EFFECT OF LOWER LEG ECCENTRIC ACTIONS ON THE NOCICEPTIVE FLEXION (R-III) REFLEX, FOOT TREMOR, AND DELAYED ONSET MUSCLE PAIN

by

JEFFREY DEAN PASLEY

(Under the Direction of Patrick J. O'Connor)

ABSTRACT

The primary purpose of this investigation was to learn whether the R-III reflex threshold, a measure of nociception that can be safely obtained from human subjects, is altered after completing lower leg eccentric exercise that causes delayed onset muscle pain. It was hypothesized that the R-III reflex threshold would decrease in the injured leg of the exercise group after eccentric exercise compared to the uninjured leg of the control group. Measures of inflammation (leg volume), pain and physiological tremor, a neurological measure not directly involved in nociception but known to be increased following eccentric exercise, were included to document that the stimulus used here induced the expected muscle injury, pain and change in non-nociceptive neurology. Initial investigations (n = 30 to 31 adults tested) examined the day-to-day reliability of lower leg volume and active foot tremor at 90^{o} and found both to be highly reliable (ICC 3,5 = .972 and .821, respectively). In the primary experiment, 22 young adult females were block randomized to either an eccentric exercise group (n=11) or a no exercise control group (n=11). Before and after the exercise or control condition (10 min, 24-, 48- and 72-hrs post) measurements were made of the R-III reflex threshold, physiologic foot tremor, leg volume and muscle pain. From 24 to 72 hours following eccentric exercise, small increases in

leg volume, pain intensity and pain affect were found as was a small decrease in foot tremor and R-III reflex threshold. Repeated measures ANOVA revealed that the range of oscillation in the minimum and maximum R-III threshold 24 to 72 hours after eccentric exercise was significantly greater than in the control condition ($F_{1,20} = 9.91$; $p = 0.005$; $\eta^2 = 0.33$; $\varepsilon = 1.00$). It is concluded that eccentric exercise inducing modest changes in indicators of muscle injury increases the range of oscillation in the R-III reflex threshold 24 to 72 hours after the exercise.

INDEX WORDS: Accelerometer, inflammation, pain affect, pain intensity, pressure algometry, water displacement, withdrawal reflex

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A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHLOSOPHY

ATHENS, GEORGIA

2007

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August 2007

DEDICATION

I would like to dedicate this dissertation to the people that matter the most in my life, my family, without which this accomplishment would not have been possible. Many thanks and appreciation goes to my father, Roger, and mother, Jean, who are the best parents a child could hope to have in life. They have always been there for me and instilled the values that make me who I am today. Thank you, Todd, for being a great brother and friend.

Lisa, words can not express the amount of appreciation I have for the patience, love, and support you have given me throughout our time together. You are my best friend and the love of my life. Jason, my son, thank you for reminding me what life is about and I hope that this may serve as inspiration to advance your education to the highest of highs. I love you all very much. Thank you!

ACKNOWLEDGEMENTS

Special appreciation goes to Dr. Pat O'Connor. Whether in the classroom, your office or in the lab, you were always there to provide support, valuable incite and direction in my educational, research and professional pursuits. Thank you very much for your efforts, I could not have asked for a better mentor and adviser.

I would like to thank Dr. Rod Dishman for being dedicated to striving for excellence. Instilling in those that pass through the Exercise Psychology Lab at the University of Georgia to be the best that they can be and that second best is not good enough. Exercise Psychology Dawgs truly are a different breed thanks to you.

Thank you, Dr. Kirk Cureton and Dr. Andrea Hohmann for putting forth the time and effort to serve on my committee, giving valuable direction throughout the completion of this project.

Special thanks go to Dr. Chris France from Ohio University for his continuing assistance with methodological issues concerning the R-III reflex and for a baseline R-III reflex script that was used in this dissertation.

I thank Simon Gray from Cambridge Electronic Design for his valuable expertise and assistance in adjustments to the script used to elicit the R-III reflex and for writing the script for the measurement of physiological tremor in this study.

I would also like to acknowledge the efforts of colleagues from the Exercise Psychology Laboratory at the University of Georgia. I would like to thank Dr. Rob Motl, for his assistance in determining the best electrodes during the early phases of developing a methodology to elicit the

R-III reflex. Thank you, Dr. Tim Puetz, for being a great friend and colleague, who I really enjoyed spending time with discussing potential research ideas. I also appreciate your willingness to be a subject when needed to perfect new scripts for the R-III reflex and tremor. Victor Maridakis, Matt Herring and Nate Thom, thank you all for being subjects at various times that proved valuable in the development of the final protocols in this dissertation.

Thank you, Dr. Kathy Simpson, for the use of the angled platform used in this investigation to induce delayed onset muscle injury.

In addition, I would like to thank Marlee Stewart and Kim Norton for their help and going the extra mile on many occasions when needed during my time at the University of Georgia.

Finally, I must thank my wife, and parents for their patience, love and continuous support throughout this entire project, without which the completion of this project would not have been possible.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Over the last several decades much has been learned about the process by which eccentric exercise causes muscle injury that leads to delayed-onset muscle pain. Although pain intensity ratings are the most frequently measured outcome variable in human studies of this type, little is known about the effect of muscle damaging eccentric exercise on the neurology that underlies delayed-onset muscle pain. Eccentric exercise may contribute to an increased incidence of accidents due to the altered neural control over movements made when muscles are injured by eccentric exercise.

This study provides new knowledge about changes in the nervous system that occur as a result of eccentric exercise that induces mild to moderate muscle injury and pain. The information about changes that occur in the nervous system ultimately is expected to contribute to improved methods for preventing and/or treating "real world" movement deficiencies that result from novel eccentric exercise. One purpose of this proposed project is to advance knowledge by examining the effect of lower leg eccentric exercise on both foot tremor and the nociceptive flexion reflex (R-III reflex). One prior investigation reported an increase in the amplitude of hand tremor following biceps brachii eccentric exercise (Saxton et al., 1995). This study proposes to extend this work to lower leg exercise and foot tremor.

The R-III reflex is a measurement tool that involves electrically exciting the nociceptive afferents of the sural nerve. The magnitude of the electrical stimulus required to stimulate the nociceptive reflex and the amplitude of the electromyographic response in the biceps femoris muscle are the measures obtained. These measurements reflect the function of the nociceptive afferents and related spinal interneurons. Since muscle injury in animals influences nociceptive afferents and related spinal interneurons (Wang, Chen, Liao, and Shyu, 2005), it is hypothesized that injurious eccentric exercise performed by humans will influence the R-III reflex. This experiment is significant because it will be among the first to objectively examine the influence of injurious eccentric exercise on nociceptive neurology of any kind.

In summary, this investigation will add to the literature by examining the influence of eccentric exercise on neurology underlying delayed-onset muscle pain. The purpose of this investigation is to determine the neurophysiological changes due to delayed onset muscle injury by assessing nociceptive changes using the R-III reflex and motor control changes using tremor. Results from this investigation could shed light on individual differences in pain ratings following eccentric exercise and may have potential for measuring the effectiveness of treatments for delayed onset muscle injury and pain. The background and rationale for the proposed research is presented in the following literature review.

Nociceptive System

Pain has been defined as an "unpleasant sensory and emotional experience associated with potential or actual tissue damage, or described in terms of such damage" (Merskey and Bogduk 1994, p. 210). Inherent in this definition are the ideas that pain is a subjective experience, pain is always an emotional experience and pain is not always directly proportional

to actual tissue damage. Nociception differs from pain. The nociceptive system is the neurobiological apparatus for providing the brain with information about actual or potential tissue damage. Nociception can occur without pain and pain can occur in the absence of evidence of tissue damage.

Sensory afferents are divided into nociceptors, primarily (type III, IV), and non-nociceptors associated with type I and II fibers. Type I and II receptors are known as low threshold mechanoreceptors. These generally do not transmit nociceptive signals through the CNS. They are large and have a fast conduction velocity due to heavy myelination. These sensory afferents are associated with the golgi tendon organs and muscle spindles and their stimulation results in signals being sent that provide proprioceptive information. A subset of type III (A-delta) and type IV (C) afferents are nociceptors which when activated by noxious pressure or chemicals suggestive of actual or potential tissue damage send signals to the dorsal horn. Afferents classified as type III (A-delta) are differentiated as high-threshold mechanoreceptive (HTM) and mechanothermal (MT) nociceptors (Price, 1999). The HTM are activated by high amounts of mechanical pressure that can potentially cause tissue damage. MT receptors respond to both mechanical pressure and heat stimuli. Type III nociceptors are wide in diameter, thinly myelinated, fast conducting (3.1-13.5 m/sec) fibers, and found in the muscle (Marchettini, Simone, Caputi, and Ochoa, 1996). Stimulating type III afferents in the muscle results in dull, cramping or aching pain. Type III fibers are considered to be responsible for epicritic or first pain that is highly localized and short in duration with a quick onset. They are sensitized by naturally occurring algesics. In contrast, Type IV receptors are activated primarily by endogenous algesics, but they also respond to heat, cold, and mechanical pressure. Type IV (C) nociceptors are unmyelinated and slower in conduction velocity (0.5-2.0 m/sec) than type III

fibers (Simone, Marchettini, Caputi, and Ochoa, 1994). Type IV afferents are found in muscle fibers and around the capillaries supplying the muscle. Stimulation of type IV nociceptors in the muscle, elicits a dull, cramping and aching type pain. Type IV fibers are considered to be responsible for protopathic or secondary pain that has diffused localization and is longer in duration, often lasting beyond the duration of the stimulus with a relatively slow onset.

Endogenous algesics that directly excite type IV fibers include adenosine, bradykinin, potassium, serotonin, and histamine. Pain typically does not occur by the excitation of only one type of nociceptor. However, certain types of stimuli primarily excite one type of nociceptor. For example, stepping on a tack (which induces a withdrawal reflex) primarily activates type III fibers. Pain that is associated with inflammation involves both an increase in pressure put on type III fibers and the chemical activation of type IV nociceptors, due to the endogenous algesics released during inflammation. The current investigation used an experimentally induced withdrawal reflex stimulated in the lower leg near where muscle inflammation occured to understand the underlying neurological changes with delayed onset muscle injury.

Peripheral mechanisms involved in inflammation

There is substantial evidence that eccentric exercise induced muscle injury results in inflammation. Teleologically, the purpose of muscle pain induced by inflammation is to motivate the organism to decrease movement and thereby prevent the injured site from being further damaged and allow time for healing.

Inflammation is a complex process. It is characterized by physical signs and symptoms including redness, heat, swelling, loss of function and pain. Redness, heat and swelling are due to an increase in blood flow into extravascular tissues. The pain associated with muscle injury is

due to both mechanical pressure and sensitization of nociceptors. This point is illustrated by the fact that pain resulting from eccentric exercise typically is not continuous but only occurs during movement or palpitation of an injured muscle or tendon. The movement or palpitation activates receptors that are sensitized by a large number of peripheral mediators of inflammation, including: bradykinin, serotonin, histamine, luekotrienes, cytokines, amines, interleukin 1 and 8, substance P, calcitonin gene related peptide, hydrogen ions, nerve growth factor, protein kinase C, and adenosine.

Bradykinin is considered one of the most potent endogenous mediators of inflammatory pain (Armstrong, Dry, Keele, and Martin, 1953). It is produced from plasma alpha2-globulins by kallikreins when factor XII of the Hagemen clotting system is activated in response to tissue injury (Mense, 1993). Release of bradykinin occurs due to tissue damage and as a result of a decrease in blood pH, as well as hypoxia and ischemia. Bradykinin contributes to increased vascular permeability, vasodilation, and stimulates immune cells (Walker, Perkins, and Dray, 1995). The administration of B_2 receptor agonists results in a decrease in pain threshold (Steranka, et al., 1988). The administration of B_2 receptor antagonists results in an increase in pain threshold (Steranka, Burch, Vavrek, Stewart, and Enna, 1988). Bradykinin induced pain is increased in the presence of other algesics, such as serotonin, and blocked by opiod receptor agonists.

Prostaglandins, arachidonic acid metabolites, are released during muscle contractions from arachidonic acid producing cells (phospholipids). Prostaglandins (e.g., PGE_2) cause pain by sensitizing nociceptive afferents. Aspirin can decrease pain by blocking the production and release of prostaglandins (Vane, 1971; Smith and Willis, 1971; Ferreiri, Moncada, and Vane, 1971).

Hydrogen ions mediate nociception directly via acid sensing ion channels and indirectly by inducing the release of bradykinen. Resting rat soleus muscle pH is more acidic after eccentric exercise (Yeung, Bourreau, Allen, and Ballard, 2002); however, early hypotheses that increased lactic acid caused delayed onset muscle pain are not supported.

Substance P and calcitonin gene related peptide (CGRP) are considered mediators of inflammatory pain in the periphery by sensitizing type IV afferents. Substance P is produced in the cell bodies of dorsal horn and afferent fibers. CGRP does not sensitize nociceptors but acts to potentiate the effects of substance P by preventing its breakdown once substance P is released and by enhancing calcium influx in the afferents. Substance P is released and binds to neurokinen 1 receptors which eventually results in PGE_2 and histamine release. PGE_2 and histamine are algesic mediators of inflammatory pain.

Glutamate and substance P are key neurotransmitters released by nociceptive afferents in the dorsal horn. Nociception can be blocked and pain behaviors reduced by blocking glutamate with NMDA receptor antagonists or by blocking substance P in the dorsal horn (Liu, Mantyh, and Basbaum, 1997).

Other mediators of inflammatory pain are mast cells, which contain serotonin (5-HT) and histamine. Degranulation of mast cells by nerve growth factor causes the release of 5-HT and histamine which decreases inflammatory pain threshold. Adenosine also is involved in the release of both 5-HT and histamine from mast cells by binding to adenosine A_3 receptors. Inhibition of the breakdown of mast cells decreases inflammatory pain (Mazzari, Canella, Petrelli, Marcolongo, and Leon, 1996).

Norepinephrine is normally not a mediator of peripheral nociception but in the presence of inflammation it can sensitize nociceptors and increase pain. The adrenal cortex and free nerve endings of the sympathetic nerves release norepinephrine.

Central sensitization is a key feature of inflammatory pain (Campbell and Meyer, 2006). Central sensitization refers to an increased excitability of brain or spinal cord neurons involved in nociceptive neural networks. The increased excitability amplifies all sensory input such that normally innocuous stimuli can become painful, noxious stimuli are perceived as more painful than usual, and/or less afferent activity is needed to cause pain. Inflammation induced increase in the sensitivity of Type IV afferents is thought to contribute to central sensitization. Inflammation induced by eccentric exercise rarely has been used to study central sensitization.

Ascending pathways

Once a muscle nociceptor is excited an action potential is propagated through an afferent pathway. The sensory afferent enters the spinal cord primarily in the dorsal root ganglion. Upon entering the spinal cord, bifurcation occurs in which both ascending and descending paths occur within the tract of Lissauer until reaching the dorsal horn. Some afferents synapse on the surface of the dorsal horn (lamina I and II) and some more deeply (lamina V). Within the dorsal horn there are short interneurons that can be excitatory or inhibitory. Projection neurons from the dorsal horn relay the information to higher brain structures, but there is substantial sensory processing that occurs within the dorsal horn (Price, 1999). In the lamina of the dorsal horn, nociceptor afferents converge on interneurons with synapses from non-nociceptive afferents. An end result can be that the type I and II fibers can inhibit type III and IV fiber activity in the dorsal horn. This is potentially relevant to the proposed research because pain ratings are usually

obtained during palpation of the muscle or during muscle and limb movement that activates non-nociceptive afferents. It is possible that this movement may inhibit pain ratings. It would be advantageous for investigators to fully describe the procedures used to obtain pain ratings, but often the procedures are poorly described.

There are three well known ascending pathways, the spinothalamic tract, spinoreticular tract, spinomesencephalic tract (Almeida, Roizenblatt, and Tufik, 2004). The spinothalamic tract (STT), ascends from lamina I and V in the dorsal horn crossing the midline of the spinal cord to the anterolateral white matter, contralateral to the noxious stimulus. The STT consists of two parts. The lateral portion is called the neospinothalamic tract in which projections ascend to the medulla and pons to ventrolateroposterior (VPL) thalamus in a somatopically-organized fashion. The VPL projects to the primary and secondary somatosensory cortex. The medial portion of the STT is called the paleospinothalamic tract and consists of projections to the reticular formation at the level of the pons and medulla to the periaqueductal grey (PAG), hypothalamus, amygdala and medial portions of the thalamus. The medial portions of the thalamus called the nucleus centralis lateralis (NCL) have projections to the premotor cortex and cerebellum.

The two additional ascending pathways are thought to be involved in the expression and regulation of emotion during pain (Almeida, Roizenblatt, and Tufik, 2004). One of those pathways is called the spinoreticular tract and include projections to the reticular formation at the level of the pons and medulla, to the medial thalamus, and ultimately to the nucleus parigigantocellularis which project to the locus coeruleus. The locus coeruleus is known to project to the limbic system and be involved in the regulation of emotion and mood. The other pathway is the spinomesencephalic tract which ascends from lamina I and V to the medulla and pons to the thalamus, PAG, and hypothalamus, having indirect connections to such structures as

the amygdala and anterior cingulate cortex. It has been established that the locus coeruleus, amydala, cingulate cortex and hypothalamus each have roles in the expression of pain behaviors such as freezing or hypervigilence. Several studies have shown that animals when placed in stressful environments exhibit freezing behavior and an increased pain threshold (Amit and Galina, 1986). Stress or fear induced hypoalgesia has been blocked by lesions in the amydala, indicating its role in regulating pain behavior.

Descending inhibitory pathways

There are at least 3 major components to the descending inhibitory pathway and they are the periacqueductal grey (PAG), rostral ventromedial medulla (RVM), and the dorsolateral pontine tegmentum (DLPT). The PAG receives input from the hypothalamus and the amygdala and projects primarily to the RVM. Cell bodies in the RVM project to the spinal cord and release 5-HT which ultimately inhibits ascending nociception pathways and decreases pain. Opiod receptors within the PAG, RVM and DLPT result in excitation of the descending inhibitory pathway, and opiods released in the spinal cord result in inhibition of nociception. The RVM and the DLPT act directly on nociceptive afferents in the dorsal horn and indirectly through interneurons.

There are occasions when a noxious stimulus can inhibit dorsal horn neurons from a different receptive field thereby inducing widespread analgesia. This process is called diffuse noxious inhibitory control (DNIC). This phenomenon only occurs with convergent interneurons. Studies with animals having a severed spinal cord at cervical levels do not show DNIC, indicating the effect involves supraspinal structures and descending pathways involving the PAG (Le Bars, Villanueva, Bouhassira, and Willer, 1992).

There have been no investigations that have assessed DNIC in relation to delayed onset muscle pain or inflammation. The current project will add to the literature by determining if DNIC is possible with DOMP as the noxious stimulus (e.g., learn whether injury to the right leg reduces the R-III threshold in the left leg).

PET scan investigations have revealed the insular cortex is stimulated during a noxious stimulation. The insular cortex receives input from the vagus nerve and baroreceptors. Electrical stimulation of the PAG decreases nociceptive responsiveness (tail flick) (Reynolds, 1969) and increases blood pressure (Lovick, 1991). Further evidence has shown that by stimulating the PAG, blood pressure is increased and hypoalgesia results. People with high blood pressure are more susceptible to a silent heart attack because they do not feel angina. This and other evidence points to the idea that mechanisms regulating the cardiovascular system are linked to the descending pain inhibitory system (Zamir and Maixner, 1986). Blood pressure is rarely considered in delayed onset muscle injury investigations. One study with normotensives found that blood pressure was unrelated to DOMP (Poudevigne, O'Connor, and Pasley, 2002). However, this finding may not generalize to people with high blood pressure. The current investigation will exclude hypertensives and take blood pressure readings each day.

Delayed-onset muscle pain (DOMP)

Delayed-onset muscle pain (DOMP) is a temporary condition of discomfort that usually begins 1 to 2 days after unaccustomed eccentric exercise and lasts up to 5-7 days. Typically it has been noted that the highest pain ratings tend to occur within one to two days following eccentric exercise. The amount of self reported soreness or pain associated with post exercise muscle damage is thought to be primarily a function of the intensity of the eccentric exercise, the

number of muscle actions performed and the recent activity history of the muscles involved. For instance, if the exercise is relatively novel, even though a person may be physically fit and considered a "trained" individual they can experience symptoms of DOMP. Other factors shown to influence the severity and time course of muscle damage include the angle of the contraction, muscle stiffness, contraction velocity and fatigue (Connolly, Sayers, and McHugh, 2003).

Three different types of muscle actions have been used to induce DOMP in the laboratory. One is eccentric exercise, which consists of muscle activation while the muscle is lengthening. Concentric exercise, defined as muscle activation while the muscle shortens in length, also has been used. Isometric exercise is another method used to induce DOMP and occurs when the muscle is activated, yet stays the same in length (i.e. no limb movement). The exercise protocol performed most frequently to induce DOMP has been eccentric exercise alone. Eccentric exercise alone is associated with greatest tension on muscle fibers. Most laboratory eccentric exercise protocols do not mimic what happens in the "real world". That is, eccentric muscle actions in the "real world" are followed by concentric muscle actions (i.e., the stretch-shortening cycle). The naturally occurring stretch-shortening cycle has been associated with a greater concentric muscle action force when compared with the amount of force generated by concentric muscle actions in isolation (Komi, 1984; Komi, 2000). Much of the literature on exercise induced muscle damage involves eccentric muscle actions performed in isolation, these results may not resemble what occurs in real world situations in which the stretch-shortening cycle occurs. More research is needed using "real world" type exercise and one of the contributions of this proposal will be the use of an exercise protocol in which the stretch-shortening cycle comes into play.

The muscle injury that occurs as a result of eccentric exercise is believed to be a mechanical disruption of the sarcomeres. Disruption of the cell membranes leads to the leaking of some of its contents into the plasma starting a chain of events that leads to an inflammatory response. The cell membrane disruption leads to a calcium influx into the cell (Brotto and Nosek, 1996). This increase in calcium within the cell leads to phospholipase A_2 being activated which leads to the synthesis of arachodonic acid. The production of arachodonic acid can lead to the production of prostagladins and thromboxanes via the cyclo-oxygenase (COX) pathway or production of leukotrienes via the lipoxygenase (LIPOX) pathway. Among the responses to the synthesis and release of prostaglandins are increased vascular permeability and direct stimulation and sensitization of type III and type IV nociceptive afferents. These responses cause an increase in nociception and pain. Leukotriene synthesis and release is responsible for increased vascular permeability and they attract neutrophils to the injured area. Neutrophils have been known to cause further muscle cell damage due to their generation of free radicals and through the release of cytoxic factors (Evans and Cannon, 1991). Various interventions have been studied to determine if free radicals impact the development of DOMP (Kaminski and Boal, 1992; Cannon et al., 1990). There is weak evidence that anti-inflammatories decrease muscle damage, reduce the time course of inflammation, and reduce free radical release to prevent further injury (Connolly et al., 2003).

Sex differences in DOMP

Throughout the pain literature there is a consensus that females living in western cultures tend to report higher pain ratings than men when exposed to an equivalent noxious stimulus (Nayak, Shiftlett, Eshun, and Levine, 2000). However there have been equivocal findings in

DOMP studies. There is evidence that men and women have similar responses to muscle damaging eccentric exercise (High, Howley, and Franks, 1989; Rinard, Clarkson, Smith and Grossman, 2000). Poudevigne, O'Connor, and Pasley, (2002) found no significant statistical differences in pain ratings between males and females, however females tended to report lower pain ratings and the authors suggest there may not have been enough statistical power to detect a significant difference due to a low sample size. Dannecker and colleagues (2003) found that there was no sex difference in pain threshold assessed after a DOMP exercise protocol in the upper arm. They did find that females reported lower pain intensities when pain was measured via a VAS. Their conclusion was that the method of pain assessment has an influence on sex differences. A confound in DOMP studies of sex differences is that groups of men and women typically differ in muscular strength. Moreover, the intensity of the exercise is rarely reported, so it's difficult to determine if a sex-related difference could be due to the absolute amount of weight lifted. The current investigation will assess DOMP threshold and intensity while moving the affected area through a standardized range of motion in a sample of young women.

Strength loss associated with DOMP

One feature of DOMP that has been debated extensively is the extent of muscle strength loss after eccentric exercise that induces DOMP. Some early evidence indicated that there was no loss in muscle strength (Ebbling and Clarkson, 1989), but more recently studies show that maximal muscle strength losses can occur from 24 hours up to a couple weeks after muscle injury. In addition, the strength loss tends to be the greatest when the muscle group is activated near full extension of the joint of the limb, and peak strength loss is approximately 24 – 48 hr post exercise. The bulk of the evidence indicates that the length of the muscle interacts with the

intensity of the eccentric exercise in influencing strength loss (Child, Saxton, and Donnelly, 1998; Newham, Jones Gosh, and Aurora, 1988). Also, the inherent passive stiffness in the muscle plays a role in the severity of DOMP symptoms. Those with greater muscle stiffness prior to eccentric exercise resulted in increased symptoms associated with DOMP (McHugh et al., 1999). Increased stiffness due to muscle damage and inflammation usually peaks 3 – 4 days post exercise and resolves in approximately 10 days.

Creatine kinase as a marker of muscle injury

An additional peripheral sign of DOMP that has been examined extensively is elevated serum creatine kinase (CK) levels. Significant increases in CK levels when compared to baseline occur from 1 to 2 days post exercise. CK levels typically peak between 3 to 6 days and return to baseline levels in 7 to 14 days. The variability in peak CK levels can be partially attributable to the exercise protocol. Downhill running has been associated with a modest increase in CK levels which peaks 12-24 hrs after the exercise (Byrnes, et al., 1985; Schwane, Johnson, Vandenakker, and Armstrong, 1983). High intensity eccentric exercise of the elbow flexors results in a large CK increase that does not begin until 48 hours post exercise and peaks much later at 4 to 6 days (Clarkson, Nosaka, and Braun, 1992). There are inherent problems with using CK levels as a marker of muscle injury due to the fact it is measured by its concentration in the bloodstream. It is a product of the amount released into the blood as a result of muscle damage and the amount removed from the blood (Clarkson and Hubal, 2002). The level of CK is used clinically in patients to determine if they've had a heart attack or have muscle damage. Use of the CK test in DOMP research has limitations because CK levels have not been highly correlated with changes in pain threshold, peak pain or even the extent of tissue or muscle damage. A CK test is useful in

providing evidence of muscle damage but that is about the extent of its usefulness. Other measures used to determine the extent of muscle damage include the measurement of lactate dehydrogenase and myoglobin. The time course of these variables also does not correlate strongly with the symptoms of DOMP.

Repeated bout effect

Little is known about the prevention of DOMP. The best prophylactic for DOMP is a prior exercise program that gradually progresses in intensity. However, even a single bout of eccentric exercise can be associated with a protective effect in which DOMP symptoms are diminished when the same exercise is performed at a later date. The repeated bout protective effect of eccentric exercise has been reported to last as long as 6 months (Nosaka, Sakamoto, Newton, and Sacco, 2001).

The nature and duration of the prophylaxis, however, is still under contention. Byrnes and colleagues (1985) found that there was a protective effect in as little as 3 weeks after the first bout of damaging eccentric exercise. Byrnes et al. (1985) and Nosaka, Clarkson, McGuiggen, and Byrne, (1991) both found that a prophylactic effect lasted 6 weeks post eccentric exercise. Nosaka et al. (1991) found that the protective effect did not last past 9 weeks. In contrast, Newham, Jones, and Clarkson (1987) produced no protective effect in DOMP symptoms with a protocol of eccentric exercise every 2 weeks for 6 weeks. The present investigation did not involve the repeated bout effect; however, participants performing heavy lower leg resistance exercise or toe raises in the previous 6 months were excluded from participation.

Muscle injury is influenced by fiber type

Muscle fiber type moderates eccentric exercise induced muscle injury. Studies have shown in both animals and humans that eccentric exercise is associated with disruptions in fast twitch muscle fibers. In animal studies, fast glycolytic (FG) tend to be the most effected fiber type, however fast oxidative glycolytic (FOG) tend to be the fiber type most affected by eccentric exercise in humans. Various animal studies have shown that eccentric exercise has been associated with approximately 1.5 times higher force generated when compared to isometric exercise (Bryne, Twist, and Easton, 2004). Human studies have shown that voluntary eccentric exercise does result in greater force production over concentric or isometric exercise, but not to the extent observed in the animal studies. The reason is that there is a protective neural control, which inhibits maximal voluntary eccentric activation from occurring to prevent the possibility of severe, irreparable muscle damage. In addition, the amount of muscle activation has been shown to be smaller during eccentric exercise as compared to concentric or isometric exercise. Therefore, it has been hypothesized that with fewer muscle fibers activated yet greater tension created, a situation is created that enhances the likelihood for muscle damage.

Subjective pain ratings associated with muscle injury

Narrative reviews have indicated there is a time course effect for pain ratings after eccentric exercise which peaks at 24 - 48 hours post-exercise. However, there seems to be large interindividual variation associated with pain ratings throughout the DOMS literature. For example, Evans, Haller, Wyrick, Parkey, and Fleckenstein (1998) found standard deviations of pain ratings to be 8.6 while the mean pain intensity ratings were 8.0 with a sample size of 16. Pain has been measured using several different methods often including scales without evidence

of their psychometric properties. More research is needed to determine possible factors associated with individual differences in pain ratings following eccentric exercise. This investigation will obtain pain intensity ratings using the well validated visual analog scale technique and expand the knowledge base by looking at the underlying neurology associated with pain ratings.

Interventions for muscle injury

Some literature has suggested that certain interventions decrease pain days following strenuous eccentric exercise, but the overwhelming majority have shown no effectiveness. There are several reasons why this may be the case. Among them, most of the exercise protocols are of an extreme nature and do not mimic the muscle injury induced by "real world" exercise. The muscle damage has afterward been so extreme that interventions may have been ineffective because of the extreme noxious stimulus. The current investigation will focus on a "real world" type of injury.

Traditional clinical treatments for DOMP have consisted of ice massage, warm water massage, hand massage, stretching, light exercise, electrical stimulation, anti-inflammatory medicines and various nutritional supplements. The evidence for the effectiveness of these treatments is inconsistent. For example, some studies found no effects with the use of NSAIDS (Donnelly, Maughan, and Whiting, 1990; Grossman, Arnold, Perrin, and Kahler, 1995) and others showed improvement with the use of NSAIDS (Hasson, et al., 1993; Connolly et al., 2003). Nontraditional treatments such as hyperbaric oxygen therapy, herbal remedies, and acupuncture have shown equivocal results. The anti-inflammatory medicines and anti-oxidant supplements have shown potential for short term improvements in pain.

Mishra, Friden, Schmitz and Lieber (1995), for example found that an anti-inflammatory drug decreased signs of DOMP short term (3 days), but led to longer term (28 days) muscle function losses compared to a no treatment control group. Supplements such as the anti-oxidants vitamin C and E have been studied with a predominantly untrained male population. The effects of these suppliments on DOMP have been small. A review by Bloomer and Goldfarb (2003) noted there is no consensus on the dosage needed for best results. Research on nutritional supplements for the attenuation of DOMP is in its infancy.

The relationship between muscle injury and muscle pain

Muscle damage does not always lead to muscle pain. For example, people with Duchenne muscular dystrophy, a muscle disease, have structural disruptions in the muscle fibers, yet have no muscle pain (Lieber and Friden, 2002). Therefore pain after eccentric exercise most likely occurs due to secondary events, such as those associated with inflammation. The timing of the secondary events also may explain the delayed time course of pain ratings after eccentric exercise.

The time course for pain often does not coincide with the time course for muscle strength loss or other muscle function disruption such as loss of motor control. This is believed to be due to the fact that mechanical damage occurs first leading to immediate strength loss. Following the muscle damage, inflammatory responses occur which include the release of various endogenous algesics explaining the reason for the delayed response of pain associated with post exercise muscle damage.

Methodological issues in DOMP research

Many of the DOMP research papers do not convey enough information so that the intensity (relative or absolute) or duration of exercise employed can be determined. For example, Bobbert, Hollander, and Huijing (1986) induced DOMS via plantar-/dorsiflexion movement. Participants were to stand with the front part of their foot on a board, then raise and lower several times. The participants were free to choose the amplitude and frequency. The authors failed to report the amplitude and frequency, therefore, the exercise intensity could not be determined. The research conducted for this dissertation used a similar protocol, however the participants' weight was measured, the position and angle of the foot was standardized, and the number of muscle actions was recorded.

Another example of poor reporting is that of Mattacola, Perrin, Gansneder, Allen, and Mickey (1997). Their procedure consisted of repeated eccentric contractions of the elbow flexors. They did report that the dumbbell began at an absolute intensity of 13.6 kg and the participants were to lower the dumbbell until failure. Once the participant failed to lower the dumbbell for 3s then the dumbbell was decreased in weight by 2.26 kg. This procedure was maintained until the dumbbell equaled 2.26 kg. The average number of repetitions to failure was not given by the authors. Thus, the overall intensity and duration of the exercise is unknown.

The descriptions of instructions for the measurement of pain also are generally poor. Typically, no anchors are given when using a visual analog scale (VAS) or there is a poor description of the anchors and many investigations do not report instructions at all. Variation in instructional sets used may influence the study outcome. The current investigation used a scripted set of instructions for pain measurement and a VAS with well described anchors.

There is clear evidence that eccentric exercise induces delayed-onset muscle soreness and that pain responses vary greatly among individuals. However, the role of nociception in DOMP is unknown.

Neuromuscular control and tremor

The vast majority of the literature on eccentric exercise has been concerned with the loss of muscle strength. Past research on muscle strength loss has been used by physical trainers to determine when it is safe for an athlete to return to play after a muscle injury. That may not tell the full story in that if the muscle tissue is healed so that the risk of further muscle damage is reduced the athlete may still be at increased injury risk because the underlying neurology may be compromised for a longer period of time. It has been reported that there is an increase in muscle activation, as assessed by iEMG, in order to perform the same amount of work after muscle damage has occurred (Komi and Viitasalo 1977; Deschenes et al., 2000). Neuromuscular efficiency is impaired for a longer duration than either muscle soreness, plasma levels of CK or strength loss when muscle damage occurs (Deschenes et al., 2000). This dissertation examined the effects of DOMP on physiological tremor, one aspect of motor control.

Research has shown neuromuscular efficiency is compromised, and impairment in overall motor control occurs following muscle damage. Perception and proprioception are influenced by muscle damage. Proprioception, which is regulated by type I and II afferent receptors, has been shown to be negatively affected by eccentric muscle damaging exercise (Brockett, Warren, Gregory, Morgan, and Proske, 1997). The perception of force generation is increased during force production in the injured limb due to the effects of muscle damage on the type I and II afferents (Saxton et al., 1995). Thus, the person may have a decreased ability to

generate force, yet feel as though they are producing more force compared to a control limb. This could be important in determining when it is safe for resumption of athletic competition or exercise following muscle damage. Strength loss may be diminished, yet motor control may not be adequately improved and the end result could be putting that person at further risk for injury.

Hand tremor is influenced by biceps muscle injury caused by eccentric exercise. Frequency of hand tremor does not change after muscle damaging eccentric exercise; however amplitude is increased immediately following eccentric exercise and 24 hours later (Saxton et al., 1995)

Tremor has been defined as "rhythmical, involuntary oscillatory movement of a body part" (Deuschl, Bain, Brin, and Ad Hoc Scientific Committee, 1998). Physiologic tremor occurs in everyone in all joints that that are unrestricted to oscillate. This physiological tremor is a result of various interactions between the motor units, reflex systems, mechanical properties of the limb involved and the CNS. Parkinson's disease like many other neuromuscular diseases results in increased amplitude of tremor. Tremor amplitude may reflect the body's readiness to move voluntarily and thus measurement of tremor could detect deficits in the neuromuscular system. This dissertation incorporated the measurement of physiological tremor of the foot as a marker of impairment in motor control.

Animal models of inflammation and persistent pain

Animal models of nociception are crucial for use in determining the effectiveness of various pharmaceutical compounds and are vital to assess the potential side effects and toxicity before trials with humans. In addition, they are quite useful in examining the pathophysiology of the nociceptive system including peripheral and central sensitization.

An example of an acute animal pain model is placing a rat on a hot plate, inducing a high intensity stimulus, and then observing the behavioral responses such as tail flick or licking the hind paw. The tail flick reflex was first used by D'Amour and Smith (1941). The hind paw latency is a withdrawal reflex initiated in response to tissue damaging heat, mechanical pressure, chemical, or electrical noxious stimuli (Walker, Fox and Urban, 1999). Numerous animal studies have used this flexor withdrawal reflex as a measurement tool to assess the effects of various treatments on the nociceptive system (Falinower, Willer, Juien, and Le Bars, 1994; Meyerson, Ren, Herregodts, and Linderoth, 1995).

Animal models of persistent pain include a formalin test in which a 10% formalin solution is injected in the paw of the rat and the number of flinches are recorded over a time period in 1 minute intervals. Another, called the mild burn model, involves subjecting an anesthetized rat to a mild burn of the paw. Mechanical and withdrawal thresholds are assessed pre and post tissue injury. Injections of various drugs are given 5 minutes prior to injury and/ or starting as soon as 30 minutes post injury for the assessment of the treatment effectiveness (Allen and Yaksh, 2004). Other animal models of chronic inflammatory pain include: Fruend's adjuvant, which attempts to mimic rheumatoid arthritis, carrageenan and turpentine, which simulate short term inflammation of joints, and UV-irradiation, which is similar to a moderate burn (Walker et al., 1999). Several animal models have been used for delayed onset muscle injury (Warren, Hayes, Lowe and Armstrong, 1993). However, the symptoms associated with mechanical hyperalgesia after human eccentric exercise have not been substantiated using those animal models (Taguchi, Matsuda, Tamura, Sato and Mizumura, 2005). The majority of delayed onset muscle injury (DOMI) animal models have concerned themselves with strength losses, strength gains, amount of muscle injury, fiber types, and the cellular mechanisms of muscle

injury (Lieber, and Friden, 2002). One animal model for DOMI allows for mechanical hyperalgesia to be assessed, thus offering the potential for neural pathophysiology to be better understood (Taguchi, Matsuda, Tamura, Sato, and Mizumura, 2005).

The most recent animal pain model used in research on inflammation is the use of knockout mice. Knockout mice which lack opioid receptor genes have been used to determine more precisely the targets of various opiate compounds and their effectiveness as treatments (Ide, Minami, Ishihara, Uhl, Sora, & Ikeda, 2006). This model has not yet been used to better understand eccentric exercise induced inflammation.

Cannabinoids modulate hyperalgesia associated with inflammation

There is well-established evidence that an endogenous cannabinoid system exists that regulates nociception spinally, supraspinally, and in the periphery (Richardson, 2000). There are at least two types of receptors known to regulate the endogenous cannabinoid system. The CB_1 receptor operates primarily in the central nervous system and the CB_2 receptor which operates primarily in the periphery. The CB_1 receptors have been found in such supraspinal nociceptive regulating centers as the PAG, dorsal raphe nucleus, the ventroposterolateral nucleus of the thalamus and the rostral ventromedulla. Some of the reasons cannabinoids have not been studied as extensively as opioids is due to the stigma associated with cannabis and the potential side effects. Recent research has focused on possible methods of eliminating some of the side effects and potentiating the benefits. Due to the class I drug classification it is difficult to perform human studies on the antinociceptive effects of cannabinoids and therefore the majority of the research has been performed with the use of animal models. The majority of early work

examined the antinociceptive effects of cannabinoids under normal conditions, typically to thermal and mechanical noxious stimuli.

More recent research has focused on the viability of cannabinoids in conditions of inflammation and persistent pain. Some of the animal models used have been knockout mice, bladder inflammation, carrageenan, and formalin models. The use of a selective CB_2 agonist known as PEA, has shown antihyperalgesic effects in some animal models and the effects are more potent when given directly in the periphery versus a systemic dose. The use of pharmaceuticals directly effecting the endogenous cannabinoid system to reduce hyperalgesia during inflammation and persistent pain states shows promise, especially when administered in the affected area. Local administration can reduce some of the side effects associated with systemic administration. It has also been shown that CB_2 receptor agonists can produce peripheral antinociception without the side effects seen by many CNS analgesics. In addition, CB_2 agonists can produce a reduction in inflammation by the inhibition of edema (Malan, Ibrahim, Vanderah, Makriyannis and Porreca, 2002). A CB_2 receptor agonist has also been shown to reduce the flexor reflex in an animal model of inflammation (Hong, et al., 2001).

An area ripe for future research would be the use of a DOMI animal model in which spinal, supraspinal and peripheral portions of the endogenous cannabinoid system could be examined. For more a more in depth review of past research on the cannabinoid system and it's potential in pain modulation please read the critical reviews by Hohmann and Suplita, (2006), Richardson (2000), Walker, Haung, Strangman, and Sanuo-Pena (2000) and Pertwee (2001).

Nociceptive flexion reflex and inflammation

In a study of neonatal Sprague-Dawley rats, inflammation induced by a 2% solution of carrageenan in the hind paw was associated with a hypersensitity to a mechanical flexion reflex. Thresholds were lower for the flexion reflex, and were increased with an epidural injection of bupivacaine. The epidural condition did not effect the tactile sensitivity in the contralateral leg (Howard, Hatch, Cole, and Fitzgerald, 2001). Based on this, one might hypothesize that inflammation associated with DOMS would result in a decrease in nociceptive reflex threshold in the ipsilateral limb.

The use of a conditioning noxious stimulus to abate behavioral, neural or reflex responses to a nociceptive system test is called nocigenic inhibition (Ness and Gebhart, 1991). Noxious pinch of the nose and colorectal distension in rats has been shown to inhibit the tail flick reflex to a heat lamp (Ness and Gebhart, 1991). In addition, research conducted with cats in which a lesion was induced in the lateral gastrocnemius has shown that inflammation significantly reduces γ-motorneuron resting activity levels and thus reduces the ability to excite the α-motorneurons involved in a reflex. Nociceptive muscle afferents were shown to inhibit γ-motorneurons (Mense and Skeppar, 1991). One advantage of inflammation having the ability to attenuate muscular reflexes is that the damaged muscle would be allowed to heal without strong muscle activation associated with a reflex. Reflexes that have been attenuated to areas with inflammation were considered stretch reflexes and not nociceptive reflexes. The DOMI would not be considered a conditioning noxious stimulus due to the inherent properties associated with this model. Pain is not perceived unless the injured muscle is activated or palpated. Thus, the present study does not lend itself to nocigenic inhibition because the injured muscle will not be

activated or palpated during the elicitation of the R-III reflex. Inflammation has a complex reaction in muscle nociceptors.

Induction of inflammation in the gastrocnemious-soleus muscle in rats led to an increased sprouting of substance P-immunoreactive fibers. Substance P is often found in most nociceptive afferents and as a result the inflamed muscle is more likely to have more afferents with nociceptive capabilities. Thus, a muscle bed with inflammation will have an increased number of muscle nociceptors activated leading to more pain when excited by a noxious stimulus (Reinert, Kaske, and Mense, 1998).

Conclusion

Based on the information reviewed in this chapter, I hypothesized that eccentric exercise induced muscle injury would result in a facilitated withdrawal reflex with higher pain ratings 24 to 48 hours post injury when compared to a (non-injury) control when excited by a noxious electrical stimulus. This study is the first to examine the effects of inflammation due to muscle damage on the nociceptive flexion reflex in humans.

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CHAPTER 2

THE NOCICEPTIVE FLEXION (R-III) REFLEX:

A POTENTIALLY USEFUL TOOL IN EXERCISE AND PAIN STUDIES[1]

[1]Pasley, J.D. and P.J. O'Connor. 2005. *International Journal of Sport and Exercise Psychology*. 3:338-351. Reprinted here with permission of publisher.

Abstract

There is a need to better understand relationships between physical activity and pain. Improvements in understanding often stem from methodological advancements. This paper provides an overview of the nociception flexion reflex. The nociception flexion, or R-III, reflex is a tool that has been fruitfully employed in studies of pain, but has not yet been used much in studies involving physical activity and pain. The methodology of the nociception flexion reflex is described and selected methodological concerns discussed. A brief description of the nociceptive system is included to provide a context for interpreting nociception flexion reflex data. Factors that have been shown empirically to modify the R-III reflex are summarized. Many of these experiments also provide validity evidence for the reflex as a measure of a portion of the nociceptive system. The relatively few sport, exercise and physical activity studies that have used the nociception flexion reflex are reviewed. Suggestions are made for future exercise and pain research that might benefit from assessing the nociception flexion reflex.

KEY WORDS: exercise, physical activity, muscle, nociception, R-III, reflex, pain

Pain is an integral part of participation in exercise and sports. Transient muscle pain often accompanies vigorous exercise in healthy people. Chest pain during physical activities afflicts many with coronary heart disease. Recreational athletes experience delayed-onset muscle pain overdoing an activity after a layoff. Sport-related injuries, such as ankle sprains and muscle strains, are common among athletes, whether they compete at a novice or an elite level.

Exercise is not only a cause of pain, but it increasingly is being shown to be an effective therapy for pain. Physical activities are routinely used by athletic trainers and physical therapists to assist in treating a wide variety of musculoskeletal ailments. Randomized controlled trial experiments show that sedentary medical patients with pain-related conditions, such as low back pain (Liddle, Baxter, and Gracey, 2004; Van Tulder, Malmivaara, Esmail and Koes, 2000), fibromyalgia (Sim and Adams, 2002), and osteoarthritis (Koltyn, 2002), report lower pain intensity after chronic treatments that involved an increase in physical activity.

There is a need to better understand the painful aspects of exercise and sports as well as the mechanisms by which acute and chronic physical activity either exacerbate or attenuate pain. This paper will focus on one tool of potential use in this regard – the nociceptive flexion reflex.

Overview of the nociception flexion reflex

Sir Charles Sherrington coined the phrase the 'flexion-reflex of the limb' to describe the withdrawal reflex in frogs, cats and dogs associated with an electrical stimulus of the sural nerve in the foot of the limb (Sherrington, 1910). Flexor reflexes in response to non-noxious stimuli provide proprioceptive and cutaneous information to the brain via Type I and Type II large diameter low threshold mechanoreceptor afferents. This information is important for motor control and posture maintenance.

Flexor reflexes in response to noxious stimuli are called nociceptive flexion reflexes (NFR). Although the NFR can be obtained in both the arms and legs, most human laboratory studies of NFR have used electrical stimulation near the ankle to excite sural nerve nociceptive afferents. The reflex is quantified using electromyographic recordings of the first hamstring (leg flexor) muscle that responds to sural nerve stimulation – the ipsilateral biceps femoris capitis brevis (Hugon, 1973). The NFR is a polysynaptic reflex that withdraws the limb from a noxious stimulus. The reflex is spinally mediated since it can be produced without inputs to or from the brain after complete spinal transection (Hugon, 1973). Under normal circumstances, the NFR is under tonic supraspinal inhibition (Sandrini, Arrigo, Bono, and Nappi, 1993; Willer, Boureau, and Albe-Fessard, 1979).

The NFR has three components - a tactile, a nociceptive, and a movement response. The tactile response, also known as the R-II reflex, has a short latency of approximately 40-70 ms after the onset of the electrical stimulation (Hugon, 1973; Willer, 1977; Skljarevski and Ramadan, 2002). The short latency of the tactile response is the result of it being transmitted by activation of low threshold large diameter Type II afferents which are faster than afferent fibers that transmit nociceptive information. The electrical current required to elicit the R-II reflex is ~5 mA when commonly used preparation techniques and stimulus parameters are employed. Using the same methods, the minimum electrical current required to elicit the nociceptive response is ~10 mA. The nociceptive response is also known as the R-III reflex because small diameter sensory afferents (Type III afferents), many of which respond solely to noxious stimuli, must be activated to elicit the reflex (Kugelberg, Eklund, and Grimby, 1960; Wiesenfeld-Hallin, Hallin, and Persson, 1984). The time window for the latency of the nociceptive response is consistent with the known speed of Type III afferents (~85 to ~150 msec). Consistent with the Gate Control

theory of pain (Melzack and Wall, 1965), there is an interaction between the tactile and nociceptive responses. An ischemic block of Type II afferents decreases the R-III threshold and increases both the R-III amplitude and pain intensity ratings (Willer, 1983). Thus, afferent tactile information inhibits the R-III reflex. The third component of the NFR is the limb movement response. This response has a latency of ~250 to 300 msec, but is typically not a dependent measure of interest in pain studies.

Pain researchers have been interested primarily in two dependent measures that can be obtained during NFR testing – the threshold and the amplitude. The most commonly reported NFR measure is the R-III reflex threshold. The threshold is obtained by determining the smallest magnitude of the electrical stimulus required to elicit a nociceptive response. Early studies showed a strong association between reported pain threshold and the R-III reflex threshold (Willer, 1977). The perception at R-III reflex threshold is a localized pain that can be described as like a pinprick or akin to a rubber band snapping on the skin.

A second measure that can be obtained is the amplitude of the EMG response following sural nerve electrical stimulation intensities at or above the R-III reflex threshold. A strong linear relationship between tolerable electrical stimulation intensities above R-III threshold (e.g., from ~10 to 15 mA) and both the amplitude of the EMG response and pain intensity ratings have been reported (Chan and Dalliare, 1989; Willer, 1977). The strong association between pain intensity ratings and the R-III reflex has led some authors to conclude that under most conditions the R-III reflex can be considered an objective measure of pain. It is important to recognize that there are circumstances in which the R-III reflex and pain ratings become uncoupled (Boureau, Luu, and Doubree, 1991; Garcia Larrea, Charles, Sindou, and Mauguiere, 1993; Leroux, Belanger, and Boucher, 1995; Anderson, Jensen, Brennum, and Arendt-Nielsen, 1995). When such dissociation

occurs the R-III reflex still provides an objective measure of a portion of the nociceptive system. It has been suggested that the cause of the dissociation may be supraspinal modulation of pain independent of spinal inputs influencing the R-III. Alternatively, the dissociation could be related to differences in the methodology by which the NFR is elicited (Skljarevski and Ramadan 2002). Several different stimulus and recording criteria have been used in association with the NFR (see Table 1). Research is warranted on whether there is a best method for eliciting the NFR.

Selected methodological issues

Those readers interesting in obtaining NFR data in their laboratory potentially may save "trial and error" time by reviewing this section and reading information on NFR methodology at a publicly available website (France, 2002). Those readers uninterested in these details can skip this section.

Stimulating electrodes. Two stimulating electrodes are placed just below and posterior to the lateral maleolus in the retromaleolar pathway of the ankle (Figure 2.1) after the surface of the skin is abraded and degreased in order to reduce bioelectrical impedance. We have found that stimulating electrode impedances of $\leq 5k\Omega$ yield significantly better NFR data than with stimulating electrode impedances between $5k\Omega$ and $10k\Omega$. Sand paper (80 grit) about the size of the tip of an index finger works well for abrading the thick skin of the foot/ankle. Alcohol swabs containing pumice, degrease the area and aid in achieving low impedance. As the skin resistance increases above $10k\Omega$ there is an exponential increase in stimulus needed to produce the NFR.

Ground electrode. A ground electrode (e.g., single silver/silver-chloride electrode, or wet Velcro strap) is placed on the lateral portion of the middle of the lower leg (halfway between the bottom of the foot and the top of the kneecap; Figure 2.1).

Recording electrodes. The NFR electromyographic responses are recorded via surface electrodes attached approximately 2 cm apart to shaved, abraded and degreased skin over the ipsilateral biceps femoris capitis brevis (i.e., short head of the biceps femoris; Figure 2.1). It can be difficult to locate this muscle due to various factors, including subcutaneous fat. One method for finding the short head of the biceps femoris is to have the test subject assume a prone position and flex their leg against ankle/foot resistance applied by the investigator (Figure 2.2). We have found that 220-grit (very fine) sandpaper is adequate for the light abrasion needed, and regular alcohol swabs are sufficient for degreasing the skin in this area. Recording electrode impedances of ≤ 10 kΩ are adequate for good quality NFR records. The EMG signal from the short head of the biceps femoris is amplified and full wave rectified when analyzed.

Stimulus. Variations in the characteristics of the transcutaneous electrical stimulus needed to elicit the R-III reflex have been reported. Typically a square or rectangular waveform is used. Trains of 5 to 10 pulses are commonly employed at a frequency of 100 to 300 cycles per second. Stimulus duration is often 1.0 millisecond and the constant current applied ranges from ~10 to ~40 mA. The size of the stimulating electrodes influences the pain intensity, and therefore needs to be reported. Smaller electrodes are associated with higher pain ratings (Alon, Kantor, and Ho, 1994). Whether an R-III reflex is elicited depends in part on interactions among the stimulus characteristics. For example, the current required to elicit the R-III using a single 0.5 msec stimulus at 100 Hz is about half that required when using an otherwise identical but shorter duration 0.1 msec stimulus. Perhaps the most commonly reported stimulus characteristics are as follows: a square wave using trains of 5 pulses at a frequency of 300 Hz with a duration of 1 msec. A current of ~10 mA will be required to elicit the R-III using this type of stimulus. When

these parameters are employed a current of ~40 mA has been suggested as the maximal stimulus that ethically can be delivered (France and Suchowieck, 1999).

During repeated presentations of an electrical stimulus to elicit multiple R-III reflexes the interstimulus interval is important. Presumably, long interstimulus intervals of greater than 5 seconds permit high reliability across trials for both threshold and amplitude measures. The length of the interstimulus interval should be varied randomly with successive stimulations because there is evidence that attending to the timing of the stimuli presented can influence the reflex. Published data addressing trial-to-trial and day-to-day reliability are scant. Type III fibers are involved in a single shock stimulus, but Hugon (1973) noted that with repeated stimuli without adequate recovery time, the larger diameter type I and II fibers contribute to eliciting the nociceptive reflex. More recently, pain researchers have been interested in better understanding temporal summation because of its role in central sensitization. Central sensitization occurs when the activation of nociceptive afferents changes the response properties of dorsal horn neurons and these changes result in an exacerbation of pain. The R-III reflex has been used fruitfully in association with repeated electrical stimulations and very short interstimulus intervals (e.g., 10-20 Hz) in order to study temporal summation (Arendt-Nielsen, Sonnenborg, and Andersen, 2000).

Brief overview of the nociceptive system

In thinking about the meaning of the R-III reflex and how it might be employed in exercise research, it is useful to appreciate the nociceptive system that transmits information about noxious stimuli throughout the body. Here we provide a very brief overview. Comprehensive treatments are available elsewhere (Wall and Melzack, 1999).

It was mentioned previously that the R-III reflex requires activation of Type III afferents. These afferents synapse primarily in laminae I, II and V of the dorsal horn of the spinal cord. An extensive amount of excitatory or inhibitory signal processing can occur within the spinal cord. The role of non-nociceptive (Type II) afferents in inhibiting the R-III reflex was mentioned previously. Also, there are brainstem nuclei that influence interneurons projecting to the alpha motorneurons, and these include the paralemniscal nucleus, locus coeruleus, subcoeruleus nucleus, red nucleus, and the paraventricular hypothalamic nucleus (Rotto-Percelay, Wheeler, Osorio, Platt, and Loewy, 1992).

Ultimately, the nociceptive signal ascends to the brain via several nerve tracts, including the spinothalamic, spinoreticular, and the spinomesencephalic tracts. These tracts project to numerous brain areas including the medial brainstem reticular formation, locus coeruleus, periaqueductal gray, hypothalamus, lateral and medial thalamus, anterior cingulate cortex and the primary and secondary somatosensory cortex.

Neurons descending to the spinal cord from the brain tonically inhibit the R-III reflex. There is a complex descending pain modulation system involving endogenous opioids that is known to both directly and indirectly inhibit both ascending nociception and pain. Brain areas that have been implicated in this system include the anterior cingulate cortex, the hypothalamus, the amygdala, the periaqueductal gray, the rostral ventromedial medulla, and the dorsolateral pontine tegmentum (Basbaum and Fields, 1978; Basbaum and Fields, 1984; Fields and Basbaum, 1999).

Factors that modify the R-III reflex threshold

Experiments have identified a host of factors that can modify the R-III reflex threshold. Many of the observed changes in R-III threshold likely are the result of increased or decreased

tonic supraspinal inhibition. When the R-III reflex threshold increases it means that a higher amount of constant electrical current is needed to elicit the reflex. One plausible explanation for a greater need of electrical current is that the magnitude of tonic supraspinal inhibition has increased.

Over 25 investigations have shown that analgesic drugs inhibit the R-III reflex threshold (Skljarevski and Ramadan, 2002). For example, morphine has dose dependent effects on increasing the R-III reflex threshold (Willer, Bergeret, and Gaudy, 1985; Willer and Le Bars, 1995; Blond, Guieu, Meynadier, Le Bars, and Willer, 1994). Also, non-steroidal anti-inflammatory drugs, such as of inodmethacin (Guieu, Blin, Pouget, and Serratrice, 1992b), ketoprofen (Willer and Harrewyn, 1987), ibuprofen (Sandrini et al., 1992) and nimesulide (Sandrini, Cecchini, Alfonsi, and Nappi, 2001) result in an increase in the R-III reflex threshold.

Non-pharmacological experimental manipulations also result in an increased R-III reflex threshold. The R-III reflex threshold is increased during hypnotic suggestion of analgesia (Kiernan, Dane, Phillips, and Price, 1995), slow wave and REM sleep (Sandrini, Manilov et al., 2001), gastric distention (Bouhassira et al., 1994), and slow rectal distension (Bouhassira et al., 1998). A recent study showed no effect of a mental task (30 s of serial addition) on the R-III reflex threshold (Terkelsen, Andersen, Molgaard, Hansen, and Jensen, 2004), and methodological differences may account for the lack of consistent findings with Willer et al., 1979 which found that the performance of a cognitive task (30 s of serial subtraction) was associated with an increase in reflex threshold. The R-III reflex threshold is higher in older adults compared to children (Sandrini et al., 1989) and increases late in the evening compared to the morning suggesting a circadian rhythm in the R-III reflex threshold (Sandrini, Alfonsi, Bono et al., 1986).

Other experimental manipulations have found a decreased R-III reflex threshold, possibly reflecting a decrease in tonic supraspinal inhibition. The R-III reflex threshold is decreased after receiving an intense (70 mA) sural nerve stimulation (Willer et al., 1979), during anticipation of an intense noxious stimulus (Willer et al., 1979), during fast rectal distention (Bouhassira et al., 1998), and during the luteal compared to the follicular phase of the menstrual cycle (Tassorelli et al., 2002). Women have decreased R-III reflex threshold compared to men (Danilov et al., 1994; Page and France, 1997; France and Suchowiecki, 1999) and obese women have decreased R-III reflex threshold compared to non-obese (Pradalier, Willer, and Boureau, 1980).

Sport and exercise research that has used the R-III reflex

Only a handful of investigators have employed the NFR in the context of physical activity, movement, exercise, or sport studies. One experiment examined the influence of a single bout of exercise on the R-III reflex (Guieu, Blin, Pouget, and Serratrice, 1992a). A small group (n=6) of young (age 18-27) high level athletes (regularly participated in national or international sporting events) were tested before and immediately after a 20 minute bout of cycle ergometry. Each participant cycled at an absolute power output of 200 watts. Heart rate increased from an average of 55 beats·min^{-1} at rest to 109 beats·min^{-1} at the end of the exercise period. A large increase (63%, effect size d=1.6) in electrical current (mean ±SD) was needed to elicit the R-III reflex after exercise (28.5±16 mA) compared to before exercise (17.5±6.8 mA). The post-exercise increase in R-III reflex threshold was observed for all six athletes, though there were substantial individual differences. The percentage increase after exercise for the six athletes was 14%, 14%, 42%, 64%, 73% and 113%. The relative exercise intensity for each participant was not reported so it is unknown whether the individual differences in R-III reflex threshold responses to exercise were related to the relative effort required due to differences in physical

fitness among the athletes. Another limitation of the investigation was that a no-exercise, test-retest control condition was not employed. Thus, if one wishes to attribute the increase in R-III reflex threshold to the 20 minute exercise bout it must be assumed that the test-retest reliability of the measure is high. The finding of an increased R-III reflex threshold generally is consistent with prior research documenting post-exercise hypoalgesia (cf., O'Connor and Cook, 1999). These R-III reflex threshold findings are worthy of independent replication since no other investigators have yet addressed this question using the R-III reflex methodology.

This same investigation did test a small control group (n=8) once under resting conditions at the same time of day the athletes were tested (Guieu et al., 1992a). The control group was of the same mean age but had a smaller percentage of women (13% vs 33%). The mean R-III reflex threshold for the control group at rest (9.93±2.57 mA) generally was consistent with what has been reported at rest by other investigators (~10 mA). The resting, pre-exercise R-III reflex threshold for the athlete group (17. 5±6.8 mA) was substantially higher compared to the controls (63% higher, effect size d~1.5). The size of this effect may be underestimated due to the smaller percentage of women in the control group. This observation also merits replication, especially since it is inconsistent with more than a half a dozen studies reporting no difference between groups of competitive athletes and non-athletes on self reported pain threshold.

Two studies have used exercise as a painful "conditioning" stimulus in order to learn whether the presence of the conditioning stimulus attenuates the R-III reflex observed during the presentation of a concomitant noxious stimulus to a different body location (France and Suchowiecki, 1999; Willer, Roby, and Le Bars, 1984). This phenomenon is known as diffuse noxious inhibitory controls (DNIC). In the larger of the two studies, 83 participants (44 women and 39 men) gripped a hand dynamometer every second for 2 minutes producing force equal to

50% of the maximum with each contraction (France and Suchowiecki, 1999). Blood flow to the forearm muscles was then occluded for 5 minutes and during this time pain intensity ratings increased from approximately 4 to 6 on a 1 to 9 scale. R-III reflex data from sural nerve stimulation were obtained before, during and immediately after the 5 minute forearm ischemia condition. The amplitude of the R-III reflex response was reduced during and after the ischemia condition compared to baseline for both the females and the males. These findings suggest that painful muscular activity in one body location may cause a transient reduction in pain experienced in other body locations.

There are a few studies that have examined the effects of physical movement on the R-III reflex. For example, changes in the R-III reflex in association with active and passive ankle movements have been investigated (Guieu and Serratrice, 1992). These experiments included the use of both vibration to increase type I afferent activity as well as skin anesthesia to eliminate skin mechanoreceptor activity. R-III reflex amplitude and pain ratings decreased in both active and passive conditions with the active condition showing the largest decrease. Vibration increased R-III amplitude and pain ratings. Skin anesthesia decreased the amplitude of the reflex during the active condition. The authors concluded that movement induced pain alleviation is associated with the activation of large cutaneous mechanoreceptors but not type Ia fibers.

Other examples of movement-related research that has involved assessments of the R-III reflex include studies of cycling movements (Anderson et al., 1995), gait (Belanger and Patla, 1987; Crenna and Frigo, 1984), and locomotion in cats and humans (Lisin, Frankenstein, and Rechtmann, 1973).

Potential use of the NFR in exercise research

The NFR has not been widely used by researchers interested relationships between pain and physical activity. It is a tool that could be used fruitfully to better understand a host of pain and exercise questions.

Chest pain. Approximately 6 million Americans will experience angina this year and this type chest pain is the primary reason that people seek medical help for heart disease. A significant number of people have myocardial ischemia without chest pain and this is known as silent ischemia. Precisely how many people have silent ischemia is difficult to quantify, but it has been estimated that it occurs in greater than one in five asymptomatic patients with type 2 diabetes (Wackers et al., 2004).

Silent ischemia is extremely dangerous. A heart attack might be caused by the completion of too intense physical activity that would have been moderated in the presence of angina. The NFR could possibly be used as a predictor of individuals at increased risk for silent ischemia during exercise. Related research hints at this potential. Individuals at increased risk for hypertension due to a family history of hypertension show a higher NFR threshold than those with a normotensive parental history (France and Suchowieki, 2001; France, Froese, and Stewart, 2002; Page and France, 1997). More research is warranted concerning the use of the NFR in understanding nociception and pain processing among patients at risk for silent heart attacks during exercise.

Exercise training to treat pain and related disorders. There is a body of scientific research showing that sedentary people with a painful medical condition report that the intensity of pain is reduced following the adoption of an exercise program. Reviews summarizing this literature have been published for low back pain (Liddle et al., 2004; Van Tulder et al., 2000),

fibromyalgia (Sim and Adams, 2002), osteoarthritis (Koltyn, 2002), and peripheral artery disease (Gardner and Poehlman, 1995). It is of interest to better understand the biological mechanisms that underlie these therapeutic effects of exercise training. The NFR could provide some information about whether reductions in pain ratings were associated with an adaptation in a portion of the nervous system known to be involved in the processing of nociceptive information. Data of this nature would provide supportive evidence that the current findings using pain ratings alone are not simply due to a response bias in answering the pain questionnaires.

Pain symptoms are not uncommon among patients with mood disorders such as depression and anxiety, and people with chronic pain often become anxious and depressed. Several pharmacological agents used to treat anxiety and depression, including amitriptyline, desipramine, fluvoxamine, moclobemide, ritanserin, and dothiepin have been found to inhibit the NFR (Amelin et al., 1998; Coquoz, Porchet, and Dayer, 1993; Sandrini, Alfonsi, De Rysky et al. 1986; Sandrini et al., 1993). Accordingly, studies concerning the influence of chronic physical activity on anxiety and depression may benefit from learning whether nociceptive processing, as indexed by the NFR, is altered in association with exercise training among patients with a mood disorder.

Delayed onset muscle pain. There is clear evidence that eccentric exercise induces delayed-onset muscle pain and that the pain ratings are highly variable between individuals. The bulk of the research on eccentric exercise has focused on either describing the time course of pain responses or determining the biological mechanisms that account for the associated loss of muscle strength. These studies rarely have examined the influence of eccentric exercise on nociceptive neurology, but such studies are needed to better understand the mechanisms for the

delayed onset muscle pain that results from eccentric exercise. More research is needed on determining the causes of the large individual differences in muscle pain. Relevant neurology, such as that assessed by the NFR, is a logical place to start. Treatments for delayed onset muscle pain have been largely unsuccessful, and limitations regarding self reported pain ratings may have contributed the negative findings. The use of the NFR could give added insight into the effectiveness of treatments for delayed onset muscle pain.

Biological mechanisms. The R-III reflex can be used in association with other psychophysiological or brain imaging tools in order to explore pain and nociception mechanistically (cf., Peyron et al., 2001). For example, in one study of 15 healthy volunteers both the R-III reflex and the Hofmann reflex were used during hypnotic analgesia (Kiernan et al., 1995). The H-reflex is a measure of the efficacy of synaptic transmission between the Ia afferent and the alpha motor neuron (Capaday, 1997). During hypnosis pain intensity was reduced by an average of ~30%, the R-III reflex was reduced by ~20% and the H-reflex, which does not index the nociceptive system, was not changed significantly. Hypnotic sensory analgesia was related to the decrease in R-III after statistically controlling for changes in the H-reflex (R^2=.51). These findings led the authors to conclude that "…hypnotic sensory analgesia is at least in part mediated by descending antinociceptive mechanisms that exert control at spinal levels in response to hypnotic suggestions."

Conclusions

In summary, the R-III reflex provides an objective measure of a portion of the nociception system. There is a body of research indicating the usefulness of the R-III reflex in pain related research. Several investigations found a strong relationship between subjective pain ratings and the R-III reflex response. This reflex has not yet been used much in physical activity

science research. However, the R-III reflex has the potential to help address several questions important to physical activity scientists.

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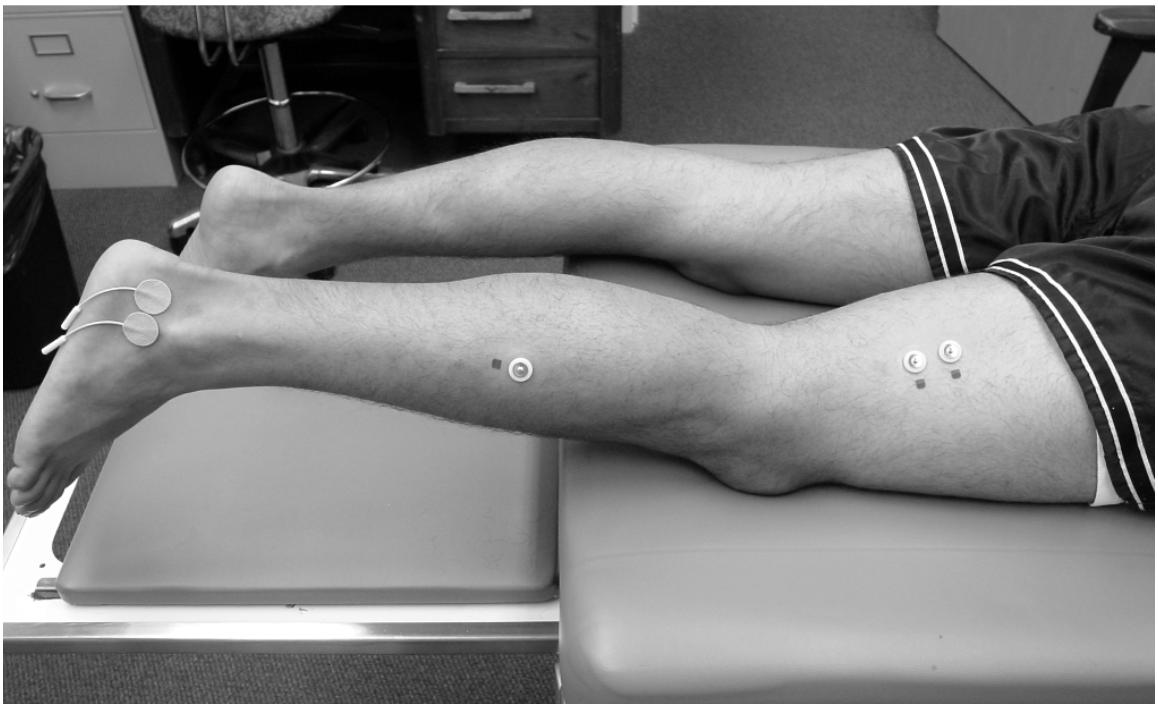

Figure 2.1: Example for placement of stimulating electrodes, ground electrode and recording electrodes (left to right).

Figure 2.2: Demonstration of the method for finding the short head of the biceps femoris muscle.

Table 2.1

Stimulus and recording criteria for the R-III reflex

Stimulus criteria for R-III reflex threshold	EMG Recording Latency	Source
3 times stimulus intensity needed for tactile sensation	85-120 msec	Hugon, 1973
lowest stimulus intensity causing a reflex on 60-70% of trials	90-130 msec	Willer, 1977
lowest stimulus intensity causing a reflex on 80-90% of trials	90-130 msec[1]	Sandrini et al., 1986
lowest stimulus intensity causing a reflex on 100% of trials	50-200 msec	Guieu et al., 1992
stimulus causing EMG to be > 1.5 SD above a baseline EMG	91-150 msec	France et al., 2002

Note: 1 = parameters were not given, however the authors referenced procedures used in Willer, 1977.

CHAPTER 3

HIGH DAY-TO-DAY RELIABILITY IN LOWER LEG VOLUME MEASURED BY WATER DISPLACEMENT: A USEFUL TOOL FOR STUDIES OF ECCENTRIC EXERCISE[2]

[2]Pasley, J.D. and P.J. O'Connor. To be submitted to *American Journal of Human Biology*

Abstract

Various methods have been used to measure change in muscle edema for several days following eccentric exercise but the day-to-day reliability of such measures in the absence of muscle injury are poorly documented. This investigation determined the day-to-day reliability of lower leg volume (soleus and gastrocnemius) measured using water displacement. Thirty young adults (15 men and 15 women) had their right lower leg volume measured by water displacement on 5 days. The participants performed normal activities of daily living and were measured at the same time of day after being seated for 30 minutes. The results revealed a high day-to-day reliability for lower leg volume. The mean percentage change in lower leg volume across days compared to day 1 ranged between 0% and 0.37%. The mean within subjects coefficient of variation in lower leg volume was 0.72% and the coefficient of variation for the entire sample across days ranged from 5.66% to 6.32%. A two way mixed model intraclass correlation (30 subjects x 5 days) showed that the lower leg volume measurement was highly reliable (ICC = .972). Foot and total lower leg volumes showed similarly high reliability. Water displacement offers a cost effective and reliable solution for the measurement of lower leg edema across days.

KEY WORDS: edema, ICC, gastrocnemius, inflammation, muscle, injury, soleus

Introduction

Muscle injury results in localized edema. Laboratory investigations of muscle injury, for example those that involve the performance of intense eccentric exercise, frequently are interested in quantifying the extent of local edema and relating it to physiological, functional or perceptual changes such as the amount of muscle fiber damage, the degree of strength loss or the intensity of pain.

There are several methods for assessing localized skeletal muscle edema including the measurement of limb circumferences (Thompson, Hyatt, De Souza, and Clarkson, 1997), limb volume (Paddon-Jones, and Quigley, 1997), bioelectrical impedance (Willoughby, Vanenk, and Taylor, 2003), computer modeling (Bednarczyk, Hershler, and Cooper, 1992), ultrasound (Nosaka and Newton, 2002), and the spin-spin relaxation time from T2-weighted magnetic resonance skeletal muscle images (Babul, Rhodes, Taunton, and Lepawsky, 2003). Each method has advantages and disadvantages.

The most frequently used method for assessing muscle edema after eccentric exercise has been limb circumference. Limb circumference measures made with a spring loaded tape measure are thought to be more reliable than those made with a conventional tape measure (Bednarcyzk et al., 1992; Greenhill, 1979). Nevertheless, most eccentric exercise studies have estimated edema by assessing limb circumference at only one limb site using a conventional tape measure. Since eccentric exercise can induce local edema at many sites within a limb, the use of a single site is a limitation which might underestimate the extent of edema. For example, when comparing 1,500 measurements of edema by tape measure to water displacement assessments in

lymphoedma patients, the tape measure method resulted in ~50% less edema compared to the water displacement method (Casley-Smith, 1994).

Magnetic resonance imaging (MRI) of skeletal muscle is a technique that is increasingly being used in studies of muscle injury. Increased blood flow and a shift of ions and water from intracellular to extracellular compartments in muscle have been proposed as contributing factors in MRI T2 changes after eccentric exercise (Nosaka and Clarkson, 1996). It is unlikely that these changes are due solely to local edema because there is evidence that it can take up to 75 days for the images to return to baseline (Shellock, Fukunaga, Mink, and Edgerton, 1991). Not all research facilities have easy access to MRI and the costs of acquiring MRI images are not trivial.

Only a few investigators have examined the influence of eccentric exercise on limb volume measured using water displacement (volumetry). One group reported a significant increase in leg volume of ~2% after eccentric exercise (Whitehead, Allen, Morgan, and Proske, 1998; Whitehead, Weerakkody, Gregory, Morgan, and Proske, 2001). Other investigators found no statistically significant change in arm volume from 24 to 144 hours post eccentric exercise of the biceps brachii (Paddon-Jones and Quigley, 1997). Limitations of both studies include that uninjured tissue was included in the analysis (e.g., the hand and the forearm with biceps brachii injury) and the statistical power was not reported. Statistical power likely was less than optimal due to small sample sizes of 8-10 subjects. The day-to-day reliability of the limb volume measure also was not quantified using accepted statistical methods. Thus, it is unclear whether the lack of consistent findings is due to measurement error associated with day-to-day variability in limb volume resulting from normal activities of daily living.

Other investigations have shown that the measurement of hand and foot edema using volumetry is highly reliable (Eccles, 1956; Engler and Sweat, 1962). For example, Moholkar and

Fenelon (2001) examined diurnal variation in edema of the feet and ankles by taking four water displacement measurements across a 9-hour period in 20 adults restricted to bed rest. Results revealed very high alpha reliabilities of r = .99 for both the right and left legs.

There appears to be no published data about the day-to-day reliability of lower leg edema among free living people. In order to determine the effect of injurious eccentric exercise on day-to-day changes in lower leg edema using water displacement there is a need to document the day-to-day reliability of the measure in free living subjects who are injury free. Thus, the primary purpose of this study was to examine the day-to-day reliability of lower leg volume (soleus and gastrocnemius). Foot volume reliability also is reported because it potentially could be used as control data in muscle injury studies and it is easily assessed when lower leg volume is measured. Total lower leg volume (lower leg plus foot) reliability also is reported in order to make comparisons to the most relevant prior research (Whitead et al. 1998; Paddon-Jones and Quigley, 1997).

Methods and Materials

Participants were recruited from Exercise Science courses at the University of Georgia. Prior to being involved in the study, each person read and signed an informed consent that summarized the procedures. The consent form was approved by the Institutional Review Board at the University of Georgia. Complete data were obtained from 15 women and 15 men. The women had a mean age of 20.7 years (SD = 3.4) and corresponding value for the men was 22.5 years (SD = 2.9). Average height of the females was 165.0 cm (SD = 4.7) and their average weight was 60.5 kg (SD = 6.3). Males were 180.2 cm (SD = 3.5) in height and had an average weight of 78.6 kg (SD = 8.3). The participants completed testing on five days with test sessions

at least 24 hours and not more than 48 hours apart. Participants were asked not to perform any strenuous exercise within two hours of each testing session. The participants sat in a chair for ~30 minutes prior to the measurement of lower leg volume during which time other measurements were made as described elsewhere (Pasley and O'Connor 2007; Pasley and O'Connor, in preparation).

Measurement of lower leg volume

Semi-permanent ink was used to make one mark on the skin near the knee (at the lateral portion of the head of the right fibula) and a second mark near the ankle (4 cm above the bottom of the lateral maleolus). Participants were asked to not wash off the marks so the same markings could be used each day.

A Lucite partial leg volumeter was used for all measurements (Volumeters Unlimited, Phoenix, AZ). Tepid water was used for all measures. Temperature was not controlled because water temperatures between 20ºC and 32ºC have small effects on volume, especially when the duration of the measurements is kept to a minimum (Boland and Adams, 1996; King, 1993). Volume measurements required < 5 minutes. Twenty separate measurements were taken in which water temperature was measured to determine the stability of the water temperature using a standard procedure. Water temperature was stable (34.3, 0.6) having a range of 34-36° C. The volumeter had a height, width and length of 61 cm, 15.5 cm and 33 cm. Participants with differing leg lengths were fit to the height of the volumeter using Lucite platforms with heights of 4.5 cm and 7 cm placed in the bottom of the volumeter as needed.

The volumeter was filled with approximately 10 gallons water until it overflowed into a pitcher placed under the spout. To reduce surface tension and enhance the consistency of the

measure, a Lucite obturator with the dimensions 7.5 cm (height) x 14 cm (width) x 17.5 cm (length) was inserted until the water stopped dripping from the spout. The pitcher was then emptied, dried and placed back under the spout of the volumeter.

Foot volume was measured next. Participants sat in a chair and inserted their right foot slowly into the volumeter up to the ink mark just above the ankle while their heel touched the back of the volumeter. Care was taken so that the leg was perpendicular to the floor and not leaning to either side. Assistance was given by the researcher so that the foot did not go deeper than the ink mark. The plastic obturator was re-inserted into the volumeter to reduce water surface tension and kept in place until the water stopped dripping from the spout (see Figure 3.1). The weight of the water was measured to the nearest 0.5 gram using a calibrated Mettler P3 3000/1g mechanical balance scale.

Lower leg volume was measured next. A dry pitcher was placed under the spout. Participants then slowly inserted their lower leg into the water until the ball of the foot reached the bottom and the heel was touching the back of the volumeter. The leg was submerged to just below the knee (see Figure 3.2). Participants were not allowed to place their foot flat against the bottom of the volumeter to avoid variations in foot pressure that might cause a difference in the amount of water displaced. The weight of the water displaced was recorded.

All displaced water weights were converted to volume using a standard equation (i.e., the density of water is 1g = 1 ml). The indentical procedure was conducted at the same time of day within each subject for all five testing sessions. The measures from the foot and the lower limb were combined to create the total lower leg volume variable.

A positive control procedure was completed to determine the accuracy of the measurement procedure. Twenty trials were completed in which a 120g weight was added to the

leg volumeter and the water displaced by the weight was then weighed. The mean weight amounted to 119.8g (0.41), indicating high stability.

Data Analysis

Data analysis was performed using SPSS 13.0. Reliability was assessed in several ways including examining means and standard deviations as well as calculating coefficients of variation (standard deviation/mean x 100%), Pearson correlations, and intraclass correlations (ICC 3, 5) (Atkinson and Nevill, 1998). The ICC model used was a two way (30 Subjects and 5 Days) mixed effects model (Subjects were considered random and Days fixed effects). The ICC model also used absolute agreement as the type of agreement and the single measures option (multiple measures of leg volume were not obtained each day). Three mixed model (2 sex x 5 day) ANOVAs were performed to assess sex-related differences in day-to-day variations in foot, lower leg and total leg volume based on the univariate F-statistic. Effect sizes were expressed as eta-squared (η^2). The Greenhouse-Geisser epsilon (ε) was used when the sphericity assumption was violated.

Results

The consistency in the mean and variability of lower leg volume across days is illustrated in Figure 3.3. Lower leg volume did not vary across days ($F_{4,120} = .81$; $p = .52$) and the associated ICC equaled .972. The coefficient of variation for each day ranged from 5.66% to 6.32%. The within subjects coefficient of variation averaged .72% (SD = .67) and ranged from .09% to 2.87%. The Pearson correlations between days (i.e., Days 1 & 2, Days 2 & 3; Days 3 &

4; Days 4 & 5) ranged between .95 and .99. The raw data for lower leg volume are reported in Table 3.1.

The consistency in mean and variability of foot volume across days is illustrated in Figure 3.4. Foot volume did not vary across days ($F_{4,120} = .63$; $p = .64$) and the corresponding ICC equaled .971. The mean percentage change across days compared to day 1 ranged between .2% and 0.65%. The coefficient of variation for each day ranged from 18.87% to 19.92%. The within subjects coefficient of variation averaged 3.01% (SD = 1.75) and ranged from .97% to 10.01%. The Pearson correlations between days ranged between .95 and .98. The raw data for lower leg volume are reported in Table 3.2.

The consistency in mean and variability of total lower leg volume across days is illustrated in Figure 3.5. Total lower leg volume did not vary across days ($F_{4,120} = 1.18$; $p = .33$) and the corresponding ICC equaled .982. The mean percentage change across days compared to day 1 ranged between .04% and 0.44%. The between days coefficient of variation ranged from 7.21% to 7.80%. The within subjects coefficient of variation averaged .86% (SD = .53) and ranged from .10% to 2.28%. The Pearson correlations between days ranged between .98 and .99. The raw data for total lower leg volume are reported in Table 3.3.

Separate repeated measures ANOVAs (Sex x Days) were performed to detect sex differences. There was a significant difference between sexes for foot ($F_{1,28} = 77.96$; $p < .001$; $\eta^2 = .736$) and total lower leg volumes ($F_{1,28} = 22.05$; $p < .001$; $\eta^2 = .441$), but there was no significant sex difference for lower leg volume ($F_{1,28} = 2.15$; $p = .15$; $\eta^2 = .071$). All sex x day interactions were not statistically significant.

Discussion

The primary novel finding of this investigation is that there is little day-to-day variability in lower leg volume using water displacement in free living young adult men and women.

The use of water displacement for the measurement of volume dates back to before 211 BC during the time of Archimedes, however, there are no prior studies in the literature reporting day-to-day reliability of lower leg volume. Research that appears to be most related to the present work showed less than 1% change from day-to-day in hand volume (Engler and Sweat, 1962). The water displacement method described here for assessing the lower leg was even more precise than the prior work on hand volume. Day-to-day changes in lower leg volume were less than 0.38% (Table 3.1). The very high day-to-day reliability means that small changes in lower leg edema induced experimentally can be detected with the water displacement method decribed here without testing large samples. Prior studies of eccentric biased exercise performed by untrained individuals found that total lower leg volume increase by an average of ~ 2.0% several days after eccentric exercise (Whithead et al. 1998; Whithead et al. 2001).

The present investigation was undertaken in preparation for subsequent studies of lower leg edema following eccentric exercise induced muscle injury. We are aware of only three studies that have used water displacement to assess edema following eccentric exercise. The findings of these studies were mixed. In one experiment, eight individuals with weight training experience performed 64 eccentric muscle actions (110% of concentric max) of their biceps brachii (Paddon-Jones and Quigley, 1997). No significant change in arm volume was found. Plausible reasons for this negative finding include a lack of muscle damage due to the prior weight lifting history of the participants, low statistical power, or the use of an unreliable water displacement technique. The two other investigations of this type reported on data from five

(Whithead et al. 1998) and seven (Whithead et al. 2001) untrained participants who performed 1800-2100 eccentric muscle actions by walking backwards on a treadmill at a speed of 3.5 $km \cdot hr^{-1}$ and an incline of 13 degrees. Both studies found a significant increase in leg volume after exercise of about 2%. No statistical information was provided about day-to-day reliability in these reports and not enough information was provided to precisely replicate the water displacement methods. The present study improves on these prior investigations by providing adequate reliability data and enough information for others to replicate the water displacement technique used.

There is little empirical evidence about the advantages and disadvantages of different techniques for assessing edema in eccentric exercise studies. Although several investigations involving eccentric exercise have obtained information about edema using different methods (Babul et al., 2003; Nosaka and Newton 2002; Nosaka, Newton, and Sacco, 2002), few studies have compared the reliability, validity and usefulness of alternative methods. It is clear that water displacement is more accurate than tape measure circumference for the measurement of surface volume when one measure of circumference is used. The accuracy of circumference estimates of edema increases and becomes more similar to that of volumetry when a series of circumference assessments are employed in conjunction with mathematical equations that model limb volume (Kaulesar Sukul, den Hoed, Johannes, van Dolder, and Brenda, 1993). By using more measurements, however, there is an increase in the amount of time it takes to assess edema and thereby eliminates one of the primary advantages gained with the circumference measurement.

One disadvantage of using the water displacement technique for measuring day-to-day change after eccentric exercise is that the specific portion(s) of the limb which changes is unknown. Changes in muscle volume may have a different percent change as compared to

volume of other areas in the affected limb. For example, MRI results show that there are regional variations in value as much as ~14% after eccentric exercise of the biceps brachii (Foley, Jayaraman, Prior, Pivarnik, and Meyer, 1999). Changes in muscle volume may be greater when assessed by MRI because of greater ability to detect regional changes (Lund, Christensen, Savnik, Boesen, Danneskiold-Samsoe, and Bliddal, 2002). Lack of specificity is a limitation of water displacement compared to some methods such as MRI. Nonetheless, it is clear that cross sectional area as assessed by MRI is a very accurate measure of edema, but that accuracy also comes with high monetary and time costs.

The time it takes to measure limb volume using water displacement is a disadvantage compared to tape measure data. It took 10-15 minutes to measure foot and lower leg volume in the present study. The method described here is reliable and faster than other water displacement techniques that might be used such as measuring total lower leg volume in a leg volumeter and foot volume in a foot volumeter and then subtracting foot volume to yield the lower leg volume of interest.

As expected significant differences were found between males and females for foot volume and total leg volume. Most of the sex effect for total lower leg volume was due to sex differences in foot size and the associated foot volume. There was no significant difference between the sexes for lower leg volume. We speculate this observation resulted from the males having a greater lower leg height and the females storing more subcutaneous fat in their lower legs.

This investigation has shown that lower leg volume can be measured reliably from day to day with uniform procedures. It also shows that foot and ankle measurements can be taken reliably from a leg volumeter and a separate foot and ankle volumeter is not needed to obtain

high day-to-day reliability. The high day-to-day stability allows researcher to detect small changes in lower leg volume as a consequence of experimental interventions such as injurious eccentric exercise. Future investigations should be used to determine the sensitivity of leg volumetry to small and moderate changes in edema induced by eccentric exercise.

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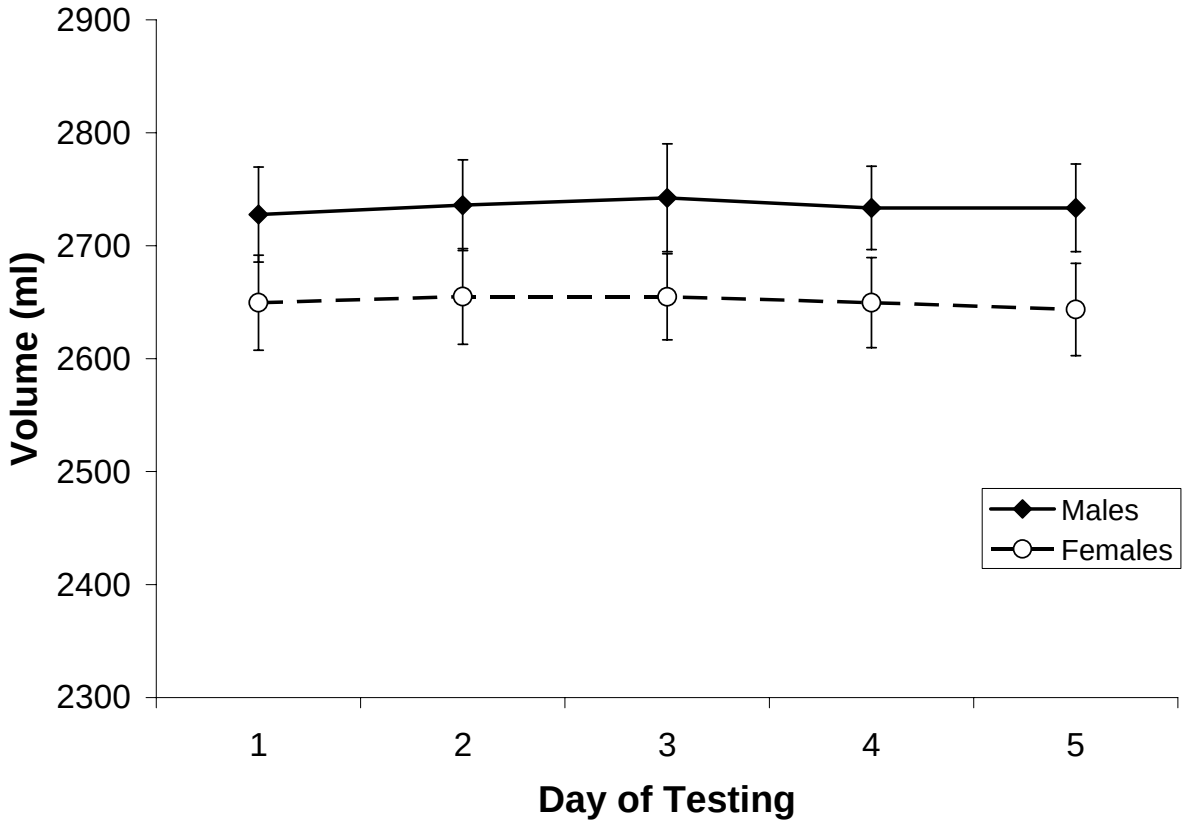
Figure 3.1: Photo of volumeter and obturator during measurement of foot volume.

Figure 3.2: Photo of lower limb position during the measurment of lower leg volume.

Figure 3.3.

Lower Leg Volume without Foot



Note: *Data are means ± SE*

Table 3.1.

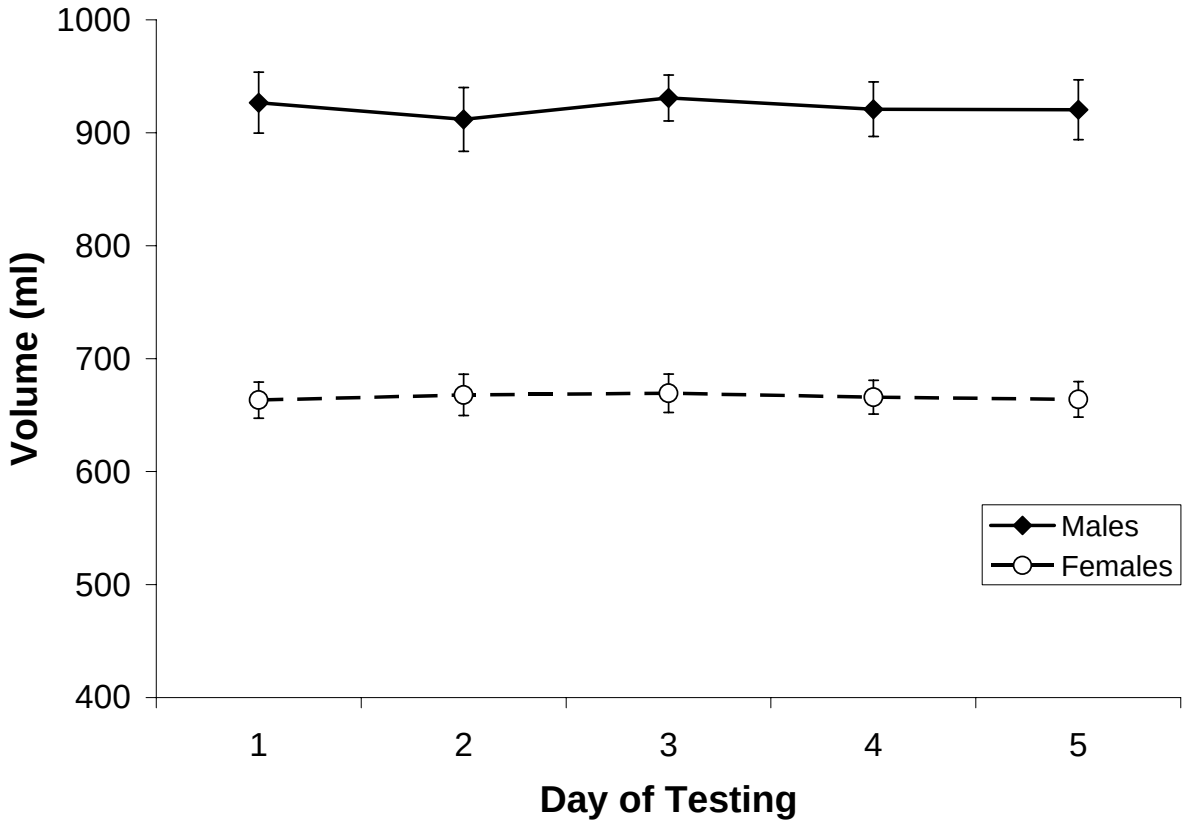
Lower Leg Volume without Foot (ml)

Females		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	CV_{within}
	1	3036	3045	3011	3011	3003	0.60
	2	2732	2713	2695	2676	2695	0.78
	3	2753	2753.5	2752	2754	2767	0.23
	4	2517	2576	2540	2527	2476	1.44
	5	2654	2627	2609	2577	2622	1.07
	6	2345	2301	2331	2344	2335	0.77
	7	2731	2725	2720	2729	2735	0.21
	8	2657	2656	2630	2660	2663	0.50
	9	2482	2604	2608	2601	2575	2.06
	10	2669	2675	2693	2681	2653	0.55
	11	2573	2576	2563	2578	2565	0.26
	12	2714	2715	2710	2714	2710	0.09
	13	2729	2727	2724	2724	2730	0.10
	14	2700	2705	2719	2735	2695	0.60
	15	2451	2428	2517	2434	2429	1.53
Males	1	2756	2735	2726	2730	2729	0.44
	2	2921	2834	2824	2821	2825	1.50
	3	2758	2761	2757	2751	2758.5	0.14
	4	2630	2639	2679	2678	2675	0.89
	5	2722	2741	2730	2737	2724	0.30
	6	2625	2717	2718	2729	2722	1.61
	7	2750	2759	2764	2747	2751	0.26
	8	2787	2793	2785	2784	2782	0.15
	9	2775	2780	2775	2775	2772	0.10
	10	2273	2288	2291	2305	2304	0.57
	11	3033	3062	3235	3017	3053	2.87
	12	2729	2742	2728	2748	2744	0.33
	13	2659	2671	2614	2670	2630	0.97
	14	2731	2739	2734	2742	2747	0.23
	15	2765	2778	2776	2769	2786	0.29
	Mean	2688.6	2695.5	2698.6	2691.6	2688.5	0.72
	SD	164.8	162.1	170.5	152.2	158.5	0.67
	SE	30.1	29.6	31.1	27.8	29.0	0.12
	CV_{days} (n=30)	6.13	6.01	6.32	5.66	5.90	
	%change		.26	.37	.11	0	

Note: CV = coefficient of variation, % change = percent change from Day 1.

Figure 3.4.

Foot Volume



Note: *Data are means ± SE*

Table 3.2.

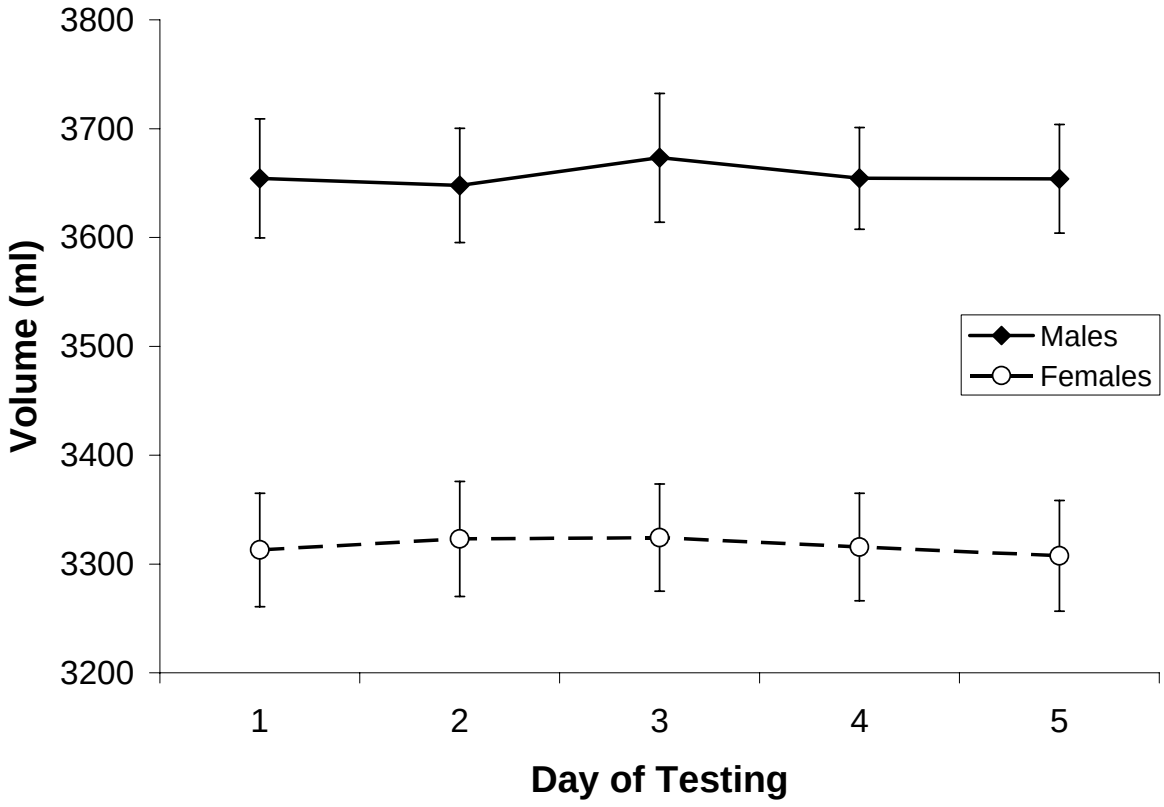
Foot Volume (ml)

Females		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	CVwithin
	1	772	752	774.5	774.5	804	2.39
	2	637	617	608	600	596	2.67
	3	642	664.5	682.5	650	627	3.26
	4	633	541	601	590	627	6.13
	5	546	581	539	604	597	5.15
	6	658	641	653	677	662	2.00
	7	690	700	706	689	691	1.07
	8	605	636	613	652	610	3.21
	9	600	596	637	627	623	2.88
	10	745	796	760	729	735	3.55
	11	680	723	700	673	667	3.33
	12	698	726	741	730	692	2.97
	13	751	744	731	747	752	1.14
	14	676	661	644	622	641	3.17
	15	617	640	651	625	636	2.09
Males	1	879	896	887	925	918	2.20
	2	787	801	859	792	813	3.57
	3	883	922	901	946	912	2.58
	4	921	956	959	903	947	2.58
	5	833	861	848	850	846	1.18
	6	826	863	882	829	853	2.77
	7	992	1041	1035	1017	1046	2.15
	8	1025	1027	1015	1039	1015	0.97
	9	1010	955	996	975	966	2.28
	10	985	956	947	976	1018	2.84
	11	1161	1027	1069	1053	1074	4.69
	12	934	918	913	908	946	1.71
	13	754	595	767	719	670	10.01
	14	958	938	943	976	910	2.60
	15	952	923	942	905	872	3.46
	Mean	795.0	789.9	800.1	793.4	792.2	3.02
	SD	158.4	153.7	151.0	150.4	154.6	1.75
	SE	28.9	28.1	27.6	27.5	28.2	0.32
	CVdays (n=30)	19.9	19.5	18.9	19.0	19.5	
	% change		0.64	0.64	0.20	0.35	

Note: CV = coefficient of variation, % change = percent change from Day 1.

Figure 3.5.

Total Lower Leg Volume



Note: *Data are means ± SE*

Table 3.3.

Total Leg Volume (ml)

Females		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	CV_{within}
	1	3808	3797	3785.5	3785.5	3807	0.29
	2	3369	3330	3303	3276	3291	1.11
	3	3395	3418	3434.5	3404	3394	0.50
	4	3150	3117	3141	3117	3103	0.62
	5	3200	3208	3148	3181	3219	0.87
	6	3003	2942	2984	3021	2997	0.99
	7	3421	3425	3426	3418	3426	0.10
	8	3262	3292	3243	3312	3273	0.81
	9	3082	3200	3245	3228	3198	2.00
	10	3414	3471	3453	3410	3388	1.00
	11	3253	3299	3263	3251	3232	0.76
	12	3412	3441	3451	3444	3402	0.63
	13	3480	3471	3455	3471	3482	0.31
	14	3376	3366	3363	3357	3336	0.44
	15	3068	3068	3168	3059	3065	1.50
Males	1	3635	3631	3613	3655	3647	0.44
	2	3708	3635	3683	3613	3638	1.06
	3	3641	3683	3658	3697	3670.5	0.59
	4	3551	3595	3638	3581	3622	0.95
	5	3555	3602	3578	3587	3570	0.49
	6	3451	3580	3600	3558	3575	1.66
	7	3742	3800	3799	3764	3797	0.69
	8	3812	3820	3800	3823	3797	0.31
	9	3785	3735	3771	3750	3738	0.58
	10	3258	3244	3238	3281	3322	1.04
	11	4194	4089	4304	4070	4127	2.28
	12	3663	3660	3641	3656	3690	0.49
	13	3413	3266	3381	3389	3300	1.89
	14	3689	3677	3677	3718	3657	0.61
	15	3717	3701	3718	3674	3658	0.72
	Mean	3483.6	3485.4	3498.7	3485.0	3480.7	0.86
	SD	267.5	259.8	272.9	251.5	260.3	0.53
	SE	48.8	47.4	49.8	45.9	47.5	0.10
	CV_{days} (n=30)	7.68	7.45	7.8	7.22	7.48	
	% change		0.05	0.43	0.04	0.08	

Note: CV = coefficient of variation, % change = percent change from Day 1.

CHAPTER 4

A METHOD TO RELIABLY ASSESS FOOT TREMOR ACROSS DAYS[3]

[3] Pasley, J.D. and P.J. O'Connor. To be submitted to *Neurophysiology*.

Abstract

This investigation determined day-to-day reliability of foot tremor. Thirty one young adults had right foot physiologic tremor measured by single plane accelerometer after being seated for 30 min on 5 separate days at least 24 hours and not more than 48 hours apart. Foot tremor was assessed in two positions: at rest and during dorsiflexion to 90º. The results revealed poor day-to-day reliability for foot tremor in a resting condition (ICC 3, 5) = .332 and adequately high day-to-day reliability for the 90º active condition (ICC 3, 5) = .821. Foot tremor can be assessed reliably across days using a single plane accelerometer.

KEY WORDS: day-to-day reliability, ICC, physiological tremor, single plane accelerometer

Introduction

Tremors are uncontrolled limb oscillations. Small physiologic tremors are normal in healthy adults, but in pathological conditions such as Parkinson's disease tremor can be a serious problem that contributes to accidents and injuries.

Mechanisms underlying tremor are incompletely understood. It has been suggested that tremor results from complex interactions between motor units, reflex mechanisms, the central nervous system and mechanical properties of the involved limb (Marsden, 1978; Saxton et al., 1995). For instance, tremor is absent in movement disorders in which the afferent part of the reflex loop is malfunctioning (Halliday and Redfrom, 1958). Also, centrally acting drugs such as alcohol can have dramatic effects on tremor (Stein and Lee, 1989), and as limb mass increases the oscillation frequency decreases (Desmedt, 1979).

Most research has focused on tremor in the upper extremities. Nonetheless, lower limb tremors are important because they are involved in motor control of the lower limb and are exacerbated in pathological conditions such as Parkinson's disease. There is a need to better understand tremors that exist in the lower extremities.

Some of the conditions that can exacerbate tremor are well recognized such as caffeine consumption (Miller, Lombardo, and Fowler 1998; Shirlow and Mathers, 1985). Other factors thought to influence tremor are less well established, including circadian variation, forces associated with the heart beat and those involving concomitant muscle actions in body areas in which tremor is not being assessed, and day-to-day variation. Determining day-to-day reliability in tremor would be useful in planning research aimed at understanding the extent to which interventions that extend over several days change tremor. For example, it has been reported that

eccentric exercise of the biceps brachii resulting in delayed onset muscle injury transiently increased tremor amplitude of the outstretched hand for 5 days following the exercise (Saxton et al., 1995). Results such as these are difficult to interpret without knowledge about the day-to-day reliability of hand tremor. Because we are interested in the effect of eccentric exercise induced lower leg muscle injury on foot tremor, the present study was the first in a series of planned investigations. The primary purpose of this experiment was to assess the day-to-day reliability of foot tremor.

Methods and Materials

Thirty one Exercise Science students at the University of Georgia were recruited as participants. Prior to being involved, each person read and signed the informed consent that summarized the procedures. The consent form had been approved by the Institutional Review Board.

Complete data were obtained from 16 women and 15 men. The women had a mean age of 20.7 years (SD = 3.4) and the men had a mean age of 22.5 years (SD = 2.9). Average height of the females was 165.0 cm (SD = 4.7) and their average weight was 60.5 kg (SD = 6.3). Males were 180.2 cm (SD = 3.5) and 78.6 kg (SD = 8.3).

Identical procedures were used in five different testing sessions that were held at least 24 hours apart. Testing sessions were not more than 48 hours apart. All testing sessions for a participant occurred at approximately the same time of day. Each participant was prepared for the assessement of foot tremor by sitting in an E-Z Leg-Up rehabilitation chair (model #4500; Rehab Seating Systems, Inc., Brookline, MA) with their shoes and socks off. The lower right leg was supported under the right calf at a leg angle of 130 degrees. The right ankle and foot were

not supported by the chair. The left foot of each participant rested comfortably on a step underneath the chair.

Physiological tremor in the right foot was measured via a piezoresistive single plane accelerometer transducer (Grass Model SPA1). The accelerometer was placed on top of the right foot parallel to the 2nd metatarsal and secured in place with ankle athletic prewrap (see Figure 4.1). The SPA1 accelerometer was attached to a Grass P511 amplifier. The amplifier was connected to a Kenwood CS-8010 digital oscillosope and a Grass Polyview 2.5 data acquisition and analysis computer software system to record the foot tremor activity. The signal was sampled at a rate of 1,000 Hz. The accelerometer was oriented so that it was sensitive to dorsiflexion or plantarflexion movements of the foot. The participants were asked to look straight ahead and avoid looking down at the foot during the recording period so as to not provide any visual feedback that might assist in maintaining steadiness of the foot. Participants were required to relax their foot for 30 seconds (see Figure 4.2) and then dorsifex their ankle and hold their foot at a 90 degree angle for 30 seconds (see Figure 4.3). These relaxation and dorsiflexion procedures were performed twice. Data in the 0-20 Hz range from both the resting and the 90° angle were analyzed for tremor amplitude using fast fourier transformation (FFT) of the signal with a Hanning window. The average of two 30-second epochs at each position were used as the criterion measure. The data were converted into milli G to facillitate comparison with a publication of interest (Saxton et al., 1995).

Data Analysis

Data analysis was performed using SPSS 13.0. Reliability was assessed in several ways including examining means and standard deviations, coefficients of variation (standard

deviation/mean x 100%), and intraclass correlations (ICC 3, 5) (Atkinson, and Nevill, 1998). The ICC model used was a two way (30 Subjects and 5 Days) mixed effects model (Subjects were considered random and Days fixed effects). The ICC model also used absolute agreement as the type of agreement and the average measures option since two tremor amplitude measures were averaged each day for both foot positions. Two mixed model (2 sex x 5 day) ANOVAs were performed to assess sex-related differences in day-to-day variations in tremor based on the univariate F-statistic. Effect sizes were expressed as eta-squared (η^2). The Greenhouse-Geisser epsilon (ε) was used when the sphericity assumption was violated.

Results

Resting foot tremor varied significantly across days ($F_{4,124} = 3.15$; $p = 0.02$) and the associated ICC equaled .332. The coefficient of variation for each day ranged from 4.42% to 13.37%. The within subjects coefficient of variation averaged 6.89% (SD = 5.20) and ranged from 0.51% to 21.84%. The percent change from Day 1 ranged from -4.10% to -7.00%. The raw data for foot tremor in a resting position are reported in Table 4.1. The consistency in the mean amplitude of foot tremor across days for 20Hz is shown in Figure 4.4.

Day-to-day variation of foot tremor in the 90º dorsiflexed condition was not statistically significantly different ($F_{4,124} = 1.22$; $p = 0.31$) and the corresponding ICC equaled 0.82. The coefficient of variation for each day ranged from 14.12% to 20.86%. The within subjects coefficient of variation averaged 9.94% (SD = 6.22) and ranged from 1.53% to 31.22%. The percent change from Day 1 ranged from 1.13% to 6.41%. The raw data for foot tremor in a 90º dorsiflexed position are reported in Table 4.2.

Two mixed model (2 sex x 5 day) repeated measures ANOVAs were performed to assess sex-related differences in day-to-day variations in tremor. Results indicated no significant interaction for Sex x Day for the resting condition ($F_{2.3,66.6} = 0.49$; $p = 0.74$; $\eta^2 = 0.02$; $\varepsilon = 0.57$). The main effect for sex was not significant ($F_{1,29} = 1.07$; $p = 0.31$; $\eta^2 = 0.04$), however the main effect for day was significant ($F_{2.3,66.6} = 3.03$; $p = 0.05$; $\eta^2 = .095$; $\varepsilon = .574$). Results for the 90° condition indicated no significant interaction for Sex x Day ($F_{4,116} = 0.84$; $p = 0.50$; $\eta^2 = 0.03$) and no significant main effect for sex ($F_{1,29} = 1.33$; $p = 0.26$; $\eta^2 = 0.04$) or day ($F_{4,116} = 1.24$; $p = .30$; $\eta^2 = 0.04$).

Discussion

The impetus for the present investigation was the absence of published data on day-to-day reliability for any lower leg tremor protocol (Braude, Charles and Barnes, 1983; Gajewski and Viitasalo, 1993; Viitasalo, and Gajewski, 1994). The primary finding of this study was that active foot tremor (90° dorsiflexion) is reliable across days but resting foot tremor is unreliable. This coincides with the literature regarding methods for measuring hand tremor. Most of the hand tremor research has required participants to activate muscles to "steady" the hand at a 180-degree angle.

Our method of using a single plane accelerometer affixed to the foot could be used to assess alterations in motor control after an injury to lower leg muscles induced in laboratory experiments. The bulk of the literature on delayed onset muscle soreness has focused on the time course needed for return to participation in sport or exercise based on the recovery of muscle strength. Recovery of motor control also is potentially important for a safe return to sport and it is less well understood than changes in strength.

Only one study has assessed changes in tremor after delayed onset muscle injury (Saxton et al., 1995). Large increases in hand tremor were found immediately (~600%) and 24 hours (~250%) after muscle damaging eccentric arm exercise compared to a pre-exercise baseline (Saxton et al., 1995). Figure 1-A of their study suggests reasonable day-to-day reliability because of stable means across days in the control arm. In agreement, Figure 4.4 of this report suggests reasonable day-to-day reliability because of stable means across days in the right foot. However, no statistical information about reliability was presented by Saxton et al., (1995) and stability in means is not an adequate measure of reliability as shown both by the data in the present report for foot tremor in the resting position and in many data other data sets (Atkinson and Nevill, 1998).

As expected there was a lack of sex-related difference in tremor amplitude. Currently there is no literature showing sex-related differences in physiologic tremor in a healthy college age group. Sex-related differences in other types of tremor have been reported, for example, with pediatric essential tremor (Louis, Fernandez-Alvarez, Dure, Frucht, and Ford, 2005).

Athletic trainers and rehabilitation professionals may find the use of foot tremor assessed via single plane accelerometer an effective tool to assist in determining recovery of patients from various foot and ankle injuries. Also, the technique could prove useful to podiatrists and other health professionals when assessing neurological damage in the feet of diabetic patients. In all these potential applications, using a method with adequately high reliability across days is needed for accurate interpretation of changes in physiological foot tremor as treatment or recovery occurs.

Future investigations should examine the use of a maximum dorsiflexion and a plantarflexion protocol in addition to the 90-degree angle. These conditions might prove to have

higher reliability and could provide more information with regard to injury and neurological damage as a result of greater activation of muscle groups and sensory afferents. One limitation of the present work is that muscle activation occurred at only one angle (90º).

In summary, we report that adequately high day-to-day reliability in foot tremor can be achieved when foot tremor is measured by accelerometry and the foot is dorsiflexed to a 90º angle. It is recommended that future studies examining the influence of a treatment on changes in foot tremor across days use an active, not a resting measure of foot tremor.

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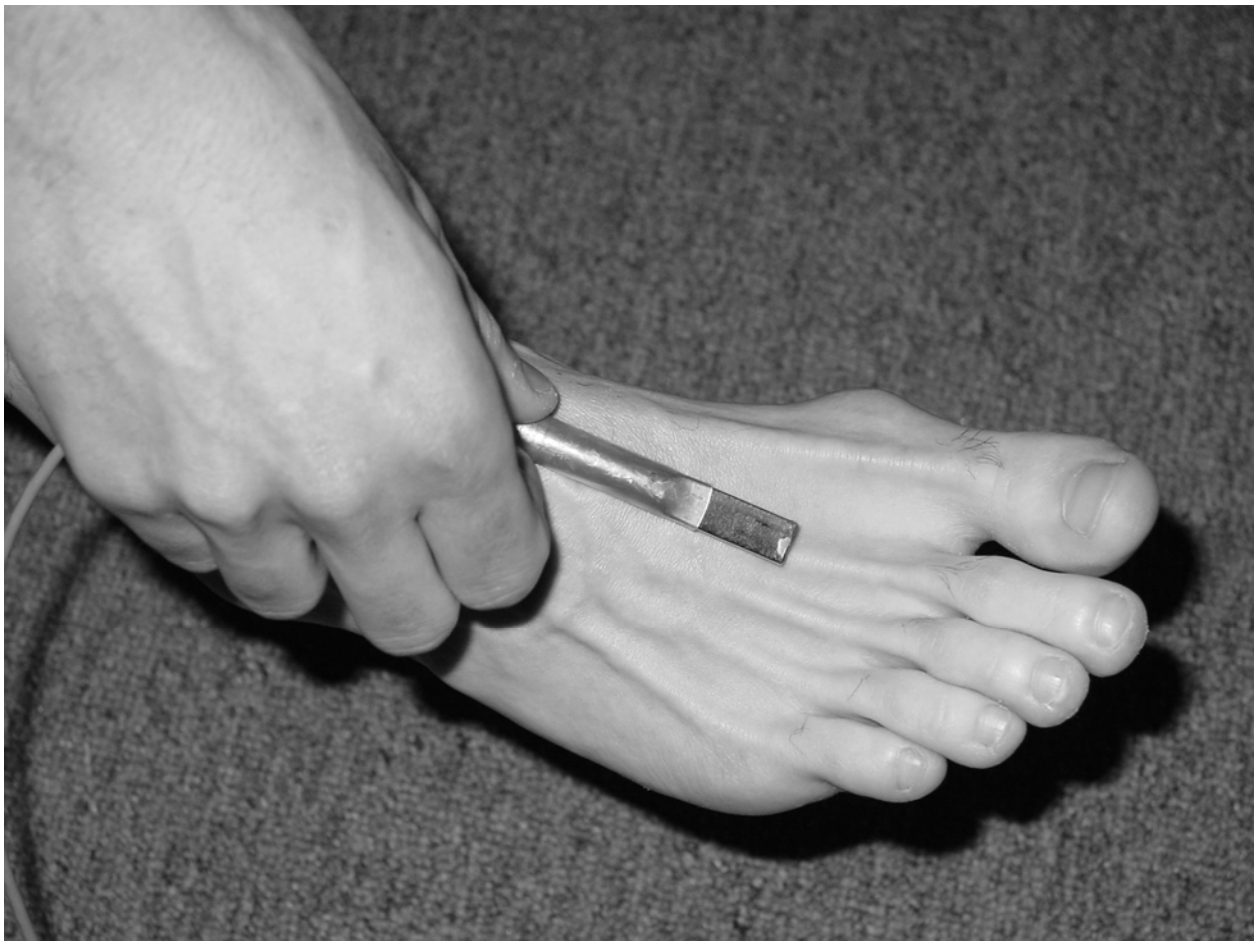


Figure 4.1: Care was taken to place the accelerometer just posterior and parallel to the second metatarsal of the right foot before wrapping it with athletic ankle wrap.

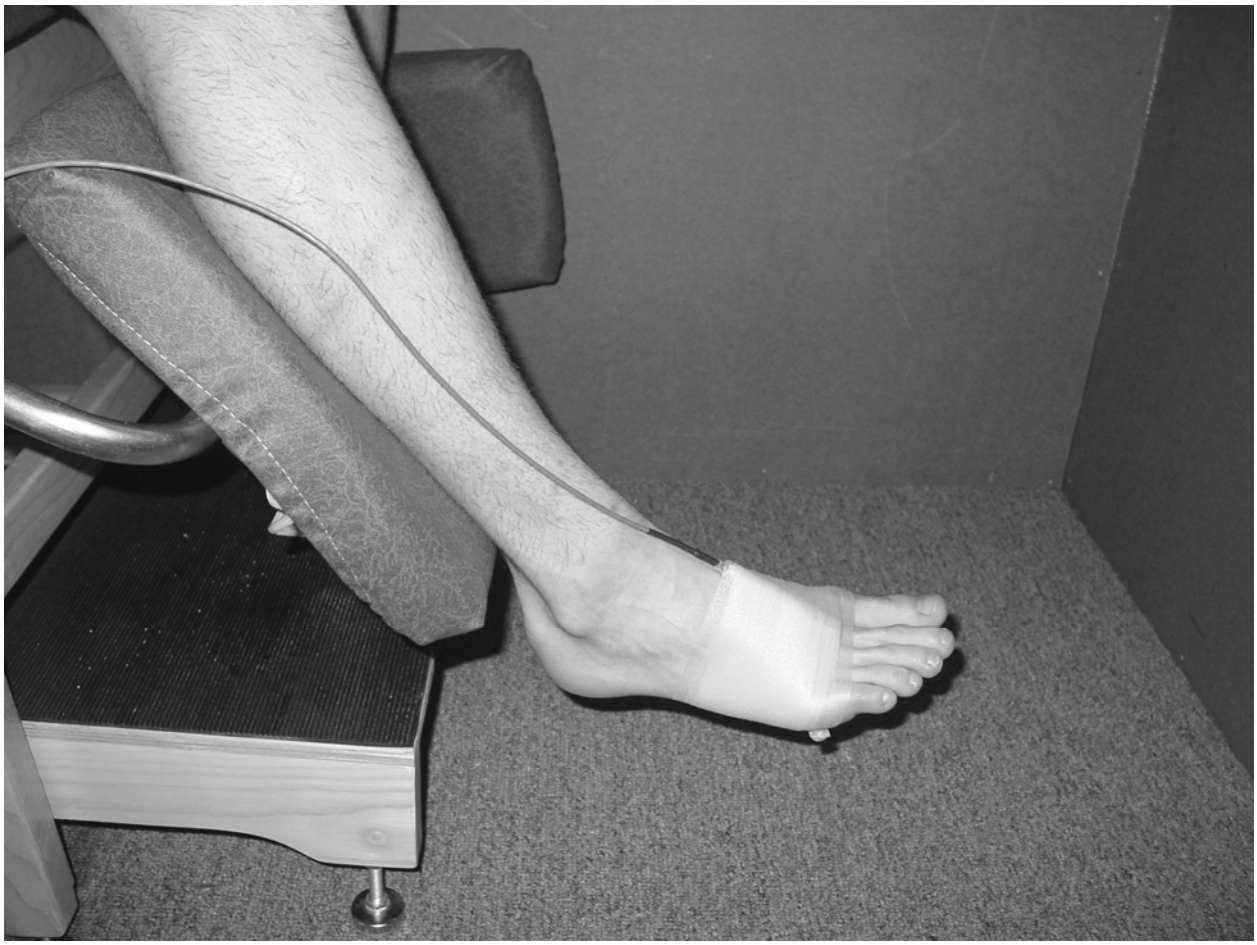


Figure 4.2: Each participant was asked to “rest” their right foot and let it hang naturally.

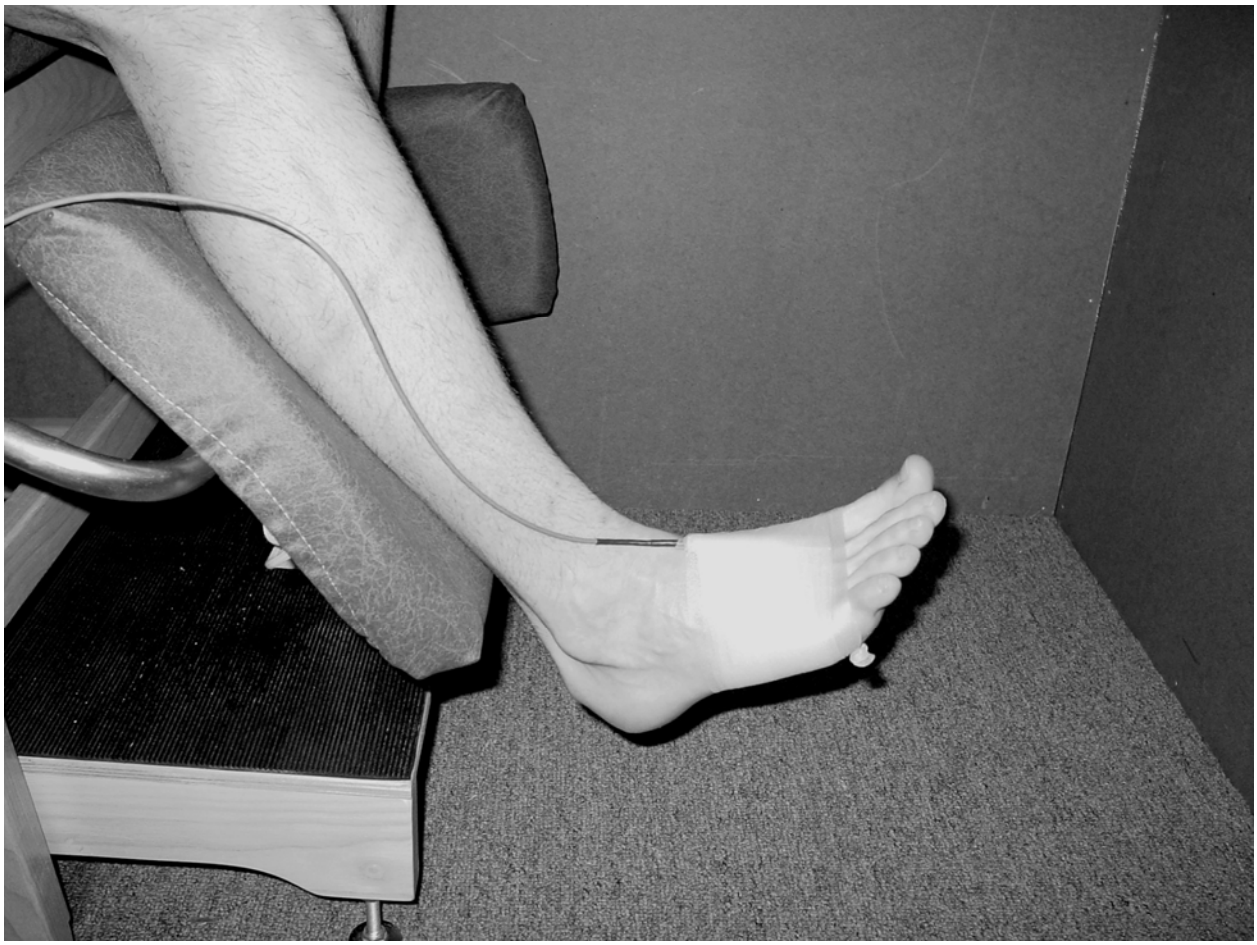
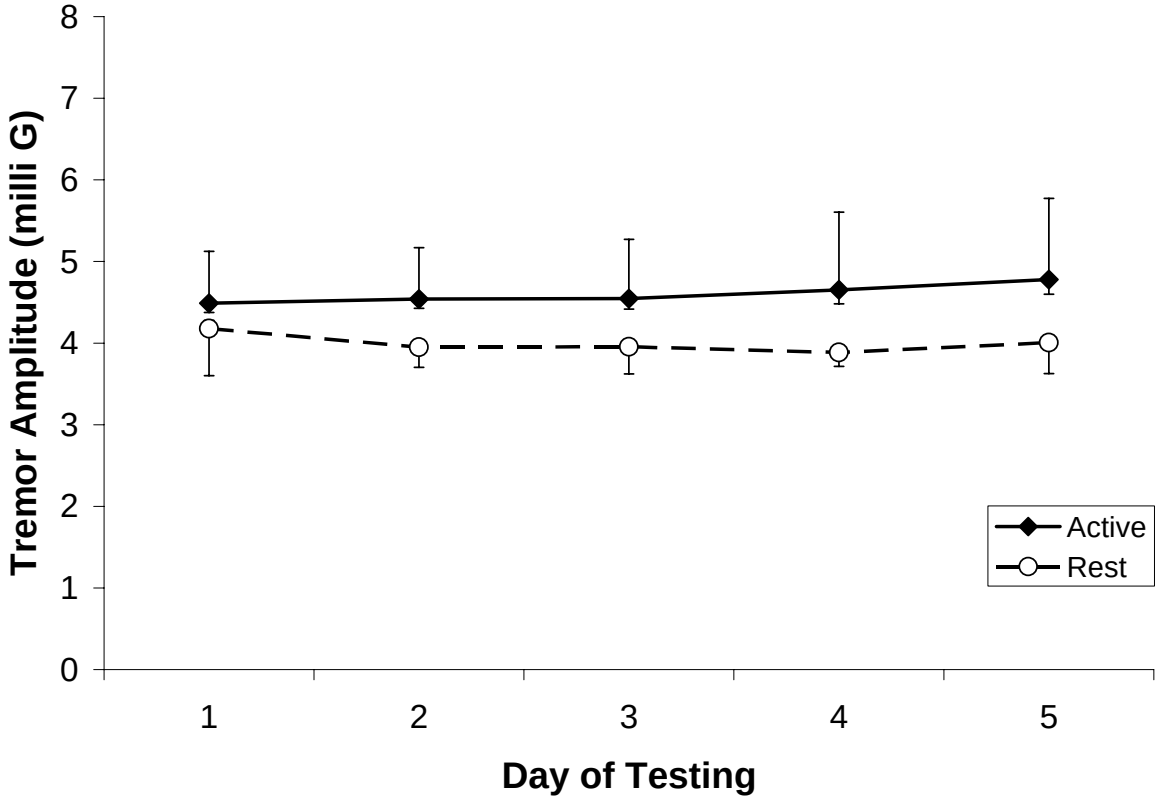


Figure 4.3: Each participant was asked to hold their right foot at a 90 degree angle.

Figure 4.4.

Foot Tremor in active 90º and resting positions



Note: *Data are means ± SE on inside and ± SD on outside of lines*

Table 4.1.

Foot Tremor Resting Condition (milli G)

Females		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	CVwithin
	1	6.08	4.11	3.90	3.83	3.98	21.84
	2	3.60	3.69	3.97	3.88	3.87	3.96
	3	4.29	3.64	3.68	4.03	3.67	7.43
	4	3.73	3.72	3.70	3.65	3.70	0.81
	5	3.77	3.84	3.82	3.68	3.69	1.98
	6	3.75	4.52	3.84	3.72	4.45	9.71
	7	4.12	3.82	3.65	3.75	3.62	5.30
	8	3.99	3.86	3.75	3.84	3.81	2.30
	9	4.05	4.22	4.21	3.95	4.79	7.70
	10	3.89	4.09	3.94	4.05	4.09	2.33
	11	5.05	3.95	4.41	3.93	4.43	10.53
	12	5.26	3.93	3.78	3.93	4.04	14.48
	13	4.70	3.86	4.83	3.88	4.77	11.17
	14	4.07	3.94	3.91	4.00	3.94	1.64
	15	3.81	4.66	3.81	4.60	3.81	10.94
	16	4.31	4.08	3.85	3.90	3.90	4.76
Males	1	3.77	3.75	3.64	3.69	3.64	1.58
	2	4.69	3.73	3.67	3.68	3.59	11.84
	3	3.66	3.58	4.88	3.88	4.80	15.19
	4	4.58	3.80	3.83	3.70	3.73	9.36
	5	4.06	4.19	3.75	3.81	3.77	5.04
	6	3.73	4.01	4.64	3.85	3.83	9.12
	7	4.16	3.95	3.76	3.94	3.76	4.22
	8	4.16	3.88	3.95	3.86	4.02	3.03
	9	3.98	4.32	3.94	3.97	3.91	4.12
	10	3.94	4.02	3.70	3.92	3.91	3.04
	11	3.87	3.69	3.80	3.90	3.80	2.10
	12	3.67	4.09	4.10	4.03	3.86	4.68
	13	3.84	3.90	3.87	3.87	3.87	0.51
	14	5.24	3.87	3.79	3.89	4.90	15.64
	15	3.78	3.82	4.37	3.88	4.32	7.10
M		4.18	3.95	3.96	3.89	4.01	6.89
SD		0.58	0.25	0.33	0.17	0.38	5.20
SE		0.10	0.04	0.06	0.03	0.07	
CVdays (n=31)		13.77	6.21	8.41	4.42	9.46	
% change			-5.44	-5.29	-7.00	-4.10	

Note: CV = coefficient of variation, % change = percent change from Day 1.

Table 4.2.

Foot Tremor Active 90º Condition (milli G)

Females		**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	CVwithin
	1	6.30	4.33	3.90	4.05	4.06	22.17
	2	3.71	4.74	4.47	4.29	4.93	10.58
	3	4.04	4.29	3.99	5.69	5.31	16.83
	4	3.94	4.29	3.89	3.79	3.96	4.74
	5	4.06	4.24	4.26	4.43	3.93	4.66
	6	4.00	4.77	3.86	4.35	4.57	8.89
	7	4.13	4.29	3.99	3.97	3.92	3.70
	8	5.21	5.66	6.16	4.89	6.36	10.97
	9	4.15	4.16	4.77	4.12	4.76	7.78
	10	3.98	4.11	4.01	4.19	4.24	2.73
	11	4.99	3.98	4.55	4.06	4.97	10.65
	12	5.28	3.93	4.18	4.26	4.65	11.81
	13	5.02	4.08	4.99	4.09	4.97	10.75
	14	4.34	4.47	4.35	4.66	4.37	3.07
	15	3.83	4.76	4.15	4.73	4.05	9.68
	16	4.68	4.86	5.37	4.60	5.76	9.83
Males	1	3.75	3.82	3.76	3.86	3.72	1.53
	2	4.81	4.21	4.19	4.61	5.12	8.71
	3	4.10	4.75	5.57	5.03	5.62	12.54
	4	4.82	4.05	5.42	4.40	3.95	13.30
	5	4.47	4.75	3.97	4.65	4.82	7.55
	6	3.78	4.01	4.99	4.00	3.95	11.62
	7	4.64	5.22	4.61	4.89	4.44	6.36
	8	4.18	4.40	4.19	4.85	4.64	6.55
	9	5.23	5.18	5.70	5.40	5.07	4.61
	10	4.40	4.77	4.40	8.33	4.90	31.22
	11	4.08	4.00	4.38	4.19	4.12	3.47
	12	4.35	4.14	4.18	4.71	4.17	5.55
	13	4.22	5.94	4.26	4.21	4.67	15.90
	14	4.80	4.09	3.87	3.82	5.17	13.87
	15	5.94	6.54	6.64	7.18	8.99	16.51
M		4.49	4.54	4.55	4.65	4.78	9.94
SD		0.63	0.63	0.73	0.95	1.00	6.22
SE		0.11	0.11	0.13	0.17	0.18	
CVdays (n=31)		14.12	13.84	15.96	20.47	20.86	
% change			1.13	1.27	3.62	6.41	

Note: CV = coefficient of variation, % change = percent change from Day 1.

CHAPTER 5

EFFECT OF ECCENTRIC EXERCISE ON THE NOCICEPTIVE FLEXION (R-III) REFLEX, FOOT TREMOR, INFLAMMATION AND MUSCLE PAIN[4]

[4]Pasley, J.D. and P.J. O'Connor. To be submitted to *Pain*.

Abstract

Hundreds of published experiments have shown that intense eccentric exercise can cause delayed onset muscle pain but little is known about the effect of eccentric exercise on the nociceptive system that underlies pain perception. The focus of this investigation was on the effect of 100 eccentric actions of the lower right leg on the nociceptive flexion (R-III) reflex. Twenty two young adult females were block randomized to either an eccentric exercise group (n=11) or a no exercise control group (n=11). Before and after the conditions (10 min, 24-, 48- and 72-hrs post) measurements were made of the R-III reflex threshold, physiologic foot tremor, lower leg volume and muscle pain. From 24 to 72 hours following eccentric exercise, small increases in leg volume (mean increase of 2.9% compared to baseline), foot tremor (mean decrease of -0.2% across three foot positions), pain intensity and pain affect (mean scores from 24 to 72 hours on a 0-100 scale were 15.5 and 14.0, respectively, indicating mild pain intensity and unpleasantness) were found as was a small decrease in R-III reflex threshold (-11.1%). Repeated measures ANOVA revealed that the range of oscillation in the minimum and maximum R-III threshold 24 to 72 hours after eccentric exercise was significantly greater than the control condition ($F_{1,20} = 9.91$; $p = 0.005$; $\eta^2 = 0.33$; $\varepsilon = 1.00$). We conclude that eccentric exercise inducing modest changes in indicators of muscle injury increases the range of oscillation in the R-III reflex threshold 24 to 72 hours after the exercise.

KEY WORDS: inflammation, pain affect, pain intensity, pressure algometry

Introduction

Novel eccentric exercise induces ultrastructural injury to skeletal muscle (Nurenberg, Giddings, Stray-Gundersen, Fleckenstein, Gonyea, and Peshock, 1992) causing stiffness and inflammation (Smith, 1991). The inflammation process includes localized swelling and the release of algesic substances (e.g., bradykinin, serotonin, adenosine) that sensitize muscle nociceptors (Tegeder, Zimmermann, Meller, and Geisslinger, 2002). Muscle pain, delayed in onset from the injury stimulus by approximately 12-24 hours, is experienced primarily in response to movement or external pressure applied to the injured area (Clarkson and Newham, 1995; Hough, 1902). Little is known about the effect of eccentric exercise on nociceptive neurology.

The nociception flexion (R-III) reflex threshold is a recognized measure of nociception that can be obtained from human subjects. A painful stimulus is required to elicit the reflex (Skljarevski and Ramadan, 2002). It is spinally mediated (Hugon, 1973) but under tonic supraspinal inhibition (Sandrini, Arrigo, Bono, and Nappi, 1993). Analgesic drugs consistently increase the R-III reflex threshold (Skljarevski and Ramadan, 2002) and the induction of inflammation in the knee joint decreases the R-III reflex threshold in the cat knee flexors (Ferrell, Wood, and Baxendale, 1988). The day-to-day reliability of the R-III reflex threshold is high (Chan and Dallaire, 1989; Willer, 1977) and it is strongly and linearly related to pain intensity ratings of the electrical stimuli used to elicit the reflex (Bromm and Treede, 1980; Chan and Dallaire, 1989; Dowman, 1991; Dowman, 1993; Willer, 1977; Willer, Roby, and Le Bars 1984). The effect of novel eccentric exercise on the R-III reflex threshold is unknown but the available evidence suggests that the R-III reflex threshold should be reduced 24 to 48 hours after

exercise. It is less clear what is expected shortly after eccentric exercise. Aerobic and anaerobic exercise and a small body of research involving forms of resistance exercise unlikely to cause delayed onset muscle injury consistently have reported a reduced sensitivity to painful stimuli shortly after exercise (Koltyn & Arborgast, 1998; Koltyn, 2000; Koltyn, 2002). This suggests that the R-III reflex threshold will increase shortly after eccentric exercise. However, if nociceptive neurological alterations are concurrent with the timing of muscle injury (rather than the timing of the delayed onset of pain) then the R-III reflex threshold will be decreased shortly after eccentric exercise.

The primary purpose of this investigation was to learn whether the R-III reflex threshold is altered after completing lower leg eccentric exercise that causes delayed onset muscle pain. It was hypothesized that the R-III reflex threshold would decrease in the injured leg of the exercise group after eccentric exercise compared to the uninjured leg of the control group. Measures of inflammation (leg volume), pain and physiological tremor, a neurological measure not directly involved in nociception but known to be increased following eccentric exercise (Saxton et al., 1995), were included to document that the stimulus used here induced the expected muscle injury, pain and change in non-nociceptive neurology.

Methods and materials

Subjects

Twenty two female participants completed four consecutive days of testing. The focus on one sex was appropriate given the large sex-related difference in R-III reflex threshold (Mylius, Kunz, Schepelmann & Lautenbacher, 2005). The participants were assigned using blocked randomization to either the eccentric exercise group (n=11) or the control (n=11) group. The

women in the control group had a mean age of 22.0 years (SD = 1.5) and the eccentric exercise group had a mean age of 22.6 years (SD = 4.6). Average height of the control group was 168.6 cm (SD = 6.1) and their average weight was 67.6 kg (SD = 7.6). Eccentric exercise group were 163.2 cm (SD = 11.7) and 62.5 kg (SD = 6.0). Sample size was determined using SPSS based on a within-subjects, repeated measures design (D'Amico, Neilands, and Zambarano (2001). Using an alpha error of .01 and assuming a correlation across repeated measures of 0.82, a sample of 10 subjects can detect a condition by time interaction effect size of 1.32 SD with a power of .80. Because no prior studies have examined the effect of eccentric contraction induced injury on the R-III reflex threshold, the expected effect size was powered based on available data with pain intensity ratings. Numerous experiments have shown a close correspondence between pain intensity ratings and R-III reflex threshold (Chan and Dallaire, 1989). Because pain intensity rating typically increase by greater than 5 standard deviations in response to novel eccentric actions (e.g., Clarkson, Nosaka & Braun, 1992), a sample size of 10 was predicted to provide adequate statistical power to detect the expected decrease in R-III reflex threshold.

The participants were recruited from flyers placed on approved bulletin boards and by announcements made in exercise science courses. Participants who reported any factor on a medical history questionnaire that was contra-indicated for performing weight lifting exercise were excluded. For example, individuals who had been injured in the past 6 months and required analgesics were not allowed to participate. Also unable to participate were diabetics, or anyone with heart ailments, sickle cell anemia, or McArdle's disease. Anyone reporting any current use of anti-anxiety, anti-depressant, or analgesic medication were excluded. Also, participants indicating they recently had experienced: coughing, back pain, swollen joints, or injuries to their

back, arms, or joints were excluded. Those reporting the use of histamines, diuretics or other dehydrating medications were excluded to reduce the risk of rhabdomyolysis.

Potential participants initially arrived at the Exercise Psychology Lab for a brief description of the project and a question and answer session so that they could decide if they wished to participate. They read the informed consent, any questions were answered by the researcher, the consent was signed, and the participant received a copy of the consent form. Then participants completed a medical history questionnaire and the investigator determined whether the exclusion criteria were met. Participants were tested during the first 12 days of their menstrual cycle because the R-III reflex threshold differs in the luteal compared to the follicular phase of the menstrual cycle (Tassorelli et al., 2002).

Procedures

Delayed-onset muscle pain inducing exercise

Eccentric exercise was performed on the first testing day. Participants in the eccentric exercise group completed two sets of 50 standing heel drops (dorsiflexions involving eccentric actions of the soleus and gastrocnemius) with the right leg. The exercise began with the participant standing on the toes and ball of her fully plantarflexed right foot. She then lowered her body in a slow and controlled fashion until her heel touched the platform. Plantarflexions by the left leg and right leg returned the body to the starting position after each heel drop. The exercise was completed on a 30 degree platform placed against a wall with 5 min of rest in between sets to reduce fatigue. Participants were allowed to balance themselves if needed by placing one hand against the wall (see Figure 5.13). This bout of eccentric exercise was expected to produce mild injury and moderate intensity pain in the lower leg (Barbiroli et al., 1993;

Tegeder et al., 2002). Those in the control condition sat at the edge of a medical examination table and performed 50 unweighted dorsiflexion and plantarflexion movements by the right foot followed by 50 unweighted plantarflexion only movements with the left foot. Then after a 5 min rest period they repeated the 50 actions performed by each foot. This was done to simulate the muscle actions performed by the eccentric exercise group, but at a low intensity that would result in no muscle injury or delayed onset pain. Before and after both the exercise and control conditions several measurements were made as described below.

Elicitation of nociceptive flexion reflex

The investigator washed his hands with soap and hot water for 30 seconds. Then the participant was prepared for skin surface measurements of biceps femoris muscle activity (hamstring muscle) of the right leg using electromyography (EMG) and percutaneous stimulation of the sural nerve according to the procedures of France and Suchowieki (1999). First, the participant repeatedly flexed the hamstring muscles and the investigator palpated the muscle to identify proper placement of the electrode position for recording. The skin surface was abraded lightly with 220 course sandpaper (very fine) in order remove dead skin cells on the skin surface. The skin surface was cleaned using alcohol swabs. Two re-usable, silver/silver chloride electrodes were attached along the midline of the biceps femoris muscle of each leg (Med Associates TD-025; St. Albans, VT). These eletrodes were cleaned after each use with antibacterial soap and hot water, then dried and kept in a sealed bag with the participants ID number on it. Potential inter-electrode variation was avoided by reusing the electrodes. The two electrodes were separated by 2 cm. These recording electrodes were attached to a Grass P511 amplifier, which was be connected to a CED micro1401 mk II digital processor linked to a Dell

computer using Spike2 version 5.16 data acquisition and analysis computer software system to record the biceps femoris muscle activity.

The researcher next placed two round carbon rubber disposable pre-gelled stimulating electrodes above the sural nerve on the lateral side just posterior to the lateral malleoulus of the right foot (EMPI 900ST 1.25" round stimulating electrodes cut to 1" diameter; Clear Lake, South Dakota). Participants walked from the prep room to an adjacent room into a Faraday cage and sat while stimulating electrodes were connected to a Digitimer DS7a constant current somatosensory stimulation unit.

After sitting for 5 min with their right arm supported at heart height, participants had arterial blood pressure measured twice in their right arm with a sphygmomanometer and stethescope (Kirkendall, Feinleib, Freis, and Mark, 1981). Participants were instructed not to talk while blood pressure was being measured. Systolic blood pressure was noted as the first sound heard and diastolic blood pressure as the last sound heard. The purpose of obtaining blood pressure data was to determine whether any participant had a hypertensive response to the experimental procedures. Hypertension is associated with a reduction in pain perception and an increased pain threshold (Ghione, Rosa, Mezzasalma, and Panattoni, 1988; Guasti, Cattaneo, Rinaldi, Rossi, Bianchi et al. 1995; Zamir and Shuber, 1980).

After blood pressure recordings, elicitation of the R-III reflex commenced. The researcher delivered a series of 5 rectangular 1 ms impulses to the sural nerve every 8-12 seconds (randomized) and the resulting biceps femoris muscle activity was recorded. The strength of the pulse delivered to the sural nerve was progressively increased to obtain tactile sensation, which typically requires a stimulus intensity of approximately 5mA. The stimulation was then increased in a stepwise method to approximately 15 mA (France and Suchowieki, 1999).

Participants provided ratings of pain intensity using a graphical numerical scale (0 to 100) after each electrical stimulus in order to supplement the objective R-III reflex data. The maximal stimulus intensity employed was 40 mA to minimize the amount of pain experienced and prevent any possibility of physical injury.

Foot tremor assessment

Each participant was then prepared for the assessement of foot tremor. Physiological tremor in the right foot was measured with a Grass Model SPA1 single plane accelerometer transducer. Each participant remained seated in the same chair used for R-III reflex elicitation. An accelerometer was placed on top of the right foot parallel to the 2nd metatarsal and secured in place with ankle athletic prewrap. The participants were then asked to look straight ahead with their eyes closed during the recording period to minimize and standardize visual feedback. Participants were required to relax their foot for 30 seconds and then hold their foot at a 90 degree angle for 1 minute. This was followed by 30 seconds of foot relaxation and a 1 min period during which the foot was plantarflexed fully. This was followed by 30 seconds of foot relaxation and a 1 minute full dorsiflexion. The procedure was completed three times (~12 min). Data in the 0-20 Hz range from the 90° angle, plantarflexion, and dorsiflexion were analyzed for tremor amplitude using fast fourier transformation (FFT) of the signal with a Hanning window. The first 10 and the last 10 seconds from each 60 second recording epoch were discarded from analysis. The average of three 40-second epochs at each foot position were used as the criterion measure. The data were converted into milli G to facillitate comparison with a publication of interest (Saxton et al., 1995).

The participants then walked to the adjacent prep room and had the stimulating and recording electrodes removed in order to assess leg volume.

Leg volume measurement

Semi-permanent ink was used to make one mark near the knee (at the lateral portion of the head of the right fibula) and one near the ankle (4 cm above the bottom of the leteral maleolus). Participants were asked to refrain from washing off the marks so the same marks could be used each day.

A Lucite partial leg volumeter was used for all measurements (Volumeters Unlimited, Phoenix, AZ). Participants with differing leg lengths were fitted to the height of the volumeter using Lucite platforms with heights of 4.5 cm and 7 cm placed in the bottom of the volumeter. The volumeter had a height, width and length of 61 cm, 15.5 cm and 33 cm. The volumeter was filled with approximately 10 gallons of tepid water until it overflowed into a pitcher placed under the spout. To reduce surface tension and enhance the consistancy of the measure, a Lucite obturator with the dimensions 7.5 cm (height) x 14 cm (width) x 17.5 cm (length) was inserted until the water stopped dripping from the spout. The pitcher was then emptied, dried and placed back under the spout of the volumeter.

Next, foot volume was measured. Each participant sat in a chair and inserted their right foot slowly into the volumeter up to the line above the ankle with their heel touching the back of the volumeter. Care was taken so that the leg was perpendicular to the floor and not leaning to either side. Assistance was given by the researcher so that the foot did not go deeper than the line drawn. The plastic obturator was re-inserted into the volumeter to reduce water surface tension and stayed in place until the water had stopped dripping from the spout. The weight of the water

was measured to the nearest 0.5 gram using a calibrated Mettler P3 3000/1g mechanical balance scale.

Lower leg volume was measured next. A dry pitcher was placed under the spout. Each participant then slowly inserted their lower leg into the water until the ball of the foot reached the bottom and the heel was touching the back of the volumeter. The leg was submerged to just below the knee. Participants were not allowed to place their foot flat against the bottom of the volumeter to avoid variations in foot pressure that might cause a difference in the amount of water displaced. The weight of the water displaced was recorded.

All displaced water weights were converted to volume using a standard equation (i.e., the density of water is 1g = 1 ml). The identical procedure was conducted at the same time of day within each subject for all 4 testing sessions. The measures from the foot and the lower limb were combined to create the total lower leg volume. The total lower leg volume was used as the criterion measure in this experiment because foot and lower leg volume both increased in response to eccentric exercise. We previously demonstrated high day-to-day reliability (intraclass correlation of .972) across five days in a study of 30 adults (Pasley and O'Connor, 2007).

Muscle pain measurement

Immediately after the 100 high resistance eccentric actions (eccentric group) or the 100 low resistance plantarflexion and dorsiflexion actions (control group) the participants rated the average intensity of the pain experienced in their right lower leg during the eccentric or control condition using a 0-100 graphical numerical scale.

Pain following eccentric exercise typically is not experienced at rest but is stimulated by skeletal muscle movement or external pressure placed on nociceptors in the injured tissue (i.e., a mechanical allodynia). Pressure was applied in three ways.

Pain Intensity Ratings. Unweighted movement was used to stimulate pain. The participants sat at the edge of an examination table with their feet off the floor and slowly dorsi- and plantarflexed their right foot through its range (~2 seconds) of motion fifteen times. Immediately after the fifteen movements the participants were asked to rate the intensity and affect of the pain in their calf muscles using a 0-100 graphical numerical scale. This procedure was then used with the left foot.

Next body weight was used to stimulate pain. The participants walked ~ 40 meters and up one flight of stairs. Next they walked down fifteen stairs at a slow paced controlled by the investigator. Immediately after, separate ratings of pain intensity and affect in the calf muscles of both legs were obtained using the 0-100 graphical numerical scale. There is a large body of evidence that valid pain intensity and affect data can be obtained with graphical numerical scales (Turk & Melzack, 2001).

Pain Threshold. Participants walked back to the lab and assumed a prone position on an examination table with their right foot plantarflexed (Weerakkody, Whitehead, Canny, Gregory, and Proske, 2001). A pressure algometer with a 1 cm sized rubber tip (Wagner Instruments FDK 20) was used to apply increasing amounts of pressure (up to 20 lbs. maximum) to a small area of the skin and underlying muscles of the lower leg. A single measurement was made in the center of the medial head of the gastrocnemius using anatomical landmarks. This location was chosen because the greatest muscle damage with the mode of exercise used in this experiment has been found in the medial head of the gastrocnemius (Fleckenstein et al., 1989). The measurement area

was marked with semi-permanent ink (Sharpie) to ensure that the same sites were tested on repeated days. Both right and left calf muscle sites were tested twice (Fisher, 1987). The amount of force used to elicit pain threshold with the algometer was recorded in lbs. Each participant was asked to report when the pressure became painful using the following instructions: "You are going to experience the sensation of pressure. When that pressure turns to pain, at that instant, tell me to stop and I will stop" (Nussbaum and Gabison, 1998). The pressure at which the participant first reported pain was recorded as the pain threshold. These methods are consistent with prior investigations of this type (Hasson, Barnes, Hunter and Williams, 1989; Hasson, Wilbe, Reich, Barnes and Williams, 1992).

Test days 2 through 4

All testing on days 2 to 4 occurred at approximately the same time of day within each subject to minimize potential circadian influences. On testing days 2 to 4, blood pressure, R-III reflex, foot tremor, leg volume and pain measurments were made one time as described above.

Statistical analysis

Preliminary analyses. Data were entered into SPSS (14.0) and checked for the accuracy of data entry. No statistical outliers ($\geq \pm 3$ standard deviations from the mean) were found for any of the dependent variables. Preliminary one-way ANOVAs showed no statistically significant group differences at baseline for any of the dependent variables. The Huynh-Feldt epsilon (ε) was used when the sphericity assumption was violated in both the preliminary and primary ANOVAs. The reliability of some of the dependent measures was analyzed using intraclass correlations (ICC 3,5; Atkinson and Nevill, 1998). The ICC model used was a two way

mixed effect model (subjects were random and trials across time were fixed). The ICC model used absolute agreement and the single measures option within SPSS.

Primary analyses. Effect sizes were expressed as eta-squared (η^2) and percentage change from baseline. The primary hypothesis of this experiment was that the R-III reflex threshold would decrease in the injured leg of the eccentric exercise group after eccentric exercise and remain unchanged in the uninjured leg of the control group. This hypothesis was tested using a two factor repeated measures ANOVA. The factors consisted of two Groups (eccentric exercise and control) and four Times (baseline, 10-min, 24-, 48- and 72-hours after eccentric exercise). A statistically significant Group by Time interaction was predicted. Contrasts of interest were examined and Bonferonni adjustments were made to minimize familywise error.

Results

Muscle Pain Measurement

During exercise lower leg pain intensity was mild for the eccentric group (24.1 ± 19.7 on the 0 to 100 scale) but significantly higher ($F_{1,20} = 15.027$; $p = 0.001$) than the control group (0.9 ± 1.9).

R-III reflex

Eccentric exercise reduced the R-III reflex threshold by 30% at 10 minutes and 23% at 24 hours after exercise (Figure 5.1). This effect was smaller than hypothesized and statistically non-significant (Group x Time interaction $F_{3.5,69.9} = 1.53$; $p = 0.21$; $\eta^2 = 0.07$; $\varepsilon = 0.87$). The main effects for Group ($F_{1,20} = 0.06$; $p = 0.81$; $\eta^2 = 0.003$) and Time ($F_{3.5,69.9} = 2.24$; $p = 0.08$; $\eta^2 =$

0.10; ε = 0.87) also were not significant as were the contrasts of interest (i.e., interaction contrast - pre vs 10 min [$\eta^2 = 0.19$; p = .17] and pre vs 24 hr [$\eta^2 = 0.09$; p = .67]).

The control group R-III reflex was highly reliable across time (ICC 3,5) = .873, p = .85 (Figure 5.2). The exercise group R-III reflex was less reliable across time than the control group (ICC 3,5) = -0.025, p = 0.11 (Figure 5.3). To explore this variability further, the highest and lowest R-III reflex thresholds obtained during the 24 to 72 hour post exercise period were examined. There was a significant Group x Oscillation (minimum and maximum R-III threshold) interaction ($F_{1,20} = 9.91$; p = 0.005; $\eta^2 = 0.33$; ε = 1.00) indicating a difference between the groups in the minimum and maximum R-III thresholds measured during the 24 to 72 hour time periods (Figure 5.4).

Blood Pressure

Blood pressure measurement was highly reliable across time in both the control group (Systolic ICC 3,5 = .878; p = 0.71; Diastolic - ICC 3,5 = .967; p = 0.08) and the eccentric group (Systolic ICC 3,5 = .878; p = 0.47; Diastolic - ICC 3,5 = .879; p = 0.46). There was no evidence of resting hypertension. The main and interaction effects were not statistically significant for either systolic or diastolic blood pressure. The blood pressure data are illustrated in Figure 5.5.

Tremor

Tremor measurement was highly reliable in the control group for all three foot positions: 90° (ICC 3,5) = .937, plantarflexed (ICC 3,5) = .912, and dorsiflexed (ICC 3,5) = .937.

90° Tremor Eccentric exercise increased 90° foot tremor by a moderate amount 24 hours post exercise, however, neither the Group x Time interaction ($F_{1.5,30.7} = 1.83$; p = 0.18; η^2 =

0.08; ε = 0.38; Figure 5.6) nor the main effects for Group ($F_{1,20}$ = 1.02; p = 0.32; η^2 = 0.049) or Time ($F_{1.5,30.7}$ = 1.96; p = 0.17; η^2 = 0.09; ε = 0.38) were statistically significant.

Plantarflexed Tremor Eccentric exercise increased plantarflexed foot tremor by a large amount 30 min after exercise. The Group x Time interaction was statistically significant ($F_{1.2,23.6}$ = 5.74; p = 0.02; η^2 = 0.22; ε = 0.30; Figure 5.7). The contrast of interest was not significant (pre vs 30 min post p = .064; η^2 = 0.259).

Dorsiflexed tremor Eccentric exercise increased dorsiflexed foot tremor by a large amount 24 hours after exercise. The Group x Time interaction was statistically significant ($F_{1.8,35.0}$ = 3.89; p = 0.04; η^2 = 0.16; ε = 0.44; Figure 5.8). The contrast of interest was not statistically significant (pre vs 24 hr p = 0.26; η^2 = 0.162).

Pain

Pain Intensity The pre- and post-exercise pain intensity data are illustrated in Figure 5.9. Eccentric exercise (right leg of the eccentric group) induced a large increase in pain intensity in response to walking down a flight of stairs. The Group x Time interaction was statistically significant ($F_{1.4,28.3}$ = 6.79; p = 0.01; η^2 = 0.25; ε = 0.35). Interaction contrasts showed that, compared to pre-exercise baseline, pain intensity was significantly increased 24 hr (p = 0.05), 48 hrs (p = 0.05) and 72 hrs (p = 0.056) after exercise.

Low intensity exercise that did not injure the control (left) leg had little effect on pain intensity as evidenced by the non-significant Group x Time interaction ($F_{1.7,33.0}$ = 0.15; p = 0.82; η^2 = 0.01; ε = 0.41). The Group ($F_{1,20}$ = 2.51; p = 0.13; η^2 = 0.11) and Time ($F_{1.7,33.0}$ = 0.12; p = 0.85; η^2 = 0.01; ε = 0.41) main effects also were not significant.

Pain Unpleasantness Scores Pre- and post-exercise pain unpleasantness data are illustrated in Figure 5.10. Eccentric exercise (right leg of the eccentric group) induced a large increase in pain unpleasantness following walking down a flight of stairs. The Group x Time interaction was statistically significant ($F_{1.3,26.1} = 4.89$; $p = 0.03$; $\eta^2 = 0.20$; $\varepsilon = 0.33$). Interaction contrasts showed that compared to pre-exercise baseline pain unpleasantness was not significantly increased: pre vs 24 hr ($p = 0.16$; $\eta^2 = 0.20$), pre vs 48 hr ($p = 0.10$; $\eta^2 = 0.23$), pre vs 72 hr ($p = .20$; $\eta^2 = 0.18$).

Low intensity exercise that did not injure the control (left) leg had little effect on pain unpleasantness as evidenced by the non-significant Group x Time interaction ($F_{1.9,38.6} = 0.43$; $p = 0.65$; $\eta^2 = 0.02$; $\varepsilon = 0.48$). The main effects for Group ($F_{1,20} = 0.86$; $p = 0.37$; $\eta^2 = 0.04$) and Time ($F_{1.9,38.6} = 1.19$; $p = 0.32$; $\eta^2 = 0.06$; $\varepsilon = 0.48$) also were not significant.

Pressure algometer pain threshold Reliability of pressure algometer pain thresholds across time in the control group were high for both the right leg (ICC 3,5) = .969 and left leg (ICC 3,5) = .958. The pressure algometer pain threshold data are illustrated in Figure 5.11.

There was a significant Group x Time interaction ($F_{2.6,51.9} = 8.77$; $p = 0.01$; $\eta^2 = 0.31$; $\varepsilon = 0.65$). Interaction contrasts showed that compared to pre-exercise baseline pain thresholds were reduced 24 hrs ($p = 0.004$; $\eta^2 = 0.41$), 48 hrs ($p = 0.008$; $\eta^2 = 0.39$) and 72 hrs ($p = 0.008$; $\eta^2 = 0.39$) after exercise.

Total Leg Volume

The total leg volume data are illustrated in Figure 5.12. Reliability of total leg volume measurements across time in the control group was high (ICC 3,5) = .997. Eccentric exercise altered leg volume to a large degree as evidenced by a significant Group x Time interaction

($F_{2.8,56.3} = 3.98$; $p = 0.01$; $\eta^2 = 0.17$; $\varepsilon = 0.70$). Interaction contrasts showed that compared to pre-exercise baseline, leg volume was increased in the injured leg at 72 hrs ($p = 0.032$; $\eta^2 = 0.31$) post exercise. The effect at 48 hrs after exercise was large but not statistically significant ($p = 0.08$; $\eta^2 = 0.24$).

Discussion

The most novel aspect of the present investigation concerned the effect of eccentric exercise on the nociception flexion reflex (R-III). To understand those results, it must be appreciated that the eccentric exercise stimulus used here caused changes in inflammation, non-nociceptive neurology and pain that were small (inflammation measure) or smaller (tremor and pain) than what has been typically produced in prior investigations involving eccentric exercise.

Inflammation, a complex physiological process involving numerous cells and chemical reactions, was inferred here from an increase in total lower leg volume. The small, statistically significant increase in leg volume of 2.9% (average change from baseline from 24 to 72 hours) found in the present experiment was similar to what has been observed in prior investigations using similar methods. For example, an average increase in leg volume over several days of ~ 2% was reported following 1 hour of eccentrically biased exercise consisting of backwards walking at a 13 degree angle (Whitehead, Allen, Morgan & Proske, 1998).

The measurements of foot tremor provided information about eccentric exercise induced changes in non-nociceptive neurology. Large, though statistically non-significant, increases in foot tremor were found in one foot position at 30 minutes (113% increase from baseline in the plantarflexed position) and a different foot position at 24 hours (67% in the dorsiflexed position) after eccentric exercise. The only prior experiment that has examined the influence of eccentric

exercise on tremor reported that 50 maximal actions of the nondominant forearm flexors performed by 12 adults significantly increased hand tremor (a 90^{o} position) by ~360% immediately following exercise and by ~100% 24 hours post-exercise (Saxton et al., 1995). We speculate that the magnitude of the effect on tremor is smaller in the present investigation because our exercise protocol likely resulted in less muscle injury. The mean pain intensity ratings, which were ~2.5 times higher in the Saxton study, support this possibility. Our findings show that the pattern of foot tremor response to eccentric exercise varied as a function of joint position. Because an increased tremor suggests a reduced ability to control posture and movement (Leger & Milner, 2000), the present findings imply that the effect of eccentric exercise on motor control and joint stability varies as a function of joint position.

As has been observed in most investigations involving novel eccentric exercise, we found that pain ratings were significantly increased 24 to 72 hours following exercise. Similar effects were found for pain threshold resulting from pressure applied to the medial head of the gastrocnemius and both the intensity and unpleasantness of the pain experienced in the calf muscles while walking down a flight of 15 stairs. The pain intensity and unpleasantness ratings here of less than 20 on a 0 to 100 scale were lower than typically reported in experiments of this type. For example, 70 maximal eccentric actions of the biceps brachii resulted in mean pain intensity ratings made in response to palpation during 24 to 72 hours post-exercise of ~5.5 on a 0 to 10 scale in a sample of 109 adults (Clarkson, Nosaka, and Braun, 1992). The pain intensity reported by those test subjects is approximately three times the intensity reported in the present investigation. In sum, the central point of the first part of this discussion is that it is important to recognize that the injury induced in the present investigation was relatively mild when considering the magnitude of the R-III reflex results.

This was the first experiment to examine the effects of eccentric exercise on the nociceptive flexion reflex in humans. Ten minutes after eccentric exercise, the R-III reflex threshold was reduced by a moderate amount (30%). Changes in the R-III threshold of the control group did not exceed 3.2% across time. It is probable that an effect of this size was not statistically significant because the sample size was too small. The sample size was selected based on the assumption that a larger effect would occur. The R-III results at 10 minutes after exercise were unexpected in part because prior investigations involving resistance exercise have reported hypoalgesia in pain tolerance (Bartholomew, Lewis, Linder, and Cook, 1996) and pain threshold (Koltyn and Arbogast, 1998) after exercise. A decrease in pain theshold after any mode or intensity of exercise appears to have never been reported (Koltyn, 2000; Koltyn, 2002; Koltyn, & Umeda, 2006). These prior investigations, however, neither attempted to produce muscle injury nor measured the R-III reflex. One of the only investigations examining the R-III reflex after any form of physical activity showed an increase in R-III reflex threshold after five minutes of gum chewing, a small muscle physical activity that is not eccentrically biased (Mohri et al., 2005).

Twenty four hours after eccentric exercise, the R-III reflex threshold was reduced by a moderate amount (23%). One possible reason that a larger mean effect on the R-III reflex was not observed at the 24 to 48 hour measurement times is the large individual variability in the time course of responses to eccentric exercise. For example, muscle pain intensity is highest 24 hours after novel eccentric exercise for some test subjects, but others report peak pain as much as two days later (Poudevigne, O'Connor & Pasley, 2002). In the present experiment, the lowest R-III threshold assessed between 24 and 78 hours post eccentric exercise occurred most frequently 24 hours post exercise. Nonetheless, 45% of our sample had an R-III minimum at either the 48 or

72 hours post injury measurement period. A primary finding of the present investigation was that eccentric exercise increased the variability of the R-III reflex threshold measured from 24 to 48 hours after exercise. The eccentric exercise group had both higher maximal R-III thresholds and lower minimum R-III thresholds during the three days following eccentric exercise (Figure 5.4). We speculate that this effect was due in part to large individual differences in the timing of multiple biological responses to eccentric exercise, not measured here, that underlie the R-III reflex.

Two plausible mechanisms by which eccentric exercise could reduce the R-III reflex threshold include central sensitization and peripheral algesics. Eccentric exercise causes muscle damage and various mediators of inflammation are released as a result. Bradykinin, serotonin, histamine, luekotrienes, cytokines, amines, interleukin 1 and 8, substance P, calcitonin gene related peptide, hydrogen ions, nerve growth factor, protein kinase C, and adenosine are all mediators of inflammation associated with tissue damage. Bradykinin, serotonin, histamine and hydrogen ions for example, are known sensitizers of nociceptive receptors. It is plausible that the decrease in R-III reflex threshold was caused by a release of various algesic substances.

Central sensitization is a key feature of inflammatory pain (Campbell and Meyer, 2006). Central sensitization refers to an increased excitability of brain or spinal cord neurons involved in nociceptive neural networks. The increased excitability amplifies all sensory input such that normally innocuous stimuli can become painful, noxious stimuli are perceived as more painful than usual, and/or less afferent activity is needed to cause pain. Inflammation induced increase in the sensitivity of Type IV afferents is thought to contribute to central sensitization. Inflammation induced by eccentric exercise rarely has been used to study central sensitization.

Our research design and test subject exclusion criteria eliminated some of the known factors that could have confounded the R-III results. The R-III effects observed in the present experiment were not moderated by sex (Mylius et al., 2005), resting systolic blood pressure (Ghione, 1996), variations in foot position (McMillan & Moody, 1986) or chronic pain conditions such as fibromyalgia (Desmeules et al., 2003). We did not control for several variables that could have introduced error into the R-III reflex threshold results. For example, the timing of the presentation of the noxious stimulus within the cardiac cycle was not controlled. It has been demonstrated that noxious stimuli presented 300 msec after the r-wave of the ECG was associated with increased R-III threshold compared to other times in the cardiac cycle (i.e., 0 and 600 msec after the r-wave) (McIntyre, Edwards, Ring, Parvin, and Caroll, 2006). In addition, the cognitive processes by which individuals cope with pain were not measured despite evidence suggesting these processes can influence the R-III reflex (Emery et al., 2006). Emotions also were not measured yet it has been shown that negative emotions enhance the R-III reflex while positive emotions inhibit the reflex (Rhudy, Williams, McCabe, Rambo, and Russel, 2005). Future experiments could more clearly determine the influence of eccentric exercise on the R-III by presenting the nociceptive stimuli in conjunction with standardized emotional stimuli (e.g., pleasant, neutral and unpleasant photographs) at a specific time in the cardiac cycle.

In conclusion, eccentric exercise that induces modest changes in indicators of muscle injury increases the range of oscillation in the R-III reflex threshold 24 to 72 hours after the exercise.

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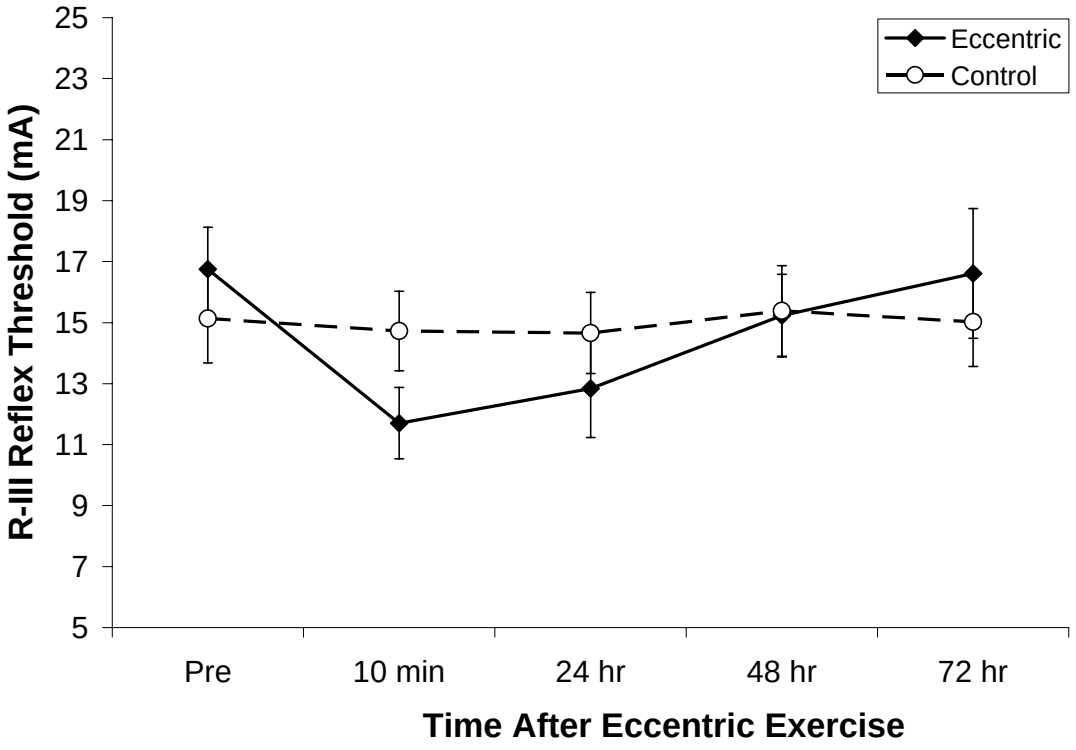
Figure 5.1.

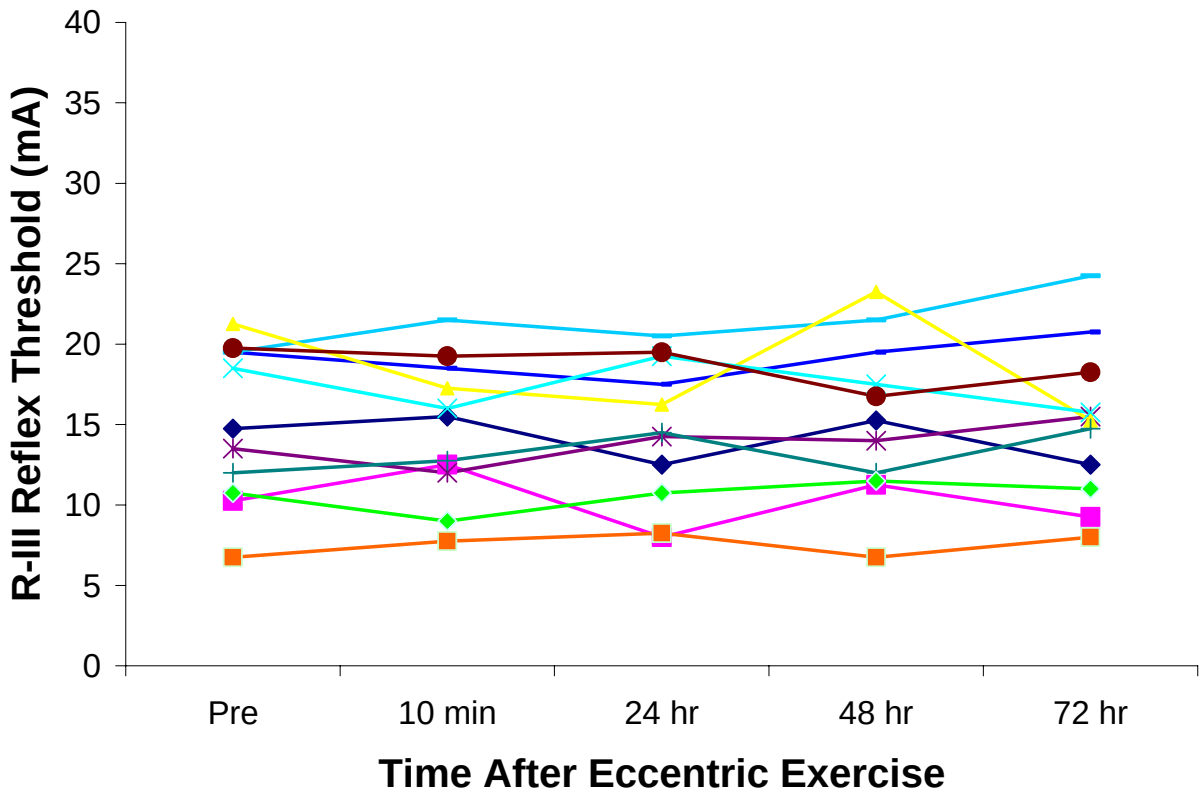
R-III reflex threshold over time



Note: *Data are means ± SE*

Figure 5.2.

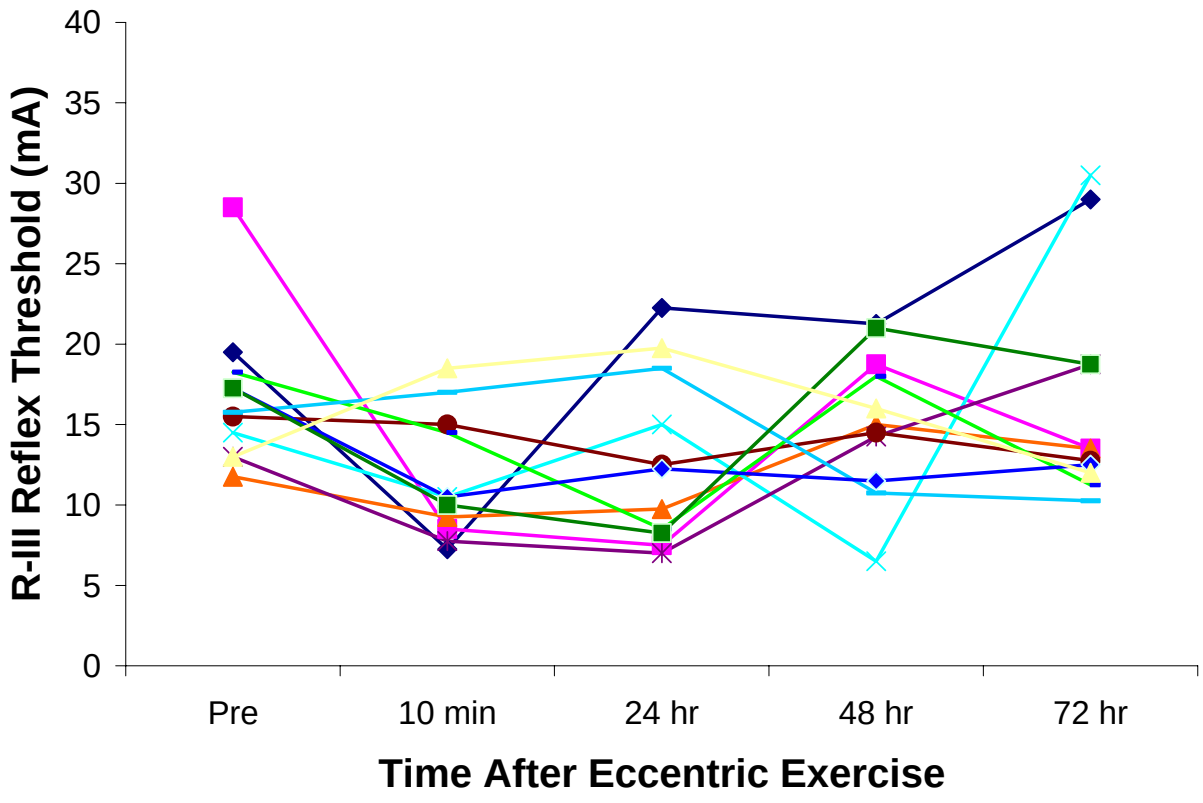
R-III reflex threshold individual scores over time for the control group



Note: *Data are means ± SE*

Figure 5.3.

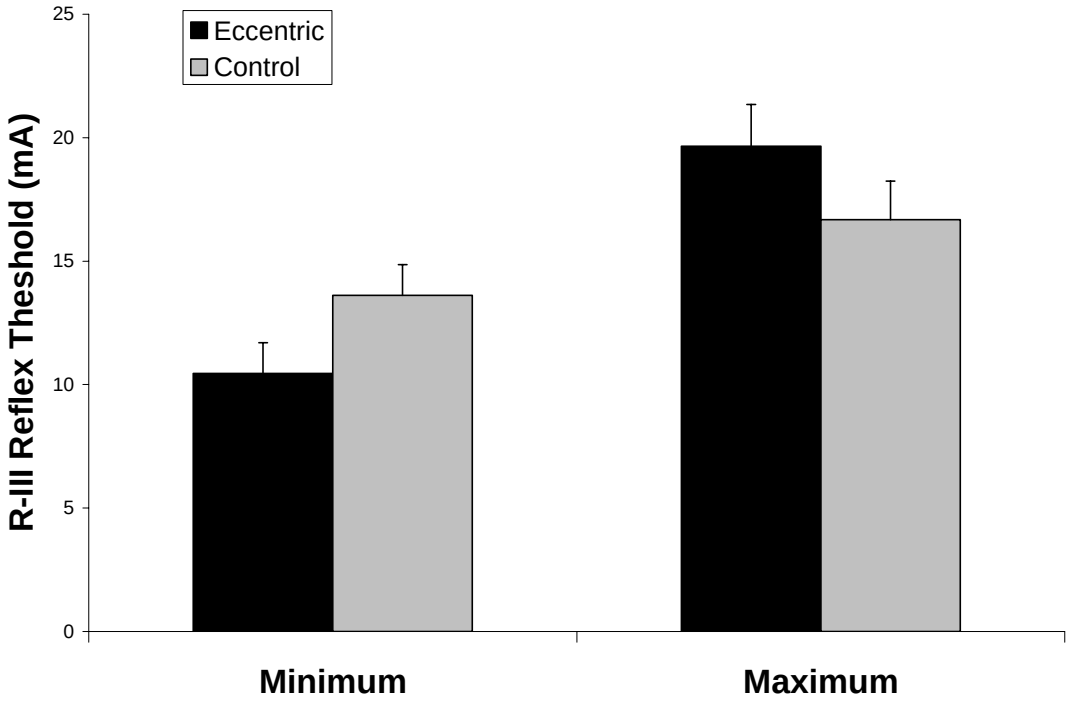
R-III reflex threshold individual scores over time for the eccentric group



Note: *Data are means ± SE*

Figure 5.4.

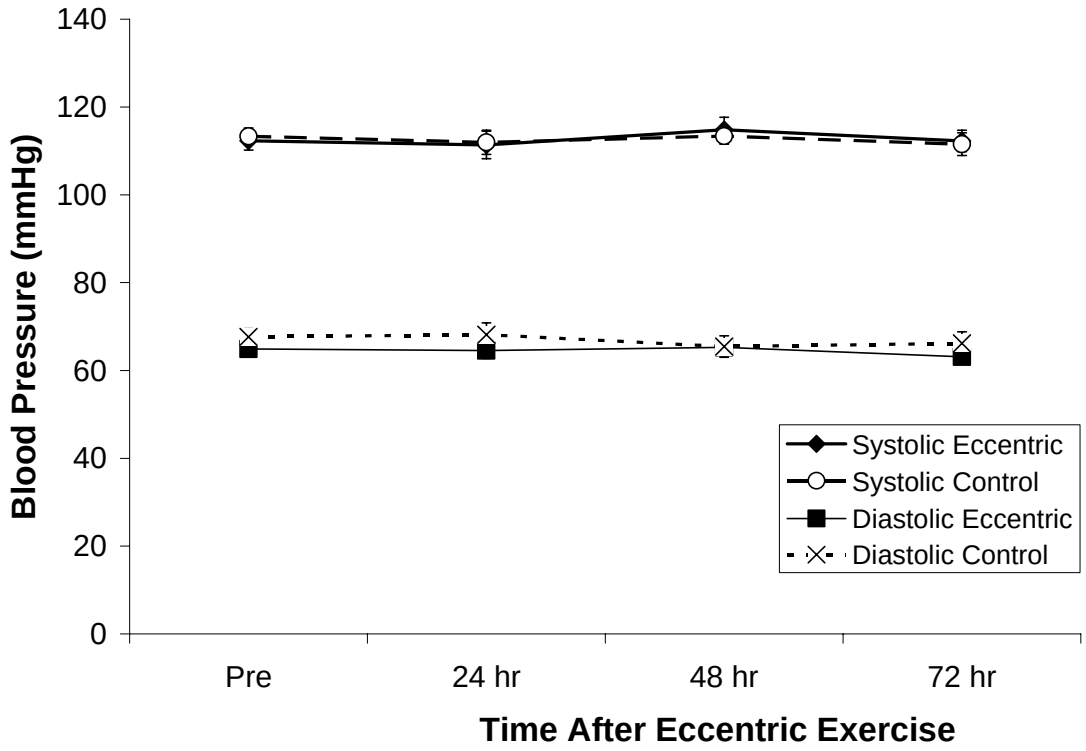
Highest and lowest R-III reflex thresholds over 3 days (24 to 72 hrs) post eccentric exercise



Note: *Data are means ± SE*

Figure 5.5.

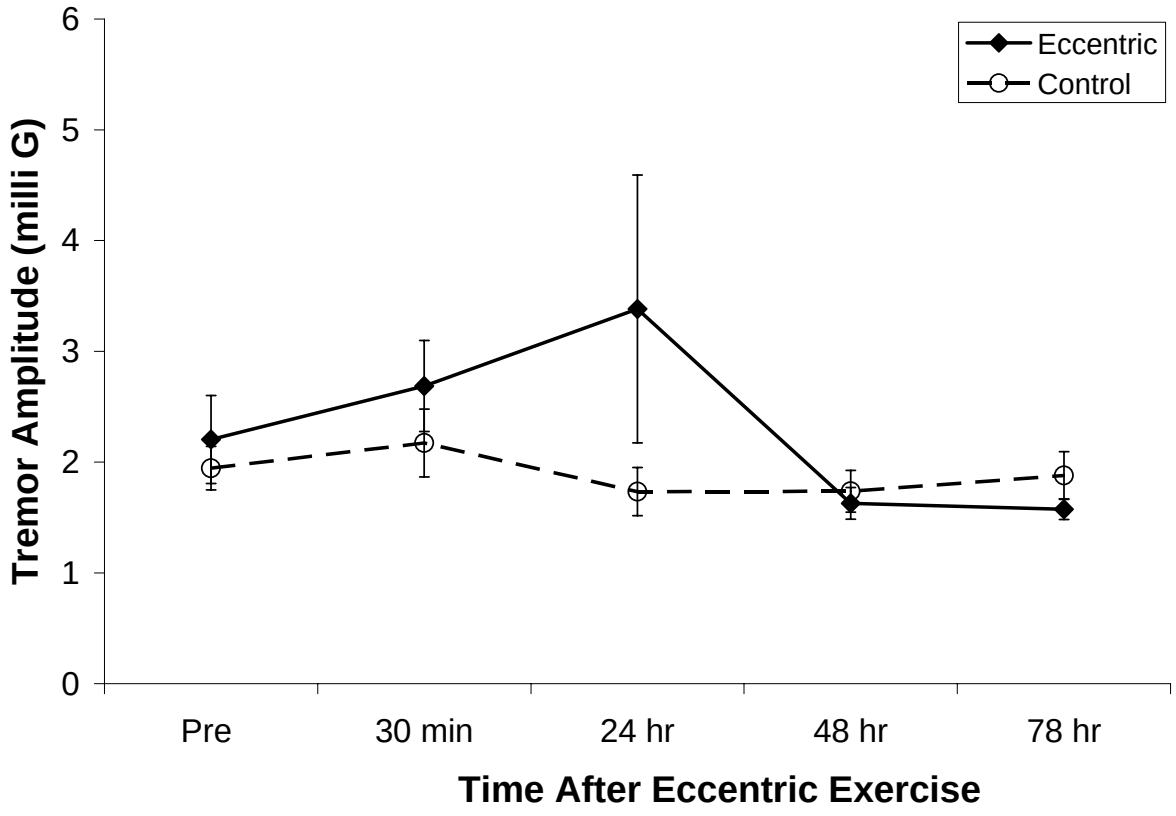
Systolic and diastolic blood pressure prior to R-III reflex elicitization over time



Note: *Data are means ± SE*

Figure 5.6.

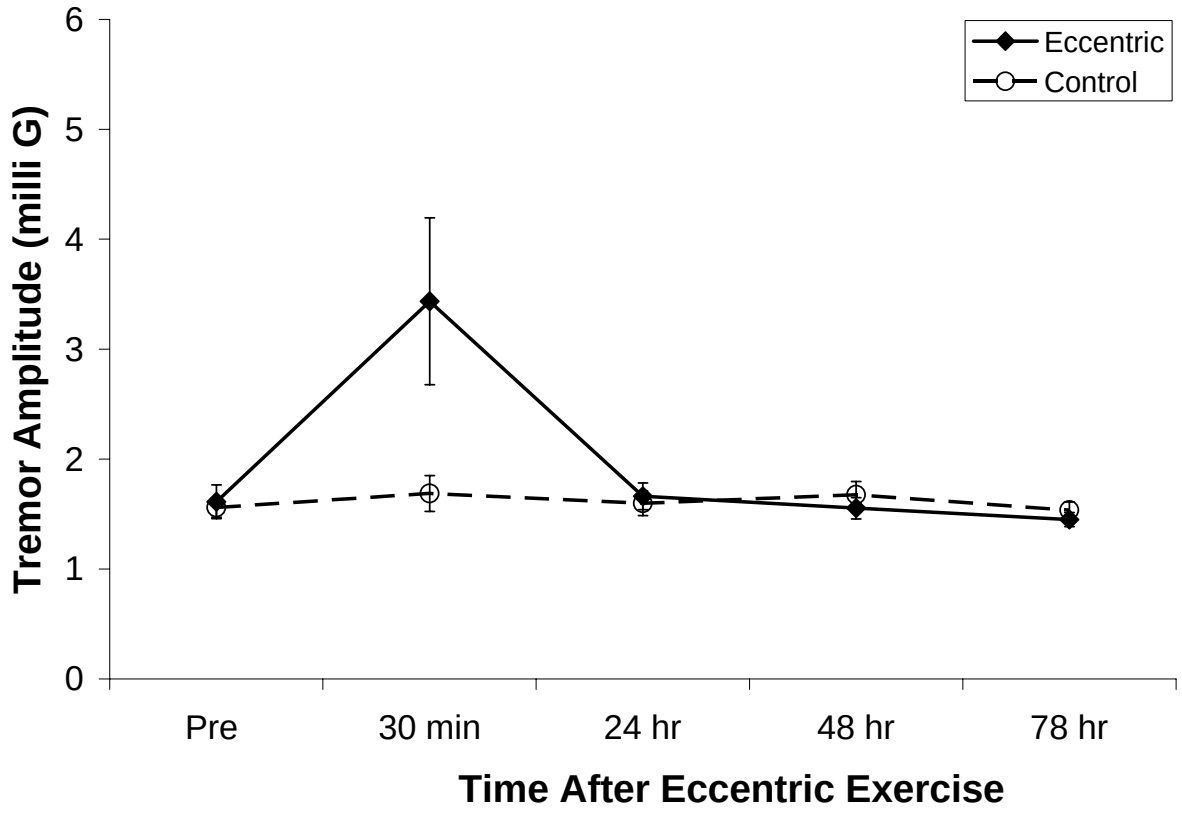
Physiologic foot tremor (90°) over time



Note: *Data are means ± SE*

Figure 5.7.

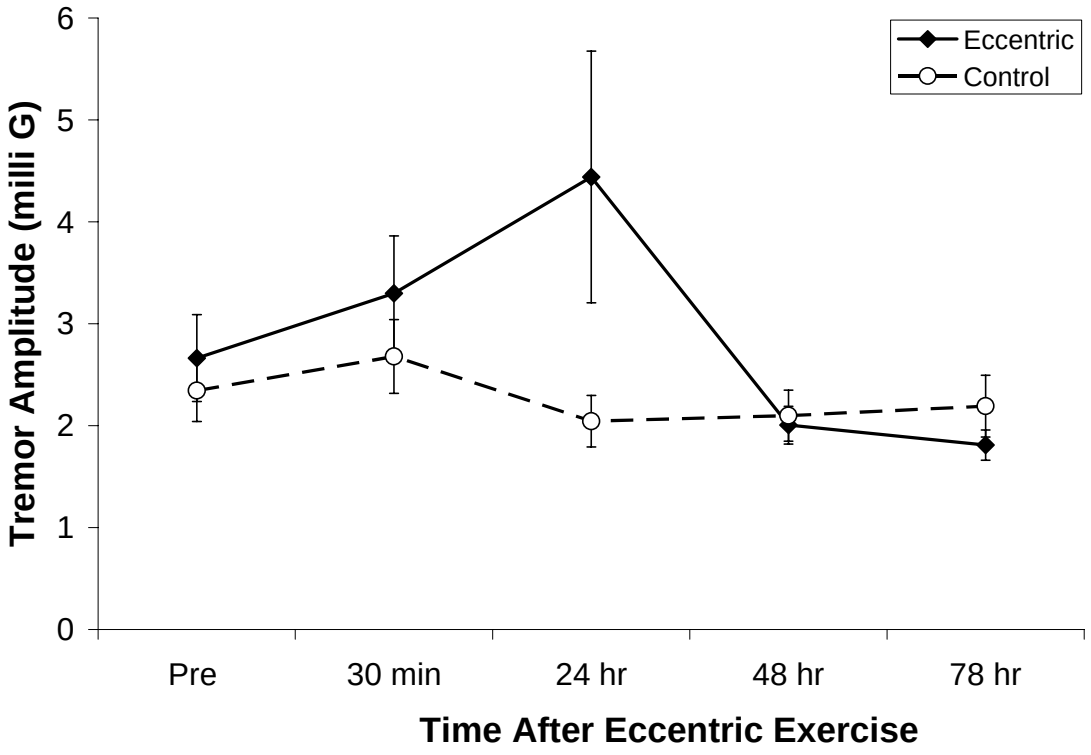
Physiologic foot tremor (plantarflexed) over time



Note: *Data are means ± SE*

Figure 5.8.

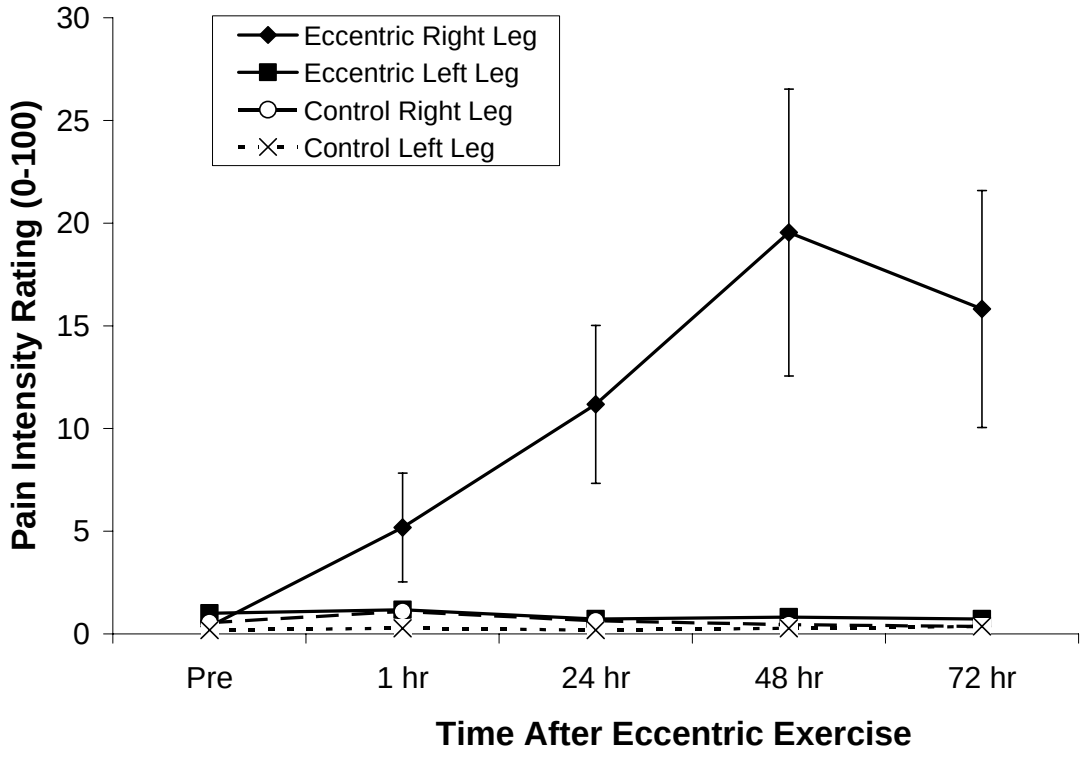
Physiologic foot tremor (dorsiflexed) over time



Note: *Data are means ± SE*

Figure 5.9.

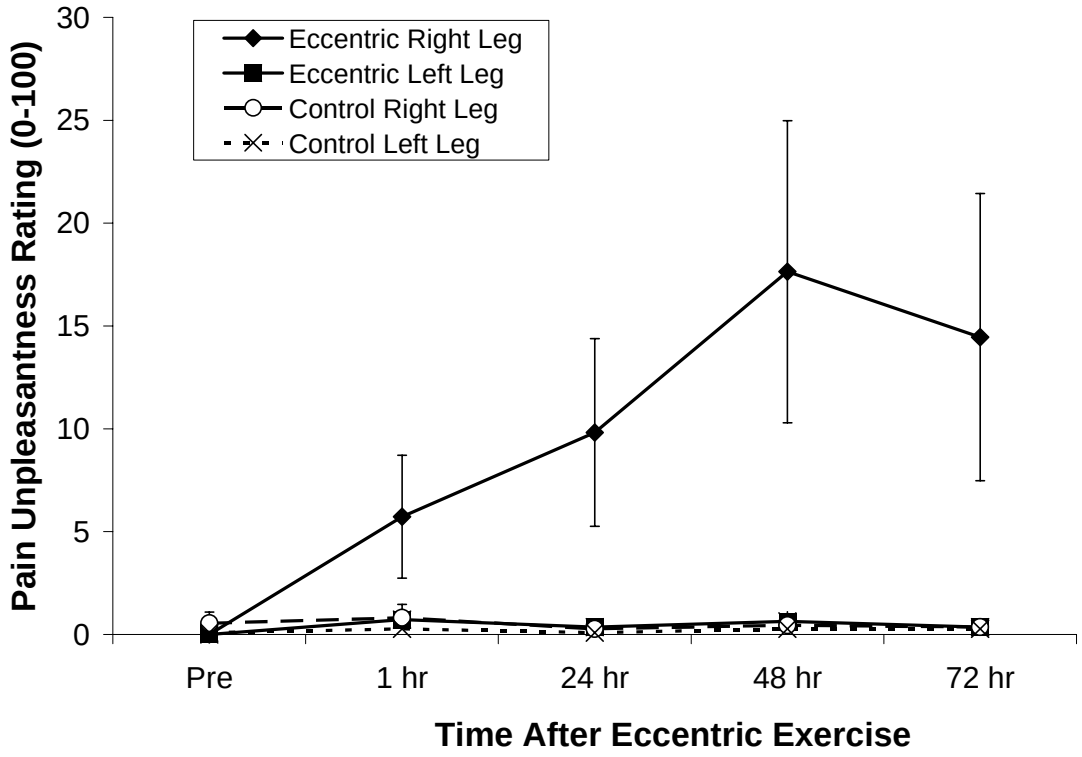
Pain intensity ratings over time in response to walking down stairs



Note: *Data are means ± SE*

Figure 5.10.

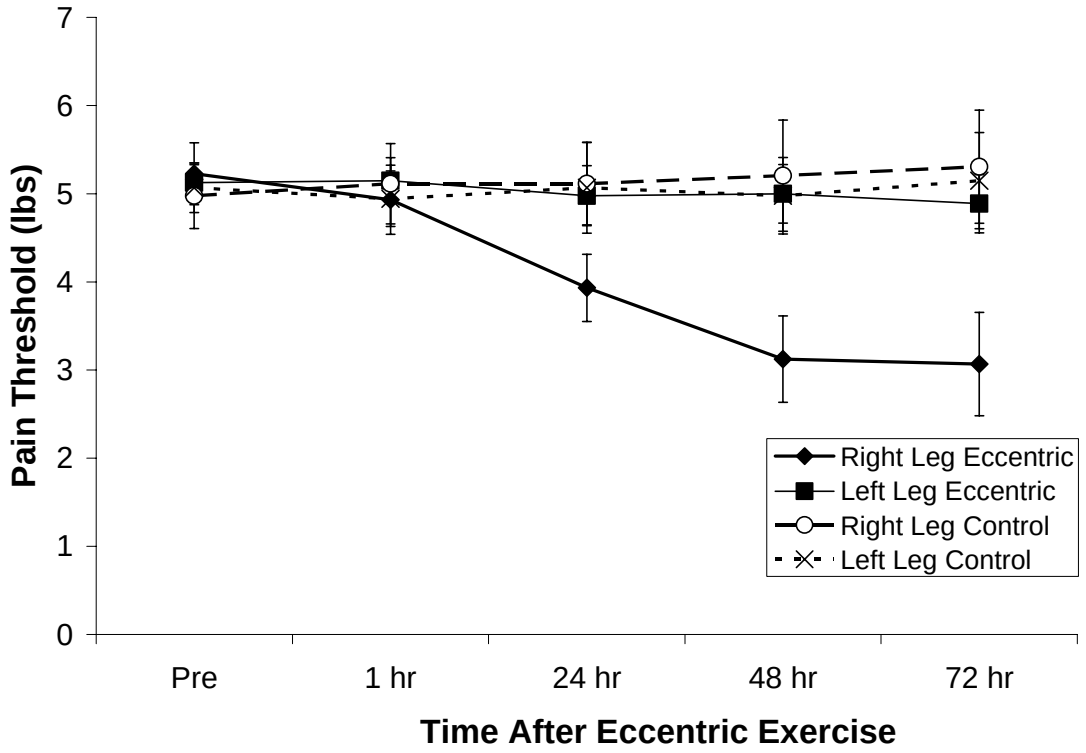
Pain unpleasantness ratings over time in response to walking down stairs



Note: *Data are means ± SE*

Figure 5.11.

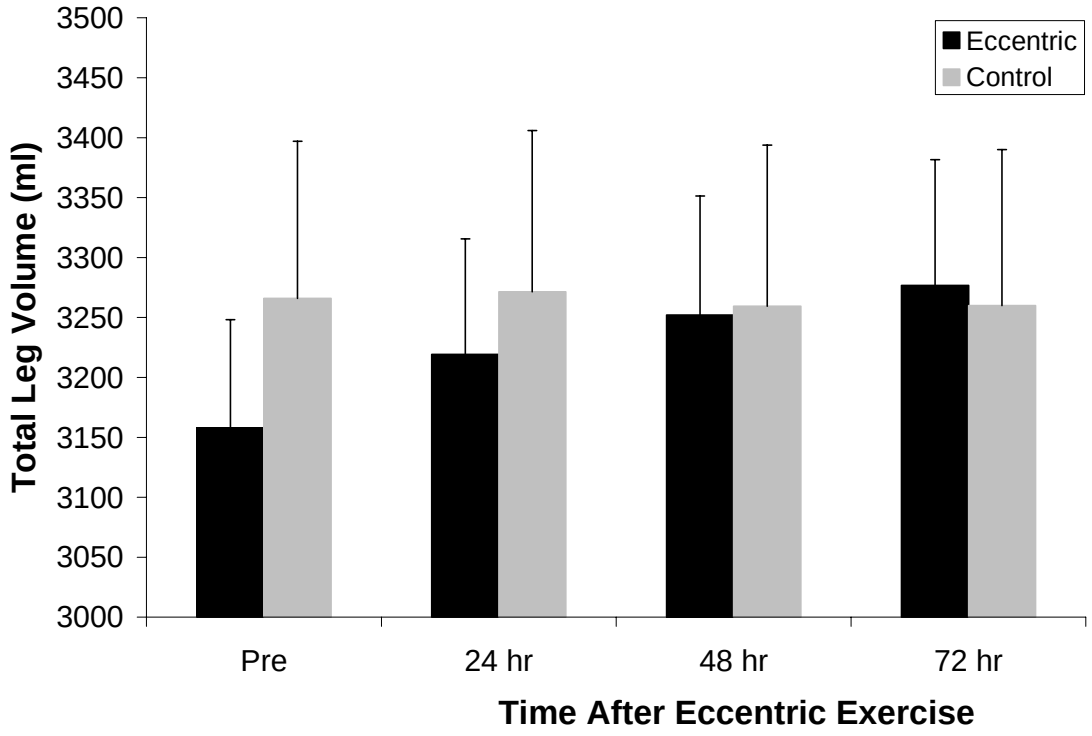
Pressure algometer induced pain thresholds over time



Note: *Data are means ± SE*

Figure 5.12.

Total leg volume over time



Note: *Data are means ± SE*

Figure 5.13: Delayed-onset-muscle-pain inducing exercise consisting of 2 set of 50 heel drops separated by 5 min rest.

CHAPTER 6

SUMMARY AND CONCLUSIONS

This dissertation examined the effects of lower leg eccentric actions on the nocicpetive flexion (R-III) reflex, foot tremor, total lower leg volume and delayed onset muscle pain. The main purpose of this dissertation was to determine the effect of muscle damaging eccentric exercise on the underlying neurology. Previous literature examining eccentric exercise has focused primarily on physiological mechanisms underlying muscle damage, but for the most part has neglected what happens to the nervous sytem after muscle damaging exercise. Measurement of the effects caused by eccentric exercise on the underlying neurology were obtained here by using the nociceptive flexion (R-III) reflex and foot tremor. The main focus of this dissertation was on the changes in the R-III reflex because no prior investigations have examined the effect of eccentric exercise on nociceptive neurology.

Physiological tremor (used as an indicator of non-nociceptive neurology) had been measured only one time in prior eccentric exercise experiments (Saxton et al., 1995), however, the day-to-day reliability of tremor was not established in that study. Similarly, water displacement (used here as an indicator of inflammation) has rarely been used in eccentric exercise experiments (Whitehead, Allen, Morgan, and Proske, 1998; Whitehead, Weerakkody, Gregory, Morgan, and Proske, 2001) and the day-to-day reliability had not been established in the prior studies. In order to determine the effects of eccentric exercise on day-to-day changes in any physiological or psychological measure, adequately high day-to-day relibility is

advantageous. The initial investigations in this line of research, therefore, examined the day-to-day reliability of leg volume and foot tremor.

As detailed in Chapter 3, lower leg volume was measured by water displacement on 5 consecutive days in 30 young adults (15 men and 15 women). The mean percentage change across days compared to day 1 ranged between 0% and 0.37% and the intraclass correlation was .972. Thus, the results revealed a high day-to-day reliability for lower leg volume. It was concluded that water displacement offers a cost effective and reliable solution for the measurement of lower leg edema across days. As detailed in Chapter 4, foot physiologic tremor was measured by single plane accelerometer after being seated for 30 min on 5 separate days at least 24 hours and not more than 48 hours apart in 31 young adults (15 men and 16 women). Foot tremor was assessed in two positions: at rest and during dorsiflexion to 90º. The results revealed poor day-to-day reliability for foot tremor in a resting condition (ICC 3, 5) = .332 and adequately high day-to-day reliability for the 90º active condition (ICC 3, 5) = .821. It was concluded that foot tremor can be assessed reliably across days using a single plane accelerometer.

After establishing high day-to-day reliability in leg volume and tremor, these measures were used in the primary experiment which was aimed at examining the influence of eccentric exercise on the R-III reflex threshold. Twenty two young adult females were randomized to either an eccentric exercise (100 heel drops) group (n=11) or a no exercise control group (n=11). Before and after the exercise or control condition (10 min, 24-, 48- and 72-hrs post), measurements were made of the R-III reflex threshold, physiologic foot tremor, leg volume and muscle pain. From 24 to 72 hours following eccentric exercise, small increases in leg volume (mean increase of 2.5% compared to baseline), foot tremor (mean increase of -0.2% across three

foot positions), pain (mean score from 24 to 72 hours on a 0-100 scale was 17 indicating mild pain) were found as was a small decrease in R-III reflex threshold (-11.1%). Repeated measures ANOVA revealed that the range of oscillation in the minimum and maximum R-III threshold 24 to 48 hours after eccentric exercise was significantly greater than the control condition ($F_{1,20}$ = 9.91; p = 0.005; η^2 = 0.33; ε = 1.00). It was concluded that eccentric exercise inducing modest changes in indicators of muscle injury increases the range of oscillation in the R-III reflex threshold 24 to 48 hours after the exercise.

Implications for Future Research:

Future investigations should examine dose-response relationships between eccentric exercise and nociception. The present results suggest both that manipulating exercise intensity and including a measure of nociception immediately following exercise could provide fruitful insights. The large non-significant decrease in R-III reflex threshold 10 min after eccentric exercise observed in the present work was an unexpected finding and warrants further attention. Potential investigations could use anti-histamines and other compounds prior to the exercise to determine the role of various algesics in alterations in pain threshold sensitivity following eccentric exercise. The role the spinal cord plays in pain threshold regulation following eccentric exercise could potentially be examined by damaging the biceps muscles in one arm and examining the influence on the R-III reflex in the ipsilateral and contralateral leg. The R-III reflex could also be used with other techniques, including EEG, MRI, and the ischemic block technique (Barlas, Walsh, Baxter and Allen, 2000) to more fully understand nociceptive changes with eccentric exercise.

Mode of the exercise needs to be examined more closely with regard to alterations in pain and nociception following exercise. The majority of the literature shows an increase in pain threshold but that work has primarily been associated with high intensity aerobic exercise. Few studies have examined the effects of resistance exercise on pain thresholds and this was the first to use eccentric muscle damaging exercise. It might be concluded from the available evidence that a short intense bout of exercise should be recommended to those people who do not like anesthetics during dental visits. However, this may not be the best advice to those who are weight lifters and prefer to use resistance exercise.

Future studies also could examine the role of peripheral opioids in R-III responses to eccentric induced muscle injury. Inflammation upregulates opioid receptors (Stein, Millan, Shippenberg & Herz, 1988). Peripherally acting opioid agonists reduce delayed onset muscle pain, and this effect is reversed by naloxone (Tegeder, Meier, Burian, Schmidt, Geisslinger, and Lotsch, 2003). Because blocking opioids also increases the R-III reflex (France, al'Abisi, Ring, France, Harju, and Wittmers, 2007), the weight of the available evidence suggests that opioids are involved in R-III responses to eccentric induced muscle injury. The present investigation has introduced more questions than it answered and it is hoped that future investigations should be directed in these research areas.

References

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APPENDICES

APPENDIX A

INFORMED CONSENT FORM

Informed Consent

I, _____(please print), agree to participate in a research study titled "The effect of lower leg eccentric exercise on the nociceptive flexion reflex and its relationship to delayed-onset muscle soreness," which is being conducted by Jeffrey D. Pasley, a doctoral student with the Department of Kinesiology at the University of Georgia, under the direction of Dr. Patrick J. O'Connor, a faculty advisor with the Department of Kinesiology at the University of Georgia. My participation is voluntary; I can refuse to participate or I can stop taking part at any time without giving any reason, and without penalty. I can ask to have information related to me returned to me, removed from the research records, or destroyed.

Purpose of the research. The primary purpose of the research is to learn about the day - to - day variation in lower leg volume, foot tremor and a leg reflex in relation to delayed-onset muscle soreness.

Benefits of participating. The benefits that I may expect to receive from participating are as follows: (1) I will learn what my resting blood pressure is; if my blood pressure is elevated above normal then the researchers will urge me to see my physician about it and (2) I will learn about the process of human research in the field of exercise science.

Incentives for participating. The incentives that I may expect o receive from participating are as follows: (1) I will receive $50 for the completion of all four testing sessions ($12.50 for each session completed, but only if the study is funded) and (2) I may be able to obtain extra course credit for participating. I understand that equal non-research options for extra credit are available.

Description of procedures. My participation in this study will involve a total of ~ 7 hours and tests will be performed on 4 separate days. Testing on day 1 will last about 2 hour and 30 min and testing on days 2, 3, and 4 will last about 1 hour and 30 min.

If I volunteer to take part in this study, I will be asked to do the following things:
1. Answer questions about my medical history, mood, and past experience with exercise (15-20 min).
2. Have my seated blood pressure measured.
3. Have my right ankle stimulated with a small electrical current. The intensity of the stimulus will be progressively increased and hamstring muscle responses will be recorded. I will provide ratings of any pain or unpleasantness I might feel after each stimulus.
4. Have measurement of physiological tremor taken from my right foot in which an accelerometer will be placed on top of my right foot. (~ 20 min)
5. Have my lower leg volume measured with a partial leg volumeter, which will require placement of my right foot and leg up to my knee in water.
6. Take part in exercise consisting of 2 sets of 50 toe raises with my right leg, with a 5 min rest between sets **or** perform 2 sets of 50 up and down movements of my feet while sitting at the edge of a medical examination table with no load on my feet.

7. Answer questions about pain intensity and unpleasantness associated with the exercise.
8. On days 2, 3, and 4 I will also complete the leg volume, foot tremor and leg reflex measurements.

Discomforts I may experience. My skin may become slightly red where the electrodes are placed. This redness usually will go away within a few hours. The risk of transmission of viral or bacterial agents will be minimized by cleaning each recording electrode after every use. The stimulation of my ankle may be unpleasant and result in brief, minor physical discomfort. The possible unpleasantness ranges from a slight tactile sensation to the experience of a rubber band being snapped on my skin. I understand that at any time, if I feel uncomfortable with the experiment, I may request that the researchers stop immediately and that I can withdraw my participation at any time without any negative consequences.

It is expected that I will experience a decrease in my ability to fully flex and extend my lower leg after performing the toe raise exercises. Also, it is expected that I will experience a small amount of lower leg swelling and discomfort and moderate pain in my calf muscles.

Potential health risks. Although not likely, it is possible that I could develop rhabdomyolysis, a condition where muscle proteins are released into the bloodstream. Although rare this condition can lead to kidney damage and failure. A primary sign of this condition is dark or red colored urine. Proper hydration prior to and following unaccustomed exercise can aid in prevention of this condition. I will be required to drink 8 oz of water prior to and following testing. I will be informed of the need for proper hydration and will be encouraged to drink water liberally thoughout the duration of the study. If these things were to happen to me than I would **immediately** contact Jeff Pasley (542-4381; 549-9276) or Patrick O'Connor (542-4382; 543-9000) and/or seek medical assistance from the UGA health center. A person trained in CPR will be present at all times in case of an emergency. In case of an emergency, the UGA police will be called and I will be taken to the UGA Health Center or the hospital of my choice. I understand that I will be responsible for the payment of any medical assistance.

Confidentiality. Any information the researcher obtains about me as a participant in this study, including my identity, will be held confidential. My identity will be coded, and all data will be kept in a secured, limited access location. My identity will not be revealed in any publication of the results of this research. The only people who will know that I am a research participant will be the members of the research team. The results of this participation will be confidential and will not be released in any individually identifiable form, unless otherwise required by law. A copy of my test results and the study will be made available to me.

Further questions. The Principle Investigator, Jeffrey Pasley, or Dr. Patrick O'Connor will be happy to answer any further questions about the research, now or during the course of the project. Jeffrey Pasley can be reached by telephone at (706) 542-4381 or (706) 549-9276. Dr. O'Connor can be reached at (706) 542-4382.

My signature below indicates that the researchers have answered all of my questions to my satisfaction, and that I consent to volunteer for this study. I have been given a copy of this form.

Jeff D. Pasley		
Name of Researcher	**Signature**	**Date**

Telephone: (706) 542-4381
Email: jpasley@uga.edu

Name of Participant	**Signature**	**Date**

Additional questions or problems regarding your rights as a research participant should be addressed to The Chairperson, Institutional Review Board Human Subjects Office, University of Georgia, 612 Boyd Graduate Studies Research Center, Athens, Georgia 30602-7411; Telephone (706) 542-3199; E-Mail Address IRB@uga.edu

APPENDIX B

MEDICAL HISTORY QUESTIONNAIRE

Self-Administered Pre-Exercise Medical History Form
EXERCISE PSYCHOLOGY LAB
UNIVERSITY OF GEORGIA, RM 102A
ATHENS, GA
706-542-4381

DEMOGRAPHIC INFORMATION

NAME_____ BIRTH DATE_____/_____/_____
Last First MI Month Day Year

AGE_____

HEIGHT _____ in___ cm___

WEIGHT _____ lb___ kg___

Sex ___ Male
___ Female

STATEMENT OF CONFIDENTIALITY

I understand that information contained on this questionnaire is regarded as confidential, and will not be released without my prior written permission. The investigators may, however, use the information for statistical and other research purposes.

_____ _____
Signature Date

1. Do you have or have you had: (Check if yes)

Had	Have		Had	Have	
___	___	Pain in heart or chest	___	___	Sickle Cell Anemia
___	___	Heart attack	___	___	McArdle's Disease
___	___	Rheumatic fever	___	___	Coughing
___	___	Diseases of the arteries	___	___	Cough on exertion
___	___	Varicose veins	___	___	Bronchitis
___	___	Heart murmur	___	___	Asthma
___	___	Any heart problems	___	___	Pneumonia
___	___	Abnormal EKG	___	___	Abnormal chest X-ray
___	___	Extra or skipped heart beats	___	___	Other lung diseases
___	___	Phlebitis	___	___	Nervous or emotional problems
___	___	Dizziness or fainting spells	___	___	Back pain
___	___	Stroke	___	___	Badly swollen ankles
___	___	Epilepsy	___	___	Swollen, stiff or painful joints
___	___	Anemia	___	___	Arthritis of arms or legs
___	___	Diabetes	___	___	Compartment syndrome
___	___	Hypertension	___	___	Tendonitis
___	___	Scarlet fever	___	___	Rhabdomyolysis
___	___	Gout	___	___	Injuries to back, arms, legs or joints

2. Explanation or comments:_____

_____

3. Date of last complete medical exam: _____Were results, normal?_____

If no, explain:_____

_____

4. Have any of your blood relatives had ANY of the following? (Give ages when ailment occurred and if fatal.)

	Mother	Father	Grandfather	Grandmother	Other
Heart attack					
Stroke					
High blood pressure					
Elevated cholesterol					
Congenital heart surgery					
Diabetes					
Obesity					

5. Have you ever smoked:

Cigarettes?	_____	Age started _____	Age quit _____	No. per day_____
Cigars?	_____	Age started _____	Age quit _____	No. per day_____
Pipe?	_____	Age started _____	Age quit _____	No. per day_____

6. Do you know of any medical problem that might make it dangerous or unwise for you to participate in vigorous exercise?_____ If yes, please explain:_____

_____

7. Do you know of <u>any other reason</u> why you should not do physical activity?

_____

8. Do you experience discomfort, shortness of breath or pain with moderate exercise?

_____

9. Does your usual job require sustained physical activity/physical labor?
___ Yes ___ No ___ Not employed ___ Not applicable (retired)

10. Is your occupation: (circle)
Sedentary (virtually no movement involved)
Inactive (small amount of movement involved)
Active (walking, light lifting involved)
Heavy work (heavy lifting and/or lots of movement)

11. Does your leisure time activity require endurance activity or lifting tasks?
___ Yes Explain_____

12. Are you currently involved in a regular exercise program? _____ If yes, indicate type and amount of exercise.

	Distance	or	Time	Frequency
Dance	_____		_____	_____
Cycling	_____		_____	_____
Walking	_____		_____	_____
Running	_____		_____	_____
Swimming	_____		_____	_____
Others	_____		_____	_____

13. Please indicate your usual activities.

	Frequency per month					Minutes per session				RPE(s)
	1-4	5-8	9-12	13-16	17+	0-20	20-40	40-60	60+	
__ Badminton	___	___	___	___	___	___	___	___	___	___
__ Baseball/softball	___	___	___	___	___	___	___	___	___	___
__ Boating	___	___	___	___	___	___	___	___	___	___
__ Bowling	___	___	___	___	___	___	___	___	___	___
__ Cycling (motor)	___	___	___	___	___	___	___	___	___	___
__ Cycling (road)	___	___	___	___	___	___	___	___	___	___
__ Cycling (stationary)	___	___	___	___	___	___	___	___	___	___
__ Dancing (aerobic)	___	___	___	___	___	___	___	___	___	___
__ Dancing (social)	___	___	___	___	___	___	___	___	___	___
__ Golf (ride)	___	___	___	___	___	___	___	___	___	___
__ Golf (walk)	___	___	___	___	___	___	___	___	___	___
__ Gymnastics	___	___	___	___	___	___	___	___	___	___
__ Hiking	___	___	___	___	___	___	___	___	___	___
__ Home repair	___	___	___	___	___	___	___	___	___	___
__ Horseback riding	___	___	___	___	___	___	___	___	___	___
__ Housework	___	___	___	___	___	___	___	___	___	___
__ Hunting, fishing	___	___	___	___	___	___	___	___	___	___
__ Jogging/running	___	___	___	___	___	___	___	___	___	___
__ Martial arts	___	___	___	___	___	___	___	___	___	___
__ Racquet/handball	___	___	___	___	___	___	___	___	___	___
__ Rope jumping	___	___	___	___	___	___	___	___	___	___
__ Rowing, canoeing	___	___	___	___	___	___	___	___	___	___
__ Sailing	___	___	___	___	___	___	___	___	___	___
__ Skating	___	___	___	___	___	___	___	___	___	___
__ Skiing (cross ctry)	___	___	___	___	___	___	___	___	___	___
__ Skiing (downhill)	___	___	___	___	___	___	___	___	___	___
__ Skiing (water)	___	___	___	___	___	___	___	___	___	___
__ Soccer/football	___	___	___	___	___	___	___	___	___	___
__ Swimming	___	___	___	___	___	___	___	___	___	___
__ Table tennis	___	___	___	___	___	___	___	___	___	___
__ Tennis	___	___	___	___	___	___	___	___	___	___
__ Volleyball	___	___	___	___	___	___	___	___	___	___
__ Walking	___	___	___	___	___	___	___	___	___	___
__ Weight training	___	___	___	___	___	___	___	___	___	___
__ Yardwork, gardening	___	___	___	___	___	___	___	___	___	___
__ Other - specify:	_____									

RPE SCALE

6

7 VERY, VERY LIGHT

8

9 VERY LIGHT

10

11 FAIRLY LIGHT

12

13 SOMEWHAT HARD

14

15 HARD

16

17 VERY HARD

18

19 VERY, VERY HARD

20

14. How would you rate your physical endurance?

low medium high

1 2 3 4 5 6 7

15. How would you rate your physical strength?

low medium high

1 2 3 4 5 6 7

16. How would you rate your overall physical fitness?

low medium high

1 2 3 4 5 6 7

17. List **all** medications you are currently taking (any physician prescribed or over the counter medication including such things as, diet pills, cold, histamines, diuretics, acne medication):_____

_____

18. List **all** dietary suppliments or herbs currently taking (any physician prescribed or over the counter):_____

_____

19. Have you ever had an exercise stress test previously? _____Yes _____No

Bicycle/Treadmill/ Other

When?_____ Results_____Max VO_2_____

Comments_____

_____

21. Have you had any lower leg injury (i.e. sprain, broken leg torn ligament, etc)? _____

If so, **when** and describe: _____

_____

22. Do you currently have any pain in your legs or have you had leg pain within the last month?

If so, please explain: _____

_____

1. If female, what was the date of the beginning of your last period? _____

2. Have you participated in lower leg strength training two to three times per week within the previous six months? Yes or No

3. Have you had any hip, knee, leg, ankle, or foot injuries in the previous 6 months? Yes or No

4. Have you taken any pain relievers within the previous 24 hours? Yes or No

5. Are you taking any medications <u>including</u> birth control? Yes or No

6. If you answered "Yes" to #4 or 5, please list the medications, the reasons for taking them, the prescribed dosage, and how long you have been taking them on a consistent basis.
   _____
   _____
   _____

7. Have you consumed any alcohol, tranquilizers, sleeping pills, antidepressants, opiates, cocaine, amphetamines, PCP, or barbiturates within the previous 24 hours? Yes or No

8. Have you consumed any antibiotics, laxatives, diuretics, neuroleptics, or theophyline within the previous 24 hours? Yes or No

9. Are you consuming any performance enhancing drugs? Yes or No

10. Are you consuming any vitamins or dietary supplements? Yes or No

11. If you answered "Yes" to #7, 8, 9, or 10, please list what you have been taking._____
    _____
    _____

12. Have you been ill within the previous week or are you currently ill (cold, flu, etc.)?

    Yes or No

13. Have you made in changes in your diet in the last month? Yes or No

14. Do you have to maintain a specific type of diet for any reason? Yes or No

15. If so, why are you having to maintain the diet?
    _____
    _____
    _____

16. Have you been diagnosed with diabetes, high blood pressure, or a chronic pain disorder? Yes or No

17. Are you currently undergoing statin or thyroid replacement therapy? Yes or No

18. Do you have any history of kidney or liver dysfunction, heart problems, or high blood pressure? Yes or No

19. Do you have any history of heat illness? Yes or No

20. Do you have any history of swelling after exercise? Yes or No

21. Do you have any history of bruising easily? Yes or No

22. Do you have a family history of muscle disease? Yes or No

23. Do you have a family history of sickle cell disease? Yes or No

APPENDIX C

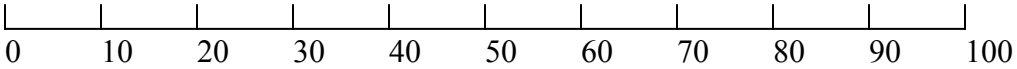
PAIN RATING QUESTIONNAIRE

Pain Intensity

How much does it hurt? Use the scale below to indicate the intensity of the pain you are feeling. A score of 0 represents "no pain". Any score above 0 represents some pain and a score of 100 represents the "highest possible pain intensity" that you can imagine.

No pain

Highest possible pain

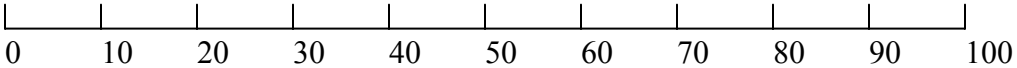


Pain Unpleasantness

How unpleasant is the pain? Use the scale below to indicate how much the pain is bothering you. A score of 0 represents "no unpleasantness". Any score above 0 represents some unpleasantness and a score of 100 represents a pain that is "as unpleasant as possible."

No unpleasantness

As unpleasant as possible



APPENDIX D

TESTING DAY MEASUREMENT FORM

ID_____

Session_____

Recording electrodes

Impedance pre_____
Impedance post_____

Impedance pre_____
Impedance post_____

Stimulating electrodes

Impedance pre_____
Impedance post_____

Impedance pre_____
Impedance post_____

Blood Pressure

Pre R-III_____ _____

DOMS Pain

R - up and down_____
L - up and down_____

R - stairs _____
L - stairs _____

R - pressure algometer_____
L - pressure algometer_____

Pain Ratings Ecc/Con_____

Weight of water

Foot _____
Lower Leg _____

Foot _____
Lower Leg _____

Day 1:

Forms - POMS, STAI
Leg Volume
R-III
Forms - POMS, STAI
Tremor
DOMP measurement
Forms - POMS, STAI
Eccentric exercise or control condition
R-III
Tremor
Leg Volume
DOMP measurement

Days 2-4:

Forms - POMS, STAI
R-III
Forms - POMS, STAI
Tremor
Leg Volume
DOMP measurement
Forms - POMS, STAI

APPENDIX E

DISSERTATION PROTOCOL CHECKLIST

Dissertation Protocol Checklist

Subject #_____ Date _____ Time _____

Preparation

___ Organize subject file

___ Turn on equipment: Grass power supply, top two P511 amplifiers, oscilloscope, Digitimer DS7a stimulator, micro1401mkII, computer-Spike5.16

___ Calibrate Grass P511 Amp (Ch. 2 Gain @ 1000: CAL. 1mV = 1V)

Procedure for calibration of P511 amplifier

1. make sure the amplifier is set at x10 and not x1000 amplification, also the line filter must be set in the out position
2. set the input/output knob to calibrate
3. choose the channel with the source knob (CH2)
4. make sure the mode knob is the same as the source knob
5. cursor knob set at V2
6. set the coupling knob to AC
7. move the mode knob to single (sweep)
8. move the display knob to store
9. move the mode knob to reset (ready to write)
10. move the CH2 knob to AC from ground **(last thing to do)**
11. adjust delta knob to line up with the sweeping signal
12. adjust the *variable volts* knob for Ch2 and adjust the sweep time appropriately
13. push G1 neg button (calibration button)
14. adjust delta to the reference line

oscilloscope settings to record R-III (*optional*)

1. * make sure the amplifier is set to use, line filter in, and x1000 amplification switch is on
2. set the Ch2 knob to ground
3. set the cursor to delta T
4. set the coupling knob to DC
5. set the delta reference knob at the onset of the signal
6. adjust the sweep time desired setting

Greet Subject – give brief tour of the lab

___ Administer informed consent (2 copies)

___ Administer medical history questionnaire, past exercise history, POMS, STAI

___ Conduct Blair's (1984) interview for 7-day physical activity history

___ Ask subject to change into exercise clothes (shorts)

___ Measure weight _____ and height _____ and age _____

R-III reflex measurement

___ Wash hands in front of participant. Put medical exam glove on right hand for sanding.

___ Prep the short head of biceps femoris EMG and stimulating electrodes (ankle)

___ Shave electrode sites (ankle and hamstring).

___ Use (100 coarse – maroon) sand paper to abrade the skin for stimulating electrode placement, moving in different directions. Tell subject: "This is used to exfoliate the skin so that we can get a better reading. It is just to take the dead skin cells and oil off. I will be vigorous the first day. Please let me know if it gets too sensitive." Use alcohol pads to clean the area.

___ **Locate sural nerve** placement for the Empi stimulating electrodes – posterior to the lateral malleolus for the anode and (2cm) distal for the cathode. Attach 2 Empi stimulating electrodes. Try to line up the electrodes so they follow the natural contour of the fossa just posterior to the lateral malleolus.

___ Prep short head of biceps femoris (EMG: two 1 cm electrodes, 2 cm apart, ~midline of the short head of the biceps femoris) – mark location with sharpie.

Locating biceps femoris electrode site: To find the biceps femoris muscle, ask the participant to bend the leg at the knee and push the foot against resistance from your hand, toward his or her head. The biceps femoris will become more visible at this point, but it is also helpful to palpate the muscle starting at the biceps tendon near the knee crease and working up. Once it is found mark the head of the biceps femoris with a dot using a black Sharpie.

___ **Biceps femoris EMG electrode placement**: The electrode placement site over the biceps femoris is located approximately 10 cm above the knee crease (~width of hand) and about one half of the way between the left side of the leg and the midline of the leg. Prepare this area in the same manner as for the sural nerve stimulating electrode. Abrade the skin with (220 coarse – light tan) sandpaper and alcohol pad until an impedance level of less than 10 KOhms is achieved. Place the electrodes on the previous dot marks which line up the center of the electrode with the head (marked).

___ Move subject into Faraday cage, adjust chair – and adjust leg angle to 120 degrees

___ Attach stimulating wires and verify impedance of biceps femoris EMG (< 10 kOhms): ___

___ Attach EMG wires and verify impedance of sural nerve stimulation (< 10 kOhms): ___

___ Instructions for procedure (R-III-reflex)

"You will receive progressively increasing intensity of stimuli [*nothing, slight tactile sensation, short sharp rubber band snapping*]. Your foot may move slightly, but keep your leg/foot relaxed and do not try to control movement. Maintain the 120 degree leg angle - do not adjust the stimulated leg around, AND be careful of the electrodes under your leg. Do not move your opposite leg; keep it rested on the black foot rest. Above all just try to remain relaxed and stable, try not to move around. Keep your eyes open, placing arms and hands on your lap."

"Do not tense the muscle in your abdomen or shoulders. Do not try to anticipate, just relax and let it happen. If at any point you wish to not continue let me know. Any questions?"

DS7a stimulator - Spike 5.16 protocol for R-III elicitization

___ Start NFR script and follow directions. Have subject verbalize pain intensity and affect after each stimulation based on pain scales posted on wall in Faraday cage.

*After EACH stimulus, obtain a VRS rating from the participant. Discontinue the procedure if the participant gives a rating of 80 (maximum tolerable). The program is designed to deliver a maximum of 40 mA. Disconnect all electrodes from the participant.

SPA1 accelerometer - Spike 5.16 protocol for Tremor

___ "Think of this as a game and the object is to remain as still as possible. I will notify you of the next stimulation by stating, for example (speak slowly), ninety degrees….and…….NOW! At that point I am recording so it is best for you to make sure that you are preparing yourself when I name the position because you need to be in position, eyes closed and facing forward and completely still. The goal is to keep your foot in the position as still as possible for 60 seconds. You also must keep your body as still as possible facing forward with your eyes closed (i.e. you can't talk or move your head or even the fingers in your hands). You will hear a buzzer at which time you can move around and open your eyes. Use that time to yawn, scratch an itch etc. If for some reason you happen to catch yourself falling asleep or you moved accidentally during a recording session let me know. We will then just repeat that 60 second trial. Any questions?"

___ Start Tremor script and follow directions.

Pressure algometry rating procedure

___ Locate the medial head of the gastrocnemius muscle of the participant by having the participant plantarflex their foot while in the prone position on the medical examination table. Mark appropriately with black Sharpie.

"You are going to feel some pressure, when that pressure turns to pain at that instant tell me to stop, and I will stop"

APPENDIX F

PERMISSION LETTER FROM THE INTERNATIONAL JOURNAL OF SPORT AND EXERCISE PSYCHOLOGY – IJSEP

West Virginia University
School of Physical Education

April 12, 2007

To: Jeff Pasley, University of Georgia

From: Matt Brann, Fitness Information Technology

Re: Copyright permission

To whom it may concern,

Fitness Information Technology, a Division of the International Center for Performance Excellence at West Virginia University, is the publisher and copyright holder of content appearing in the *International Journal of Sport and Exercise Psychology* (*IJSEP*). As publishers, we grant Jeff Pasley permission to use any copyrighted material from his article titled "The Nociception Flexion (R-III) Reflex: A Potentially Useful Tool in Exercise and Pain Studies" that appeared in *IJSEP*, Volume 3, Number 3, pp. 338-351.

Best regards,

Matt Brann
Editor

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