Effect of prior eccentric exercise on muscle lactate accumulation during high intensity exercise

by

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I hereby certify that the research constituting this thesis, except where otherwise stated or acknowledged, is the original work of the author.

I also certify that this work has not been submitted in whole or in part to this University or to any other educational institution for marking and assessment either previously or concurrently.

Signatures have been redacted for privacy

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ABSTRACT

Muscle H^+ accumulation may be the primary causal factor in limiting high-intensity exercise (HIE) performance. High muscle H^+ concentrations (or low muscle pH) inhibit the activity of rate-limiting enzymes in glycolysis, leading to compromised ATP resynthesis and Ca^{++} release from the sarcoplasmic reticulum, subsequently affecting the excitation-contraction coupling. As such, any impairment in the ability of the muscle to remove H^+ during the HIE bout may lead to premature fatigue and a decrease in exercise performance. It is proposed that eccentric-induced muscle damage caused by unaccustomed eccentric muscle contractions may lead to higher muscle lactate and H^+ concentrations during HIE and an impaired ability to remove these metabolites after completion of the exercise bout. Impaired clearance of muscle lactate and H^+ may be a direct effect of damage to the primary inter-cellular (and intra-cellular) transporters of lactate and H^+, the monocarboxylate transporters (MCT), which reside on the sarcolemma.

Eight male college-aged subjects performed either a muscle-damaging eccentric resistance exercise bout (ECC) or a maximal concentric (CON) bout of 100 maximal isokinetic repetitions. Two days following the resistance bout, subjects performed a graded HIE cycling bout to exhaustion. Muscle samples were taken from the right vastus lateralis prior to the HIE bout, immediately post-HIE, and 10 minutes following the HIE bout. Muscle lactate concentration, muscle pH, and muscle buffer capacity (β_m) were calculated.

The ECC bout led to considerable muscle soreness (8 ± 2 arbitrary units vs 0 ± 0 for CON) and raised concentrations of plasma creatine kinase (ECC: 847 ± 330 U/L; Con: 226 ± 40 U/L). There was no significant interaction between ECC and CON across time in muscle

lactate concentration, muscle pH, in-vivo β_m or in-vitro β_m. However, ECC did show a trend toward lower muscle lactate and H^+ concentrations both immediately post-HIE and 10 minutes post-HIE than did CON. Both groups showed similar rate of recovery of muscle lactate concentration over the 10 minutes post-HIE. There were no significant differences between groups in exercise performance or economy.

The slightly lower accumulation of lactate and H^+ during the HIE bout following ECC, without a subsequent change in either the rate of recovery post-HIE or in the β_m, suggests that eccentric-induced muscle damage may lead to lower production of lactate and H^+ during HIE. No conclusions can be made regarding the effect of eccentric-induced muscle on lactate and H^+ clearance. The impaired ability of eccentrically damaged muscle to produce lactate and H^+ during subsequent HIE may represent reduced glycolytic potential and is possibly related to lower resting muscle glycogen levels. These findings have implications for athletes training for short-term high-intensity events, who need to minimize unaccustomed eccentric exercise immediately prior to competition.

INTRODUCTION

The causes of muscle fatigue that limits short-term high-intensity ('anaerobic') exercise (HIE) performance are still unknown. While the etiology of fatigue is influenced by the intensity, duration, and nature of the exercise, possible causes for failure to maintain sufficient muscle contraction and force output have been hypothesized to relate to a wide variety of metabolic factors. These include ATP and phosphocreatine availability, muscle hydrogen ion (H^+) accumulation, muscle lactate (La^-) accumulation, inorganic phosphate (P_i) accumulation, and decreased intra-muscular potassium (K^+).

The role of La^- accumulation and pH changes on muscle fatigue during and following HIE is still not well understood. Accumulation of intra-cellular H^+, leading to decreased muscle pH, has been proposed to play a major role in fatigue generated during short-term HIE bouts. High muscle H^+ concentrations inhibit the activity of phosphofructokinase and phosphorylase, key enzymes in glycolysis, thereby limiting ATP resynthesis. In addition, H^+ can act to directly inhibit Ca^{++} release from the sarcoplasmic reticulum, subsequently affecting the excitation-contraction process.

Similarly, La^-, once considered a relatively inert by-product of exercise metabolism, has recently been implicated in the fatigue process. Intra-muscular accumulation of La^- inhibits caffeine- and Ca^{++}-induced Ca^{++} release from the sarcoplasmic reticulum (Dutka & Lamb, 2000; Favero et al, 1997), again possibly affecting the excitation-contraction process.

These findings give rise to the theory that the ability of the working muscle to either 1) buffer the H^+ produced or to 2) transport La^- and H^+ out of the muscle cell has a major bearing on the ability to limit or delay fatigue during HIE. An increase in either the muscle buffer capacity (β_m) or the transport capabilities of the working muscle cell could lead to improved HIE performance.

Transport of La^- and H^+ out of the muscle, either into the mitochondria for oxidation or into the blood for transport to other tissues, is performed by the monocarboxylate transporter (MCT). These carriers therefore play a major role in the clearance of these metabolic by-products from the working muscle. MCT-1, found mainly in skeletal mitochondria as well as in the sarcolemmal membrane, is thought to act primarily on transporting La^- (and H^+) into the mitochondria for oxidation, especially in oxidative muscle

fibers. MCT-4, found only in sarcolemmal membrane, transports La^- and H^+ across the sarcolemma into the blood. Changes in expression or activity of MCT in the working muscle, such as a result of training, is related to a corresponding change in rate of La^- transport. As such, increased MCT expression or activity following training may help to increase HIE performance, through reduced intra-muscular La^- and H^+ accumulation.

The metabolic role of La^-, H^+ and pH in muscle fatigue during exercise bouts following eccentric-induced muscle damage has not been closely studied. Unaccustomed eccentric exercise has been implicated in structural muscle damage, leading to decreased muscle function and strength, inflammation, release of muscle enzymes and delayed-onset muscle soreness (Proske & Morgan, 2001; Morgan & Allen, 1999). In particular, disruption of the sarcolemmal membrane and structural damage to the sarcomere has been noted following unaccustomed eccentric exercise.

It is possible that disruption to the sarcolemma, brought about by eccentric muscle contraction, can have a pronounced effect on sarcolemmal transport proteins. Eccentric muscle activity has been seen to reduce the content of muscle glucose sarcolemmal transporter proteins (GLUT-4), whereas other muscle proteins were unaffected (Asp et al., 1995; Kristiansen et al., 1997).

Similarly, the expression or activity of MCT-1 and MCT-4, both sarcolemmal transport proteins, could be adversely affected by the disruption to the sarcolemma as a result of unaccustomed eccentric contraction (Pilegaard & Asp, 1998). By limiting the action of sarcolemmal La^-/H^+ carriers, eccentric-induced muscle damage may act to lower sarcolemmal La^-/H^+ transport capacity and thereby reduce the ability of the working muscle to clear La^- and H^+. Research involving rats has shown reduced sarcolemmal La^-/H^+ transport capacity and a lower muscle buffer capacity (β_m) following eccentrically stimulated contraction, suggesting that muscle pH regulation may be impaired after unaccustomed eccentric activity (Pilegaard & Asp, 1998; Yeung et al., 2002).

Considering the role that La^- and H^+ potentially have in the fatigue process during intense exercise, eccentric-induced muscle damage could have a negative effect on muscle contraction and exercise performance in exercise bouts following the unaccustomed eccentric bout. The purpose of this study is analyze the response of skeletal muscle pH regulation, La^-

accumulation and β_m both prior to and during a HIE bout, after having induced muscle damage by an unaccustomed bout of eccentric exercise.

Statement of the Problem

What effect does unaccustomed eccentric exercise, and subsequent eccentric-induced muscle damage, have on 1) La^- and H^+ concentrations, 2) pH regulation, and 3) β_m in the working muscle during a single bout of short-term, HIE exercise?

REVIEW OF LITERATURE

Muscle Lactate and H^+ Production and Clearance

In working skeletal muscle, lactate (La^-) concentrations are regulated by the relationship between the rate of lactate production and rate of lactate removal. Accumulation of La^- in the working muscle occurs when the rate of La^- production exceeds the rate of removal. Regulation of La^- levels is a continuous process, with an alteration in either the rate of production or the rate of removal influencing muscle La^- concentration. The concept of a specific 'lactate threshold', commonly used by exercise scientists to note a change in blood La^- concentration, does not note the exercise intensity at which La^- production begins but the intensity at which rate of La^- production exceeds the rate of removal.

Recently, it has been proposed that La^-, rather than being a 'dead-end' for glycolysis and an inhibitor of muscle performance, is a useful metabolic intermediary because it can be exchanged rapidly among tissues (Gladden, 2001). Once formed by the working muscle, La^- can be used as an energy source, both by nearby oxidative muscle cells, such as the heart or other skeletal muscle, or possibly even within the working muscle itself. Thus, the 'intra-cellular shuttle' of La^- within the working muscle cell and 'inter-cellular shuttle' of La^- to other nearby working skeletal muscle, other tissues and organs, allows the glycolytic and oxidative pathways to be viewed as linked, as opposed to distinct, alternative pathways (Brooks et al., 1999a). Lactate also acts as a gluconeogenic precursor in the liver.

Similarly, muscle pH homeostasis is a balance between rate of H^+ production, via H^+ influx and metabolic production by dissociating from lactic acid, and rate of H^+ clearance, via intra-muscular buffers and sarcolemmal transport systems. Just as a concentration gradient forms for La^- across the muscle cell membrane during exercise, so to it is believed that an intra-muscular to venous pH gradient develops. Recent advances in techniques to allow measurement of interstitial pH continuously during exercise in human muscle have confirmed that intra-muscular pH decreases in relation to exercise intensity (Street et al., 2001). Previously, muscle pH changes during exercise had been inferred from venous measures however, the relationship between venous pH and muscle pH is complicated by the venous blood coming from inactive tissues mixing with blood from the working muscle (Radegran & Saltin, 1998). After high intensity exercise, muscle and blood (venous) pH are

reduced from normal to levels ~6.7 and ~7.1, respectively (Bansgbo et al., 1993), with peak acidification seen ~1 minute post-exercise (Street et al., 2001). However, acidosis is relatively transient with a return, in an exponential manner, to normal pH possible in as little as 10 minutes following intense exercise, with or without active recovery (Bangsbo et al., 1993).

Changes in intra-muscular pH during exercise may be an important signal in blood flow regulation (Street et al., 2001), with acidosis having a direct effect vasodilatory effect on vascular smooth muscle and may even modulate vascular K^+ channels (Davies, 1990).

Lactate / H^+ Production

Lactate production is mediated by glycolysis, whereby muscle glycogen and blood glucose is broken down to the metabolic by-product pyruvate and then to lactic acid. Two molecules of lactic acid are produced for every glucose molecule consumed. Lactic acid is formed from pyruvate through the action of the enzyme lactate dehydrogenase (LDH), along with the reduction of nicotinamide adenine dinucleotide (NADH). This formation is shown by the reaction: $\text{Pyruvate} + \text{NADH} + H^+ \rightarrow \text{Lactate} + NAD^+$

The regeneration of NAD^+ in the reaction of pyruvate to lactate is crucial to sustain glycolytic pathway activity by cycling back to reform NADH in the oxidation of glyceraldehyde-3 phosphate to 1,3 bisphosphoglycerate. At physiological pH (~7.4), lactic acid virtually fully dissociates into lactate and H^+, due in part to its low dissociation constant ($pK_a = 3.86$). This is shown in the reaction: Lactic acid (HLa) $\rightarrow La^- + H^+$

Lactic acid production is dependent upon a number of factors, in particular (but not limited to) oxygen availability (Myers & Ashley, 1997). Even at rest, when oxygen availability is sufficient to maintain ATP concentrations via oxidative phosphorylation, lactate can still be produced (Myers & Ashley, 1997). Production is dependent on a competition for pyruvate and NADH between LDH, the NADH shuttles (malate-aspartate and the glycerol-phosphate), and the La^-/H^+ transporters (monocarboxylate transporters; MCT) (Gladden, 2001). Since LDH has the highest V_{max} of any enzyme in the glycolytic pathway and the K_{eq} for pyruvate to lactate is far in the direction of La^-, La^-/ H^+ production is

an inevitable result of glycolysis, especially during rapid glycolysis as seen with intense exercise.

With increased exercise intensity, rate of glycolysis is increased. However, the common thought that O_2 deficiency is necessary for La^-/H^+ production and accumulation is incorrect. In addition to an increased demand for oxygen by the working muscle, changes in the concentrations of metabolic intermediaries, such as a reduced muscle ATP, increased ADP, increased P_i, and increased ammonia, lead to activation of PFK. Activation of PFK, the rate-limiting enzyme in glycolysis, speeds up glycolysis and therefore production of La^-/H^+. Similarly, increases in muscle Ca^{2+}, P_i, AMP, and circulating epinephrine have been implicated in the activation of phosphorylase, leading to increased glycolytic rate and La^-/H^+ production. All of these effects on La^-/H^+ production can occur independent of O_2 limitation (Gladden, 2001). Significant quantities of La^- are produced even under post-absorptive and post-prandial conditions in resting individuals (Brooks, 1986).

Net production of HLa accelerates in the transition from rest to high intensity exercise, where there is a rapid increase in energy demand. The sudden acceleration in production is due to 1) an acceleration in glycolysis during the initial period of the exercise bout before the oxidative pathway can meet the energy demands of the exercise task and 2) the maximal glycolytic capacity of the working muscle exceeds the maximal oxidative capacity, a ratio that is highly dependent on the predominant fiber type of the muscle (Juel & Halestrap, 1999).

Lactate / H^+ Clearance

Lactate

The clearance of both La^- and H^+ from the working muscle cell is essential for maintaining cellular homeostasis. Lactate clearance from the working muscle is mediated by the transport of La^- 1) into the mitochondria for oxidation, 2) to the liver for gluconeogenesis, 3) or to other tissues, cells and organs for oxidation. Lactate is a metabolic end-product and needs to be converted back to pyruvate, either in the cytosol or the mitochondria, before it can be metabolized. Dissociation from HLa into La^- and H^+ at physiologic conditions

impedes its movement out of the muscle cell, as free La^- moves relatively slowly across the plasma membrane (Juel, 1997).

Clearance of La^- and H^+ from the working muscle cell involves a La^-/H^+ cotransporter. In skeletal muscle, the monocarboxylate transporters MCT-1 and MCT-4 act to transport La^- and H^+ into either the mitochondria for oxidation or out of the muscle cell into the blood. These transporters reduce the accumulation of lactate in the working muscle as well as lessen the drop in pH, both of which have been implicated as a mechanism involved in the fatigue process limiting intense-exercise performance.

Transport into the muscle cell mitochondria

Recent evidence suggests that La^- and H^+ formed in the cytosol during intense exercise can be taken up directly by the mitochondria of the working muscle cell, via the actions of MCT-1, and oxidized (Gladden, 2001). Mitochondria contain an internal pool of LDH (Brooks et al., 1999b), the catalytic enzyme in the conversion of lactate to pyruvate, as well as MCT-1 in the inner mitochondrial membrane. The mitochondrial La^-/H^+ transporter, MCT-1 and mitochondrial LDH seem to work in unison, allowing La^- to be oxidized. The internal pool of LDH in the mitochondria, rather than cytosolic LDH, is essential for mitochondrial uptake and oxidation of La^-. Fully oxygenated cells, while still undergoing glycolysis and producing La^- and H^+ (due to the large free energy change and the equilibrium constant for LDH being far in the direction of the product), do not accumulate La^- and H^+ due largely to mitochondrial oxidation of La^-.

Brooks (2002) has proposed the concept of an 'intra-cellular shuttle' of La^- from the working muscle cell cytosol into the mitochondria for oxidation thus, La^- is cleared without leaving the cell. The 'intra-cellular shuttle' of La^- and H^+ into the mitochondria of the working muscle cell for oxidation, besides reducing intra-muscular acidosis as a result of dissociation of H^+ with La^- production, has the additional benefit of shuttling reducing equivalents (NADH) into the mitochondria. Lactate flux is favored by the La^- concentration gradient between the cytosol and mitochondria, in addition to the high NADH and low H^+ concentrations within the mitochondria when the electron transport chain is activated (Brooks et al., 1999a).

The utilization of La^- as a substrate for energy production plays a significant role in exercise metabolism. Working skeletal muscles not only produce La^- but also consume La^- from other tissues, such as other working muscles, via the blood stream (Stanley et al., 1986; Brooks, 1991). During steady-state submaximal exercise, working skeletal muscle first releases La^- on a net basis when exercise starts but then switches to net La^- uptake as the exercise bout continues (Brooks, 2000).

It is estimated that ~75-80% of La^- ends up being oxidized, either by the working muscle itself or by other working muscle, tissues and organs, with much of the remainder used for gluconeogenesis by the liver (Brooks, 2000). Lactate may be the predominant substrate for the heart, most probably due to the high mitochondrial density in the smooth muscle of the heart and the prevalence of heart-type lactate dehydrogenase (LDH-H4, LDH-1) (Brooks et al., 1999a). Both during exercise, and especially as the muscle respiratory rate declines during recovery from exercise, La^- becomes the preferred substrate for gluconeogenesis in the liver (Brooks, 1986b; Stanley et al., 1988). Surprisingly, La^-, not glucose, is the preferred substrate for glycogen synthesis by the liver (Brooks, 1998).

Transport to the liver and other tissues

Besides clearance via oxidation within the working muscle cell, La^- and H^+ are also transported out of the muscle cell into the blood. Lactate and H^+ are taken to either the liver, to be used as a precursor for gluconeogenesis, or to other working muscles, tissues and organs for oxidation. Brooks (2002) proposed the concept of a 'cell-to-cell shuttle' of lactate and H^+ out of the working muscle cell for use by other tissues. 'Cell-to-cell shuttling' of La^- and H^+ occurs between white-glycolytic and red-oxidative fibers within a working muscle, between working skeletal muscle and the heart, and between tissues of net La^- release and gluconeogenesis (Brooks, 2002).

The rate of La^- removal from the muscle is strongly related to the muscle La^- concentration gradient. A high correlation has been noted between muscle La^- and venous La^- both before ($r = 0.92$) and after ($r = 0.85$) training (Bonen et al., 1998). Bangsbo et al. (1993) found a strong linear relationship between La^- release and the muscle La^- gradient, for muscle La^- concentrations ranging from 0-45 mM. However, as the gradient reaches higher levels,

the La^- release appears to slow, indicating that a saturation point could exist at high intramuscular La^- concentrations that limit the rate of La^- transport across the sarcolemma. Alternatively, increased blood lactate concentration during moderate-intensity exercise increased La^- oxidation, while also sparing blood glucose and lowering glucose production, indicating that exogenous La^- can be taken up by working muscle in times of increased energy demand (Miller et al., 2002).

Lactate (and H^+) clearance is also affected by plasma volume. Increased extra-cellular water acts to increase the concentration gradient toward diffusion of La^- out of the muscle cell (Green et al., 1997). It has been postulated that swelling of the muscle fiber, as seen as a result of muscle contraction during exercise, could play a role in muscle fatigue. The production of HLa and other metabolic by-products during HIE increases the osmolality of the muscle fiber, leading to influx of extra-cellular water via diffusion into the fiber. This swelling, while having no great effect on the intracellular ionic potential, could possibly affect the propagation of the action potential in the T-tubule and the sarcoplasmic reticulum (Dutka & Lamb, 2000).

H^+ ion

Muscle pH regulation is regulated by a number of transport systems, with different transport systems performing at rest and during exercise. The main transporters involved in muscle pH regulation at rest, through clearance of H^+, are the Na^+/H^+ exchange system, with the assistance of the Na^+-dependent and Na^+-independent Cl^--bicarbonate (HCO_3^-) transport systems (Juel, 1998). During exercise, the dominant transporter of H^+ from the muscle cell is the La^-/H^+ transporter (MCT), with the Na^+/H^+ exchange system providing only a fraction of H^+ release (Juel, 1996; Juel, 1998). Passive influx of H^+ is counteracted by these transport systems, allowing continuous clearance of H^+ from the muscle cell.

The capacity of the Na^+/H^+ exchange system increases following high-intensity training in rat skeletal muscle but is unaffected by endurance training (Juel, 1998b). Na^+/H^+ exchange system activity was independent of fiber type following an acute applied acid and La^- load (Juel, 1995), but expression of the Na^+/H^+ exchange isoform 1 (NHE1) was highest in type IIb muscle fibers following 3 weeks of endurance training and negatively correlated

to type I and type I+IIa fiber distribution, even though NHE1 content increased, ranging from 29-36%, in all muscle fiber types (Juel, 2000). The enhanced initial rate of Na^+/H^+ exchange during exercise seen following high-intensity training seems to be linked to the elevated buffer capacity reported in vivo, indicating that adaptive changes in the Na^+/H^+ exchange system may be of importance for pH regulation in association with HIE (Juel, 1998b).

At low pH, in association with a high muscular lactate concentration, removal of H^+ is primarily due to the La^-/H^+ transporter. During recovery from an applied load of 40mM La^- and pH 6.5, the capacity of the La^-/H^+ transporter (240 nmol H^+/ mg protein/ minute) to transport H^+ was observed to be 3 times greater than the sum of both the Na^+/H^+ exchange system (33 nmol H^+/ mg protein/ minute) and the Cl^--$HCO3^-$ transport systems (47 nmol H^+/ mg protein/ minute) capacities combined in vesicles from mixed muscles (Juel, 1995). Capacity to remove H^+ by the La^-/H^+ transporter was fiber-type dependent, with greater clearance rates seen in red fibers than in vesicles from white muscle fibers. These observations indicate that pH regulation during exercise is more effective in oxidative (red) muscle fibers than in glycolytic (white) fibers.

However, the activity of the La^-/H^+ transporter is mainly driven by the lactate gradient rather than by the H^+ gradient. Muscle transport of H^+ is insensitive to changes in blood pH in the range of 0.02-0.08 pH units (Bangsbo et al., 1997). Thus, while the La^-/H^+ transporter acts to regulate muscle pH during and following intense exercise, it is only slightly sensitive to muscle pH. In contrast, the Na^+/H^+ exchange system is strongly activated by a reduced muscle pH, thus better suited for pH regulation at rest when a La^- gradient is absent.

Metabolic alkalosis, such as induced by Na bicarbonate ($NaHCO_3$) ingestion, has been proposed as a possible agent in restoring muscle pH to normal following intense exercise. By buffering the HLa produced, alkalytic agents such as bicarbonate can limit intra-muscular acidosis. Induced muscular alkalosis during incremental exercise has been seen to increase blood La^- concentration (Hollidge-Horvat et al., 2000; Jones et al., 1977; Hood et al., 1988), presumably as a result of increased La^- efflux from the muscle. It is possible that an increase in muscle La^- production could at least in part contribute to a raised blood

concentration of lactate. Induced metabolic alkalosis has been seen to increase lactate production via an acceleration of glycogenolysis (Hollidge-Horvat et al., 2000).

During moderate intensity exercise (60% VO_2max), Hollidge-Horvat et al. (2000) found both pyruvate production and oxidation to be significantly elevated following induced alkalosis, while La^- production was unchanged from that of control subjects. Increased pyruvate production and oxidation indicates that alkalosis aids glycolysis, presumably via maintenance of suitable conditions for PFK activity and entry of the glycolytic end-product pyruvate into the tricarboxylic acid (TCA) cycle for oxidation. Both PFK, the rate-limiting enzyme in glycolysis and the active form of pyruvate dehydrogenase (PDH_a), the mitochondrial enzyme that catalyzes the decarboxylation of pyruvate and therefore reflects the rate of pyruvate entry into the TCA cycle, increase following induced alkalosis.

The inherent difficulties in studying the relative rates of production and clearance of both La^- and H^+ within the working muscle tissue, due in large part to these processes being arranged in series and parallel, has led to reductionist techniques. These techniques include isolation of cell membranes and sarcolemmal giant vesicles, which have become standard in the study of La^- kinetics (Brooks, 2000).

Monocarboxylate Transporters (MCT)

Characteristics

The clearance of La^-/H^+ from the working muscle during exercise is primarily regulated by a proton-linked protein MCT (Juel, 1998). Transport of these metabolic by-products across the sarcolemmal and mitochondrial membranes involves the binding to an MCT.

The MCT is a 12 transmembrane-spanning (TM) helix with intracellular C- and N-termini and a large intracellular loop between TM segments 6 and 7 (Poole & Halestrap, 1997). It is believed that the N-terminal domains may play a role in energy coupling, via H^+ or Na^+ co-transport, membrane insertion and/or maintenance of protein structure, while the C-terminal domains may be involved in determination of substrate specificity (Garcia et al., 1994). Transport involves first the binding of the proton to the MCT and then the La^- ion.

The MCT trans-locates across the membrane to release the La^- and H^+ ion on the other side of the membrane. The process is reversible with equilibrium reached when:

$$[Lactate]_{in} / [Lactate]_{out} = [H^+]_{out} / [H^+]_{in}$$

However, equilibrium is unlikely to ever be reached in muscle. Return of the unloaded MCT intra-cellularly to again bind a proton and La^- ion is the rate-limiting step for net La^-/H^+ clearance. The transporter is bi-directional, allowing La^- and H^+ flux either into or out of the muscle cell along concentration gradients (Brown & Brooks, 1994).

Transport across the sarcolemmal and mitochondrial membranes via MCTs is saturable and stereo-specific with a 1:1 coupling between La^- and H^+ (Juel & Halestrap, 1999). Transport is stereo-specific for the L(+) isomer of La^-, with uptake rates for the D(-) isomer remaining linear at concentrations from 1-200 mM La^- (Roth & Brooks, 1990). The K_m of MCT-4 for $_L$-lactate is about sixfold higher than that of MCT-1 (Manning-Fox et al., 2000). The MCT is also responsible for transporting pyruvate and the ketone bodies acetoacetate, β-hydroxy-butyrate and acetate, as well as other short-chain fatty acids, monocarboxylates, and 2 or 3 oxo-acids (Poole & Halestrap, 1993). Again, MCT-4 shows a higher K_m values for these substrates, especially with α-keto acids, in the order of 20-100 times greater than for MCT-1 (Manning-Fox et al., 2000). The 1:1 coupling of La^- and H^+ in their movements across the sarcolemmal membrane enables the distinct processes of transport and the subsequent metabolism of La^- to be linked without an imbalance of protons. Other than H^+, there are no known phosphorylation sites or allosteric binding agents on the MCT.

Lactate transport via MCT's seems to be both pH- and temperature-sensitive. Lowering pH on the same side of the membrane as La^- is added stimulates transport, primarily through decreasing the K_m for La^-. Similarly, by raising pH on the other side of the membrane, La^- transport increases due to an increase in the V_{max} of transport, likely increasing the rate at which the unloaded MCT re-orientates within the membrane (Juel & Halestrap, 1999). Lactate uptake increases with increasing temperature, with a threefold higher rate of uptake seen at 37°C than at 25°C (Roth & Brooks, 1990). Rate of transport does not seem to be affected directly by either Cl^- or HCO_3^- flux.

Monocarboxylate transporters are inhibited reversibly by cinnamate, thereby blocking oxidation of both La^- and pyruvate by mitochondria, and inhibited irreversibly by the sulfhydryl reagent p-chloromercuribenzene. Monocarboxylates with longer branched aliphatic or aromatic side chains, such as α-cyano-4-hydroxycinnamate (CHC), act as inhibitors by binding to the MCT but not releasing from the MCT following translocation. Lactate transport at low acid load (1 mM) is inhibited by protein-modifying reagents such as p-chloromercuriphenyl-sulfonic acid, N-ethylmaleimide and $HgCl_2$ during pH-stimulated conditions (Roth & Brooks, 1990). Lysine and arginine reagents, and anion transport inhibitors, such as stilbene disulphonates, also act to inhibit MCTs (Poole & Halestrap, 1993). The affinity of MCT-4 for many of these inhibitors appears to be less than for MCT-1 (Manning-Fox et al., 2000). LDH is inhibited by oxamate, leading to blockage of lactate, but not pyruvate, oxidation (Brooks et al., 1999b).

Skeletal muscle contains both MCT-1 and MCT-4, while heart muscle contains only MCT-1, with MCT-4 disappearing from the heart after the first 10 days of life (Hatta et al., 2001). Differences in MCT-1 and MCT-4 expression indicate that they have different roles in La^- and H^+ transport. Differences in expression have been noted both among muscles, muscle fiber types, different sites within the skeletal muscle and different rates of expression following training. Among six rat fast-twitch muscles, a strong inverse relationship ($r = -0.94$) was noted between MCT-1 and MCT-4 content (Bonen et al., 2000). Both types of transporter have been noted in plasma membranes, t-tubules, SR and intra-cellular membranes (Bonen et al., 2000). However, MCT-1 appears confined mainly to the plasma membrane, while MCT-4 expression is significant in the plasma membrane, t-tubules and SR. Expression of MCT-1 in the heart and both MCT-1 and MCT-4 in skeletal muscle appears to be regulated by pre-translational processes, while MCT-4 expression can also be regulated post-transcriptionally (Hatta et al., 2001).

MCT-1

MCT-1 expression is greatest in slow twitch or oxidative muscle fibers. A positive, linear relationship exists between MCT-1 content and proportion of type I fibers in skeletal muscle (Pilegaard et al., 1999a), with the oxidative fiber content (% slow twitch oxidative +

% fast-twitch oxidative glycolytic) (r = 0.91), with citrate synthase activity (r = 0.82), and with an increased capacity to remove lactate from the circulation (r = 0.90) (McCullagh et al., 1996). A strong negative relationship (r = -0.98) has been noted between MCT-1 content and fast-twitch glycolytic fiber proportion (Bonen et al., 2000), and with whole body LDH activity (r = -0.80), an index of glycolysis (McCullagh et al., 1996). Increased expression of MCT-1 in the soleus muscle parallels the increase in slow-twitch oxidative muscle fibers in this muscle in the first few months of life (Hatta et al., 2001). It is also known to be abundant in erythrocytes, mitochondria and sarcolemmal membranes (Brooks et al., 1999a). These findings give rise to the notion that MCT-1 is crucial for lactate and H^+ clearance from highly oxidative muscles.

Baker et al (1999b) suggest that the major role of MCT-1 is to transport lactate into the mitochondria for oxidative metabolism. This notion is based on the evidence suggesting that the MCT-1 increase as a result of training takes place mainly in 'oxidative' skeletal muscles, comprising mainly slow twitch oxidative muscle fibers, rather than 'glycolytic' muscle, comprising predominantly fast-twitch fibers. MCT-1 expression in muscle fiber correlates with the muscle's mitochondrial content. Highly oxidative muscles, such as soleus, display high MCT-1 content. In contrast, muscle with high glycolytic fibers, such as semimembranosus and semitendinosus, contain very little MCT-1 (McCullagh et al., 1996). However, evidence exists to suggest MCT-1 content may not differ between types I and type II fibers within a given muscle but does vary highly between different muscles (Pilegaard et al., 1999a), which may be reflective of the MCT-1 content in type II muscle sarcolemma.

In support of this theory, Brooks et al. (1999b) have further proposed the existence of an "intra-cellular lactate shuttle". MCT-1 is believed to 'shuttle' lactate from the cytosol into the mitochondria, for conversion back to pyruvate, via the enzyme LDH, and subsequent oxidation in working skeletal and heart muscle. Evidence for the proposed 'shuttle' stem from the fact that isolated mitochondria readily consume exogenous lactate (Brooks et al., 1999b) and working muscle and heart consume and oxidize La^- (Brooks et al., 1999a). Because MCT-4 is not expressed in the mitochondria, it is believed that MCT-1 is the sole intra-cellular shuttle transporter.

MCT-4

In contrast, MCT-4, while found in all fiber types, is predominantly expressed in fast twitch fibers (Pilegaard et al., 1999a; Bonen et al., 2000). MCT-4 protein content is correlated with percent fast-twitch glycolytic fiber ($r = 0.88$) in rat skeletal muscle (Bonen et al., 2000). Inter-individual variation in MCT-4 content is relatively high, as compared with inter-individual variation in MCT-1 content in homogenous samples (Pilegaard et al., 1999a; Dubouchard et al., 2000). This transporter is expressed in skeletal muscle sarcolemma but is not present in mitochondria indicating that it may be the constitutive sarcolemmal La^- transporter (Brooks et al., 1999; Dubouchard et al., 2000).

According to this theory, MCT-4 is important for La^- and H^+ clearance from highly glycolytic muscles, via cell-to-cell exchange. This is supported by evidence showing MCT-4 to be the only MCT expressed in human white blood cells, which are highly glycolytic and net HLa exporters (Wilson et al., 1998). Also, MCT-4 expression is repressed at the same time as glycolysis is reduced in both heart and skeletal muscle (Hatta et al., 2001). MCT-4, but not MCT-1, is also found in an intracellular (endosomal) pool, possibly representing an intra-cellular storage compartment of MCT-4 to be recruited during intense exercise, when La^-/H^+ production increases (Bonen et al., 2000). Clearly, the different sites for expression of these two transporters is an important factor in the role that they are believed to play in La^- and H^+ kinetics.

Manning-Fox et al. (2000) propose that the low affinity of MCT-4 for pyruvate may act to prevent loss of pyruvate from the muscle cell, allowing NADH to be re-oxidised and glycolysis to continue. By acting to remove La^- and not pyruvate from the working muscle cell, glycolysis and ATP production is maintained. The low affinity of MCT-4 for other substrates, such as ketone bodies, is also consistent with its presence in glycolytic tissues that do not oxidize or produce these metabolites. However, the relatively low affinity of MCT-4 for $_L$-lactate is surprising as the low affinity leads to lower efficiency in removal from the muscle and cause HLa accumulation. The authors hypothesize that muscle La^- accumulation may play a critical role in the fatigue process, and acting as a 'feedback loop' to prevent HLa accumulation in the blood.

While only MCT-1 is found to act at the mitochondria, both MCT-1 and MCT-4 act to transport La^- across the sarcolemma (Juel & Halestrap, 1999; Dubouchard et al., 2000). These two transporters act to create a "cell-to-cell lactate shuttle", releasing La^-/H^+ from the working muscle for exchange with other cells, tissues and organs, in particular as a gluconeogenic precursor for the liver and for oxidation in the heart smooth muscle. Both MCT-1 and MCT-4 are expressed at the sarcolemma (Dubouchard et al., 2000).

Lactate clearance rate appears to be directly related to the content of MCT-1 and MCT-4 in the working muscle (Green et al., 2002). In particular, a strong correlation exists between MCT-1 and La^- transport in skeletal muscle, particularly in highly oxidative muscles (Baker et al., 1998). A strong negative correlation ($r = -0.85$) has been seen between mitochondrial MCT-1 content and net La^- release at rest (Dubouchard et al., 2000), indicating that by facilitating intra-muscular lactate transport into the mitochondria for oxidation, MCT-1 depresses net La^- release from the muscle into the blood. However, Dubouchard et al. (2000) showed a strong positive correlation between sarcolemma MCT content and net La^- release during exercise, but saw no relationship between MCT content with either La^- oxidation or clearance from the muscle. These findings indicate that MCT-1 is a crucial transport carrier for La^- across both the sarcolemmal and mitochondrial membranes.

By acting as the main sarcolemmal transporter of La^-/H^+ from working skeletal muscle, MCT-4 expression may reflect the capacity of the muscle for efflux, while MCT-1 expression correlates with capacity for influx for oxidation (Bonen et al., 2000). Thus, the transporters have differing roles and the expression is regulated independently in skeletal muscle. These findings indicate that the differences between MCT-1 and MCT-4 in expression and role are similar to that shown by glucose transporters (GLUT-1 and GLUT-4) in skeletal muscle

Muscle Buffer Capacity (β_m)

A buffer system consists of an acid and its dissociated elements. This system acts to minimize the effects of acids, or bases, on muscle and plasma pH. Intra-cellular physiochemical buffers include P_i, protein-bound histidine residues, carnosine, HCO_3^- and

PCr. Changes in pH during the first few seconds of contraction can be entirely prevented by proton consumption via net PCr hydrolysis (Adams et al., 1990).

The role of the HCO_3^- system in buffering the dissociated H^+ is described in the following equation: $H^+ + HCO_3^- \rightarrow H_2CO_3 \rightarrow H_2O + CO_2$

Thus, HLa^- production leads to production of CO_2, which is then eliminated from the blood at the lungs. Ventilatory regulation of blood pH, by altering the CO_2 content of the blood, acts in unison with the muscle buffering systems to minimize the effects of an acid load on the body.

While β_m tends to be positively related to training for high-intensity exercise bouts, evidence suggests β_m is not a major limiting factor in the performance of short-term high intensity exercise. During short-term high intensity exercise, involving both isometric and dynamic tasks, an elevated β_m was associated with a lesser decrement in muscle pH during exercise but did not lead to improved exercise performance (Mannion et al., 1995). Dynamic exercise performance, using a modified Wingate test, was in fact associated with a high muscle La^- concentration and low muscle pH post-exercise, indicating that β_m, high muscle La^- or low muscle pH may not be major limiting factors in exercise performance during short-term, high intensity tasks. However, in trained competitive cyclists, β_m correlated ($r = -0.82$) with short-duration time trial performance, but not with time to fatigue at 150% peak power output (Weston et al., 1997). Muscle buffer capacity has been seen to have only a small, non-significant relationship ($r = 0.42$) with relative area of type II fibers within the quadriceps in young, healthy adults (Mannion et al., 1995).

Rate of La^-/H^+ production is dependent upon β_m, which comprises the creatine kinase reaction rate, muscle protein buffering, HCO_3^- concentration, La^- efflux, and increases in metabolites with acid dissociation constant values within the physiological range (Richardson et al., 1998). The effective β_m has been estimated to range from 20-30 slykes (mmol/L/pH unit) (Kemp et al., 1993).

Effect of Training

Muscle Lactate and pH Regulation

The impact of training, either endurance, speed (high-intensity) or strength, on muscle pH regulation and muscle H^+ and La^- production/clearance has not been clearly determined. It is clear that La^-/H^+ accumulation in the working skeletal muscle is lowered following endurance training. Measurement limitations have to date precluded agreement as to which process, a decrease in La^- production and/or an increase in La^- clearance, dominates after training to reduce La^- accumulation at a given workload (Green et al., 2002). Lactate production by the working muscle is reduced following training as a result of improved matching of glycolytic and PDH fluxes and independent of changes in O_2 consumption (Putman et al., 1998). Bergman et al. (1999) determined that both an increase in La^- clearance and a decrease in La^- production resulted from endurance training however, the relative importance of each process in the training-induced decrease in La^-/H^+ accumulation in the working skeletal muscle is still to be deduced.

Muscle activity does have a major effect on La^- transport in skeletal muscle. Following periods of inactivity, such as with denervation (Pilegaard & Juel, 1995; McCullagh & Bonen, 1995) or hindlimb unweighting (Dubouchard et al., 1996), muscle La^- transport is decreased. Denervation acted to lower La^- transport by 15% and 41% after 1 day and 21 days respectively in red muscle fibers, as compared with contra-lateral muscle fibers (Pilegaard & Juel, 1995).

In contrast, exercise training has a positive effect on La^- transport, with the magnitude of the increase related to the intensity of training (Pilegaard et al., 1993). Athletes tend to display a higher La^- transport capacity than both untrained and trained subjects while bicyclists showed a higher La^- transport capacity in vastus lateralis muscle fibers than runners, possibly due to specific adaptations to training in this muscle (Pilegaard et al., 1994). These results indicate that a high volume of training, including high intensity sessions, can improve La^- transport capacity.

Lactate transport in sarcolemmal vesicles is modified following as little as a single bout of exhaustive exercise (Dubouchard et al., 1999; Tonouchi et al., 2002), with the nature of the changes in La^- transport dependent upon the applied La^- concentration. A short (10

min), intense period of muscle contraction, resulting in an external La^- concentration of 20 mM led immediately to an increase in the rate of La^- uptake (64%) into sarcolemmal giant vesicles, however no changes in La^- uptake were seen when a 1 mM La^- external concentration was applied (Tonouchi et al., 2002).

An increase in La^- transport has been noted following a progressive endurance-training program (Pilegaard et al, 1993), after repeated bouts of moderate-intensity running (McDermott & Bonen, 1993), and following high-intensity treadmill endurance training in rats (Baker et al., 1998). Pilegaard et al. (1993) noted improvements of 58% and 76% following 7 weeks of moderate (~90% VO_2max) and high intensity (112% VO_2max) interval treadmill training respectively. However, no changes in La^- uptake have been noted following either a moderate-intensity running program (Baker et al., 1998) or low-intensity (~50% VO_2max) swimming (Pilegaard et al., 1993). The positive effects of training are transient, with the improvements in La^- transport capacity reversed in as little as 5 weeks of inactivity following training (Pilegaard et al., 1993).

The effect of training on β_m is equivocal. It is evident that muscle buffer capacity (β_m) is strongly influenced by altitude training. Increases in β_m of between 5-17% have been seen following endurance training involving either living at, or training at, moderate altitude (1500-3000 meters above sea level) (Gore et al., 2000). With acclimatization to altitude, muscle La^- concentrations during and following a bout of exercise are typically lowered, indicating either decreased La^- production or improved La^- clearance from the working muscle (Hahn & Gore, 2001).

High-intensity, submaximal interval training, comprising 6 sessions in 4 weeks, has shown to increase β_m in well-trained competitive cyclists (Weston et al., 1997). Sharp et al. (1986) showed 8 weeks of sprint training to increase β_m while anaerobically-trained subjects are noted to display a higher β_m than untrained subjects (Sahlin & Henriksson, 1984). However, β_m was unaffected by 8 weeks of knee extensor training using varying intensities, with both trained and untrained muscle having a β_m of ~45 slykes (mmol/kg wet weight/pH unit) (Pilegaard et al., 1999b).

MCT Expression

The mechanism behind a possible training effect on La^- clearance from the working muscle is thought to be an increase in muscle MCT-1 and MCT-4 content. In both heart and skeletal muscle, La^- uptake is only increased when MCT content is increased (Baker et al., 1998). An increase in both MCT-1 and MCT-4 content can result from as little as a single sub-maximal exercise session, with MCT content remaining elevated for at least 6 days (Green et al., 2002). The increase in both MCT-1 and MCT-4 content corresponded to a decrease in muscle La^- concentrations seen during sub-maximal exercise performed 2, 4 and 6 days following a sub-maximal exercise bout (Green et al., 2002).

Short-term endurance training (7 days x 2 hrs/day at 65% VO_2max) modestly increased MCT-1 (18%), in association with reduced muscle and venous La^- concentrations during exercise performed after training (Bonen et al., 1998). Short-term chronic muscle contraction, over a 1-3 week duration, further stimulates MCT expression. Chronic muscle contraction (24 hrs/day x 7 days) led to up-regulated MCT-1 expression in direct proportion ($r = 0.96$) to the corresponding increase in the rate of La^- uptake (McCullagh et al., 1997). MCT-1 expression increased (+78%) in red muscles after both 1 and 3 weeks of chronic contraction, and increased (+191%) after 1 week in white muscle but not after 3 weeks (Bonen et al., 2000b). However, Bonen et al. (2000b) did not note a change in MCT-4 expression in either red or white muscle after 1 or 3 weeks of chronic contraction, with MCT-4 mRNA even transiently lowered (~15%) in both muscle fiber types in the first week. The increase in MCT-1 expression, but not MCT-4 expression, was still sufficient to increase La^- uptake from the circulation (Bonen et al., 2000b).

The rise and plateau in MCT-1 expression with chronic muscle contraction over an extended period indicates a 'ceiling' in the level of expression is reached after a critical amount of contractile activity has been performed. Bonen et al. (2000b) further noted that stimulation of 3 hr/day for 7 days was sufficient to induce maximal MCT-1 expression in both red and white muscle, however extending the duration period (24 hr/day for 7 days) did not produce a further increase and further prolonging the stimulation (24 hr/day for 21 days) reduced MCT-1 content. The authors proposed that the 'ceiling' in MCT expression seen in

this study may be due to muscle remodeling interfering with MCT-1 expression and related enzyme activity.

Both MCT content and rate of La^-/H^+ transport was increased following 8 weeks of knee extensor training of varying intensity (Pilegaard et al., 1999b). Rate of La^-/H^+ transport, determined in sarcolemmal giant vesicles, was 12% higher in trained muscle samples, as compared to untrained muscle. The content of MCT-1 and MCT-4 was 76% and 32% higher, respectively, in trained muscle. Interestingly, while both La^-/H^+ release from the working muscle and muscle pH at the end of an intense exhaustive knee extension bout were similar for both trained and untrained subjects, the estimated muscle intra-cellular-to-interstitial gradients of La^- and H^+ were lower in trained muscle. The results from Pilegaard et al. (1996b) indicate that muscle adapts to the demands of intense training through an improved ability to transport La^-/H^+ from the working muscle. The higher MCT content following training can in part explain the increases seen in La^-/H^+ transport. However, since the relative contributions of MCT-1 and MCT-4 to total La^-/H^+ transport are not known, it is unclear whether the relative changes in MCT-1 and MCT-4 content are reflective of similar relative changes in La^-/H^+ transport. The authors further hypothesize that, since the free diffusion of La^-/H^+ at a given muscle La^-/H^+ gradient is unlikely to be altered by training, the relative change in MCT-mediated transport of La^-/H^+ is larger than that observed in this study.

In contrast to these findings showing a strong positive relationship between improved La^-/H^+ transport and MCT expression with training, Eydoux et al. (2000) found endurance training to have no effect on lactate transport capacity, despite an increase in MCT-1 content in the working skeletal muscle and heart. Alternatively, Tonouchi et al. (2001) saw improved La^- uptake despite a concomitant decrease in MCT content immediately following a short (10 minutes), intense bout of muscle contraction. While the rate of La^- uptake into sarcolemmal giant vesicles increased by 64% at an external La^- concentration of 20 mM, plasma membrane content of MCT-1 and MCT-4 were reduced by ~10% and ~25% respectively immediately following the exercise bout. Further trials, using a more modest rate of muscle contraction, again saw reduced MCT-4 content (~20%) following the 10 minutes of intense muscle contraction, but not reduced MCT-1 content. The authors speculated that rate of La^-

uptake was improved due to, not an increase in MCT content, but an improvement in the intrinsic activity of the MCTs.

Changes in MCT expression appear to be dependent upon training intensity in a similar fashion to the changes in La^- uptake noted with differing training protocols. While 3 wks of high-intensity treadmill training led to improvements in both La^- uptake and MCT-1 expression in heart and skeletal muscle, moderate training only increased La^- uptake and MCT-1 expression in heart muscle with no changes seen in skeletal muscle (Baker et al., 1998). These findings indicate that heart muscle may have a lower threshold of training intensity required to stimulate MCT expression and subsequent improvement in La^- uptake. Alternatively, detraining can lead to down-regulation of MCT expression. Expression of both MCT-1 and MCT-4 was decreased by 40-60% following 3 weeks of denervation of rat hind limb muscle, with the decrease in parallel with the decrease in La^- efflux (Wilson et al., 1998).

Dubouchard et al. (2000) noted an increase of MCT-1 content at both the sarcolemmal and mitochondrial membranes following endurance training. Mitochondrial MCT-1 content increased ~80% in the working muscle after 9 weeks of cycling endurance training, independent of an increase in total mitochondrial density. Content of MCT-1 was increased in both sarcolemma-enriched (+60%) and mitochondria-enriched fractions (+78%) of muscle preparations, supporting the notion that MCT-1 plays a role as a La^-/H^+ transporter at both the mitochondria and the sarcolemma. Expression of MCT-1 following training is highly associated to citric synthase (CS) activity. Dubouchard et al. noted a strong positive correlation ($r = 0.83$) between changes in MCT-1 content and changes in muscle CS activity following 9 weeks of endurance training. These findings indicate that increases in mitochondrial content and density with training, as reflected by CS activity, are accompanied by proportional increases MCT-1 content at the mitochondrial membrane.

Endurance training has a variable effect on MCT-4 content, with greater inter-individual variability noted in the response to training (Dubouchard et al., 2000; Wilson et al., 1998). Dubouchard et al. (2000) noted MCT-4 content was only increased in sarcolemma-enriched fractions of muscle preparations (+47%), with no changes in content seen in either mitochondria-enriched or total muscle preparations. Similarly, Wilson et al.

(1998) saw no changes in MCT-4 expression following chronic stimulation of the hindlimb, despite a 2- to 3-fold increase in MCT-1. In general, expression of MCT-4 following endurance training is less pronounced than expression of MCT-1. The difference in expression between MCT-1 and MCT-4 is logical since endurance training acts to increase mitochondrial density, where MCT-4 is absent.

Chronic hypo-baric hypoxia, or exposure to high altitude, has a variable, tissue-specific effect on MCT expression. McCelland & Brooks (2002) saw no change in MCT-1 expression in heart muscle, either in mitochondria-enriched fraction or whole muscle preparations. In contrast, MCT-4 expression was increased (+34%) in whole muscle samples from the heart. Both MCT-1 and MCT-4 expression was suppressed in plantaris whole muscle samples and unchanged in samples from the soleus, gastrocnemius and the liver. These inconsistent findings were tentatively attributed to tissue-specific adaptations to hypoxia with regional differences in oxygenation.

Besides an increase in MCT content and MCT activity following endurance training, the increase in rate of La^- clearance could also be attributed to increases in blood flow within the working muscle mediated by increased plasma volume (Green et al., 2002).

In conclusion, it seems that training, in particular endurance training, may positively affect both La^-/H^+ transport capacity and expression of MCT in the muscle sarcolemma (MCT-1 and MCT-4) and mitochondrial membrane (MCT-1). These changes are functionally significant. However, the response in La^-/H^+ transport and MCT expression to training programs involving short, high-intensity exercise (such as sprint or strength training) is still unclear. Similarly, there is no evidence for any short- or medium-term hormonal regulation, or short-term regulation by allosteric modulation or phosphorylation on either La^-/H^+ transport or MCT expression.

Mechanisms for Muscle Fatigue: Short-term, High-intensity Exercise

The mechanisms for muscle fatigue during exercise, characterized by a failure to maintain force production, are not yet clear. Determining the mechanism/s involved is a complex problem considering multiple factors, such as the frequency, intensity and duration of the exercise as well as the fiber type distribution of the fatiguing muscle are involved.

However, the etiology behind fatigue during sub-maximal continuous exercise is different from that associated with high-intensity maximal exercise. In most cases involving well-motivated subjects, the causes of fatigue lie within the muscle itself (Bigland-Ritchie, 1981; Fitts, 1994).

Superior performance during short-tern high-intensity exercise has been associated with high muscle La^- concentration and low muscle pH, each in turn dependent upon relative type II fiber area (Mannion et al., 1995). However, contradictory evidence showing trained subjects to have both a lower muscle La^- concentration and a higher muscle pH after contraction to fatigue than in untrained subjects (Sahlin & Henriksson, 1984) suggests that this issue is not clearly defined. In the majority of cases, the central nervous system and the neuromuscular junction do not act to limit muscle performance (Fitts, 1994).

Theoretically, the muscle cellular mechanisms which control force include: 1) the Ca^{++} concentration surrounding the myo-filaments, 2) the sensitivity of the myo-filaments to Ca^{++}, and 3) the force produced by the cross-bridges characterized by the maximum Ca^{++}-activated force. As Ca^{++} concentration rises, Ca^{++} binds to the inner surface of the t-tubular charge sensor to increase charge and subsequently release Ca^{++} as well as opening SR Ca^{++} channels that are not voltage-regulated. As intra-cellular Ca^{++} concentration increases further, a 'feedback loop' acts to close the channels at both the t-tubule and in the SR. Therefore, at the cellular level, Ca^{++} release from the SR and its concentration around the contractile elements is believed to greatly influence force production and the rate of fatigue. The late stages of fatigue appear to be partly caused by reduced SR Ca^{++} release (Fitts & Balog, 1996). By minimizing the negative effects certain metabolites have on Ca^{++} release by the SR, it may be possible to delay fatigue and improve exercise performance. Metabolites known to, or believed to, directly affect Ca^{++} release from the SR include La^-, H^+, P_i and K^+.

Muscle H^+ Accumulation / Lowered Muscle pH

Although the central mechanism behind muscle fatigue during high-intensity exercise is unclear, the accumulation of H^+ ions, as a result of degradation of muscle glycogen to lactic acid and subsequent dissociation into La^- and H^+, may play a major role in the fatigue

processes. Accumulation of H^+ ions in the muscle is thought to negatively affect muscle force production in a number of ways:

1. H^+ directly inhibits the excitation-contraction process by preventing Ca^{++} release from the SR (Myers & Ashley, 1997). This has been proposed to be the major limiting factor in short-term maximal exercise performance. Williams and Klug (1995) hypothesize that the control of intracellular Ca^{++} is crucial to delaying fatigue. However, low pH has been seen to have no effect on Ca^{++} release from the SR release channel (Lamb et al., 1992) or on t-tubular charge movement (Fitts & Balog, 1996).
2. H^+ inhibits the enzymes PFK and phosphorylase, the rate-limiting enzymes involved in muscle glycogenolysis (Myers & Ashley, 1997). At a pH of 6.4, glycolysis is almost completely stopped.

Since the La^-/H^+ transporter only increases transport of La^-/H^+ by 3- to 4-fold, La^-/H^+ does accumulate during exercise and fatigue is induced. It is hypothesized that this may be an in-built regulatory mechanism of the human body, acting to protect against acidosis during intense exercise. The intra-muscular accumulation of HLa and reduced muscle pH inhibits glycolysis and the excitation-contraction process, leading to fatigue before the HLa already cleared from the muscle into the blood overwhelms the pH regulatory mechanisms of the body.

Muscle Lactate Accumulation

Following intense exercise, where muscle La^- concentrations can reach 30 mM, a strong inverse relationship exists between La^- concentration and both force production and recovery from fatigue (Fitts, 1994). While this relationship could be strongly linked to the concomitant increase in H^+ concentration occurring with La^- production, or even to increases in P_i and Mg^{++} concentration, La^- could affect the excitation-contraction process in its own right. This follows the findings of Hogan et al. (1995) where increased muscle La^- concentration led to lower force production, even without an accompanying pH change.

Lactate, at muscular concentrations ranging from 10-30 mM, has been seen to inhibit the ability of caffeine and Ca^{++} to induce Ca^{++} release from the SR in muscle fibers (Dutka & Lamb, 2000; Favero et al, 1997). Concentrations of 10-30 mM La^- are commonly seen during

and following high-intensity exercise. The magnitude of the inhibitive effect on caffeine-induced Ca^{++} release was seen to be dependent on the La^- concentration in the muscle, ranging from ~25% decrement in Ca^{++} release at 10 mM La^- up to ~37% Ca^{++} release decrement at 20 mM lactate concentration (Favero et al., 1997; Dutka & Lamb, 2000). Lactate has also been seen to inhibit Ca^{++} release activated by H_2O_2 and Ag^+ (Favero et al., 1995). The authors hypothesize that, since La^- inhibits Ca^{++} release induced by such a diverse group of activators, it is likely that the site at which La^- binds to the Ca^{++} release channel is critical to normal function and that Ca^{++} release is inhibited by La^- independent of the form of the physiological trigger.

Considering the role Ca^{++} plays in the contraction process, acting to bind to troponin and allowing the cross-bridge cycling between the actin and myosin filaments, a reduction in the SR release of Ca^{++} from caffeine-induced and Ca^{++}-induced Ca^{++} release channels could theoretically lead to inhibition of the contraction process and a decrease in muscle force. These tests were performed in vitro, therefore caution should be taken before interpreting these results to intact muscle, especially during exercise bouts. These results with SR vesicles and isolated release channels do not show whether La^- interferes with in vivo Ca^{++} release, which involves activation of Ca^{++} release channels by the voltage sensor receptors in the adjacent t-tubular system (Melzer et al., 1995).

Williams & Klug (1995) argue that the majority of energy consumed during muscle contraction is directly linked to Ca^{++} ions (myosin ATPase activity) and SR Ca^{++} sequestration (SR Ca^{++}-ATPase activity). Inhibition of SR Ca^{++} release and peak intra-cellular Ca^{++} concentration would lead to down-regulation of both myosin ATPase and SR Ca^{++}-ATPase activity, thus conserving energy and reducing force production. Thus, Ca^{++} concentration in the working muscle cell may be a critical mechanism in reducing force production during fatigue.

However, the impact of La^- potentially inhibiting the Ca^{++}-induced and caffeine-induced Ca^{++} release during intense exercise is thought to be relatively minor, since La^- has been seen to have only a minimal inhibitory effect on the normal voltage-sensor Ca^{++} release channels, even with very high La^- concentrations (Dutka & Lamb, 2000; Posterino & Fryer, 2000). The normal voltage-sensor Ca^{++} release channels play a greater role in providing Ca^{++}

for the excitation-contraction process. These sensors are similarly are not majorly affected by H^+ (Lamb et al, 1994). Maximal Ca^{++}-activated force decreased by only 3% and 2% when an acid load of 15 mM and 30 mM La^-, respectively, was applied to rat muscle fibers (Dutka & Lamb, 2000), while in chemically skinned rabbit fibers, maximal Ca^{++}-activated force decreased by 2-8% and 0-4%, depending on the ion substitution used, when acid loads of 15 mM and 30 mM respectively were applied (Andrews et al., 1996).

Similarly, Posterino & Fryer (2000) demonstrated that $_L$-lactate ion has negligible effects on either voltage-dependent Ca^{++} release or SR Ca^{++} handling, leading to only a modest (~5%) inhibitory effect on the E-C process and muscle contraction, irrespective of the intensity of contraction This indicates that La^- accumulation, in its own right, has only a small inhibitory effect, if any, on the excitation-contraction coupling process and therefore does not seem to play a major role in muscle fatigue during intense exercise bouts. Dutka & Lamb (2000) hypothesise that the inverse correlation between muscle La^- concentration and force production is therefore likely to be the result of other metabolic changes occurring during intense exercise, such as increased P_i and to a lesser degree increased H^+ concentration or to increased fiber swelling.

Inorganic Phosphate (P_i) Accumulation

While the most popular theory regarding the mechanism for fatigue during short-term, high intensity exercise focuses on H^+ and La^- accumulation in the working muscle, other metabolites may in fact be involved, at least in part. A recent hypothesis by Allen and Westerblad (2001) regarding a mechanism for fatigue of the working muscle revolves around accumulation of P_i. The concentration of P_i in the myoplasm increases substantially during fatigue, from a resting level of ~1-5 mM up to as high as 30-40 mM during intense contraction. This is a result of the breakdown of PCr to creatine and P_i in an effort to maintain sufficient intra-cellular ATP levels.

The accumulation of myoplasmic P_i has been noted to reduce Ca^{++} release from the SR, just as La^- and H^+ have been reported to do. Allen and Westerblad (2001) contend that the myoplasmic P_i can move into the SR during fatigue and cause precipitation of Ca & P_i within the SR. This insoluble precipitate is thought to buffer the Ca^{++} within the SR and

greatly increase the Ca^{++} uptake capacity of the SR. In fatigued muscle, not only does the Ca^{++} release from the SR slow considerably, therefore negatively affecting the contraction process and promoting fatigue, but the store of releasable Ca^{++} in the SR also declines in a manner mirroring the decline in Ca^{++} release. Furthermore, in muscle fibers with inoperative creatine kinase (CK), the rise of myoplasmic P_i is absent during exercise and the decline in Ca^{++} release from the SR is delayed.

Lowered Intra-cellular Potassium (K^+)

Another recent hypothesis toward a mechanism for fatigue involves extra-cellular K^+ accumulation, as a result of a loss of intra-cellular K^+ during muscle contraction. Since intra-cellular K^+ is important for cell excitability, it is proposed that a decrease in muscle cell K^+ could play a role in the fatigue process during exercise. In electrically stimulated rat soleus muscle, high extra-cellular K^+ led to loss of titanic force. However, when either HLa, propionic acid or carbonic acid (from addition of high CO_2) was applied, the effects of elevated K^+ accumulation were counter-acted (Neilsen et al., 2001). Thus, it is possible that HLa may actually have a role in delaying of fatigue by minimizing the harmful effects of decreased intra-cellular K^+. Similar results were seen following one-legged knee extensor exercises, with extra-cellular K^+ increasing with increasing intensity of contraction (Juel et al., 2000). There appears to be a limit to which intra-cellular K^+ can decrease before the associated depolarization begins to limit the action potential frequency (Fitts & Balog, 1996).

Eccentric-induced Muscle Damage

Eccentric contractions involve lengthening the muscle under tension, producing greater force with greater neural efficiency than concentric contractions. Eccentric contractions performed by muscle unaccustomed to forced lengthening typically result in transient, repairable skeletal muscle damage. Damage is often seen following an initial session of resistance exercise, such as when beginning resistance training or following an extended period of detraining.

It is well known that unaccustomed eccentric muscle contraction leads to structural damage within the muscle, decreased muscle strength and function, delayed soreness, and

inflammation. In general, high-intensity contractions through a full range of motion tend to produce greater muscle damage. Early function loss is thought to be a result of either changes in the excitation-contraction coupling (Warren et al., 2001) or disruption at the level of the sarcomere (Morgan & Allen, 1999; Proske & Morgan, 2001).

Characteristics

Eccentric-induced muscle damage is characterized in a number of forms including:

Structural Damage

Disruption of muscle fibers has been noted by a variety of changes in structural characteristics. Most notable is broadening, streaming and often total disruption of Z-discs (Beaton et al., 2002; Friden & Lieber, 1998), leading to the suggestion that the Z-disc may be the weak link in the myofibrillar contractile chain. Other notable signs of structural damage include myofibrillar and sarcolemmal disruptions, such as the widening of the A-band (Friden & Lieber, 1998), misalignment of sarcomeres, regional disorganization of the myofilaments and cytoskeletal proteins, and t-tubule damage (Morgan & Allen, 1999). Muscle content of structural proteins titin and nebulin are both reduced as a consequence of eccentric-induced muscle damage (Trappe et al., 2002). This internal damage is hypothesized to ultimately lead to sarcolemma disruption (Proske & Morgan, 2001). T-tubule damage can result in leaking of Ca^{++} into the muscle cell, resulting in elevated intracellular Ca^{++}.

Serum and plasma (and sometimes urinary) levels of specific muscle proteins are used as indicators of muscle damage. Creatine kinase, LDH, myoglobin and slow myosin heavy-chain fragments are commonly used indices of eccentric-induced muscle damage, with plasma CK being the most commonly used (Warren et al., 1999). Plasma CK activity increases ~48 hrs post-ECC and can take up to 7 days to reach peak levels (Balnave & Thompson, 1993; Nosaka & Clarkson, 1995; Eston et al., 1996; Pearce et al., 1998; Hortobagyi et al., 1998). Plasma CK correlates poorly with measures of muscle function, in particular strength (Ebbeling & Clarkson, 1989), indicating separate distinct mechanisms are involved in the process of eccentric-induced muscle damage. Rate of protein synthesis begins to rise ~48 hrs post-exercise and elevates by ~80% at day 5 post-exercise (Lowe et al., 1995).

Neuromuscular Performance Decrements

Following unaccustomed eccentric exercise, an immediate decrement in maximal strength is seen. Maximal strength tends to decrease by 30-60%, depending on the severity of the eccentric bout (Hortobagyi et al., 1998; Golden & Dudley, 1992; Cleak & Eston, 1992; Nosaka & Clarkson, 1995; Howell et al., 1993; Pearce et al., 1998). The magnitude of this decrement is similar for both concentric and eccentric contractions (Golden & Dudley, 1992). Recovery of strength is slow, relative to the recovery of other indices of eccentric-induced muscle injury. In certain instances, complete recovery of strength has not been seen even after 10 days post-eccentric bout (Cleak & Eston, 1992; Howell et al., 1993). Similarly, motor coordination and proprioception both appear to be negatively affected in eccentric-induced muscle damage (Pearce et al., 1998) possibly due to damage to the muscle spindles and tendon organs that regulate muscle length and tension (Brockett et al., 1997).

Delayed-onset Muscle Soreness

Delayed-onset muscle soreness generally appears in the muscle unaccustomed to eccentric exercise ~24 hrs following the bout. Peak soreness is usually experienced ~48-72 hrs post-exercise and gradually subsides over the course of 7 days. Soreness is prevalent mainly in the musculo-tendinous junction initially, before gradually spreading throughout the muscle (MacIntyre et al., 1995). Musculo-tendinous soreness is further evidence that damage brought about by eccentric exercise is structural in nature and occurs to muscle fibers and/or connective tissue (Ebbeling & Clarkson, 1989). The fibers immediately proximal to the musculo-tendinous junction are oriented obliquely, making them most vulnerable to the tension generated (Friden et al., 1986). Connective tissue, being less elastic than muscle tissue, may be more susceptible to damage.

Mechanism/s

Eccentric-induced muscle damage is a direct result of mechanical stress as a consequence of lengthening the muscle fiber under tension. Structural damage, both to the contractile elements and to the surrounding connective tissue, leads to inflammation and soreness.

Morgan (1990) proposed that eccentric contractions cause some sarcomeres in each myofibril to extend to very long lengths. As such, some sarcomeres do not properly interdigitate upon relaxation. Subsequent eccentric contractions stretch these sarcomeres, placing great tensile stresses on neighbouring myofibrils. Consequently, adjacent sarcolemma and SR fail. However, this proposal does not explain the role of inflammation in the recovery process or the mechanism behind adaptation to eccentric contractions.

Armstrong et al. (1991) expanded on the model proposed by Morgan (1990), noting that damage to the sarcolemma leads to alterations in the permeability of the membrane and basal lamina. Consequently, excessive amounts of Ca^{++} enter the fiber down its electrochemical gradient. If sarcolemmal membrane integrity is badly disrupted, the ATPase pumps, acting to transport Ca^{++} out of the cytoplasm, are overwhelmed and free cytosolic Ca^{++} concentration rises.

This reaction to damage of the sarcolemma (and SR) may help to explain the sudden decrement in muscle function and significant muscle shortening (Chleboun et al., 1998) seen immediately following unaccustomed eccentric exercise. Ca^{++} release from the SR plays a major role in the contraction process. As such, failure of the SR to provide sufficient Ca^{++} release for force generation will lead to a decrease in force produced. Accumulation of intracellular Ca^{++} may be the mechanism causing activation of proteolytic and phospholypolytic pathways that degrade structural and contractile proteins respectively. Inflammation gradually appears over the course of 2 to 6 hours post-eccentric bout when phagocytic cells such as macrophages become active at the damage site.

While inflammation has been implicated as the major cause for the progression and severity of eccentric-induced muscle damage, evidence suggests this may not be the case. A comparison of the time-courses of inflammation with other indices of eccentric-induced muscle damage, such as DOMS, loss of muscle strength and passive muscle shortening, shows significant differences (Cleak & Eston, 1992; Chleboun et al., 1998; Howell et al., 1993). In addition, anti-inflammatory drugs have no effect on soreness, or any other measure of muscle damage such as strength, passive muscle shortening or muscle protein release (Pizza et al., 1999; Bourgeois et al., 1999).

Eccentric-induced Muscle Damage and La^- Clearance / pH Regulation

Preliminary evidence using rats suggests muscle pH regulation is compromised following eccentric-induced muscle injury (Pilegaard & Asp, 1998; Yeung et al., 2002). Pilegaard & Asp (1998) saw a decreased rate of La^- transport 2 days after intense eccentric contractions by the gastrocnemius and soleus muscles as compared with the contra-lateral control leg muscles. The magnitude of the decrement in La^- transport rate was fiber-type dependent, with white gastrocnemius muscle fibers (29% decrease) showing a more severe decrement in rate of La^- transport following eccentric-induced muscle damage than red gastrocnemius fibers (13% decrease). Similarly, a reduced physiochemical buffer capacity (13% & 9% for white and red gastrocnemius fibers respectively) was noted 2 days following intense eccentric contractions. The soleus muscle fibers were unaffected by the eccentric-contraction protocol, with no changes seen in either rate of La^- transport or physiochemical buffer capacity.

Muscle La^- concentrations during recovery from intense isometric contractions 2 days post-eccentric bout was also compromised following eccentric-induced muscle damage. The decrease ranged from 33-41% immediately post-isometric contractions and 30-32% 3 minutes post-isometric contractions for the white gastrocnemius, red gastrocnemius, and soleus fibers, as compared with the control muscle fibers. Total release of La^- from the muscle fibers during the entire period of isometric-stimulation and 5 minutes recovery was 19% for eccentric-induced damaged muscle fibers. However, there did not appear to be any difference in muscle pH nor release of H^+ between eccentric-induced muscle damage and the control fibers at any time point either prior to, or during recovery from, the isometric contractions applied 2 days post-eccentric bout.

These results indicate two main points:

1) Sarcolemmal La^-/H^+ transport capacity is compromised by eccentric-induced muscle damage. The magnitude of eccentric-induced muscle damage, and subsequent compromisation of La^-/H^+ transport capacity, appears greater in fast twitch or glycolytic (white) muscle fibers, as compared with slow twitch or oxidative (red) fibers.

2) Production of La^- may be compromised due to a reduced glycogenolytic potential. Lower La^- production and subsequent dissociation of H^+ was theorized to be the cause of

lower muscle La^- concentrations seen during recovery from the isometric contractions, even given the impaired ability of the sarcolemmal La^-/H^+ transport in the damaged muscle fibers as indicated by the decreased rate of La^- transport and the lower total release of La^- from the damaged fibers.

However, these results also raise a more intriguing question. Why is muscle pH unaffected by eccentric-induced muscle damage, even though both La^-/H^+ transport and La^- production appear to be negatively affected? Are the decrements in both La^-/H^+ production and La^-/H^+ clearance from a muscle fiber similar in magnitude, therefore canceling each other out and creating no net change in muscle pH?

Interestingly, Yeung et al. (2002) noted a lower resting pH following eccentric-induced muscle damage as compared with performance of solely isometric contractions. In addition, a slower rate of recovery from acidosis, induced by application and removal of either 20mM or 40mM ammonium chloride (NH_4Cl), following eccentric-induced muscle damage was noted. The pH in eccentrically-contracted muscle recovered at a rate of 0.013 ± 0.002 U/min after application of an acid load as compared with a rate of 0.022 ± 0.003 U/min for isometrically-contracted muscle. Half-time recovery from the acid load was 23.3 ± 1.9 min and 15.9 ± 1.7 min for eccentrically-contracted and isometrically-contracted muscle, respectively. Rate of recovery from the applied acidosis was further slowed by application of a Na^+/H^+ exchanger (NHE) inhibitor, amiloride. Interestingly, no difference was noted in the estimated buffering power between eccentrically contracted (76.8 ± 12.2 mM) and isometrically contracted (73.1 ± 11.2 mM) muscle. This range of estimates for buffering power is higher than noted previously (Aickin & Thomas, 1977), which the authors assumed was linked to the differing methodology, cell preparation and temperature used.

Yeung et al. (2002) believe it is clear that the underlying cause for compromised pH regulation, La^- removal and buffer capacity following eccentric-induced muscle damage is due to either 1) an increase in La^-/H^+ production during subsequent exercise, 2) a reduced ability to clear the La^- and H^+ from the working muscle cell, via either reduced La^-/H^+ transport or a reduced physiochemical buffer capacity, or 3) a combination of both mechanisms.

Both authors hypothesized that the more likely explanation is a reduced ability to clear La^-/H^+ from the working muscle cell. Yeung et al. (2002) base this assumption on the evidence provided by a slower rate of recovery from an acid load seen with eccentric-induced muscle damage, while Pilegaard & Asp (1998) showed eccentric-induced muscle damage to have a lower La^- transport and a lower physiochemical buffer capacity. The possibility that the first hypothesis, an increase in La^-/H^+ production, may be the underlying cause for compromised pH regulation was contradicted by Pilegaard & Asp (1998) showing the muscle La^- concentration is actually reduced in eccentric-induced muscle damage, even with an accompanying lower La^-/H^+ clearance.

It is further hypothesized that the impairment of La^-/H^+ clearance could be a result of impaired La^-/H^+ transport across the sarcolemma or mitochondrial membranes. Sarcolemmal damage, as a result of eccentric-contraction, may lower MCT content or limit the ability of the MCT's to transport both La^- and H^+ across the sarcolemmal membrane into the extracellular fluid.

Yeung et al. (2002) however hypothesize that damage to the Na^+/H^+ exchanger (NHE), rather than the MCT, may be the main cause for the compromised pH regulation. The NHE is the main transporter involved in muscle pH regulation at rest, working along with the Na^+-dependent and the Na^+-Cl^--HCO_3^- transport systems to maintain homeostasis in skeletal muscle (Juel, 1998). This hypothesis is based on the negative effect amiloride, an inhibitor of the NHE, has on the rate of recovery from acidosis. Possible reasons put forward by the authors for impaired pH regulation following eccentric-induced muscle damage include 1) a direct reduction of NHE activity by a local metabolite, paracrine or autocrine influence, 2) damage to t-tubules, resulting in sealing over of some t-tubules so that some NHEs cannot contact the extra-cellular solution, 3) increased permeability of the surface membrane to protons, so that the efflux of the NHE is counteracted by the inward flux of H^+, or 4) an increase in intracellular Na^+ associated with membrane damage, leading to a reduced inward Na^+ gradient and, therefore, a reduction in H^+ efflux.

However, the findings of Yeung et al. (2002) are based on testing using an acid load applied during resting conditions, where the NHE is dominant in the role of pH regulation within the skeletal muscle. The relevance of the findings from Yeung et al. (2002) to acidosis

formed as a result of intense exercise, where the La^-/H^+ transporting MCT-1 and MCT-4 are the major facilitators of pH regulation and the NHE is relatively inactive (Juel, 1998), is unclear.

Neither of the studies to date that focused on eccentric-induced muscle damage and muscle La^-/H^+ clearance measured MCT expression or content. However, expression of GLUT-4, the muscle glucose transporter with similar properties to the MCT-1 and MCT-4, is decreased following eccentric-induced muscle damage (Asp et al., 1995a; Asp et al., 1995b). Total GLUT-4 content was decreased in both white and red gastrocnemius fibers 1 and 2 days after an eccentric exercise bout (Asp et al., 1995b). In a similarly designed study, GLUT-4 content was depressed by 32% and 34% from the control muscle at days 1 and 2 following an eccentric bout of one-legged knee flexion, and had returned to normal by days 4 and 7 (Asp et al., 1995a). Neither study analyzed changes in rate of glucose transport, however, it can be assumed that glucose transport had been compromised while GLUT-4 content was reduced.

METHODS

Participants

Characteristics

Eight healthy, male college-age subjects (mean age: 22; height: 181 ± 7 cm; weight: 80 ± 14 kg) participated in this study. Subjects were non-resistance trained, having not participated in a formal resistance exercise program within 3 months prior to commencement of the study. Participants were predominantly drawn from the student population of Iowa State University. Subjects were required to give informed consent to participate in this study. Confidentiality of test results was guaranteed and freedom to withdraw from participation at any time and for any reason was assured. Subjects were free of any medical condition that may have hindered exercise performance.

Diet & Lifestyle Requirements

Although no set diet guidelines were enforced for the participants during the course of the study, all participants were instructed to avoid alcohol, smoking and drugs and to avoid strenuous exercise. Moderate, constant intensity exercise, such as leisurely walking and bicycling was allowed. Subjects were asked to record any exercise or physical activity performed in the 3 days leading up to the HIE bout in each trial. Each subject had similar activity patterns across trials over this time period.

Instruments

Eccentric / Concentric Exercise bouts

A Biodex isokinetic dynamometer was used for the muscle-damaging ECC and the CON bout. The Biodex dynamometer (Biodex Medical Systems, Shirley, NY) allows all muscle actions to be performed under conditions in which variables such as contraction speed and resistance, and limb and joint positioning, can be controlled and manipulated.

Standardization of Subject Positioning

The aim of the positioning of the participant was to promote full knee range-of-motion (ROM) while limiting both hip flexion or extension (at the ends of the range of

motion) and the desire of the individual to generate additional force via upper body movement. Knee extension required the participant to be seated and secured with hip flexion at ~90^0. The knee joint was aligned with the axis of rotation of the lever arm of the dynamometer. Lever arm length was adjusted in accordance with the lower leg length of the individual. Mechanical stops were engaged to prevent excessive range of motion for both extension and flexion at the knee joint. The subject's upper body, waist and lower thigh were strapped securely to minimize confounding effect of leverage and hip flexor muscle activity.

All participants performed a sub-maximal warm-up before performing the ECC and CON bouts. The sub-maximal warm-up consisted of 5-8 concentric contractions at a speed of 30^0/sec, graduating from approximately 50% to 90% of maximal voluntary contraction. Subjects were closely monitored during the initial testing session to determine proper positioning of the individual's knee joint in relation to the axis of rotation of the lever arm. Seat height and back angle, the ROM and the length of the lever arm were noted for each individual during the initial trial and repeated in the subsequent exercise bout.

Exhaustive, High-intensity Exercise bout

The HIE bout required subjects cycling on a Lode Excalibur Sport cycle ergometer (Lode Medical, Groningen, NL). Seat height was recorded and used for the repeat trial. Gas exchange was analyzed using the Physiodyne Max II Metabolic Cart system (Physio-dyne Instrument corp., Quogue, NY).

Blood and Muscle Sampling

Samples of exercised muscle were taken during biopsy, under the local anesthesia Xylocain. A trained, qualified practitioner performed all muscle biopsies. Muscle samples were taken from the vastus lateralis of the right leg. Samples were immediately frozen in liquid nitrogen and stored at -80^0 C awaiting later assay. All samples were dissected free of blood and connective tissue before analysis. Samples were analyzed for pH, La^- concentration, and β_m.

A single blood sample was taken immediately prior to the HIE bout (Pre-HIE) via venu-puncture. Samples of 10 mL were drawn from the antecubital vein and analyzed for CK concentration.

The pre-HIE sample were drawn after resting for 15 minutes in the supine position. The post-HIE samples were drawn first immediately following completion of the test with the subject still sitting on the bike (Post-HIE 0min) and then, 10 minutes later, while lying in the supine position (Post-HIE 10min).

Procedures

Testing Sessions

Eccentric Bout & Concentric bout

All subjects performed a muscle-damaging bout of unaccustomed maximal eccentric leg extensions. Each bout consisted of 100 total repetitions (10 sets x 10 repetitions) at an angular velocity of 60^0/sec throughout the contraction. Maximal resistance was required throughout the range of motion (~90^0). Eccentric contractions involve resisting the Biodex lever arm from the point of maximal leg extension through to full flexion, while conversely, concentric contractions involve pushing against the lever arm from full leg flexion through to full extension. The leg was passively returned to the starting position following each repetition at an angular velocity of 60^0/sec, thus each repetition took ~1.5 seconds with ~1.5 seconds recovery between repetitions. Recovery between sets was 30 seconds. Verbal encouragement and feedback was given throughout the bout to help ensure maximal effort is given on each repetition.

The peak torque (Nm) and total work (J) performed by each individual in each set was recorded and used to determine parity across trials. While it was not expected that each subject would perform a similar amount of work over the 100 repetitions across the two trials, it was hoped that the ECC bout would be able to elicit moderate to severe muscle damage and soreness and that the CON would act as the control group with no soreness or muscle damage expected.

Exhaustive High-intensity Bout

Following the muscle-damaging ECC bout, all subjects completed an exhaustive, high-intensity cycling bout (HIE). This was performed 2 days (~48 hrs) after the ECC bout, when most symptoms and markers of eccentric-induced muscle damage tend to be at peak levels. A progressive and continuous VO_2max protocol over a 10-15 minute span was used. Subject's workload was incrementally increased every 2 minutes until fatigue, with the starting workload set at 50 W.

Study Design

Dependent Measures

The dependent variables measured and analyzed were:

- Muscle pH
- Muscle buffer capacity (β_m)
  - In-vivo
  - In-vitro
- Muscle La^- concentration
- Plasma Creatine Kinase (CK)
- Delayed-onset muscle soreness (DOMS)

Muscle pH and β_m

Muscle pH was measured using a small glass electrode after homogenization of freeze-dried muscle samples in a non-buffered solution containing 145mM KCl, 10mM NaCl, and 5mM sodium fluoride (Pilegaard et al., 1999).

Two measures of β_m were calculated:- I) in-vivo and II) in-vitro. In-vivo β_m was calculated from the change in muscle pH and La^- concentration observed from rest (pre-HIE) to exhaustion (immediately post-HIE). The equation used was the ratio of the change in muscle La^- from rest to exhaustion divided by the change in muscle pH. The in-vitro (non-HCO_3 physiochemical) muscle buffer capacity (β_m) was calculated from the change the pH of the muscle sample when adjusted by adding 20uL of 0.04 N HCl. Both measures of β_m are expressed as mmol/ kg/ pH unit and are termed 'slykes'.

Muscle Lactate Concentration

A perchloric acid extract of a 5-10 mg muscle sample was incubated, frozen and then centrifuged. The La^- was extracted from the muscle sample while it was still frozen, due to the confounding influence of muscle enzymes at room temperature. The sample was analyzed using enzymatic fluorometry to determine La^- concentration (Lowry, 1972).

Plasma Creatine Kinase

Blood samples were centrifuged to separate plasma from the red blood cells. The supernatant was analyzed using a spectrophotometer for CK concentration.

Delayed-Onset Muscle Soreness (DOMS)

Muscle soreness was measured subjectively using a linear 10-point scale for the knee extensors of each participant. The muscle soreness scale (Appendix D) ranges from 0 (no pain or discomfort) to 10 (extreme pain and discomfort). Participants gave a verbal indication of the level of soreness experienced after viewing the scale.

Study Timeline

Day 1: ECC (or CON) bout

Day 3: Blood & muscle sample (Pre-HIE) → HIE bout → Muscle samples (Post-HIE 0min & Post-HIE 10min)

A cross over design was used. Control trials consisted of a concentric bout of exercise (CON) in place of the ECC bout. All subjects completed the testing cycle twice, completing both the ECC trial and CON trial. Approximately 28 days separated each testing cycle by the individual to minimize the effect of a possible training adaptation from the HIE bout and residual effects from the ECC bout.

Analysis

All data are presented as mean ± standard error (SE). Muscle pH, muscle La^- concentration and muscle H^+ concentration (nM) was analyzed using a between (ECC & CON)-by-within (Pre-ECC, Post-HIE 0 min & Post-HIE 10 min) ANOVA with repeated

measures on trials. By applying the Bonferroni technique to adjust α, experimenter-wise error was minimized. Where significance for a particular variable was found, post hoc analysis using Scheffe technique was performed. In vivo β_m, in vitro β_m, rate of La^- recovery, VO_2peak, peak workload, VO_2 at 200 W, RER at 200 W and plasma CK were analyzed using a dependent t-test, looking at comparisons of group means. Significance was set at the $P < 0.05$ level for all comparisons. Muscle soreness was analyzed by a non-parametric Friedman repeated measures ANOVA on ranks.

RESULTS

Figure 1 shows plasma CK for ECC and CON groups two days after the resistance exercise bout. It is clear from the figure that unaccustomed ECC action (mean: 847.6 ± 330.5 U/L) led to higher levels of CK compared with the CON group (mean: 226.6 ± 40 U/L), although the difference was not significant ($p<0.089$) due to the large variation inherent with the ECC group. The ECC group ranged from 63-2156 U/L with 4 subjects having plasma CK concentrations less than 150 U/L while 3 subjects had levels above 1700 U/L. In contrast, the CON group only ranged from 95-357 U/L.

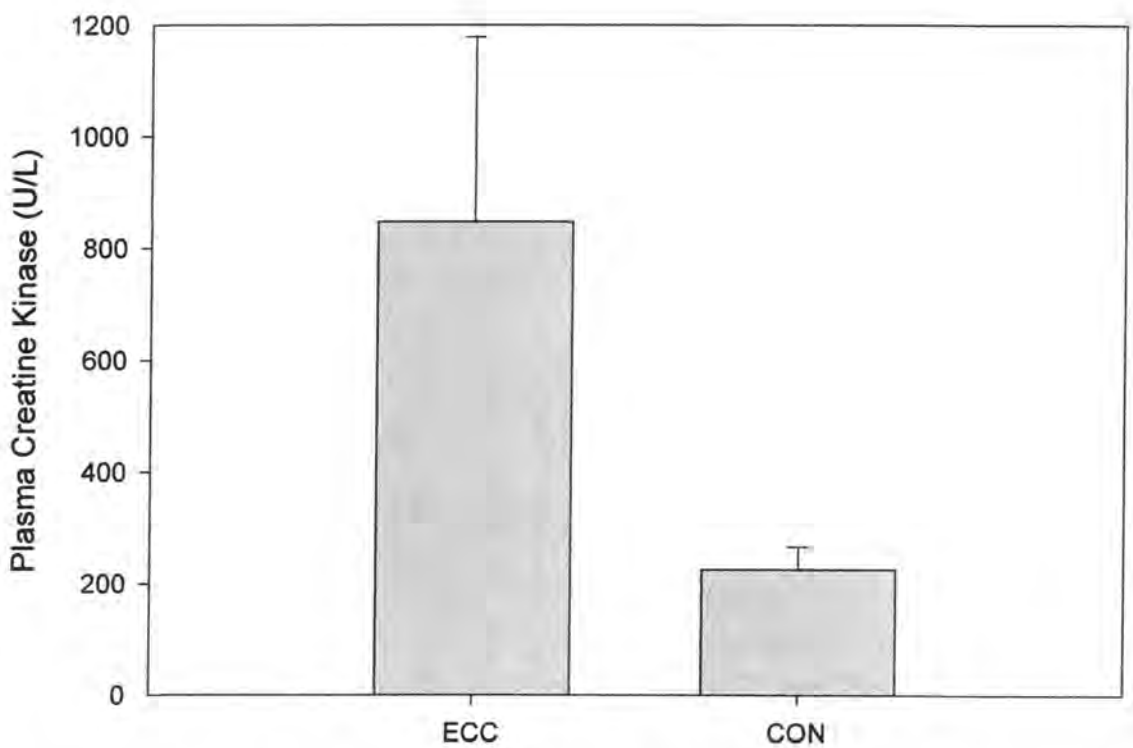

Figure 1. Mean ± SE plasma creatine kinase (CK) two days after performing either eccentric (ECC) (n=8) or concentric (CON) (n=8) resistance exercise.

The plasma CK results reflect the results seen in the other measure of eccentric-induced muscle damage, rating of muscle soreness. Rating of muscle soreness values are

shown in Table 1. The ECC group displayed significantly (p=0.0047) greater muscle soreness two days following the resistance bout than the CON group, with all eight subjects estimating their soreness levels to be 5 (some soreness / discomfort) or above. Two subjects estimated their soreness to be the maximum of 10 (extreme soreness / discomfort) following the ECC bout. In contrast, no subjects believed any soreness existed in the affected muscle two days following the CON bout.

Table 1. Mean ± SE values for rating of muscle soreness, VO_2peak, workload at VO_2peak, and VO_2 at 200W two days after performing either eccentric (ECC) or concentric (CON) resistance exercise.

	Muscle Damage	**HIE bout Performance & Economy**			
Trial	**Muscle soreness (arbitrary units)**	**VO_2 peak (ml/kg/min)**	**Workload at VO_2 peak (W)**	**VO_2 at 200 W (Index of economy)**	**RER at 200 W**
ECC (n=8)	8 ± 2	49.5 ± 2.4	308 ± 17	33.6 ± 1.7	1.04 ± 0.03
CON (n=8)	0 ± 0	50.9 ± 2.1	313 ± 18	33.8 ± 2.0	1.00 ± 0.02
Significant difference?	Yes (p=0.0047)	No	No	No	No

No significant differences were noted between groups in either VO_2peak, workload at VO_2peak, VO_2 at a workload of 200W, or RER at 200W as shown in Table 1. Mean VO_2peak values of ~50ml O_2/kg/min, as noted in this study, are typically seen in healthy college-aged males.

While muscle La^- concentrations were raised significantly (p<0.0001) both immediately following and 10 minutes following the HIE bout, as compared to pre-HIE values, no significant differences were noted between ECC and CON group means or as an interaction across trials. It is clear from Figure 2 that, while no significant differences were noted between the ECC and CON groups, it does appear that there is greater muscle La^-

accumulation in the CON group both immediately post- (CON: 18.9 ± 2.2 umol/g; ECC: 15.7 ± 1.5 umol/g) and 10 minutes post-HIE (CON: 14.6 ± 1.1 umol/g; ECC: 11.2 ± 1.5 umol/g). A moderate effect size (ES=0.5) for the immediately post-HIE data and a large effect size (ES=1.1) for the 10 minutes post-HIE data gives support to there being greater muscle La^- accumulation in CON than in ECC at these time points.

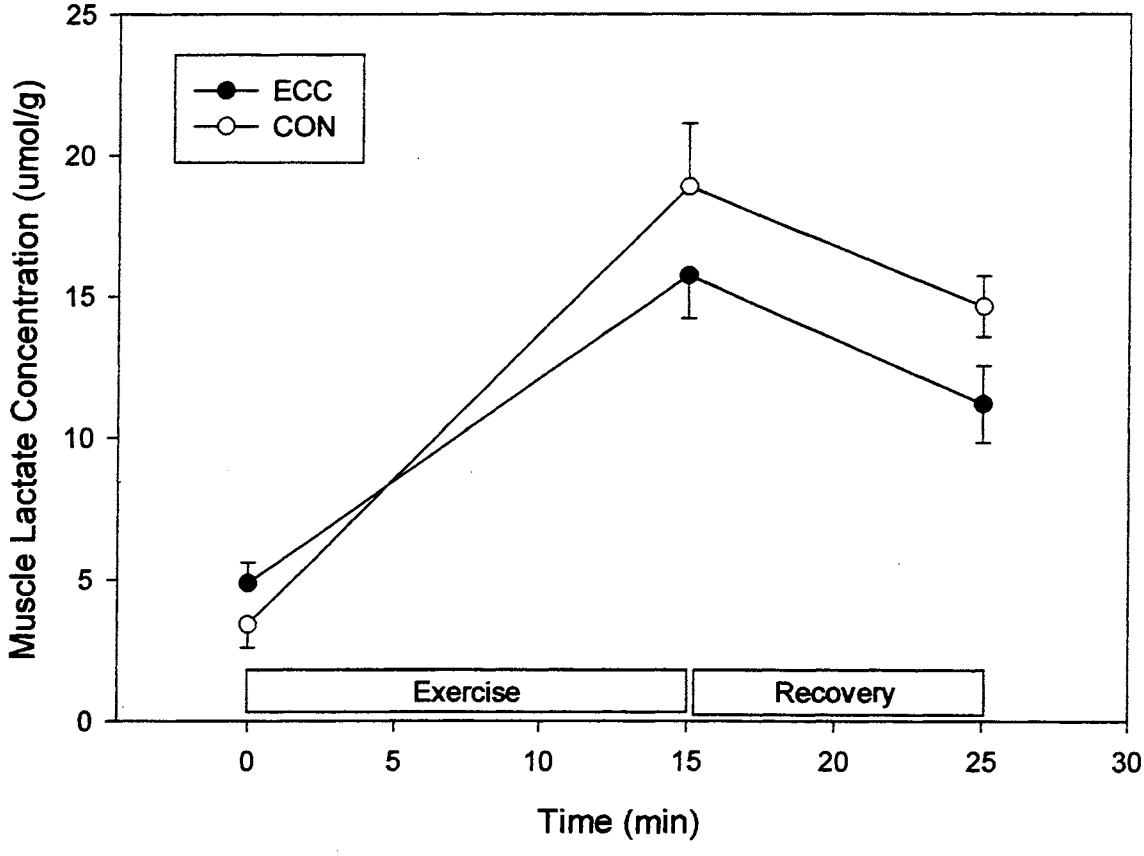

Figure 2. Mean ± SE muscle lactate concentration before, immediately following, and 10 minutes following graded exercise to exhaustion two days after performing either eccentric (ECC) (n=8) or concentric (CON) (n=8) resistance exercise.

Since both ECC and CON displayed similar resting La^- concentrations prior to the HIE bout (4.8 ± 0.7 umol/g and 3.4 ± 0.8 umol/g respectively), it appears that the CON group had the greater rise in muscle La^- accumulation during the exercise bout leading to the higher

levels immediately post-HIE. However, both ECC and CON showed similar rate of recovery in muscle La^- levels in the 10 minutes post-HIE.

Both groups' muscle La^- levels dropped in the 10 minute recovery period, by 22 ± 16% for the ECC trial and 13 ± 11% for the CON trial. There was no significant difference in the rate of muscle La^- recovery between trials due in large part to large inter-individual variation. The rate of recovery of muscle La^- over the 10 minute recovery period ranged from -35%-70% for the ECC trial and –45%-37% for the CON trial, with the negative values denoting an actual increase in muscle La^- concentration over the 10 minute recovery period. Two subjects in each of the ECC and CON trials showed an increase in muscle La^- during the recovery period.

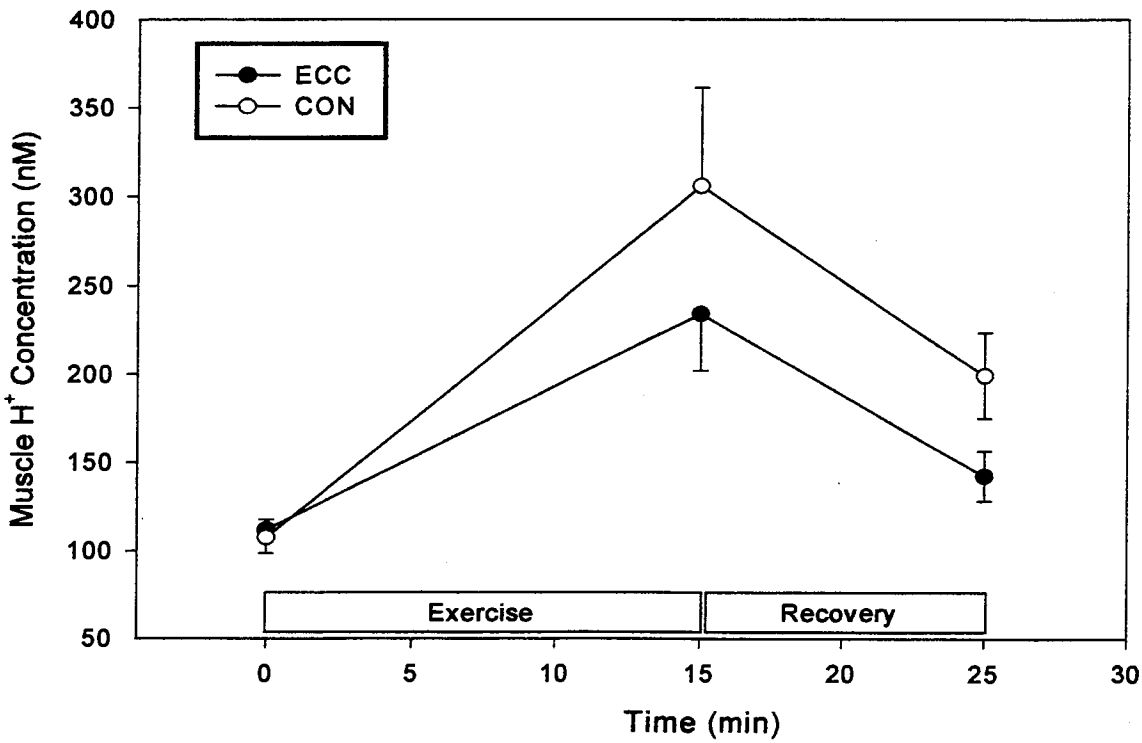

Figure 3. Mean ± SE muscle H^+ concentration before, immediately following, and 10 minutes following graded exercise to exhaustion two days after performing either eccentric (ECC) (n=8) or concentric (CON) (n=8) resistance exercise.

In a similar trend as the muscle La^- concentration, the CON trial showed a distinct rise in muscle H^+ concentration during the exercise bout, compared to the ECC group. While there was no significant interaction between trial and time, it is clear from Figure 3 that the CON trial showed greater muscle H^+ concentrations at both immediately post- (CON: 305.9 ± 55.5; ECC: 234.1 ± 32.1) and 10 minutes post-HIE (199.2 ± 24.2; ECC: 142.3). This was reflected by effect sizes of 0.5 and 0.8 for immediate post-HIE and 10 minutes post-HIE respectively.

Just as seen with muscle La^- concentration, the CON group displayed a greater rise in muscle H^+ concentration (2.8 times pre-HIE values) during the exercise bout than the ECC group (2.1 times pre-HIE values), however, both groups showed a similar rate of H^+ removal during the 10 minute recovery period. The CON group had a 35% drop in muscle H^+ concentration over the 10 minute recovery period while the ECC group saw a 39% drop. This translates into a rise in pH of 0.21 units and 0.22 units for CON and ECC groups respectively over the 10 minute recovery period, after initially dropping by 0.47 units and 0.32 units, respectively, during the bout. Neither group had returned to pre-HIE values for muscle H^+ concentration (and thus muscle pH) even after 10 minutes of passive recovery from the HIE bout.

Table 2. Mean muscle pH before, immediately following, and 10 minutes following graded exercise to exhaustion two days after performing either eccentric (ECC) (n=8) or concentric (CON) (n=8) resistance exercise.

Trial	Pre-HIE	Immediate post-HIE	10min post-HIE
ECC	6.95	6.63	6.85
CON	6.98	6.51	6.70

Measurement of β_m included calculations based on both in vivo and in vitro data. No significant differences were seen between ECC and CON groups in either measure of β_m. Figure 4 shows the β_m calculated by in-vivo methods for ECC and CON groups, two days after the resistance exercise bout. The ECC group (mean: 32.6 ± 2.0 slykes) had a smaller range of in-vivo β_m values (28-37 slykes) compared to the CON group (mean: 37 ± 8.6

slykes; range: 19-70 slykes). Both groups had 3 (out of 8) missing data points due to invalid muscle pH or La^- values, thus lowering the power of the performed test. Figure 5 shows the β_m calculated by in-vitro methods for ECC and CON groups, two days after the resistance exercise bout.

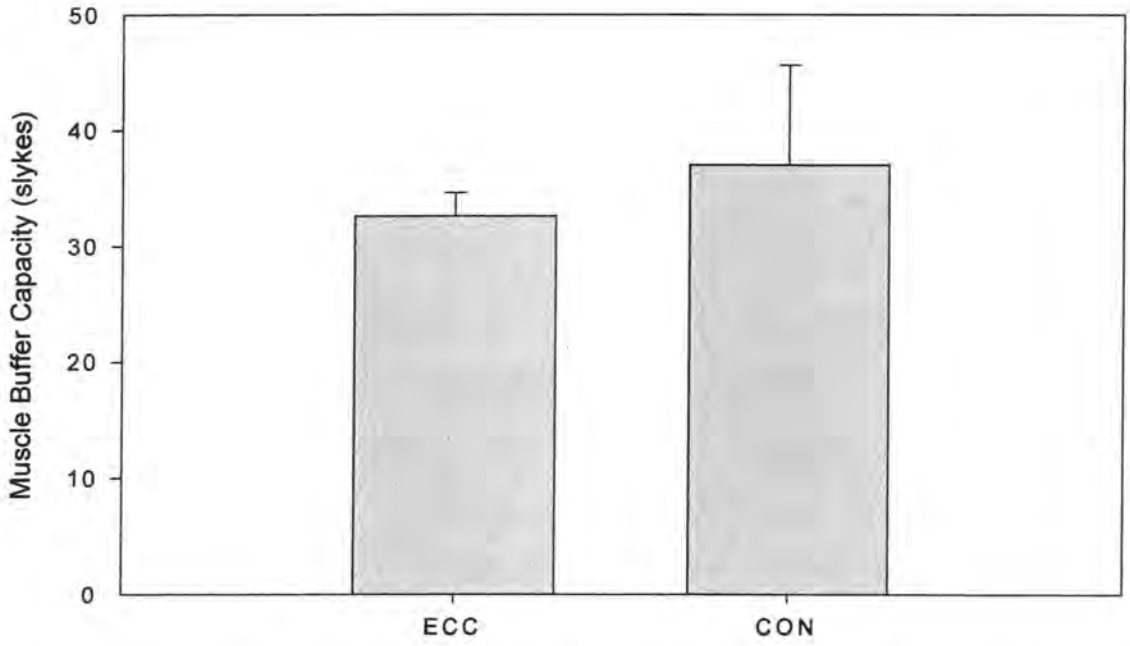

Figure 4. Mean ± SE in vivo muscle buffer capacity two days after performing either eccentric (ECC) (n=5) or concentric (CON) (n=5) resistance exercise.

While mean in vitro β_m values were larger (by 46%) than those calculated in vivo, the lower SE for each group and the lack of missing data points allowed for a more sensitive measurement than the in-vivo calculation. In contrast to the in-vivo calculation where the CON group had a marginally higher β_m, the ECC (mean: 53.1 ± 2.7) showed a marginally higher β_m than the CON group (mean: 48.6 ± 3.3).

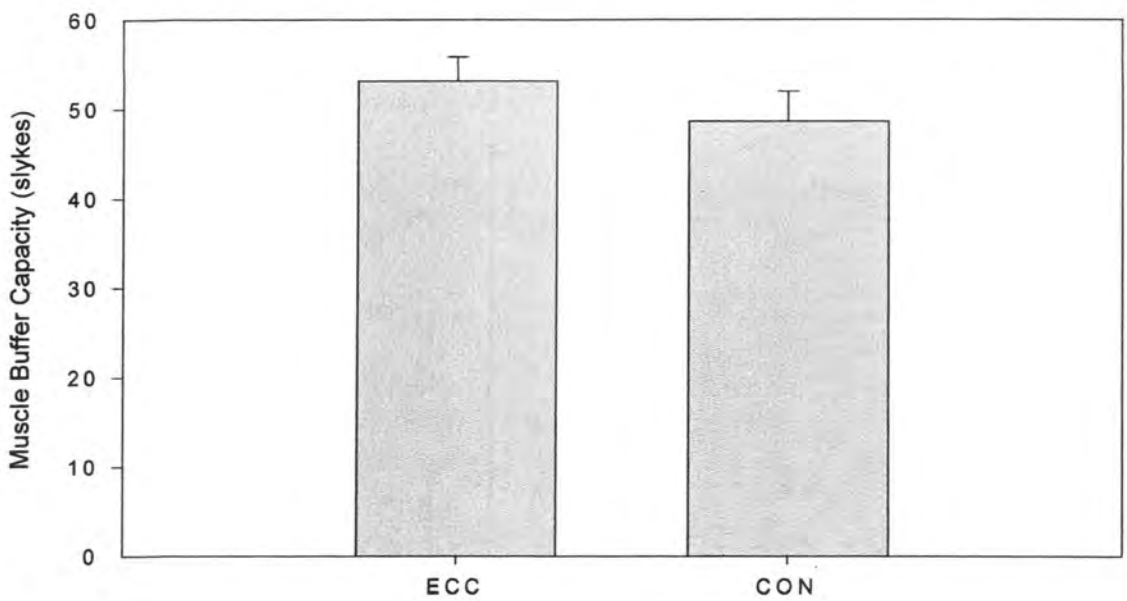

Figure 5. Mean ± SE in vitro muscle buffer capacity two days after performing either eccentric (ECC) (n=8) or concentric (CON) (n=8) resistance exercise.

DISCUSSION

While there were no significant differences noted in muscle La^- concentrations, muscle pH or the measures of β_m either between ECC and CON trials or as an interaction with time, the moderate-to-high effect sizes noted both immediately following HIE and during recovery from HIE for muscle La^- and H^+ concentrations give an indication of the relationship between eccentric-induced muscle damage and the La^-/H^+ production/removal capabilities of the working muscle. The main findings from this study are a trend toward a smaller rise in muscle La^- concentration and a smaller drop in muscle pH in eccentrically damaged muscle during subsequent exhaustive HIE. These results are in conjunction with no change in either β_m or exercise performance and economy parameters.

The lower accumulation of muscle La^- and H^+ during the exercise bout in eccentrically damaged muscle, without an accompanying change in exercise performance, may be attributed to either a decrease in production of both La^- and H^+ during the bout or an improved ability to remove these metabolites from the muscle.

Because there appeared to be no difference between ECC and CON trials in rate of lactate recovery during the 10 minutes post-HIE or in the trans-membrane flux capabilities as indicated by the similar in vivo β_m between groups, it appears that eccentric-induced muscle damage does not affect removal of La^- and H^+ from the muscle cell. Thus, from the data in this study, it appears that the lower muscle La^- accumulation and higher muscle pH both at the end of HIE and during recovery seen with eccentric-induced muscle damage is mainly due to a decrease in production of these metabolites during the exercise bout.

Care needs to be taken with this assumption due to a number of factors. Firstly, no direct measures of La^- (or H^+) transport were made in this study. Measures of MCT content or activity from the muscle samples may, in time, help to paint a broader picture of the clearance kinetics of these metabolites. Secondly, the two measures used as an indicator of La^- (and H^+) flux from the muscle, the rate of La^- recovery during the 10 minutes post-HIE and the in-vivo β_m, are indirect and may not be sensitive enough to allow a valid and reliable insight into clearance kinetics of the muscle cell. The rate of La^- recovery following the exercise bout also incorporates continuing La^- and H^+ production thus making judgments on clearance capabilities unclear.

The other measure used to indirectly make assumptions regarding La^- and H^+ clearance capabilities, the in vivo β_m, is also highly dependent upon factors other than trans-membrane flux of H^+. Therefore, no firm conclusions can be made from this study regarding the effect of eccentric-induced muscle damage on La^- and H^+ transport capabilities.

The experimental hypothesis stated that eccentric-induced muscle damage leads to greater muscle La^- and H^+ accumulation during the HIE bout and that the rate of recovery of these metabolites back to pre-exercise concentrations would be slower during the 10 minutes recovery period. The premise for this hypothesis was a likely impaired ability to clear La^- and H^+ from the muscle, most probably as a result of the damage to the sarcolemma and to the MCTs that are bound to the sarcolemma. Reduced MCT content or activity as a result of eccentric-induced muscle damage would theoretically lead directly to impaired La^- and H^+ clearance from the working muscle.

The hypothesis of impaired La^- and H^+ clearance with eccentric-induced muscle damage was based primarily on findings from the two major studies to have previously investigated the effect of eccentric-induced muscle damage on La^- and H^+ kinetics. Pilegaard et al. (1998) saw a decrease in both the sarcolemmal La^- and H^+ transport capacity and the physiochemical β_m in eccentric-damaged muscle. The authors noted a decrease in La^- transport rate of 29% in white gastrocnemius muscle and 13% in red gastrocnemius muscle that had undergone unaccustomed eccentric contractions compared to control rat muscle during a subsequent intense exercise bout. Muscle release of La^- during recovery from the exercise bout was also compromised. The results were in combination with significantly lower muscle La^- concentrations both immediately following and during recovery from intense exercise seen in the ECC group, complicating the issue. A lower muscle La^- concentration leads to lower La^- gradients across the sarcolemma and therefore a lower driving force for La^- efflux. Pilegaard et al. were thus not able to deduce to what extent the reduced La^-/H^+ transport capacity per se contributed to the lower release of La^- from the eccentrically damaged muscle.

Although current data and findings from previous studies indicate that transport capacity may be compromised with eccentric-induced muscle damage, we cannot discount that damage to the sarcolemma may lead directly to increased passive flux of these

metabolites. This theory may help to explain the lower concentrations of both La^- and H^+ immediately following and during recovery from HIE. Sarcolemma damage, leading to 'holes' in the membrane, would theoretically allow for greater potential for La^- and H^+ to 'leak' out through the areas in the membrane that are disrupted, leading to lower accumulation.

A further confounding factor in the effect of eccentric-induced muscle damage on La^- and H^+ transport is the possibility of the associated inflammation raising extra-cellular fluid volume and aiding passive flux of these metabolites down the concentration gradient out of the muscle cell. To date, no study has analyzed this issue.

The assumption that eccentric-induced muscle damage leads to slightly lower production of La^- and H^+ during HIE without any accompanying change in the ability to clear La^- and H^+ from the muscle is plausible from a physiological standpoint but does not support the initial experimental hypothesis.

It is important to note that Pilegaard et al. did find eccentric-induced muscle damage to result in significantly lower muscle La^- concentrations both immediately post- and 3min post-supramaximal isometric contractions as compared with control muscle. Muscle La^- concentrations in ECC-white gastrocnemius, ECC-red gastrocnemius, and soleus muscle samples were lower immediately after (34%, 41% and 33%, respectively) and 3 minutes after (32%, 30%, and 32%, respectively) the supra-maximal isometric stimulation. In addition, ECC-white gastrocnemius muscle had significantly higher muscle pH immediately post-isometric contractions than the CON muscle, although there were no other significant differences in muscle pH between any ECC group and the CON group at any other time points.

Pilegaard et al. (1998) explained these conflicting findings by proposing that eccentric-induced muscle damage leads to lower La^- (and H^+) production during subsequent intense exercise, indicating that the muscle damage may lower the glycogenolytic potential of the muscle. The authors further hypothesized that the reduced glycogenolytic potential may be due to sub-normal muscle glycogen concentrations. Eccentric-induced damage has been associated with sustained decreased muscle glycogen content (Asp et al., 1995a; Doyle et al., 1993; Widrick et al., 1993).

Asp et al. (1998), in a study similar in design to this study, noted significantly lower resting muscle glycogen concentrations in eccentric-exercise thigh muscle (402 mmol/kg dry wt) than in control thigh muscle (515 mmol/kg dry wt). This difference was further widened following two-legged CON exercise (190 mmol/kg dry wt vs 379 mmol/kg dry wt for ECC and CON respectively) despite identical power and duration of exercise by the two legs. These findings were in association with higher La^- release at rest prior to the concentric exercise in the eccentric-exercised leg but lower La^- release just before termination of the exercise bout. Maximal work capacity was also lower (by 23%) in the eccentric thigh compared with the control thigh.

The authors concluded that eccentric-induced muscle damage leads to an increase in resting glycogenolysis, leading to the higher rate of La^- release seen at rest. During concentric exercise at a given power output, eccentrically damaged muscle is working at a higher relative workload, resulting in increased glycogen utilization and, in turn, decreased endurance. The decreased rate of La^- release seen during late exercise may be a sign of glycogen depletion and may be a contributing factor in the development of fatigue.

Yeung et al. (2002) saw a lower resting pH in eccentrically-damaged muscle in addition to a slower rate of recovery from induced acidosis. However, the authors concluded that, since the rate of recovery from induced acidosis was slower, it is more likely due to a decrease in H^+ clearance rather than an increase in H^+ production.

Two measures of β_m were made in this study, with the aim of analyzing both the physiochemical buffering properties and the degree of trans-membrane flux of H^+ from the muscle. In vivo calculation of β_m takes into account changes from the pre-HIE to immediate post-HIE muscle samples, thus encompassing both the physiochemical buffer properties of the muscle sample in addition to the trans-membrane flux of La^- and H^+ out of the muscle cell, either into the blood or into the mitochondria for oxidation. Pre-HIE and immediate post-HIE muscle samples are assumed to be homogenous, allowing the physiochemical buffer properties of the muscle samples to be investigated. In contrast, in vitro measurement of β_m involves a single (pre-HIE) muscle sample being subjected to an applied acid load in a controlled environment. Therefore, the trans-membrane flux of La^- and H^+ cannot be investigated, allowing only an analysis of the physiochemical buffer properties.

In this study, neither measure of β_m, either in vivo or in vitro, showed any significant differences between eccentrically damaged muscle and that of undamaged muscle. In the case of in vitro β_m, this finding supports the hypothesis that the physiochemical buffering properties of muscle should not be greatly affected by eccentric-induced muscle damage. Physiochemical buffering properties include the activity of P_i, HCO_3^- and histidine on H^+ removal. The concentrations or activity rates of these compounds should not be greatly affected by the type of muscle damage inflicted by unaccustomed ECC contractions. However, the finding by Pilegaard et al. (1998) of a significant decrease in physiochemical β_m with eccentric-induced muscle damage is surprising and conflicts the findings of this study, where no significant change in in vitro β_m was seen or even expected. Pilegaard et al. believed that this finding was due to release of muscle enzymes to plasma but did not expand on this theory. The authors also ascribed the fact that eccentric-induced muscle damage lead to lower muscle La^- accumulation both during and following intense exercise but did not lead to higher muscle pH at these time-points was due to the compromised physiochemical β_m.

The finding from this study that eccentric-induced muscle damage had no effect on in-vivo β_m, which additionally takes into account the trans-membrane flux of La^- and H^+, is surprising and does not support the experimental hypothesis. The measurement of in-vivo β_m was based on the rationale that trans-membrane flux of La^- and H^+ would be compromised as a result of eccentric-induced muscle damage. It was hypothesized that eccentric-induced muscle damage would have a negative effect on the sarcolemma-bound MCTs, either through a decrease in content or activity. This would lead to a decrease in transport of both La^- and H^+ across the sarcolemma from the working muscle cell out into the circulation.

It is possible that the inability to find any significant effect of eccentric-induced muscle damage on trans-membrane flux of La^- and H^+ may be due to an increase in intra-cellular removal of these metabolic intermediates by the working muscle cell mitochondria, rather than transport out of the cell into the blood. An increase in mitochondrial oxidation of La^- may plausibly explain the slightly lower muscle La^- concentration and higher muscle pH seen immediately post-HIE in the ECC group without a corresponding increase in trans-membrane flux of these metabolites.

The lack of physiological significance being achieved by eccentric-induced muscle damage on β_m, either by in-vivo or in-vitro calculations, may in part be attributed to the lack of sensitivity of the testing procedures. In-vivo calculations are based on the validity and reliability of both the pH and the lactate measurements in pre-HIE and immediate post-HIE muscle samples. As such, error in the measurement of either or all of the pre-HIE muscle pH, pre-HIE muscle lactate, immediate post-HIE muscle pH or immediate post-HIE muscle lactate samples will be magnified in the resulting calculation of in-vivo β_m. Conversely, in-vitro calculation of β_m involves adding a known quantity of acid to the pre-HIE muscle sample and measuring the change in pH, error in either measurement of pH can affect the subsequent calculation of β_m.

Interestingly, the in vitro measures of β_m, which focus purely on just the physiochemical properties of the muscle, were higher for both ECC and CON groups than the in-vivo calculation of β_m, which encompasses the physiochemical properties and the trans-membrane flux of La^- and H^+. The mean in vivo β_m values seen in this study (CON: 37 slykes) were slightly below those seen by Sharp et al. (1986), where the average pre-sprint training β_m was 44 slykes. Although both studies involved college-aged males, the larger sample size used by Sharp et al. (n=15) aids in raising the power of their results. The relatively low in vivo β_m seen for both groups in this study may in part be attributed to the high resting muscle La^- concentrations. Resting muscle La^- concentrations typically are ~1-2 mmol, however mean levels of 4.87mmol and 3.41mmol for ECC and CON respectively were noted in this study.

In contrast, the mean in vitro β_m values from this study (ECC: 53.2 slykes; CON: 48.6 slykes) were well above that noted by Pilegaard et al. (1998), where mean β_m was ~37 slykes for eccentrically damaged rat muscle and ~40 slykes for control rat muscle samples. Yeung et al. (2002) also noted no significant difference between eccentrically damaged rat muscle and control rat muscle in estimated buffering power after a bout of isometric contraction. However, Yeung et al. measured the change in NH_4^+ concentration with a given change in pH, as opposed to the change in La^- concentration used to measure β_m in the other studies, and was measured only 10 minutes after the eccentric bout. These intra- and inter-study

differences in β_m may reflect either limitations in the testing procedures or may be an indication that the present methods for calculating β_m may be not sensitive enough to be able to compare results from either different measures of β_m or results from different studies.

In summary, eccentric-induced muscle damage leads to lower muscle accumulation of La^- and H^+ during HIE and these levels are still depressed during recovery from that seen in undamaged muscle. This may be a result of a lower rate of production of HLa, via reduced glycogenolytic potential, or by raised passive flux of these metabolites out of the muscle through the damaged membrane.

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APPENDIX A

Informed Consent Form - Study

INFORMED CONSENT DOCUMENT

Research project title: Effect of prior eccentric exercise on muscle lactate accumulation during high intensity exercise

Principal Investigators: Andrew Keech, Graduate Assistant
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Rick Sharp, Professor
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250 Forker Bldg
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This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

The purpose of this study is to examine the effect unaccustomed eccentric (letting weights down slowly – also called negative lifts) muscle action has on subsequent high-intensity exercise performance. In particular, we wish to look at the effect eccentric muscle actions have on lactic acid and hydrogen ion build-up within the working muscle during the subsequent high-intensity bout. This study will add to the knowledge base regarding the production and clearance of lactic acid from muscle that has performed unaccustomed eccentric exercise. No one has ever examined the effect that this special type of muscle action has on subsequent high-intensity exercise performance, yet this information is important in understanding development of peak performance potential in people who may participate in competitive sports. You are being invited to participate in this study because you are a healthy, young college student at Iowa State University.

DESCRIPTION OF PROCEDURES

If you agree to participate in this study, your participation will last for 1.5 months comprising a total of 4 testing sessions here at Forker building. Each testing session should last ~ 1hr.

This study will involve performing a single bout of resistance exercise (100 consecutive contractions of knee extension) on a Biodex isokinetic dynamometer - a special machine that can control the speed of movement and measure how much force you produce. This bout of exercise involves performing just eccentric contractions and is aimed to make your thigh muscles sore. Two days later, when some muscle soreness has appeared, you will perform a VO2max test on a cycle ergometer (stationary cycling) to determine your maximal aerobic

capacity. This test involves about 12-15 minutes of cycling on a stationary bike. After a 4 minute warmup period, you will cycle at a very easy workload for 3 minutes and workload will be increased by 40 watts every 2 minutes until you feel that you can no longer maintain the pedal cadence at 70 rpm. During this test you will breathe through a breathing valve that will direct your expired air into the gas analyzers for measurement of oxygen uptake. During the VO2max test, we also will take one venous blood sample immediately before the test and muscle biopsy samples before the VO2max test, immediately after the VO2max test, and at 10 minutes after the VO2max test. The blood sample will be used to measure the level of CPK (a marker enzyme that leaks out of muscle whenever damage to the muscle cell membrane occurs). The muscle biopsy samples will be used to measure levels of lactic acid and pH that accumulate in the muscle during the VO2max test and how quickly they recover after the test.

Four weeks after this first testing session, you will return to repeat the testing. However, instead of doing the eccentric resistance exercise two days before the VO2max test, you will perform concentric resistance exercise (100 consecutive contractions). Half of the subjects will perform the eccentric contractions in their first testing session while the other half will perform the concentric contractions first.

Testing Session 1	Testing Session 2
Day 1 100 Eccentric Contractions	Day 1 100 Concentric Contractions
Day 3 Muscle Biopsy Venous Blood Sample VO2max Test Post-Exercise Biopsy 10 Minute Post-Ex Biopsy	Day 3 Muscle Biopsy Venous Blood Sample VO2max Test Post-Exercise Biopsy 10 Minute Post-Ex. Biopsy

RISKS

While participating in this study you may experience the following risks:

- Muscle soreness, as a result of the eccentric bout. This soreness will peak ~24-48hrs after the bout and will subside after about 1 week. This soreness should not affect your ability to perform your average daily tasks but you notice it mostly in walking down stairs.
- All exercise bouts in this study require either maximal efforts or a sustained effort until fatigue. As such, exercise of this nature involves the risk of an adverse reaction, such as fainting, dizziness and nausea. Care will be taken to prevent these adverse reactions through careful monitoring of your condition through each exercise bout and recovery period. You may stop an exercise bout at any stage if you begin to feel unwell. In addition, all exercise testing will be monitored by study personnel who are trained in emergency first aid and CPR.
- Muscle sampling involves making a ¼-inch incision into your leg, while under local anesthesia, and extracting a piece of muscle using a biopsy needle. The muscle

sample is about the size of a sunflower seed. This procedure may be slightly uncomfortable, especially when done for the first time. Some muscle soreness may be felt for 2 days following the procedure.

- Blood sampling involves inserting a needle into a forearm vein to draw ~10mL of blood (one test-tube full). A slight pin-prick will be felt when the needle is inserted and slight discomfort might be felt when blood is being withdrawn.

BENEFITS

If you decide to participate in this study there may be no direct benefit to you. It is hoped that the information gained in this study will benefit society by helping to understand the effect of eccentric-muscle actions on exercise performance. Many coaches and athletes use strength programs, requiring a high level of eccentric muscle action, even immediately before competition. This study may give insight into the validity of performing unaccustomed eccentric exercise immediately before competition, and how it affects performance.

COSTS AND COMPENSATION

You will not have any costs from participating in this study. We will pay you $100 for participating in this study. If, for any reason, you decide to discontinue your participation in this study, your compensation will be pro-rated at $25 per visit.

PARTICIPANT RIGHTS

•All test results and findings are strictly confidential. Your identity will not be disclosed in any reports or publications that may result from this research.

• Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

RESEARCH INJURY

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance

CONFIDENTIALITY

Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal

government regulatory agencies and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy your records for quality assurance and data analysis. These records may contain private information. To ensure confidentiality to the extent permitted by law, the following measures will be taken:

- Subjects will be assigned a unique code and letter and will be used on forms and in statistical analyses.
- Only Dr. Sharp and Andrew Keech will have access to study records.
- All hard-copy data files will be stored in a locked filing cabinet while computer files of data will be password protected.
- If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study. If you require more information regarding this study, please do not hesitate to contact me, either through the Department of Health & Human Performance (Forker building) or at home (ph: 268 - 1762). Alternatively, I can be e-mailed at **akeech@iastate.edu** Contact information for the faculty member supervising this research is: Dr. Rick Sharp (294 – 8650; rlsharp@iastate.edu). If you have any questions about the rights of research subjects or research-related injury, please contact the Human Subjects Research Office, 2810 Beardshear Hall, (515) 294-4566; meldrem@iastate.edu or the Research Compliance Officer, Office of Research Compliance, 2810 Beardshear Hall, (515) 294-3115; dament@iastate.edu

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SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____ _____

(Subject's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

APPENDIX B

Informed Consent Form – Muscle Biopsy

INFORMED CONSENT – MUSCLE BIOPSY

Research project title: Effect of prior eccentric exercise on muscle lactate accumulation during high intensity exercise

Principal Investigators: Andrew Keech, Graduate Assistant
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Evenings: 268-1762

Rick Sharp, Professor
Dept. of Health & Human Performance
250 Forker Bldg
294-8650, rlsharp@iastate.edu

This is a research study. Please take your time in deciding if you would like to participate. Please feel free to ask questions at any time.

INTRODUCTION

Participation in this research involves muscle biopsy. This is a well-established procedure for obtaining muscle tissue to use in measurement of metabolic responses to exercise. The needle-biopsy procedure was introduced in the early 1960's as a method to study human muscle function during exercise (Bergstrom, 1962). Here at Iowa State University, we have been using the needle biopsy technique since 1982 in our studies of metabolic responses to exercise.

DESCRIPTION OF PROCEDURES

In this research, we are proposing to obtain biopsy samples from your vastus lateralis muscle (front thigh muscle) before and at two time points after performing a VO2max test. The procedure involves cleaning and numbing the skin over the area where the biopsy will be taken. We use about 1.5 cc of 2% Xylocaine injected under the skin to anaesthetize the skin. The injection of the Xylocaine usually causes a mild burning sensation lasting for a few seconds. Once the area is numbed, a ¼ inch incision is made with a scalpel. A sterile gauze is held firmly over the incision for a couple of minutes to stop the small amount of skin bleeding that usually accompany the incision. The biopsy is then taken by inserting the biopsy needle into the incision and into the muscle. The biopsy needle is hollow and has a blade that slides down the inside of the needle. Once the biopsy needle is in the muscle, the blade is pulled back allowing muscle to bulge into the inside of the needle, and the blade is pushed back down, trapping the small piece of muscle inside the needle. The needle is then withdrawn and the muscle sample is frozen in liquid nitrogen for later analysis.

After the biopsy is performed, we put a sterile gauze over the incision and hold tight pressure for five minutes to stop any bleeding. Then, once the bleeding has stopped, the incision is closed with a butterfly bandage or a steri-strip. It is then covered with another larger bandage that we ask you to leave on and keep dry for three days.

In this study, muscle biopsies will be taken before and after a VO2max test. The first biopsy will done as described above, but the subsequent biopsies will be taken from the same incision as the first biopsy. This means that although three biopsies will be taken, there will only be one incision. By the time you come in for your second experimental trial one month later, the first biopsy site will be completed healed.

In this study, all muscle biopsies will be performed by Rick Sharp, Ph.D. (Professor, Health & Human Performance) who has performed approximately 500 biopsies since 1982.

RISKS

- While injecting the Xylocaine, you will experience a burning or stinging sensation in your skin for a few seconds.
- When the biopsy needle is inserted into the muscle, most people feel as if the muscle is being stimulated to contract, almost like that feeling right before you get a cramp.
- On the day or two after the biopsy, some people (about one-half) report a mild tightness and/or soreness of the muscle like that experienced after a heavy weight workout. This discomfort can be minimized by staying active (avoid long periods of sitting) and by applying ice over the site. This is normal and is a function of the healing process.
- There is a risk of infection of the incision. All of the instruments and procedures we use in the biopsy procedure are sterile. Nevertheless, it is possible that the incision could become infected while it is healing. For this reason, we ask that you keep the bandages on for three days and dry. In the more than 500 biopsies we have performed to date, no infections have been reported.

BENEFITS

If you decide to participate in this study there may be no direct benefit to you. It is hoped that the information gained in this study will benefit society by helping to understand the effect of eccentric-muscle actions on exercise performance. Many coaches and athletes use strength programs, requiring a high level of eccentric muscle action, even immediately before competition. This study may give insight into the validity of performing unaccustomed eccentric exercise immediately before competition, and how it affects performance.

COSTS AND COMPENSATION

You will not have any costs from participating in this study. We will pay you $100 for participating in this study. If, for any reason, you decide to discontinue your participation in this study, your compensation will be pro-rated at $25 per visit.

PARTICIPANT RIGHTS

•All test results and findings are strictly confidential. Your identity will not be disclosed in any reports or publications that may result from this research.

• Your participation in this study is completely voluntary and you may refuse to participate or leave the study at any time. If you decide to not participate in the study or leave the study early, it will not result in any penalty or loss of benefits to which you are otherwise entitled.

RESEARCH INJURY

Emergency treatment of any injuries that may occur as a direct result of participation in this research is available at the Iowa State University Thomas B. Thielen Student Health Center, and/or referred to Mary Greeley Medical Center or another physician or medical facility at the location of the research activity. Compensation for any injuries will be paid if it is determined under the Iowa Tort Claims Act, Chapter 669 Iowa Code. Claims for compensation should be submitted on approved forms to the State Appeals Board and are available from the Iowa State University Office of Risk Management and Insurance.

CONFIDENTIALITY

Records identifying participants will be kept confidential to the extent permitted by applicable laws and regulations and will not be made publicly available. However, federal government regulatory agencies and the Institutional Review Board (a committee that reviews and approves human subject research studies) may inspect and/or copy your records for quality assurance and data analysis. These records may contain private information. To ensure confidentiality to the extent permitted by law, the following measures will be taken:

- Subjects will be assigned a unique code and letter and will be used on forms and in statistical analyses.
- Only Dr. Sharp and Andrew Keech will have access to study records.
- All hard-copy data files will be stored in a locked filing cabinet while computer files of data will be password protected.
- If the results are published, your identity will remain confidential.

QUESTIONS OR PROBLEMS

You are encouraged to ask questions at any time during this study. If you require more information regarding this study, please do not hesitate to contact me, either through the Department of Health & Human Performance (Forker building) or at home (ph: 268 - 1762). Alternatively, I can be e-mailed at **akeech@iastate.edu** Contact information for the faculty member supervising this research is: Dr. Rick Sharp (294 – 8650; rlsharp@iastate.edu). If you have any questions about the rights of research subjects or research-related injury, please contact the Human Subjects Research Office, 2810 Beardshear Hall, (515) 294-4566;

meldrem@iastate.edu or the Research Compliance Officer, Office of Research Compliance, 2810 Beardshear Hall, (515) 294-3115; dament@iastate.edu

****

SUBJECT SIGNATURE

Your signature indicates that you voluntarily agree to participate in this study, that the study has been explained to you, that you have been given the time to read the document and that your questions have been satisfactorily answered. You will receive a copy of the signed and dated written informed consent prior to your participation in the study.

Subject's Name (printed) _____

_____ _____

(Subject's Signature) (Date)

INVESTIGATOR STATEMENT

I certify that the participant has been given adequate time to read and learn about the study and all of their questions have been answered. It is my opinion that the participant understands the purpose, risks, benefits and the procedures that will be followed in this study and has voluntarily agreed to participate.

_____ _____

(Signature of Person Obtaining Informed Consent) (Date)

APPENDIX C

Medical History Questionnaire

MEDICAL HISTORY QUESTIONNAIRE

Please respond to the following items as accurately as possible. This information will be used by the investigator to ensure a safe exercise environment and to determine if there are any contraindications to exercise or participation in this study. All information will remain confidential unless further professional consultation is warranted.

A) Personal Information

Name_____ Age_____
Tel.#_____
Height_____ Weight_____ Male or Female (circle)

B) Medical Information

1. How would you describe your recent general health?
_____Excellent _____ Good _____ Fair _____ Poor

2. Place an X in those boxes that describe symptoms or disorders which you have been diagnosed to have. If possible, also indicate the date of the diagnosis.

_____ high blood pressure	_____ arthritis	_____ chest pain
_____ irregular heart beat	_____ epilepsy	_____ heart attack
_____ heart murmur	_____ anemia	_____ migraine
_____ asthma	_____ back trouble	headaches
_____ hay fever or other allergies	_____ dizziness/	_____ diabetes
_____ other _____	fainting spells	

3. Describe any surgery that you have had within the last two years:_____
_____.

4. Have you ever sustained an injury or experienced any type of chronic pain that has been diagnosed as due to physical activity or sports participation?
_____ Yes _____ No
If yes, please describe_____
How long ago?_____

5. Do you smoke cigarettes? _____ Yes _____ No

6. Are you presently taking any of the following medications?

_____ drugs to control blood pressure	_____ drugs for asthma
_____ drugs to regulate heart rate	_____ drugs for diabetes
_____ drugs for allergies	_____ thyroid hormone
_____ cortisone	_____ prednisone

Indicate the name(s) of those drugs_____
Also note the dosage and frequency of use_____

7. How long has it been since your last physical examination?
_____ less than 1 year _____ 1-2 years_____ 2-3 years

_____ more than 3 years

8. Have any of the above symptoms, disorders or injuries limited your physical activity in the past? _____
In what way?_____

C) Family Medical History

1. Have any of your blood relatives been diagnosed as having any of the following symptoms/disorders? (Include grandparent, parents, brothers, sisters)

_____ heart attack, under age 50
_____ stroke, under age 50
_____ high blood pressure
_____ hyperlipidemia (high cholesterol)
_____ obesity
_____ other _____
_____ asthma or hay fever
_____ congenital heart disease
_____ heart surgery
_____ diabetes

D) Exercise Information

1. List and give the date of any supervised exercise or sports program that you have participated in recently_____

2. Are you currently participating in a regular program of physical activity?
_____ Yes _____ No.
If yes, how often do you exercise per week (on average)?

_____ 1-2 days/wk
_____ 3-4 days/wk
_____ 5-6 days/wk
_____ every day

For how long do you exercise each day?

_____ < 30 min/day
_____ 60-90 min/day
_____ > 120 min/day
_____ 30-60 min/day
_____ 90-120 min/day

What types of activities are regularly included in your program?

_____ jogging
_____ cycling
_____ weight lifting
_____ recreational sports (basketball, racquetball, volleyball, tennis, etc)
_____ other _____
_____ calisthenics
_____ swimming
_____ aerobic dance

How long have you been in your present program?

_____ less than 1 month
_____ 1-3 months
_____ 3-6 months
_____ 6 months to 1 year
_____ more than 1 year

3. How would you categorize your current physical fitness level?

_____ superior _____ good _____ average _____ below average
_____ poor

4. Is there any reason why you think your activity should be limited in this research project?_____

_____

_____

I attest that all of the above information is accurate to the best of my knowledge
Signed_____ Date_____

APPENDIX D

Delayed Onset Muscle Soreness (DOMS) – Scale of Severity

Delayed Onset Muscle Soreness (DOMS)
Scale of severity

0	**No soreness**
1	
2	**Slight sensation of soreness**
3	
4	**Some soreness / discomfort**
5	
6	**Moderate soreness/ discomfort**
7	
8	**Severe soreness / discomfort**
9	
10	**Extreme soreness / discomfort**

APPENDIX E

Raw Data

Subject	Trial	Time	H as nM	pH	Lactate
1	1	0	114.8	6.94	6.51
1	1	1	204.2	6.69	13.5
1	1	2	106.5	6.97	9.28
1	2	0	105.9	6.97	5.05
1	2	1	234.5	6.63	11.72
1	2	2	234	6.63	17.03
2	1	0	129	6.89	6.01
2	1	1	342.8	6.46	22.03
2	1	2			
2	2	0	104.7	6.98	2.63
2	2	1	148	6.83	13.11
2	2	2	139.2	6.86	11.18
3	1	0	131.8	6.88	
3	1	1	120.2	6.92	14.23
3	1	2	153.4	6.81	16.21
3	2	0	113	6.95	3.32
3	2	1			
3	2	2	138	6.86	9.85
4	1	0	97.7	7.01	4.78
4	1	1			
4	1	2	208.9	6.68	14.63
4	2	0	74.1	7.13	0.91
4	2	1	321.5	6.49	22.15
4	2	2	154.9	6.81	13.92
5	1	0			7.22
5	1	1	138	6.86	9.31
5	1	2	138	6.86	12.62
5	2	0	119	6.92	4.05
5	2	1	549.5	6.26	23.73
5	2	2	346.7	6.46	17.42
6	1	0	92	7.04	4.16
6	1	1	242.7	6.61	16.17
6	1	2	131.8	6.88	9.91
6	2	0	87.1	7.06	1.96
6	2	1	403.4	6.39	23.84
6	2	2	210.1	6.68	17.06
7	1	0	105.9	6.97	1.37
7	1	1	274.5	6.56	16.62
7	1	2	164.9	6.78	10.08
7	2	0			1.47
7	2	1	336.7	6.47	24.66
7	2	2	180.5	6.74	17.47
8	1	0	112.2	6.95	4.05
8	1	1	316.2	6.5	18.35
8	1	2	92.4	7.03	5.58
8	2	0	149.3	6.83	7.93
8	2	1	148	6.83	12.94

		8	2	2	190.5	6.72	13.19		

Subject	Trial	CK	DOMS	VO2peak	Wpeak	Econ	BC (in vivo)	BC (in vitro)	La % rec
1	1	111.6	5	44.3	263	35.7	28	53.21	31.23
1	2	144.4	0	49.6	250	37.4	19.3	58.45	-45.34
2	1	2156	7	39.1	325	25.5	37.7	57.33	
2	2	356.9	0	39.8	304	22.9	69.9	38	14.76
3	1	143.9	7	59.8	366	34.3		47.4	-13.86
3	2	227.2	0	60.4	375	35.7		55.29	
4	1	63.1	7	58.2	383	28.9		53.7	
4	2	357.4	0	53.6	396	30.5	33.3	36.19	37.19
5	1	1780.4	10	50.9	304	38.7		38.4	-35.56
5	2	349	1	55.2	304	37.8	29.6	58.47	26.6
6	1	107.8	6	49.4	275	36.4	28.5	54.33	38.74
6	2	95.6	0	52	296	35.4	32.9	43.2	28.46
7	1	475.9	10	47.1	304	30.8	36.9	63.75	39.33
7	2	108.6	0	47.2	325	30		43.4	29.14
8	1	1942.4	8	47.4	250	38.3	31.8	57.19	69.58
8	2	173.7	0	49.6	254	40.4		56.12	-1.96

APPENDIX F

Statistical Analysis

Two Way Repeated Measures Analysis of Variance on Two Factors
General Linear Model

Dependent Variable: Muscle H^+ Concentration (nM)

Normality Test: Failed (P = 0.0001)

Equal Variance Test: Failed (P = <0.0001)

Source of Variance	DF	SS	MS
Subject	7	18287.4	2612.5
Trial	1	12429.0	12429.0
Trial x Subject	7	102186.4	14598.1
Time	2	172354.3	86177.2
Time x Subject	14	63761.5	4554.4
Trial x Time	2	479.3	239.7
Residual	9	49098.6	5455.4
Total	42	427882.9	10187.7

Source of Variance	F	P
Subject	0.1898	0.9758
Trial	0.8556	0.3856
Trial x Subject		
Time	18.6330	<0.0001
Time x Subject		
Trial x Time	0.0439	0.9572
Residual		
Total		

Dependent Variable: Muscle Lactate Concentration

Normality Test: Failed (P = 0.0001)

Equal Variance Test: Failed (P = <0.0001)

Source of Variance	DF	SS	MS
Subject	7	38.419	5.488
Trial	1	0.532	0.532
Trial x Subject	7	161.918	23.131
Time	2	824.223	412.112
Time x Subject	14	198.710	14.194
Trial x Time	2	57.598	28.799
Residual	10	154.685	15.468

Total	43	1925.384	44.776

Source of Variance	F	P
Subject	0.2470	0.9481
Trial	0.0240	0.8807
Trial x Subject		
Time	28.6718	<0.0001
Time x Subject		
Trial x Time	1.8618	0.2054
Residual		
Total		

One Way Repeated Measures Analysis of Variance - Plasma Creatine Kinase

Normality Test: Passed (P = 0.2078)

Equal Variance Test: Failed (P = 0.0088)

Group	N	Missing
1.000	8	0
2.000	8	0

Group	Mean	Std Dev	SEM
1.000	847.6	934.9	330.5
2.000	226.6	113.2	40.0

Power of performed test with alpha = 0.0500: 0.3033

The power of the performed test (0.3033) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Source of Variance	DF	SS	MS
Between Subjects	7	3429041.8	489863.1
Between Treatments	1	1542750.3	1542750.3
Residual	7	2779075.6	397010.8
Total	15	7750867.7	

Source of Variance	F	P
Between Subjects		
Between Treatments	3.89	0.0893
Residual		
Total		

One Way Repeated Measures Analysis of Variance – VO2peak

Normality Test: Passed (P = 0.1092)

Equal Variance Test: Passed (P = 0.9592)

Group	N	Missing
1.000	8	0
2.000	8	0

Group	Mean	Std Dev	SEM
1.000	49.5	6.86	2.42
2.000	50.9	6.07	2.14

Power of performed test with alpha = 0.0500: 0.1081

The power of the performed test (0.1081) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Source of Variance	DF	SS	MS
Between Subjects	7	554.37	79.20
Between Treatments	1	7.84	7.84
Residual	7	32.26	4.61
Total	15	594.47	

Source of Variance	F	P
Between Subjects		
Between Treatments	1.70	0.233
Residual		
Total		

One Way Repeated Measures Analysis of Variance – Workload (W) at VO2peak

Normality Test: Passed (P = 0.4231)

Equal Variance Test: Passed (P = 0.6436)

Group	N	Missing
1.000	8	0
2.000	8	0

Group	Mean	Std Dev	SEM
1.000	308.8	47.5	16.8

2.000	313.0	51.8	18.3

Power of performed test with alpha = 0.0500: 0.0487

The power of the performed test (0.0487) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Source of Variance	DF	SS	MS	
Between Subjects	7	33754.8		4822.1
Between Treatments	1	72.3	72.3	
Residual	7	806.8		115.3
Total	15	34633.8		

Source of Variance	F	P
Between Subjects		
Between Treatments	0.627	0.454
Residual		
Total		

One Way Repeated Measures Analysis of Variance – Index of Economy (VO2 at 200W)

Normality Test: Passed (P = 0.3409)

Equal Variance Test: Failed (P = 0.0226)

Group	N	Missing
1.000	8	0
2.000	8	0

Group	Mean	Std Dev	SEM
1.000	33.6	4.73	1.67
2.000	33.8	5.64	1.99

Power of performed test with alpha = 0.0500: 0.0487

The power of the performed test (0.0487) is below the desired power of 0.8000.
You should interpret the negative findings cautiously.

Source of Variance	DF	SS	MS	
Between Subjects	7	368.819		52.688
Between Treatments	1	0.141		0.141
Residual	7	10.374		1.482
Total	15	379.334		

Source of Variance	F	P
Between Subjects		
Between Treatments	0.0949	0.767
Residual		
Total		

Paired t-test: Buffer Capacity (in-vivo)

Normality Test: Passed (P = 0.4643)

Group	N	Missing
1.000	8	3
2.000	8	3

Group	Mean	Std Dev	SEM
1.000	32.58	4.56	2.04
2.000	37.00	19.24	8.61

Difference	-9.30	20.9	12.1

t = -0.771 with 2.00 degrees of freedom. (P = 0.5212)

95 percent confidence interval for difference of means: -61.2 to 42.6

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.5212)

Power of performed test with alpha = 0.0500: 0.0578

Paired t-test: Buffer Capacity (in-vitro)

Normality Test: Passed (P = 0.5215)

Group	N	Missing
1.000	8	0
2.000	8	0

Group	Mean	Std Dev	SEM
1.000	53.16	7.54	2.67
2.000	48.64	9.40	3.32

Difference 4.52 14.9 5.26

t = 0.860 with 7.00 degrees of freedom. (P = 0.4181)

95 percent confidence interval for difference of means: -7.91 to 17.0

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.4181)

Power of performed test with alpha = 0.0500: 0.0500

Paired t-test: % Lactate Recovery

Normality Test: Passed (P = 0.4897)

Group	N	Missing
1.000	8	2
2.000	8	1

Group	Mean	Std Dev	SEM
1.000	21.6	38.8	15.8
2.000	12.7	28.6	10.8
Difference	21.3	56.5	25.3

t = 0.842 with 4.00 degrees of freedom. (P = 0.4473)

95 percent confidence interval for difference of means: -48.9 to 91.5

The change that occurred with the treatment is not great enough to exclude the possibility that the difference is due to chance (P = 0.4473)

Power of performed test with alpha = 0.0500: 0.0503

RM ANOVA on Ranks – Muscle Soreness

Friedman Repeated Measures Analysis of Variance on Ranks

Group	N	Missing
1.000	8	0
2.000	8	0

Tested	8	0

Group	Median	25%	75%
1.000	7.00	6.50	9.00
2.000	0.00	0.00	0.00

Chi-square= 8.00 with 1 degrees of freedom. P(est.)= 0.0047 P(exact)= 0.0078

The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = 0.00781)