FATIGUE DURING HIGH INTENSITY EXERCISE: THE INTERACTION BETWEEN pH AND THERMAL STRESS

By

Melissa Marie Rogers

Bachelor of Science
California Polytechnic State University, Pomona
Pomona, CA

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Thesis Approved:

Joel B. Mitchell Ph.D.
Major Professor

David E. Upton Ph.D.
Committee Member

Melody D. Phillips Ph.D.
Committee Member

Paulette Burns, Ph.D., Dean
College of Nursing and Health Sciences

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DEDICATION

I would like to dedicate this thesis to my parents who have always supported me and given me the strength to realize that anything is possible with perseverance and commitment.

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CHAPTER ONE
INTRODUCTION

Background

Intense exercise is associated with a high production of lactate (LA) with a concomitant elevation in acidity, whereby the pH may fall ~0.5 pH units within the exercising muscles (6, 16, 63). Decreased pH plays a role in fatigue by inhibiting phosphofructokinase, which plays a key role in glycolysis. In fact, at a pH of 6.4, glycolysis can cease completely (6, 41). Further, evidence from numerous experimental approaches suggests that an elevated muscle hydrogen ion concentration ($[H^+]$) could also depress muscle function by: 1) reducing the transition of the cross-bridge from the low-to the high-force state; 2) inhibiting maximal shortening velocity; 3) inhibiting myofibrillar ATPase; 4) reducing cross-bridge activation by competitively inhibiting calcium (Ca^+) binding to troponin C; and 5) reducing Ca^+ re-uptake by inhibiting the sarcoplasmic ATPase (24, 41).

For much of the 20th century, LA was largely considered a dead-end waste product of glycolysis associated with hypoxia, and it was considered a major cause of muscle fatigue (16, 24). Lactate, as the dissociated form of lactic acid, however, is present in the blood at varying levels during all types of activities depending on the intensity and duration of the exercise. In addition, recent research indicates that LA is a by-product of glycolysis that can be utilized via inter- and intracellular lactate shuttles that enable the conversion of LA to glucose for energy (16). Lactate is either removed by oxidation in the muscle fibers or is released to the blood and removed by other cells according to the cell-cell lactate shuttle, which is facilitated by monocarboxylate transporters (MCT) (34). During submaximal exercise, LA levels remain near resting

values until a threshold is achieved. Lactate threshold (LT) is defined as the work rate at which an increase in blood LA occurs (28). This LT corresponds to a level of exercise intensity at which LA is produced at a rate greater than removal or utilization by the lactate shuttles. Exercise intensities below the LT can be sustained for extended periods of time; whereas, exercise above the LT produces fatigue relatively quickly. Since LA itself is not a metabolically harmful substance it is likely that the increase in $[H^+]$ is at least partially responsible for the fatigue incurred at suprathreshold intensities (16, 41). Since H^+ come from multiple sources, there may not always be a perfect relationship between lactate and $[H^+]$.

Although numerous studies have been conducted to examine the LT, few investigators have assessed pH responses as a function of exercise intensity, nor has the possibility of a threshold in tissue pH (pHT) been explored extensively. In a recent review, Cairns (16) described research indicating that a pHT exists based on the muscle pH and force relationship whereby a critical pH was identified that significantly impacts force production (16). The pHT can be defined as a level of work above which muscle fatigue associated with a decrease in tissue pH occurs (28). Because an abundance of protons $[H^+]$, are derived from the dissociation of lactic acid, a decline in muscle pH and the pHT may coincide with the LT.

Many factors influence the acid-base balance and transport of H^+, including 1) LA/ H^+ co-transporter, 2) bicarbonate (HCO_3^-), and 3) Na^+/ H^+ exchanger. During moderate and high intensity exercise, the LA/ H^+ co-transporter and HCO_3^- are the most critical in regulating $[H^+]$ in the muscle cells. In fact, the H^+ associated with lactic acid dissociation are buffered in the blood to a large extent by the HCO_3^- system (28, 36). As

a result, there is an inverse relationship between blood LA and HCO_3^- during exercise. A contributing factor to buffering capacity is the inherent characteristics of the muscle tissue as indicated by the close correlations between Type II muscle fibers and buffering capacity (38). Putman et al (54) reported that HCO_3^- decreased progressively during incremental cycling before training, and the decrease was attenuated after training. In addition, post-training values were higher in both the arterial and venous compartments during 15-minutes of cycling at 65 and 75% VO_2max. Buffering responses during high intensity exercise are also impacted by training as indicated by the fact that sprint-trained athletes generally have been shown to possess a higher buffering capacity than endurance-trained or sedentary subjects (38).

In response to training, LA production in skeletal muscle is modified such that less LA is formed compared to untrained subjects at a given absolute workload (33). This results in the LA curve being shifted towards the right indicating that blood LA levels are lower for a given submaximal intensity, resulting in greater workloads at LT. Research shows that endurance training increases mitochondrial density, and it induces an increase in skeletal MCT1 expression (53). The MCT1 protein is largely found in oxidative muscles and is a major contributor to intramuscular LA transport (9, 28). Research by Pilegaard et al. (53) and Putman et al. (54), indicates that the training-induced elevation in MCT density was associated with a reduction in muscle lactate during exercise. These findings suggest a possible mechanism for training-induced shifts in the LT since these researchers also reported elevated rates of LA/ H^+ transport in some highly trained athletes.

Training has been shown to also reduce pH displacement during exercise. The enhanced buffering capacity associated with training results in improved performance and resistance to fatigue (38). Improvements in the LA/ H^+ co-transporter systems in response to training are primarily responsible for maintaining pH near resting levels for a longer period of time prior to reaching pHT. This conclusion is supported by animal research involving the LA/ H^+ co-transport system in rat skeletal muscle in which high and moderate-intensity treadmill training for 7 weeks induced a significant increase in the transport capacity, whereas low-intensity swim training had no effect (52).

Exercise in a hot environment is associated with an accelerated onset of fatigue due to heat storage and the accompanying systemic and metabolic changes that occur compared to exercise in a neutral environment. During extreme climatic conditions there is competition for cardiac output, with the need for an increased skin circulation and heat dissipation in addition to the metabolic requirements of the exercising muscle (37). While there are many factors that could lead to early muscle fatigue during exercise in the heat, researchers have yet to completely elucidate the interaction between metabolic and thermoregulatory factors. Glycolytic metabolism is altered during submaximal exercise in the heat such that there is a greater reliance on glycogen during endurance exercise in hot environments compared to room temperature environments (26, 30, 57). Also, LA concentrations are higher, along with an elevated intramuscular temperature, compared with similar exercise in a cooler environment (57). These responses are indicative of a shift toward an increased anaerobic metabolism that would result in a greater H^+ accumulation; thus, the earlier onset of fatigue in the heat may be associated with a greater and more rapid reduction in tissue pH.

Purpose

The primary purpose of this study is to determine the relationship between acid-base factors associated with muscular fatigue and thermal stress relative to high intensity exercise performance in endurance-trained athletes. Specifically, the effect of completing high intensity (80 and 100% of VO_2max) time-to-exhaustion cycle ergometer rides in hot (38^oC) and cool (10^oC) environments on pH, blood bicarbonate, lactate, and core temperatures will be examined. A secondary purpose is to observe the relationship between LT and pHT and relate these responses to high intensity performance.

Hypotheses

1) Time-to-exhaustion will be significantly reduced and lactate levels will be elevated at both exercise intensities performed in the hot compared to cool environment.

2) Blood pH will be similar at exhaustion, regardless of the intensity or condition.

3) Blood HCO_3^- will be lower at both exercise intensities performed in the hot compared to cool environments.

4) Core temperature (Tc) will be higher when exercising in the hot versus cold environments and when performing exercise at 100% versus 80% VO_2max, regardless of the environmental condition.

5) Potassium (K^+) concentrations will be significantly elevated in the 100% versus 80% VO_2max trials.

Project Significance

Few studies have been conducted to assess the interaction between heat-induced fatigue and acid-base balance during exercise in thermally stressful environments. It will be of value to further characterize how thermal stress impacts LA accumulation,

decreases in pH, Tc, and their respective relationships to exercise performance. A greater understanding of these relationships will provide further insight into the metabolic and/or thermal factors associated with fatigue. The findings will be of value for both exercise scientists and practitioners interested in mechanistic and applied performance issues, respectively.

CHAPTER TWO
LITERATURE REVIEW

High Intensity Exercise Metabolism

During short duration, high intensity exercise, muscles rely on the rapid release of energy from adenosine triphosphate (ATP) to accomplish work and maintain homeostasis. A finite amount of ATP is stored in muscle tissues, and additional ATP is rapidly synthesized during exercise. Via a process referred to as hydrolysis, the combination of ATP and water, adenosine diphosphate (ADP), inorganic phosphate, and free energy are released and muscle is able to generate the necessary contractile force. The two primary energy systems that provide the majority of the energy during high intensity exercise are the ATP-Phosphocreatine (ATP-PCr) system, and anaerobic glycolysis. Via a variety of reactions, these two metabolic pathways are responsible for the phosphorylation of ADP back to ATP.

During muscle contraction associated with supramaximal intensity and durations of 10 to 15 seconds, the primary contributor to the energy needs of the muscle is the ATP-PCr system, also known as the immediate energy system. Creatine phosphate (CP or PC) is an additional high energy phosphate compound that is stored within the cell. It is found at concentrations of approximately three times that of ATP and provides bond energy for resynthesis of ATP. Creatine kinase is the enzyme responsible for catalyzing the reaction of PC and ADP in order to produce ATP. Phosphagen availability is limited; thus, fatigue during supramaximal exercise is associated with substantial drops in ATP levels and near depletion of PC.

Once the ATP-PCr system is depleted of creatine phosphate, glycolysis becomes the primary mechanism by which muscles receive energy during supramaximal exercise

lasting up to approximately one minute. Glycolysis is a series of chemical reactions, beginning with glucose-6-phosphate and ending with either the conversion of pyruvate to lactate (anaerobic glycolysis), or the entry of pyruvate into the mitochondria (aerobic glycolysis). Glucose-6-phosphate is derived from blood glucose or stored glycogen. During high intensity exercise, glycogen is the primary source providing substrate to the glycolytic system. As exercise continues beyond approximately one minute, oxidative processes in the mitochondria gradually contribute proportionately greater amounts of energy. As will be explained later in this chapter, glycogen utilization, or the rate of glycolysis, is elevated during exercise in hot environments. Research by Starkie et al (50) confirmed that increased muscle temperature augments net muscle glycogen utilization during submaximal exercise. These findings are also correlated with high lactate concentrations ([LA]); however, most of the research regarding fatigue and environmental conditions has involved submaximal modes of exercise.

Fatigue

Muscular fatigue can be defined as the inability to maintain a given exercise intensity. There are many factors that play a role in the development of fatigue during exercise, including factors that are identified by location (peripheral and central) and factors related to the mechanism of fatigue (neuromuscular and metabolic). The rate at which fatigue occurs for a given exercise intensity is also influenced by the training status of an individual and is often related to the environment in which the activity is performed; the latter issue will be discussed in later sections.

Neuromuscular. Skeletal muscles contract due to the presence of action potentials caused by changes in the polarity within the cell due to increased permeability of sodium (Na^+).

Motor neurons deliver the action potential to the skeletal muscles through saltatory conduction, whereby the impulse travels from one node of Ranvier to another at a high velocity due to the presence of myelinated nerve fibers. The motor neuron divides into many branches at the skeletal muscle site with each final branch leading to a neuromuscular junction for a single muscle fiber. Within the neuromuscular junction, neurotransmitters, such as acetylcholine, are released from the terminal axon into the junction. The binding of acetylcholine to its receptor causes activation of Na+ and potassium (K^+) diffusion at the motor end plate and the continual propagation of the nerve signal to the interior of the cell via the T-tubules. Finally, calcium (Ca^+) is released from the sarcoplasmic reticulum, then binds to troponin to cause initiation of the conformational change between actin and myosin, resulting in muscular contraction (14).

Peripheral fatigue can be defined as the inability of the muscle to respond to neural input. In addition, neuromuscular fatigue can occur due to errors in the signal conduction. Performing high-intensity exercise results in alterations between the Na^+-K^+ pump, leading to reduced membrane excitability and the inability to maintain action potentials. Reduced membrane excitability can be referred to as the inability of the sarcolemma and T-tubule to translate the neural discharge command into repetitive action potentials (56). The ability of the sarcolemma and T-tubules to conduct repetitive action potentials is intimately dependent on active transport of Na^+ and K^+ following an action potential, which is mediated by Na^+-K^+ ATPase (56). During exercise, Na^+ diffuses to the intracellular space while K^+ diffuses outside the cell; however, decreased activity of the Na^+-K^+ ATPase pump during exercise results in higher concentrations of extracellular K^+, causing reduced membrane excitability (56). Therefore, inhibition or reduced

activity of any one of the numerous processes involved in the propagation of an action potential will result in the inability of the muscle to respond to a neural impulse.

An alternative to peripheral fatigue is the idea of central fatigue limiting exercise performance. This hypothesis states that the brain plays a role in motor control and has the ability to vary the amount of work rate and metabolic demands by altering the number of skeletal muscle motor units recruited during exercise. A “central governor” regulates the mass of skeletal muscle recruited during exercise through motorneuron pool recruitment, a consequence of which would be to protect the heart from ischemia during maximal exercise (49).

Metabolic. Fatigue related to metabolic factors is associated with the depletion or accumulation of specific metabolites that play a role in providing energy, or inhibition of the production of energy for muscular contraction during exercise. Depletion of substrates such glycogen and glucose play a causal role in fatigue during prolonged exercise while depletion of phosphagens leads to muscular fatigue during high intensity exercise. On the other hand, metabolic fatigue is also associated with ionic changes during high intensity exercise, such as the accumulation of protons (H^+) within the working cells and an increase in K^+ in the interstitial spaces. These metabolic and ionic changes are most pronounced during high intensity exercise, which is also associated with the rapid development of fatigue (38).

<u>Protons.</u> Proton accumulation leads to a decrease in the cellular pH that ultimately causes a reduction in functional capacity. During exercise, pH can decrease by as much as 0.5 units within the exercise muscles. Decreased pH plays a role in fatigue by

inhibiting enzyme function, including phosphofructokinase, which plays a key role in glycolysis. In fact, at a pH of 6.4, glycolysis can cease completely (6, 41). Further, evidence from numerous experimental approaches suggests that an elevated muscle hydrogen ion concentration ([H+]) could also depress muscle function by: 1) reducing the transition of the cross-bridge from the low-to the high-force state; 2) inhibiting maximal shortening velocity; 3) inhibiting myofibrillar ATPase; 4) reducing cross-bridge activation by competitively inhibiting Ca^{+} binding to troponin C; and 5) reducing calcium re-uptake by inhibiting the sarcoplasmic ATPase (24, 41). Further effects of H^{+} accumulation associated with muscular fatigue will be discussed later on in the section termed "Acidosis".

<u>Potassium.</u> Accumulation of K^{+} in skeletal muscle interstitium during intense exercise has been suggested to cause fatigue in humans. Conversely, a lowering of interstitial K^{+} during exercise has been suggested to be associated with an increased performance (58). The primary determinant of $[K^{+}]$ in the cell is the concentration of nondiffusible anions (62). During high intensity exercise, K^{+} is released from the intracellular to the extracellular space in the contracting muscles and further into the blood stream (40, 43). Potassium is released to the extracellular spaces via voltage-dependent K^{+} channels activated during the propagation of action potentials (43, 58).

Interstitial K^{+} values at exhaustion have been observed between 10-12 mmol/L in human skeletal muscle. For example, Mohr and colleagues (40) observed K^{+} concentrations ~12 mmol/L at exhaustion. Also, venous plasma K^{+} concentrations may reach 7 mmol/L during high-intensity exercise (58). The capacity for K^{+} re-uptake is exceeded during muscle activity, leading to the accumulation of K^{+} in the interstitium

(58). Research suggests that high levels of K^+ accumulation might play a role in muscle fatigue by reducing the excitability of the cell membrane and also decreasing contractility (38, 43).

In addition, metabolic acidosis has been suggested as a possible mechanism for exercise hyperkalemia; however, K^+ elevations occur within the early stages of moderate- to high-intensity exercise without the presence of acidosis. In addition, research evaluating the relationship between LA and K^+ kinetics are contradictory. Some researchers indicate that the two variables are correlated with muscle fatigue while others disagree that any relationship exists due to evidence that the kinetics might be different for LA compared to potassium. For example, results from recent research show that K^+ concentrations return to normal values immediately following the cessation of exercise while LA continues to be released from previously active myocytes for a longer period of time (62). Wasserman and colleagues (62) reported that K^+ concentrations were lower than their resting value in the 1-2 minutes recovery period, after which complete K^+ homeostasis was achieved following 5-minutes of recovery. Also, Nielsen et al (43) observed rapid recovery of K^+ concentrations whereby interstitial K^+ reached a resting level after 4.5 minutes, regardless of training status. These results differed from LA during the recovery period in that LA remained elevated for 15 minutes following exercise at 85% VO_2peak, thus supporting conclusions that K^+ and LA release do not parallel each other.

In contrast, results from other studies indicate that K^+ homeostasis requires 20 min following a 3-to-5-minute exhaustive exercise bout (39). Harmer and colleagues (27) also reported that K^+ did not return to resting levels for 5 to 20 minutes following a

supramaximal cycle test to exhaustion. These results are similar to the recovery kinetics of LA in which the metabolite is released for an extended period of time following exhaustive exercise and can take up to 30 minutes to return to baseline levels.

The Na^+-K^+ pump, and its inability to keep up with intracellular K^+ loss during exercise, is considered the primary mechanism for hyperkalemia. Because the Na^+-K^+ pump is responsible for the maintenance of high intracellular K^+ concentrations and low Na^+ concentrations, the change in membrane potential during muscular contraction and failure of the pump to keep pace with the high loss of K^+, leads to hyperkalemia in the extracellular spaces (62). The K^+ recovery can be explained by the high capacity of the Na^+-K^+ pump, which starts to normalize interstitial K^+ as soon as the contraction-induced release of K^+ stops (58).

Another possible mechanism for hyperkalemia is suggested by the linkage between PC hydrolysis and K^+ release during exercise. As previously mentioned, the major source of high-energy phosphate at the beginning of exercise is derived from the hydrolysis of PC. As PC splits into creatine and inorganic phosphate, there is a rapid reduction in the nondiffusible anions in the contracting muscle cells because PC is a highly dissociated acid at the cell pH. When it hydrolyzes, it is converted into a neutral molecule, creatine, and dibasic phosphate. This reaction results in alkalinization of the cell prior to the acidifying effects of carbon dioxide (CO_2) accumulation and lactic acidosis during high intensity exercise. Following this initial phase, excess intracellular K^+ diffuses to the extracellular spaces while protons move across the cell membrane into the intracellular spaces. The final step in the PC hypothesis involves the accumulation of

intracellular H^+ due to an increase in the dissociation of carbonic acid and donation of H^+ to the cell (62).

All of these reactions occur within the first 30 seconds of exercise and correspond to a concomitant increase in bicarbonate (HCO_3^-) and cellular pH. The hydrolysis of PC is a reaction that is virtually complete during the period of increasing oxygen consumption, within the first 3 minutes of exercise, and the decrease in PC is sustained during the entire course of exercise to a level depending on the work intensity (62). Bendahan and colleagues (7) reported that during the initial seconds of exercise there was a decrease in PC that was linearly associated with a pH increase from rest to the end of exercise when performing a maximal bout of finger flexion exercise over an18 second duration (8). During the 3-minute recovery period, pH was reduced as a result of PCr resynthesis, supporting the buffering role of PC at the beginning of exercise. Finally, there are two main similarities between K^+ and PC that support the PC hydrolysis hypothesis for exercise hyperkalemia: 1) K^+ remains elevated throughout exercise while PC continually drops depending on exercise workload and 2) intracellular K^+ is high during the initial alkalinizing period.

Training response. Extracellular K^+ increases in response to high intensity exercise are attenuated following a period of training (38). Nielsen and colleagues (43) observed the same response after 7 weeks of intense one-legged knee-extensor training. Interstitial K^+ concentrations increased more rapidly and were higher throughout exercise at 30 W in the control leg (CL) compared to the trained leg (TL). Furthermore, during a 60 and 70 W incremental exercise test, interstitial [K^+] were higher in the CL than the TL; whereas, no difference existed at the point of fatigue. McKenna and colleagues (38)

also showed post-training attenuations of K^+ in response to maximal exercise. Potassium was analyzed at rest, during the final 10 seconds of exercise, and in the recovery period. Prior to training, subjects started with a value of 4.4 mmol/L (venous blood) and increased to 8.2 following maximal exercise. In the recovery period, K^+ actually decreased below the resting value, an indication that cessation of exercise causes marked increases in the transport of K^+ across the cell membrane. In addition, the recovery of K^+ occurred within 2-5 minutes following exercise. Finally, research suggests that these training adaptations take place without any changes in the Na^+-K^+ pump (62).

Lactate. Lactate is a metabolic by-product of glycolysis produced at varying levels depending on the intensity and duration of an activity. It has long been thought that LA accumulation was a causal factor in muscle fatigue. Early studies involving LA resulted in the assumption that a point exists during exercise in which oxygen consumption fails to meet the metabolic demands of the body, leading to an increased reliance on anaerobic glycolysis and the accumulation of LA (41). These early research studies observed the accumulation of LA with increasing exercise intensity and that LA was highest at or following exhaustion (49). However, these studies failed to provide direct evidence of the cause-and-effect relationship between [LA] and muscle fatigue (41). Rather, early studies base these assumptions on correlations, such as that previously mentioned. Furthermore, a current study by Nielsen and colleagues (44) revealed that infusion of 20mM lactic acid failed to demonstrate any inhibitory effect on muscle contractility. In addition, recent studies have observed the presence of LA in resting cells, which have lead to the questioning of LA and its role in muscle fatigue (24). Recent research reveals that LA is a metabolic substrate that can be utilized by various tissues within the body,

such as skeletal and cardiac muscle, liver, and kidney; in fact, LA is the preferred fuel for cardiac and skeletal muscle tissues (13, 24). In addition, recent studies conclude that LA is produced during fully aerobic conditions (13, 41). Finally, [LA] within cells and blood are subject to a ratio of production and removal of this metabolite. Lactate can be oxidized by two mechanisms according to the lactate shuttle hypothesis: 1) intracellular lactate shuttle and 2) intercellular lactate shuttle.

Lactate Threshold. During submaximal exercise, [LA] remain near resting values because the LA that is produced is being removed at a rate equal to the rate of production. As the intensity of exercise increases, however, LA begins to accumulate due to the inability of the shuttles to remove LA at a rate equal to the rate of production. Lactate threshold (LT) is defined as the work rate at which an increase in blood LA occurs (28). The LT corresponds to a level of exercise intensity at which LA is produced at a rate greater than removal or utilization by the LA shuttles. Researchers and coaches often observe the relationship between LA and workload in endurance athletes. Theoretically, athletes with a LT corresponding to a greater percentage of their maximal oxygen uptake may exercise at a higher work rate without accumulation of blood LA compared to those with a lower LT (9).

Determination of Lactate Threshold. Even though numerous studies have evaluated LT, there is not a clear methodology for the determination of the LT. For example, many studies define the LT as an increase in lactate of 1 mmol/L concentration with an increase in workload (21); however, the measured value in the blood is a reflection of the metabolic stress from the previous stage, and not the workload at which

the increase in LA occurred. Baldari et al examined maximal LA steady state (MLSS), or the maximal workload that can be maintained for a prolonged period of time without an accumulation of lactate (17), using two different individual anaerobic threshold (IAT) workload values. The IAT is defined as the metabolic rate where the elimination of LA from the blood is maximal and equal to the rate of diffusion of LA from exercising muscle to blood so that higher exercise intensities lead to progressively higher LA values (4). The two IAT measurements included: 1) measured IAT (IAT_m) and 2) antecedent IAT (IAT_a). The IAT_m value corresponded to the workload related to the stage at which LA accumulation occurred while the IAT_a value was associated with the workload of the stage prior to LA accumulation. Following the preliminary test to determine IAT workloads, subjects were required to maintain a 30-min treadmill run at the given workloads while LA was measured every 5 minutes to evaluate the response at the various intensities. The results indicated that exercising at the workload corresponding to IAT_m lead to a significant increase in LA accumulation compared to the IAT_a, which proved to elicit a workload at which a MLSS could be maintained. Therefore, based on these results, the LT should be analyzed in such a way that the metabolic stress causing the inflection in the LA curve be utilized to express the workload at LT.

Lactate Shuttle Hypothesis. Lactate produced in the muscles during exercise is transported across the muscle cell membrane by diffusion and more importantly by facilitated transport (22). Recent research indicates that LA is a by-product of glycolysis that can be utilized via inter- and intracellular LA shuttles that enable the conversion of LA to glucose for energy (15). Lactate is either removed by oxidation in the muscle fibers or is released to the blood and removed by other cells according to the cell-cell LA

shuttle, which is facilitated by monocarboxylate transporters (MCT) (13, 34, 60). The process of transporting LA within the cell requires the presence of lactate dehydrogenase (LDH) within the mitochondria of the oxidizing cell, along with MCT (13, 20). Brooks et al (13) observed that LA and pyruvate oxidation was blocked with the inhibition of muscle MCT transporters, while inhibition of oxamate, an LDH inhibitor, blocked the oxidation of LA, but not pyruvate.

There are two predominate LA transporters within human skeletal muscles, MCT1 and MCT4 (20, 24, 49). It has been proposed that MCT1 plays a major role in the intracellular and intercellular LA shuttles because it occupies both the sarcolemmal and mitochondrial domains, whereas MCT4 is primarily involved in the intercellular LA shuttle because it occupies the sarcolemmal domain (13, 20). In addition, MCT1 is found mainly in slow-twitch fibers while MCT4 is found mainly in fast-twitch fibers (13, 22, 60). Finally, there is an inverse relationship between the percentage of slow-twitch muscle fibers and the LA gradient. This relationship is primarily due to the high expression of MCT1 in slow-twitch fibers that allows for greater intracellular oxidation of LA.

The "intracellular lactate shuttle" refers to the ability of cytosolic LA to be transported to the mitochondria by MCT, where it is oxidized and utilized as a source of energy. Isolated heart, skeletal muscle, and liver mitochondria were shown to oxidize LA directly (13). In skeletal muscles there is a high correlation between MCT1 and 1) LA uptake, 2) heart-type LDH content, and 3) citrate synthase activity (3). This indicates that MCT1 is a primary transporter of LA across the sarcolemmal membrane. By facilitating intramuscular LA exchange and oxidation, MCT1 depresses net muscle LA

release (20). As will be explained later in this section, MCT1 expression is elevated in response to training, which allows for greater oxidation of LA and a decrease in LA release. This results in a negative correlation between MCT1 content and net LA release during exercise (20, 60).

As mentioned previously, the "intercellular lactate shuttle" refers to the ability of LA to be released into the blood and consumed by other cells for oxidation. This hypothesis proposed that LA was able to transfer from its site of production to neighboring cells and a variety of organs (e.g. liver, kidney, and heart), where its oxidation or continued metabolism could occur (49) The cell-to-cell LA shuttle hypothesis is supported by demonstration of LA exchange between producing and consuming cells and tissues (13). For example, Brooks et al (15) showed that arterial [LA] are markedly elevated early during exercise, but after a few minutes, net LA release declines even though arterial LA concentrations remain elevation. Therefore, exercising muscle cells are producing LA, which is released into the circulation and consumed by neighboring muscle cells where it is utilized as a metabolic substrate. According to this hypothesis, LA is more a metabolic intermediate than an end product of glycolysis (20).

<u>Chronic vs. Acute Changes in Response to Exercise.</u> In response to training, LA production in skeletal muscle is modified such that less LA is formed compared to untrained subjects at a given absolute workload (20, 33). This decline in blood [LA] after a period of training can be observed even in highly trained athletes, and in the absence of any changes in VO_2max (16). In addition to reduced production of LA, endurance training results in an enhanced ability to clear LA from the exercising cells at low but not high power outputs (15, 20, 49, 60).

High capacities for LA clearance in trained subjects resulting in lower muscle and blood LA accumulation despite higher rate of work are attributable to higher mitochondrial densities and enhancement of the intracellular LA shuttle due to greater expression of MCT1 (15). For example, research by Juel et al (34), Pilegaard et al. (53), Putman et al. (54), and Thomas et al (61) indicates that the training-induced elevation in MCT expression was associated with a reduction in muscle LA during exercise. Juel et al (34) showed that 7-8 weeks of training lead to greater MCT1 content while there was a tendency for MCT4 to increase, although it did not reach a level of significance. In addition, Pilegaard and colleagues observed a 70% elevation in MCT1 concentrations in the trained leg compared to the untrained leg. Similarly, Baker and colleagues (3) further support findings from the previous studies, in which the researchers observed that increased LA uptake was found when MCT1 increased. In a moderately trained group, however, no changes were observed in MCT1 content in the soleus, red and white gastrocnemius, and extensor digitorum longus, while higher intensity training led to 70 and 94% increase in the soleus and red gastrocnemius, respectively. Furthermore, Everston and colleagues concluded that MCT1 expression was unchanged following a period of high intensity training, while the protein expression was reduced in the moderately trained group. In addition, MCT4 expression remained unchanged in both groups following the training period. Finally, the high intensity training was more effective in raising the running speed at LT and in improving the running performance compared to the moderate training group. This most likely occurred due to the relationship between LA and protein transport expression in which the [LA] following exhaustive treadmill running positively correlated with MCT1 concentration.

Few researchers have examined the influence of acute exercise on protein transport expression; however, Bishop and colleagues (11) showed that MCT1 and MCT4 expressions were significantly decreased following a bout of high-intensity exercise, (24 and 26%, respectively). Others have observed the opposite results when examining the acute effects of exercise on protein expression following prolonged exercise (11). The few studies conducted thus far indicate that MCT expression is elevated following acute exercise.

Finally, these findings suggest a possible mechanism for training-induced shifts in the LT since these researchers also reported elevated rates of LA-H^+ co-transport in some highly trained athletes. Elevations in MCT1 expression tend to improve LA transport and clearance; thus leading to enhanced performance by improving the workload accomplished at LT. For example, data by Everson and colleagues (22) supported previous findings that oxidative fibers, trained muscle fibers, and chronically stimulated muscle fibers have more MCTs and can transport more LA than less oxidative fibers, untrained, and unstimulated control fibers.

Acidosis

Lactic acidosis is a common term to describe the marked decrease in pH during exercise in which the production of lactic acid causes the release of a H^+, leaving the final product to be the acid salt LA (55). Lactic acidosis as a result of exercise has long been associated with and termed a causal factor of muscular fatigue. As mentioned previously, however, LA is present during all types of activity and is unlikely to be the causal factor in fatigue. It is most likely the accumulation of H^+ from lactic acid and other sources that leads to decreased muscular pH, resulting in the inability to maintain force production.

An additional key concept is that the cause of metabolic acidosis is not merely proton release, but an imbalance between the rate of H^+ release and the rate of H^+ buffering and removal (55).

Intense muscular contraction is associated with high rates of ATP hydrolysis and a resulting increase in ADP, inorganic phosphate, and H^+ with a concomitant decrease in cellular pH (35). In fact, high intensity exercise can reduce blood and cellular pH by as much as 0.5 units, from 7.4 to 6.9, due to the accumulation of H^+ (16, 63). In a recent review by Cairns (16), plasma pH can fall from ~7.4 to 6.9-7.0; however, an acidosis of 0.1-0.2 pH is much more common. The decreases in pH correlate with changes in muscle force such that low pH decreases fiber force and slows the muscle-shortening velocity in a fiber-type-dependent manner, implicating pH as a causative in fatigue (35). Therefore, removal of H^+ from skeletal muscle seems to be important to maintain force production (61).

Buffering. Many factors influence the acid-base balance and transport of H^+, including 1) LA-H^+ co-transporter, 2) HCO_3^-, 3) CP hydrolysis, 4) amino acids, 5) proteins, and 6) Na^+- H^+ exchanger (32, 55). All of these buffering systems either bind or consume H^+ in order to prevent intracellular acidosis. When the rate of H^+ production exceeds the capacity of the numerous buffering systems, however, metabolic acidosis will occur within the exercising skeletal muscle.

During moderate and high intensity exercise, the LA-H^+ co-transporter and the HCO_3^- buffering system are the most critical in regulating [H^+] in the muscle cells. In fact, H^+ associated with lactic acid dissociation is buffered in the blood to a large extent by the HCO_3^- system leading to the nonmetabolic production of CO_2 (28, 36). As a

result, there is an inverse relationship between blood LA and HCO_3^- during exercise (58). For example, McKenna et al (38) showed that [HCO_3^-] decreased while LA increased during maximal cycling exercise, and bicarbonate and LA continued to decrease or increase, respectively, throughout a 5-minute recovery period.

The LA coupled H^+ production is the largest single contributor to the intramuscular pH change during intense muscle activity in which a close correlation has been reported between LA accumulation and intramuscular pH associated with high intensity exercise (33). The LA-H^+ co-transports H^+ and LA ions in a 1:1 manner in which the transport rate is influenced by the gradients for both substrates (32). The LA-H^+ co-transporter system is mainly involved during intense exercise because of a required high LA gradient in order to transport H^+ across the sarcolemma (32, 33). Westerblad and Allen (63) observed that inhibition of LA transport resulted in a more pronounced acidification of the muscle tissue and accelerated the development of fatigue in isolated mouse skeletal muscle, whereas inhibition of the other membrane transporters had no effect on fatigue.

A contributing factor to buffering capacity is the inherent characteristics of the muscle tissue as indicated by the close correlations between Type II muscle fibers and buffering capacity (35). Juel (31) observed that the total H^+ transport capacity is higher in slow-twitch than fast-twitch fibers, indicating a more efficient pH regulation in the oxidative fibers. The main reason for this is that the LA-H^+ co-transport capacity in slow-twitch fibers is ~50% higher than that in fast-twitch fibers.

Buffering due to H^+ transport capacity is elevated in response to moderate-to-high intensity training (32) and is greater in trained compared to untrained subjects (51). The

results of studies examining the transport capacity in rat skeletal muscles show that transport capacity in enhanced following high intensity training, whereas no change is observed in response to low intensity training (51). In addition, it seems that the improvement in transport capacity is more pronounced at low than at high [LA], suggesting that a decrease in K_m is also involved. Pilegaard et al (51) showed that transport capacity was greater in trained subjects in which this enhancement was most likely due to a greater percentage of Type I muscle fibers. Results from this study showed that the LA transport capacity was correlated with the relative occurrence of Type I muscle fibers. Also, improvement in transport capacity was negatively correlated with the fatigue index.

The HCO_3^- system is also responsive to training as indicated by the fact that Putman et al (53) reported that HCO_3^- decreased progressively during incremental cycling before training, and the decrease was attenuated after training. In addition, post-training values were higher in both the arterial and venous compartments during 15-minutes of cycling at 65 and 75% VO2max. Buffering responses during high intensity exercise are also impacted by training as indicated by the fact that sprint-trained athletes have generally been shown to possess a higher buffering capacity than endurance-trained or sedentary subjects (11, 38). In contrast, however, McKenna and colleagues (38) failed to observe a significant difference in [HCO_3^-] following a period of sprint training. In fact, HCO_3^- was slightly lower in response to exercise and during the recovery period in response to training.

pH Threshold. Although numerous studies have been conducted to examine the LT, few investigators have assessed pH responses as a function of exercise intensity, nor has the

possibility of a threshold in tissue pH (pHT) been explored extensively. In a recent review, Cairns (16) described research indicating that a pHT exists based on the muscle pH and force relationship whereby a critical pH was identified that significantly impacts force production (16). The pHT can be defined as a level of work above which muscle fatigue associated with a decrease in tissue pH occurs (28). Because an abundance of H^+ are derived from the dissociation of lactic acid, a decline in muscle pH and the pHT may coincide with the LT. There is conflicting evidence as to which threshold occurs first. Systrom et al. (59) studied pHT, LT, and ventilatory threshold (VT) during plantar flexion, and reported thresholds corresponding to 66.4, 78.6, and 75.0% of maximal oxygen uptake, respectively. On the other hand, Iwanaga et al. (28) observed that the work rate at pHT was significantly larger than that at LT. These results suggest a lack of association between the two thresholds such that intracellular pH can be kept near resting levels, even though blood LA accumulation had already occurred. Finally, based on the findings from the present study, there appears to be a HCO_3^- threshold (BCT) in which a reduction of [HCO_3^-] will result in the inability to maintain pH; thus, likely playing a role in muscular fatigue. This threshold is similar to that of the pHT in which previous research regarding the concept has yet to be examined and a valid method for determining this threshold has yet to be defined.

Thermoregulation

Normal core temperature (Tc) in humans ranges from 36.5-37.5° C at rest, and is regulated by controlling the rate of heat gain and loss. Heat loss is largely dependent upon the temperature gradient between the skin and the external environment; it is also dependent on the amount of cooling provided by the heat exchange processes: radiation,

evaporation, conduction, and convection (37). The hypothalamus increases the rate of heat production when Tc falls and increases the rate of heat loss when Tc increases. During exercise, the rise in Tc is related to the exercise intensity and external environment. For example, there is a linear relationship between workload and Tc when the thermal environment is held constant; and, a proportionate increase in Tc with increases in environmental temperature at a given workload (14). Although there is a substantial amount of literature on thermoregulation during exercise, most of the research pertaining to heat gain during exercise in varying environments has been conducted with prolonged, submaximal trials. Few studies have examined the effect of exercise in a hot environment and the consequent thermoregulation during supramaximal exercise. Some researchers suggest that brief bouts of maximal exercise may not be affected by heat stress to the same extent as prolonged, continuous physical activity (37). On the other hand, there is evidence that high intensity exercise in a hot environment can produce rates of heat gain of 0.15-0.20 ^{o}C /min. (39). Such rates of increase, when carried out over even a few minutes of exercise, could lead to a very rapid onset of hyperthermia. Hyperthermia associated with high intensity exercise may impact energetics and other aspects of muscle function in ways that are different from those that have been observed in lower intensity, long duration exercise.

As a result of the thermal strain encountered during exercise in a hot environment, fatigue during prolonged exercise occurs earlier at higher environmental temperatures (23). Exercising in hot environment requires more blood flow to be directed toward the skin to aid in the dissipation of heat with a concomitant reduction in blood flow available for the working tissues (37). This often results in the blood supply not meeting the

metabolic demands which leads to a greater reliance on energy being derived from anaerobic glycolytic metabolism. Numerous researchers have reported an increased rate of glycogen utilization during exercise in the heat compared to that in a neutral environment (23, 48, 57). Some investigators have indicated that [LA] are higher following exercise in the heat (21, 48) compared to those in either cold or neutral environment while others have observed no difference in this metabolite due to environmental stress (37, 47). The increase in glycogen use would provide a logical explanation for increased LA levels; however, there are a variety of other possible causes that center around the balance between the production and removal of LA that results in varied accumulation rates. Impairment of the LA transporters as a result of thermal stress, as suggested by Oyono-Enguelle et al could lead to decreased LA removal (47). In addition, decreased blood flow to the exercising muscles during heat exercise has been previously observed. Central blood volume may decrease and cause a decrease in cardiac filling pressure. The result is increased heart rate in an attempt to compensate for the fall in stroke volume. At high intensity exercise, however, increased heart rate cannot offset the deficit, so fatigue occurs at an accelerated rate. Research indicates that increased blood flow to the skin is a primary factor for Tc regulation that results is less blood being distributed to the muscles, causing reductions in stroke volume and elevations in heart rate that might ultimately lead to reduced cardiac output. For example, Gonzalez-Alfonso et al (25) observed the influence of submaximal heat exercise, while wearing a water temperature perfused jacket to control for rates of heat gain, on skin blood flow. The authors reported that cardiac output was reduced in proportion to the rate of heat gain while heart rate was elevated in proportion to the rate of heat gain. In addition,

arteriovenous mixed O_2 difference was significantly higher in when comparing the greatest rate of heat gain compared to the lowest rate of heat gain and the control, indicating that more blood was being distributed to the periphery and less was available to the working muscles. Finally, changes in plasma volume also influence the amount of blood available for circulation. During exercise, plasma volume decreases due to increased plasma filtration in response to elevated blood pressure (13). In addition to affecting blood volume, changes in plasma volume result in alterations of the many metabolic and ionic substances within the blood during exercise. Hemoconcentration during exercise causes marked changes in metabolites that must be taken into consideration.

Parkin et al. (48) examined the influence of ambient temperature on human skeletal muscle metabolism during submaximal exercise and showed that [LA] were significantly greater in the hot trials compared to the cold trials. Starkie et al (57) took the question one step further by testing the relationship between muscle temperature and glycogen utilization. Using a water perfused cuff model, in which a temperature regulated cuff (hot = 50-55 ^{o}C and cold = 0 ^{o}C)) was wrapped around each limb over the vastus lateralis, muscle temperature was significantly greater in the hot leg compared to cold leg trials. Their findings led to the conclusion that increased muscle temperature was associated with elevated glycogen utilization during the hot trials (208 compared to 118 mmol/kg for hot and cold, respectively. The results of this study, however, failed to show the expected difference in post-exercise [LA] between the two trials. The lack of difference in the LA results is supported by the research by Maxwell et al (37) who compared exercise trials in hot and cold environments while varying the temperature

utilized during a warm-up period. They manipulated the warm-up and exercise temperatures leading to three trials: 1) cold/cold (CC), 2) cold/hot, and 3) hot/hot, where the first environment is that of the warm-up and the second represents the experimental exercise environment. The experimental protocol included a 20-min exercise/stretching warm-up followed by repetitions of 20-s sprints, with 100-s recovery, at increasing speed until volitional exhaustion. Performance was significantly higher in the CC trial than in the CH and HH trials. Rectal and mean skin temperatures were also significantly higher in the hot experimental trials compared to the cold only trial. In addition, performance was shown to be significantly related to LA values after the sprints, even though there were no significant differences in LA between conditions.

Drust et al (19) examined the effects of 40-minutes intermittent cycle exercise (15s intervals with 15s unloaded exercise) at an intensity eliciting 60% VO_2max prior to performing five 15s maximal sprints separated by 15s in hyperthermic and neutral environments on thermoregulation and the associated metabolic responses, such as LA, K^+. At 38 minutes, LA and K^+, concentrations had increased significantly from resting values, but there were no significant differences between hyperthermic and control groups., The post-repeated sprint LA levels were significantly greater than the previous sampling period, but were similar between groups at this time point. On the other hand, the $[K^+]$ were slightly higher in the control group following repeated sprints; however, this was a trend that failed to reach significance. Power output during the hyperthermia trial was significantly reduced while muscle temperature was elevated compared to the control trial. Therefore, the authors concluded that fatigue was unrelated to metabolic factors and most likely attributed to elevations in muscle temperature.

There is evidence in the literature that there is a critical Tc and/or muscle temperature at which fatigue occurs. This would suggest that regardless of the environment, the exercise intensity and duration, or other contributing factors, individuals will experience fatigue at the same critical temperature. The majority of the work in this area has focused on Tc as the critical temperature of interest. Drust et al (19) reported that, despite beginning exercise at different Tc, exhaustion occurred at the same Tc and muscle temperature for all trials. In addition, time to exhaustion was significantly shorter during the higher than during the lower rate of heat storage, which also parallels results from the current study. Nybo and colleagues (46) observed that hyperthermia was not associated with impaired muscular function performance; rather, impaired central activation accounted for the reduction in maximal voluntary force development during prolonged isometric contractions. Gonzalez-Alonso et al (25) also examined the critical Tc concept by studying the rate of increase in body temperature on heat exhaustion during prolonged exercise by having trained subjects wear a water perfused jacket. Subjects exercised in a hot environment at a workload equivalent to ~66% VO_2max until volitional exhaustion. In addition, time to exhaustion was significantly shorter during the higher (H) than during the lower (C) rate of heat storage in which TTE occurred at 28-minutes for H and 63-minutes for C. Therefore, the authors concluded that fatigue occurred at a similar Tc value and was inversely related with rate of heat gain.

Conclusions Regarding the Interaction Between High Intensity Exercise and Thermal Stress on Muscular Fatigue

Based on the previous literature regarding thermal stress and exercise, it is apparent that elevations in core temperature play a role in muscular fatigue and is supported by

evidence that performance is reduced when exercising in hot environments. Exercising in hot environments, especially during submaximal exercise, results in marked changes in core temperature that result in the redistribution of blood flow towards the periphery, leading to less blood availability to the working muscles and the inability to meet the metabolic demand at high intensity exercise. In addition, research indicates that exercising in a hot environment might enhance performance to a certain extent due to minimal elevations in muscle temperature that result in elevated force production. Finally, some researchers suggest that muscular fatigue occurs at a specific core temperature.

CHAPTER THREE
METHODS

Subjects

Fourteen endurance trained athletes aged 21-40 years were recruited from cycling and triathlon groups in the Fort Worth and TCU communities via informational flyers, e-mails, and word of mouth. Inclusion criteria included: 1) endurance trained athletes (>100 miles/week cycling) currently competing in local/national road races or triathlons; and 2) a maximal aerobic capacity (VO_2max) greater than 3.5 $L*min^{-1}$ (Table 1). Subjects completed a university-approved informed consent and a medical history questionnaire. The medical history questionnaire included basic background information regarding conditions that are contradictions to strenuous exercise. Eleven of the 14 subjects completed all experimental testing.

TABLE 1: Subject Characteristics

	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	% Body Fat	VO_2max (L/min)	W_{max} (Watts)
Mean±SD	32.56± 4.89	185.43± 7.67	82.51± 8.75	13.79± 3.63	4.23± 0.49	340.33± 33.74

Table 1: Subject characteristics. Maximal oxygen consumption and workload were assessed during the preliminary graded exercise test on a cycle ergometer. Values represent mean ± SD (n=14).

Experimental Design

This experiment was conducted using a repeated measures, three factor, and condition by environment by time design. Subjects completed four experimental trials requiring that each subject complete a time-to-exhaustion ride at: 1) 80% VO_2max in a

cold environment (C80); 2) 80% VO_2max in a hot environment (H80); 3) 100% VO_2max in a cold environment (C100); and 4) 100% VO_2max in a hot environment (H100), in a randomized, counterbalanced order. The temperatures for the cold and hot environments were 10.17 and 36.61 ^{o} C with relative humidities of 25.85 and 57.05%, respectively. During each time-to-exhaustion cycle ergometer ride, blood samples, core temperature (Tc) responses, and cardiorespiratory measurements were taken at regular intervals.

<u>Preliminary Testing.</u> Subjects entered the Exercise Physiology Laboratory on the preliminary test day and completed all documents (medical history questionnaire and informed consent) along with preliminary anthropometric measurements. The same day, those meeting the inclusion criteria completed an incremental exercise cycle ergometer test to assess aerobic capacity (maximal oxygen consumption- VO_2max) and to determine the lactate threshold (LT), pH threshold (pHT), and bicarbonate ($HCO3^-$) threshold (BCT) for each subject.

Anthropometric Measurements. Height and weight were measured for all subjects. In addition, to assess percent body fat, a 7-site skinfold test was used with measurements taken from the chest, midaxillary, triceps, subscapular, abdominal, suprailiac, and thigh regions. The average of three attempts was recorded for each region.

VO_2max test. All exercise tests were performed on a Monark cycle ergometer (Ergometer 894E, Monark Exercise AB, Vansbro, Sweden) that was equipped with a digital display that allowed for the monitoring of cycle cadence. Subjects wore a Polar heart rate monitor (Polar Electro E600, Polar Electro Inc, Lake Success, New York) around their chest and were fitted with a mouthpiece in order to collect and analyze

respiratory gas exchange. The first four stages of the test were three minutes in duration, beginning at an intensity of ~85 W and increasing 45 W per stage. The stages subsequent to the first four were 2-minutes in duration with 45 W increases in load that were continued until voluntary termination. Oxygen consumption was measured continuously via a computer-based on-line gas analysis system (TrueOne 2400 Metabolic, Parvo Medics, Inc, Sandy, Utah) while subjects breathed via a mouthpiece and one-way valve (2700 Series, Hans Rudolph, Inc, Kansas City, Missouri). Heart rate was also monitored continuously via a sensor that fed the heart rate signal to the gas analysis system. A valid VO_2max was determined by achieving two or more of the following criteria: 1) Heart rate equivalent to 220-age; 2) RER > 1.1; 3) a plateau in VO_2, or 4) a blood lactate greater than 8.0 mM. Each subject was allowed to determine their cycling cadence that was most comfortable, within a range of 85-95 RPM. This cadence was recorded and then utilized for all subsequent trials in order to control for workload.

Lactate, pH, and Bicarbonate Thresholds Prior to the VO_2max test, a flexible catheter was inserted into an antecubital vein and maintained patent with a flush of sterile physiological saline. Blood samples were collected prior to exercise, at the end of each stage, and following a 3-minute active recovery period. Samples were analyzed for lactate, pH, and $HCO3^-$ to determine an individual lactate threshold (LT), pH threshold (pHT), and bicarbonate threshold (BCT) for each subject. The LT corresponded to the workload that elicited an increase in 1 mmol/L from the previous stage and/or a level greater than 4 mmol/L. Based on the concept that further increases in workload will result in muscle acidosis, the pHT and BCT were determined on an individual basis by observing the deflection and inflection points on a graph, respectively. The LT, pHT, and

BCT were expressed in both absolute terms and as a percentage of VO_2max. Using the data from the VO_2max test, individual prediction equations were generated for each subject based on the relationship between work load and VO_2. These equations were used to determine the work loads for the warm-up rides and the experimental trials. All prediction equations had an R^2 of at least 0.98.

Learning Trial. A minimum of two days following the preliminary test day, subjects reported to the laboratory to undergo a learning trial. This trial matched that of the H80 experimental trial and was conducted in the same manner, absent of any temperature or blood analysis. After a five minute warm-up, subjects entered the environmental chamber and were fitted with a mouthpiece and heart rate monitor. A four minute period elapsed prior to beginning the time-to-exhaustion trial. During the trial, subjects were encouraged to continue cycling at their given cadence until volitional exhaustion and/or a reduction of 10 RPM over a 20 second time period. Heart rate, oxygen consumption, and time-to-exhaustion were recorded. The primary purpose of this trial was to acclimate the subjects to the type of trial and effort that they were expected to give on the experimental test days.

Experimental Testing

Subjects reported to the laboratory in a fasted state at least 3 days following the learning day. After resting in a supine position for 15 minutes, a catheter was inserted in an antecubital vein and a baseline blood sample was collected. Subjects wore a Polar heart rate monitor around their chests, and were fitted with a mouthpiece for respiratory gas analysis during the experimental trials. An esophageal thermistor connected to a digital telethermometer (Model 8502-12, Cole-Parmer Instrument Company, Vernon

Hills, Illinois) was also inserted through the nasal passage to a depth corresponding to 25% of the subject's height. After baseline temperatures had been recorded, the subjects were given a 5-minute warm-up at room temperature on a Monark cycle ergometer at an intensity corresponding to 60 percent of their VO_2max. Following the warm-up, subjects entered the environmental chamber where there was a 4-minute time delay prior to beginning each trial. Subjects were allowed to get there cadence to their optimal level without any resistance in a 10-second period before beginning the time-to-exhaustion ride. Subjects were required to maintain the given workload until exhaustion, which was defined as a drop in the required cycling cadence by 10 RPM for more than 20 sec. During each trial, respiratory gas data and heart rate was monitored continuously using the equipment described previously in the preliminary testing section.

Blood Sampling and Analysis. Blood samples were obtained before exercise (after 15 min of supine rest), every 5 minutes during exercise, and at 3 minutes of recovery for analysis of pH, lactate, bicarbonate, hematocrit, and hemoglobin. Following each blood sample, the catheter was rinsed with sterile physiological saline to prevent clotting of the catheter. Lactate was assayed using an enzymatic, spectrophotometric assay. Briefly, 0.5 ml whole blood was pipetted into 8% perchloric acid and centrifuged for 10-minutes at 23˚C. The supernatant was stored in -80˚C for later analysis. The enzymatic assay requires the mixing of 25 μL supernatant with 1 mL of reagent cocktail containing a glycine-hydrazine buffer, lactate dehydrogenase, and NAD^+. The mixture is incubated for 45-min to complete the reaction based on the conversion of lactate to pyruvate with the simultaneous mole-to-mole conversion of NAD^+ to NADH, with the latter read at 340 nm against a reagent cocktail blank. The molar extinction coefficient of NADH is then

used to derive lactate concentrations. All samples were measured in triplicate. Blood pH and bicarbonate levels were determined using a blood gas and electrolyte analyzer (ABL 77 Series, Radiometer, Copenhagen, Denmark). On this system, bicarbonate is a derived value based on the following equation: $cHCO_3^-(P) = 0.23 \times pCO_2 \times 10^{(pH-pKp)}$ mmol/L, where $pK_p = 6.125 - \log[1+10^{(pH-8.7)}]$ and pCO_2 is expressed in kPa. The derived value, $cHCO_3^-(P)$ includes ions of hydrogen carbonate, carbonate and carbamate in the plasma. Hematocrit and hemoglobin were measured using the microcapillary tube and cyanometthemoglobin methods, respectively. These data were used to calculate the percent change in plasma volume according to the equation of Dill and Costill (18). Lactate, bicarbonate, and potassium concentrations were corrected for exercise-induced plasma volume shifts.

Statistical Analysis.

Data from the experimental trials were analyzed using either a two-factor or a three-factor repeated measures analysis of variance (ANOVA). The first factor was "intensity" and had two levels: 80 and 100% of VO_2max; the second factor was "environment" and also had two levels: hot and cold; and for those variables that were sampled multiple times within a condition, the third factor was "time" with a variable number of levels depending on the sampling frequency. A Newman Keuls post hoc analysis was used to isolate the location of differences detected by the ANOVA. Using a correlation matrix, Pearson correlations were conducted between all dependent variables. Backwards, step-wise multiple regression analyses were performed for each condition to determine the contributions of the various dependent variables to performance (TTE). The predictor variables used in the multiple regression analysis were pH, lactate,

bicarbonate, potassium, ventilation, and core temperature. An alpha level of $p < 0.05$ was accepted as significant for all analyses.

CHAPTER FOUR
RESULTS

Fourteen competitive male triathletes and/or cyclists volunteered for the study. Their descriptive characteristics are shown in Table 1 of the methods section. Subjects had a mean lactate threshold (LT) equivalent to 76% VO_2max. The lactate concentration ([LA]) at LT and exhaustion during the VO_2max test was 3.07 mmol/L and 8.76 mmol/L, respectively. The pH threshold (pHT) was at a slightly higher intensity, equivalent to 81.41% VO_2max that corresponded to an average pH of 7.30. At exhaustion, pH decreased to a value of 7.18. Finally, bicarbonate (HCO_3^-) threshold (BCT) occurred at an intensity similar to pHT (81.8% VO_2max), while [HCO_3^-] was 24.6 and 20.8 mmol/L at threshold and exhaustion, respectively.

TABLE 2: Threshold Data from the VO_2max Test

SUB.	LA (mmol/L) @LT	LT %VO2max	[LA]max (mmol/L	pH@ pHT	pHT %VO2 max	pHmax	[HC03] mmol/L @BCT	BCT %VO2 max	$HCO3^-$ max (mmol/L)
1	4.65	76.73	12.40	7.30	89.40	7.13	26.00	89.40	19.40
2	3.60	75.24	8.96	7.31	85.34	7.18	23.50	85.34	21.50
3	3.36	66.06	11.60	7.30	66.06	7.18	22.40	66.06	15.40
4	4.61	82.40	9.85	7.25	82.37	7.20	24.10	82.33	22.60
5	2.24	62.38	9.57	7.31	77.81	7.16	26.00	96.46	21.20
6	4.13	74.95	8.94	7.32	87.58	7.26	22.50	87.58	19.50
7	3.02	80.09	9.42	7.30	80.00	7.15	24.00	80.09	20.00
8	3.13	82.60	9.41	7.29	82.60	7.16	22.80	82.60	15.00
9	2.45	75.00	8.80	7.32	95.37	7.25	23.60	86.11	18.60
10	2.47	75.00	9.42	7.30	87.38	7.23	28.00	75.00	23.80
11	2.67	78.89	8.95	7.31	78.89	7.17	27.40	78.89	22.80
12	2.26	80.15	4.34	7.29	80.15	7.16	24.00	80.15	21.90
13	2.09	77.75	5.28	7.29	77.75	7.19	27.50	77.75	25.70
14	2.29	77.49	5.75	7.29	69.05	7.14	26.60	77.49	23.80
MEAN±SD	3.07± 0.89	76.05± 5.69	8.76± 2.24	7.299± 0.02	81.41± 7.72	7.18± 0.04	24.6± 2.08	81.8± 7.24	20.8± 3.08

Table 2. Lactate, pH, and bicarbonate values at threshold, % of VO2max at threshold, and maximal values following the preliminary graded exercise test. Values represent the mean ± SD (n=14).

Cardiorespiratory Data

A significant intensity x time interaction (p=0.00) was present for oxygen consumption. The post hoc analysis indicated that VO_2 was greater in H100 and C100 compared to H80 and C80 at all time points (Figure 1) Also, a significant interaction (p=0.000) was present for ventilation (VE). Post hoc analysis also revealed that VE was greater in H100 and C100 compared to H80 and C80 at all time points (Figure 2). In addition, a significant environment x time interaction (p=0.001) was present for VE. The post hoc test indicated that ventilation was greater in both hot conditions compared to both cold conditions at 3-MIN and FINAL, but not at 2-MIN (Figure 2). Finally, a significant intensity x time interaction (p = 0.00) was present for heart rate. Post hoc analysis showed that heart rate was greater during H100 and C100 compared to H80 and C80 at 2-MIN and 3-MIN, but not at FINAL (Figure 3). Oxygen consumption, VE, and HR increased across time during all of the experimental trials. Oxygen consumption, VE, and HR values represent the average taken over a one minute period; therefore, the 2-min value is indicative of the cardiorespiratory variable from 1-2 minutes of exercise, 3-min represents the average between 2-3 minutes, and FINAL represents the variable average over the last minute of exercise.

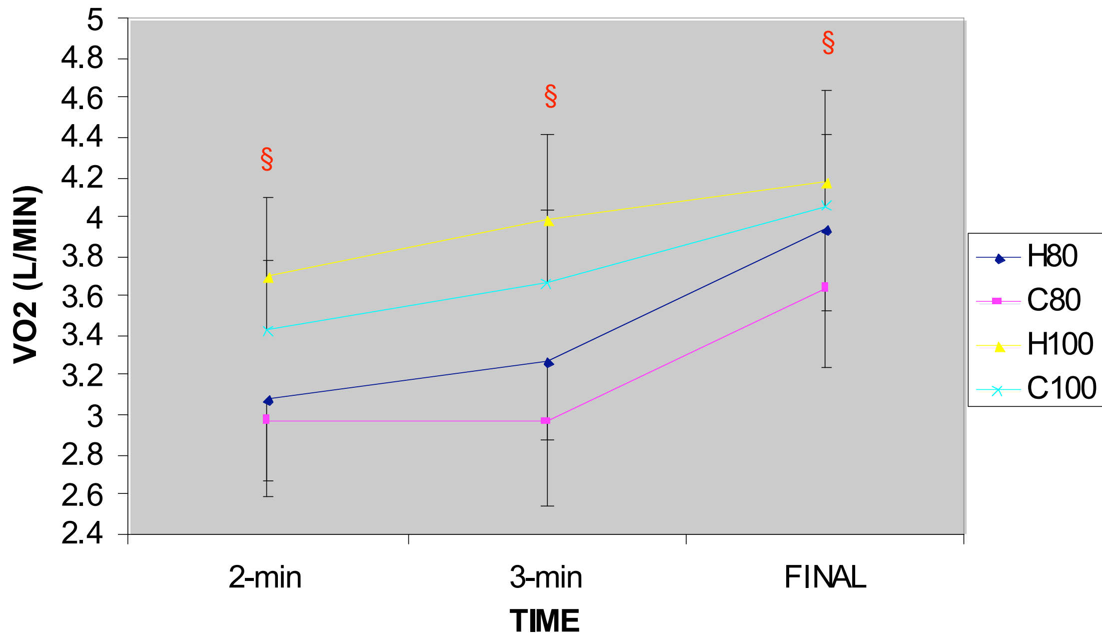

Figure 1. Changes in VO_2 during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The § indicates that oxygen consumption was significantly greater during H100 and C100 vs. H80 and C80. For Figures 1-3, the 2-min value is indicative of the cardiorespiratory variable from 1-2 minutes of exercise, 3-min represents the average between 2-3 minutes, and FINAL represents the variable average over the last minute of exercise. Note: the time interval between 3-min and FINAL varies depending on the duration of the TTE for each condition (see Figure 4). Values represent the mean ± SD (n=11).

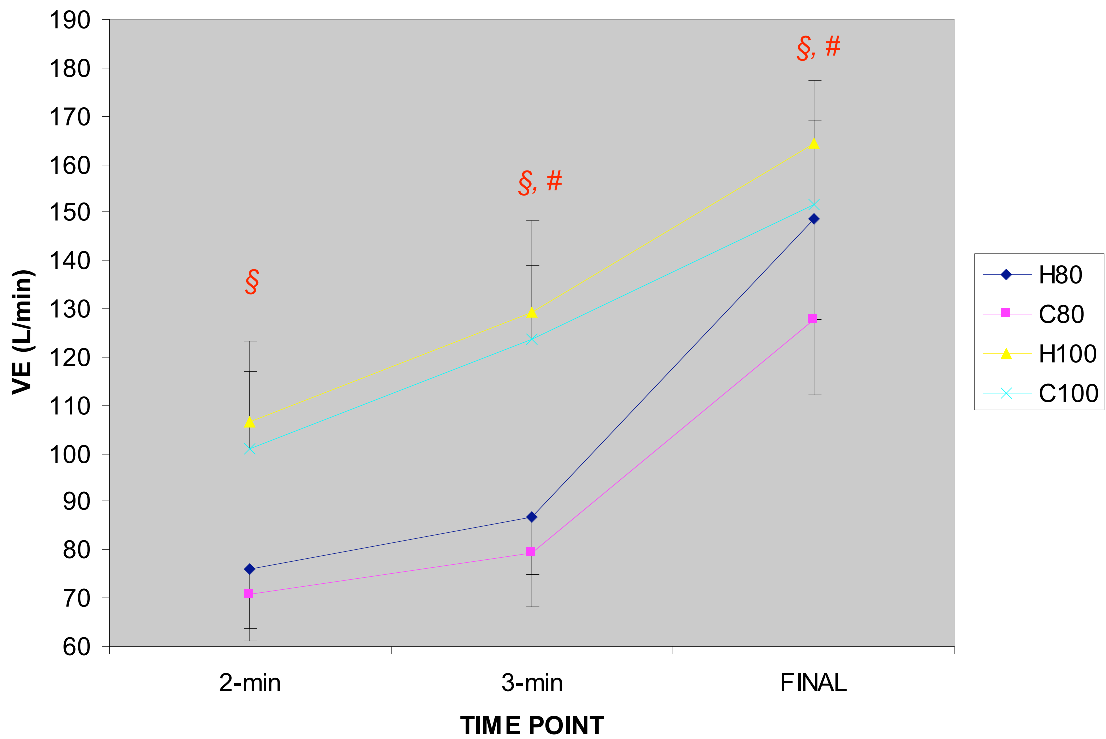

Figure 2. Ventilation responses during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The # indicates that ventilation was greater during H100 and C100 vs. H80 and C80. The § indicates that ventilation was greater in H80 and H100 vs. C80 and C100 ($p<0.05$). Values represent the mean ± SD (n=11).

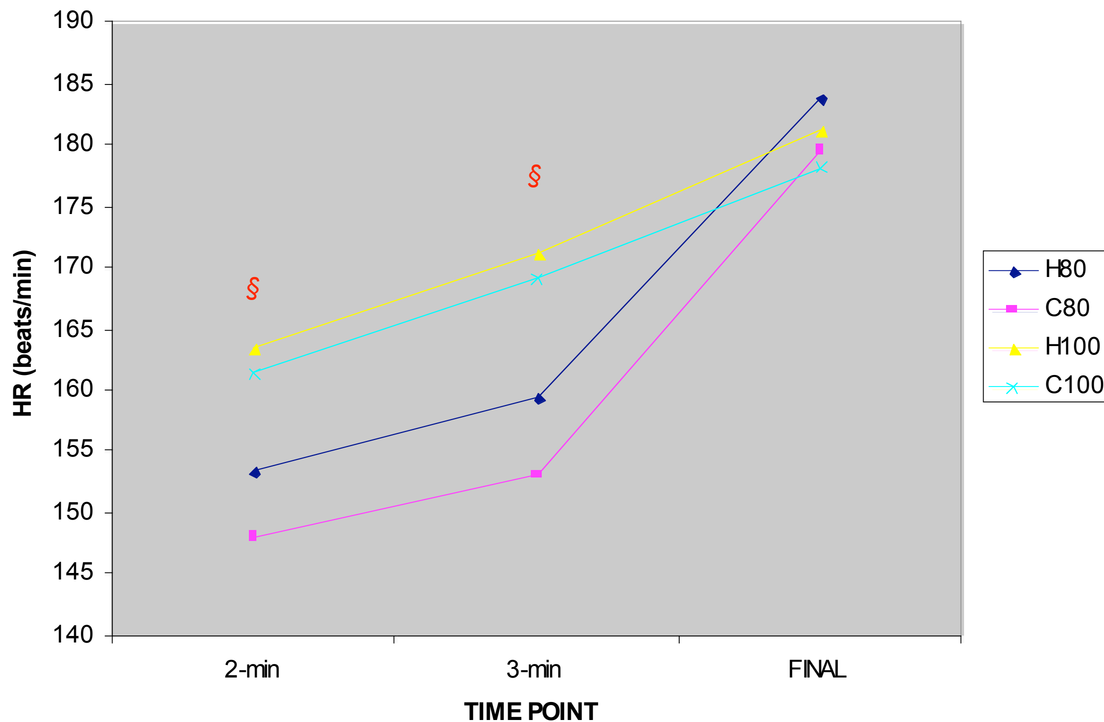

Figure 3. Heart rate responses during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The § indicates that heart rate was significantly greater in H100 and C100 vs. H80 and C80 ($p < 0.05$). Values represent the mean ± SD (n=11).

Time-to-Exhaustion

A significant environment x intensity interaction (p=0.000) was present for time-to-exhaustion (Figure 4). Post hoc analysis indicated that performance was significantly different in all trials except when exercising at 100% in both environments.

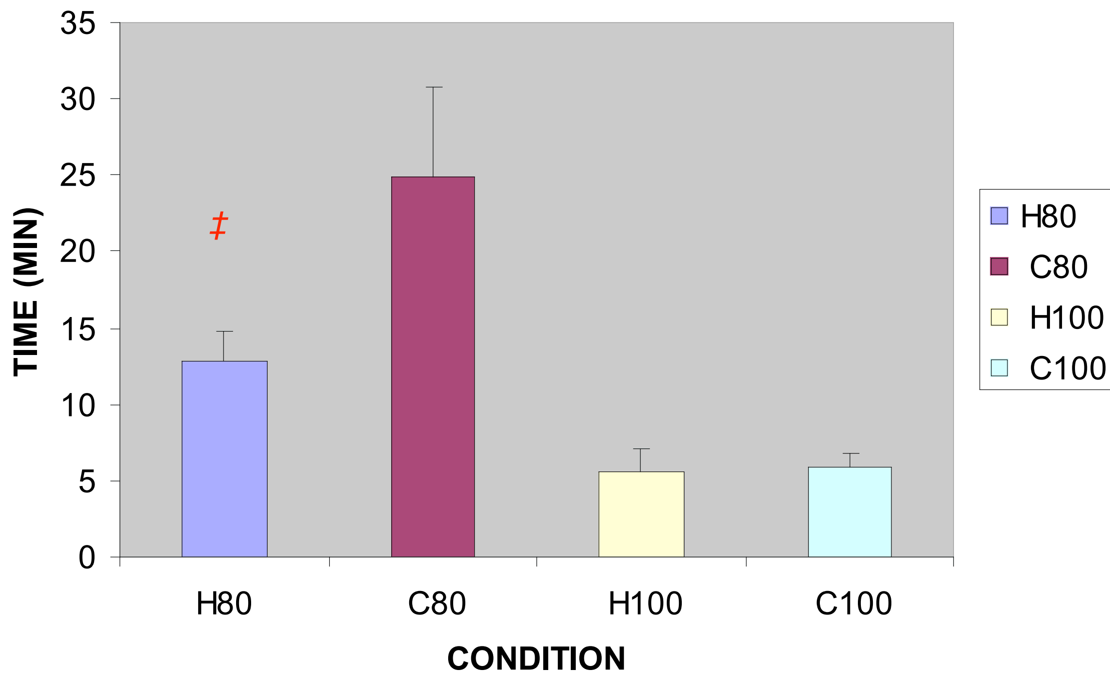

Figure 4. Time-to-exhaustion in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The ‡ indicates that performance was significantly different between all trials except when comparing H100 and C100. Values represent the mean ± SD (n=11).

pH

Prior to exercise, pH was similar at all time points (Figure 5). A significant intensity x environment x time interaction (p=0.015) was present for pH. Post hoc analysis indicated that pH at EXH was progressively lower from C80 to H80 to H100 with C100 the lowest of all conditions. At REC, post hoc analysis indicated that pH followed the same pattern as EXH except that the H100 and C100 conditions were not different. When analyzing the H80 and C80 trials separately, an environment x time interaction (p = 0.035) was present for pH. Post hoc analysis indicated that pH was significantly lower in H80 compare to C80 at 10 min, EXH, and REC (Figure 6). The

time point, PRE, refers to the baseline value, EXH refers to the value immediately post-exercise, and REC refers to the sample taken at 3-minutes post-exercise.

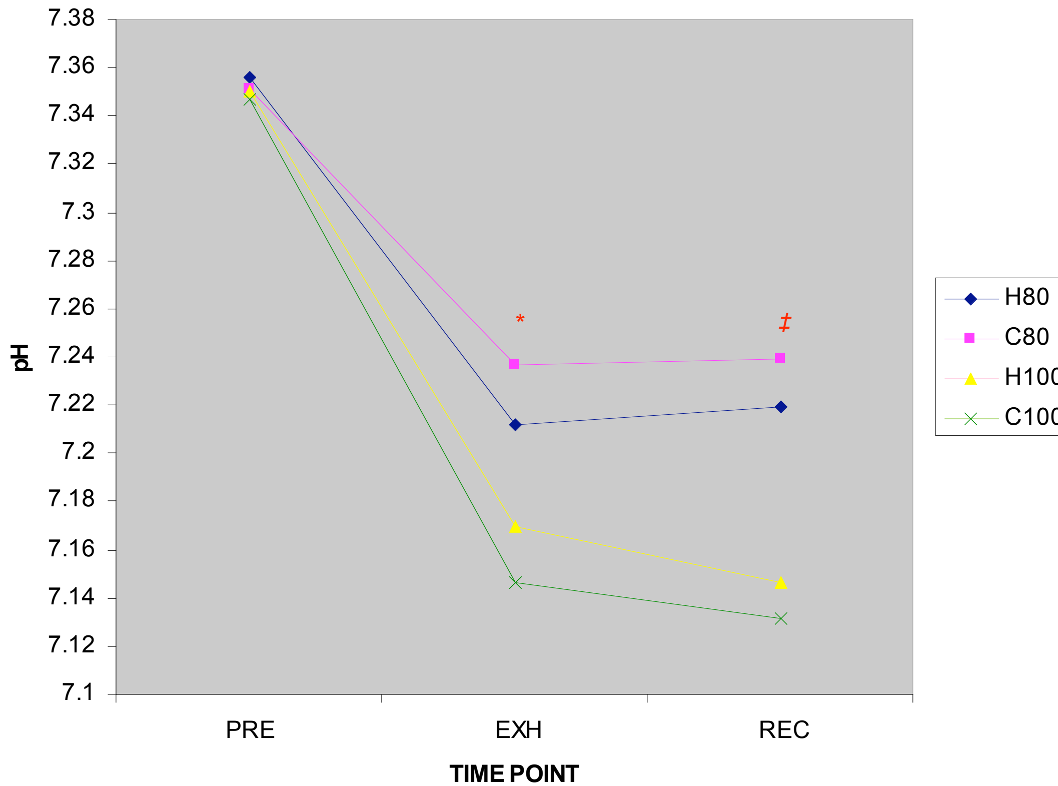

Figure 5. Blood pH response during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The * indicates that pH was significantly different among all trials. The ‡ indicates that pH was significantly different among all trials at REC, except when comparing the H100 and C100 conditions. For Figures 5-12, the time point PRE refers to the baseline value, EXH refers to the value immediately post-exercise, and REC refers to the sample taken at 3-minutes post-exercise. Note: the time interval between PRE and EXH varies depending on the duration of the TTE for each condition (See Figure 4). Values represent the mean ± SD (n=11).

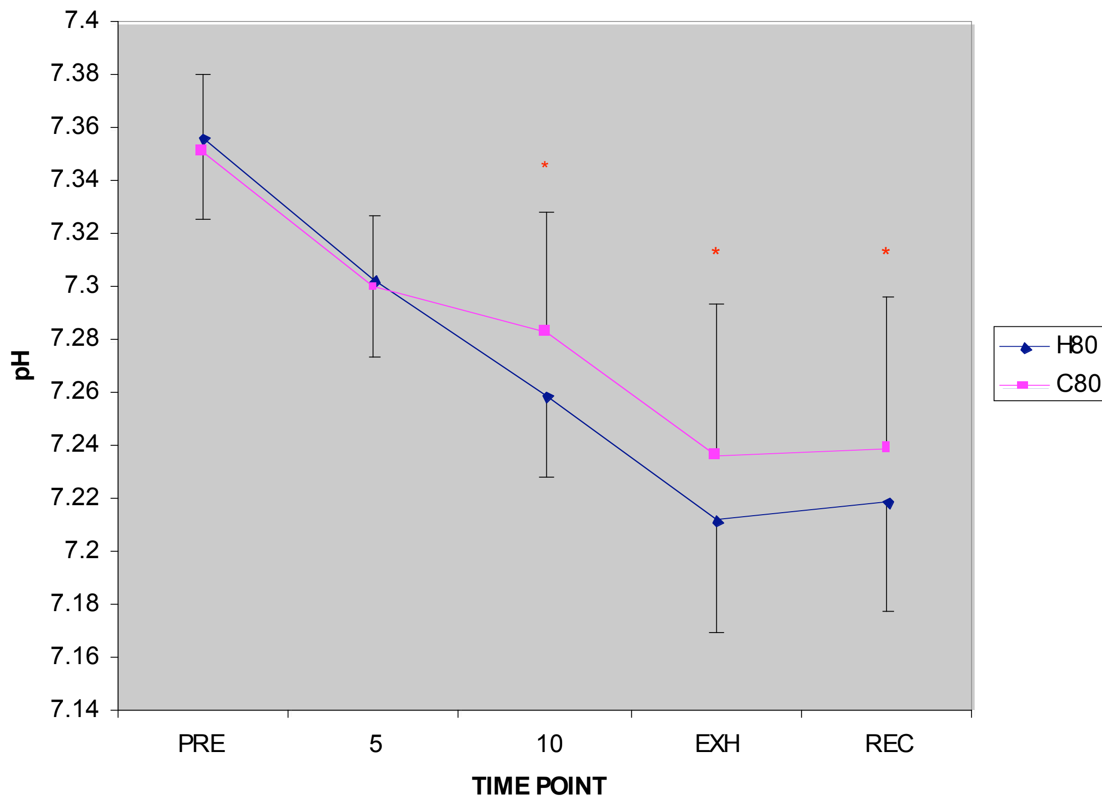

Figure 6. Blood pH response during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80% of VO2max. The * indicates that pH was significantly lower in H80 vs. C80 ($p < 0.05$). Values represent the mean ± SD (n=11).

Lactate

A significant intensity x time (p=0.027) was present for corrected lactate concentration (Figure 7). Post hoc analysis indicated that lactate was greater in the H100 and C100 conditions compared to the H80 and C80 conditions at REC only. In addition, a significant environment x time interaction (p=0.044) was present at REC whereby lactate was greater in H80 and H100 compared to a C80 and C100.

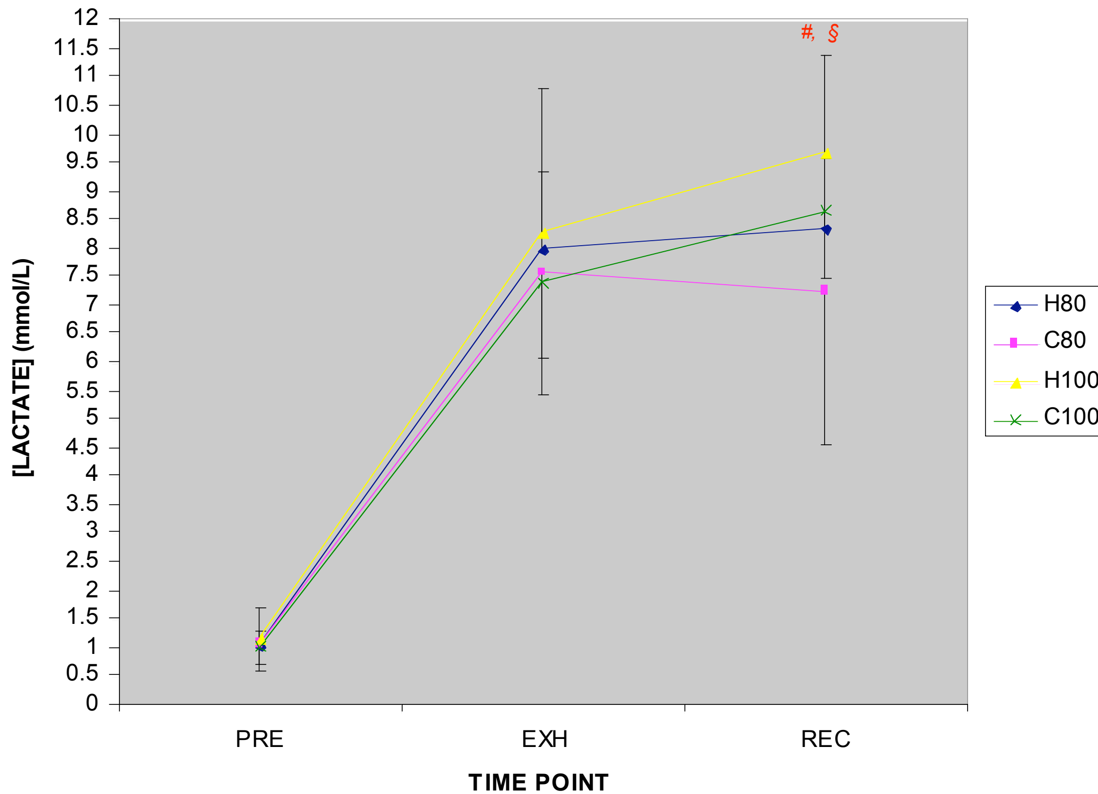

Figure 7. Lactate responses during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The # indicates that lactate was significantly higher at REC in H100 and C100 vs. H80 and C80. The § indicates that lactate was significantly higher at REC in H80 and H100 vs. C80 and C100 ($p < 0.05$). Values represent the mean ± SD (n=11).

A significant environment x time interaction (p=0.005) was present for lactate when comparing the effect of environment at 80% of VO_2max. Post hoc analysis indicated that lactate was higher in the H80 compared to C80, but only at 5-min and REC (Figure 8).

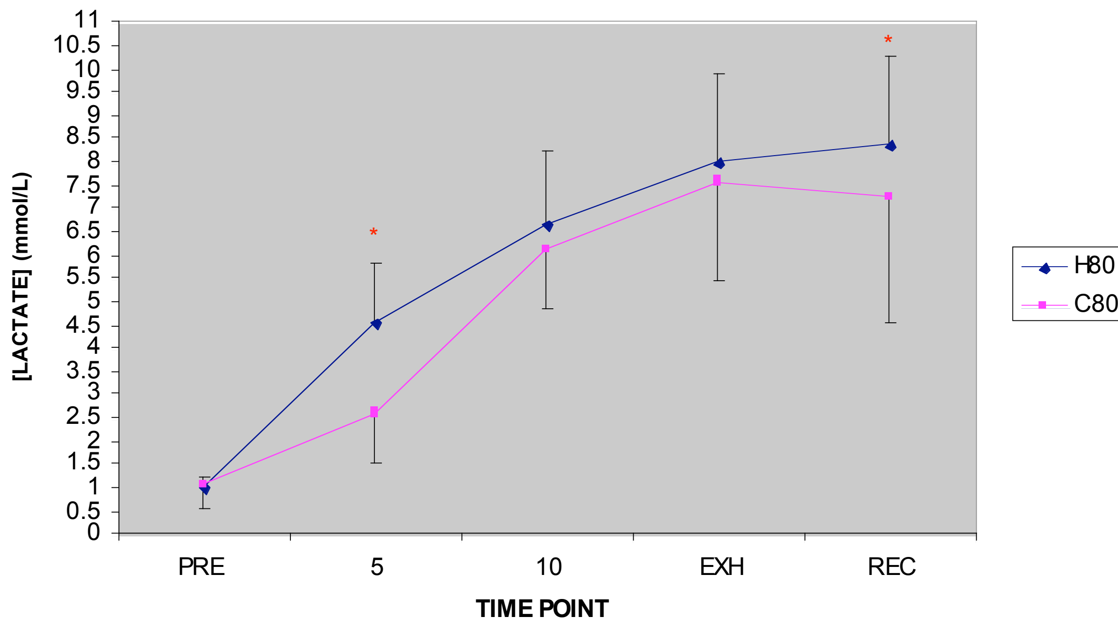

Figure 8. Lactate responses during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80% VO_2max. The * indicates that lactate was significantly higher at 5-min and REC in H80 vs. C80 ($p<0.05$). Values represent the mean ± SD (n=11).

Bicarbonate

A significant intensity x environment x time interaction (p=0.038) was present for bicarbonate. Bicarbonate was similar prior to exercise for all trials and progressively decreased in response to exercise. Post hoc analysis indicated that bicarbonate was different in all trials at EXH, except when comparing H80 and C80. In addition, the post hoc analysis indicated that bicarbonate was different in all trials at REC, except when comparing H80 and H100 (Figure 9). Bicarbonate was highest at REC in the C100 trial, followed by C80, H100, and H80. Finally, a significant environment x time (p=0.000) was present for bicarbonate when comparing the effect of environment at 80% VO_2max.

Post hoc analysis indicated that bicarbonate was significantly higher in the C80 compared to H80 at 5-, 10-MIN, and REC, but not at PRE and EXH (Figure 10).

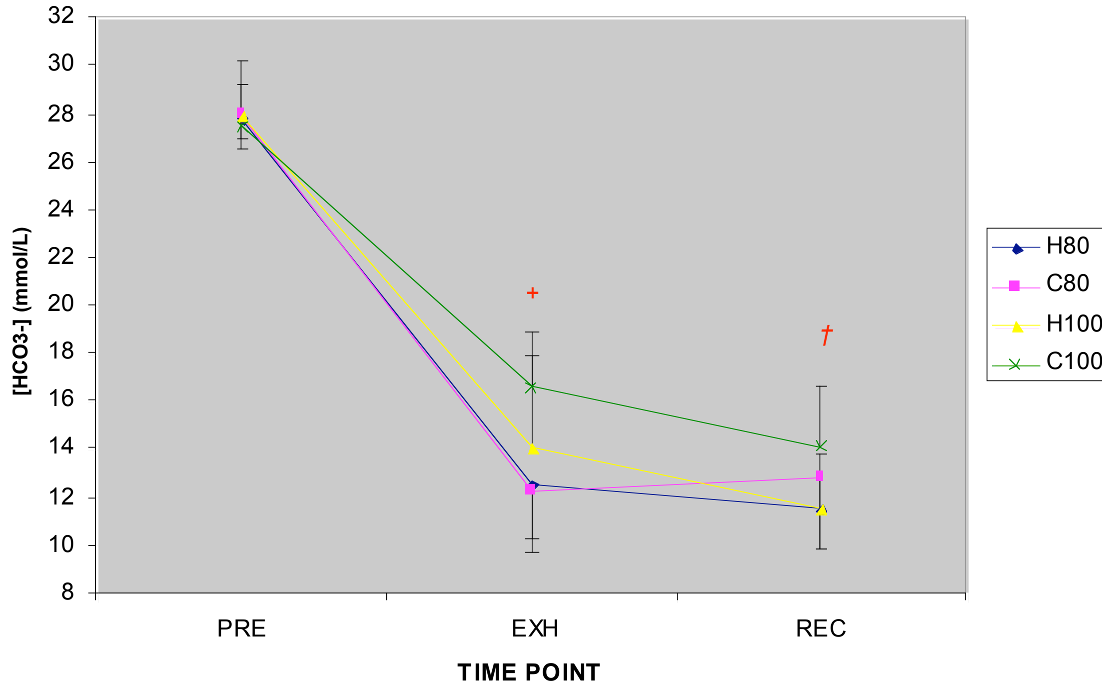

Figure 9. Bicarbonate responses during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80- and 100% VO_2max. The + indicates that bicarbonate was significantly different at EXH for all trials, except when comparing H80 and C80. The † indicates that bicarbonate was significantly different for all trials, except when comparing H80 and H100 ($p<0.05$). Values represent the mean ± SD (n=11).

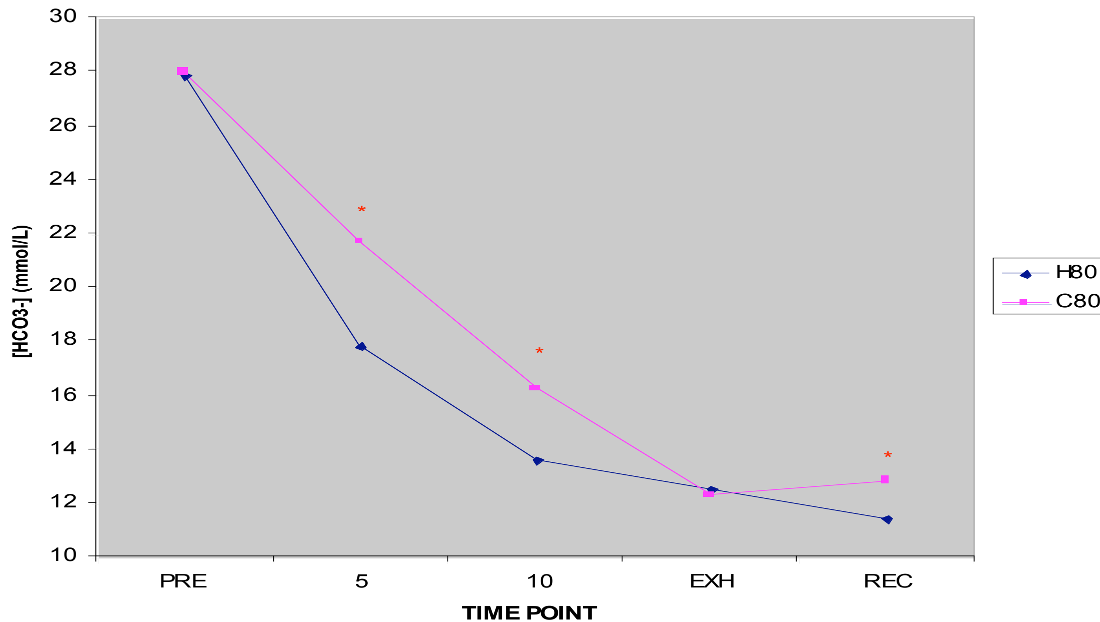

Figure 10. Bicarbonate response during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80% VO_2max. The * indicates that bicarbonate was significantly higher at 5-, 10-min, and REC in C80 vs. H80 ($p<0.05$). Values represent the mean (n=11).

Potassium

A significant environment x time interaction (p=0.030) was present at EXH for potassium. Post hoc analysis indicated that potassium was significantly higher at exhaustion in the H80 and H100 compared to the C80 and C100 trials.. Potassium concentrations were highest at exhaustion in H100, followed by H80, C100, and C80, respectively. At REC, potassium concentrations progressively decreased from H80 to H100 to C100 to C80; however, there were no significant differences in potassium concentration between all trials at this time point (Figure 11).

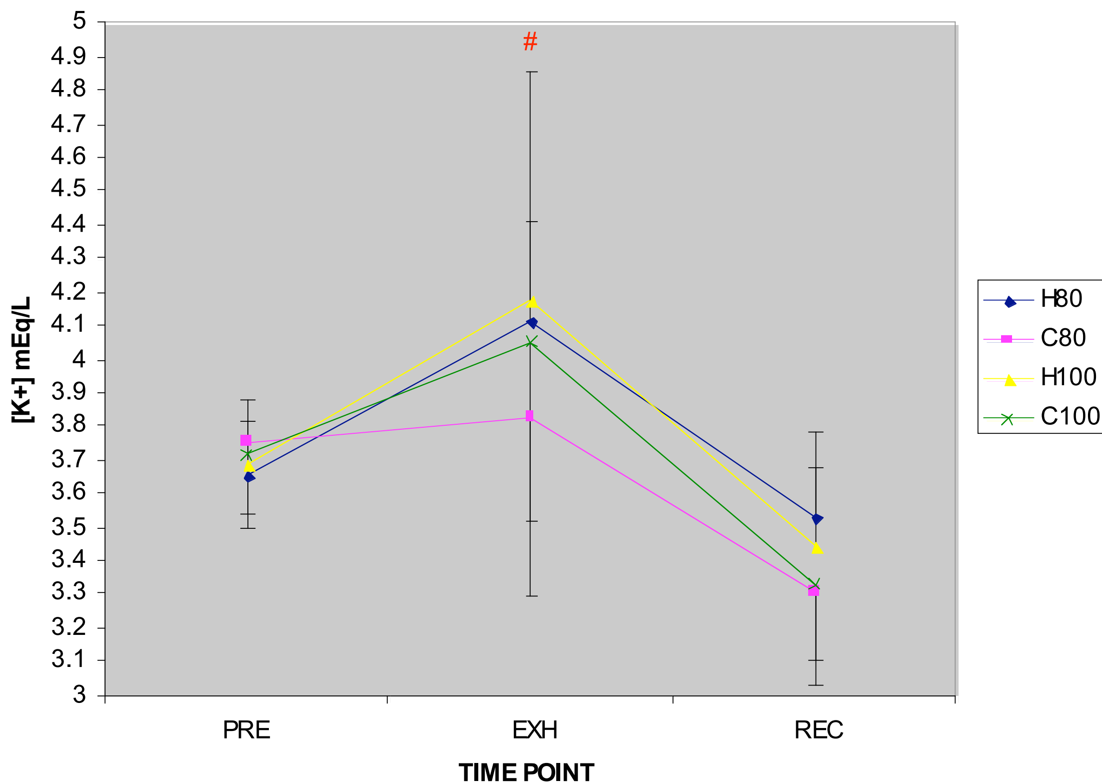

Figure 11. Potassium response during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The # indicates that potassium was significantly higher at EXH in H80 and H100 vs. C80 and C100 ($p<0.05$). Values represent the mean ± SD (n=11).

Core Temperature

A significant intensity x time interaction (p=0.000) was present for absolute Tc. The rate of heat gain was similar for all trials during the first 3-minutes; however, post hoc analysis indicated that Tc was significantly greater in H80 and C80 compared to H100 and C100 at EXH and REC. In addition, a significant environment x time interaction (p=0.010) was present for absolute Tc. Post hoc analysis indicated that Tc was significantly higher at REC in H80 and H100 compared to C80 and C100 (Figure

12). Furthermore, absolute Tc decreased during the recovery periods in C80 and C100; whereas, there was no change or a slight increase in the H80 and H100 trials.

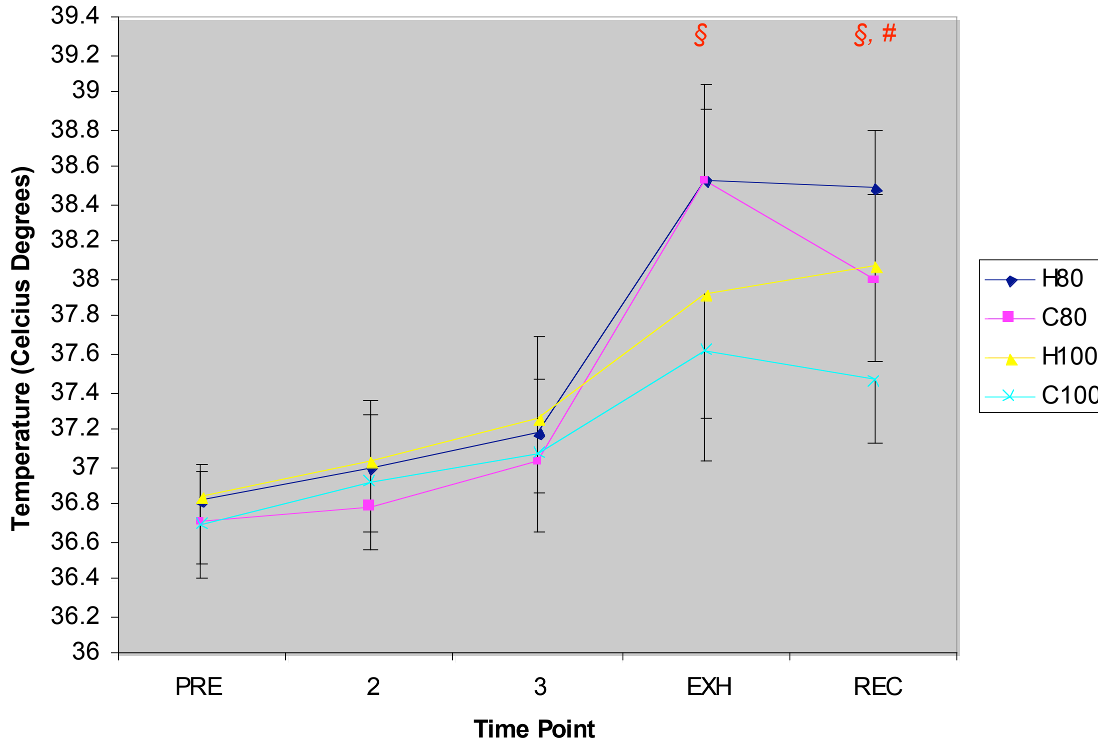

Figure 12. Absolute Tc response during time-to-exhaustion trials in hot (37◦C, 26% RH) and cold environments (10◦C, 57% RH) at 80 and 100% VO_2max. The # indicates that Tc was significantly higher at REC in H80 and H100 vs. C80 and C100. The § indicates that Tc was significantly higher at EXH and REC in H80 and C80 vs. H100 and C100 ($p<0.05$). Values represent the mean ± SD (n=11).

Change in Core Temperature

A significant intensity main effect (p=0.005) was present for change in Tc. Change in Tc was significantly higher for H80 and C80 compared to H100 and C100. In addition, a significant environment main effect (p=0.001) was present for change in Tc (Figure 13). Change in Tc was significantly greater in H80 and H100 compared to C80 and C100.

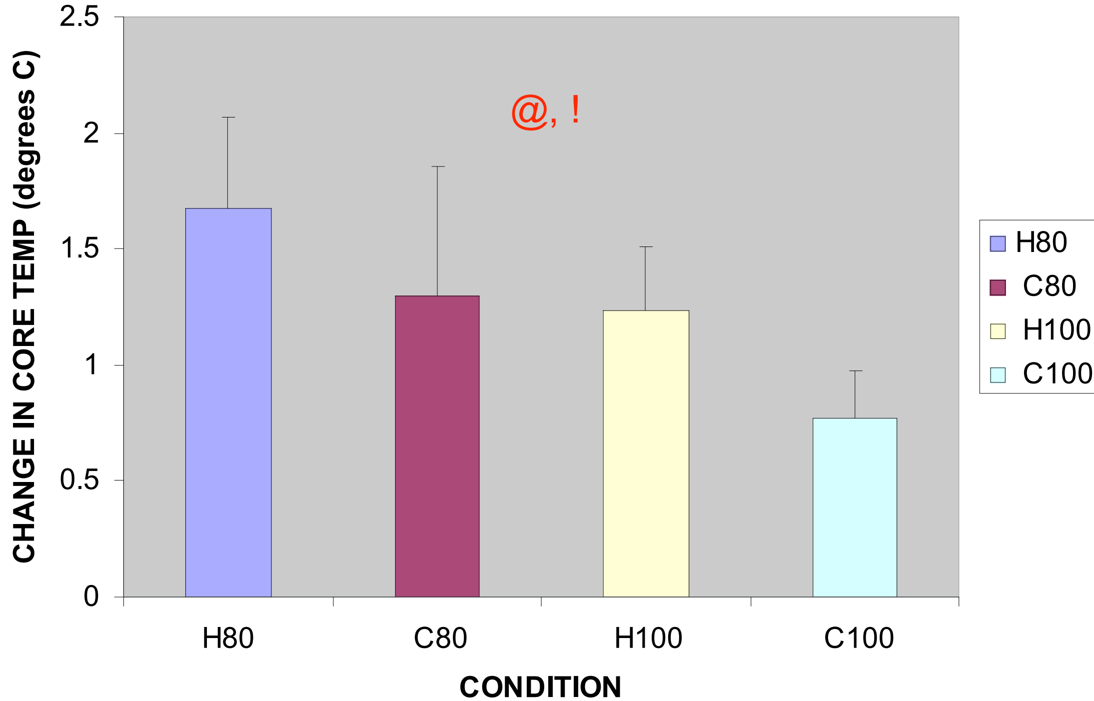

Figure 13. Change in Tc response during time-to-exhaustion trials in hot (37∘C, 26% RH) and cold environments (10∘C, 57% RH) at 80 and 100% VO_2max. The @ indicates that H80 and C80 are greater than H100 and C100 (intensity main effect). The ! indicates that H80 and H100 are greater than C80 and C100 (environment main effect) ($p<0.05$). Values represent the mean ± SD (n=11).

Correlations

The pairwise Pearson correlations indicated that no relationships existed between the primary dependent variables in H80 (Table 3a). Pearson correlations indicated that VE/lactate, VE/Tc, and lactate/K+ were positively related to one another during C80 ($p<0.05$) (Table 3b). In addition, VE/HCO3-, lactate/$HCO3^-$, and $HCO3^-$/Tc were negatively related to one another ($p<0.05$).

TABLE 3a- Pearson Correlations: H80							
	VE	**pH**	**Lactate**	**$HCO3^-$**	**K^+**	**TTE**	**Tc**
VE							
pH							
Lactate			NO SIGNIFICANCE P= 0.115				
$HCO3^-$							
K^+							
TTE							
Tc							

Table 3a. Pearson correlation for H80. None of the dependent variables indicated a relationship (p=0.115).

TABLE 3b- Pearson Correlations: C80							
	VE	**pH**	**Lactate**	**$HCO3^-$**	**K^+**	**TTE**	**Tc**
VE							
pH							
Lactate	0.684/ 0.020						
HCO3-	-0.810/ 0.003		-0.839/ 0.001				
K^+			0.698/ 0.017				
TTE				-0.645/ 0.032			
Tc	0.758/ 0.007						

Table 3b. Pearson correlation for C80 (r/p). VE/lactate, VE/Tc, and lactate/K+ were positively correlated ($p<0.05$). VE/$HCO3^-$, lactate/$HCO3^-$, and $HCO3^-$/TTE were negatively correlated.

In addition, Pearson correlations indicated a significant negative relationship between pH/lactate and lactate/$HCO3^-$ during H100 ($p<0.05$) (Table 3c).

TABLE 3c- Pearson Correlations: H100							
	VE	**pH**	**Lactate**	**$HCO3^-$**	**K^+**	**TTE**	**Tc**
VE							
pH							
Lactate		-0.837/ 0.001					
$HCO3^-$			-0.723/ 0.012				
K^+							
TTE							
Tc							

Table 3c. Pearson correlations for H100 (r/p). pH/lactate and lactate/$HCO3^-$ are negatively correlated with one another ($p<0.05$).

Finally, in C100, Pearson correlations indicated a negative correlation between pH/lactate and $HCO3^-$/TTE and a positive correlation between K+/TTE (Table 3d).

TABLE 3d- Pearson Correlations: C100							
	VE	**pH**	**Lactate**	**$HCO3^-$**	**K^+**	**TTE**	**Tc**
VE							
pH							
Lactate		-0.611/ 0.046					
$HCO3^-$							
K^+							
TTE				-0.610/ 0.046	0.602/ 0.050		
Tc							

Table 3d. Pearson correlations for C100 (r/p). pH/$HCO3^-$ and $HCO3^-$/TTE are negatively correlated, and K^+/TTE are positively correlated.

TABLE 4- Multiple Regression Prediction of Time-to-exhaustion					
	Independent variable	Included	Adjusted R^2	Variable probability	Regression probability
H80	Tc	included	0.720	0.067	0.017*
	$HCO3^-$	included		0.006*	
	Lactate	included		0.030*	
	pH	included		0.075	
	VE	excluded			
	K+	excluded			
C80	pH	included	0.213	0.104	0.086
	K^+	excluded			
	$HCO3^-$	excluded			
	Tc	excluded			
	VE	excluded			
	Lactate	excluded			
H100	Tc	included	0.746	0.011*	0.005*
	$HCO3^-$	included		0.049*	
	pH	included		0.066	
	Lactate	excluded			
	VE	excluded			
	K^+	excluded			
C100	VE	included	0.858	0.003*	0.002
	$HCO3^-$	included		0.001*	
	Lactate	included		0.002*	
	pH	included		0.001*	
	K^+	excluded			
	Tc	excluded			

Table 4. Multiple regression for all experimental trials. A backwards, step-wise multiple regression was used to predict time-to-exhaustion using six predictor variables.

Using an alpha to include of 0.10, the backwards, step-wise multiple regression (Table 4) revealed that lactate and $HCO3^-$ were significant predictors of TTE in the H80 trial ($R^2 = 0.72$, p = 0.017). Tc and pH were included in the final regression but failed to reach significance individually. In the C80 condition, pH was the only independent variable in the final regression; however, the regression was not significant ($R^2 = 0.213$, p = .086). For H100, Tc, $HCO3^-$, and pH were included as predictors of TTE ($R^2 = 0.746$, p = 0.005); however, pH was not significant individually. In the C100 condition, VE,

HCO_3^-, lactate, and pH were included as significant predictors of TTE ($R^2 = 0.858$, $p = 0.002$).

CHAPTER FIVE:
DISCUSSION

The primary finding of this study was that pH was significantly different at exhaustion (EXH) among all trials, except when comparing H100 and C100. These results failed to support our hypothesis which stated that pH would be similar at fatigue, regardless of intensity and environment. Interestingly, differences in pH at EXH occurred without concomitant changes in lactate (LA) for this time point. In addition, significant elevations in core temperature (Tc) were seen when comparing the longer, less intense bouts of exercise (H80 and C80), suggesting that Tc played a prominent role in performance during these trials while pH was an important factor in time-to-exhaustion (TTE) for the high intensity trials (H100 and C100), regardless of environment.

Cardiopulmonary Responses

Oxygen consumption (VO_2) increased throughout the experimental trials and varied depending on the exercise intensity. As expected, VO_2 was significantly higher in H100 and C100 vs. H80 and C80; however, when expressed as a percentage of VO_2max, the final VO_2 during both H80 and C80 was considerably higher than the predicted value as subjects were exercising at 92% and 86% VO_2max for H80 and C80, respectively. This difference was not observed in the 100% experimental trials (96.05%). The greater percentage of VO_2max observed at EXH can be attributed to elevations in Tc that resulted from a greater thermoregulatory strain and a greater metabolic demand in the 80% trials. This finding is similar to that reported by Arngrimsson et al (2) in subjects who completed 20-minutes of submaximal treadmill exercise in 25, 35, 40, and 45 ^{o}C environment prior to an exhaustive incremental test in the same environmental conditions

and showed an increase in relative intensity expressed as a percentage of VO_2max, along with elevated heart rate (HR) when exercising in 35, 40, and 45 °C compared to the 25 °C conditions. These changes were associated with findings that VO_2peak was reduced when exercising in a hot environment (2). Although the VO_2 slow component, defined as a gradual increase in oxygen uptake during prolonged steady state exercise, is not completely understood, the cost of thermoregulation may be a contributing factor. As Tc increased, more blood needed to be distributed to the periphery to aid in thermoregulation and to the exercising muscles in an attempt to maintain the given workload. The lack of a significant difference in heart rate at FINAL among all trials in combination with a greater VO_2 at this time point in H100 and C100 compared to H80 and C80 suggests that either decreased stroke volume and/or arteriovenous O_2 difference in the 80% trials were likely factors causing a lower VO_2 without a concomitant difference in heart rate.

In the present study, heart rate (HR) was significantly higher at 2-MIN and 3-MIN during 100% vs. 80%, but there was no significant difference between trials at EXH. These data suggest that muscular fatigue corresponded to a similar maximal heart rate (HRmax) for all trials, regardless of exercise intensity or environment. Given that VO_2 was higher at EXH during the 100% trials, the similarity in HR suggests that either stroke volume (SV) or arteriovenous oxygen difference was higher in the H100 and C100 conditions compared to the H80 and C80 conditions at exhaustion. The higher Tc in the H80 and C80 conditions would be in line with the usual thermoregulatory increase in subcutaneous blood flow and the concomitant reduction in venous return that would produce a reduction in SV and a concomitant elevation in HR in the lower intensity conditions.

The lack of a greater HR in the hot vs. cold is in contrast to a vast amount of evidence that exercising in hot environments at the same absolute workload leads to an increased cardiovascular load associated with the increases in Tc. In the present study HR was only influenced by exercise intensity, not environment; therefore, our HR results are different from those reported by Drust et al (19), Maxwell et al (37), and many others. The lack of difference in HR in the C100 and H100 conditions can be explained by the simple fact that the effort was maximal; thus, there was no HR reserve that could be impacted by the thermal stress of the H100 condition. In addition, the duration was short enough to preclude substantial cardiovascular drift. In the H80 versus C80 comparison, the explanation is not as clear cut. There was a tendency for a greater HR in H80, but it did not reach significance. It is likely that a more thorough analysis of the HR data taking into account multiple time points and the longer duration of the C80 condition would produce the expected difference in HR that is typically observed in hot environments.

The greater ventilation (VE) measured in the 100% compared to the 80% trials can be attributed to the higher absolute exercise intensity performed. In addition, metabolic factors such as PCO_2 and decrements in pH are plausible factors causing increased VE during the 100% trials. Furthermore, exercising in a hot environment lead to significant elevations in VE compared to the cold trials, a result that is indicative of metabolic factors controlling the ventilatory rate. Since pH was lower in hot vs. cold trials, and plays a role in the humoral control of VE, pH and PCO_2 are likely factors associated with higher ventilatory rates in the hot vs. cold trials, especially late in the bouts of exercise when hyperventilation was most prevalent. The role of VE in the

respiratory buffering associated with the bicarbonate reaction will be discussed in greater detail in a later section.

pH Responses

The primary purpose of this investigation was to observe the relationship between TTE and pH in varying levels of exercise intensity and environment. The lack of similarity in the pH levels observed at EXH suggests that muscular fatigue did not occur at a similar level of cellular acidosis across the two intensities and the two environments; thus, the cause of fatigue was likely different either as a function of intensity or environment. When exercising at maximal intensity, however, the pH levels were similar; thus, pH likely played a greater role in fatigue during the 100% trials compared to the 80% trials. Previous researchers have reported that decrements in interstitial pH of 0.5 units can lead to decreased functional ability of the active muscles, resulting in muscular fatigue. In the present study, however, pH decreased between 0.12-0.22 units, which is a considerably smaller decline compared to the previously reported decrements of 0.5 units in response to high intensity exercise. These differences can be explained due to the location of pH measurement since we measured pH in whole blood compared to Bangsbo et al (6) who assessed intracellular pH changes. In a recent review, Cairns (16), reported that plasma pH changes were similar to the results in the present study. In addition, pH was stated to be lowest within a few minutes following exercise, which is consistent with the current findings in which pH either remained constant or decreased during the recovery period.

Intense muscular contraction is associated with high rates of ATP hydrolysis and a resulting increase in ADP, inorganic phosphate, and H^+ with a concomitant decrease in

cellular pH (35). Numerous researchers have alluded to the role of H^+ accumulation and reduced pH as a causal factor in fatigue. It is believed that the accumulation of H^+, which is the result of production being greater than removal, depresses muscle function by: 1) reducing the transition of the cross-bridge from the low-to the high-force state; 2) inhibiting maximal shortening velocity; 3) inhibiting myofibrillar ATPase; 4) reducing cross-bridge activation by competitively inhibiting calcium (Ca^+) binding to troponin C; and 5) reducing Ca^+ re-uptake by inhibiting the sarcoplasmic ATPase (24, 41). These disruptions in muscular contractility occur during high intensity exercise associated with marked reductions in cellular pH. In the present study, change in blood pH was 0.137, 0.111, 0.204, and 0.208 for H80, C80, H100, and C100, respectively. The present findings indicate that similar changes in muscular function might have occurred during the 100% trials due to significantly reduced pH, while there are likely other factors, such as temperature and HCO_3^-, playing a more important role in the 80% trials.

At exhaustion, pH was significantly different for all trials while there was no difference in LA at this time point. This suggests that H^+ were released by sources other than LA and that there is a dissociation between LA and acidosis. Even though LA is the largest contributor to H^+ accumulation (33), its well supported that other sources contribute H^+ since as much as one third of H^+ originate from sources other than LA such as phosphagen degradation (33). Lactate levels in the blood are themselves a result of a balance between production and removal; thus, given that H^+ are subject to this same balance, albeit due to different biochemical processes, it is not surprising that changes in LA and pH do not correspond. Furthermore, the production of LA consumes two H^+, thus retarding acidosis (55). Therefore, since H^+ are produced and buffered through a variety

of biochemical processes, and LA aids in this buffering, it is not surprising that recent evidence fails to support the role of LA and muscular fatigue. In the present study, pH was an important contributor to muscular fatigue in the 100% trials, in which there was no difference in blood pH at EXH for H100 and C100. Lactate, on the other hand, was significantly different between H100 and C100, indicating that other sources and/or processes, either via buffering and/or production, were contributing to the presence of H^+ that resulted in similar responses for these high intensity trials.

The H^+ produced in the cell are buffered via a variety of co-transport and cellular buffers. Research indicates that H^+ can be transported out of the cell via LA-H^+ co-transport, Na^+-H^+ transport, and can be consumed by HCO_3^- to aid in intracellular buffering (6, 33). The primary mechanisms for H^+ buffering are the LA- H^+ co-transport and HCO_3^- systems. Researchers agree that H^+ is widely buffered by the HCO_3^- reaction, such that there is an inverse relationship between LA and HCO_3^- during exercise (38). Furthermore, co-transport of LA and H^+ occurs in a 1:1 ratio, in which the rate of transport is related to the gradient of these substrates. The LA- H^+ co-transport is considered to be a major contributor to H^+ buffering since inhibition of other H^+ co-transport systems does not alter exercise performance. In the present study, LA progressively increased during exercise, and continued to during the recovery period, while HCO_3^- steadily decreased in an inverse fashion. In addition, as stated previously, there was no significant difference in pH for H100 and C100 while there were significant differences in LA and HCO_3^- at fatigue, suggesting that many factors play a role in the production and removal of H^+.

Multiple regression analysis was used to predict TTE using different dependent variables. The goal of the multiple regression analysis was to determine which, if any, of the dependent variables contributed to the independent measure, in this case, TTE. In the present study, six dependent variables were used to predict TTE, in which all of the variables are physiologically related. Therefore, failure of the multiple regression to correspond completely with the findings of the ANOVA could be due to the relationship between the variables and the inability of the statistical method to tease apart these differences as they are related to TTE. Regardless of the limitations of the multiple regression analysis, pH was the only measure included in the final analysis for all four trials; however, pH was only a significant individual predictor in the C100 experimental trial; however, pH approached significance as an individual predictor in H100 ($p=0.066$). Therefore, the multiple regression analysis provides a slightly different interpretation of the association between pH and TTE compared to our ANOVA results. The possibility that pH was not a significant factor in the cause of fatigue is supported by the findings of Bangsbo et al. (6) who reported that pH was not the only factor associate with muscular fatigue. Even though numerous researchers agree that reduced cellular pH leads to reduced force production and fatigue, others suggest that accumulation of additional by-products (such as K^+, inorganic phosphate, and LA) causes the inability to maintain the required workload (6, 40, 43, 58. 62). It is important to clarify that these findings do not rule out pH as a cause of fatigue, they simply suggest that low pH is not the only factor playing a causal role in muscular fatigue. In the current investigation, however, we failed to observe a consistent relationship between other metabolic factors such as K^+ and LA accumulation and muscular fatigue. Bangsbo et al (6) compared the relationship between

lactate, H^+, pH, and fatigue in a high lactate trial during which subjects performed an intermittent arm exercise prior to an exhaustive leg extensor exercise, and a control trial which involved only the exhaustive leg extensor exercise. The conclusion of their study was that fatigue occurred at a different pH between the two trials, again suggesting that low pH is not the only factor causing fatigue during intense muscular contraction. This conclusion is supported by the results of the current investigation.

Bicarbonate Responses

As mentioned previously, HCO_3^- plays a role in cellular buffering during exercise, and in conjunction with respiratory compensation, aids in the prevention of acidosis. The HCO_3^- buffering system alone does not have a large capacity; however, when combined with the ventilatory response to changes in CO_2, the bicarbonate buffering system is the most important in preventing detrimental pH reductions. The large reduction in HCO_3^- in combination with a pronounced hyperventilation in the current study suggests that the HCO_3^- reaction was active in all the trials, regardless of environment or intensity. There was, however, a significantly higher HCO_3^- in the C100 trials compared to all other conditions with a concomitantly lower pH. This unexpected finding suggests that subjects were unable to utilize their HCO_3^- supply, leading to greater reductions in pH and possibly contributing to muscular fatigue. Reduced VE during this trial could explain why HCO_3^- usage was not maximized, resulting in a lack of difference in performance between the H100 and C100 conditions. The final VE in C100 was, however, only slightly lower than H100, so it may not have been a large enough difference to impact the HCO_3^- buffering. Since environmental conditions are known to alter blood flow, it is possible that differences in blood flow somehow altered the HCO_3^-

reaction; however, this is unlikely due to the fact that a hot environment typically reduces skeletal muscle blood flow; thus, in the C100 condition it could be assumed that muscle blood flow was better maintained. In comparison to the H100 condition, the presumed maintenance of muscle blood flow in the C100 condition would have actually enhanced HCO_3^- availability, thus increasing proton buffering activity. Results from the multiple regression analyses indicate that HCO_3^- played a role in predicting TTE in H80, H100, and C100 since it was a significant contributor when predicting TTE in these trials and was the most prominent dependent variable in predicting TTE.

Our results paralleled research by McKenna et al (38) in which [HCO_3^-] decreased throughout maximal cycling exercise. The current results clearly demonstrate a relationship between the ability to utilize HCO_3^- and the maintenance of pH closer to resting levels, which ultimately is associated with enhanced performance demonstrated by a longer TTE in 80% trials with a concomitant lower change in blood pH throughout these trials. In addition, the continued decline in [HCO_3^-] during the 3-min recovery period was similar to that observed by McKenna et al (38), suggesting that HCO_3^- buffering prevented further declines in pH during the recovery period. Although we did not continue to monitor VE during recovery, it likely remained elevated allowing for HCO_3^- buffering to continue. In the present study, HCO_3^- and LA were inversely related during the recovery period as LA continued to increase while HCO_3^- continued to decrease, a result that is similar to the findings of McKenna and colleagues (38). The results from the multiple regression indicate that HCO_3^- played an important role in TTE; in fact, HCO_3^- was found to be a significant contributor to TTE in H80, H100, and C100, The ANOVA results indicated that HCO_3^- responded differently as a function of intensity

and environment, and may have differentially impacted the maintenance of cellular pH; and, these results tend to be in line with those of the multiple regression. For example, HCO_3^- was lower in H80 and C80 compared to H100 and C100 at EXH when the pH was higher than in the 100% trials. This further supports findings that the ability to utilize the bicarbonate buffering system can delay muscular fatigue by preventing significant changes in muscular pH.

Lactate Responses

Lactate has long been considered a metabolic factor associated with muscular fatigue, although recently, it has been realized that this molecule does not play a causal role. The early assumptions were based on the presence of high LA concentrations following exhaustive exercise, but failed to show a cause-and-effect relationship between LA accumulation and muscular fatigue. This lack of a causal relationship became particularly apparent once the work of Brooks and others (15, 16, 55) showed that LA is an oxidizable substrate that can be used by active and inactive tissues not only during recovery, but also during exercise itself. In the present study, we hypothesized that LA would be different at exhaustion in all trials; however, LA was similar at exhaustion for all experimental trials. Based on significant intensity by time and environment by time interactions, LA was, however, significantly greater both as a function of higher intensity and the hot environment at REC.

When measuring blood LA, there is a time delay that needs to be accounted for due to the transit time of LA being transported from the cell, where it is produced, to the blood. Based on this transit delay, we can assume that the LA level at REC is indicative of the level within the cell at EXH. Therefore, even though H80 and C80 were

significantly longer exercise trials that resulted in a greater total work output, the higher LA levels in H100 and C100 were likely due to a greater rate LA production combined with a lower rate of removal compared to the H80 and C80 trials. The greater rate of production was obviously due to an increased rate of glycolysis since it is the primary metabolic pathway providing ATP during high intensity exercise. The short duration of the H100 and C100 trials likely reduced the time available for clearance of LA in active and inactive tissues, thus contributing further to the imbalance between production and removal.

The significantly higher LA levels at REC when comparing H80 and H100 to C80 and C100 are consistent with the findings of Parkin and colleagues (48) who examined muscle metabolism during submaximal exercise in hot, neutral, and cold environments. They reported that LA was significantly elevated in exercise in the hot compared to neutral and cold environments. In contrast, research by Starkie et al (57) and Maxwell et al (37) failed to find differences in LA when comparing muscle metabolism following exercise in hot and cold environments. Starkie and colleagues (57) examined the influence of hot (HL) compared to cold (CL) water-perfused cuffs, on [LA] and glycogen utilization. The results indicated that increases in muscle temperature were associated with increased glycogen utilization; however, there was no difference in [LA] between the two trials. Maxwell et al (37) compared exercise trials in hot and cold environments while varying the temperature utilized during a warm-up period to produce three conditions: 1) cold/cold (CC), 2) cold/hot, and 3) hot/hot, where the first environment is that of the warm-up and the second represents the experimental exercise environment. The experimental protocol included a 20-min exercise/stretching warm-up followed by

repetitions of 20-s sprints, with 100-s recovery, at increasing speed until volitional exhaustion. Performance was significantly higher in the CC trial than in the CH and HH trials. Rectal and mean skin temperatures were also significantly higher in the hot experimental trials compared to the cold only trial. Despite these different temperature and performance results, there were no significant differences in LA between conditions. Therefore, the conclusions from these studies fail to support the findings from the present investigation; however, this could be due to the time point at which the blood was analyzed. As previously mentioned, blood LA at the recovery time point is indicative of the concentration within the exercising muscles at EXH, whereas the LA concentration at EXH is indicative of the LA level prior to fatigue based on the concept of a transit delay between production and clearance.

The multiple regression analysis using LA at EXH supports the lack of significant differences in LA at EXH detected by the ANOVA. Lactate at EXH was only a significant predictor for TTE during the H80 and C100 trials. It is possible that if we had used REC LA in the multiple regression analysis we might have seen a different predictive value of LA. Regardless of the statistical analysis and its interpretation, elevated LA at either EXH or REC is most likely simply associated with fatigue and not a causal factor. When exercise intensity is high leading to greater dependence on glycolysis, or when removal is lower due to environmentally-induced changes in blood flow that might impact removal, greater LA accumulation tends to occur.

Potassium Responses

Potassium (K^+) was significantly higher at exhaustion in both hot compared to both cold environments; however, the post-exercise K^+ elevations were much lower than

those reported in the literature. For example, recent studies have observed marked elevations in K^+ following exercise; in some cases K^+ can increase to 12 mmol/L within skeletal muscle (40) and 7 mmol/L in venous blood (58). In the present study, K^+ was 3.7 at baseline and increased to 4.04 mmol/L at EXH. The blunted levels in the present study compared to those reported in the literature are likely due to the fact that we corrected for plasma volume changes. Intense exercise causes significant hemoconcentration that would exaggerate the increase in the extracellular K^+ levels. Absolute $[K^+]$ were 5.19 mmol/L at EXH, which is substantially greater than the corrected K^+ values and comparable to the literature values.

Regardless of the absolute levels based the presence or absence of plasma volume corrections, of greater relevance to the questions addressed in this study, numerous previous studies have suggested that K^+ plays a role in muscular fatigue (6, 27, 38, 40, 48, 58, 62). It is believed that increases in $[K^+]$ during exercise lead to decreased membrane excitability, resulting in reduced force production (38, 43, 58). In the present study, however, the ANOVA results showed that K^+ was significantly higher in the hot vs. cold trials at EXH only, and multiple regression analysis failed to indicate the contribution of K^+ as a factor in TTE. Potassium was excluded in the final analysis for predicting TTE for all four experimental trials. These results suggest that K^+ is not a good predictor of TTE and is unlikely to play an important role in muscular fatigue under the conditions examined in this investigation.

An additional issue that is of interest relative to K^+ metabolism is the time course of post-exercise recovery. In the present study, K^+ declined to ~3.4 mmol/L during the 3-min recovery, which is similar to the K^+ kinetics reported in the literature; however, there

is controversy as to the recovery kinetics of K^+ (43, 58, 62). Previous researchers have observed fast recovery of K^+ to baseline following exercise. Wasserman and colleagues (62) reported that K^+ concentrations were lower than their resting value in the 1-2 minute recovery period, after which complete K^+ homeostasis was achieved following 5-minutes of recovery. Also, Nielsen et al (43) observed rapid recovery of $[K^+]$ in which interstitial K^+ reached a resting level after 4.5 minutes. On the other hand, Harmer and colleagues (27) reported a slower return of K^+ homeostasis following a supramaximal cycle test to exhaustion with K^+ returning to values similar to that of rest between 5 and 20 minutes of recovery. Our data agree with those studies in which a rapid recovery was reported, and it is possible that had we sampled more frequently and for a longer recovery period, we would have observed the same profile as reported by Wasserman et al (62).

Core Temperature

Core temperature (Tc) responses during the experimental trials were consistent with those reported in the literature. In response to high intensity exercise, elevations in Tc up to 41.0° C have been reported following exercise in hot vs. cold environments, resulting in an accelerated onset of fatigue (37). Our Tc results parallel research by Drust et al (19) and Maxwell et al (37), in which repeated sprints in hot vs. cold environments lead to significantly greater elevations in Tc in the hot environment compared to the cold environment.

Of importance to our primary question, Tc was significantly higher following exercise in H80 and C80 compared to H100 and C100; thus, lower intensity combined with a longer duration produced the greatest heat storage. The failure to observe higher Tc during the H100 and C100 trials does not parallel research in which Tc is elevated

when exercising at greater workloads. However, most of these studies compared relatively low workloads, between 50-70%VO_2max, over longer periods of time. These lower workloads can be sustained for a significantly longer duration, resulting in a greater impact of intensity on Tc. The current study, however, consisted of relatively shorter trials in which the lower intensity (80%) trials were significantly longer than the higher intensity (100%) trials, resulting in a slower rate of change in Tc, but a greater absolute change compared to the high intensity trials.

The primary explanation for the present findings is that the greater duration of the 80% compared to the 100% trials allowed for the development of a greater imbalance between heat production and heat removal. When analyzing any heat accumulation data it is important to take into account both the work rate, which was constant in this study, and the total work produced since a greater total work is also associated with a greater total amount of heat production. Because of the longer TTE in the 80% trials, and the longer TTE in C80 compared to H80 subjects completed different amounts of total work when comparing the various conditions. The identical Tc at EXH in the H80 and C80 condition was a result of a more effective thermoregulatory response in the C80 condition that eventually was offset by the substantially greater heat production associated with the longer TTE.

Rates of heat gain of 0.15-0.20 ^{o}C/min have been reported during high intensity exercise in the heat (39). The present study showed higher rates of heat gain during exercise at 100% vs. 80%, and as expected, the rate of heat gain was significantly higher when exercising in the hot vs. cold environments. Specifically, the rate of heat gain was 0.13, 0.01, 0.22, and 0.13 ^{o}C/min for H80, C80, H100, and C100, respectively. Clearly,

exercising at a higher intensity lead to a greater rate of heat gain, even though H100 and C100 resulted in significantly lower Tc at exhaustion compared to H80 and C80. Although the rates of heat gain in the 100% trials were greater, other factors such as pH, possibly curtailed exercise before greater heat storage could occur. As mentioned earlier, pH was significantly lower in the 100% trials, with no significant difference between H100 and C100. The results from the multiple regressions also indicate that Tc played a role in performance in the hot trials, but not in the cold trials. Even though temperature is a contributor to fatigue in the hot environments, the regression shows that it is not the only factor since pH and HCO_3^- are also predictors of TTE during these trials.

Finally, research by Drust et al (17) and Nybo et al (45) suggests that an absolute elevation in core Tc might be responsible for central fatigue. The hypothesis is based on the idea that fatigue occurs at a specific Tc due to impairments of the CNS to activate the motor units, resulting in lower force production and the inability to maintain the required workload. Drust et al (17) reported that, despite beginning exercise at different Tc, exhaustion occurred at the same Tc and muscle temperature for all trials. In addition, time to exhaustion was significantly shorter during the higher than during the lower rate of heat storage, which also parallels results from the current study. Nybo and colleagues (46) observed that hyperthermia was not associated with impaired muscular function performance; rather, impaired central activation accounted for the reduction in maximal voluntary force development during prolonged isometric contractions. Although we do not have data to address the central fatigue issue, we did not identify a specific Tc, either in absolute terms or expressed as a change in temperature, at which fatigue occurred. Therefore, based on the ANOVA results, our hypothesis stating that Tc would be greater

following exercise in a hot environment was supported. The other hypothesis, however, which stated that Tc would be significantly elevated in response to H100 and C100 compared to H80 and C80 was not supported. As previously stated, these findings are likely due to the duration of the 80% trials that resulted in a greater total workload completed compared to H100 and C100. Therefore, Tc likely played a more important role in the longer, less intense trials while pH and other metabolic factors were more important during the shorter, more intense trials. The multiple regression results tended to support the ANOVA since Tc was included in the final prediction for H80 and H100; however, it was only a significant predictor for TTE in H100.

Threshold Analysis

The preliminary data indicated that the pH threshold (pHT) and bicarbonate threshold (BCT) occurred simultaneously, at a greater exercise intensity compared to the lactate threshold (LT). The individual thresholds occurred at 76.05, 81.41, and 81.8% VO_2max for LT, pHT, and BCT, respectively. Numerous studies have evaluated LT as it corresponds to exercise intensity, but few have examined the relationship between LT and pHT. In a recent review, Cairns (16) described research indicating that a pHT exists based on the muscle pH and force relationship whereby a critical pH was identified that significantly impacts force production (16). The pHT can be defined as a level of work above which muscle fatigue associated with a decrease in tissue pH occurs (28).

In the present study, LT was determined using the standard method of an increase in [LA] of 1 mmol/L with a subsequent increase in exercise intensity. Because of the scarcity of research dealing with pHT and BCT, there is not a standard method of determining these thresholds; thus, in order to arrive at an estimation, graph deflection

points have to be observed. Based on this method, there is obviously substantial room for error. Nevertheless, in the present study, pHT and BCT were determined on an individual basis and expressed as a function of exercise intensity. It appears that the pHT corresponded to a reduction in pH of approximately 0.05 units. Our results support research by Iwanaga et al (28) in which the work rate and the percentage of VO_2max at pHT were significantly greater than LT. These results indicate that pH and HCO_3^- can remain near resting levels, even in the presence of increasing LA; suggesting that LA is not the only factor contributing to H^+ accumulation, resulting in reduced pH and muscular fatigue. The current data also suggest a strong relationship between pH and HCO_3^- in which the inability to utilize HCO_3^- for further buffering results in lower pH. The BCT appeared to take place at a level corresponding to a reduction in bicarbonate of 3.75 mmol.

The fact that LT was less than the other thresholds, and if pH and HCO_3^- are metabolically more significant than LA, it may be that the measurement of these factors as a function of exercise intensity would provide a more accurate assessment of critical performance intensities. That is, LT may provide a lower relative intensity at which prolonged steady state exercise can be maintained than would be determined by using either pHT or BCT. The vast research on LT supports its use as an accurate predictor of sustainable intensities; however, the possible value of pHT and BCT should not be overlooked.

Strengths and Limitations

A number of factors associated with this study and its design can be considered as strengths of the study that enhance the interpretive value of the data. The current study

was conducted in a repeated measures, counterbalanced order. In addition, subjects performed an acclimation trial prior to the experimental trials to prevent a learning curve between trials. Also, all subjects were highly trained, endurance athletes accustomed to cycle ergometer exercise. Finally, subjects were instructed to abstain from strenuous physical activity for 24-h prior to the trials and to maintain a similar dietary composition during the same time period. These controls were an attempt to maintain consistency between trials and prevent extraneous variables, such as glycogen content and muscle soreness, from playing a role in exercise performance between trials.

Despite the aforementioned strengths, there are a number of factors that can be considered weaknesses. Limitations of the current study include the lack of muscle pH measurements, compared to blood pH, that would clarify the relationship between the multiple factors involved in muscular fatigue. Measurement of muscle temperatures would also improve the assessment of causal factors since elevated tissue temperatures may impair contractile function. In addition, continuation of more frequent blood sampling during the recovery period would allow for us to elucidate more clearly the rate of recovery for pH and other variables. Furthermore, it would allow us to clarify the controversy that currently exists in the literature for the recovery of K^+ to resting levels. A final factor that must be considered is that the study was conducted at a time of year when the subjects were not acclimated to the heat. Heat acclimation may have produced different responses than those observed in the present study.

Conclusion

Many factors play a role in muscular fatigue, depending on the intensity and environmental conditions. The current investigation observed differences for pH at

exhaustion between all trials, implying that pH is not the only factor playing a role in muscular fatigue. Furthermore, this is supported by the multiple regression results in which pH was only a significant predictor for fatigue in C100. In addition, LA is unlikely a major contributor to decrements in performance based on the differences observed at REC. This suggests that fatigue occurred regardless of variations in [LA]. Furthermore, the present study failed to show any relationship between K^+ and muscular fatigue. Finally, it seems that there is a strong relationship between HCO_3^- and pH. The ability to utilized HCO_3^- resulted in a prolonged TTE while the inability to consume HCO_3^- lead to lower pH and a decreased TTE, as observed in C100.

Thermal stress also plays a role in muscular fatigue. In hot environments, pH was significantly lower at REC while HCO_3^- was lower for all time points, except EXH. Also, LA was higher in hot vs. cold during 80% at 5-min and EXH, supporting current research in which exercising in a hot environment results in higher rates of glycolysis leading to greater elevations in LA, and associated decreases in pH, compared to exercise in cold and/or neutral environments.

Practical Applications

The results of this study suggest that pHT is a better testing variable compared to the widely used LT to predict performance. Many coaches test individual LT in order to assess fitness and develop a program specialized for a given athlete. The present results, however, indicate that utilization of this testing method might not optimize performance due to the greater workload observed at pHT compared to LT. In addition, relative threshold intensities should be determined by the workload that can be maintained for a prolonged period of time, especially when testing endurance athletes. Finally, results

from the experimental trials can be used by the athletic community to guide coaches and athletes by showing the relationship between the primary factors associated with muscular fatigue and how they vary depending on the environmental condition and exercise intensity. For example, a program designed for a sprinter should take into consideration factors such as pH and HCO_3^-, whereas endurance athletes have other factors, such as glycogen depletion, change in Tc, and hydration, that are more likely to be associated with fatigue. Finally, coaches and athletes should also consider the changes in relative intensity as it relates to exercising under various ambient temperatures. For example, relative intensity, as expressed in terms of $\%VO_2max$ is higher when exercising in hot environments. Therefore, relative to their threshold values (LT, pHT, or BCT), athletes could exercise at a lower absolute intensity in a hot environment, and training-wise, could receive the same benefits as a higher intensity workout in a neutral environment. Considering these applications will allow the athlete to continually improve without overtraining.

Future Directions

Future research should be conducted to:

1) examine the role of HCO_3^- and pH to solidify the relationship between these variables and performance;

2) compare the relationship between H^+ accumulation, pH, HCO_3^-, and LA in response to high intensity exercise in varying thermal environments;

3) observe the kinetics of K^+ during an extended recovery period to clarify the rate of return to homeostasis following exercise; and

4) Develop a reliable method for determining pHT and BCT that can be utilized by coaches to maximize athletic performance.

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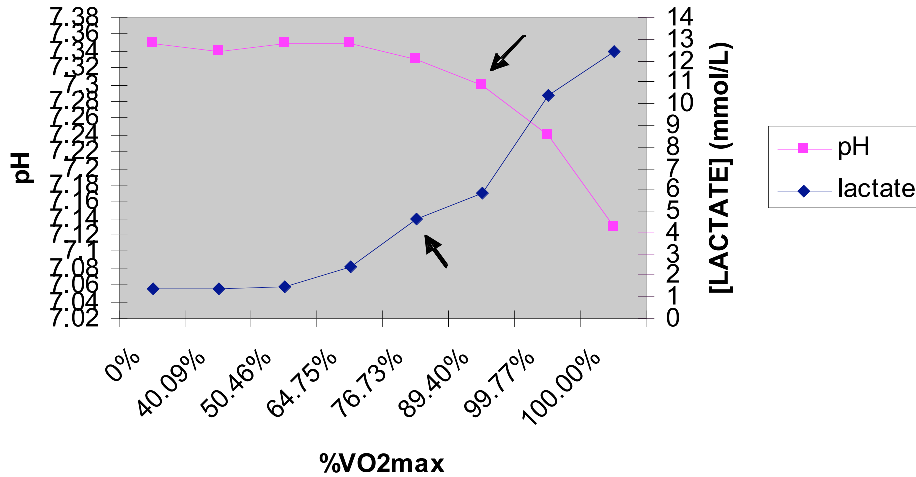
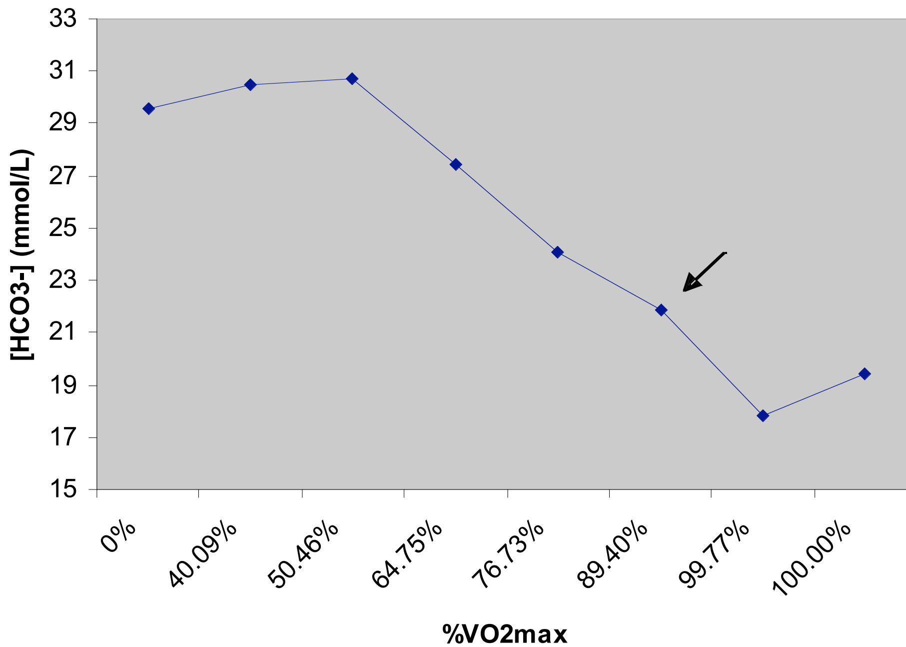
APPENDIX ONE

Subject 1

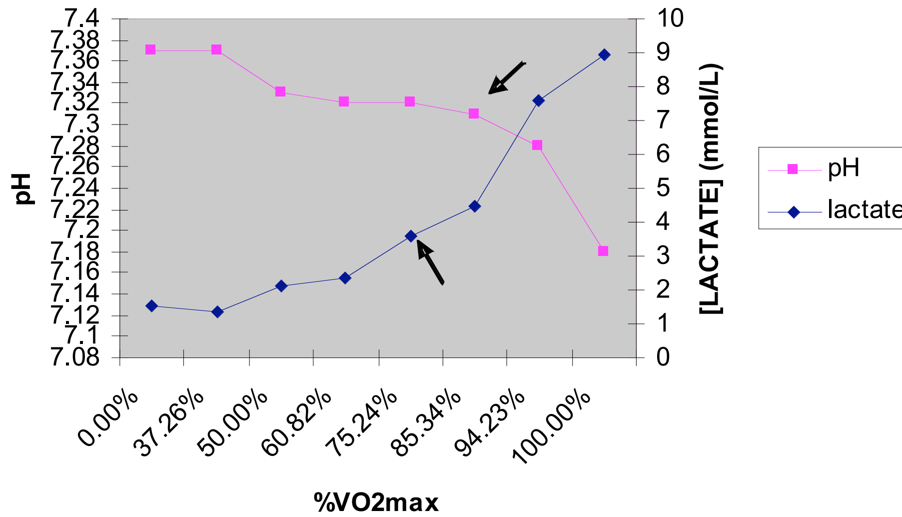


Appendix Figure 1. Lactate threshold (LT) and pH threshold (pHT) data for Subject 1. LT occurred at 76.73% VO_2max. LA concentration ([LA]) was 4.65 mmol/L at LT. pHT occurred at 89.4% VO_2max and at a value of 7.3.

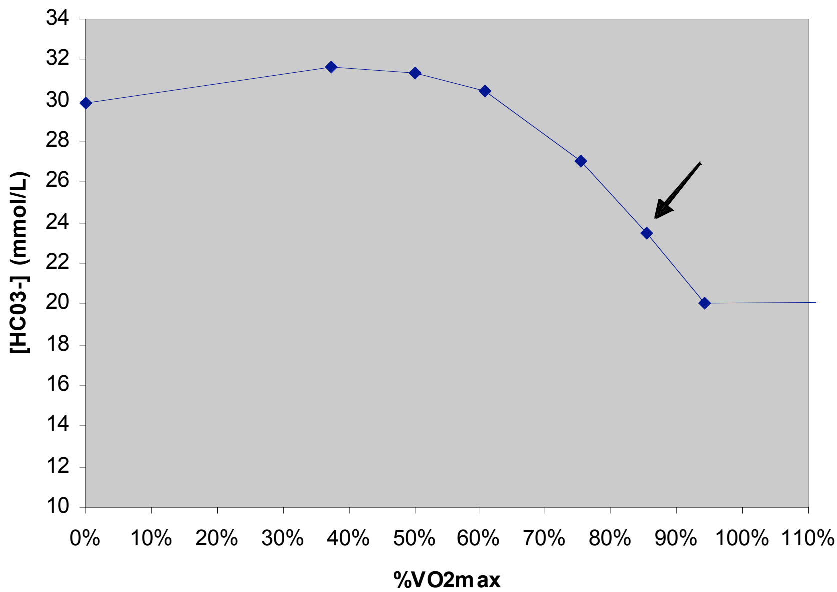


Appendix Figure 2. Bicarbonate threshold (BCT) for Subject 1. BCT occurred at 89.4% VO_2max. HCO_3^- concentration ($[HCO_3^-]$) was 22.00 mmol/L at BCT.

Subject 2

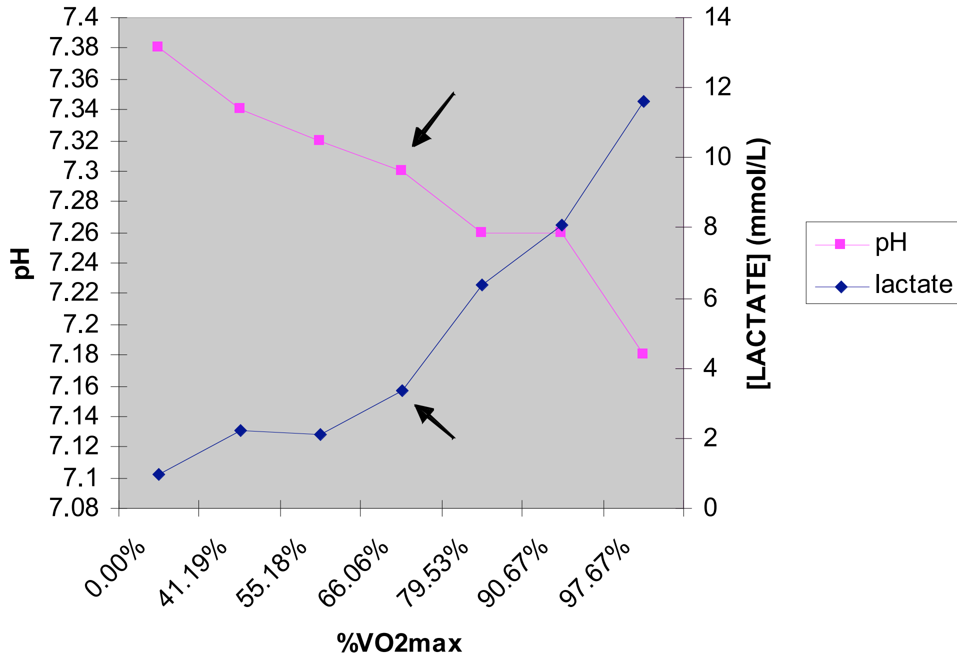


Appendix Figure 3. LT and pHT for Subject 2. LT occurred at 75.24% VO_2max. [LA] was 3.60 mmol/L at LT. pHT occurred at 85.34% VO_2max and at a value of 7.31.

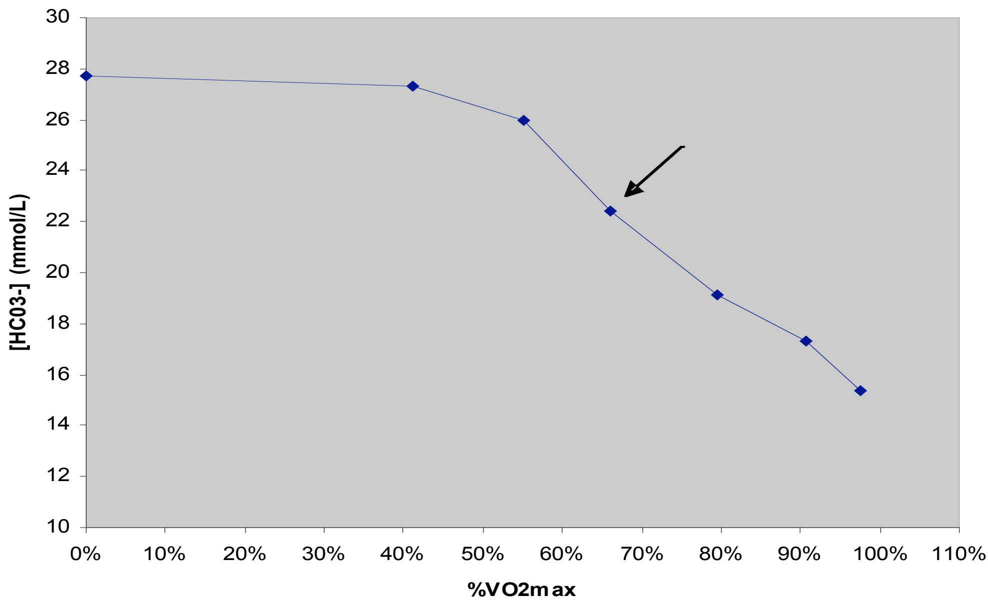


Appendix Figure 4. BCT for Subject 2. BCT occurred at 85.34% VO_2max. $[HCO_3^-]$ was 23.5 mmol/L at BCT.

Subject 3

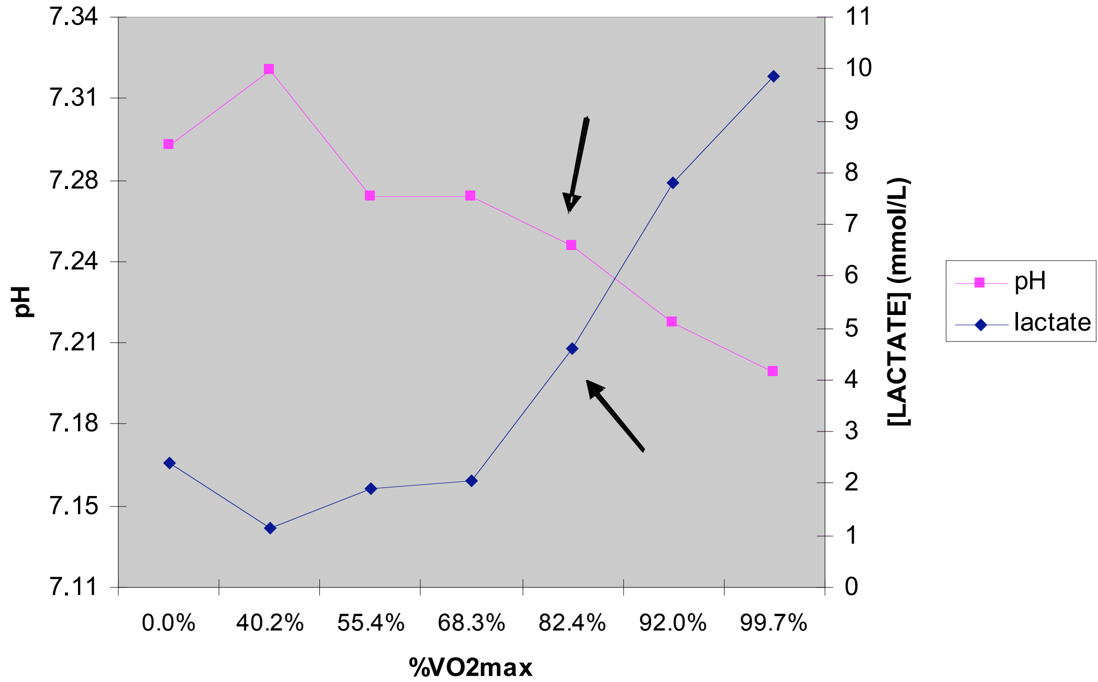


Appendix Figure 5. LT and pHT for Subject 3. LT occurred at 66.06% VO_2max. [LA] was 3.36 mmol/L at LT. pHT occurred at 66.06% VO_2max and at a value of 7.3.

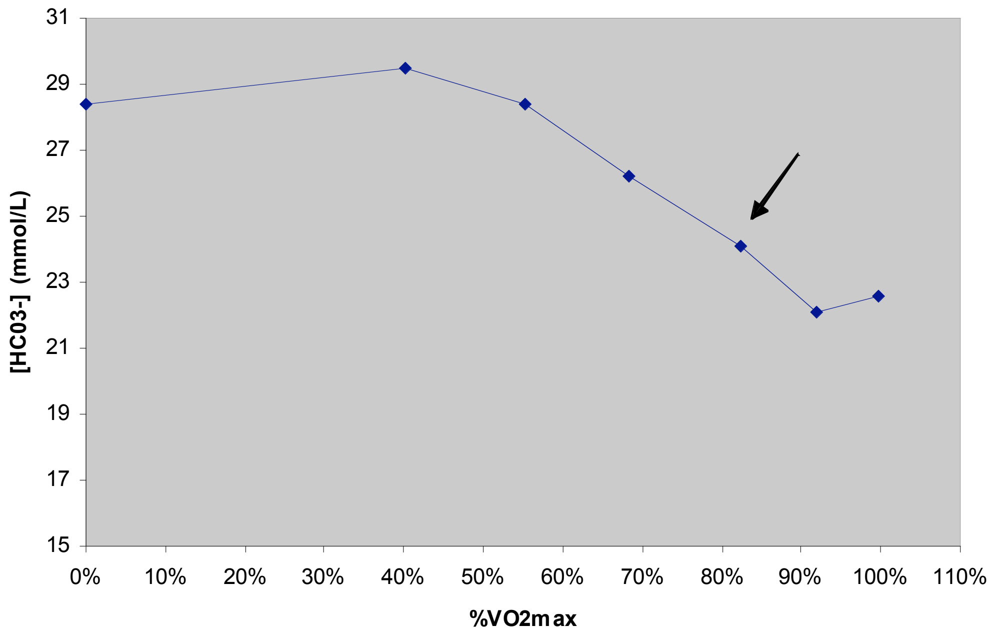


Appendix Figure 6. BCT for Subject 3. BCT occurred at 66.06% VO_2max. $[HCO_3^-]$ was 22.40 mmol/L at BCT.

Subject 4

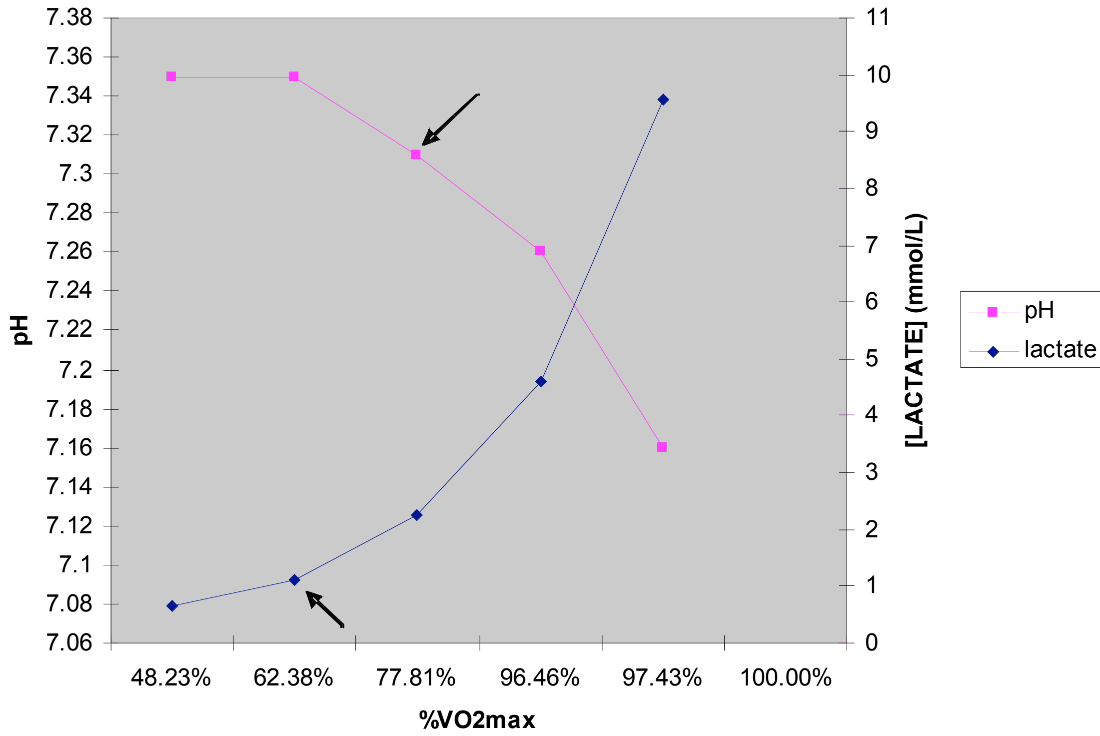


Appendix Figure 7. LT and pHT for Subject 4. LT occurred at 82.4% VO_2max. [LA] was 4.61 mmol/L at LT. pHT occurred at 82.4% VO_2max and at a value of 7.25.

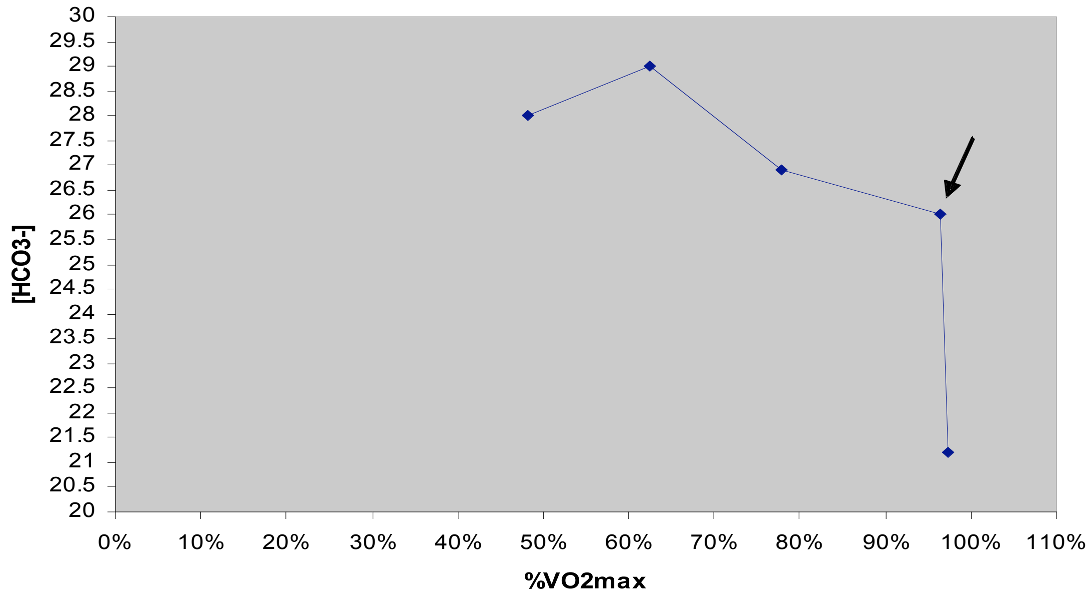


Appendix Figure 8. BCT for Subject 4. BCT occurred at 82.33% VO_2max. [HCO_3^-] was 24.1 mmol/L at BCT.

Subject 5

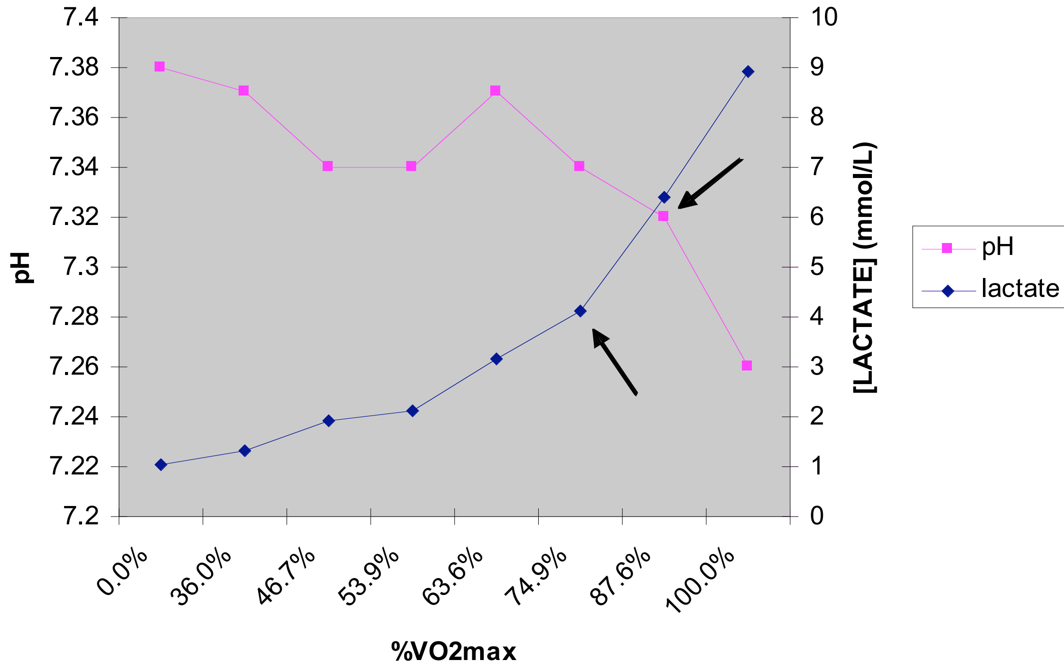


Appendix Figure 9. LT and pHT for Subject 5. LT occurred at 62.38% VO_2max. [LA] was 2.24 mmol/L at LT. pHT occurred at 77.81% VO_2max and at a value of 7.31.

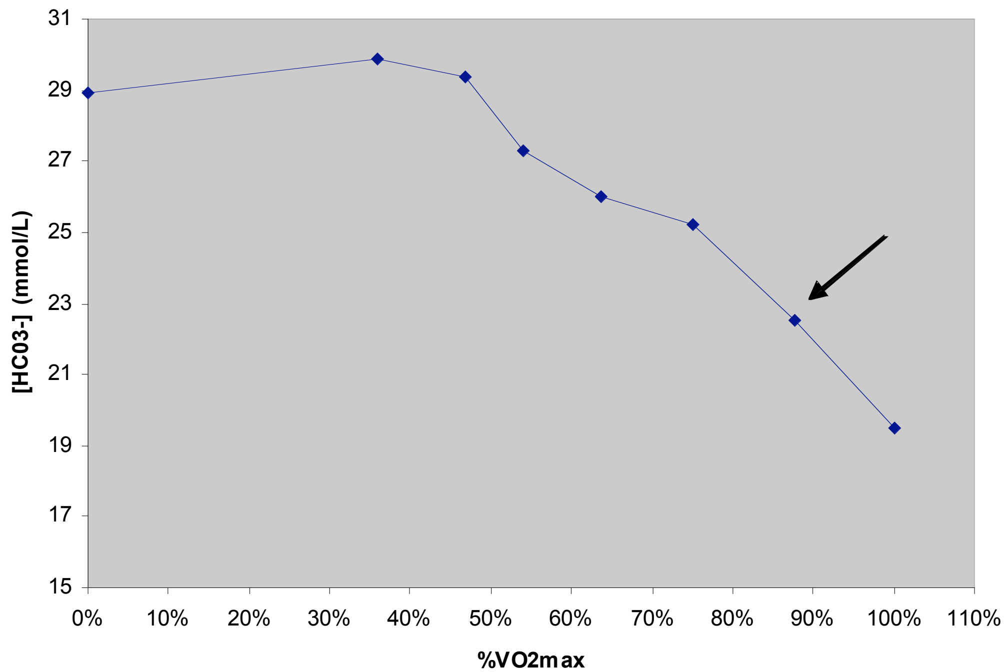


Appendix Figure 10. BCT for Subject 5. BCT occurred at 96.46% VO_2max. $[HCO_3^-]$ was mmol/L at BCT.

Subject 6

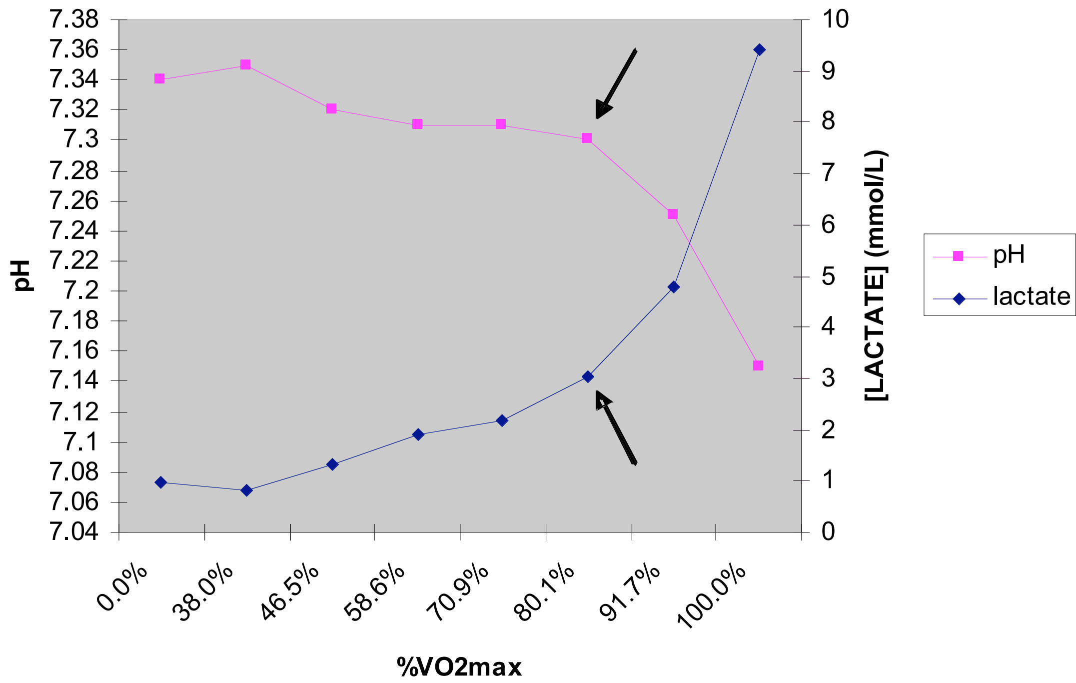


Appendix Figure 11. LT and pHT for Subject 6. LT occurred at 74.95% VO_2max. [LA] was 4.13 mmol/L at LT. pHT occurred at 87.58% VO_2max and at a value of 7.32.

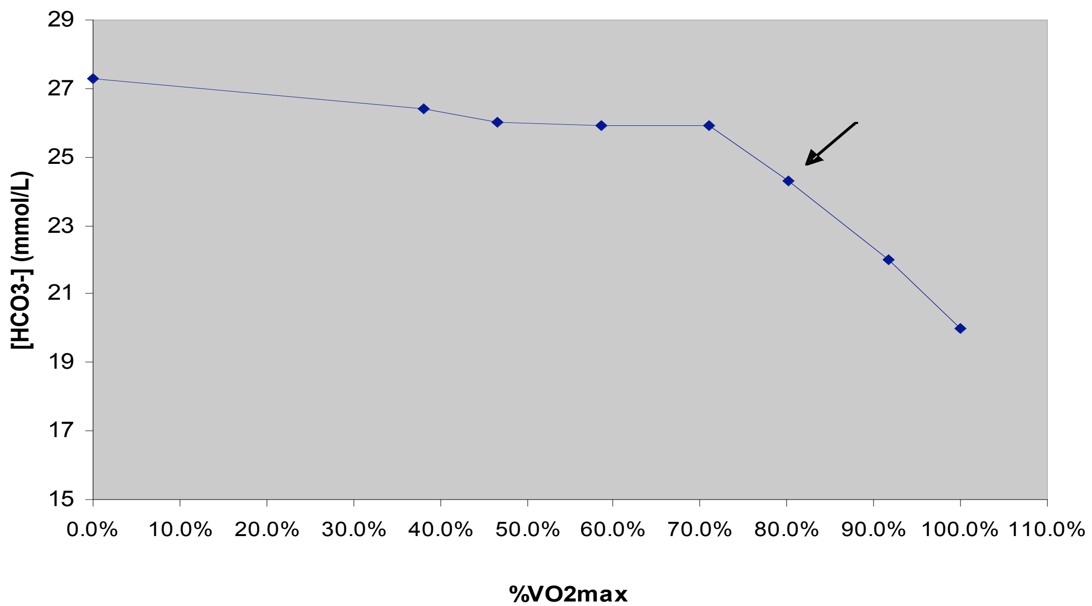


Appendix Figure 12. BCT for Subject 7. BCT occurred at 87.58% VO_2max. [HCO_3^-] was 22.5 mmol/L at BCT.

Subject 7

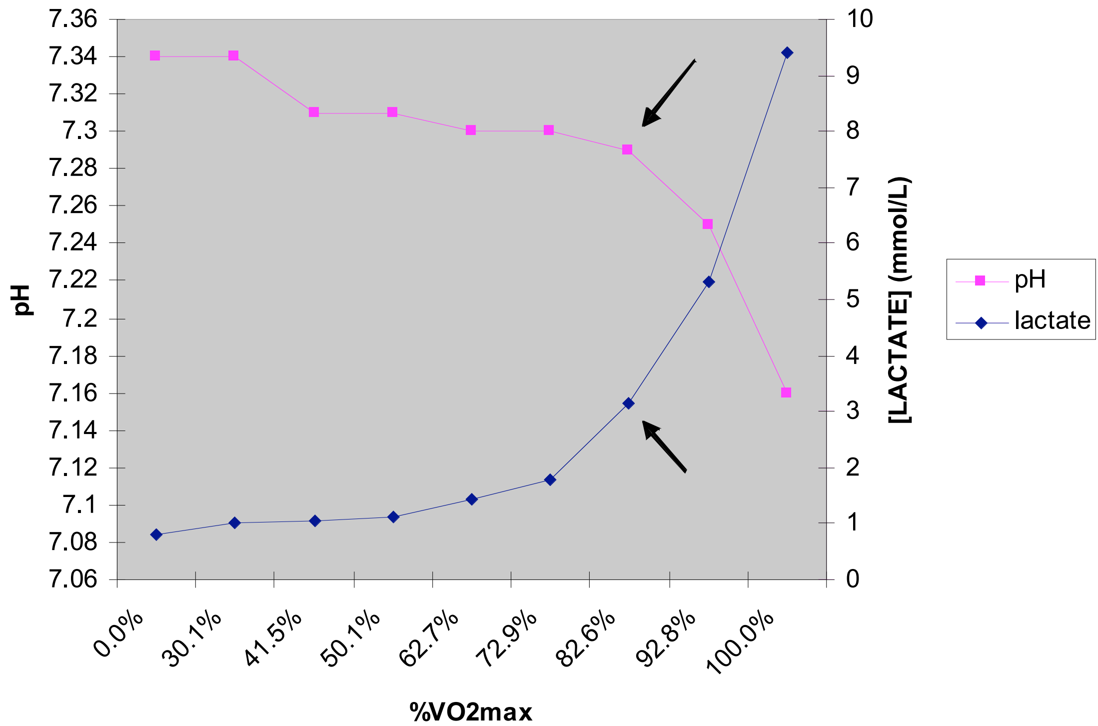


Appendix Figure 13. LT and pHT for Subject 7. LT occurred at 80.09% VO_2max. [LA] was 3.02 mmol/L at LT. pHT occurred at 80.00% VO_2max and at a value of 7.30.

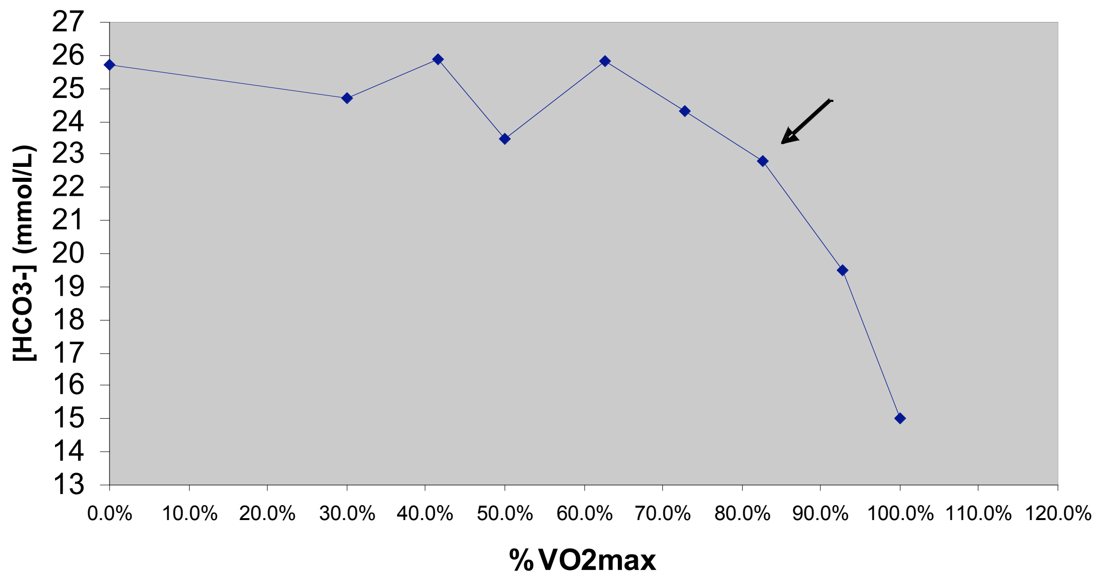


Appendix Figure 14. BCT for Subject 7. BCT occurred at 80.09% VO_2max. [HCO_3^-] was 24.00 mmol/L at BCT.

Subject 8

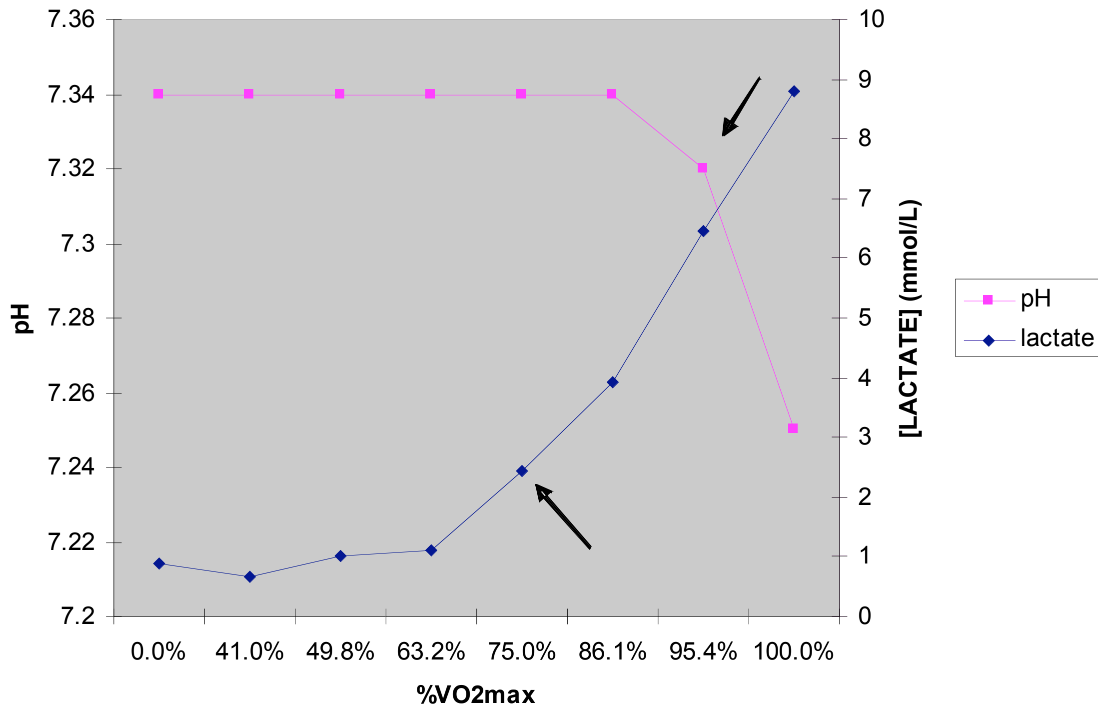


Appendix Figure 15. LT and pHT for Subject 8. LT occurred at 82.60% VO_2max. [LA] was 3.13 mmol/L at LT. pHT occurred at 82.60% VO_2max and at a value of 7.29.

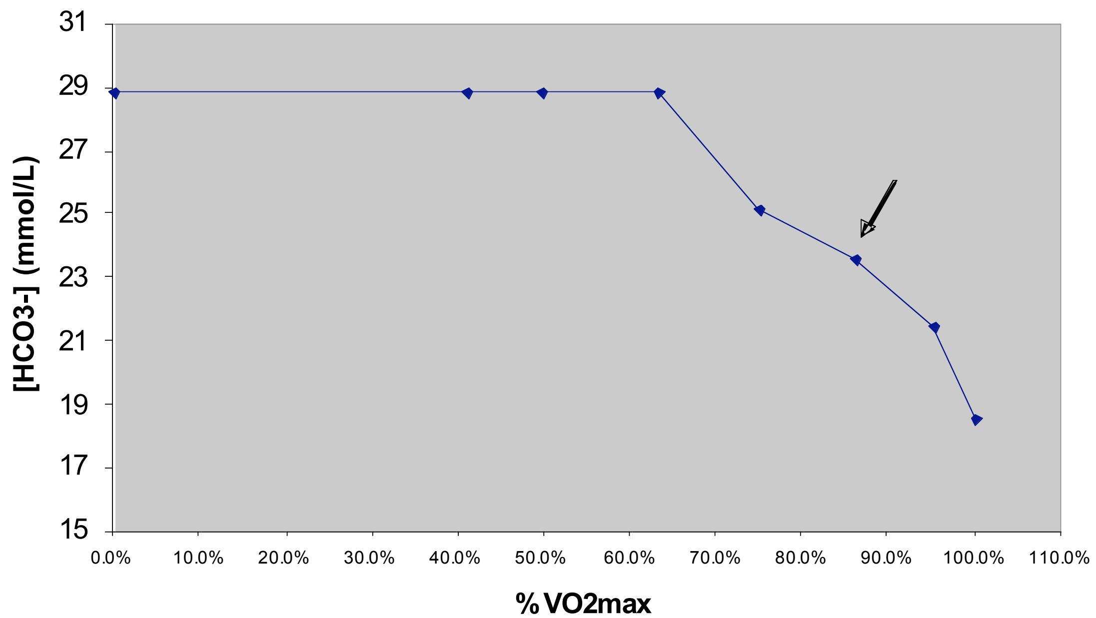


Appendix Figure 16. BCT for Subject 8. BCT occurred at 82.60%VO_2max. $[HCO_3^-]$ was 22.80 mmol/L at BCT.

Subject 9

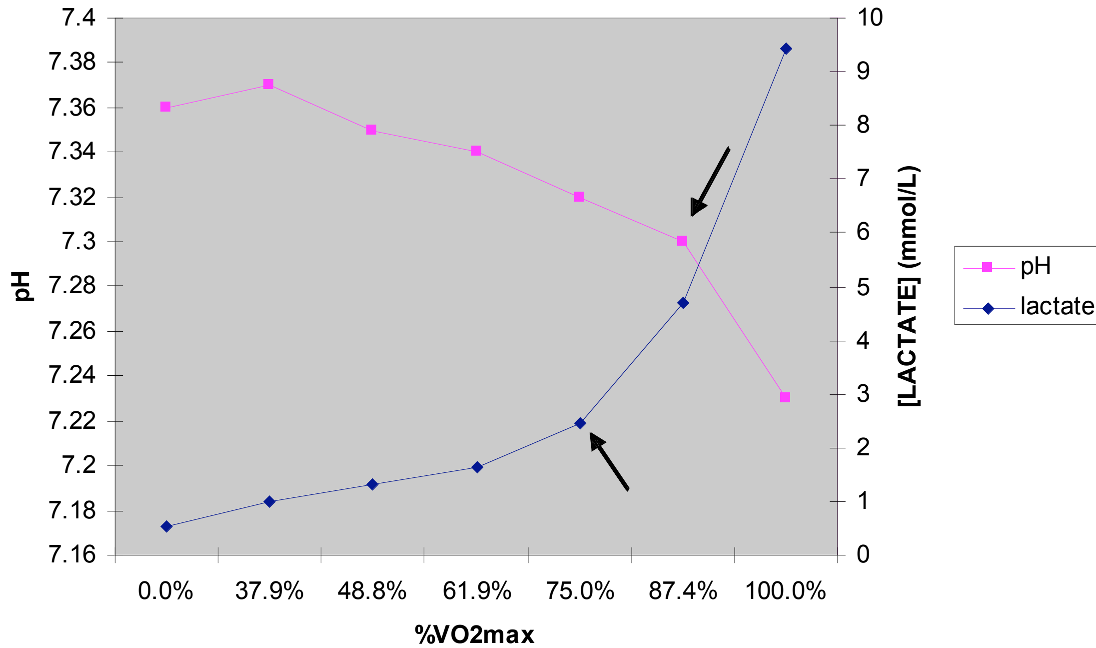


Appendix Figure 17. LT and pHT for Subject 9. LT occurred at 75.00% VO_2max. [LA] was 2.45 mmol/L at LT. pHT occurred at 95.37% VO_2max and at a value of 7.32.

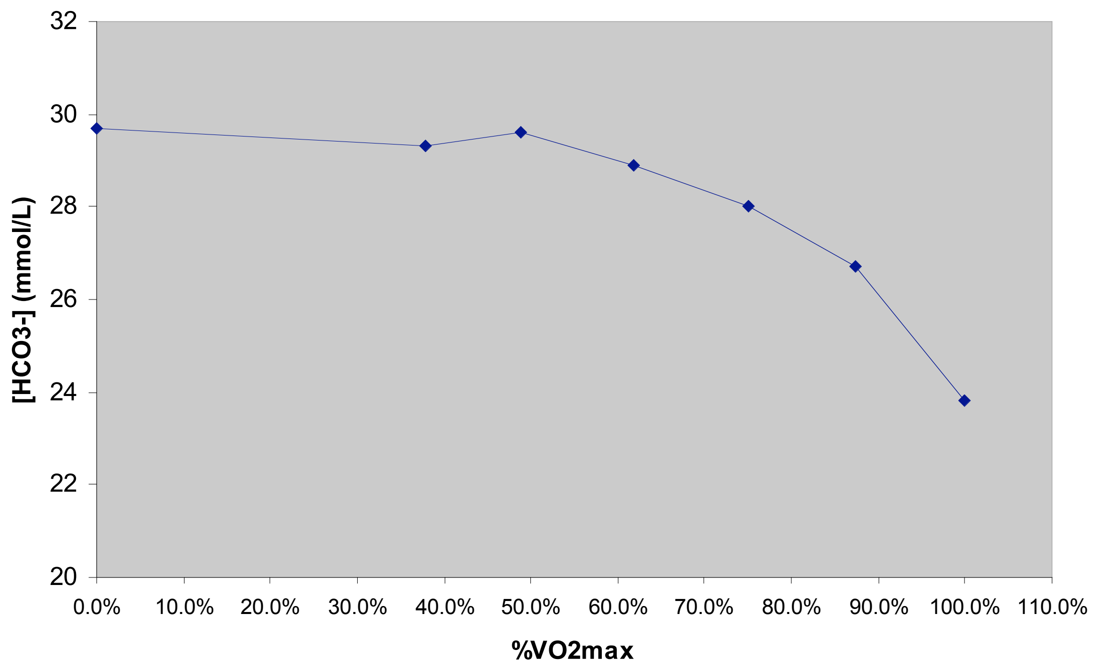


Appendix Figure 18. BCT for Subject 9. BCT occurred at 86.11% VO_2max. [HCO_3^-] was 23.60 mmol/L at BCT.

Subject 10

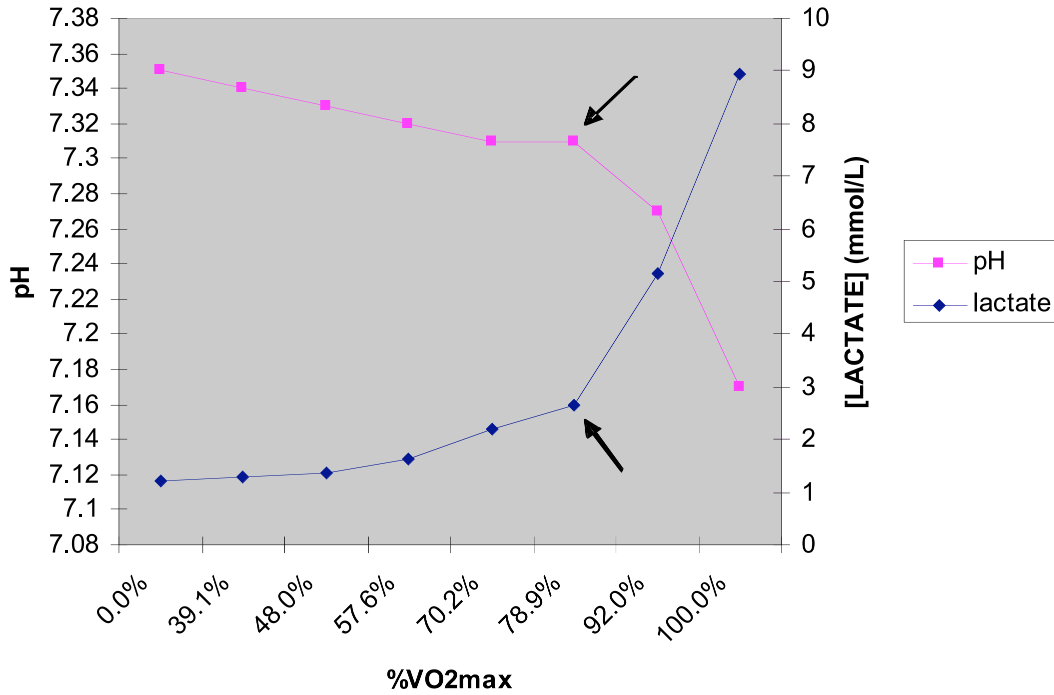


Appendix Figure 19. LT and pHT for Subject 10. LT occurred at 75.00% VO_2max. [LA] was 2.47 mmol/L at LT. pHT occurred at 87.38% VO_2max and at a value of 7.30.

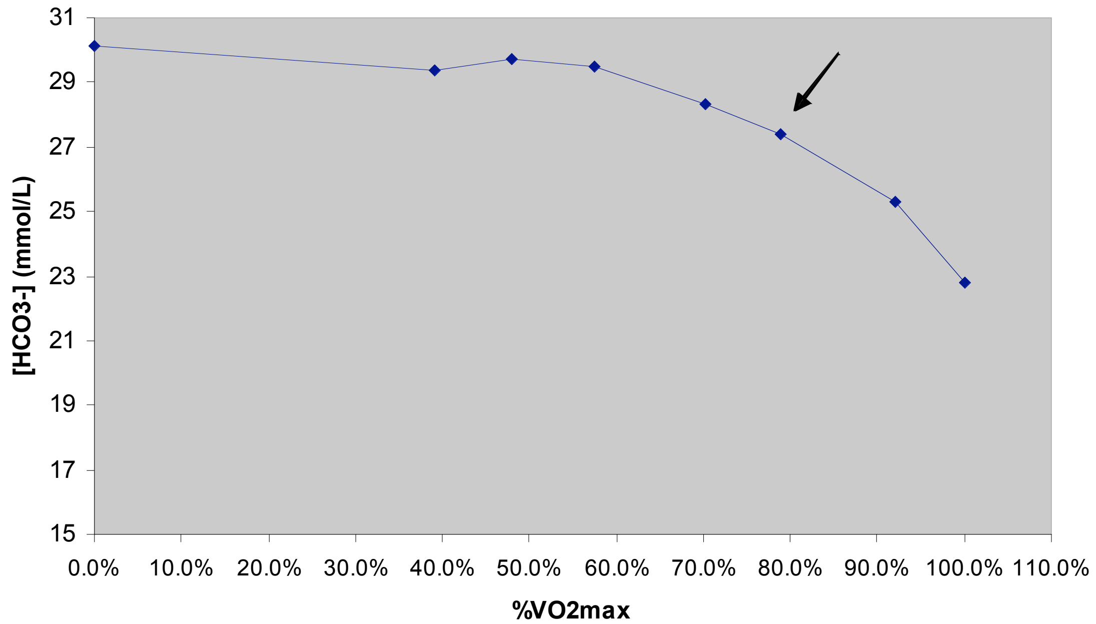


Appendix Figure 20. BCT for Subject 10. BCT occurred at 75.00% VO_2max. $[HCO_3^-]$ was 28.00 mmol/L at BCT

Subject 11

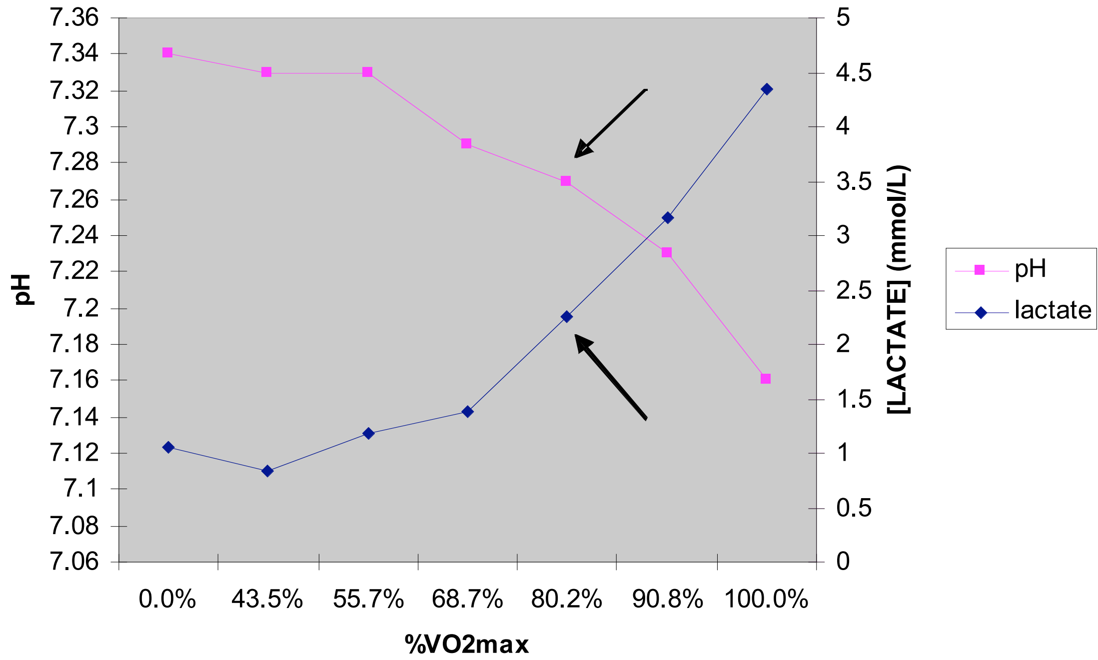


Appendix Figure 21. LT and pHT for Subject 11. LT occurred at 78.89% VO_2max. [LA] was 2.67 mmol/L at LT. pHT occurred at 78.89% VO_2max and at a value of 7.31.

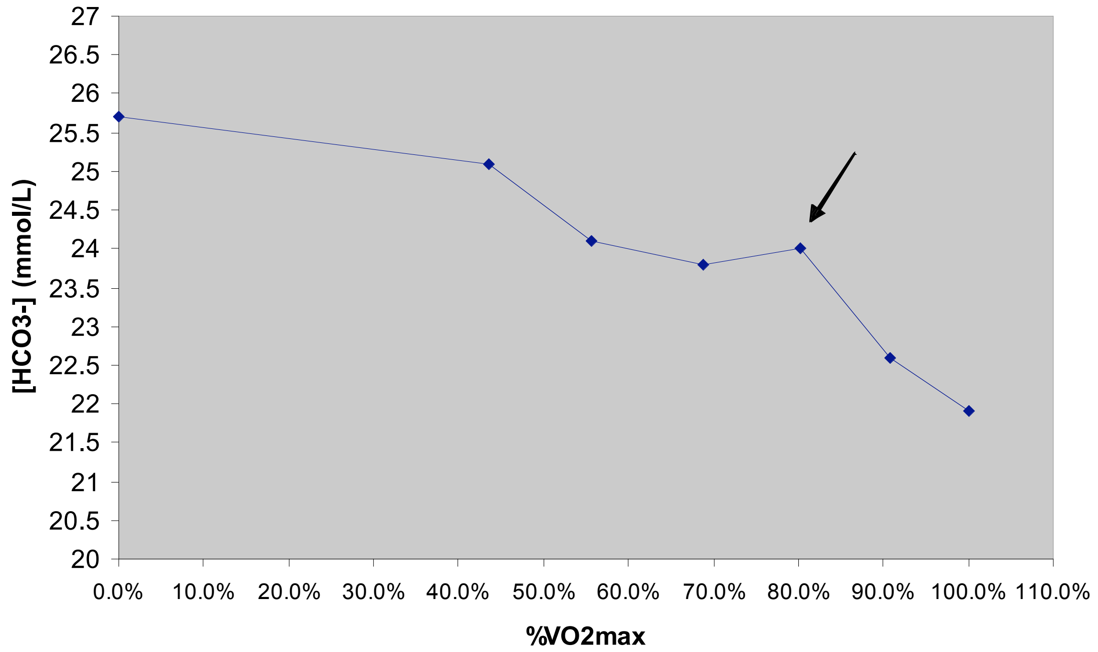


Appendix Figure 22. BCT for Subject 11. BCT occurred at 78.89% VO_2max. [HCO_3^-] was 27.4 mmol/L at BCT.

Subject 12

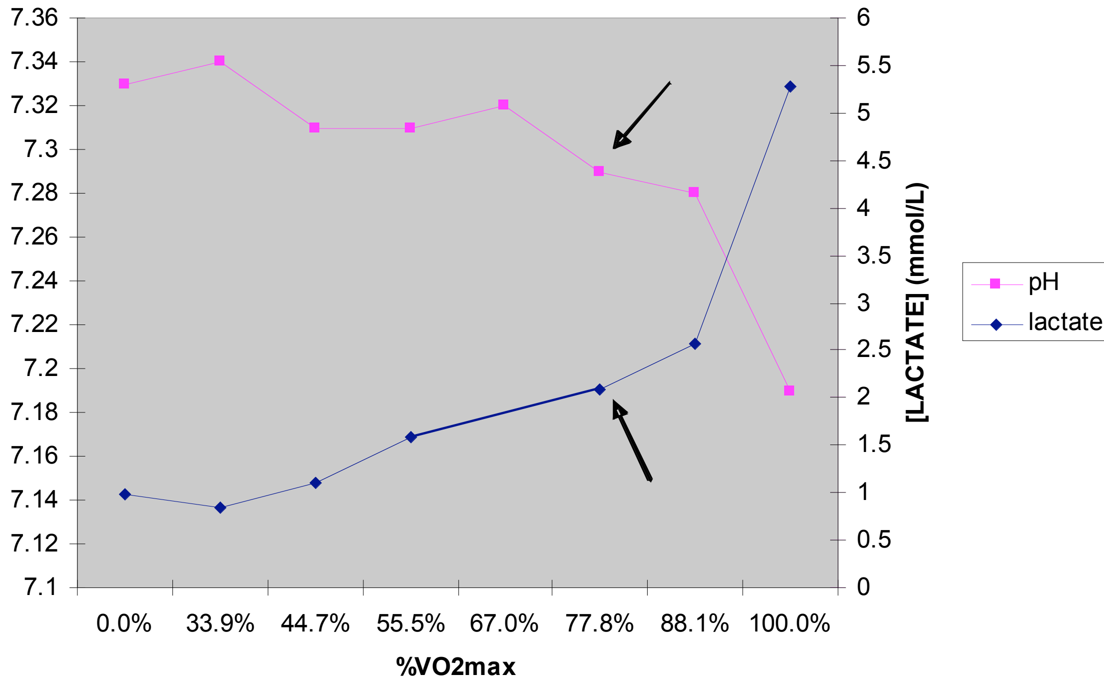


Appendix Figure 23. LT and pHT for Subject 12. LT occurred at 80.15% VO_2max. [LA] was 2.26 mmol/L at LT. pHT occurred at 80.15% VO_2max and at a value of 7.29.

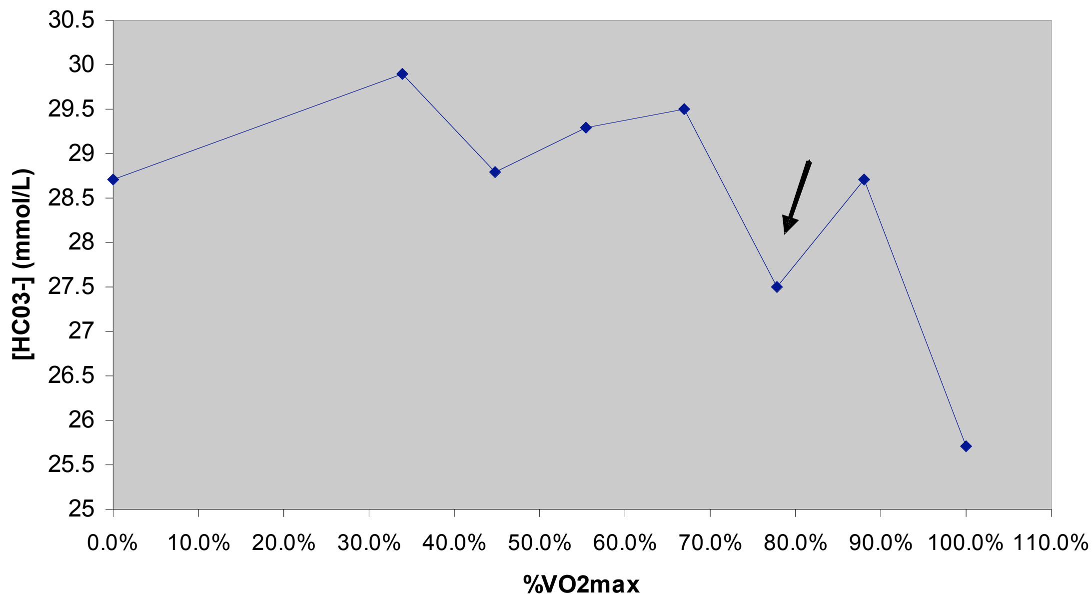


Appendix Figure 24. BCT for Subject 12. BCT occurred at 80.15% VO_2max. $[HCO_3^-]$ was 24.00 mmol/L at BCT

Subject 13

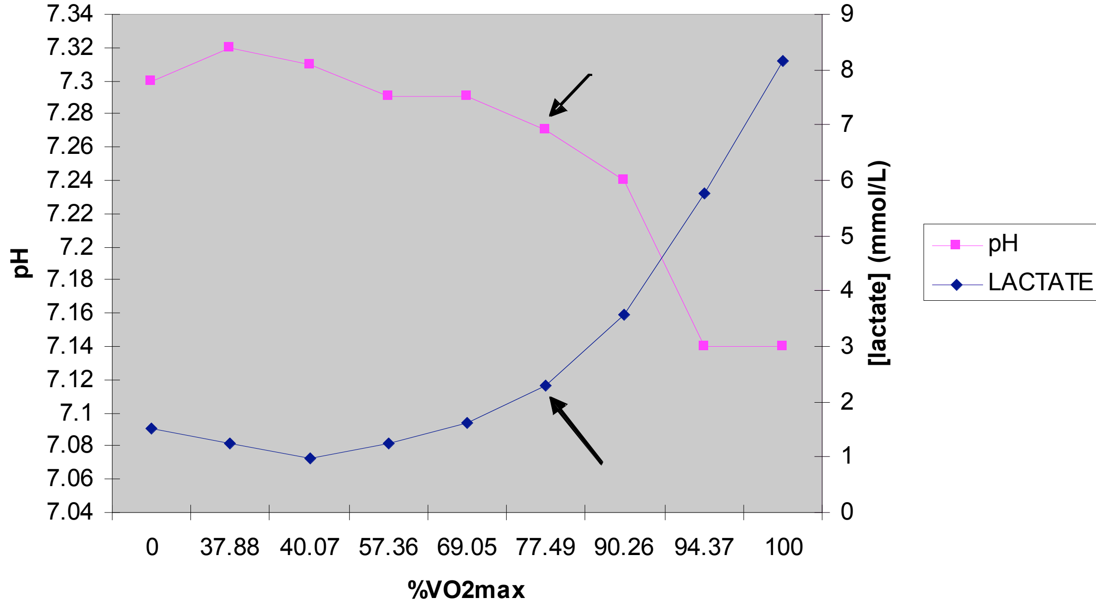


Appendix Figure 25. LT and pHT for Subject 13. LT occurred at 77.75% VO_2max. [LA] was 2.09 mmol/L at LT. pHT occurred at 77.75% VO_2max and at a value of 7.29.

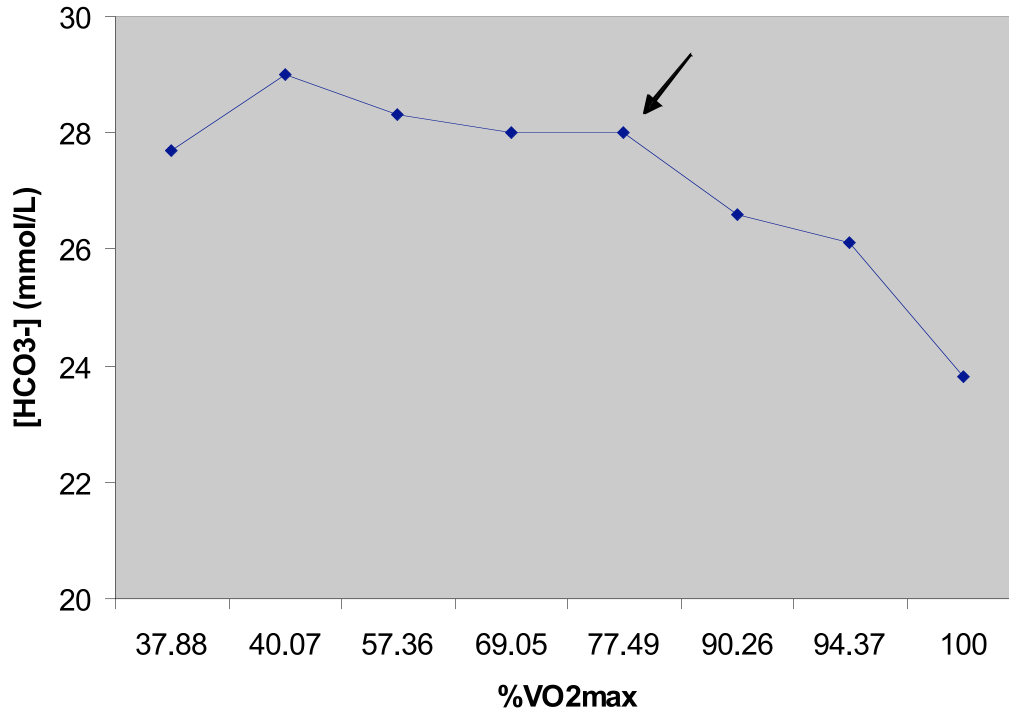


Appendix Figure 26. BCT for Subject 13. BCT occurred at 77.75% VO_2max. [HCO_3^-] was 27.50 mmol/L at BCT.

Subject 14



Appendix Figure 27. LT and pHT for Subject 14. LT occurred at 77.49% VO_2max. [LA] was 2.29 mmol/L at LT. pHT occurred at 69.05% VO_2max and at a value of 7.29.



Appendix Figure 28. BCT for Subject 14. BCT occurred at 77.49% VO_2max. $[HCO_3^-]$ was 26.60 mmol/L at BCT.

ABSTRACT

FATIGUE DURING HIGH INTENSITY EXERCISE: THE INTERACTION BETWEEN pH AND THERMAL STRESS

Melissa M. Rogers, M.S.
Department of Kinesiology
Exercise Physiology Laboratory
Texas Christian University

Thesis Advisor: Joel B. Mitchell, Ph.D.

Background: Intense exercise is associated with a high production of lactate (LA) with a concomitant elevation in acidity, whereby the pH may fall ~0.5 pH units within the exercising muscles. Exercise in a hot environment is associated with an accelerated onset of fatigue due to heat storage and the accompanying systemic and metabolic changes that occur compared to exercise in a neutral environment. While there are many factors that could lead to early muscle fatigue during exercise in the heat, research has yet to completely elucidate the interaction between metabolic and thermoregulatory factors. **Purpose:** The primary purpose of this study was to compare the effects of high intensity, time-to-exhaustion (TTE) cycling exercise (80- and 100% VO_2max) in hot (38°C) and cold (10°C) environments on pH, LA, bicarbonate (HCO_3^-), K^+, and core temperature (Tc). The secondary purpose of this study was to determine the relationship between the pH threshold (pHT), lactate threshold (LT), and bicarbonate threshold (BCT). **Methods:** Prior to data collection, VO_2max tests were performed to assess levels of aerobic fitness (VO2max > 3.5 L/min.), and to determine experimental workloads. A learning trial was performed in the hot environment at 80% VO2max prior to the experimental trials. Subjects completed four separate TTE cycling trials: 1) Hot (35°C) 80% VO2max (H80), 2) Cold (10°C) 80% (C80), 3) Hot 100% (H100), and 4) Cold 100% (C100) in a randomized, counterbalanced order. Trials were separated by a minimum of 3 days. Subjects were fitted with an esophageal probe prior to exercise in order to assess Tc. A catheter was placed in a forearm vein for blood sampling. A 5-min warm-up was completed at room temperature prior to beginning the exercise trial. Blood samples were taken at baseline, every 5-min during exercise, post-exercise, and 3-min post-exercise. A blood gas analyzer was used to measure whole blood pH, HCO_3^-, and K^+ (Radiometer, ABL77). Lactate (LA) was measured using a spectrophotometric, enzymatic assay. VO_2 (open circuit spirometry with automated gas analysis) and heart rate (telemetry-- Polar Heart Watch) data were obtained during the trials. Repeated measures 2x2x3 and 2x2x5 ANOVAs (Condition x Intensity x Time) were used to determine differences between experimental trials and Newman-Keuls post hoc analyses were used to determine where the differences occurred between the trials. Alpha was set at $P < 0.05$. Backwards, step-wise multiple regression was used to predict TTE using multiple dependent variables as predictors. **Results:** Time-to-exhaustion (TTE) was significantly different between all trials except H100 and C100. In addition, pH was significantly different at REC for all trials except H100 and C100 while LA was significantly different between trials at REC. Bicarbonate was lowest at EXH in H80 and C80, but there was no difference between these two trials; however, HCO_3^- was significantly lower for H80 and H100 at REC. Potassium was significantly higher at EXH when performing exercise in a hot environment. Finally, Tc was higher at EXH for H80 and C80 compared to H100 and C100. **Conclusions:** Core temperature likely played a greater role in muscular fatigue during the longer, less intense trials (H80 and C80), while pH and HCO_3^- may have been more important factors during the shorter, more intense trials (H100 and C100).

CURRICULUM VITAE

Melissa Rogers
6401 Wildwood Circle N #101
Fort Worth, TX 76132
(949) 291-4491
m.m.rogers@tcu.edu

Education

M.S. Texas Christian University: Kinesiology, Exercise Physiology (2007). Thesis title: "Fatigue During High Intensity Exercise: The Interaction Between pH and Thermal Stress"

B.S. California Polytechnic State University, Pomona: Kinesiology, Exercise Science (2005). Senior Research Project: "Trained vs. Untrained: Differences in the Lactate Curve and Blood Pressure in Response to Exercise"

Academic Experience

- ***Graduate Assistant (TCU: Fall 2005-present)***
  - Teaching undergraduate physical activity courses (jogging and body conditioning) and personal fitness.
    - Developing individualized fitness programs based on personal goals and current fitness level.
    - Teaching important concepts in health and well-being: current recommendations based on the ACSM guidelines, nutrition to optimize health and performance, and proper technique when lifting weights to prevent injury.
  - Laboratory Research Assistant
    - Experience in exercise testing subjects, blood pressure assessment, numerous assay procedures to include: glucose, hematocrit, hemoglobin, lactate, immunology markers, creatine kinase, etc.

Vocational Experience

- **Physician Assistant: Health Corp.- Dr. Herman Falsetti; Irvine, CA (Summer 2006)**
  - Stress testing patients with graded exercise protocols on the treadmill, cycle ergometer, and stationary bicycle; skinfold measurements; blood pressures assessment at rest and during exercise; analyzing anaerobic threshold and prescribing exercise programs to improve health and fitness; pulmonary function testing; and monitoring EKG readings to assure patient safety during exercise.

References

Dr. Joel Mitchell 817-257-7665
Texas Christian University
Department of Kinesiology Chair

Dr. Melody Phillips 817-257-6865
Texas Christian University

Dr. David Upton 817-257-7665