduct of anaerobic respiration. Theoretically, when an extended steady-state protocol is at too high of a workload for a subject, they will need to use both aerobic and anaerobic respiration to produce the necessary amount of ATP. This will lead to a buildup in lactate in the muscle. This lactate has long been thought to have an inhibitory effect on muscle contraction. However, Westerblad et al. note that "the temporal connection between impaired contractile function during fatigue and reduced pH is not always present" (Westerblad et al. 2002), noting however that "an alternative mechanism by which lactic acid formation may impose a limit on performance is during long-lasting types of exercise in which glycogen depletion is a key factor" (Westerblad et al. 2002), in which lactate causes an increased rate of glycogen store depletion because "the total amount of ATP produced from the stored glycogen is lower than with complete aerobic breakdown" (Westerblad et al. 2002). The connection between lactate threshold, acidosis, and muscle fatigue is explored more in depth later as well.

The role of pH

Intro to pH

The pH scale is a measurement of the concentration of hydrogen ions in a solution. It is represented by a logarithmic scale from one (acidic) to fourteen (basic), with seven being neutral. Chemical reactions are often heavily impacted by the pH in which they take place. One clear example is with hydrolysis reactions, as hydrogen ions produced through hydrolysis can build up and increase the energy requirement for further reactions to occur. The human body has a wide range of pH conditions, and each is optimized for its specific location. Digestive enzymes are optimized for the acidic conditions they are found in, while other reactions such the synthesis of ATP in liver mitochondria have multiple optimum pH values, corresponding to the different enzymes present (Myers and Slater 1957). Specifically in skeletal muscles, there are a few main factors which influence intramuscular pH. One of the largest factors has been thought to be lactic acid, which was thought to be produced during anaerobic respiration and dissociate into lactate and a hydrogen ion. However, modern research has discredited this idea, as lactate is produced, not lactic acid. The acidifying effects of lactate are still under debate. Potassium ions, creatine phosphate synthesis, and the bicarbonate buffer system also impact muscular pH. Blood pH tends to stay around 7.35 during rest, but may change during exercise as metabolites are produced and moved into the blood (Soller et al. 2007). It is important to note that while blood pH may often be a good predictor of intramuscular pH, it has been shown that exercise under certain circumstances can lead to significant differences between venous and intramuscular pH (Soller et al. 2007).

Old Ideas on pH

In the past, it has often been standard practice to teach that pH is at least partially the cause of muscular fatigue. There were a number of theories on mechanisms through which this could occur, including troponin C becoming less available for calcium binding, chloride ion permeability of muscle membrane decreasing, interruption of ATP hydrolysis during cross bridge cycling, or lessening the amount of calcium released from the sarcoplasmic reticulum.

One of the difficulties in such research was finding a practical method of studying pH in muscle during exercise. In 1939, Dubuisson helped to pioneer a method for measuring pH on the surface of skeletal muscle using an electrometer and a galvanometer. In this study, he recognized that past use of this technology had been "recording not only the changes in pH, but also variations in tissue polarization" (Dubuisson et al. 1939). Dubuisson claimed to have improved on these methods, such that there was "an excellent correlation" between "this recording technique [and] the results of chemical analyses made immediately after the experiment on the same muscles" (Dubuisson et al. 1939). While previous methods had only allowed pH measurement by chemical analysis at a single time point, Dubuisson helped to pioneer the idea of measuring pH throughout the duration of exercise.

During the period of time from the 1930s through the 1970s, *in vitro* animal muscle was a major focus of muscular fatigue research. Following in the steps of Dubuisson, Disteche in 1960 went on to examine pH during both muscle twitch and tetany. In this study, tortoise muscle at 22C was examined during "single isometric twitches" (Disteche 1960). Using the technology of Dubuisson, Disteche claimed that

by “knowing the cellular carbon dioxide/bicarbonate ratio, the pK of carbonic acid, and the retention factor which accounts for other buffer systems”, one would be able to solve for the amount inorganic phosphate split from ATP by calculating the amount of hydrolysis of phosphate bonds from the remaining pH change in the muscle(Disteche 1960). In this experiment, Disteche found that during tetany the muscles he was examining reached a steady state, where he claimed the “absorption of H+ overcompensates the H+ liberation after a few stimuli” (Disteche 1960). Disteche ended up concluding that there was a “good qualitative agreement between the H+ production and the heat production during tetanus” (Disteche 1960). Overall, this experiment was useful for improving the method of measuring pH in muscle during exercise, and for demonstrating the relationship between muscular activation and pH.

In June of 1967, Carter et al. improved further on this technique by using multiple-barreled electrodes. In the past, intracellular pH had been measured through indirect techniques, such as the Disteche study above where much had to be controlled for in order to estimate actual pH. According to their study, they used single barreled electrodes for the “determination of resting potential and intracellular pH with a minimum of cellular injury”, double barreled electrodes which by use of a reference were able to measure intracellular pH independent of transmembrane potential, and triple barreled electrodes which allowed for measurement during “controlled hyperpolarization or depolarization of the cell membrane” (Carter et al. 1967). During this study, Carter et al. found that they could only replicate pH values in previous experiments with “inadequately insulated electrodes” (Carter et al. 1967). They went on to examine rat thigh muscles *in vivo* while the exposed muscle was “continuously

perfused with castor oil" at 37C (Carter et al. 1967). They ended up concluding that "H+ of intracellular and extracellular fluid was in electrochemical equilibrium at all levels of [transmembrane potential]" which they claimed implied that "the determinants of intracellular pH are the transmembrane potential and the blood pH" (Carter et al. 1967). This study not only discounted some results from past studies, but made the assertion that there were large external factors influencing intracellular pH in skeletal muscle.

In 1967, Hutter and Warner examined chloride conductance in frog Sartorius muscle using a similar micro-electrode technique. They noted that while skeletal membrane potential had once seemed insensitive to outside chloride ion concentration, research in the 1950s had shown that membrane potential was largely influenced by chloride concentrations at certain pH levels (Hutter and Warner 1967). By artificially controlling the pH of the solution that the frog muscle was kept in, they attempted to show a correlation between acidic conditions and muscle fatigue. According to their data, they found that alkaline conditions improved chloride conductance, and vice versa (Hutter and Warner 1967). They went on to conclude that "even moderate extracellular accumulation of hydrogen ions could produce an appreciable reduction in chloride permeability", continuing on to suggest that in situations of muscle fiber depolarization and swelling, "a simultaneous fall in pH would produce a useful retardation of only slowly reversible osmotic changes" (Hutter and Warner 1967). Because chloride ions are closely tied to membrane excitation in skeletal muscle, decreased permeability of these ions in acidic conditions could theoretically lead to muscular fatigue.

In a 1980 study by Stevens, frog Sartorius muscles were isolated and exposed to baths of either pH 7 or pH 8 at 22°C (Stevens 1980). The muscles were then electrically stimulated, and force production was measured. Stevens found that "presoaking in pH 8 saline increased time to 50% fatigue by almost 50% in experiment A and 35% in experiment B" (Stevens 1980). This led to the conclusion that "the present experiments demonstrate that one important factor [in fatigue] is the pH of the external environment" (Stevens 1980). This study agreed with many others at the time, which as a combined body of evidence seemed to strongly support the idea that pH played a major role in muscle fatigue.

However, research contradictory to these ideas also arose during this time, such as Kindermann et al. in 1977. In this experiment, a bicarbonate and Tris-buffer combination was given to males during a 400m run (Kindermann et al. 1977). The results suggested that "run time, maximal lactate concentration and heart rate remained unchanged after the buffer infusions" (Kindermann et al. 1977). However, the change in pH was only measured to be 0.1 after the infusion, and only 10 subjects were used. Still, Kindermann et al. concluded that "the importance of pH as the performance limiting factor must be questioned" (Kindermann et al. 1977). While this study was far from disproving the role pH played in muscular fatigue, it suggested that the results of past studies on pH may not translate to *in vivo* implications.

More recently, these past studies have been criticized for a number of reasons. Very few studies took place in human models, and very few took place *in vivo*. Many of the studies used extreme pH values that could never be obtained within the body to get their results. In order to keep animal muscles stable *in vitro*, it was necessary to perform

many of these experiments at temperatures far below physiological levels. Stackhouse et al. bring up a number of studies which show that “when muscle is studied at temperatures that are close to the normal body temperatures of living organisms, the effect of a decreasing pH on maximum isometric tension and shortening speed is greatly reduced” (Stackhouse et al. 2001).

Modern research on pH causing fatigue

While the causes of muscular fatigue are still up for debate, research in the past twenty years has largely shifted to the viewpoint that pH does not play a major direct role in causing muscular fatigue. Improvements in technology and methodology have led to more research *in vivo,* and many results from past studies have been discounted due to flawed methodology. Still, it is accepted that acidosis does have some physiological impacts in muscle. As Allen et al. stated in 1995, pH “reduces maximal Ca(2+)-activated force and Ca2+ sensitivity, slows the maximal shortening velocity and prolongs relaxation. However, acidosis is not the only metabolic change in fatigue which causes each of the above” (Allen et al. 1995). While these functions are accepted, there is contradictory research on the extent of these impacts as far as influencing fatigue.

A review of literature on pH and muscle fatigue reveals that a significant number of sources stating the fatigue inducing effects of muscular acidosis are physiology textbooks. It is difficult to find an article on pH and fatigue that does not begin with some variation of ‘Intracellular acidosis has long been thought to be a cause of muscle fatigue’, many citing a range of textbooks over the past 60 years (Stackhouse et al. 2001). Peer reviewed articles strongly supporting the role of acidosis in muscular

fatigue tend to be outdated, while more modern research on pH influence on fatigue takes a more cautious approach to the extent of the effects. Still, there is a large body of modern research which suggests that pH has at least some inhibitory effect on muscular performance.

In a 2012 review of biomarkers to use for the determination of muscular fatigue, multiple different biomarkers are discussed, including lactate and pH. This study first mentions the fatigue inducing effects of acidosis by stating “multiple mechanisms of fatigue, of which the most important include: 1. Acidosis and depletion of ATP…” before continuing on to other biomarkers unrelated to pH (Finsterer 2012). This paper makes the important distinction that the exact causes of muscle fatigue are currently unknown, and that it is necessary to look at multiple possibilities. However, it supports the idea that acidosis causes fatigue by stating “even minimal decrease in muscle pH interferes with cross-bridge binding and ATPase activity due to competitive binding and reduced enzyme function” (Finsterer 2012). It continues on to state that “decreased intracellular pH may additionally impair oxidative enzyme activity and may adversely affect ryanodine receptor function” (Finsterer 2012). It is clear that even in 2012, the idea that pH causes muscle fatigue is both prominent and has not been categorically disproved. This paper further enforces the correlation between lactate threshold and muscle fatigue by stating “lactate appears to be a promising biomarker of muscle fatigue if workload conditions are standardized” (Finsterer 2012).

In a 2004 study by Robergs et al., the argument is made that there is no evidence for lactic acidosis, or any link between the supposed lactic acidosis and any metabolic acidosis (Robergs et al. 2004). There is a systematic review of the biochemistry behind

lactate production which will be discussed later upon examining the temporal link between lactate threshold, acidosis, and muscle fatigue. The work even goes on to state that lactate production retards metabolic acidosis (Robergs et al. 2004). However, the part of this study which is relevant to this section is where the authors conclude that "if muscle did not produce lactate, acidosis and muscle fatigue would occur more quickly and exercise performance would be severely impaired" (Robergs et al. 2004). Whether or not the statement is true, there is a clear implication that acidosis causes muscular fatigue.

While many of the more recent studies which insinuate pH plays a role in causing muscular fatigue are based on past research, there is little novel data supporting that connection. Instead, the focus of a majority of current research on the topic either supports the idea that pH does not play a significant role in causing muscular fatigue, or the idea that muscular acidosis actually helps to prevent muscular fatigue. A 2014 study by Siegler et al. specifically examines "the effect of pH on fatigue" on human muscle *in vivo* (Siegler et al. 2015). Eight males performed three trials of submaximal isometric contractions, under a control condition as well as acidosis induced by ingested ammonia chloride and alkalosis induced by ingested sodium bicarbonate (Siegler et al. 2015). While muscular pH was not actively measured during the exercise protocol, Siegler et al. worked off of past research which suggested that blood pH would have an impact on both "central and peripheral factors associated with fatigue and force production" (Siegler et al. 2015). This study found that "calf fatigue associated with intermittent, isometric contractions to task failure is unaffected by alterations in pH" (Siegler et al. 2015). There is a serious limitation when applying results of systemic acidosis induced

by ingestion to the function of acidosis within exercising muscle. However, two strengths of this study are that it occurred both *in vivo* and in a human subject. This heavily suggests that while acidosis may have a multitude of intramuscular effects, the combined effect may well be neutral.

In a 2004 study by Pedersen et al., intracellular acidification is acknowledged as a commonly thought cause of muscle fatigue. Pedersen then goes on to detail an experiment which implies that acidosis protects against muscle fatigue. In this experiment, rat extensor digitorum longus was prepared such that the muscle could be stimulated at any individual step of the excitation-contraction coupling process (Pedersen et al. 2004). By doing this, Pedersen et al. were able to demonstrate that "force responses to [action potentials] in the T system elicited by electrical stimulation did display pH dependence" (Pedersen et al. 2004). This data suggested that "intracellular acidosis protects against the loss of force caused by depolarization" which may be due to "enhanced excitability of the T system" (Pedersen et al. 2004). In order to elucidate this mechanism, Pedersen et al. performed an additional experiment in which superphysiological levels of Cl- were introduced to the rat muscle in order to enhance the effects of chloride ions on depolarization of the T system. This setup resulted in significantly larger drops in force in alkaline than acidic conditions (7.1 and 6.6 respectively) (Pedersen et al. 2004). They concluded by stating that "in the presence of Cl-, intracellular acidosis increases the excitability of the T system in depolarized muscles[sic] fibers, thus counteracting fatigue at a critical step in ECC" (Pedersen et al. 2004). This experiment suggests that muscular acidosis helps prevent muscle fatigue by decreasing Cl- permeability.

In a 2007 study, Lindinger cites previous research both by Sjogaard and within their own lab that suggests that fatigue is due to decreased sarcolemmal excitability. They state that fatigue is likely a mechanism to prevent damage that would occur to muscle cells if contraction continued. This paper reviews past literature to find that "acidosis counteracted the effects of increased extracellular potassium" on the sarcolemma in rat muscle (Lindinger 2007). Lindinger went on to come to the conclusion that "increased extracellular acidity…similar to that seen during high intensity exercise" was able to combine with the effects of adrenaline to "stabilize membrane excitability" (Lindinger 2007), supporting the idea that "extracellular lactate accumulation has a protective effect on muscle excitability" (Lindinger 2007). They finished by acknowledging limitations, such as non-physiological temperatures and a limitation of the full range of physiological mechanisms due to *in vitro* research (Lindinger 2007). This experiment suggests that muscular acidosis helps to prevent muscular fatigue by decreasing the fatigue-causing effects of potassium ions.

It is clear that there is still debate on the exact intramuscular effects of acidosis in relation to muscle fatigue. Current literature seems to be moving towards the position that pH does not play a major role in causing muscle fatigue. However, in our lab we are striving to anticipate muscle fatigue in exercising subjects, not to understand its cause. For this reason, it may be more pertinent to examine the temporal connection between acidosis, lactate threshold, and muscular fatigue. Even if acidosis were to have a preventative effect on muscular fatigue, if there were a significant correlation between when acidosis occurs and the onset of muscle fatigue, we would be able to use that connection to help prevent muscle fatigue in exercising subjects.

Temporal connection

Before tackling the issue of a temporal connection between acidosis and fatigue, it seems relevant to discuss the correlation between lactate threshold and acidosis. As previously discussed, lactate threshold is closely related to anaerobic threshold. From a physiological standpoint, switching from only aerobic respiration to aerobic and anaerobic respiration is likely to mark the onset of muscle fatigue, or at least a point very close to it if there were a period of time where anaerobic respiration could be buffered. If this were true, then using pH to determine when a subject crossed their lactate threshold would allow a simple method of preventing subjects from experiencing muscle fatigue; as soon as a subject demonstrated that they had crossed their lactate threshold by a corresponding drop in pH, exercise intensity could be decreased to prevent fatigue and subject dropout. For this reason, it is relevant to discuss acidosis and fatigue in the context of lactate threshold, and to analyze if such a connection exists.

Both sides of the argument on whether lactate causes or prevents acidosis agree on a couple of physiological concepts. First is the idea that anaerobic respiration does not produce lactic acid, but lactate. Second is the idea that muscles play not only an important role in lactate production, but also in lactate clearance. However, the disagreement comes in the form of both the number of hydrogen ions produced during lactate production and the physiological function of the lactate molecule itself. We previously discussed a 2004 study by Robergs et al. which made the claim that lactate production actually had a retardant effect on acidosis in muscles (Robergs et al. 2004). Robergs et al. supported this claim by analyzing the biochemical processes behind lactate production. They claimed that every two lactate molecules produced released a

single hydrogen ion (Robergs et al. 2004). They went on to claim that “research also clearly discredits the interpretation of acidosis as being caused by lactate production”, saying that it was instead largely due to “nonmitochondrial ATP turnover” (Robergs et al. 2004). If true, this study would prevent the use of pH measurement as a determinant of lactate threshold.

In a 2005 paper, Lindinger et al. took direct issue with the Robergs paper and set out to disprove its assertions, stating Robergs’ argument “hinders interpretation and detracts from the demonstration of lactate- production independent of increasing intracellular [H+]” (Lindinger et al. 2005). They went on to argue that the Robergs paper violated both “conservation of mass and maintenance of electroneutrality in solutions” (Lindinger et al. 2005). The argument essentially hinges around the fact that lactate “is a strong acid anion that fundamentally alters the behavior of water” (Lindinger et al. 2005). Because of this, they argue that “the accumulation of lactate- within skeletal muscle directly contributes to intracellular acidosis” (Lindinger et al. 2005). If true, this paper would suggest that we can indeed assume intracellular acidosis is related to lactate production, and therefore lactate threshold.

When presented with conflicting evidence, it is often useful to look at the effects of believing the incorrect interpretation in order to determine the proper course of action. If we incorrectly assume that lactate threshold and acidosis are correlated, we risk misattributing an onset of acidosis to lactate threshold and muscular fatigue, or missing the point at which the subject reaches their lactate threshold and begins experiencing fatigue. If we incorrectly assume that there is no correlation between acidosis and lactate threshold, we are then unable to state that sudden acidosis indicates

both lactate threshold and the onset of muscular fatigue. While literature seems to indicate a correlation between acidosis and lactate threshold, I believe it is safest to operate under the assumption of no correlation and instead examine the temporal connection between acidosis and muscular fatigue independent of lactate threshold. If such a useful connection exists, whether the acidosis is caused by lactate threshold or by another cause becomes irrelevant.

There is a 2001 paper by Stackhouse et al. by the title of “challenging the role of pH in skeletal muscle fatigue” which provides an excellent introduction into the topic of pH, suggested mechanisms by which acidosis could cause fatigue and research which refutes each one, as well as a section titled “lack of temporal association” (Stackhouse et al. 2001). In this section, the following three studies are brought up as evidence, as well as a fourth which is not free for public access.

The definition of a lack of temporal connection between acidosis and muscle fatigue used in the Stackhouse paper is borrowed from another paper by Saugen et al., who state “increases or decreases in metabolite levels [which] do not occur at the same time as increases or decreases in force-generating capacity” (Saugen et al. 1997). This definition limits temporal association to only existing in the case of an exact temporal correlation between acidosis and fatigue. In this 1997 study, eight subjects performed one-legged repetitive isometric knee extensions at 40% of maximum contraction for six seconds at a time until exhaustion. While this was happening, pH and other metabolites were measured every nine seconds. pH was measured using P-NMR, or Phosphorous-31 nuclear magnetic resonance. They noticed an immediate uptick in pH at the onset of exercise, which they hypothesized to be due to hydrolysis of phosphocreatine (Saugen

et al. 1997). After that, there was a steady drop in pH as exercise continued. However, there was a lot of variability, as some subjects experienced rapid drops in pH, while others experienced much smaller, steadier declines, as can be seen in Figure 1 (Saugen et al. 1997).

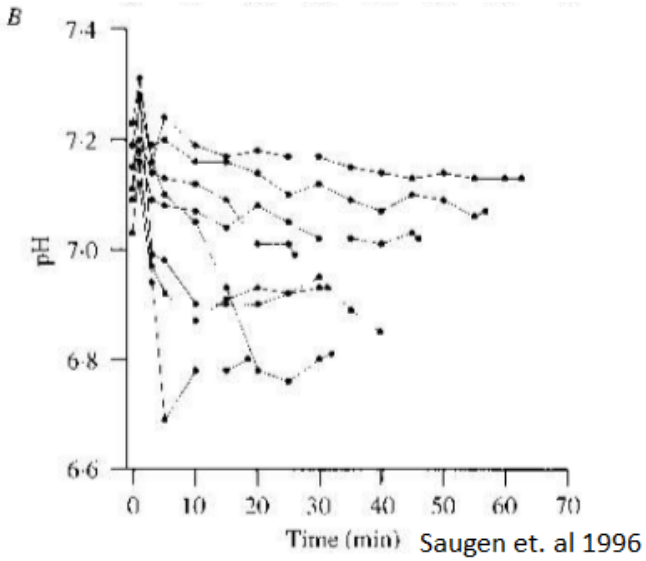


Figure 1: pH and time during one-legged repetitive isometric knee extensions

While some subjects did not experience significant changes in pH, the group as a whole went from a resting pH of 7.13±.02 to a pH at 25% of exercise duration of 7.00±.06, with an insignificant decrease in pH from that until exhaustion, as can be seen in Table 1 (Saugen et al. 1997).

	PCr (%)	ATP (%)	pH
Rest	100	100	7·13 ± 0·02
Exercise time			
25 %	51 ± 10	101 ± 5	7·00 ± 0·06
50 %	40 ± 10	109 ± 7	6·99 ± 0·05
75 %	34 ± 9	107 ± 8	6·95 ± 0·05
Exhaustion	35 ± 9	102 ± 8	6·95 ± 0·04

Exercise time is given as a percentage of endurance time. PCr and ATP levels are presented as percentages of their respective pre-exercise values. All high-energy phosphate levels were first calculated relative to the total phosphate peak area (P_i + PCr + γATP; see Methods). Saugen et. al, 1996

Table 1: pH decreases during exercise

They noted in their data that "in some subjects RIE could be continued for 10-15 min. . .without further changes in pH or ATP" (Saugen et al. 1997). In the discussion, they go on to suggest that the rest intervals between contractions "enable[d] sufficient aerobic ATP resynthesis, in keeping with previous results. . . showing a very moderate rise in muscle lactate" (Saugen et al. 1997). This research does not indicate any sort of useful temporal connection between pH and muscle fatigue. However, this may be due to exercise intensity being only at 40% of maximum effort, as there may be no transition into anaerobic respiration. Because this experimental protocol required 40% max effort and for the subject to maintain contraction for six seconds before resting for two, it may give significantly different results than dynamic knee extension with 65% max effort, no isotonic portion, and shorter rest periods between contractions.

Wong et al. specifically looked at patients with chronic fatigue syndrome when determining a temporal relation between acidosis and fatigue. 22 CFS patients were compared to 21 healthy adults in a protocol of "dynamic, graded, plantar flexion" with a "constant repetition rate of 30 cycles/min against resistance that was increased at 2 kg/min" until exhaustion (Wong et al. 1992). P-NMR was used to acquire a range of data, including pH. They went on to conclude from their data that "the degree of change in PCr, Pi, and pH from rest to peak dynamic exercise was quantitatively large, and equal, in both study groups" (Wong et al. 1992). While the focus of this study was to determine differences between healthy populations and CFS patients, there was evidence of a significant decrease in pH right before exhaustion when compared to rest.

Additional evidence against a temporal connection between acidosis and fatigue comes in a 1993 study by Degroot et al., in which five subjects performed an exercise protocol of “maximal isometric foot plantar flexion sustained for 4 minutes” (Degroot et al. 1993). P-NMR spectra were obtained in order to analyze an array of metabolites. Results showed that “[H+] decreased immediately after the onset of exercise, but then rose steadily until the end of exercise to a value of . . . pH6.47±.04” compared to a control value of pH 7.09, as can be seen in Figure 2 (Degroot et al. 1993).

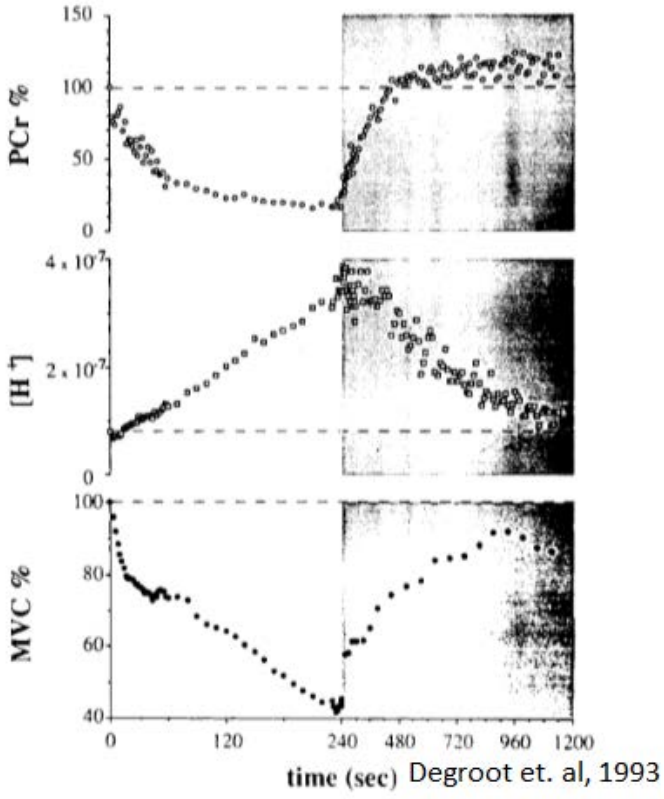


Figure 2: H+ concentration and time during maximal isometric foot plantar flexion

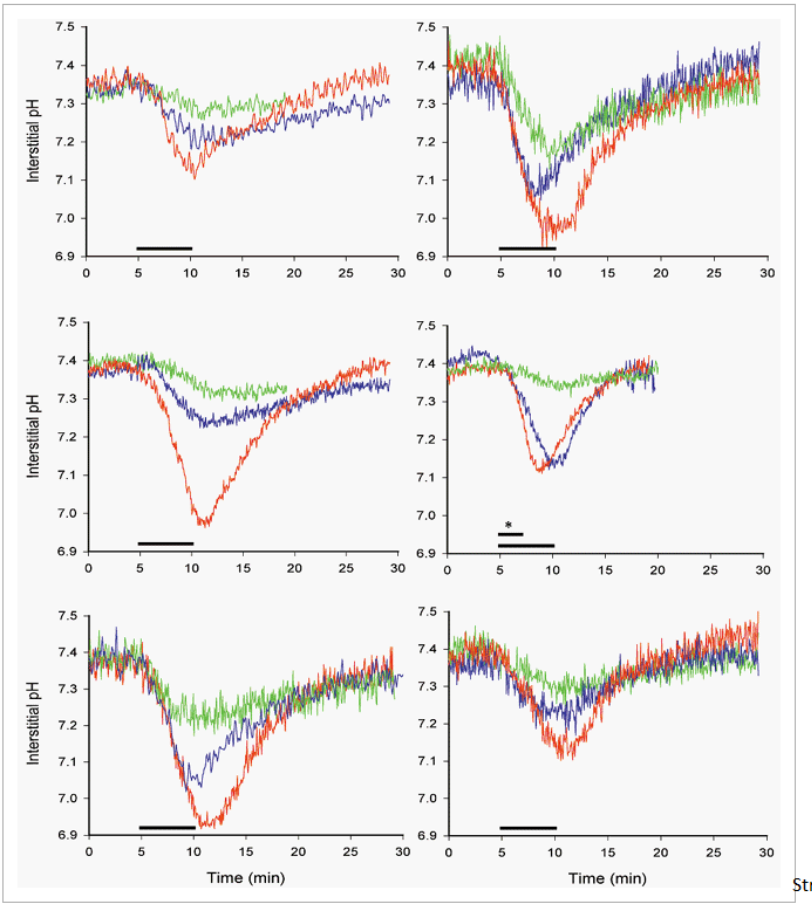
Notably, there was a significant increase in pH during the first 20 seconds of exercise (likely due to PCr hydrolysis), with a concurrent steady decline in MVC. Overall, this study found that “although the decline of force and increase in [H+] may

be associated later during exercise, during the initial 10 seconds force declines while [H+] decreases" (Degroot et al. 1993).

Returning to the Stackhouse paper from above, the three previous papers are used to make the assertion that "the results of these studies. . . demonstrate that in certain phases of fatiguing exercise, there is a clear lack of temporal association between changes in pH and changes in force" (Stackhouse et al. 2001). Using the - definition given in the Saugen paper of a lack of temporal association being "increases or decreases in metabolite levels [which] do not occur at the same time as increases or decreases in force-generating capacity"(Saugen et al. 1997), it is clear that this is a reasonable conclusion; all three papers showed that changes in pH did not exactly align with changes in force production, especially at the onset of and recovery from exercise. However, all three papers showed a significant drop in pH between rest and peak exercise. In addition, all three studies were performed at exercise intensities other than steady state exercise. In fact, the Degroot paper specifically states that "one long-standing hypothesis has been that fatigue occurs as a result of a rise in intracellular [H+], and this has been supported by various studies which have employed steady state or prolonged exercise", before going on to explain why this experiment would be different than ones which utilized steady state protocols (Degroot et al. 1993). With these papers, we can come to a couple important conclusions. Firstly, the time course of pH does not exactly follow muscle fatigue, especially at onset or recovery from exercise. Secondly, as examined above, acidosis is likely not the cause of muscle fatigue. However, there does seem to be a significant reduction in pH at peak exercise when compared to rest. Even if acidosis does not cause muscle fatigue and does not

always occur at the exact same time as muscle fatigue, if there is a general correlation between acidosis and fatigue at any time-point during exercise, it may still be useful for preventing fatigue during a steady state protocol.

The next step is then to examine the limited data on pH during steady state exercise in order to determine if there is a usable connection between pH and muscle fatigue. A study by Street et al. in 2001 conducted a study with a protocol much closer to steady-state conditions. Six subjects “performed one-legged knee extensor exercise” and “were required to maintain a cadence of 60 r.p.m for 5 min duration at each workload” for workloads of 30, 50, and 70W (Street et al. 2001). pH was studied using microdialysis throughout the exercise protocol. At rest, mean interstitial pH was 7.38. Street et al. found that “exercise induced a reduction in muscle interstitial pH in all six subjects and at all intensities”, as well as “a correlation between power output and peak acidification” in each subject (Street et al. 2001).



Street et. al, 2001

Figure 3: Individual continuous pH response during one legged knee extensor exercise

Figure 3 shows each subject's individual pH response to exercise, where it is evident that pH decreases according to power output throughout the entire duration of exercise which does not end in exhaustion (Street et al. 2001). Figure 4 shows the relationship between power output and interstitial pH (Street et al. 2001).

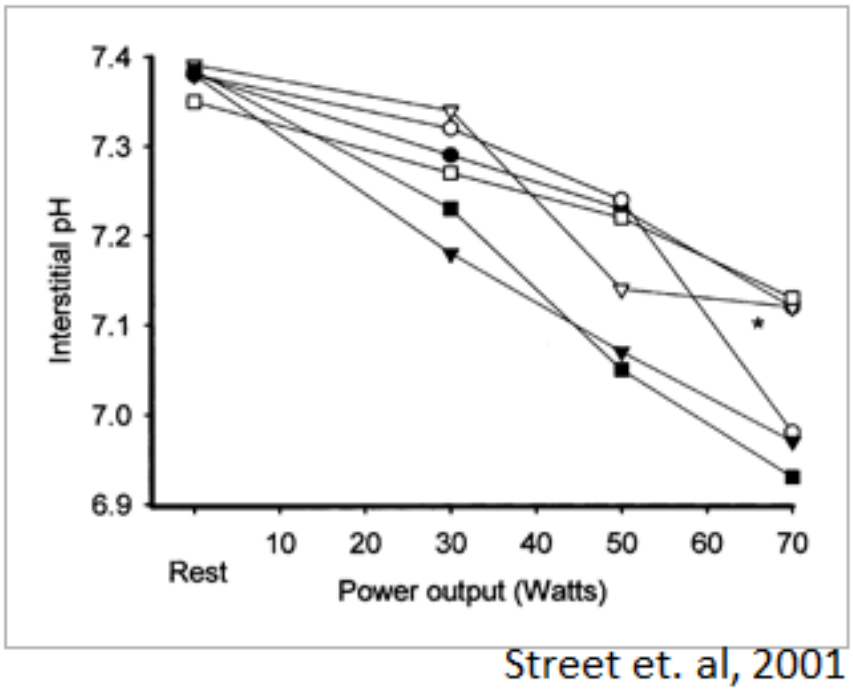


Figure 4: pH and power output during one legged knee extensor exercise

The paper concludes by stating “the present study demonstrated that interstitial pH is continuously decreasing during muscle activity”, noting that “pH was correlated with power output” (Street et al. 2001). The main issues preventing this study from being directly applicable to our needs are the small sample size, non-relative workloads, and the fact that exercise was not performed until fatigue (except for one trial for one subject). Because of these limitations, we are unable to see if there are significant changes in pH right before exhaustion during steady state exercise. Still, this study provides evidence that pH is continually decreasing during steady state exercise, and suggests that pH measurement could be a useful tool for determining muscle fatigue, especially if these results are replicable within subjects.

Further evidence of the use of pH in steady state exercise is presented in a 1985 paper by Wilson et al., in which “nine patients with chronic congestive heart failure” and eight controls were put through an exercise protocol involving steady state exercise

of the forearms (Wilson et al. 1985). P-NMR was used to measure pH levels throughout the protocol, which consisted of “wrist flexion every 5 sec for 7 min” at 1, 2, and 3 J. They found that “exercise resulted in a decrease in pH only at 0.6W”, or only at the highest workrate. This can be seen in Figure 5 (where the dashed line represents CHF patients and the solid represents control), where pH remains fairly even between rest and the lower workrates in healthy patients, but then drops significantly for the highest workrate (Wilson et al. 1985). In the lower half of Figure 5, fatigue is ranked on a subjective perceived scale by the subject, with 0 being no fatigue and 4 being highest.

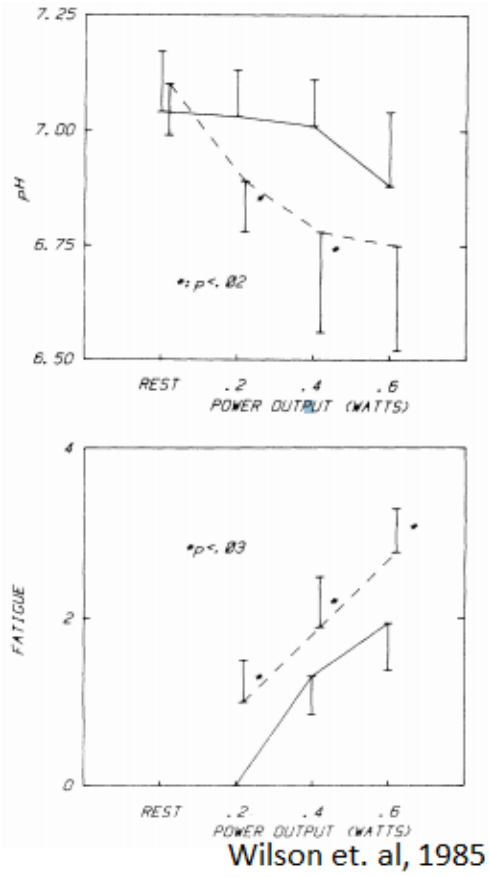


Figure 5: pH, power output, and fatigue during steady state wrist flexion

Fatigue increases with workload as expected, and correlates nicely with the drop in pH after exercise (Wilson et al. 1985). Due to the use of P-NMR, this study does not

have continuous measurement of pH throughout the exercise protocol. However, it does suggest that there may be a steady-state workload which leads to a significant drop in pH relative to lesser workloads. This is very promising for future research on the connection between a measurable sudden acidosis event and the onset of fatigue.

One of the most promising studies occurred in 1988, by Miller et al. In this study, the exercise protocol consisted of both a 4 minute sustained maximum voluntary contraction and an intermittent protocol, of which the latter is more relevant to our work (Miller et al. 1988). The intermittent protocol consisted or contracting and relaxing the adductor pollicis once every 10 seconds for 5 minutes at 75% of their MVC. This was repeated eleven times without rest periods, with each trial occurring at a different contraction/relaxation split (6s contraction and 4s rest, 3s contraction 7s rest, etc.) (Miller et al. 1988). Miller et al. state that "analysis of 1-min spectral blocks indicated that a steady state was almost always reached after 1 min" (Miller et al. 1988). After analyzing the data, the conclusion was that "during intermittent exercise, pHi gradually dropped to 6.55±.03 [compared to a resting pH of 7.08±.04] and then gradually returned to control values by 40 min" (Miller et al. 1988). In Figure 6 where triangles represent pH and squares represent MVC, it is evident that during the steady state protocol, pH and MVC displayed qualitatively similar changes throughout the protocol (Miller et al. 1988). Specifically, a significant drop can be seen in both from rest to the onset of exercise. From there, pH steadily drops along with MVC until the cessation of exercise, at which point pH begins to recover and MVC quickly follows (Miller et al. 1988). The lag that MVC experiences in recovery supports the lack of exact temporal association, but the overall qualitative connection is clear.

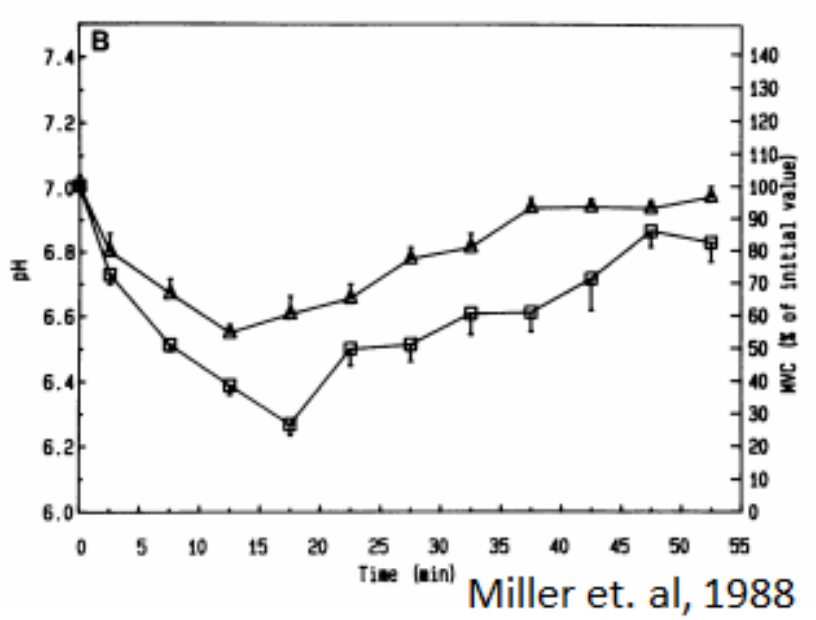


Figure 6: pH over time during steady state adductor pollicis contraction

This correlation is even more evident in Figure 7 (Miller et al. 1988).

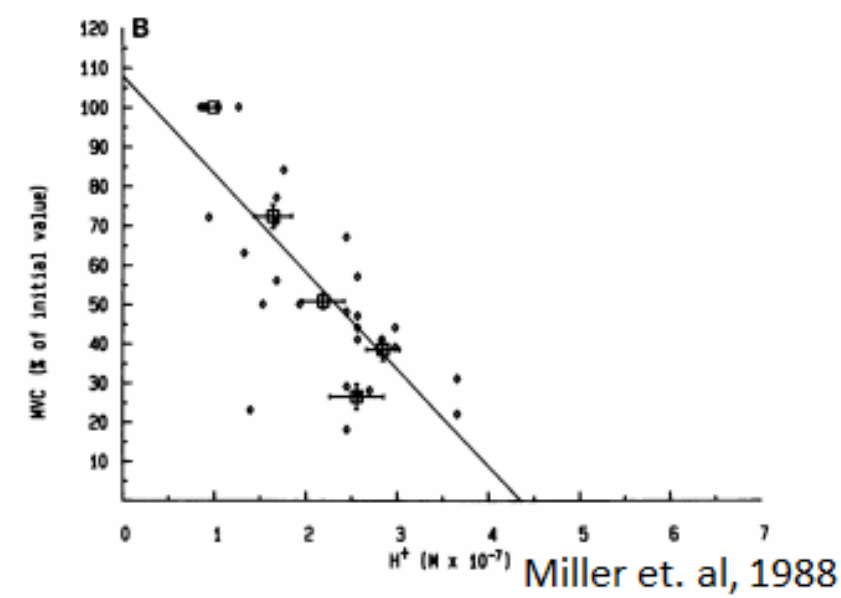


Figure 7: Maximal volumetric contraction and hydrogen concentration during steady state adductor pollicis contraction.

A regression analysis was performed between the two variables, and an r value of 0.77 was found, indicating a strong linear relationship between MVC and pH (Miller

et al. 1988). This study indicates that while acidosis may not cause fatigue or even be directly temporally associated with it, there seems to be a strong linear relationship between acidosis and fatigue during steady state exercise protocols. This is extremely encouraging for future research, as it makes clear that measurement of pH may well lead to useful extrapolations to fatigue.

These three papers examining acidosis during steady state exercise provide a significant body of evidence suggesting that pH measurement may be a useful tool during this type of exercise. While there are a lot of unknowns, it seems clear that for a portion of steady state exercise, pH most likely decreases at a steady rate. Further, it appears evident that higher workloads lead to a more significant decrease in pH than lower workloads. Finally, although causation and temporal association have not been shown, it seems as though pH is closely related to muscle fatigue as determined by continuous measurement of maximal volumetric contraction. Assuming the technology is practical and available, it seems to be worthwhile to more closely examine continuous pH response to steady state exercise at levels which may induce fatigue.

Methods of measuring pH

There are a couple goals we must have when determining the best technology and methodology to measure pH during steady state exercise. First is the potential for nearly continuous measurement; more frequent measurements allow for a greater chance of sensing significant changes in pH at any given time point. The second is minimal invasiveness in order to both allow for use during most experiments and to improve subject experience. The third is demonstrated, repeatable accuracy in measuring the intended value. The fourth is minimal equipment needed; technology that is prohibitively expensive or requires special staff to operate will not be practical to use during a standard exercise protocol.

Two techniques used in the past to measure pH are venous blood samples and muscle biopsies. In the Street paper discussed previously, the argument is made that since venous blood is a mixture of metabolites from the working muscle and blood returning from other non-working muscle, there must be some difference between blood and interstitial pH (Street et al. 2001). It goes on to state that due to differences found between blood and interstitial lactate levels, “it may, therefore, also be expected that interstitial to venous pH gradients exist during exercise”, concluding this section with “it can be hypothesized that the exercise-induced changes in interstitial and venous blood pH are different and that the changes in venous blood pH underestimate the local interstitial pH changes” (Street et al. 2001). While venous blood draws are simple to perform, venous blood lacks accuracy in estimating interstitial pH and is impractical to take at regular short intervals throughout exercise. For this reason, venous blood draws are not practical for pH monitoring during steady state exercise. Similarly, muscle

biopsies have been taken from exercising muscle and analyzed to determine pH levels. However, high accuracy in measuring interstitial pH is traded for not allowing continuous measurement, being highly invasive, and requiring special staff and equipment. For these reasons, muscle biopsy should not be considered a practical method of pH measurement during steady state exercise.

A large number of the studies which occurred in the 20th century used glass microelectrodes to measure pH. As discussed in the history of pH measurement section earlier in this paper, this technique was used in human as well as other animal muscle, and has gone through a long period of technique refinement. There is a discussion of the method of using mircoelectrodes "for measuring potential or determining the free concentration of cytosolic constituents" in a paper titled "using microelectrodes" (Halliwell et al. 1987). While the methodology of using microelectrodes has existed for a century, nearly every subsequent paper does something to improve upon the errors of past studies. This paper documents the five main sources of error when using microelectrodes as follows:

> "(1) Varying tip potentials of the ME. (2) Varying junction potentials. (3) Asymmetry of electrode reference potentials and their dependence on salt concentration in the bath solution. (4) Inadequate amplifier frequency responses when monitoring fast signals. (5) Errors in potential measurement when injecting current because of 'bridge balance' or ME resistance change, or due to too high a switching rate when using the discontinuous current injection method" (Halliwell et al. 1987)

The benefits of this technology are that the shortcomings are well understood and documented so that use of the equipment will likely give results that closely align with what is actually meant to be measured. Techniques such as the single/double/triple barrel microelectrode as discussed in the Carter paper in the section on pH causing

fatigue exist which make the technology highly adaptable to specific needs (Carter et al. 1967). The main issue is that study of intramuscular pH requires exposure of the exercising muscle for accurate measurement. Surface and skin membrane potential measurements have been taken, but relationships between those and intracellular measurements are far from conclusive. For this reason, this technique has not been used for exercising human muscle in the past, and is likely not practical for pH monitoring during steady state exercise.

A technique which improves upon the weaknesses of venous blood draw (non-continuous, not an accurate measurement of intracellular pH) is microdialysis. With this method, after injection of a local anesthetic a microdialysis probe is introduced into the exercising muscle using an introducing needle, which is subsequently removed. A pH-sensitive dye can be mixed with a saline solution and infused through the microdialysis probe. When used in conjunction with a spectrophotometer, constant monitoring of intracellular pH can occur. This method was used to great effect in the Street paper discussed earlier. This study improved upon past methods of microdialysis which actually showed alkalization during muscle activity by adding a bicarbonate buffer to the perfusate at physiological levels, or about 25 mM (Street et al. 2001). This method has the benefit of providing a constant stream of data for analysis, allowing for greater temporal accuracy than any other current method (Street et al. 2001). Microdialysis is somewhat invasive, but does not require highly specialized training or inhibit normal exercise. However, this method has not been used extensively in the past, so it is difficult to know if current techniques provide perfectly accurate results. In exercise protocols which do not require invasive equipment, introducing anesthesia and a probe

inside the exercising muscle seems to be excessive for our purposes. However, the method does seem to be very effective and is worth consideration in certain situations.

Many of the more recent studies have utilized P-NMR, or phosphorous-31 nuclear magnetic resonance. In the Miller paper discussed previously, P-NMR was used specifically to measure human intramuscular pH during steady state exercise. According to Maryellen Nerz-Stormes of Bryn Mawr college, NMR "is based on the fact that when a population of magnetic nuclei is placed in an external magnetic field, the nuclei become aligned in a predictable and finite number of orientations. For ^{1}H there are two orientations" (Nerz-Stormes 2009). If you picture a compass, there are two stable positions for the needle; with the south end facing north as natural (the alpha form) or with the north end facing so perfectly north that the forces pushing it to either side balance out (the beta form). By putting energy into the system, the nuclei are switched to the beta form, and upon returning to the alpha orientation create a measurable change in the magnetic field. This can be used to calculate H+ ion concentration assuming you can account for the other molecules present in the sample (Nerz-Stormes 2009). NMR using phosphorous is specifically used because it is easier to interpret than other methods of NMR. In the Miller study, P-NMR provided useful, significant data when examining intramuscular pH (Miller et al. 1988). However, P-NMR requires around 30 seconds for each scan, during which time the muscle being examined can't be moved. Additionally, P-NMR requires access to a superconducting magnet to generate the required magnetic field, such as an MRI machine. P-NMR has a lot of use in determining the role pH plays in the body and in the muscle. However, it is not a practical method of pH monitoring during steady state exercise.

Near Infrared Spectroscopy is not a new technology. However, due to the complex nature of its functioning, new uses are still being discovered. Spectroscopy depends on chemical interactions within molecules. If a molecule is made of two atoms, the atoms are bonded to each other through an interaction of forces. These forces can be thought of as a spring between the atoms. This spring naturally stretches and relaxes, creating a vibration within the molecule. Adding more atoms creates a large number of unique ways in which the molecule can vibrate (Rupawalla et al. 2013). As with anything that can vibrate, there are specific resonant frequencies, in which the internal vibration of the molecule combines with external vibrations to cause a greatly magnified effect (Rupawalla et al. 2013). Near Infrared Spectroscopy uses electromagnetic waves near the infrared range of wavelengths. By projecting these waves onto a molecule and going through a range of wavelengths, it is possible to determine which wavelengths cause the molecule to resonate (Rupawalla et al. 2013). Because every molecule has different forces causing different internal vibrations, it is then possible to look at which wavelengths caused resonance, and reverse engineer what molecule is present (Rupawalla et al. 2013).

This technology was first utilized in the 1950s for chemistry applications. However, it has been recently found that the same technology could be used to measure pH levels in the muscle (Soller et al. 2008). Because pH of interstitial fluid is dependent on the relative concentrations of hydrogen ions and carbon dioxide, analyzing which molecules are present in the sample using the method described in the previous paragraph allows for analysis of interstitial pH (Soller et al. 2008). In a paper entitled "Noninvasive determination of exercise-induced hydrogen ion threshold through direct

optical measurement", Soller et al. used many of the concepts discussed above in order to determine if there is an H+ threshold during exercise which occurs at the same time as anaerobic threshold (Soller et al. 2008). Soller notes that "Near-infrared light passes through skin and subcutaneous fat and can be used to noninvasively measure metabolic parameters in muscle" (Soller et al. 2008). The experiment was designed around the hypothesis that "this methodology can be extended to determine a threshold based on the accumulation of hydrogen ions in the muscle interstitial fluid during graded exercise, the H+ threshold" (Soller et al. 2008).

The exercise protocol consisted of both handgrip dynamometry with eight subjects and cycle ergometry with ten subjects. The handgrip protocol involved "four 5-min bouts" of "2-s contractions with intervening 1 s of relaxation", with workload determined by MVC (Soller et al. 2008). The cycle protocol involved "a graded exercise test. . . to maximal exertion" with 3 minute stages at 75 rpm and an increase of 50W per stage (Soller et al. 2008). During both protocols, NIRS data was collected and blood samples were taken to determine lactate levels. As can be seen in figure 8, a very close relationship was found between NIRS H+ measurements and invasive controls, with an r^2 value of 0.722 (Soller et al. 2008).

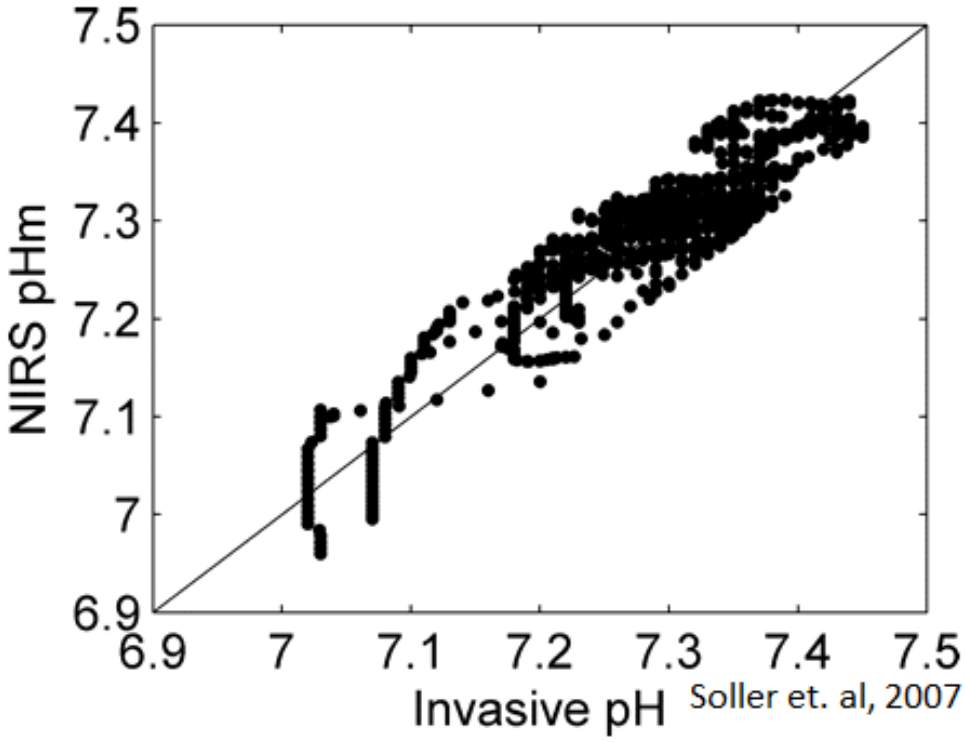


Figure 8: Comparison between pH measured by NIRS and by traditional invasive methods

In Figure 9, there are two things of note in the data of a single subject. First, there is a clear qualitative H+ threshold that occurs, with a sudden increase in H+ concentration at a certain exercise rate. Second, there is a clear qualitative connection between this H+ threshold and the lactate threshold (Soller et al. 2008).

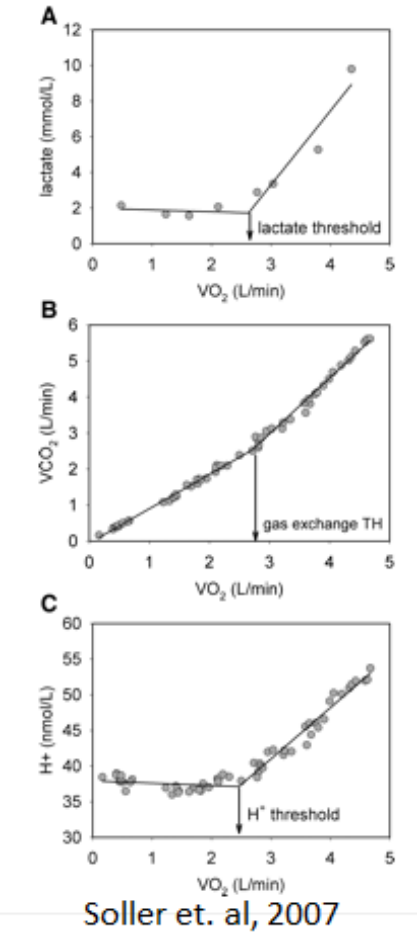


Figure 9: Proposed H+ threshold measured by NIRS in an exercising individual

This data is only from a single subject and a general conclusion can't be made, but the data is promising for future research. Soller et al. further found that H+ threshold and LT were highly correlated with an r^2 value of 0.946 (Soller et al. 2008). They conclude by stating that "an accurate mathematical model was established relating pHm to near-infrared spectra of exercising muscle during handgrip dynamometry and that the resultant model can be used during graded cycle exercise to measure [H+]" (Soller et al. 2008).

NIRS has been shown to provide fairly continuous measurement, be minimally invasive, accurately determine intramuscular pH, and do so without any prohibitive

equipment or training. In addition, it has been used to determine the exact mechanism that we are looking for in our research, namely an H+ threshold that is related to both lactate threshold and possibly the onset of muscle fatigue. For these reasons, NIRS is the gold standard for monitoring pH during steady state exercise. The main drawback is that because this is a novel use of this technology, it is relatively unproven. If H+ thresholds are repeatable within subjects, we can theoretically use this technology to make sure that no subject crosses their lactate threshold, preventing subject dropout and confounding variables during aerobic steady-state exercise.

Future directions for research

The Soller study discussed above is a very promising proof of concept, but additional research must be done. To determine a future direction for research, we have to ask what we know for sure, what we suspect, and what still needs to be tested. As I have systematically shown in this paper, there are a few facts that must be accepted until future research disproves them. First, evidence does not support the idea that acidosis is the primary cause of muscle fatigue. Second, evidence does not support an exact temporal connection between acidosis and lactate threshold. Third, evidence does not support an exact temporal connection between acidosis and muscle fatigue. However, it has also been shown that for the purposes of monitoring pH during steady state exercise to prevent muscle fatigue, an exact association is not needed, only a correlation between pH and muscle fatigue. We further know that for these purposes, past techniques of pH measurement like venous blood draws, muscle biopsies, and glass microelectrodes are impractical options. We have seen how P-NMR and microdialysis can both have limited use depending on the situation. We also know that preliminary

data suggests that NIRS is the most promising way forward for pH monitoring during steady state exercise.

Due to a knowledge of physiology and past research, we suspect that at a certain steady-state exercise level, some physiological threshold is crossed which begins the process of muscle fatigue. We further suspect that this threshold is related to the switch between aerobic respiration to aerobic and anaerobic respiration. While not necessarily caused by or exactly in time with the anaerobic threshold, it would make sense if this threshold were at least somewhat correlated with the lactate threshold. Further, we suspect that around the time that this physiological threshold is crossed and muscle fatigue begins to set in, there may be a sudden build-up in H+ concentration, regardless of the cause and effect. Research by Soller et al. suggests that we can not only measure this build-up with NIRS, but according to our other suspicions we could then use this H+ threshold to prevent muscle fatigue in subjects.

There is much that still needs to be tested in the future based on these ideas. One of the first directions of research should be to determine if H+ thresholds are present during exercise protocols that we run in the lab, including dynamic knee extension and cycle ergometry protocols. This can be accomplished by running standard exercise protocols with a NIRS sensor attached, and looking for a sudden inflection in H+ concentration. If H+ thresholds are found within groups of subjects, the next step will be to determine if this data is useful for single subjects, by creating a method of determining when a subject has hit their H+ threshold. A steady state H+ concentration will need to be established in each subject, with a protocol to determine if a specific value lies above or below the H+ threshold. If these experiments are successful, the next

step would be to find a correlation between H+ threshold and muscle fatigue. This could be accomplished with a cycle ergometry study, with one group of subjects exercising at a level just above their H+ threshold, and a control group exercising at a level just below their H+ threshold. If the experimental group fatigues significantly faster than the control group, it can then be concluded that NIRS is a useful tool to prevent subjects from experiencing fatigue during steady state exercise. If this is found, NIRS will prove to be an invaluable resource for not only our lab, but any lab involved in steady-state sub-anaerobic threshold exercise protocols.

Conclusion

It has been systematically shown that the causes for muscle fatigue are still up for debate. However, evidence supports the idea that pH is neither causally nor exactly temporally associated with fatigue. However, evidence does suggest that pH measurement may still be useful for determining the onset of muscle fatigue during steady state exercise. There are a variety of methods that exist for measuring pH in the body, of which the most promising for our purposes are microdialysis, P-NMR, and NIRS. Of the three, NIRS appears to be the best choice for further research. Future research should focus on establishing the functionality of NIRS for determining the onset of muscle fatigue in individuals performing steady-state exercise protocols.

Bibliography

Allen, DG, H. Westerblad, and J. Lannergren. "The Role of Intracellular Acidosis in Muscle Fatigue." *Adv Exp Med Biol* 384 (1995): 57-68. NCBI. Web. 12 July 2016.

Cairns, S. P., V. Ruzhynsky, and J.-M. Renaud. "Protective Role of Extracellular Chloride in Fatigue of Isolated Mammalian Skeletal Muscle*." AJP: Cell Physiology* 287.3 (2004): n. pag. Web.

Carter, Norman, Floyd Rector, David Campion, and Donald Seldin. "Measurement of Intracellular PH of Skeletal Muscle with PH-sensitive Glass Microelectrodes." *The Journal of Physiology* 46.6 (1967): 920-33. Web.

Clausen, Torben, and Ole Baekgaard Nielsen. "Potassium, Na ,K -pumps and Fatigue in Rat Muscle." *The Journal of Physiology* 584.1 (2007): 295-304. Web.

Contessa, Paola, Alessio Puleo, and Carlo J. De Luca. "Is the Notion of Central Fatigue Based on a Solid Foundation?" *Journal of Neurophysiology* J Neurophysiol 115.2 (2015): 967-77. Web.

Davis, J. Mark, and Stephen P. Bailey. "Possible Mechanisms of Central Nervous System Fatigue during Exercise." *Medicine & Science in Sports & Exercise* 29.1 (1997): 45-57. Web.

DeGroot M, Massie BM, Boska M, et al. “Dissociation of [[H.sup.+]] from fatigue in human muscle detected by high time resolution [sup.31]P-NMR.” *Muscle Nerve*. 1993;16:91-98.

Denadai, Benedito Sérgio, Euripedes Barsanulfo Gonçalves Gomide, and Camila Coelho Greco. "The Relationship Between Onset of Blood Lactate Accumulation, Critical Velocity, and Maximal Lactate Steady State in Soccer Players." *The Journal of Strength and Conditioning Research* 19.2 (2005): 364. Web.

Distèche, A. "PH Changes during Muscle Twitch and Tetanus." *Nature* 187.4743 (1960): 1119-120. Web.

Dubuisson, M., (1939), “Studies on the chemical processes which occur in muscle before, during and after contraction.” *The Journal of Physiology*, 94 doi: 10.1113/jphysiol.1939.sp003694.)

Finsterer, Josef. "Biomarkers Of Peripheral Muscle Fatigue During Exercise." *BMC Musculoskeletal Disorders* 13.1 (2012): 218-230. *Academic Search Premier*. Web. 31 July 2016.)

Hall, John E. *Guyton and Hall Textbook of Medical Physiology*. South Asian Edition ed. New Delhi: Elsevier, 2013. Print.

Halliwell, James, Michael Whitaker, and David Ogden. "Using Microelectrodes." (n.d.): n. pag. UT Dallas. Web. http://www.utdallas.edu/~tres/microelectrode/microelectrodes_ch01.pdf

Halliwill, John. "HPHY Department of Human Physiology." John Halliwill. University of Oregon, n.d. Web. 18 July 2016.

Hutter, O. F., and Anne E. Warner. "The pH Sensitivity of the Chloride Conductance of Frog Skeletal Muscle." *The Journal of Physiology* 189.3 (1967): 403–425. Print.)

Jennett, Colin Blakemore; Sheila, and "fatigue (in Physiology)." *The Columbia Encyclopedia*, 6th Ed.. 2016. "Fatigue." Encyclopedia.com. HighBeam Research, 01 Jan. 2001. Web. 18 Aug. 2016.

Kindermann, W., J. Keul, and G. Huber. "Physical Exercise after Induced Alkalosis (bicarbonate or Tris-buffer)." E*uropean Journal of Applied Physiology and Occupational Physiology*. 37.3 (1977): 197-204. Web

Lindinger, M. I., J. Kowalchuk, and G. Heigenhauser. "Applying Physicochemical Principles to Skeletal Muscle Acid-base Status." *AJP: Regulatory, Integrative and Comparative Physiology* 289.3 (2005): n. pag. Web.

Lindinger, M. I. (2007), "Combating muscle fatigue: extracellular lactic acidosis and catecholamines". *The Journal of Physiology*, 581: 419. doi:10.1113/jphysiol.2007.132209

Mclester, John R. "Muscle Contraction and Fatigue." *Sports Medicine* 23.5 (1997): 287-305. Web.

Miller, R. G., M. D. Boska, R. S. Moussavi, P. J. Carson, and M. W. Weiner. "31P Nuclear Magnetic Resonance Studies of High Energy Phosphates and PH in Human Muscle Fatigue. Comparison of Aerobic and Anaerobic Exercise." *Journal of Clinical Investigation*. 81.4 (1988): 1190-196. Web.).

Myers, D. K., and E. C. Slater. "The Enzymic Hydrolysis of Adenosine Triphosphate by Liver Mitochondria. 1. Activities at Different pH Values." *Biochemical Journal* 67.4 (1957): 558–572. Print.

Nerz-Stormes, Maryellen. "The Basics Nuclear Magnetic Resonance Spectroscopy." *Organic Chemistry Study Aides*. Bryn Mawr College, n.d. Web.

Ørtenblad, Niels, Håkan Westerblad, and Joachim Nielsen. "Muscle Glycogen Stores and Fatigue." *The Journal of Physiology* 591.18 (2013): 4405-413. Web.

Pedersen, T. H., Nielsen, O. B., Lamb, G. D., & Stephenson, D. G. (2004). “Intracellular acidosis enhances the excitability of working muscle.” *Science*, 305(5687), 1144-7

Robergs, R. A., F. Ghiasvand, and D. Parker. "Biochemistry of Exercise-induced Metabolic Acidosis*." AJP: Regulatory, Integrative and Comparative Physiology* 287.3 (2004): n. pag. Web.

Rupawalla, Mustafa, Harpreet Singh, Randeep Johl, Korey Reid, and Molly Mulleague. "Infrared Spectroscopy." *Chemistry LibreTexts*. N.p., 02 Oct. 2013. Web. 19 Aug. 2016

Saugen E, Vollestad NK, Gibson H, et al. “Dissociation between metabolic and contractile responses during intermittent isometric exercise in man.” *Exper Physiol*. 1997;82:213-226

Siegler, J.C., Marshall, P., Pouslen, M.K. et al. *Eur J Appl Physiol* (2015) 115: 565. doi:10.1007/s00421-014-3027-2

Soller, Babs R., R. Donald Hagan, Michael Shear, J. Matthias Walz, Michelle Landry, Dulce Anunciacion, Alan Orquiola, and Stephen O. Heard. "Comparison of Intramuscular and Venous Blood PH, PCO 2 and PO 2 during Rhythmic Handgrip Exercise." *Physiol. Meas. Physiological Measurement* 28.6 (2007): 639-49. Web.

Soller, B. R., Y. Yang, S. M. C. Lee, C. Wilson, and R. D. Hagan. "Noninvasive Determination of Exercise-induced Hydrodgen Ion Threshold through Direct Optical Measurement." *Journal of Applied Physiology* 104.3 (2008): 837-44. Web.

Stackhouse, Scott, Darcy Reisman, and Stuart Binder-Macleod. "Challenging the Role of PH in Skeletal Muscle Fatigue." *Physical Therapy* 81.12 (2001): 1897-903. Web.

Stevens, E. Don. "Effect of PH on Muscle Fatigue in Isolated Frog Sartorius Muscle." *Canadian Journal of Physiology and Pharmacology* 58.5 (1980): 568-70. Web.

Street, D., Bangsbo, J. and Juel, C. (2001), “Interstitial pH in human skeletal muscle during and after dynamic graded exercise” *The Journal of Physiology*, 537: 993–998. doi:10.1111/j.1469-7793.2001.00993.x

Wilson JR, Fink L, Maris J, Ferraro J, Power-Vanwart J, Eleff S, et al. “Evaluation of energy metabolism in skeletal muscle of patients with heart failure with gated phosphorus-31 nuclear magnetic resonance”. *Circulation* 1985; 71:57-62

Westerblad, Hakan, David Allen, and Jan Lannergren. "Muscle Fatigue: Lactic Acid or Inorganic Phosphate the Major Cause?" *Physiology* 21.1 (2002): 17-21. *American Physiological Society*. Web.

Wong R, Lopaschuk G, Zhu G, et al. “Skeletal muscle metabolism in the chronic fatigue syndrome: in vivo assessment by [sup.31]P-nuclear magnetic resonance spectroscopy”. *Chest*. 1992;102:1716-1722