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Is a High Core Temperature during Exercise Associated with Greater Post-exercise Muscle Damage?

by

Luke Weyrauch

A Thesis

Submitted to the Graduate Faculty of

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Abstract

Muscle damage is a common consequence of exercise, particularly eccentric exercise. During eccentric exercise, mechanical damage to the muscle cell occurs, which leads to a variety of metabolic changes in and around the muscle cell inducing more damage. It is possible that adding heat to muscle-damaging exercise may augment the amount of damage. This research sought to answer that question. Seven young men (age 24.7 ± 3.57) engaged in an eccentric exercise protocol of the elbow flexors previously proven to elicit muscle damage³, first with a thermoneutral core temperature, and two weeks later with an elevated core temperature ($38.3 \pm 0.19^{\circ}\text{C}$). Max voluntary contraction (MVC), subjective muscle soreness, and creatine kinase (CK) were measured as indirect indicators of muscle damage before, and 48 hours post-exercise. No significant differences existed between trials in any of the variables measured ($p > 0.05$). The eccentric exercise protocol failed to induce a significant elevation in CK in either trial ($p > 0.05$). The results of this study indicate that added heat to eccentric exercise does not influence the amount of muscle damage. Furthermore, CK does not seem to be the best marker of muscle damage due to its high variability among subjects engaging in identical exercise. It is recommended that different methods of measuring muscle damage be employed in the future.

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Chapter I: Review of Literature

Exercise represents a disruption to the body's homeostasis. Sometimes this disruption in homeostasis is large enough to evoke significant cellular changes, both structurally and biochemically in exercising muscle. These homeostatic disruptions can result in muscle damage. There are two common components to muscle damage: mechanical and metabolic. The mechanical component is a direct result of the exercise being performed, in which mechanical stress can cause lesions in muscle cell membranes and disrupt the microstructure of the skeletal muscle cell. This mechanical damage gives rise to a variety of metabolic responses, some of which amplify the damage while others attempt to attenuate it. Many of the metabolic responses increasing the damage are initiated by increased intracellular calcium. To counter this, the main metabolic response which attenuates muscle damage is a purposeful increase in heat shock proteins.

An increase in core and skeletal muscle temperature during exercise can potentially augment the metabolic component of muscle damage, primarily by increasing enzyme activity. However, heat also has the potential to increase heat shock protein expression. In effect, heat may be a stimulus for both sides of the metabolic component of muscle damage. The relative strength of the stimulus between these two mechanisms will determine if heat will increase or decrease muscle damage.

Previous literature investigating, in part, the role of heat in muscle damage is equivocal. The primary reason for no clear answer is that this question was not the focus of their research; rather it was just a subtopic. Because the role of heat was not the primary focus, study methods employed were not able to adequately answer the question of whether heat induces greater muscle damage or not. Different absolute workload between trials, timing of trials, and subject pools were not ideal to answer this question. Therefore, the purpose of this review is to highlight work done on muscle

damage and identify variables that might affect how heat influences muscle tissue damage from exercise.

Muscle Damage

Exercise can be considered either non-damaging or damaging. In non-damaging exercise, there is no evident structural or functional damage, whereas in damaging exercise structural and functional damage is clearly present¹. In damaging exercise, the initial muscle damage is incurred during the exercise, and will be termed mechanical damage. The ensuing muscle damage and muscle soreness experienced days after is due to the metabolic response brought on by the original mechanical damage during the exercise.

Eccentric contractions, in which the opposing torque is greater than the muscular torque, in particular produce high amounts of muscle damage^{2,3}. The mechanical stress on the muscle damages several structures within the muscle cell, primarily the Z-line of the sarcomere^{4,5}. During an eccentric contraction, it is theorized that myosin heads are forcibly detached from actin without ATP splitting⁶. Since less myosin is bound to actin, intermediate filaments must take a greater role in maintaining the structure and integrity of the sarcomere. This increased stress on the intermediate filaments is partially responsible for the visible muscle damage seen in electron micrographs^{4,5,7}. The most well-known intermediate filaments are proteins associated with the Z-line: desmin, vimentin, synemin, and titin^{5,7,8}. Additional structures have been identified to get damaged through eccentric contractions including the sarcolemma, T-tubules, myosin, actin, and the cytoskeleton^{9,10}.

Fridén and his group were some of the first to look at human electron micrographs of skeletal muscle following eccentric exercise. After a group of men completed an eccentric bout of cycling exercise, muscle biopsies were taken from the vastus lateralis one hour, three days, and six days after the exercise. In total, 666 micrographs were randomly chosen to assess muscle damage

identified by Z-line streaming, broadening, or total disruption. Of those samples taken one hour after the exercise, 32% exhibited muscle damage. After three days this increased to 52%, and dropped to 12% after six days. Investigators credited the rise in visible muscle damage from one hour to three days to “metabolic violence” including increased intracellular calcium and subsequent calcium mediated activation of protease enzymes⁴.

This increase in intracellular calcium and activation of protease enzymes is part of a complex web of events that ultimately amplifies the muscle damage incurred by the mechanical event. The start of this process is the increased sarcolemma and T-tubule permeability due to tears in the membrane caused by the eccentric contractions⁹. This increased membrane permeability allows calcium to leak into the cell down its electrochemical gradient. This increased intracellular calcium will initiate several responses which contribute to an increase in muscle damage^{8,9,11-13}. One of these responses is the activation of the enzyme phospholipase^{9,11}. Active phospholipase cleaves phospholipids producing lysophospholipids and free fatty acids, both of which disrupt membrane structure. This will further increase sarcolemma and T-tubule permeability, further increasing the leakage of calcium into the cell, and thereby augmenting the calcium-mediated mechanisms⁹.

Several calcium-dependent protease enzymes exist in the muscle cell, the most well-known being the cysteine protease calpain. Calpains are located around the Z-disk and I-band of the sarcomere, and are normally inactive. Once calcium concentration increases in the area, it binds to and activates calpain⁸. Calpain acts on several proteins associated with the Z-disk, most notably desmin, titin, troponin, and tropomyosin^{8,9,12}. Past research has investigated calpain response to damaging exercise^{8,12}. One day after 30 minutes of downhill treadmill running, intramuscular calpain mRNA was significantly increased (220%), indicating greater calpain activity. Fourteen days after the exercise, intramuscular desmin content was 250% higher, suggesting increased calpain stimulates

significant remodeling of the Z-disk associated proteins¹². Similarly, after heavy eccentric exercise of the knee extensors, intramuscular calpain activity was significantly elevated for up to eight days after exercise⁸. Clearly, a large component of the stress response to exercise is calpains, whose activity is mediated by calcium.

Calcium is a potent activator of several enzymes involved in oxidative pathways including α -ketoglutarate dehydrogenase, isocitrate dehydrogenase, pyruvate dehydrogenase and ATP synthase¹³. This is an important consideration in muscle damage due to the production of reactive oxygen species (ROS) from oxidative pathways, primarily superoxide. At rest, the ROS produced from oxidative pathways is balanced by the antioxidant system. During exercise this balance can be offset so that ROS production becomes too great for the antioxidant system. This results in free ROS which will cause oxidation of lipids and proteins^{9,14}. Lipid oxidation will result in lipid peroxides, which will disrupt membrane fluidity and increase membrane permeability. This increased membrane permeability will further increase calcium entry into the cell, intensifying the calcium mediated mechanisms⁹. Since calcium increases oxidative phosphorylation, an increase in intracellular calcium will lead to greater ROS production, leading to protein damage (oxidation) and increases in membrane permeability^{9,13}.

Muscle damage can be categorized into a mechanical component and a metabolic component. It is initiated by mechanical tearing of protein structures and the sarcolemma/T-tubule. Following this membrane permeability increase, calcium concentration in the cell goes up, which is the key piece to further muscle damage induced by the metabolic component. Calcium will further increase membrane permeability through stimulating phospholipase, induce calpain to degrade proteins, and increase ROS production by increasing oxidative pathway activity. These actions will ultimately lead to an increase in muscle damage^{8,9,11-13}. Mechanisms causing muscle damage is only

one side of the story. The other side, mechanisms preventing muscle damage, specifically heat shock proteins, will be discussed in a later section.

Several methods exist to measure muscle damage of which the majorities are indirect measures. The only real direct measure is by taking a muscle biopsy and analyzing the ultrastructure for signs of Z-line streaming, or disruption to T-tubules, myofibrils, the sarcolemma, or the cytoskeleton. Indirect measures of muscle damage include muscle strength, subjective quantification of muscle soreness, joint range of motion, limb circumference, electromyography, markers of collagen breakdown, and different blood markers (myoglobin, lactate dehydrogenase, and creatine kinase)¹⁰. None of these indirect markers are perfect and are all subject to some degree of variability. One of these measures, creatine kinase (CK), will be investigated further along with a discussion of variables that can affect muscle damage as measured by CK. After that, a review of muscle soreness will be briefly covered.

Creatine Kinase

One of the most common methods of assessing muscle damage is measuring serum creatine kinase (CK). CK is an enzyme involved in ATP production. In the cytosol, its primary role is removing a phosphate from phosphocreatine and adding it to ADP, thereby forming ATP. Mitochondrial CK does the opposite: the CK removes a phosphate from ATP derived from oxidative phosphorylation and attaches it to a creatine molecule, forming phosphocreatine^{11, 15}.

Cytosolic CK is made of two polypeptide subunits. There are two types of subunits: M type (muscle) and B type (brain). Therefore, three different isoenzymes of cytosolic CK exist: CK-MM (skeletal muscle), CK-BB (brain), and CK-MB (cardiac muscle)^{11, 15}. The CK in skeletal muscle cytosol is 98% MM, and 2% MB. Two types of mitochondrial CK exist: a sarcomeric type and a non-sarcomeric type. Additionally, CK exists as a macroenzyme (a complex of CK and immunoglobulin), which is only

present during disease states¹⁵. For the purposes of the present research, only the CK-MM isoenzyme is of concern, as this is what is measured to assess muscle damage. From this point forward, when CK is mentioned it is the MM isoenzyme that is being discussed, unless otherwise noted.

When the sarcolemma is damaged as a result of exercise, CK leaks out of the permeable membrane and into interstitial fluid. From there it is picked up by the lymphatic system and is then dumped into the blood. Resting CK levels have been reported to be between 35-175 U/L¹⁵ and between 60-400 U/L¹¹. After resistance exercise, CK levels peak in the range of 300-6,000 U/L¹¹ and can reach upwards of 3,000,000 U/L with extreme rhabdomyolysis¹⁵. Evaluating the increase in CK can be useful for indirectly measuring muscle damage. However, the viability of CK as a marker of muscle damage has been questioned, as the CK response is so variable between individuals even if they have similar characteristics and engage in identical exercise^{11, 15}.

The reason for this phenomenon has tried to be elucidated through genetic variation between individuals. There have been several genetic polymorphisms linked to individuals classified as “high responders” (those who have a relatively higher CK response to exercise), with the best evidence coming from a polymorphism in the gene coding for the protein α -actinin-3. This actin binding protein is an important structural component of the Z-line in skeletal muscle. It is believed that this protein has a large role in maintaining the structural integrity of the sarcomere. Therefore, those with the polymorphism in the gene coding for α -actinin-3 have less structural stability during eccentric contractions, and would be more susceptible to muscle damage which would be reflected in CK making them “high responders”¹¹. This is evidenced by those with the polymorphism having higher CK values, as well as other markers of muscle damage, compared to those without the polymorphism after completing the same eccentric exercise protocol¹⁶.

Individual variation aside, other variables will affect muscle damage and the CK response including gender, age, training status, and exercise type^{2, 3, 17-20}. Males seem to have greater increases in CK than females^{2, 17, 19}. Twenty four hours after completing a marathon, CK levels in men were 3,467 U/L while CK levels in females were just 683 U/L¹⁷. This CK value for men was close to the CK value for men taken 30 hours post marathon (2,213 U/L) in a separate investigation¹⁸. Similar results are seen with resistance exercise: 24 hours after six sets of squats at 90% of one rep max, CK in men was 540 U/L while in women CK was 160 U/L¹⁹. After 50 maximal eccentric contractions of the elbow flexors, CK in men peaked at 11,918 U/L whereas in women CK peaked at 6,750 U/L².

The reason for this discrepancy between genders is thought to be due to the antioxidant properties of estrogen, thereby inhibiting the permeability changes in the cell membrane¹¹. However, research investigating differences in estrogen receptor expression on granulocytes after damaging eccentric exercise contradicted this. Put simply, greater expression of estrogen receptors on granulocytes would indicate a greater protective effect against the exercise. No difference existed between men and women, suggesting estrogen does not directly protect against muscle damage and cannot explain the difference¹⁹. Another possible explanation is that males have higher CK activity, therefore more intramuscular CK and a resulting greater absolute release of CK into the blood^{2, 17}.

Younger individuals tend to have a smaller increase in CK relative to older individuals. After completing maximal eccentric contractions of the elbow flexors, young men (19.4 years) had a CK peak of approximately 8,750 U/L, whereas older men (48.0 years) peaked at 12,500 U/L. However, this difference was not significant due primarily to the huge variance among individuals in each group. The authors hypothesized that the reduction in muscle mass and strength would result in the older group being more susceptible to muscle damage. The older men used in the study were not a

good representation of “general” middle-aged individuals, according to the authors. This may account for the lack of difference and contradictory results to previous research investigating age as a factor in muscle damage³. Other research has found the opposite, in that younger individuals have higher CK compared to older individuals. They claim that since fast-twitch muscle fibers are more susceptible to damage, and older adults have less fast twitch fibers, it stands to reason that they would have less muscle damage compared to their younger counterparts¹¹. The potential age difference in muscle damage seems to be dependent on how old the individuals are in the studies investigating this effect.

Untrained individuals tend to have greater CK responses to exercise compared to trained individuals. After a single 300 meter sprint, trained men had a higher peak CK value compared to untrained men. However, the trained group had a higher resting CK value (168 U/L) than the untrained group (88 U/L). So while trained individuals had a higher peak, the change from rest was greater in the untrained men. The trained men also had an earlier peak (2 hours post-exercise) compared to the untrained men (24 hours post-exercise). Samples were only taken up to 24 hours, so the actual peak may have been missed for both groups. Authors attributed the different CK response to the exercise to the trained athletes having a greater antioxidant defense system, thereby decreasing membrane permeability and subsequent CK leakage, and also to the enhanced ability to resynthesize ATP from glycolytic pathways as a result of their training. As a result, the untrained men would be expected to have, and did have, a greater rise in CK from pre- to post-exercise²⁰. Further evidence of this is seen in a group of men performing 300 maximal eccentric contractions of the knee extensors. Those who reported to be sedentary had CK values ranging from 13,000-25,000 U/L, while the average peak of those subjects reporting to be physically active was below 1,000 U/L²¹.

As previously described, the type of exercise will influence the amount of muscle damage, and therefore affect the CK response. The most important variable seems to be unfamiliarity of the exercise. If the exercise is something a person doesn't do regularly, they will experience greater muscle damage than those who do engage in that exercise regularly, as evidenced by trained sprinters having a smaller increase in CK compared to untrained subjects after sprinting²⁰. Further evidence of this is that the upper limbs are more susceptible to muscle damage than the lower limbs. Sedentary men performed maximal eccentric contractions of the elbow flexors and extensors, and the knee flexors and extensors. CK was significantly higher following the elbow exercises compared to the knee exercises. Additionally, knee flexor exercise elicited a significantly greater CK response compared to knee extensor exercise. These differences are credited to more daily activity of the legs compared to the arms, and the knee extensors compared to the knee flexors²².

It has been proposed that CK is more useful as a qualitative measure of *whether* muscle damage has occurred, rather than a quantitative measure to assess exactly *how much* muscle damage has occurred¹¹. Despite this and all the variables affecting CK, it is still arguably the most common marker of indirect muscle damage used in this area of research. Most often, CK is thought to be superior to myoglobin or lactate dehydrogenase, other fairly common measures of indirect muscle damage. Of the different methods to measure muscle damage, CK seems to be the best and most practical current option available¹⁰.

Muscle Soreness

Virtually everyone has engaged in exercise at some point in their life, and experienced pain and soreness the following days. This is referred to as delayed onset muscle soreness (DOMS). This pain usually peaks sometime between 24-72 hours, and then subsides until it disappears five to seven days after. This is, of course, dependent on the volume and intensity of exercise²³. The first

identification of this delayed soreness dates back to 1900, when Theodore Hough experimented with finger flexion. He engaged in a fatiguing finger flexion protocol, and then experienced great soreness the following day. From his experiment he came up with the novel idea that fatigue experienced during exercise is a separate entity from the discomfort experienced days after the exercise. He proposed that the pain experienced with movement during the days following exercise was due to breaking of adhesions formed during the repair process²⁴.

The exact mechanism for DOMS is still not fully understood. Several theories exist explaining DOMS: lactic acid accumulation, muscle spasm, microtrauma, connective tissue damage, inflammation, and electrolyte and enzyme flux²⁵. Rather than covering all of these theories, the most widely accepted and reasonable cause for DOMS will be presented. First, the mechanism that provides the actual sensation of pain/soreness must be covered briefly.

Of the three types of peripheral afferent nerve fibers in the nervous system (A, B, and C), only A and C exist in the skeletal muscle. The free endings of these fibers are located in the connective tissue between muscle cells, with a higher concentration around capillaries, arterioles, and the musculotendinous junctions. Type A (specifically type A-delta) are myelinated afferent fibers and are responsible for transmitting “sharp” pain. Type C fibers are unmyelinated, and transmit pain signals that are more “dull.” Given this, the type C afferent fibers are responsible for the sensation of DOMS. These type C fibers are sensitive to several stimuli: chemical, mechanical, and thermal. An individual type C fiber is usually sensitive to just one of these stimuli, but some can be sensitive to multiple²³.

A prerequisite for muscle soreness seems to be mechanical damage leading to tearing of the sarcolemma^{23, 26-28}. The increased permeability results in intracellular components being leaked into the interstitium and plasma. This leakage will draw monocytes to the area, and they will

subsequently convert to macrophages. Macrophages will activate mast cells, leading to histamine production, one of the chemicals that is capable of stimulating type C afferent nerve fibers. Other chemicals responsible for type C fiber stimulation include potassium and bradykinin, both of which would be present in large amounts in the interstitium following muscle damage²³. Since the repair process takes time, and monocytes remain at the site of injury for at least 48 hours, it makes sense that DOMS persists for days after exercise²⁷. Additionally, the swelling that accompanies muscular injury may increase pressure and stimulate mechanoreceptor type C fibers, while the increase in temperature accompanying injury would potentially stimulate the heat sensitive fibers²⁸. So it appears that a variety of stimuli exciting the type C fibers are responsible for the sensation of DOMS.

Exercise in the Heat

All metabolic processes within the body produce heat. Put simply, there is a conversion of metabolic energy to mechanical and thermal energy. In a perfect system, 100% of the metabolic energy would be converted to mechanical energy. However, this process is not very efficient, and an estimated 30-70% of this metabolic energy is lost as heat (thermal energy). During exercise, heat production increases significantly due to contraction of skeletal muscle. Under normal conditions, the body's thermoregulatory processes are very adept at handling this heat increase. However, when this same exercise is done in a hot environment, problems arise that lead to premature fatigue²⁹. The investigation regarding the influence of heat on exercise has been around for nearly 100 years, dating back to 1916. Early hypothesized mechanisms for heat causing premature fatigue included production of "fatigue substances" through "abnormal metabolism," circulatory changes, and depression of neurological activity³⁰. All three of these mechanisms are still under investigation today as possible means for premature fatigue when exercising in the heat.

Before these mechanisms are investigated further, the thermoregulatory processes must be understood. The human body is excellent at maintaining its homeostatic core temperature (~37°C). Fundamentally, core temperature is determined by two things: heat production and heat loss. As alluded to above, heat is constantly produced through the conversion of metabolic energy to mechanical energy. This heat is lost primarily in two ways: heat transfer within the body, and heat transfer with the environment. Within the body, heat can be moved through the tissue itself or, more importantly, through the vasculature. Both of these methods within the body are dependent on heat gradients. For instance, in the cold there is a large gradient between the core and the periphery, allowing heat to move easily down this gradient²⁹.

Heat transfer with the environment is dependent on skin blood flow, and the ambient temperature and humidity. In a thermoneutral environment, radiation accounts for the majority of heat loss, approximately 60%. Conduction makes up approximately 18% of heat loss, while evaporation takes up the remaining 22%³¹. The amount of evaporation is largely determined by cutaneous blood flow. Under resting conditions, the skin receives an estimated 5-10% of cardiac output. Under significant heat stress, the skin can receive up to 50-70% of cardiac output³². This highlights the importance of heat transfer with the environment, especially under hot conditions. Heat transfer within the body will be minimized in the heat, as the heat gradients become very small, resulting in heat transfer with the environment becoming the primary means of maintaining a homeostatic core temperature.

Of the methods of heat exchange with the environment, radiation, convection, and conduction become minimal, as they are also dependent on a heat gradient, and can in fact result in heat gain if the environment is hotter than the individual. This leaves the majority of the heat loss to be handled by evaporation. For every gram of water evaporated, 0.58 kilocalorie of heat is lost^{29, 31}.

So for every liter of sweat evaporated, approximately 580 kilocalories of heat are lost. To put this in perspective, the leg at rest produces approximately 0.12 kilocalorie of heat per minute²⁹. If evaporation were the only means to lose this heat, about 1 gram of water would need to be evaporated every five minutes. This heat production will obviously increase markedly during exercise as energy demands increase, leading to a greater demand for evaporative heat loss. If heat production exceeds heat loss, a subsequent elevation in core temperature will occur.

At homeostasis, core temperature is around 37°C²⁹. A person is considered to be in hyperthermia if core temperature rises above 37.5-38.3°C, and in heat stroke at temperatures exceeding 40.6°C³³. A high temperature can be damaging to enzymes and other protein structures, neural tissue, and organs as a whole³³⁻³⁵. A core temperature of 40-41°C is often suggested as being a danger level, and anything above this results in bodily harm. However, a higher core temperature can be reached without damage occurring. For instance, patients passively heated to a core temperature of up to 42°C for one hour had no tissue damage³⁴. During exercise, core temperature usually does not reach this point, as fatigue usually occurs between 39-40°C. Fatigue can be prolonged and can occur at higher core temperatures, especially with dopamine or caffeine supplementation, or if the person is highly motivated, for instance if they are in a high-level competition³⁶. For example, during a marathon, an individual reached a core temperature of 41.9°C with no detriment to running pace³⁷.

Given that an individual is unlikely to reach core temperatures where damage occurs during exercise, the temperature of the exercising muscle becomes of greater concern. Since directly measuring muscle temperature is an invasive procedure, the question of whether core temperature (which is much more easily measured) correlates with muscle temperature must be answered. As one would expect, core temperature does indeed correlate with exercising muscle temperature³⁸⁻⁴⁰.

Men engaging in an intermittent running protocol to exhaustion in the heat exhibited a significant rise in both core temperature (37.25 to 39.6°C) and vastus lateralis temperature (34.7 to 40.2°C)³⁸. A similar pattern was observed in twelve men performing 40 minutes of cycling exercise in the heat. Core temperature increased from 37.2 to 39.6°C while vastus lateralis temperature went from 36.0 to 40.7°C³⁹. In a third similar experiment, thirteen men engaging in cycling exercise in the heat saw increases in core temperature from 37.2 to 39.7°C and in vastus lateralis temperature from 36.5 to 40.4°C⁴⁰.

None of these muscle temperatures are in the dangerous range, so it is unlikely that there was tissue damage resulting directly from heat. This means that temperature was not at a level to cause damage to enzymes, so the increase in temperature actually increased enzyme activity. Most enzymes have a Q_{10} value of 2, meaning that for every 10°C increase in temperature, a doubling of reaction velocity is reached when there is saturating amounts of substrate present⁴¹. Putting this into perspective, men performing an intermittent running protocol had a resting muscle temperature (vastus lateralis) of approximately 35°C, and by the end of exercise this increased to around 40°C³⁸. Following the Q_{10} value for enzymes, this 5°C increase in temperature would equate to a 50% increase in enzyme activity.

It should be noted that there is obviously a limit to this Q_{10} value. There comes a point when enzyme activity begins to decrease rather than increase with increasing temperature. This point will be different from enzyme to enzyme, and even among isoenzymes. For example, lactate dehydrogenase (LDH) has 5 different isoenzymes with different temperature stabilities. LDH-1, found in the heart, remains active up to 55°C, whereas LDH-5, found mostly in skeletal muscle and liver, has lost virtually all activity at 55°C³⁵.

This increase in enzyme activity is part of one of the three causes for premature fatigue mentioned at the beginning of this section: abnormal metabolism. With an increase in enzyme activity there will be an increase in reactive oxygen species (ROS). Just as with increased calcium, as explained earlier, increased temperature will increase the activity of enzymes involved in oxidative phosphorylation leading to greater production of ROS, particularly superoxide^{13,41}. The increased ROS will oxidize proteins within the muscle cell, disrupting their activity⁴². This may lead to less efficient energy production and cross-bridge cycling, thereby inducing fatigue more quickly.

In addition to ROS, greater reliance on glycolytic pathways over oxidative pathways for energy production may play a role in premature fatigue⁴³⁻⁴⁵. Well-trained men performing cycling exercise with both legs having a water perfused cuff on (one at 0°C the other at 55°C) had muscle biopsies taken of the vastus lateralis for glycogen measurement. The muscle temperature of the leg perfused with hot water (37.5°C) was significantly higher than the leg perfused with cold water (30.8°C). The leg perfused with hot water had significantly less glycogen, and therefore significantly greater glycogen utilization (same glycogen content pre-exercise), compared with the leg perfused with cold water (208 mmol/kg, 118 mmol/kg respectively)⁴³. Similarly, men cycling at two different temperatures (20°C and 3°C) had significant differences in glycogen utilization. During the trial at 20°C, muscle temperature was significantly higher (39.1°C) than the trial at 3°C (35.4°C), and glycogen utilization was also higher (196 mmol/kg, 142 mmol/kg, respectively)⁴⁴. It stands to reason that an increase in glycolytic metabolism would lead to a decrease in oxidative metabolism. Young and colleagues documented this, as men exercising in the heat (49°C) had a lower aerobic metabolic rate than when they exercised in a thermoneutral environment (21°C). Additionally, oxygen consumption was greater in the thermoneutral environment, while respiratory exchange ratio was greater in the hot environment⁴⁵.

One of the mechanisms responsible for this change in energy contribution from the glycolytic and oxidative pathways is thought to be a reduced blood flow to skeletal muscle during exercise in the heat⁴⁵. Less oxygen availability would favor glycolytic pathways over oxidative pathways. A second proposed mechanism is the increase in epinephrine seen with exercise in the heat. The adrenal glands release more epinephrine during hyperthermia, which would stimulate glycolytic pathways³³. In support of this, Morris et al. found significantly higher epinephrine and norepinephrine when men completed an intermittent running protocol in the heat compared with a neutral environment. Additionally, epinephrine levels were significantly correlated with glycogen utilization³⁸.

As mentioned earlier, changes in the circulation are in part responsible for premature fatigue in the heat. During exercise in the heat, there is a battle for blood flow between the skin and the exercising skeletal muscles. The skeletal muscles require blood flow for oxygen delivery, and removal of CO₂ and other waste products of metabolism. Under thermoneutral conditions, blood flow is not compromised and this O₂ delivery and CO₂/waste removal works effectively to keep the skeletal muscle working at a given intensity. In the heat, there is a decrease in this skeletal muscle blood flow resulting in greater accumulation of waste products and subsequent detriments to skeletal muscle energy production and ultimately function, resulting in premature fatigue. The reason for this decrease in skeletal muscle blood flow is the increase in skin blood flow. As noted earlier, blood flow to the skin under rest accounts for 5-10% of cardiac output but with exercise in the heat this can reach as high as 70%³². As one can easily gather, this increased blood flow to the skin is necessary for thermoregulation. Since evaporation becomes virtually the sole method for heat loss during exercise in the heat, blood flow to the skin must increase³¹.

The third mechanism for premature fatigue in the heat is depression of neural activity. Essentially this boils down to the brain not sending out the same impulses to the exercising skeletal muscle in the heat as it would in a thermoneutral environment³⁶. Evidence of this can be seen in a group of men who exercised to reach a core temperature of 40°C, and then performed a maximal voluntary contraction of both the exercised muscles (knee extensors) and unexercised muscles (forearm), and received electrical stimulation of these same muscles. Both the exercised and unexercised muscles produced significantly more force during the electrically stimulated contraction compared to the maximal voluntary contraction, indicating reduced neural output from the brain to the involved muscles⁴⁶.

The question then becomes was it the elevated core temperature that caused this decrease in neural output, or could it have been an elevation of brain temperature? Brain temperature is normally slightly higher than core temperature (about 0.2-0.3°C), so a high core temperature during exercise would mean an even higher brain temperature³⁶. As it is difficult to differentiate between core and brain temperature in humans ethically, this question was investigated in goats. Thermo-elements were implanted in the brains of these goats to increase hypothalamic temperature (42°C) independent of core temperature. Likewise, in a separate experiment, core temperature was elevated (43°C) independently of brain temperature. The goats walked on a treadmill with a cable connecting them to the treadmill by their head. How much the cable was “pulling” the goats along the treadmill was measured using a strain gauge. No differences existed in the amount of strain between the conditions, but in the heated brain condition, more goats refused to complete the 60 minute exercise compared to the heated core condition. Obviously, caution should be taken interpreting results from animal studies and applying them to humans. That being said, these results

suggest that an elevated brain temperature plays a role in decreasing neural output to skeletal muscle, or at least in stopping exercise⁴⁷.

Exercise produces, through skeletal muscle metabolism, a large amount of heat. Under normal conditions, this heat is adequately dealt with through thermoregulatory processes. During exercise in the heat these thermoregulatory processes are impaired, and core temperature homeostasis is compromised. The attempt to maintain this homeostatic core temperature results in premature fatigue during exercise in the heat. In future bouts this premature fatigue does not occur as rapidly, in large part due to heat shock proteins, which as the name suggests, are stimulated by heat, among other things.

Heat Shock Proteins

The term heat shock protein (HSP) refers to a family of protein molecules whose primary function is maintaining cellular homeostasis by facilitating repair from a damaging insult and protecting against future insults. In an unstressed cell, HSPs act as molecular chaperones. They help correctly fold newly synthesized proteins, transport these proteins to their destination, prevent improperly folded proteins from clumping together, and facilitate refolding of denatured proteins. When the cell is stressed, where there is an increase in denatured/damaged proteins, the role of HSPs is essentially the same. HSPs bind to these unfolded proteins and facilitate their refolding¹.

There are several types of HSPs, but only a few will be discussed here. The different types of HSPs are categorized by their molecular mass. For instance, HSP60 has a molecular mass of 60kDa. The most commonly studied HSP is the HSP70 family. Of the four major isoforms of HSP70, the two most prevalent isoforms are the cognate form (abbreviated as HSC70, "C" being cognate) and the inducible form, usually called HSP70 (sometimes referred to as HSP72). As the names suggest, HSC70 is found in the cell regularly under all conditions, while HSP70 content is more variable. The primary

role of the HSP70 family is as a molecular chaperone and in cytoprotection. It carries out both of these roles in much the same way: ensuring correct protein folding and translocation, preventing protein aggregation, and refolding misfolded/damaged proteins. HSP60 carries out virtually the same roles, except in the mitochondrial matrix as opposed to the cytosol and nucleus where HSP70 is primarily found⁴².

HSP27 and α B-crystallin are two smaller HSPs whose primary function is protection from contraction-induced damage. Both of these HSPs translocate to cytoskeletal and myofibrillar proteins following damaging contractions. It's believed this translocation is indicative of these HSPs' involvement in remodeling and stabilization of the cytoskeleton and Z-line^{1, 12}.

As a substantial component of the repeated bout effect^{7, 48}, HSP expression is increased after a damaging bout of exercise^{12, 14, 49, 50}. The mechanism for this increase is relatively simple. Under unstressed conditions, HSPs are bound to a separate protein molecule known as a heat shock factor (HSF). When there is an abundance of unfolded/damaged proteins in stressed conditions, the HSP dissociates from the HSF to handle the damaged proteins. This is allowed due to the affinity of HSPs for damaged proteins being higher than for HSFs. This allows the now-free HSF to enter the nucleus. There, it will trimerize with two other HSFs, become hyperphosphorylated, and bind to a heat shock element (HSE), a short nucleotide sequence located on the HSP gene. The binding of the HSF complex to the HSE initiates transcription for HSP RNA, which will ultimately lead to the production of HSPs. So, the more damaged proteins present, the greater the stimulus for HSP synthesis³⁷.

The increase in HSPs in response to exercise has been well documented in the literature^{12, 14, 49, 50}. The majority of this research has focused primarily on the HSP70 family. Seven men performing single leg cycle ergometer exercise had muscle biopsies taken from the vastus lateralis of the exercised leg at one, two, three, and six days post-exercise to measure for HSP70 and HSP60.

HSP70 began to increase two days post-exercise, but did not reach significance until six days post-exercise. HSP60 was significantly higher three days post-exercise only. For both HSP70 and HSP60, there was substantial variation between subjects⁴⁹. Similar results were obtained for ten men performing single leg isometric contractions of the knee extensors who had muscle biopsies taken from the vastus lateralis at one, two, three, and six days post-exercise. HSP70 was significantly elevated one day post-exercise, and remained significantly elevated through six days post-exercise. Just as with the previous study, there was substantial variation in the HSP70 response of individual subjects¹⁴.

Morton and colleagues investigated several HSPs in eight men performing 45 minutes of treadmill running. Muscle biopsies were taken to assess HSP content at one, two, three, and seven days post-exercise. HSP70 was significantly elevated at two and seven days post-exercise, but no other time points. HSC70 and HSP60 were not significantly elevated at any time point, although the peak (at differing time points) for each was significantly higher than pre-exercise values. HSP27 and α B-crystallin did not increase at any time point, nor was there a difference between peak values and pre values. Similarly to the previous two studies, there was high variation in HSP levels between subjects⁵⁰. In contrast to the results for HSP27 and α B-crystallin obtained from Morton's research, Féasson and associates identified a significant increase in both HSP27 and α B-crystallin after 30 minutes of downhill treadmill running. Both HSP27 and α B-crystallin were significantly elevated just one day post-exercise, and remained significantly elevated after 14 days, when the last muscle biopsy was taken¹².

Major factors stimulating the increase in HSPs are thought to include mechanical damage to cellular proteins, reactive oxygen species, and heat. Mechanical damage to muscular proteins seems to be one of the more clearly documented factors stimulating a rise in HSPs. The fact that the affinity

of HSPs for damaged proteins is greater than that for HSFs gives evidence to mechanical damage being an important factor for HSP increase¹. Further evidence is that after 45 minutes of non-damaging treadmill running (no significant rise in CK), HSP27 and α B-crystallin did not increase⁵⁰. However, after 30 minutes of damaging downhill treadmill running (significant increase in CK), these same two HSPs were significantly elevated one day post-exercise and remained significantly elevated for 14 days¹². Furthermore, women engaging in damaging resistance exercise had significantly higher HSP72 increases compared to women engaging in non-damaging treadmill running. The women performing resistance exercise had significantly higher CK than the women engaging in the treadmill running⁵¹.

No direct evidence for reactive oxygen species (ROS) causing a rise in HSPs exist, although indirect evidence does. Sedentary men engaging in cycling exercise had a significant increase in HSP70 and HSP60 six and three days after the exercise, respectively. The researchers did not measure ROS, but they did measure superoxide dismutase, an enzyme involved in breaking down the ROS superoxide. Superoxide dismutase was significantly elevated at one, two, and three days post-exercise, indicating that there was a significant increase in superoxide⁴⁹. The exact mechanism for ROS inducing an increase in HSPs is not understood. The HSF and its binding to HSEs within the nucleus seems to be the modulatory mechanism for increasing HSPs. It's thought that ROS *indirectly* increases HSF binding to HSEs. ROS will oxidize proteins, which will lead to HSPs dissociating from HSFs to go deal with the damaged proteins. This allows the now free HSFs to enter the nucleus, bind to HSEs, and induce HSP synthesis⁴².

As the name implies, HSPs would be expected to be increased under heat stress. This appears to be the case, as midway through treadmill running in the heat (33°C) women had significantly higher HSP72 than women running in normal heat (23°C)⁵¹. However, whether heat in

itself induces increases in HSPs is questionable. Seven men had one leg passively heated by immersion in a hot (45°C) water bath for one hour, while the other leg was kept out of the bath. Muscle biopsies were taken from both legs for measuring HSP content before, and two and seven days after the heating protocol. Core temperature and muscle temperature of the submerged leg increased significantly (37.4 to 38.9°C, 35.9 to 39.5°C, respectively) while that of the non-submerged leg did not change. No significant increase in HSP70, HSC70, HSP60, HSP27 or α B-crystallin was observed in either limb⁵². In support of this finding, no difference in DNA binding to HSFs was observed between exercise in a hot environment (40.3°C) and a thermoneutral environment (20°C)⁵³.

So while heat alone doesn't seem to induce HSP synthesis (at least a short exposure to heat), its effects on other factors increasing HSP synthesis likely play a role. As discussed earlier, heat increases enzyme activity⁴¹ therefore increasing reactive oxygen species¹³, both of which ultimately stimulate HSP synthesis^{42, 49}. Therefore, in an exercise bout, it is fair to say that heat does not *directly* stimulate HSP synthesis, but rather does so *indirectly*.

Proposed Mechanism for Heat Causing Additional Muscle Damage

Both exercise and heat, individually, have the capacity to cause muscle damage. If exercise were done in the heat, wouldn't it stand to reason that greater muscle damage would be present? The limited research available investigating exercise in the heat and its effects on muscle damage do not necessarily suggest this^{51, 54-57}. However, the methodology of these studies has been problematic for answering the question of whether exercise in the heat causes greater muscle damage compared to exercise in a thermoneutral environment. It should be noted that the primary question seeking to be answered by these researchers was not necessarily whether heat causes greater muscle damage. These methodology limitations are present for answering this question of heat and muscle damage, but not necessarily for answering the researchers' primary question.

The basic design of these studies was the same: exercise was done in two different environments, one warmer than the other, separated by a certain number of days if subjects completed both trials⁵⁴⁻⁵⁶. If the subjects were separated into different groups, the groups completed the exercise in different temperatures^{51,57}. The biggest design limitation was that in all but one of the experiments, the amount of work done during exercise was not kept consistent between the different temperature conditions⁵⁴⁻⁵⁷.

Research by Nybo and colleagues is a prime example of this, in which elite soccer players played an entire soccer match once at 21.1°C and once at 42.8°C separated by one week. The researchers kept track of the distance covered and the amount of high-intensity running (running speed >14km/hr) during the two matches. As one would expect, the athletes ran significantly further and engaged in significantly more high intensity running in the cooler environment. This resulted in a significantly elevated CK after the soccer match played in the cool environment compared to that in the hot environment⁵⁴. The question of whether exercise in the heat causes additional muscle damage cannot be answered as the amount of work completed between trials was significantly different. Three of the other aforementioned studies followed similar design limitations with regard to heat⁵⁵⁻⁵⁷.

Hamzehkolaie, Roshan and Hosseinzade (2013) had additional limitations in their procedures and introduced a different type of potential error. Two trials were completed by different subjects. This is acceptable if the number of subjects in each group is very large, which would help alleviate the individual variation, but there were only nine subjects in each group⁵¹. Given the huge person-to-person variation in CK^{11,15}, results from their study are probably not very valid.

Due to various methodological issues, the results from these studies vary. In some cases, CK was significantly higher after the exercise trial in the heat^{51, 55}, while in another the opposite was reported⁵⁴. The remaining two studies did not find a significant difference in CK^{56, 57}.

A few of the authors did shed light on a possible mechanism for why exercising in the heat would cause greater muscle damage than exercise in a thermoneutral environment^{51, 56}.

Hamzehkolaei and colleagues suggested the increased muscle temperature will result in greater reactive oxygen species production, leading to greater muscle damage⁵¹. Maresh et al. hypothesized that since heat increases anaerobic metabolism, more CK would be released by the muscle⁵⁶. They did not elaborate on this point, however. What they were presumably inferring may be that since glycolytic pathways are less efficient at deriving ATP from substrate compared to oxidative pathways, greater reliance on glycolytic pathways in the heat would ultimately lead to decreased ATP. A possible contributing mechanism for CK release is a decrease in ATP. A decrease in ATP would lead to a decrease in the sodium-potassium ATPase pump and sarcoplasmic reticulum calcium ATPase pump activity. This would lead to increased intracellular calcium, activating several pathways which would eventually lead to increased membrane permeability and CK release¹⁵.

Arguably the largest possible contributor to heat causing increased muscle damage is heat's effect on enzyme activity. As previously described, increases in temperature will increase enzyme activity⁴¹. This includes enzymes involved in oxidative phosphorylation. Increased oxidative phosphorylation will result in increased formation of free radicals, primarily superoxide¹³. This increased number of free radicals will increase lipid peroxidation, leading to increased membrane permeability⁹. As discussed earlier, this increased membrane permeability is a key step in the development in muscle damage. The more permeable the cell membrane, the more calcium will flow into the cell, activating several pathways that will lead to further increases in membrane

permeability, activation of proteases, and allosteric activation of enzymes in oxidative phosphorylation. Further, heat will increase the activity of phospholipase, leading to greater membrane permeability. This will all ultimately lead to greater breakdown of muscle protein and increased membrane permeability leading to increased CK leakage^{9,13}.

This added heat stress on top of exercise would theoretically induce greater muscle damage than exercise alone. However, the flip side of added heat stress must be considered as well: stimulation of HSPs. As described earlier, heat shock proteins work to reduce muscle damage, in effect increasing the amount of stress necessary to induce damage. Heat, along with calcium, protein degradation and reactive oxygen species, stimulate the production of heat shock proteins¹. So the question regarding added heat stress is will it increase one side of the muscle damage production/prevention model more than the other? Will enzyme function be increased to a greater extent than heat shock proteins, thereby increasing muscle damage? Or will the opposite be true? Answers to these questions have not been clearly answered in the literature.

Summary

Whether added heat to eccentric exercise further augments the amount of muscle damage is dependent on one thing: will the added heat stimulate processes that increase muscle damage (increased enzyme activity of calpain, phospholipase, and enzymes in oxidative energy pathways) more than processes that inhibit muscle damage (heat shock proteins)? It is really this balance that will determine whether differences, if any, exist when performing eccentric exercise in a thermoneutral environment compared to a hot environment.

Chapter II: Proposal

Muscle damage is a common side effect of strenuous exercise. Ample research has been done documenting eccentric exercise as being a large producer of muscle damage. This is due to the mechanical damage associated with eccentric contractions. In eccentric contractions, there is less motor unit recruitment for a given load relative to concentric contractions. This results in a greater load per fiber in eccentric contractions leading to higher susceptibility for muscle damage⁶. This mechanical damage results in tears in the sarcolemma and damage to the microstructure of skeletal muscle^{9,10}. This mechanical damage gives rise to secondary muscle damage, namely metabolic responses.

These metabolic responses include increases in intracellular calcium due to increased membrane permeability, and subsequent activation of calcium dependent processes. Calpain activation, phospholipase activation, and increased reactive oxygen species (ROS) production through increased activity of enzymes in oxidative phosphorylation are the primary calcium-mediated processes which further augment muscle damage^{8,9,11-13}. An additional metabolic response to mechanical damage is the increased expression of heat shock proteins (HSPs). HSPs are normally bound to heat shock factors (HSFs) within the muscle cell. When damage to skeletal muscle proteins occurs due to eccentric contractions, the HSPs dissociate from the HSFs to bind and repair the damaged proteins. This results in HSFs being able to translocate into the nucleus where they will bind to heat shock elements (HSEs), regions of the HSP promoting gene. This binding will ultimately increase the HSP content within the muscle cell, protecting against future muscle damaging exercise¹.

Exercise in the heat has the potential to amplify this damage resulting from eccentric contractions. The mechanism of heat accomplishing this is mainly explained by heat's effect on

enzyme activity. Heat will increase the activity of enzymes by a factor of 2 for every 10°C increase in temperature⁴¹. Therefore, heat would potentially increase phospholipase activity causing increased membrane permeability^{9, 11}. Enzymes in oxidative phosphorylation would also increase, leading to increased ROS production, further increasing membrane permeability and damaging muscle proteins^{9, 13, 14}. On the flipside, heat will also increase HSP expression, which would combat metabolic muscle damage^{1, 13, 41, 42, 49}.

Prior research investigating the effect of heat on muscle damage has not clearly answered this question. This is due to the methods employed by these researchers not being targeted to answer this question; muscle damage was just a secondary variable they measured^{51, 54-57}. The main research design problem was unequal work between the two conditions. For instance, soccer players played a match in a cool environment and a hot environment, and ran significantly further and engaged in more high intensity running in the cooler environment. This resulted in the cool trial yielding significantly greater muscle damage than the hot trial⁵⁴.

Therefore, the aim of the present study is to control for the amount of work done between trials as closely as possible to correct for this limitation. If the amount of work between the two trials is the same, muscle damage would be expected to be identical. When heat is added to one trial, specifically an increase in core/muscle temperature, any differences in muscle damage observed could more confidently be said to have been due to heat rather than differences in workload.

Purpose

The purpose of this research is to determine if eccentric muscle-damaging exercise in the heat causes greater muscle damage than the same exercise done in a thermoneutral environment. More specifically, the influence of an elevation in core/muscle temperature on muscle damage will

be investigated. This influence will be measured through creatine kinase (CK), max voluntary contraction, and subjective muscle soreness.

Hypothesis

The following null hypotheses will be tested:

- 1) There will be no difference in CK following exercise in the heat relative to the same exercise in a thermoneutral environment.
- 2) There will be no difference in maximal voluntary isometric contraction following exercise in the heat relative to the same exercise in a thermoneutral environment.
- 3) There will be no difference in subjective muscle soreness following exercise in the heat relative to the same exercise in a thermoneutral environment.

Limitations

- 1) Given the nature of the study, a limited number of participants is anticipated, making it a challenge to find significant differences that may be present.
 - 2) Subjects may gain adaptations from the first exercise bout which will protect against muscle damage in the second bout (repeated bout effect^{7,48}), despite precautions taken.
 - 3) In order to minimize the adaptations referred to in 2), trials will not be counter-balanced.
- All subjects will complete the control trial first followed by a 14 day recovery.

Methods

Pilot Testing

In order to determine a reasonable core temperature to reach during the heat trial, several days of pilot work were done. One subject engaged in cycling exercise (average of 150 watts) for 40 minutes in the heat (~38°C) while wearing clothing covering the majority of his body preventing sweat evaporation, and attained a core temperature of 38.5°C, but was pretty exhausted and

nauseas at the end. A different subject did this same cycling exercise with greater attention placed on preventing sweat evaporation and reached a core temperature of 38.5°C in 18 minutes.

Thermoregulation will differ between subjects, but it is clear that preventing sweat evaporation will be a key component in raising core temperature.

Subjects

Given the nature of the study, criteria for subject inclusion will be limited. The subjects will all be male, over 18 years old, and have no health conditions that will limit their participation in strenuous exercise. Activity level will not necessarily be controlled for, but individuals who engage in intense resistance exercise of the elbow flexors will be excluded. For instance, individuals completing more than 5 sets of bicep curls to failure more than once a week will not be included.

Instruments

- Cycling exercise in the heat trial will be done on a Monark 828E cycle ergometer.
- An SPX model 402A space heater will be used to increase room temperature during the cycling exercise in the heat.
- A polar heart rate monitor will be worn by subjects during the cycling exercise in the heat.
- A YSI rectal thermoprobe and YSI skin thermometer will be connected to a Fisher Scientific digital thermometer to measure core and bicep skin temperature, respectively, during both trials.
- Creatine kinase will be measured using a Roche Diagnostics Reflotron.
- A Block Scientific osmometer model 3MO will be used to measure urine osmolality.
- A visual analog scale (100mm line) will be used to assess muscle soreness.

- Uniaxial load cells hooked up to SIMI software will be used to quantify maximal voluntary isometric contraction.

Procedures

Subjects will first sign an informed consent form and fill out a PAR-Q questionnaire before beginning participation in the research. Answering “yes” to any of the questions on the PAR-Q will disqualify them from participating. All subjects will complete two trials. The first trial completed by all participants will be the control trial (trial 1). The second trial for all participants will be the trial done in the heat (trial 2). Upon arrival to the testing location, subjects will be tested for maximal voluntary isometric contraction (MVC) of the elbow flexors of the dominant or non-dominant arm. Half of the subjects will perform trial 1 with their dominant arm, while the other half will perform trial 1 with their non-dominant arm. The opposite arm will be used in trial 2. In determining MVC, subjects will kneel behind a chair, and have the back of the chair in their armpit of the arm being measured. The upper arm will rest against the back of the chair, with the elbow flexed to 90 degrees. Subjects will grasp a handled rope which will be tied to a 200 pound load cell. A second rope will go from the load cell to an immovable anchor. Therefore, the amount of force exerted by the subject will be measured by the load cell. SIMI software will be used with a sampling frequency of 100Hz to quantify the output from the load cell. Prior to MVC measurement, the load cell will be calibrated using three different weights within the range of the load cell to establish a calibration curve.

A small (30 μ l) blood sample will be collected and analyzed for creatine kinase (CK) using a Roche Diagnostics reflotron prior to the eccentric exercise protocol. Before trial 2, a urine sample will be obtained and measured for urine osmolality using a Block Scientific osmometer. Urine

osmolality must be below 600 mOsm/kg for the subject to be eligible to participate at that time. If urine osmolality is above 600 mOsm/kg, the subject will have to come back another time for testing.

For trial 2, subjects will wear a heart rate monitor during the cycling exercise. Subjects will be asked to insert a rectal thermometer 13cm beyond the anal sphincter. An inked line on the probe will be used to insure proper placement, and then the probe will be connected to a Fisher Scientific digital thermometer for constant measurement of core temperature. Additionally, a skin thermometer will be placed on the bicep of the arm to be exercised. The subject will wear long pants, a long sleeve shirt, long socks to cover the ankle, gloves, and a plastic sweat suit during the cycling exercise. Additionally, a hooded sweatshirt will be worn or a towel will be wrapped around the subject's head so only skin on the face is exposed to the environment. A small room will be heated to around 37-40°C (98.6-104°F) in which the cycling exercise will take place. Subjects will bike on a cycle ergometer starting at 100W for as long as it takes to get to a core temperature of 38.5°C. If 100W becomes too difficult, wattage will be decreased to a comfortable level. Every three minutes during the cycling exercise, heart rate, RPE, core temperature, and bicep skin temperature will be recorded. Based on pilot work, the rise in core temperature to 38.5°C should take roughly 20 minutes. If the subject is unable to reach a core temperature of 38.5°C, anything $\geq 38^\circ\text{C}$ will be accepted. If the subject cannot attain this, they will be dropped from the study.

Confirmed through pilot testing, once the target core temperature is reached, subjects will come out of the hot room, but will leave all clothing on to ensure core temperature does not drop too much before the eccentric exercise is completed. Subjects will begin the eccentric exercise 3 minutes after completion of the cycling exercise. Core temperature will be monitored for the duration of the eccentric exercise. If core temperature drops below the core temperature achieved

during the cycling, this will be noted, but the subject will not be forced to complete the trial again or raise the core temperature back up.

The same eccentric exercise protocol will be used for trials 1 and 2, with the only difference being the arm that is exercised. Subjects will be in the same position as they were for testing MVC. A dumbbell corresponding to 80% of the subject's isometric MVC specific to the exercised arm will be used for the elbow flexor exercise. Six sets of five repetitions will be completed, with each repetition lasting five seconds. Full eccentric range of motion will be completed for each repetition. Three seconds will separate each repetition, and two minutes will separate each set. The subject will only be going through the eccentric portion of the exercise. The researcher will catch the weight at the bottom and lift it back up so no loaded concentric action is done by the subject. In total, the elbow flexors will be under tension for three minutes, while the entire exercise will take slightly under 15 minutes. Core temperature and bicep skin temperature will be recorded immediately following each set.

Forty eight hours after a trial, subjects will return to the testing location. Here they will rate their soreness/discomfort on the visual analog scale, get their blood drawn for CK measurement, and perform another MVC. This visual analog scale is a 100mm line where 0mm is no soreness/discomfort at all, while 100mm is maximal soreness/discomfort. In the event CK is out of range on the reflotron (max value of 1700 U/L), a second blood sample will be drawn and diluted using a lactated ringer's solution. Trials will be separated by at least 14 days (from the day of exercise).

Data Analysis

A paired samples t -test will be used to determine if any significant differences ($p < 0.05$) are present between the two trials. Specifically, the change in each variable from pre- to 48 hours post-exercise will be compared between trials.

Chapter III: Manuscript

Introduction

Muscle damage resulting from exercise represents a substantial disruption to the skeletal muscle's homeostasis. These disruptions take shape in the form of both structural and biochemical changes. More specifically, the structural changes give rise to subsequent biochemical changes. Given this, muscle damage can be broken into two parts: a mechanical component and a metabolic component. The mechanical component is a direct result from the exercise. Tearing of the cellular membrane, demolition of the intracellular scaffolding, and disruption to contractile proteins are all direct results of the mechanical stress of the performed exercise^{4, 5, 7, 9, 10}. This mechanical damage leads to a variety of biochemical changes within and around the muscle cell. Some of these changes amplify the muscle damage, while other changes attempt to repair the damage. The majority of these metabolic responses which cause damage are mediated by a rise in intracellular calcium concentration^{8, 9, 11-13}. Stemming from the same mechanical damage as the increased intracellular calcium, a purposeful rise in heat shock proteins (HSPs) attempts to counter the damaging metabolic changes.

Mechanical muscle damage is greatest when the exercise being performed is eccentric in nature. Each individual muscle fiber is generating more force compared to concentric contractions. This greater force places an increased demand on the muscle fibers, resulting in more mechanical damage. In theory, during eccentric contractions the myosin head is forcibly detached from the actin without ATP splitting⁶. Since less myosin is bound to actin, greater demand is placed on intermediate filaments such as desmin, titin, vimentin, and synemin, thereby damaging these structures^{5, 7, 8}. Other structures that are damaged during eccentric contractions include the sarcolemma, T-tubules, myosin, actin, and the cytoskeleton^{9, 10}. Early work done by Fridén and colleagues was some of the

first to observe electron micrographs of skeletal muscle after eccentric exercise^{4,5}. In these electron micrographs, an evident disruption to the Z-line can be observed. Interestingly, of the 666 micrographs looked at, 32% exhibited noticeable damage one hour post-exercise, but three days later this increased to 52%⁴. What was the cause of this delayed increase in visible damage?

The answer resides in something smaller than an electron micrograph can see: biochemical changes within and around the skeletal muscle cells, or as Fridén and colleagues stated: “metabolic violence”⁴. This “violence” starts with the changes in intracellular calcium. Due to tears in the sarcolemma and T-tubules, calcium can more freely travel down its electrochemical gradient into the cell⁹. The increased intracellular calcium leads to several metabolic responses which are responsible for amplifying the initial mechanical muscle damage^{8,9,11-13}. One of these responses involves activation of the enzyme phospholipase, which will ultimately disrupt membrane structures leading to greater permeability of calcium ions^{9,11}. Another response is the activation of calcium-dependent proteases, most notably calpain, leading to degradation of protein structures^{8,9,12}. The third major response is the stimulation of several enzymes involved in oxidative pathways, which will lead to greater reactive oxygen species (ROS) production thereby increasing lipid and protein oxidation, the former of which will further increase membrane permeability^{9,13,14}. These calcium-mediated responses are a rather vicious cycle that will continue to amplify one another further and further. So why doesn't this cycle just spiral out of control until the muscle is completely ripped apart and degraded?

In short, there are mechanisms which combat this increasing muscle damage, the most noteworthy being HSPs. The term HSP refers to a family of protein molecules whose primary purpose is maintaining cellular homeostasis. They accomplish this by completing tasks such as refolding damaged proteins, ensuring correct placement of newly synthesized or refolded proteins, and

preventing protein aggregation^{1, 12, 42}. It is well established that HSP concentration increases after exercise and can remain elevated for up to 14 days^{12, 14, 49, 50}. A large stimulus for their increased activity is the amount of damaged proteins in the cell. Simply put, the more damage to proteins, the greater HSP response³⁷. So presumably what happens is, initially, the calcium-mediated responses are greater than the HSP response. Over the next few days, depending on the severity of the exercise which would determine the initial level of calcium-mediated response intensity, the HSP response would overcome the calcium-mediated responses, leading to repair of the tissue.

The magnitude of muscle damage can be measured a variety of ways: directly via muscle biopsy, and indirectly via changes in muscle strength, subjective quantification of muscle soreness, joint range of motion, limb circumference, electromyography, markers of collagen breakdown, and different blood markers (myoglobin, lactate dehydrogenase, and creatine kinase)¹⁰. Arguably the most common method assessing muscle damage is with creatine kinase (CK). Creatine kinase is an enzyme involved in ATP production, specifically moving a phosphate from phosphocreatine to an ADP molecule or moving a phosphate from an ATP molecule to creatine, depending on the enzymes location^{11, 15}. Despite its robust use as a marker of muscle damage, the validity and reliability of CK have been questioned by some due to its extreme variability even among individuals of similar characteristics who engage in identical exercise^{11, 15}. It's proposed that CK should be used as a *qualitative* measure as opposed to a *quantitative* measure, due to its high variability¹¹. With that being said, CK still seems to be the best and most practical current option available for measuring muscle damage¹⁰.

The effects of eccentric exercise resulting in muscle damage are well established, but the added effect of heat is yet to be determined. More specifically, whether or not the heat of the muscle being exercised will impact the magnitude of muscle damage is in question. Previous

literature on this subject has yielded mixed results^{51, 54-57}. However, research on this topic did not have its methods set up in a way that sought to answer this question directly. The primary question was not whether heat induced greater muscle damage; rather it was discussed simply because muscle damage and heat were both measured. For instance, the effects of ambient temperature on soccer performance were observed, and a variable measured was CK. Creatine kinase was significantly higher during the soccer match in the more temperate conditions (21.1°C) compared to the soccer match in the heat (42.8°C). However, the distance ran was significantly higher in the temperate conditions, as was the amount of high-intensity running⁵⁴. Essentially, the amount of work performed in each trial was not the same, so saying that the added heat produced less muscle damage cannot accurately be said because the amount of work was different, which will obviously greatly impact the amount of muscle damage. Therefore, the purpose of the present study is to evaluate whether added heat during exercise, specifically an elevated core temperature, results in greater muscle damage compared to the same exercise done with a thermoneutral core temperature.

Methods

Subjects

Seven male university students (age 24.7 ± 3.57 ; height $1.79 \pm 0.12\text{m}$; weight $77.2 \pm 15.0\text{kg}$) served as subjects for this research. Subjects varied in activity level, with three being regular resistance exercisers, one being a regular endurance exerciser, and the remaining three engaging in less than 150 minutes of physical activity per week. All subjects signed an informed consent (Appendix A) and successfully passed a Physical Activity Readiness Questionnaire (Appendix B). One of these subjects (a regular resistance exerciser) dropped out of the study.

Exercise Protocol and Testing Procedure

Subjects reported to the testing laboratory on four separate occasions. Two of these occasions involved engaging in an eccentric exercise protocol. Trial 1 was completed with a thermoneutral core temperature, while trial 2 was completed with an elevated core temperature. All subjects completed trial 1 first, and opposite arms were used during the two exercise trials, so as to minimize the repeated bout effect^{7, 48}. Half of the subjects completed trial 1 with the dominant hand, and the other half completed trial 1 with the non-dominant hand.

Upon determination of maximal voluntary contraction (MVC) of the elbow flexors of the arm to be exercised, a dumbbell equating to 80%MVC, rounded up to the nearest 2.5lbs was used for the eccentric exercise protocol. The eccentric exercise protocol consisted of 6 sets of 5 repetitions of kneeling, single arm eccentric biceps curls. Each repetition was 5 seconds in duration, from the starting position (elbow fully flexed) to the ending position (elbow nearly fully extended). The researcher lifted the dumbbell back to the starting position after each repetition in order to ensure minimal concentric muscle action was completed by the subject. Three seconds of rest separated each repetition, and 2 minutes of rest separated each set. Before the first set, and after each subsequent set, a measure of core temperature and biceps skin temperature of the exercising arm was taken. In trial 1, only four of the seven subjects had core temperature measured during the eccentric exercise protocol.

During the exercise protocol, subjects kneeled behind a straight backed chair upon which they rested the elbow of the exercising arm. The opposite hand was allowed to grasp the chair for balance during the repetitions. Subjects were allowed to stand, stretch out, and walk around the lab during the 2 minutes of rest between sets. In the event that the subject could not lower the weight

by himself, the researcher would provide assistance to ensure the 5 seconds were met. Verbal encouragement was provided throughout.

During trial 1 subjects completed the eccentric exercise protocol without any warming up of the core, however, subjects were encouraged to stretch out and warm up the arm to be exercised, although no formal warm-up protocol was followed. During trial 2, subjects went through the core temperature elevation protocol, rested for 3 minutes, and then completed the eccentric exercise protocol. Upon arrival for these two eccentric exercise trials, subjects rated their muscle soreness and CK was measured. Fourteen days separated each eccentric exercise trial.

The other two occasions where subjects came in were 48 hours after the eccentric exercise protocol was completed. During these occasions, MVC, muscle soreness, and CK were measured.

Core Temperature Elevation Protocol

During trial 2, subjects engaged in a core temperature elevation protocol prior to completing the eccentric exercise protocol. This consisted of cycling on a cycle ergometer (Monark 828E) at a self-selected power output in a hot environment while wearing restrictive clothing. After adjusting the seat height appropriately, subjects picked a comfortable power output that they thought they could easily maintain for at least 30 minutes. If the workload became too difficult during the protocol, subjects were allowed to decrease the workload to a more comfortable setting.

The hot environment was achieved by placing a space heater (SPX model 402A) in a small room (1.37x3.66m) and increasing temperature to approximately 37-40°C. Humidity was kept around 24%. In some instances, the space heater malfunctioned leading to lower than desired temperatures. In these cases, a hot shower was turned on in the room to increase humidity to approximately 64%.

Subjects wore long pants, a long sleeve shirt, long socks to cover the ankle, and plastic gloves during the cycling exercise. Additionally, a hooded sweatshirt was worn or a towel wrapped around

the subject's head so only skin on the face was exposed to the environment. To prevent cooling of the body through evaporation, a plastic sweat suit was worn over the rest of the clothing.

Prior to entering the hot room, and every 3 minutes during the cycling, measures of heart rate (polar heart rate monitor), rating of perceived exertion (Borg 6-20 scale), core temperature, and bicep skin temperature were recorded. Subjects were asked to cycle until core temperature reached 38.5°C. If they couldn't continue and core temperature hadn't reached at least 38.0°C, they were eliminated from the study. This only happened with one of the subjects.

Core and Skin Temperature Measurement

To measure core temperature, subjects inserted a YSI rectal thermoprobe 13cm beyond the anal sphincter. An inked line on the probe was used to insure proper depth. A YSI skin thermometer was taped to the middle of the muscle belly of the biceps of the arm being exercised. Both the rectal thermoprobe and skin thermometer were connected to a Fisher Scientific digital thermometer for constant measurement of both core and biceps skin temperature, respectively.

Creatine Kinase (CK)

A small (30 µl) blood sample was collected and analyzed for CK using a Roche Diagnostics Reflotron. Blood samples were analyzed immediately after collection.

Maximal Voluntary Contraction

To determine maximal voluntary contraction (MVC) of the elbow flexors, a properly calibrated 200 lb uniaxial load cell was hooked up to SIMI software using a sampling frequency of 100 Hz. Subjects were positioned identical to how they would be positioned for the eccentric exercise protocol, with the elbow set at 90 degrees. Subjects grasped a handled rope which was tied to the load cell. A second rope tied the load cell to an immovable anchor, therefore, isometric MVC was measured. Subjects were instructed to slowly ramp up the force of their contraction, and then hold it

maximally for 2-3 seconds. Subjects were closely watched by the researcher to ensure they didn't cheat by leaning back or going into an eccentric contraction.

Data were exported to Excel, where it was converted to pounds and the maximum value was taken as MVC. For trials 1 and 2, a dumbbell corresponding to 80% of this MVC, rounding to the nearest 2.5 lbs, was chosen as the dumbbell to be used during the eccentric exercise protocol.

Muscle Soreness

Muscle soreness (MS) was assessed using a visual analog scale. Subjects placed a vertical mark on a 100mm line as to how sore the exercised arm felt from "no soreness/discomfort" to "maximal soreness/discomfort". The distance between the "0" (no soreness/discomfort) to the marked line was measured, and quantified 0-100.

Statistical Analysis

A paired samples *t*-test was used to determine if any significant differences ($p < 0.05$) were present between the two trials using Microsoft Excel software for the following dependent measures: CK, MVC and MS. Specifically, the change in each variable from pre- to 48 hours post-exercise was compared between trials.

Results

Eccentric Exercise Protocol

The percent of MVC used as resistance between the two trials was not different ($79.92 \pm 1.45\%$ trial 1; $80.42 \pm 1.54\%$ trial 2; $p = 0.6661$). Of the four subjects who had it measured, core temperature did not significantly increase (0.0 - 0.2°C increase from beginning to end) during the eccentric exercise protocol in trial 1. The biceps skin temperature was significantly higher at every time point in trial 2 compared to trial 1, and core temperature continued to rise throughout the eccentric exercise protocol in trial 2 (Table 1).

Core Temperature Elevation Protocol

Average room temperature was $35.7 \pm 1.70^{\circ}\text{C}$. In two instances the space heater malfunctioned and room temperature failed to get above 34°C , in which case humidity was increased to 58% in one case, and 70% in the other by means of turning on a hot shower in the room. Subjects started at either 100 or 150W, and all but one of the subjects decreased the workload during the cycling exercise. Average cycling time was 23.6 ± 5.87 minutes. Core temperature increased from 37.3 ± 0.26 to $38.3 \pm 0.19^{\circ}\text{C}$ from beginning to end of the cycling exercise. Only two subjects were able to reach the desired 38.5°C . One of the subjects failed to reach 38.0°C , and was subsequently dropped from the study. Biceps skin temperature increased from 34.4 ± 0.89 to $38.4 \pm 0.34^{\circ}\text{C}$.

Table 1

Biceps skin and core temp (mean \pm SD $^{\circ}\text{C}$) pre-eccentric exercise, and after each set of eccentric exercise.

	Pre	Set 1	Set 2	Set 3	Set 4	Set 5	Set 6
Skin Biceps Temp-Trial 1	31.8 ± 0.25	32.4 ± 0.59	33.0 ± 0.70	33.3 ± 0.67	33.5 ± 0.63	33.7 ± 0.69	33.9 ± 0.81
Skin Biceps Temp-Trial 2	37.4 ± 0.72	37.1 ± 0.81	37.0 ± 0.86	36.8 ± 0.96	36.6 ± 1.11	36.4 ± 1.14	36.5 ± 0.95
<i>p</i>	0.00001	0.00005	0.00017	0.00044	0.00115	0.00226	0.00214
Core Temp-Trial 1 (<i>n</i> = 4)	37.4 ± 0.27	37.4 ± 0.27	37.4 ± 0.29	37.5 ± 0.26	37.5 ± 0.24	37.5 ± 0.24	37.5 ± 0.25
Core Temp-Trial 2 (<i>n</i> = 6)	38.4 ± 0.20	38.4 ± 0.18	38.5 ± 0.18	38.6 ± 0.20	38.7 ± 0.21	38.7 ± 0.20	38.8 ± 0.18

Indicators of Muscle Damage

No significant differences existed in CK, MVC or MS from pre- to post-exercise between the two trials (Table 2). The eccentric exercise protocol failed to induce significant increases in CK in either trial 1 ($p = 0.97$) or trial 2 ($p = 0.63$). Ratings of MS increased significantly in both trial 1 (p

=0.007) and trial 2 ($p = 0.03$). Decreases in MVC approached significance in trial 1 ($p = 0.06$), but were insignificant in trial 2 ($p = 0.28$).

Table 2

Changes from pre- to 48 hours post-exercise in maximum voluntary contraction (MVC) of the exercised elbow flexors, muscle soreness (MS) of the exercised arm, and creatine kinase (CK) Given p values are differences in changes pre- to post-exercise between trials.

	Trial 1	Trial 2	p
MVC (kg)	-2.69 \pm 2.49	-1.28 \pm 2.34	0.3558
MS (0-100)	40.0 \pm 20.8	32.7 \pm 24.4	0.1903
CK (U/L)	2.33 \pm 63.0	47.5 \pm 209	0.5345

Discussion

The main findings of this research include: 1) no differences existed in any of the variables measured between the two trials, suggesting added heat does not influence the magnitude of muscle damage, and 2) the exercise protocol failed to induce significant increases in CK. These results were contrary to what was expected. Based on the hypothesized mechanism for heat stimulating metabolic processes leading to muscle damage, it was expected that greater CK increases, greater detriments to MVC of the elbow flexors, and greater reports of MS would be evident after the trial with the elevated core/muscle temperature.

No between-trial differences were observed in any of the indirect measures of muscle damage (Table 2). This is contrary to prior research investigating muscle damage differences when exercising in the heat versus a thermoneutral environment. While none of these research studies had a primary objective of answering the question of whether heat induces greater muscle damage, their results will still be compared for lack of direct research of this question.

Elite male soccer players playing two soccer matches, one in the heat (42.8°C) and one in a thermoneutral environment (21.1°C), exhibited significantly different CK responses 48 hours after the two matches. Mean CK was significantly higher in the thermoneutral environment (575 U/L) compared to the hot environment (380 U/L). These differences were due more to the significant differences in total work performed (distance run and “high-intensity” running) than to the environmental conditions⁵⁴. Had there been a significant heat factor, perhaps CK in the hot trial would have been higher or, at the very least, equaled that of the thermoneutral environment. In the current study, no difference existed between the heat and control trial regarding CK.

In contrast to results found by Nybo and colleagues⁵⁴, Hammouda et al. discovered higher CK levels after exercise in a hot environment rather than in a thermoneutral environment⁵⁵. Fifteen young soccer players performed two yo-yo tests in a randomized fashion, once in the early morning when it was cooler outside, and once in the afternoon when it was hotter outside. While no ambient temperatures were provided for the two conditions, the core temperature (measured orally) of subjects was significantly higher in the afternoon trial ($36.9 \pm 0.3^\circ\text{C}$) compared to the morning trial ($36.1 \pm 0.2^\circ\text{C}$). Mean CK increased significantly more from pre- to post- exercise in the evening trial ($20.3 \pm 5.08\%$ increase) compared to the morning trial ($16.1 \pm 3.52\%$ increase). Subjects ran significantly further during the evening than the morning⁵⁵. Once again, differences in CK cannot be attributed to temperature differences due to the unequal work performed in each trial.

In a third study involving repetitive box lifting in either a hot (38°C) or thermoneutral (23°C) environment, the CK response was measured 24 and 48 hours post exercise⁵⁶. During the thermoneutral trial, subjects lifted significantly more boxes compared to the hot trial (i.e., more work performed). Despite differences in amount of work performed, post-exercise CK was not significantly different between trials⁵⁶. One can speculate on the possibility that the significantly higher core

temperature during the heat trial made up for the greater work performed in the thermoneutral trial, thereby eliciting no difference in post-CK. However, according to results of the current study, it is likely that added heat did not play a role in augmenting muscle damage.

While no differences existed in the variables measured, observing other variables may lead to more conclusive results. During the eccentric exercise protocol in trial 2, several subjects reported feeling weaker and more fatigued. Additionally, subjects reported that the exercise felt more difficult during trial 2. This is beyond the scope of this study, but perhaps this is indicative of the central fatigue hypothesis⁴⁶. Due to the high variability in CK and MVC, perhaps a different method of assessing muscle damage would reveal differences between trials, such as electron microscopy from a muscle biopsy or some other subjective measure of fatigue.

Regarding MS, all but one subject experienced some degree of MS. Interestingly, MS tended to be higher after trial 1 compared to trial 2, although differences were not statistically significant. This may be due to subjects never having experienced the amount of MS resulting from such eccentric exercise before, and/or it seemed extreme to them at the time. After trial 2, which all subjects completed second, the same amount of soreness may have been present, but subjects perceived it to be not quite as bad because it was not a new feeling to them.

The eccentric exercise protocol employed in the present research was modeled after a protocol established to produce large increases in CK³. In their research, Lavender and Nosaka had subjects perform 6 sets of 5 repetitions of eccentric contractions of the elbow flexors. Rest between repetitions was 3 seconds, and rest between sets was 2 minutes. All of these parameters were identical to methods employed in the current study. Contrary to Lavender and Nosaka, who used a dumbbell corresponding to 40% of the subject's isometric MVC, a dumbbell corresponding to 80% of the subject's isometric MVC was used in this research. Given the greater load used in the current

study, it was expected that the CK response might be greater or, at the very least, be equal to those results of Lavender and Nosaka. This was not the case. Forty-eight hours after the eccentric exercise, subjects with similar characteristics used in the current study had CK levels of approximately 1,400 U/L³. In the present study, 276 U/L was the average 48 hour post-CK in the two trials.

Other research observing the CK response after eccentric elbow flexor exercise elicited similar results to that of Lavender and Nosaka. In a more extreme eccentric exercise protocol, where subjects performed 50 maximal eccentric contractions, CK was over 10,000 U/L in males four days post-exercise². In an even more rigorous eccentric exercise protocol, subjects performed 70 maximal eccentric contractions of the elbow flexors (14 sets of 5 repetitions) each lasting 3 seconds. Values for CK measured four days later approached 10,000 U/L⁵⁸. In another study, subjects performed 6 sets of 5 repetitions of 90% MVC eccentric contractions of the elbow flexors, with each repetition lasting 5 seconds—a procedure nearly identical to that used in the current study. A 48 hour post-exercise CK measurement yielded results close to 3,000 U/L⁵⁹. Contrary to these results, research by Serinken and colleagues yielded results more similar to the present study⁶⁰. Subjects engaged in a slightly less vigorous eccentric exercise protocol, performing 20 eccentric contractions of the elbow flexors at 80% of 1RM, with each repetition lasting 2-3 seconds. Mean CK after 48 hours was approximately 340 U/L, fairly similar to the 276 U/L found in the current study. Interestingly, a similar model of assay was used to assess CK: a Roche Diagnostic Reflotron Plus⁶⁰.

As stated earlier, CK is a highly variable measure of muscle damage^{11, 15}. A prime example of this was illustrated when 11 men performed 300 maximal eccentric contractions of the knee extensors, with each repetition lasting 3 seconds. In eight of these subjects, the peak CK activity was 965 U/L, probably not quite as high as one would expect given the extreme nature of the exercise

performed. In the remaining three subjects, peak CK activity was approximately 19,000 U/L²¹. Why can there be such extreme variability in CK between subjects performing the exact same exercise?

Several variables have been proposed to explain this high variability, the foremost being activity level of subjects involved. In the previously mentioned research involving eccentric leg extension exercise, the only measured characteristic difference between the “high-responders” and “moderate-responders” was self-reported activity level²¹. Furthermore, the high-responders all had lower expression of the protein PIIINP (N-terminal propeptide of procollagen type III), a protein involved in the remodeling of the cellular matrix after damaging exercise^{8, 21}. Perhaps the more well-trained individuals have a higher baseline level of PIIINP, allowing for repair of damage *sooner* than their less-trained counterparts, leading to less CK leakage out of the muscle cell.

Another example of trained individuals having a low CK response is seen in wheelchair basketball players. After eccentric exercise of the elbow flexors (4 sets of 5 repetitions lasting 2-3 seconds with a load of 80% 1RM), CK reached 340 U/L. While this was a significant increase from baseline, the magnitude of the increase was not very large and was quite different than values reported in previous research involving the eccentric loading of the elbow flexors^{2, 58-60}. Since subjects were wheelchair-bound, they presumably use their arms for locomotion rather than legs as an able bodied person would. This high-volume training of the arms may make them less susceptible to muscle damage when the arms are eccentrically exercised.

Genetic differences between individuals may explain some of the variability seen in CK response as well. The most notable genetic difference is that of a gene coding for the protein α -actinin-3¹¹. This actin binding protein is important in maintaining the structural integrity of the Z-line of skeletal muscle. Individuals with a polymorphism in the gene coding for this protein would have less support of the Z-line during eccentric contractions, which would lead to greater muscle damage

and subsequent greater CK release¹¹. Indeed, this was the case in a study conducted by Vincent and colleagues. Nineteen men, 10 with the polymorphism and nine without, completed 20 maximal eccentric contractions of the knee extensors. Twenty-four hours after the exercise, those with the polymorphism had substantially higher CK values (~650 U/L) than those without the polymorphism (~300 U/L), although these differences were insignificant ($p = 0.10$).

Given the high variability of CK, and the cause of this variability seeming to be at the molecular level, using CK as a tool to quantify the amount of muscle damage is not recommended. It may be useful in circumstances where subjects are not trained, have been tested for the α -actinin-3 coding-gene polymorphism, and other genes thought to be responsible for variability in CK. However, all of this subject screening would be a costly hassle. The best method for quantifying muscle damage appears to be through the use of muscle biopsy, and subsequent electron microscopy. While this is an invasive, and not entirely perfect method⁵⁸, it seems to be far superior to CK and any other indirect measure of muscle damage.

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Appendix A

Informed Consent

Participant _____

Saint Cloud State University

Informed Consent Form

Is a high core temperature during exercise associated with greater post-exercise muscle damage?

INTRODUCTION

You are invited to be in a research study that seeks to determine whether a high body temperature induces greater muscle damage (soreness following exercise) than a lower body temperature. Muscle damage (soreness) usually is a result of lowering weights during lifting. A high body temperature, or more specifically a high muscle temperature, may increase this muscle damage soreness following lifting.

BACKGROUND

The purpose of this study is to determine whether a higher body temperature during exercise elicits greater muscle damage than a neutral body temperature during the same exercise.

PROCEDURES

All participants will be tested between January 26th and April 20th. Participants will perform two eccentric exercise trials separated by 9 days. During one of these trials, participants will engage in a body temperature elevation protocol. Participants will return to the testing location 48 hours after each trial for measurement of creatine kinase, muscle soreness, and maximal voluntary isometric contraction.

If you agree to be in this study, we would ask you to complete the following things:

- You must be well hydrated on testing days.
- Finger stick blood collection for creatine kinase analysis- [four separate days]
- Rating of muscle soreness on a visual analog scale [four separate days]
- Determination of maximal voluntary isometric contraction of the elbow flexors [four separate days]
- In a private room, insert a reusable, bleach-sterilized rectal probe 10cm (4 in) into the anus for determination of body temperature [two separate days]
- With the probe in place, cycle at 150 watts for 15-20 minutes in a hot environment while wearing restrictive clothing to raise body temperature to ~38.5°C (101.3°F) [one day]
- Engage in an eccentric exercise protocol (30 eccentric contractions of the elbow flexors using a dumbbell equivalent to ~80% of maximal voluntary contraction) [two separate days]

RISKS

To ensure subjects are fully hydrated before testing, urine osmolality will be tested and if it reaches a level of 800 milliOsmols or greater then no testing will be allowed that day. Small

blood samples will be obtained to assess creatine kinase levels via a finger stick [~1/100th of a teaspoon]. This may cause minimal discomfort. Blood collection will be conducted by a trained professional. The rectal probe being used in this study is a soft, flexible 3/16" diameter line with a protective tip thus the risk of bowel perforation is extremely small. Some muscular discomfort and/or soreness will be experienced following the eccentric exercise protocols, but this will diminish in one to several days.

Core temperature rises very slowly during exercise in a warm environment like the one used in this study. That is also the reason for the need to use protective clothing to prevent most sweat from evaporating during the trial. Pilot testing has shown core temperature will rise to approximately 38.5°C (101.3°F) after 20-40 minutes of cycling exercise in the designed warm room. There is little chance your core temperature will exceed 38.5°C (101.3°F). However, if it does, participants may take a cool shower in the same room in which they will exercise. Several measures are in place to ensure participant safety. A researcher will be watching the participants at all times and continuously monitoring core temperature, heart rate, and perceived exertion. If a participant appears to be unable to maintain the desired workload, they will be stopped and removed from the warm room and monitored until back to a normal body temperature.

BENEFITS

Your participation in this study will help contribute to our understanding of exercising in the heat and its effects on muscle soreness. The information gathered from this research may be beneficial to athletes exercising in hot environments, and help influence current training practices of coaches and these athletes.

CONFIDENTIALITY

The records of this study will be kept confidential through coding of the data by the last four digits of your SCSU I.D. number. Research records will be kept in a password secured document. Only the researchers will have access to the records. In any reports or public presentations, no information will be included that would make it possible to identify a participant.

VOLUNTARY NATURE OF THE STUDY

Your participation in this research study is completely voluntary. You may stop participating at any time without penalty or costs of any kind. Your decision whether or not to participate will not affect your current or future relationship with Saint Cloud State University.

CONTACTS AND QUESTIONS

The researchers conducting this study are Luke Weyrauch, Dr. David Bacharach, and Dr. Glenn Street. You may ask any questions you have now. If you have questions later, you may contact them at:

Luke Weyrauch: welu1301@stcloudstate.edu 763-498-2351

David Bacharach: dwbacharach@stcloudstate.edu

Glenn Street: gmstreet@stcloudstate.edu

A copy of this form will be provided to keep for your records.

STATEMENT OF CONSENT

I have read the above information. I had the opportunity to have my questions answered. I consent to participate in the research.

I attest that:

- I have volunteered to take part in this project and understand I can stop participating at any time.
- I am at least 18 years of age.
- I have no known medical condition or physical injury that will prevent me from participating in exercise.
- I am satisfied that the results will be stored securely.
- I know the results will be published, but they will not be linked to me.
- I am aware of the possible risks and discomfort.
- I agree to inform the researcher immediately if I am in pain, or if I feel uncomfortable.
- I have answered "no" to all the questions on the Physical Activity Readiness Questionnaire.

I have read this form and I understand it. I agree to take part in this project.

Signature _____ Date _____

Printed name _____

Appendix B

Physical Activity Readiness Questionnaire

PHYSICAL ACTIVITY READINESS QUESTIONNAIRE (PARQ)

For most people, physical activity should not pose any problem or hazard. PARQ has been designed to identify the small number of adults for whom physical activity might be inappropriate or those who should have medical advice concerning the type of activity most suitable for them.

Please read the questions below and check the appropriate answer.

Yes No

- | | | |
|---|---|---|
| — | — | 1. Has your doctor ever said you have heart trouble? |
| — | — | 2. Do you frequently suffer from pains in your chest? |
| — | — | 3. Do you often feel faint or have spells of severe dizziness? |
| — | — | 4. Are you aware of having a sickle trait? |
| — | — | 5. Has a doctor ever told you that you have a bone or joint problem such as arthritis that has been aggravated by exercise, or might be made worse with exercise? |
| — | — | 6. Is there a good physical reason not mentioned here, why you should not follow an activity program even if you wanted to?* |
| — | — | 7. Are you over 35 and not accustomed to vigorous exercise? |

*Please pay particular attention to question #6 regarding the elbow and shoulder.

If you answer yes to one or more of the above questions, you cannot participate in any research study involving exercise.

Referenced from ACSM's Guidelines for Exercise Testing and Prescription, Fifth Edition.

Return this form to the Human Performance Laboratory as instructed.