1-5-2018

Downhill running impairs activation and strength of the elbow flexors

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Presentations

Poster Presentations

Brandenberger, K.J., Ingalls, C.P., Rupp, J.C., & Doyle, J.A. Exogenous supplementation of melatonin does not affect 20 mile cycling time trial performance. American College of Sports Medicine Annual Conference, June 1, 2013, Indianapolis, Indiana.

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Brandenberger, K.J., Henes, S., & Doyle, J.A. Nutrient intake during a 24 hour ultramarathon. American College of Sports Medicine Annual Conference, June 1, 2014, Orlando, FL. Accepted for presentation.

ABSTRACT

DOWNHILL RUNNING IMPAIRS ACTIVATION AND STRENGTH OF THE ELBOW FLEXORS

by

Kyle J. Brandenberger

Under the Direction of Dr. J. Andrew Doyle

PURPOSE: The aim of this study was to determine if knee extensor injury induced by 1 h of downhill running attenuated force production in uninjured skeletal muscle (e.g., elbow flexors). METHODS: Recreationally active subjects (n = 12) completed a two group (injury vs control) repeated measures design with the injury group running downhill for 1 h and the control group performing only the measurement procedures. Strength and percent voluntary muscle activation were measured using an isokinetic dynamometer and electrical stimulation of the elbow flexors and knee extensors before and after a fatigue protocol at the following time points in relation to the downhill run: 15 min pre, 15 min post, 24 h post, and 48 h post. Blood samples were collected at the same time points to measure IL-1β and TNF-α concentrations. RESULTS: Knee extensor strength was significantly reduced by 53.5±9.9% immediately post-injury and remained reduced for up to 48 h in the injury group. Elbow flexor strength was significantly reduced immediately and 24 h post-injury by 13.2±3.9% and 17.3±4.0% respectively in the injury group. Elbow flexor electrically stimulated strength was not found to be different at any time point (P = 0.561). Elbow flexor activation was significantly reduced compared to control at

24 and 48 h post-injury by 22.9±9.1% and 13.5±5.7% respectively. No differences were observed in IL-1β or TNF-α between groups. CONCLUSION: A 1 h downhill run significantly injured the knee extensors. The elbow flexor muscles remained uninjured based on electrically stimulated strength, but voluntary strength of these muscles was impaired due to reduced activation. This suggests an injury to the knee extensors can impair strength in uninjured muscles by reducing voluntary activation. The mechanism behind this reduction remains undetermined.

INDEX WORDS: Downhill running, Muscle injury, Central nervous system, Inflammation

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Kyle J. Brandenberger

A Dissertation

Presented in Partial Fulfillment of Requirements for the

Degree of

Doctor of Philosophy

in

Kinesiology

in

The Department of Kinesiology and Health

in

the College of Education and Human Development

Georgia State University

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2017

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DEFINITIONS

Adenosine Triphosphate (ATP): A high energy molecule containing three phosphate groups used in various cellular processes to provide energy.

Creatine Phosphate (PCr): A high energy molecule used by cells to produce energy anaerobically.

Cyclooxygenase (COX): An enzyme responsible for synthesizing prostaglandin E2.

Graded Exercise Test (GXT): An aerobic fitness test where the subject's expired gas is measured for oxygen consumption. Subjects run on a treadmill and the speed is increased at fixed intervals until the subject cannot maintain their speed.

Injury: A state of long-lasting impaired function resulting from physical damage to organs/tissue/cells in a biological system.

Interleukin 1β (IL-1β): A pro-inflammatory cytokine which binds to the IL-1 receptor.

Interleukin 1receptor antagonist (IL-1ra): a competitive inhibitor of IL-1β which binds to the IL-1 receptor and prevents signaling.

Maximal voluntary contraction (MVC): A muscle contraction in which the subject voluntarily produces the maximum amount of force they can achieve.

Nuclear Factor Kappa B (NF-κB): A transcription factor that is sensitive to reactive oxygen species.

Peak Oxygen Consumption (VO2peak): The highest oxygen consumption observed during a maximal effort GXT.

Prostaglandin E2 (PGE2): A messenger particle synthesized by Cyclooxygenase.

Sarcoplasmic Reticulum (SR): An organelle within a muscle cell which stores and releases calcium.

Tumor Necrosis Factor alpha (TNFα): A pro-inflammatory cytokine.

Twitch Interpolation (TI): A technique used to quantify the percentage of muscle activated in a voluntary contraction.

Voluntary Muscle Activation (VMA): The percentage of muscle that can be activated by a subject without direct outside stimulation of the nervous system compared with activation when directly stimulating the target nerve.

CHAPTER ONE

REVIEW OF LITERATURE

Introduction

Fatigue can be very broadly defined as a decline in force production not due to physical injury of the working muscle. Numerous situations can result in fatigue, and the cause of declining force production is often multifactorial. In order to more fully understand fatigue, it is necessary to isolate the different causes to determine why force production declines in a given scenario.

Peripheral Fatigue

Peripheral fatigue refers to a decrement in a given muscle fiber's ability to maximally produce force. Peripheral fatigue has been attributed to changes within the muscle that reduce the muscle fiber's ability to produce maximal force. The muscle fiber relies on the release of calcium from the sarcoplasmic reticulum (SR) as a signal to initiate muscle contraction (S Ebashi & Endo, 1968; Riiegg, 1988). Sufficient calcium must be present in the muscle cytosol to bind to troponin C, which leads to a conformational shift, exposing the myosin binding site on actin (Setsuro Ebashi, Endo, & Ohtsuki, 1969). Since calcium is crucial to the development of force by a muscle fiber, anything that significantly decreases SR calcium release would lead to a reduction in maximal force production of the individual fiber and thereby decreasing the overall force production capability of the muscle (Riiegg, 1988).

Metabolic changes

During prolonged, intense muscle contractions, a number of metabolic changes occur in the muscle. Accelerated use of ATP and depletion of the creatine phosphate (PCr) stores leads to increased levels of free creatine and inorganic phosphate (Pi). While free creatine has not been linked to any change in force production the associated rise in Pi has been shown to have a direct effect on the calcium stores in the SR by causing calcium to precipitate (D. Allen & Westerblad, 2001; Westerblad, Allen, & Lännergren, 2002). The reduced SR calcium leads to a reduced calcium transient, which in turn leads to reduced force production as fewer cross bridges are formed (D. Allen & Westerblad, 2001; D. G. Allen, Kabbara, & Westerblad, 2002). Early studies on skinned fibers suggested a significant role for acidosis on reductions in force production (Cooke, Franks, Luciani, & Pate, 1988; Donaldson, Hermansen, & Bolles, 1978; Metzger & Moss, 1987). However, it was subsequently determined that the effects of pH on muscle force production become less significant at physiological temperatures (Pate, Bhimani, Franks-Skiba, & Cooke, 1995). More recent research has even suggested that reductions in pH can preserve force production (O. B. Nielsen, Paoli, & Overgaard, 2001).

Glycogen Depletion

Glycogen depletion has been associated with fatigue in numerous studies (Jacobs, Kaiser, & Tesch, 1981; Tsintzas, Williams, Boobis, & Greenhaff, 1996; Wagenmakers et al., 1991). It was originally thought that depletion of muscle glycogen caused fatigue consequent to reduced ATP resynthesis, but the evidence suggests that ATP concentrations are maintained even when glycogen is depleted (J. Nielsen, Schrøder, Rix, & Ørtenblad, 2009; Stephenson, Nguyen, &

Stephenson, 1999). Some evidence now suggests that depletion of glycogen reduces calcium release from the ryanodine receptor through an unknown signaling mechanism (Chin & Allen, 1997; Ørtenblad, Nielsen, Saltin, & Holmberg, 2011).

Oxidative Stress

Some evidence exists that acute exercise is capable of increasing oxidative stress (Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005; M. B. Reid, Shoji, Moody, & Entman, 1992), and that training blunts this increase in oxidative stress by upregulating gene expression via Nuclear Factor Kappa B (NF-κB) signaling (Gomez-Cabrera, Domenech, & Viña, 2008; Miyazaki et al., 2001). The specific role of oxidative stress in fatigue is incompletely understood. It appears that some basal level of oxidative stress is needed for adequate force generation (Michael B Reid, Khawli, & Moody, 1993), but increasing oxidative stress beyond a certain level impairs force development via reducing calcium release and possibly altering calcium sensitivity of the contractile proteins (Michael B Reid, 2008).

Central Fatigue

The role of the central nervous system in fatigue has been recognized for over a hundred years since it was first demonstrated that mentally challenging work could affect physical performance (Mosso, 1904). Central fatigue can be defined as a transient reduction in the ability of the central nervous system (CNS) to voluntarily fully recruit motor units (Gandevia, 2001). Voluntary recruitment of motor units occurs when the motor cortex generates an action potential that travels down the spinal cord through alpha motor neurons to activate a muscle. The spinal cord then integrates the level of stimulation from the brain with peripheral signals from muscle

afferents to select the appropriate level of motor unit recruitment (J. B. Nielsen, 2004). Evidence suggests that CNS dysfunction can occur at the supraspinal level due to suboptimal output from the motor cortex (Gandevia, Allen, Butler, & Taylor, 1996; Taylor, Allen, Butler, & Gandevia, 2000; Taylor, Todd, & Gandevia, 2006), or at the spinal level due primarily to altered afferent feedback from the group III and IV afferents (Amann & Dempsey, 2008; Amann et al., 2008; Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009) (see Figure 1.1).

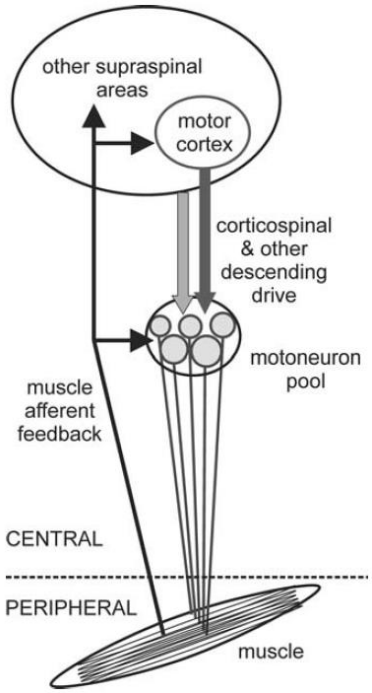

Figure 1.1. Central versus peripheral fatigue. Depicting the different sites in the neuromuscular system necessary for force development. Fatigue can occur due to dysfunction in any one of these sites leading to reduced force production (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016)

Supraspinal Fatigue

The precise mechanisms that lead to development of central fatigue are not entirely understood at present. The first proposed mechanism for central fatigue suggested that alterations in brain serotonin were responsible for reductions in force generating capability (Romanowski & Grabiec, 1973). This was later expanded upon to suggest a role for free fatty acids displacing tryptophan from albumin and increasing brain uptake of free tryptophan which is converted into serotonin within the brain (Acworth, Nicholass, Morgan, & Newsholme, 1986; Chaouloff, Kennett, Serrurrier, Merino, & Curzon, 1986; Curzon, Friedel, Katamaneni, Greenwood, & Lader, 1974; Newsholme, 1987; Pardridge, 1979). However, studies on serotonin's role in central fatigue have had conflicting results, suggesting that other mechanisms may play a role (Roelands & Meeusen, 2010).

There is some evidence to suggest that dopamine, another neurotransmitter plays a significant role in the initiation of force production. A meta-analysis of examining dopamine reuptake inhibitors to treat cancer related fatigue in 2010 found a significant (effect size = 0.28) improvement in fatigue when measured using a subjective rating scale (Minton, Richardson, Sharpe, Hotopf, & Stone, 2011). However, previous researchers have suggested that dopamine alterations are a downstream consequence of the inflammation occurring in the brain, with the proposed pathway below (see Figure 1.2) (Bower & Lamkin, 2013). Supporting this conclusion, treatment with dopamine elevating medications have mixed results on reducing fatigue in Parkinson's patients (J. S. Lou et al., 2003; Mendonça, Menezes, & Jog, 2007; Valko et al., 2010).

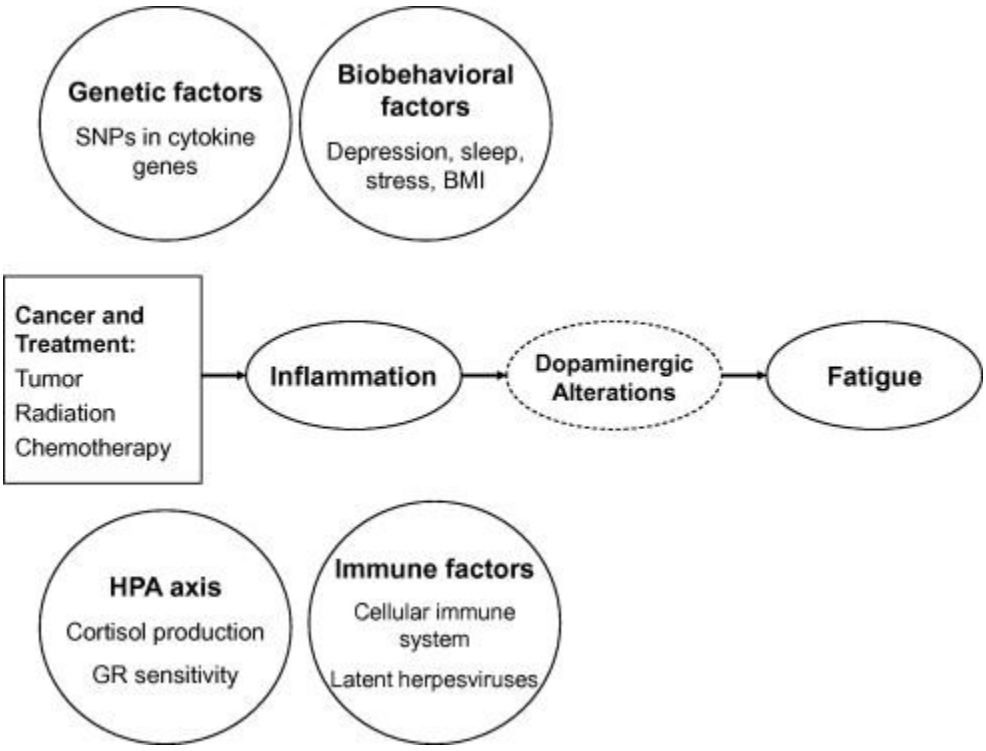

Figure 1.2. Inflammation alters dopamine levels resulting in fatigue. Inflammation from systemic causes related to cancer can impair dopamine signaling leading to fatigue (Bower & Lamkin, 2013).

Modulating Force

The motor unit is comprised of the alpha motor neuron and the muscle fibers it innervates. A motor unit is the lowest functional unit of the neuromuscular system capable of producing force (Taylor et al., 2016). The central nervous system is capable of modulating force production via two approaches, both involving the activation of the motor unit (J. H. Friedman et al., 2007; Heckman & Enoka, 2012). The first approach is to change the number of motor units recruited.

Motor units are normally recruited based on size, with the smallest motor units recruited first (J. H. Friedman et al., 2007; Heckman & Enoka, 2012). Once a given motor unit is recruited, the force can be altered by changing the rate at which action potentials are delivered to the motor unit (Taylor et al., 2016). During a brief contraction, the rate of action potentials can vary considerably, but functionally muscles tend to peak between 10-40 Hz of activation (Fuglevand, Lester, & Johns, 2015; Mottram, Heckman, Powers, Rymer, & Suresh, 2014; Taylor et al., 2016). During fatiguing tasks involving MVCs, motor unit firing rates have been consistently shown to decline (Bigland-Ritchie, Dawson, Johansson, & Lippold, 1986; Dalton, Harwood, Davidson, & Rice, 2010; Taylor et al., 2016; Woods, Furbush, & Bigland-Ritchie, 1987). This reduction in motor unit firing rate is likely attributed to reductions in neural drive, local changes in the motoneurons, of inhibitory feedback mechanisms (Heckman & Enoka, 2012; Taylor et al., 2016). During submaximal fatiguing contractions firing rates are extremely variable with intensity, type of task performed, muscle location, muscle architecture, and training status influencing changes (Taylor et al., 2016). Following a bout of eccentric exercise, it appears that rate coding increases, as the CNS attempts to compensate for losses in force (De Ruiter, Elzinga, Verdijk, Van Mechelen, & De Haan, 2005; Piitulainen, Holobar, & Avela, 2012).

Motor Neuron Excitability

The alpha motor neurons play an integral role in the development of force, because they are the last link in the nervous system necessary for activation of a particular motor unit. The alpha motor neuron and the muscle fibers that make up the motor unit form a one to many relationship making the (Taylor & Gandevia, 2008) firing of the motor unit reflect the firing

alpha motor neuron in a one-one ratio (Taylor et al., 2016). The excitability of the motor neuron is influenced by corticospinal drive, neurotransmitters, afferent nerve input and the current level of activation of the given motor neuron (Taylor et al., 2016) (see Figure 1.3) . Evidence suggests that during fatiguing contractions the motor neurons are less responsive to excitation, which would reduce their firing rate leading to the recruitment of additional motor units (Carpentier, Duchateau, & Hainaut, 2001; Johnson, Edwards, Van Tongeren, & Bawa, 2004; Taylor et al., 2016).

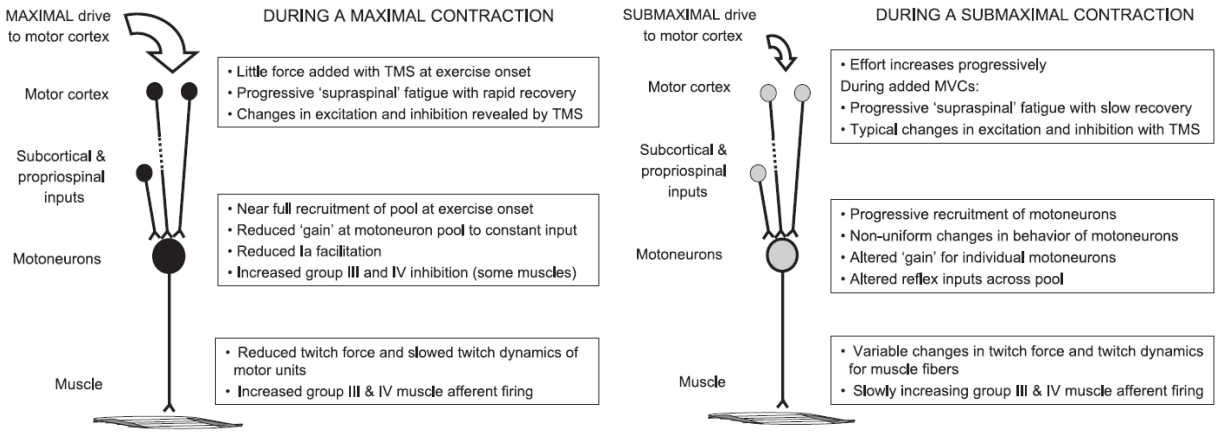

Figure 1.3. Motor unit recruitment varies with intensity. A summary of the differences in motor unit recruitment for maximal versus submaximal contractions (Taylor & Gandevia, 2008).

The role of serotonin in fatigue was previously discussed in the context of the central nervous system. However, researchers have suggested that serotonin plays a complex role in the excitability of the motor neurons (Taylor et al., 2016). Evidence suggests that low levels of serotonin increase motor neuron excitability when confined to the somatodendritic membrane, but when levels of serotonin are high enough to spill over onto the axonal initial segment, motor neuron excitability is decreased (Cotel, Exley, Cragg, & Perrier, 2013). This likely occurs via

the distribution of 5-HT2 excitatory and 5-HT1A inhibitory serotonin receptors (Cotel et al., 2013). Studies suggest that serotonin is released in a graded response to force (Wei et al., 2014) , but at least during rhythmic activities of running with a sufficient time frame the level of serotonin release will decline, taking 30-45 minutes to recover (Fornal, Martín-Cora, & Jacobs, 2006; Gerin, Becquet, & Privat, 1995).

Motor neuron excitability can also be altered via afferent feedback (Enoka et al., 2011; Taylor et al., 2016). The motor neuron receives input from excitatory sources (descending drive, and muscle spindle afferents) and inhibitory sources (Golgi tendon afferents, Renshaw cells, and group III/IV afferents) (Taylor et al., 2016). Of the inhibitory sources, only the group III/IV afferents are thought to play a significant role in force loss during fatigue. (Amann & Dempsey, 2008; Gandevia, 2001; Hilty et al., 2011; P. G. Martin, Smith, Butler, Gandevia, & Taylor, 2006; Pettorossi, Torre, Bortolami, & Brunetti, 1999; Woods et al., 1987). The muscle spindle afferents promote motor neuron excitation. During a sustained contraction, there is a decline in muscle spindle activity, leading to reduced motor unit excitation (Macefield, Hagbarth, Gorman, Gandevia, & Burke, 1991). From studies of the short latency H-reflex, comprising a spinal loop, there is evidence to suggest that muscle spindle firing primarily leads to changes at the spinal level that in turn lead to presynaptic inhibition of the motor neuron pool (Duchateau, Balestra, Carpentier, & Hainaut, 2002; Duchateau & Hainaut, 1993). In contrast there is a differential response on the long latency reflex, with no change during maximal efforts, but a decline at submaximal efforts (Duchateau et al., 2002) (see Figure 1.4).

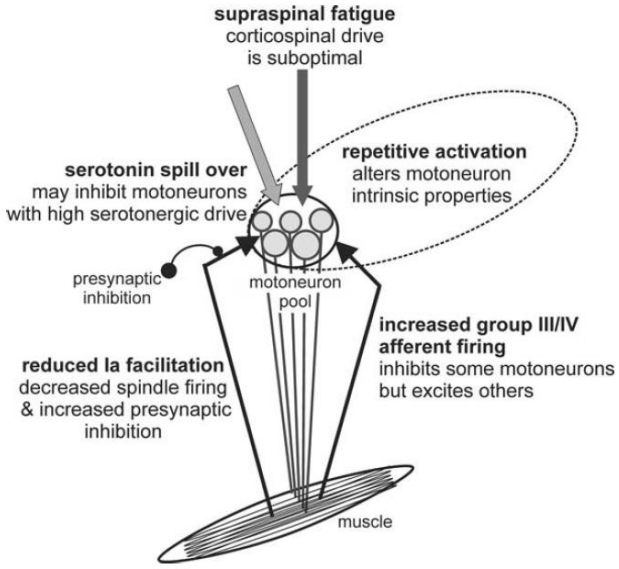

Figure 1.4. Motor Neuron excitability. Depicting the different factors affecting motor neuron excitability (Taylor et al., 2016).

Group III and IV Afferents

Sensory nerves that innervate skeletal muscle can be activated by contractions. These sensory nerves are classified based on function, and physical properties into group I, II, III or IV. The group III and IV afferent nerves are thought to play an important role during fatiguing contractions. The group III afferents are largely responsive to mechanical stimulation from muscle contraction, and the group IV afferents are sensitive to metabolites (Taylor et al., 2016). These group III/IV afferents play a significant role in relaying information to the central nervous system for the regulation of cardiopulmonary response to exercise. Evidence suggests that these afferents influence the rapid increase in cardiopulmonary output during exercise (Amann et al., 2010; Kaufman & Forster, 1996). Since O_2 delivery to the working muscles limits development of fatigue in the working muscles (Amann & Calbet, 2008), the faciliatory effects of group III/IV afferents on the cardiopulmonary system could be thought to reduce peripheral fatigue

(Taylor et al., 2016). Additional studies suggest that fatiguing byproducts, such as hydrogen and Pi, can activate group III and IV which alter spinal function and reduces motor unit recruitment to possibly avoid the development of severe peripheral fatigue (Amann & Dempsey, 2008; Amann et al., 2008; Amann et al., 2009). It is thought that group IV afferents can alter central motor drive since the information from group IV afferents is communicated to the brain (Amann, 2011; Gandevia, 2001). This inhibition can also affect recruitment of distant limbs (Sidhu et al., 2014). A 2004 study suggests that intramuscular inflammation due to skeletal muscle injury can sensitize group IV afferents leading to reduced voluntary muscle activation within the same muscle (Marqueste et al., 2004) (see Figure 1.5).

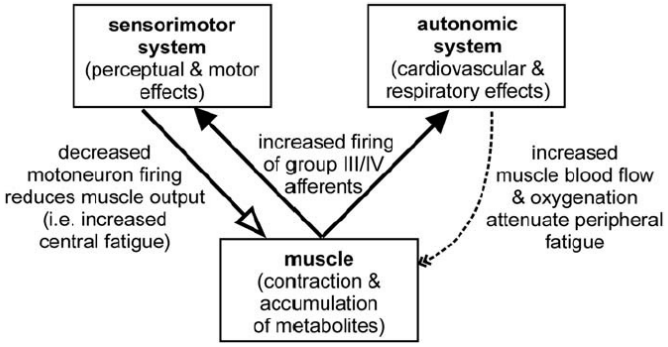

Figure 1.5. Muscle afferents interactions. Depicting the complex role of group III and IV afferents in the development of central and peripheral fatigue (Taylor et al., 2016).

Central vs Peripheral Fatigue

As the previous sections illustrate, fatigue likely occurs through a combination of overlapping mechanisms that include both peripheral factors intrinsic to the individual muscle fibers, and central factors involving the brain, spinal cord and alpha motor neurons. While the cellular environment of a muscle cell can be altered through exhaustive exercise and lead to a loss of the intrinsic force production of the muscle (Fitts, 1994), The central nervous system also clearly

plays a role in determining the level of motor unit recruitment. Peripheral mechanisms also do not explain the loss of force observed in submaximal exercise below thresholds thought to produce excessive fatiguing byproducts, which is distinguished by a decrease in voluntary muscle activation (V. Martin et al., 2010). The interplay between peripheral mechanisms of fatigue and central factors is complex, and the extent to which each mechanism contributes to the overall development of fatigue depends on the task and conditions of the performance (Taylor et al., 2016).

Measuring Fatigue

Previous researchers have suggested that measuring fatigue is difficult because the very concept of fatigue is difficult to define (Eidelman, 1979; Muscio, 1921). Despite this difficulty, there are a number of methods used to measure fatigue. One of the earliest methods developed to study fatigue involved a subjective rating scale based on subject observation and feedback (Krupp, LaRocca, Muir-Nash, & Steinberg, 1989; Pearson, 1957; Pearson & Byars Jr, 1956; Yoshitake, 1971). While useful, these rating scales are subjective and lack the precision needed to determine either the cause or magnitude of any fatigue present. In spite of the drawbacks, fatigue rating scales are still employed in numerous studies (Barsevick et al., 2004; Given, 2002; Jean-Pierre et al., 2010; Lower et al., 2009) to assess fatigue. Despite the apparent subjectivity of fatigue, there are more objective measures commonly used to quantify the magnitude of fatigue present in subjects.

Numerous studies have examined fatigue using tests to fatigue, where a subject performs an exercise at a constant work rate until they can no longer maintain the desired work rate (E.

Coyle et al., 1983). The benefit to this type of measurement is that it mimics competitive activities and is easily translatable to performance. It has been used extensively to examine metabolism and fuel utilization (Coggan & Coyle, 1987; E. Coyle et al., 1983; E. F. Coyle, Coggan, Hemmert, & Ivy, 1986). This method has also been used to study the effects of inflammation on fatigue in animal models (Carmichael et al., 2006). Unfortunately, the drawback to this method is its inability to determine whether the fatigue occurs in the peripheral muscle or the central nervous system.

Another method of measuring fatigue is to measure maximal voluntary contraction (MVC). Measuring force production during a MVC has advantages when studying central fatigue because, force generated during an MVC involves the entire pathway from the brain to the skeletal muscle (Taylor & Gandevia, 2008). The drawback to this procedure is that the results of any changes in MVC strength may not directly translate into functional/performance applications since movements in daily activity or sport rarely involve MVC type of muscle action. However, and MVC remains the best way to test both the peripheral muscle and the central nervous system. Therefore, an MVC can be used to assess the function of the entire nervous system from brain to the alpha motor neurons.

Measuring Activation

The relationship between muscle activation and electrical current has been known for several centuries (Galvani & Aldini, 1792). The first recorded EMG were reported in 1849 (Du Bois-Reymond, 1849), and subsequently, the technique has been refined through the use of various technology to record an analyze the small electrical signals due to the movement of ions

involved in muscle contraction (Fridlund & Cacioppo, 1986; Kamen & Caldwell, 1996). Measurements of activation relying on EMG signals suffer from two major drawbacks. First, the voltages recorded are very small in the μV range, making them highly susceptible to interference from outside sources which would need to controlled (Fridlund & Cacioppo, 1986). Second, there is no way to ascertain based on the voltage of the muscle whether the there is any portion that remains inactive. Third, the reliability of EMG measurements decreases with increasing intensities of exercise, making them unreliable for measurements during a maximal voluntary contraction (Dankaerts, O'Sullivan, Burnett, Straker, & Danneels, 2004; Mathur, Eng, & MacIntyre, 2005; Yang & Winter, 1983).

Twitch interpolation is a technique used to quantify the amount of muscle voluntarily activated during a contraction (Shield & Zhou, 2004). During a maximal contraction, a stimulus is applied to the either the nerve innervating the muscle, or directly to the muscle belly with surface electrodes. The additive force of the twitch is then compared to a twitch induced during relaxation from the MVC, known as a control twitch. The following equation is then used to estimate the percentage of voluntary muscle activation during the maximal contraction (Shield & Zhou, 2004).

Voluntary Activation percentage = (1 – Superimposed Twitch Torque / Control Twitch Torque) X 100 (G. Allen, Gandevia, & McKenzie, 1995).

The control twitch torque in conjunction with the superimposed twitch torque can be used to quantify the level of dysfunction/fatigue within the muscle (Park et al., 2008). The superimposed twitch torque represents the amount of muscle not activated during the voluntary

contraction (Shield & Zhou, 2004). Combining these measures can be used to determine how much of the fatigue is due to central activation or changes within the muscle.

This technique can be applied to muscle injury to determine the relative contribution of central and peripheral mechanisms to the force loss associated with injury. Changes in the control twitch torque would represent changes intrinsic to the muscle fiber. Whereas, changes in the superimposed twitch torque relative to the control twitch torque would represent changes in central activation. If the injury is broadly affecting motor unit recruitment, then the ratio of superimposed twitch torque to control twitch torque will be increased in uninvolved limbs. Questions remain of whether the muscle injury is communicated into the brain producing widespread changes, and what is the most likely mechanism for this communication?

Inflammation and Signaling

Inflammation is most commonly diagnosed and defined by the symptoms: redness, swelling, heat, and pain (A. Scott, Khan, Cook, & Duronio, 2004). It has been shown that these symptoms are the result of chemical messengers that broadly activate immune processes throughout the body (Rocha e Silva, 1978). There are a variety of different chemical messengers employed in this process that have both redundant and differential effects (Watkins, Maier, & Goehler, 1995). Tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) are secreted early in the inflammatory process and are referred to as "pro-inflammatory" cytokines because of their role in upregulating inflammatory mediators such as nitric oxide and matrix metalloproteinases (Pedersen et al., 2001). Interleukin 6 is also secreted early in the inflammatory response, but appears to have both pro-inflammatory and anti-inflammatory effects

(Barton, 1997). Another set of cytokines including interleukin 1 receptor antagonist (IL-1ra), soluble TNF-α receptors, TGFβ, interleukin 4, interleukin 10, and interleukin 13 have anti-inflammatory effects (Cannon & Pierre, 1998; Pedersen et al., 2001).

Inflammation Communication across the Blood Brain Barrier

Originally it was thought that the brain was relatively immune privileged (Pachter, de Vries, & Fabry, 2003), meaning that the blood brain barrier protected the brain from experiencing the effects of inflammation. However, recent research in mice and humans has provided good evidence that peripheral inflammation induced in mice via downhill running (Carmichael et al., 2010), in human fetuses via lipopolysaccharide (Lee, Liu, Dickson, Brosnan, & Berman, 1993), and various models of human disease (Gabay, Lamacchia, & Palmer, 2010), can communicate across the blood brain barrier to induce inflammation in the brain itself. This process is thought to be activated by inflammatory signaling of the molecule IL-1β which can bind to the IL-1 receptor activating brain microglial cells, which then secrete prostaglandin E_2 (PGE2) (Carmichael et al., 2010; Gabay et al., 2010; Harrington, 2012; Lee et al., 1993). It is also thought that prolonged signaling through the IL-1 receptor can result in dysfunction of the blood brain barrier itself and lead to infiltration of immune cells and inflammatory signaling molecules directly into the brain. Evidence for this comes from an *in-vitro* model of endothelial cells (Labus, Häckel, Lucka, & Danker, 2014).

Cerebral Inflammation and Sickness Behavior

Cerebral inflammation has been shown in mice to induce a variety of behavior changes (fatigue, loss of appetite, reduced work capacity, etc...) often referred to collectively as sickness

behavior. These behavior changes tend to peak 2-6 h following an inflammatory stimulus in mice (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008), which matches the time frame observed in studies of IL-1β signaling inhibition in humans showing a reduction in feelings of fatigue after inhibiting IL-1 signaling (Fitzgerald, LeClercq, Yan, Homik, & Dinarello, 2005; Verbsky & White, 2004).

Role of IL-1β in Fatigue

Studies have shown that IL-1 signaling impairs voluntary activity and time to fatigue in mice following a bout of downhill running (Carmichael et al., 2005; Carmichael et al., 2006). This concept is also supported by studies of voluntary activity after injection with an immune stimulating substance in the absence of any injury, which have shown that reductions in voluntary activity are strongly correlated with Il-1β levels in the brain (W. Sheng, Hu, Ding, Chao, & Peterson, 2001; W. S. Sheng, Hu, Lamkin, Peterson, & Chao, 1996). Taken together, these studies indicate that brain inflammation is communicated from the periphery in both injury and sickness, and this brain inflammation causes a form of fatigue in mice. This phenomenon has been linked specifically to brain macrophage like cells, which appear to be key in the reduction of time to fatigue in treadmill running following eccentric exercise induced muscle injury in mice (Carmichael et al., 2010). This phenomenon has then been applied to human clinical fatigue, feelings of fatigue associated with sickness and disease that is associated with inflammation, but without clear, direct evidence that this pathway is conserved in humans.

The evidence for IL-1β's role in fatigue in humans largely comes from clinical studies of conditions that involve elevated systemic inflammation. Numerous studies have shown that

conditions associated with inflammation including: Multiple sclerosis (Leocani et al., 2001; Ng, Miller, Gelinas, & Kent-Braun, 2004; Sheean, Murray, Rothwell, Miller, & Thompson, 1997), rheumatoid arthritis (Bearne, Scott, & Hurley, 2002; O'Reilly, Jones, Muir, & Doherty, 1998), fibromyalgia (Nørregaard, Bülow, Vestergaard-Poulsen, Thomsen, & Danneskiold-Samsøe, 1995), and chronic fatigue (Schillings et al., 2004; Siemionow, Fang, Calabrese, Sahgal, & Yue, 2004), have a diminished capacity to voluntarily recruit motor units. While these studies do not clearly show a causative effect of inflammation on reduced voluntary motor unit recruitment, they do suggest that such an effect may occur, which other reviewers have suggested (Zwarts, Bleijenberg, & Van Engelen, 2008). The work of T.D. Noakes suggests that the brain can integrate numerous signals including, inflammation, temperature, blood glucose and psychological factors to alter motor cortex function and ultimately affect motor unit recruitment (Noakes, 2011). If inflammation can induce these behavior changes in humans, it will likely affect motor unit recruitment, and maximal force production.

Force Loss after Muscle Injury

Skeletal muscle injury often occurs following novel eccentric muscle actions. This injury can lead to functional deficits in the muscle which are disproportionate to the extent of physical damage within the muscle fiber (Warren, Ingalls, Lowe, & Armstrong, 2002). It is generally accepted that muscle injury leads to a loss of force production due to physical disruptions within the cell of the force generating structures, and the structures involved in transmitting signals from the nerve into the interior of the cell, also known as excitation contraction (E-C) uncoupling (Warren et al., 2002). E-C uncoupling accounts for most of the early force loss (0-3 days) after injury, whereas a frank loss of contractile proteins becomes more prominent 3-28 days after

injury (Warren et al., 2002). There is evidence that skeletal muscle injury results not only in a reduced intrinsic force producing capability, but also in what is classically thought to be central fatigue. While Marqueste et al., 2004 dealt with the effects of intramuscular inflammation, the literature suggests that prolonged eccentric exercise results in elevated plasma levels of IL-1β, IL-6 and TNF-α (Moldoveanu, Shephard, & Shek, 2001; Peake, Nosaka, & Suzuki, 2005) which might further impair muscle force production at the central level. A few studies have already suggested that muscular function is partly related to IL-1β at the peripheral level (Marqueste et al., 2004) and the central level (Carmichael et al., 2005; Carmichael et al., 2006) following muscle injury.

Fatigability after Muscle Injury

There is also evidence that exercise induced muscle injury increases the fatigability of the injured muscle (Asp, Daugaard, Kristiansen, Kiens, & Richter, 1998; Doncaster & Twist, 2012). Although this increased fatigability has been thought to be attributed primarily to alterations in the metabolic properties of the muscle after injury (Asp et al., 1998), this would not explain the progressive decline in EMG signal observed in other studies during fatiguing contractions (Hedayatpour, Falla, Arendt-Nielsen, & Farina, 2008). Interestingly, this same phenomenon has been documented in patients with multiple sclerosis (Sheean et al., 1997). One of the factors contributing to fatigue in multiple sclerosis is increased systemic inflammation (Gold & Irwin, 2009; Heesen et al., 2006; Trapp & Nave, 2008). Studies also suggest that inflammation induced by the exercise protocols may affect the basal ganglia, which contain the brain's reward centers (Calabrese et al., 2010; Tellez et al., 2008).

Inflammation Alters the Brain Reward Centers

One possible explanation for increased fatigability is that inflammation may alter the function of the Substantia Nigra, known as the reward center, of the brain and thereby alters the incentive to endure the pain associated with fatigue. This alteration has been observed in humans during MRI scans of people after receiving a typhoid fever vaccine, a method of inducing inflammation (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008). Interestingly, the subjects in that study also displayed psychomotor slowing, a possible sign of fatigue. This interaction with the brain reward center has been shown in numerous other studies (Croisier, Moran, Dexter, Pearce, & Graeber, 2005; Herrera, Tomas-Camardiel, Venero, Cano, & Machado, 2005), and this region has also been linked to motor dysfunction as a result of enhanced IL-1β signaling (Ferrari et al., 2006). Reviewers have even suggested that IL-1β signaling is key to the pathophysiology of Parkinson's Disease (Godoy, Tarelli, Ferrari, Sarchi, & Pitossi, 2008; Nagatsu, Mogi, Ichinose, & Togari, 2000). Parkinson's is also associated with abnormal central fatigue, defined as the inability to initiate physical tasks (J. Friedman & Friedman, 1993; J. H. Friedman et al., 2007; Karlsen, Larsen, Tandberg, & Jørgensen, 1999; J. S. Lou, Kearns, Oken, Sexton, & Nutt, 2001). Alterations in the Substantia Nigra have been shown to reduce integrated force during a 30 second fatiguing contraction via a loss of motor unit recruitment (J.-S. Lou, 2005). Reducing the brain's perceived reward from performing a painful sustained contraction leads to a progressive loss in motor unit recruitment,

Exercise Induced Muscle Injury Leads to Elevated IL-1β

While some researchers suggest that most of the IL-1β generated due to eccentric muscle injury is retained within the injured muscle (J. Peake et al., 2005), the levels of IL-1β in plasma are necessarily much smaller than those of other inflammatory cytokines because it becomes cytotoxic above picomolar concentrations (Moldoveanu et al., 2001). This means the circulating levels of IL-1β remain close to the detection threshold (Moldoveanu et al., 2001). Due to this, results in the literature concerning IL-1β plasma concentrations following exercise have been inconsistent (Moldoveanu et al., 2001; Suzuki et al., 2002). That some studies have shown elevated plasma IL-1β levels following marathon running (Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998), 5k runners (Netea et al., 1996) cycling (Haahr et al., 1991; Lewicki, Tchorzewski, Majewska, Nowak, & Baj, 1988), and eccentric cycling (Evans et al., 1986) suggests there may be small significant elevations in IL-1β following higher intensity (>75% VO2max) exercise. As pointed out previously in the literature (Nieman et al., 1998), while IL-1β is difficult to measure in plasma, its elevation in both muscle (Cannon et al., 1989; Fielding et al., 1993) and urine (Sprenger et al., 1992) post injury suggests elevated levels are present in the blood.

Endurance Exercise Leads to Elevations in Pro-Inflammatory Cytokines

Most studies have shown that prolonged endurance exercise (≥60 min) results in elevated plasma TNFα (Brenner et al., 1999; Dufaux & Order, 1989; Espersen et al., 1990; Moldoveanu, Shephard, & Shek, 2000; Ostrowski, Rohde, Asp, & Pedersen, 1998; Rokitzki, Logemann, & Keul, 1994; Sprenger et al., 1992) Shorter protocols tend to not illicit elevated plasma TNFα levels (Brenner et al., 1999; Der Meer, 1998; Natelson et al., 1996), but one study using a 5 km run was able to detect levels of TNFα after administration of lipopolysaccharide (Netea et al., 1996). Studies of the effects of endurance exercise on circulating IL-6 are more consistent, with studies showing consistent elevations in IL-6 of exercise ≥ 30 min (Brenner et al., 1999; Moldoveanu et al., 2000; Ostrowski, Rohde, Asp, et al., 1998; Ostrowski, Rohde, Zacho, et al., 1998; Ullum et al., 1994), with eccentric exercise resulting in significantly more IL-6 (Bruunsgaard et al., 1997; Nieman et al., 1998). Increases in IL-6 (25 to 100 fold increases) tend to be larger than increases in either TNFα or IL-1β (~2.5 fold increase) (Ostrowski, Rohde, Asp, et al., 1998) making it a more easily detectable marker of overall inflammation.

Detecting systemic IL-1β in Plasma and Urine

In order to circumvent the detection problem, it may be beneficial to test for IL-1β in the urine because the concentrations would be higher and thus easier to detect. Studies of plasma IL-1β have found basal levels < 10 pg/mL and post injuries levels typically below 100 pg/mL (Ostrowski, Rohde, Zacho, et al., 1998; L. L. Smith et al., 2007), while studies of urine yield results in the 100 – 300 pg/mL (Sprenger et al., 1992). In urine there is a delay in the time that

significant levels are detected. The literature seems to indicate that a delay of 3 h following the onset of exercise is needed before significant levels of IL-1β appear in the urine (se Figure 6.6).

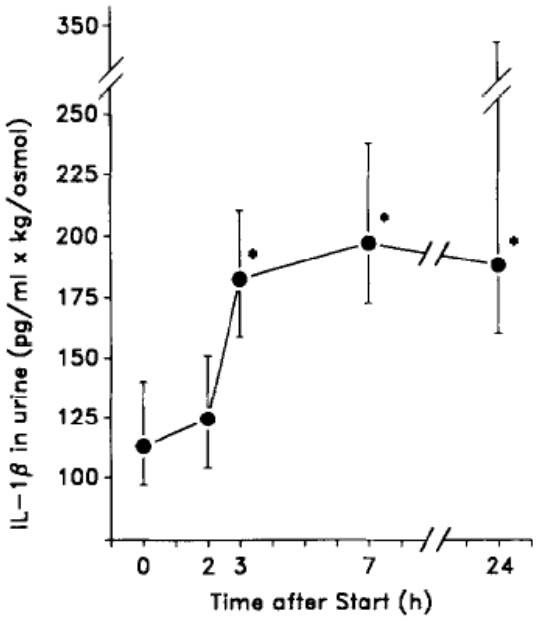

Fig 1.6. Concentration of IL-1β detected in urine from the start of a 2 h run. Levels of IL-1β were assessed in urine before, immediately after (2h) and at the other time points indicated (Sprenger et al., 1992).

Exercise Intensity, and Training Status Influences IL-1β levels

Evidence suggests that higher intensity exercise produces significantly more inflammation than moderate or low intensity exercise of the same duration (J. M. Peake, K. Suzuki, M. Hordern, et al., 2005). In support of this, studies employing a race format with subjects attempting to achieve the fastest time possible tend to produce significant levels of IL-6 (Ostrowski, Rohde, Zacho, et al., 1998; Sprenger et al., 1992). Evans et al., 1986 found that untrained men had significantly more inflammation than trained men; however, the workload between groups was a fixed 200 W, making it difficult to determine whether changes in relative work rate were responsible for the reduced inflammation in the trained group (Evans et al.,

1986). It is likely that the results of Evans et al., 1986 reflect the importance of relative intensity on the inflammatory response. Most studies showing significant elevations in IL-1β used relative work intensities between 75-80% of VO_2max (Cannon et al., 1989; Pournot et al., 2011; J. P. Scott et al., 2013). Therefore, any study attempting to induce a uniform inflammatory response should scale the intensity to a percentage of maximal effort instead of a fixed work rate.

Exercise Duration influences IL-1β levels

Cox et al., 2007 performed a series of trials with 18 well trained males. There was a 30 min trial at 60% VO2max, a 60 min trial at 65% VO_2max, and an interval trial with 6 sets of 3 min running at 90% VO2max. The long trial was found to increase IL-6 by 527.1% compared to 65.1% (interval trial) and 42.8% (30 min trial) (Cox, Pyne, Saunders, Callister, & Gleeson, 2007). This would suggest that duration is a key determinant in the level of inflammatory response. A number of studies have attempted to induce inflammation using a short duration (2-5 min) plyometric workout, but have failed to show any significant increases in IL-1β (Chatzinikolaou et al., 2010; Isaacs, 2012; Tofas et al., 2008). The magnitude of injuries in these plyometric studies is often very small and in some cases fails to significantly alter muscle function (Chatzinikolaou et al., 2010; Tofas et al., 2008). Studies with longer injury protocols, from 45 min (Cannon et al., 1989; Evans et al., 1986; Pournot et al., 2011), 60 min (Haahr et al., 1991; J. P. Scott et al., 2013), and longer (Ostrowski, Rohde, Zacho, et al., 1998; Sprenger et al., 1992) have shown significant elevations in IL-1β suggesting that longer protocols may induce more inflammation. However, results have been inconsistent with manipulating duration alone with other studies showing no change after an hour of downhill running (L. L. Smith et al.,

2007). The literature as a whole suggests that longer duration protocols tend to produce significant elevations of plasma IL-1β, but shorter protocols do not.

Differential effects of whole body vs single joint exercise on IL-1β levels

Isolated single joint exercises produce significantly less inflammation than whole body endurance exercise protocols (J. M. Peake, K. Suzuki, M. Hordern, et al., 2005; J. M. Peake, K. Suzuki, G. Wilson, et al., 2005; Petersen et al., 2001; L. Smith et al., 2000; Thompson et al., 2004). Taken together, these studies suggest that the amount of muscle mass involved in the injury is important to the magnitude of the inflammatory response. Specifically, the greater the amount of muscle involved in the injury, the larger the inflammatory response will be (see Table 1.1).

Table 1.1. Summary of different injury protocols and their resultant inflammatory markers

Study	Protocol	Time Length	training status	Sample size	MVC Change	IL-6	IL-1β
Sprenger et al., 1992	20 km run (95-120 min)	95-120 min	trained (large range)	n = 22		3 fold peak increase in plasma returned to baseline in 24 hours	Urine levels elevated 3, 7 and 24 hrs post exercise
Chatzinikolaou et al., 2010	50 hurdle jumps, 50 drop jumps	short	52.1±6.4	n = 12	No significant change detected	2 fold increase at 24 hours	Undetectable expect immediately post exercise
Tofas et al., 2008	96 hurdle jumps, 96 box jumps	2-5 min	untrained	n = 18	No significant change detected		
Isaacs AW, 2012	10 sets of 10 high jumps	short	untrained	n = 39		Slight significant increase	
Pournot et al., 2011	Simulated Trail run up to 80% VO2max	45 min	62±1.18	n = 8			Significantly elevated 1 h post exercise
Cannon et al., 1989	45 min downhill run 16% incline 75% VO2max	45 min		n = 5			Elevated intramuscular levels up to 5 days
Smith et al., 2007	60 min downhill run 13.5% incline	60 min	47.1±3.6	n = 10		Up to 29 pg/mL	No change detected
Evans et al., 1986	45 min eccentric cycling 250 W	45 min					Elevated 3h post exercise in untrained males
Ostrowski et al., 1998	Copenhagen Marathon run time 3:17:03 ± 00:07:39	~197±7min	58.8	n = 16		Significant increase from 1.5±0.7 pg/mL to 94.4±12.6 pg/mL	Significant increase from 0.61±0.24 pg/mL to 0.92±0.26 pg/mL immediately post exercise
Haahr et al., 1991	200W for 60 min	60 min	43.1-59.7	n = 10			Significantly elevated 2h post exercise
Vyver et al., 2013	12X5 min 15km/h 10% grade	60 min	50.7±1.1	n = 18		2 fold increase for 4 hours	No Change detected
Scott et al., 2013	60min @ 60% run to exhaustion @ 75%	~2 hrs	67.9-55.5	n = 10			2.5 fold increase end of exercise

Mechanism of Fatigue in an Uninjured Muscle Following Eccentric Injury of Another Muscle Group

The studies above provide evidence that eccentrically biased exercise of sufficient duration and intensity could be communicated to the central nervous system via inflammation. Prior studies suggest that longer duration, high intensity exercise can lead to elevated circulating pro-inflammatory cytokines. There is evidence that acute inflammation is communicated to the central nervous system via two avenues: sensitization and activation of group IV afferents, and activation of brain macrophage like cells which in turn secrete inflammation directly into the brain. Sensitization of the group IV afferents can lead to a reduction in MVC by reducing the number of recruited motor units. The areas of the brain affected involve the cortex and subcortical regions which may also affect motor unit recruitment by altering the excitability of the motor cortex. The evidence also suggests that the Substantia Nigra, or the reward center of the brain is specifically activated and may sensitize the brain to fatigue. Taken together, it is possible that inflammation from exercise induced muscle injury results in decreased peak torque and voluntary activation for 24-48 h post injury, but these ideas have never been directly tested. Two key questions need to be addressed. First, is the CNS's ability to activate uninjured muscle affected after an acute muscle injury? If the answer to this question is yes, the second question that needs to be addressed is whether inflammatory cytokines are related to any changes observed in CNS function? This review suggests that such information could be communicated via circulating pro-inflammatory cytokines.

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CHAPTER TWO

INTRODUCTION

Skeletal muscle injury often occurs following novel or intense eccentric muscle actions. This injury can lead to functional deficits in the muscle which are disproportionate to the extent of physical damage within the muscle fiber (Warren, Ingalls, Lowe, & Armstrong, 2002). It is generally accepted that muscle injury leads to a loss of force production due to physical disruptions of the force generating structures within the cell, and the structures involved in transmitting signals from the nerve into the interior of the cell, also known as excitation contraction (E-C) uncoupling (Warren et al., 2002). In a mouse model of muscle injury, E-C uncoupling accounts for most of the early force loss (0-3 days) after injury, whereas a frank loss of contractile proteins becomes more prominent 3-28 days after injury (Warren et al., 2002).

The strength of a given muscle depends upon the size of the muscle, the number of actomyosin cross bridges, and the level of activation from the central nervous system (Frontera & Ochala, 2015), among other factors. There is evidence that skeletal muscle injury results not only in a reduced intrinsic force producing capability, but also impairment of activation of the injured muscle (Behrens, Mau-Moeller, & Bruhn, 2012; Deschenes et al., 2000; Komi & Viitasalo, 1977). This suggests that information of the injury is communicated to the central nervous system (CNS), which then alters motor unit recruitment or rate coding. This information could be transmitted to the central nervous system via two separate mediums.

The first medium capable of transmitting this information is blood. Muscle injury results in the release of numerous intracellular proteins into systemic circulation (McKune, Semple, &

Peters-Futre, 2012). This is thought to result in elevated pro-inflammatory cytokines in the systemic circulation (Nieman et al., 1998; Suzuki et al., 2002). Previous studies suggest that pro-inflammatory cytokines in circulation can communicate exercise-induced muscle injury to the brain, resulting in reduced CNS function (Carmichael et al., 2005; Carmichael et al., 2006). Since this medium would not convey the specific location of the injury to the central nervous system, any effects would likely be widespread throughout the body.

The second medium that could transmit information of muscle injury to the central nervous system is afferent nerves. Group III/IV afferent nerves have been shown to relay information of muscle fatigue to the central nervous system, resulting in reduced activation of both the working muscle (Amann & Dempsey, 2008; Amann, Proctor, Sebranek, Pegelow, & Dempsey, 2009), and muscles distant from the site of fatigue (Sidhu et al., 2014). Group IV afferent nerves are thought to be sensitized following muscle injury by pro-inflammatory cytokines retained within the injured muscle (Marqueste et al., 2004).

This study had three main objectives. The first objective was to determine whether exercise-induced muscle injury could affect the strength of a muscle distant from the site of injury. The second objective was to determine whether any dysfunction in the distant muscle was due to reductions in CNS function, and the third was to examine whether plasma cytokines were related to any changes observed. Answering these questions improves our understanding of the CNS's role following muscle injury, and could lead to insights of mechanisms contributing to widespread neuromuscular dysfunction.

PROCEDURES

Study Design

The overall design of this study was to induce injury in leg muscles with a downhill running protocol in a set of subjects, and to determine whether injury of the knee extensors affected subsequent function of uninjured elbow flexor muscles. Measurements of strength and activation of knee extensors and elbow flexors were taken before, 15 min, 24 h, and 48 h after downhill running. Subjects then performed a fatigue protocol with the knee extensor and elbow flexor muscles to examine how strength and activation following muscle injury are impacted in a fatigued state. Urine and blood samples were taken pre, post, 24 h post, and 48 h post injury to assess markers of inflammation. A control group did not undergo the injury protocol, but provided strength and activation measurements of the knee extensors and elbow flexors over the same time period for comparison (Warren, Hermann, Ingalls, Masselli, & Armstrong, 2000). For a summary of the procedures see Figure 2.1.

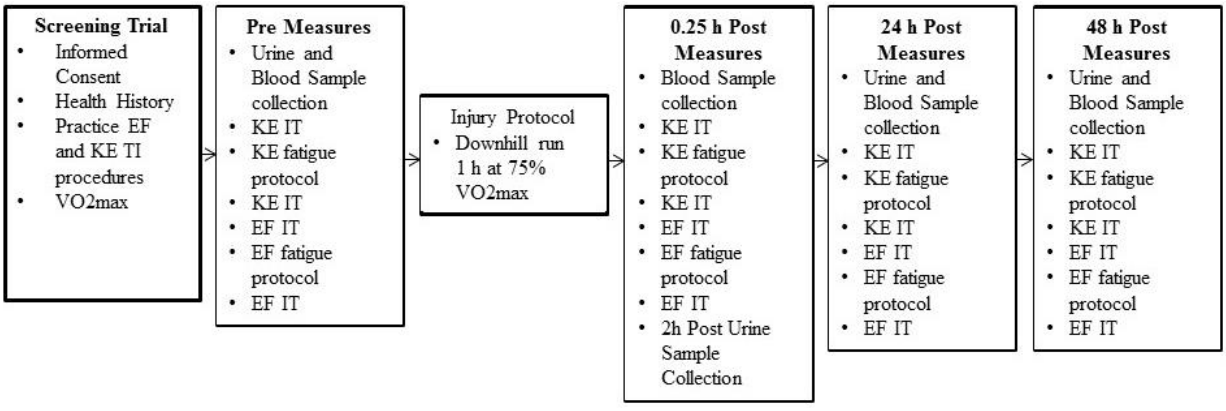

Fig 2.1. An outline of the testing protocol. KE IT refers to the Knee Extensor Interpolated Twitch. EF IT refers to the Elbow Flexor Interpolated Twitch. Urine and blood samples were stored and measured for IL-1β content.

Subjects

Recreationally active subjects who were not specifically training for running were recruited for this study. Based on a health history form, subjects who either failed to meet the ACSM guidelines for low risk (Thompson, Gordon, & Pescatello, 2009), ran more than 10 miles per week, or participated in plyometric training were excluded from testing. A power analysis was performed using G-power 3.1 (Universitat Kiel, Germany) for a repeated measures ANOVA on biceps MVC peak force. The effect size was estimated at .2, and the correlation of repeated measures estimated at .9 giving an estimated sample size of 14. The measured effect in the elbow flexors was larger than estimated ($\eta^2 \geq 0.241$) allowing for a smaller sample size of 12 subjects, while maintaining statistical power. For a summary of subject characteristics see Table 2.1.

Table 2.1 Subject Characteristics

	Injury (n = 6)	Control (n = 6)
VO2peak (ml/kg/min)	49.2 ± 12.18	44.2 ± 6.98
Height (cm)	175.6 ± 6.05	174.4 ± 12.28
Weight (kg)	80.2 ± 5.44	73.3 ± 13.66
Age (yr)	27.3 ± 7.69	27.6 ± 8.48

Values are mean ± SD.

Screening Trial

Subjects reported to the Applied Physiology Lab for testing. Subjects first provided their informed consent, and then filled out a health history form to ensure all subjects were low risk according to the ACSM risk stratification procedures (Thompson et al., 2009). Subjects were then escorted to a separate lab containing a Kin-Com III (Chattecx: Chattanooga, TN) and asked to perform a practice set of the twitch interpolation and fatigue inducing procedures for the knee extensors and elbow flexors. Subjects that failed to demonstrate an ability to adequately perform the twitch interpolation procedure were excluded from further testing. Subjects were then weighed without shoes and asked to perform a graded exercise test (Appendix 5). Gas exchange variables were measured throughout the test using a True Max 2400 Metabolic Measurement System (Parvomedics: East Sandy, UT). The highest 15 second average of VO_2 was used as the subject's VO_2peak. Subjects were then scheduled for the remaining testing sessions. The injury protocol took place within 1-2 weeks of the subject's screening trial.

Injury Protocol

Subjects were asked to run downhill on a treadmill for one hour to injure the knee extensor muscles. A downhill run at 10% grade for 60 minutes at a speed equivalent to 75% of VO_2peak at 0% grade was selected to induce injury because this protocol resulted in a significant injury, produced a large inflammatory response, and could be employed with untrained subjects (McKune, Smith, Semple, Mokethwa, & Wadee, 2006; Smith et al., 2007).

Twitch Interpolation Protocol

Twitch interpolation was used to quantify changes in voluntary muscle activation to determine whether changes in strength were due to the inability to maximally activate skeletal muscle, ie., central fatigue. A constant current, high voltage Digitimer DS7AH stimulator (Digitimer: Hertfordshire, UK) was used to stimulate the elbow flexor and knee extensor muscles. To test the elbow flexor muscles, the subjects were seated in a CB-6 Arm Curl Bench (Valor Fitness: St. Petersburg, FL) in close proximity to a Kin-com III set up to measure torque at 90° of elbow flexion. To test the knee extensors, subjects were seated in a Kin-com III set up in the standard configuration to measure knee extensor torque (Manual) with 110^0 of hip flexion and 70^0 of knee flexion. The subject's leg, chest, and waist were strapped down for knee extensor measurements. The placement of the seat, lever arm and servo-motor were recorded to standardize within subject measurements between trials. A constant current, high voltage Digitimer DS7AH stimulator (Digitimer: Hertfordshire, UK) was used to stimulate the knee extensors and elbow flexors. Stimulation electrodes (UniPatch 6696SS, Wabasha, MN) were placed over the proximal belly and the distal tendon of the biceps for elbow flexor measurements (Magnus, Barss, Lanovaz, & Farthing, 2010). Stimulation electrodes (UniPatch 617SB, Wabasha, MN) for the knee extensors were placed over the distal vastus medialis muscle and the proximal vastus lateralis muscle near the anterosuperior iliac spine. For continuity, the position of the electrodes was outlined in permanent marker (Park et al., 2008). A series of control twitches was delivered to the muscle starting at 60mA for the elbow flexors, 100mA for the knee extensors, and increased by 20 mA, until two consecutive increases in amperage failed to increase the peak torque of the twitch. This current was recorded and used throughout the trial to stimulate the muscle. (Magnus et al., 2010; Park et al., 2008).

The subjects were asked to perform a 3 second maximal voluntary contraction (MVC), and an electrical stimulation was applied 2.5 seconds into the contraction. Two paired stimuli were then applied to the muscle at 2 and 4 seconds after the MVC; the resultant torque was averaged and used for analysis. The subjects performed this procedure six times, with 1 minute of rest between contractions. The three interpolated twitch measurements with the highest force were selected and averaged to determine resting voluntary activation. Voluntary activation was estimated using the following equation: Voluntary Activation percentage = (1 – Superimposed Twitch Torque / Control Twitch Torque) X 100 (Allen, Gandevia, & McKenzie, 1995). The interpolated twitch technique is depicted graphically in Figure 2.2.

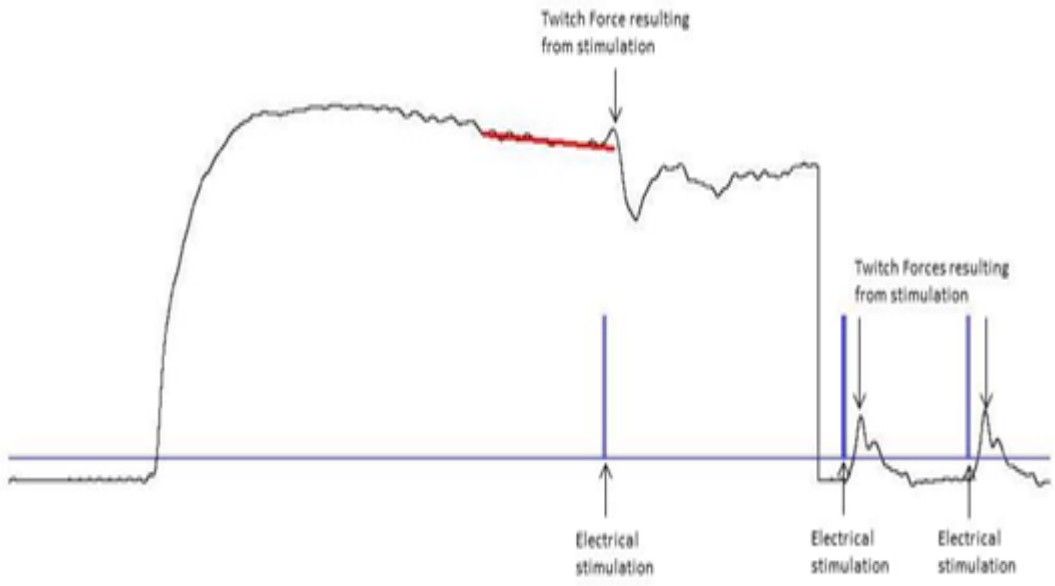

Figure 2.2. A visual representation of the interpolated twitch procedure. Representative force tracing shows a 3 second MVC with twitch interpolation procedure. The resultant twitch height during the MVC contraction is compared the twitch height while the muscle is relaxed to determine the level of activation in the muscle.

Fatigue Protocol

A procedure to fatigue the elbow flexors and the knee extensors was conducted in a similar fashion to the procedure described previously in the literature (Collier, Hardy, Millard-Stafford, & Warren, 2015). Subjects performed five sets of 10 isotonic concentric contractions of the knee extensors or elbow flexors on a KinCom III dynamometer. Subjects were given 5 s of rest between contractions and 20 s of rest between sets. For elbow flexor testing, the concentric action was done at 30^0/s over a 75^0 range beginning at maximal extension (Hansen, 1967). For knee extensor testing, the concentric action was done at 30^0/s over a 45^0 range beginning at 90^0 flexion. The isotonic load was set at 55% of peak force for each day. The subject was required to complete each contraction, and if unable to complete a contraction the researchers provided the minimum amount of assistance needed to complete the contraction.

Urine Samples

Urine samples were collected prior to other pre-injury measurements, 2 h post injury, 24 h post injury, and 48 h post injury. The time frame of the urine samples was chosen based on previous research showing that IL-1β has a delayed appearance in the urine of 3 h after the start of exercise (Sprenger et al., 1992). Immediately after collection, a 5 ml sample of the urine was centrifuged at 1,000g for 10 min at 4^0 C to remove leukocytes and debris (de Reijke, de Boer, Kurth, & Schamhart, 1996; Thomas, Sexton, Benson, Sutphen, & Koomen, 2010). The centrifuged urine samples were then apportioned into 1.5 ml aliquots for freezing and storage at -80 ^{0} C for later analysis.

Blood Samples

Blood samples were collected via venipuncture prior to other pre-injury measurements, immediately post injury, 24 h post injury, and 48 h post injury. Blood samples were drawn into 4 mL Vacutainers (BD: Franklin Lakes, NJ) treated with 7.2 mg of EDTA. The blood was centrifuged under refrigeration at 2,000 x g for 15 minutes. The plasma sample was then transferred to a clean tube and apportioned into 1.5 mL aliquots for storage at -80^0 C for future analysis (Smith et al., 2007).

Determination of Plasma IL-1β content

IL-1β levels were measured in plasma samples using an ultra-sensitive ELISA kit (Life Technologies Corp.: Frederick, MD) with a range of 0.3 to 20 pg/mL and a sensitivity of < 1 pg/mL. The following procedure was used for quantifying IL-1β content: 50 μL of incubation buffer was added to each well, except for chromogen blank wells. Researchers added 100 μL of samples or controls to their appropriate wells. The plate was then covered and allowed to incubate at room temperature for 3 h. The liquid in the wells was then removed and each well was washed 4 times. Then 100 μL of biotin conjugate was added to all wells except the chromogen blanks. The plate was then covered and allowed to incubate at room temperature for 1 h. The liquid in the wells was then removed and each well was washed 4 times. Then 100 μL of Streptavidin-HRP working solution was added to each well except the chromogen blanks. The plate was then covered and allowed to incubate for 30 min at room temperature. The liquid in the wells was then thoroughly removed and washed 4 times. Researchers added 100 μL of

stabilized chromogen to each well, which began to turn blue in color. The plate was then incubated for 30 min. Then 100 μL of stop solution was added to each well and the color changed from blue to yellow. The absorbance was read using a 450 nm filter after blanking the plate reader against the chromogen blanks. The absorbance of the standards was used to create a standard curve, which was used to determine the IL-1β concentrations for the unknown samples.

Determination of Plasma TNF-α content

TNF-α levels were measured in plasma samples using an ultra-sensitive ELISA kit (Life Technologies Corp.: Frederick, MD) with a range of 0.5 to 32 pg/mL. The following procedure was used for quantifying TNF-α content: Either 100 μL controls or 50 μL of standard diluent buffer and 50 μL of sample was added to the appropriate wells, except for chromogen blank wells. Then 50 μL of biotin conjugate was added to each well except the chromogen blanks. The plate was then covered and allowed to incubate at room temperature for 3 h. The liquid in the wells was then removed and each well was washed 4 times. Researchers added 100 μL of Streptavidin-HRP working solution to each well except the chromogen blanks. The plate was then covered and allowed to incubate for 30 min at room temperature. The liquid in the wells was then removed and the plate was washed 4 times. Then 100 μL of stabilized chromogen was added to each well, which began to turn blue in color. The plate was then allowed to incubate for 30 min. Researchers added 100 μL of stop solution to each well and the color changed from blue to yellow. The absorbance was read using a 450 nm filter after blanking the plate reader against the chromogen blanks. The absorbance of the standards was used to create a standard curve, which was used to determine the TNF-α concentrations for the unknown samples.

Measuring Subjective Pain

Subjects were given two visual analog scales labeled “Thigh Pain” and “Upper Arm Pain”. Then subjects were asked to rate their current level of pain at rest using a tick mark to indicate on a scale of 0-100 mm. A score of 0 mm was labeled as “No Soreness” and a score of 100 mm was labeled as “Very, Very Sore”. The tick marks were then measured and recorded for future analysis (Cleather & Guthrie, 2007).

Upper Arm and Thigh Circumference

Sites for standard upper arm and thigh circumference measurements were located on the subjects based the ACSM guidelines (Thompson et al., 2009). A standard Gulick Measurement tape was used for all measurements. Circumferences were then recorded in cm. Three measurements were taken at each site during every trial (pre-injury, immediate post-injury, 24 h post-injury, and 48 h post-injury). The average of these three measurements was used for data analysis.

Statistics

All statistical analysis was performed using SPSS version 24 (IBM: Armonk, NY) with a significance level of 0.05. Values in the results are reported as means ± SEM. A set of two-way repeated measures ANOVAs were conducted for both the resting conditions on the following variables: knee extensors strength, knee extensors voluntary activation, knee extensors electrically evoked force, elbow flexors strength, elbow flexors voluntary activation, elbow flexors electrically evoked force, plasma IL-1β, plasma TNF-α, thigh pain, thigh circumference,

upper arm pain, and upper arm circumference with 4 within factor measurements (pre, post, 24 h post and 48 h post) and a between factor (injury vs control). separate two-way repeated measures ANOVAs were conducted for both the resting and fatigued conditions on the following variables: knee extensors strength, knee extensors voluntary activation, knee extensors electrically evoked force, elbow flexors strength, elbow flexors voluntary activation, elbow flexors electrically evoked force with 4 within factor measurements (pre, post, 24 h post and 48 h post) and a between factor (rested vs fatigued). If the assumptions of sphericity were violated, then a Huynh-Feldt correction was applied. When significant interactions were detected a simple main effects test with a Benjamini & Hochberg false discovery rate correction was used to determine where the differences lay.

RESULTS

Subjects

A total of 12 subjects completed the research protocol. One additional subject completed the entire procedure but was later excluded from the results due to an inability to complete the downhill running protocol.

Overall Findings

Knee extensor strength was significantly reduced by 53.5 ± 9.9%, 46.0 ± 7.0% and 44.6 ± 8.1% immediately, 24h and 48h after 1 h of downhill running, respectively ($P \leq 0.006$). Knee extensor electrically stimulated strength was significantly reduced 49.7 ± 7.7%, 30.8 ± 6.3%, and 27.4 ± 6.8%, immediately, 24h and 48 h after the downhill run, respectively. There was a 23.4 ± 7.9%, 25.8 ± 8.8% and 24.5 ± 7.9% change in activation of the knee extensors immediately, 24h and 48 h after the downhill run, but these changes were not significant ($P \geq 0.075$). Elbow flexor strength was significantly reduced by 13.2 ± 3.9%, 17.3 ± 4.0%, and 9.0 ± 3.3% immediately, 24h and 48h after the downhill run ($P = 0.019$). Elbow flexor activation was reduced by 16.2 ± 5.1%, 20.9 ± 6.7%, and 12.9 ± 4.5% in the injury group, but these changes were only different when compared to the control group ($P = 0.045$). Electrically stimulated force was not impaired in the elbow flexors following the downhill run ($P = 0.631$). Plasma IL-1β ($P = 0.235$) and TNF-α ($P = 0.456$) were not different in the injury group when compared with the control group.

MVC Strength of the Knee Extensor Muscles

Knee extensor strength prior to the fatiguing protocol was not different at rest between the control and injury groups ($P = 0.533$). In the control group, there was no significant change in knee extensor

strength from baseline at any timepoint (P > 0.394). After 1 h of downhill running, the injury group experienced an immediate decline in strength relative to the preinjury values (P < 0.001). Strength in the injury group was significantly reduced below baseline for 48 h (P < 0.001). Decline in force for 48 h after the downhill run indicates muscle injury. The changes in knee extensor strength are depicted below in Figure 2.3.

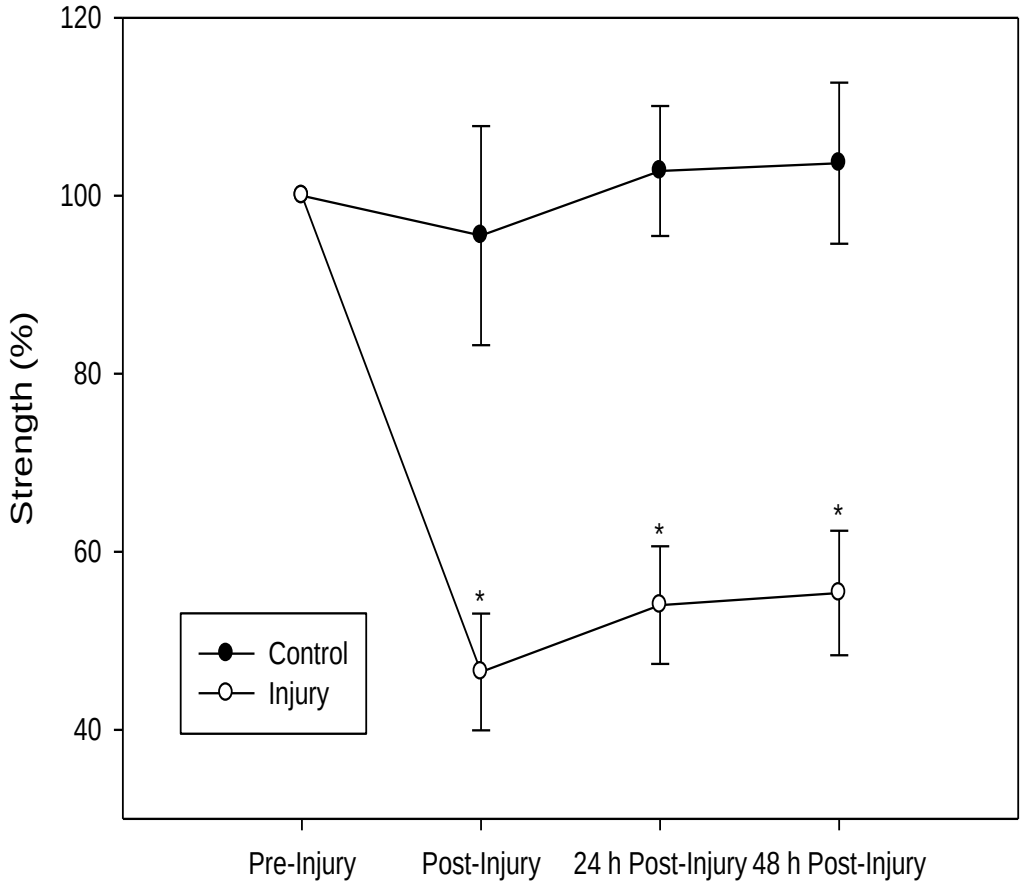

Figure 2.3. Knee extensor force in relation to an injury inducing downhill run. Subjects (n = 12) performed MVCs at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to pre-injury values. Knee extensor force was found to be significantly reduced at all time points after injury (P < 0.05) when compared to control. Significant effects are denoted with (*).

In the control group, knee extensor strength was compared in the rested and fatigued states. Overall, there was a 17.3 ± 9.8% change in strength following the fatigue protocol, but this change was not found to be significant (P = 0.108). No significant changes in strength occurred over time (P = 0.762). The changes in control group knee extensor strength are depicted in Figure 2.4.

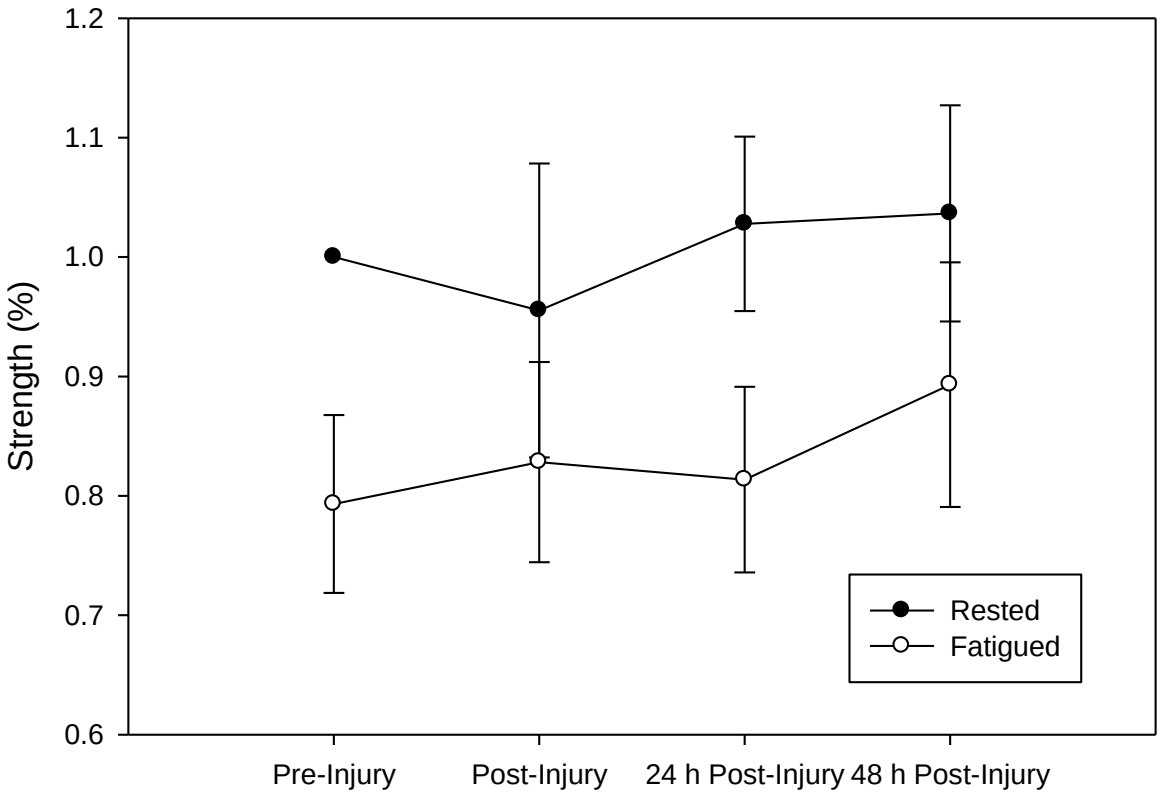

Figure 2.4. Knee extensor strength normalized to non-fatigued pre-injury values of the control group before and after a fatiguing protocol in relation to an injury inducing downhill run. Subjects (n = 6) performed MVCs immediately after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Knee extensor force was not found to be significantly different based on fatigue within the control group (P = 0.108). Values are reported as mean ± SEM.

In the injury group, knee extensor strength was compared in the rested and fatigued states. Overall, there was a significant 45.3 ± 5.7% change in strength following the injury protocol, which persisted for up to 48 h ($P < 0.001$). Compared with the rested values, there was an overall change of 9.7 ± 4.9% following the fatigue protocol, but this change was not significant ($P = 0.075$). The changes in control group knee extensor strength are depicted in Figure 2.5.

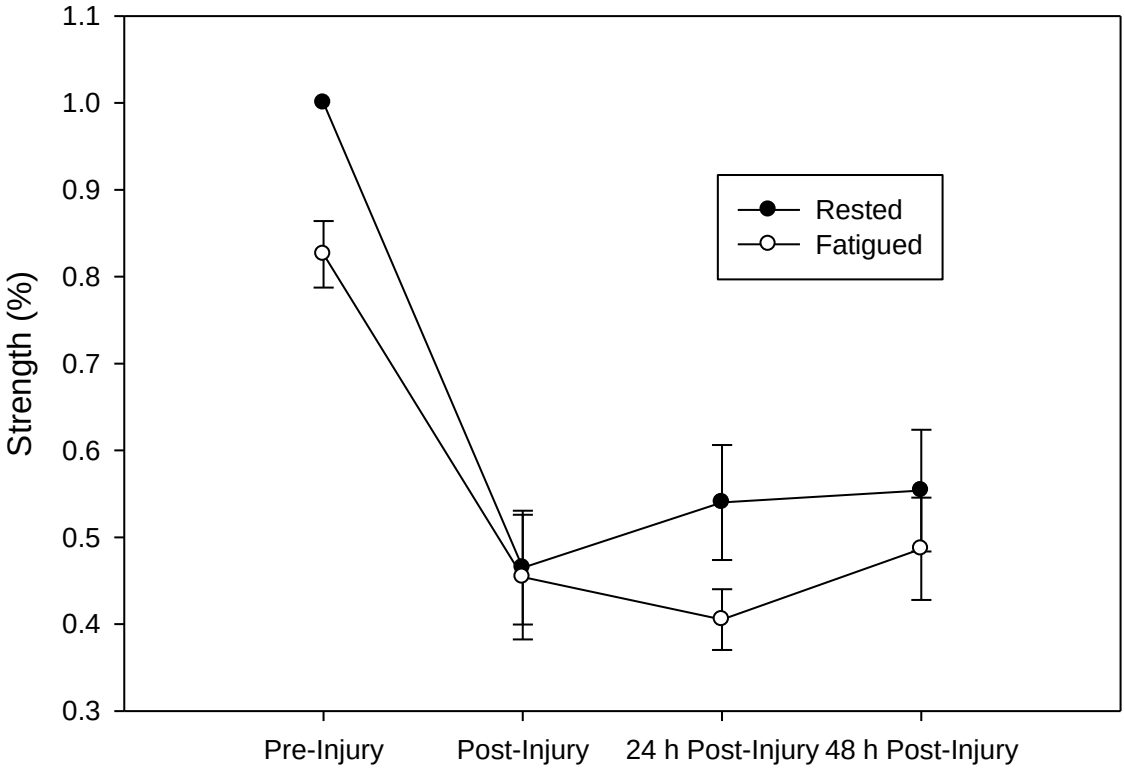

Figure 2.5. Knee extensor strength of the injury group before and after a fatiguing protocol in relation to an injury inducing downhill run. Subjects (n = 6) performed MVCs immediately after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to non-fatigued pre-injury values. Knee extensor force was found to be significantly lower than pre-injury values at all time points post-injury ($P < 0.025$). Significant effects are denoted with (*). Values are reported as mean ± SEM.

Twitch Force of the Knee Extensor Muscles

Knee extensor electrically stimulated strength was not different between the control and injury groups prior to the downhill run (P = 0.549). There was no change across any time point in electrically stimulated force over time relative to the pre-injury values for the control group (P ≥ 0.293). The electrically evoked strength relative to the pre-injury values was significantly reduced immediately (P = 0.010) and 24 h post-injury (P = 0.022), but the strength loss was no longer significantly reduced by 48 h post-injury (P = 0.091). The changes in knee extensor electrically stimulated force are depicted in Figure 2.6.

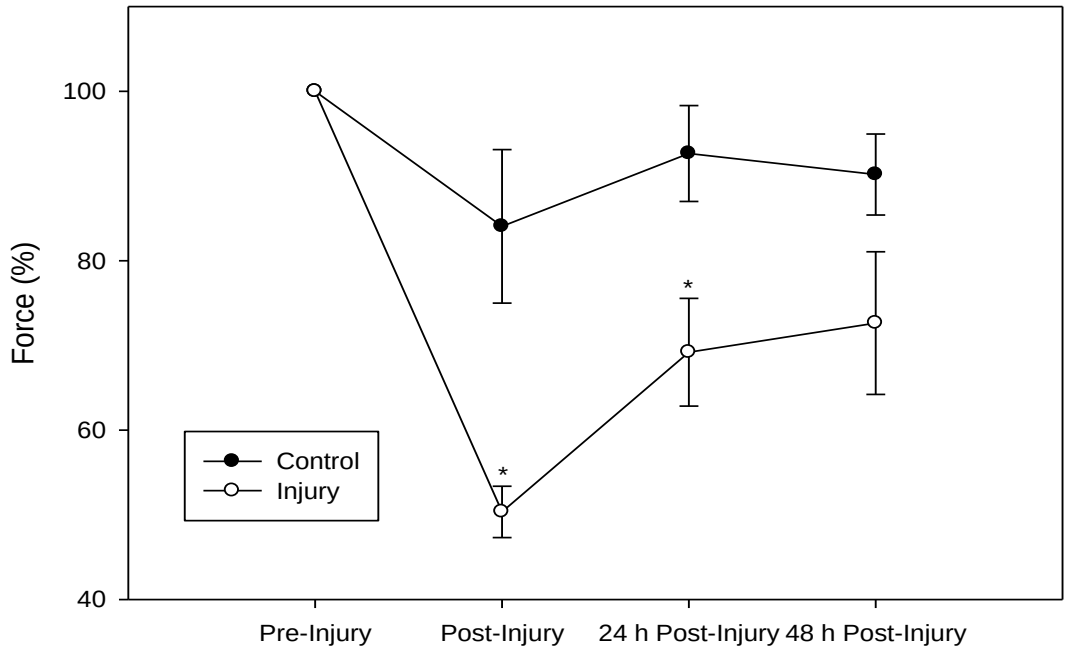

Figure 2.6. Knee extensor electrically stimulated force at rest in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Knee extensor electrically stimulated force was measured in subjects (n = 11) immediately prior to a fatiguing protocol was performed using the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to pre-injury values. Knee extensor electrically stimulated force at rest was found to be significantly reduced at 24 h post-injury (P = 0.010) and 48 h post-injury (P = 0.022) compared to control. Significant effects are denoted with (*). Values are reported as mean ± SEM.

In the control group, knee extensor electrically simulated strength was compared between the rested and fatigued states. Overall, there was a 1.3 ± 7.7% change in electrically stimulated strength following the fatigue protocol, but this change was not found to be significant (P = 0.872). Significant changes in electrically stimulated strength occurred over time (P = 0.004), but post-hoc testing did not detect differences at any time point. There was no significant interaction (P = 0.841). The changes in knee extensor electrically stimulated force following a fatiguing protocol are depicted below in Figure 2.7.

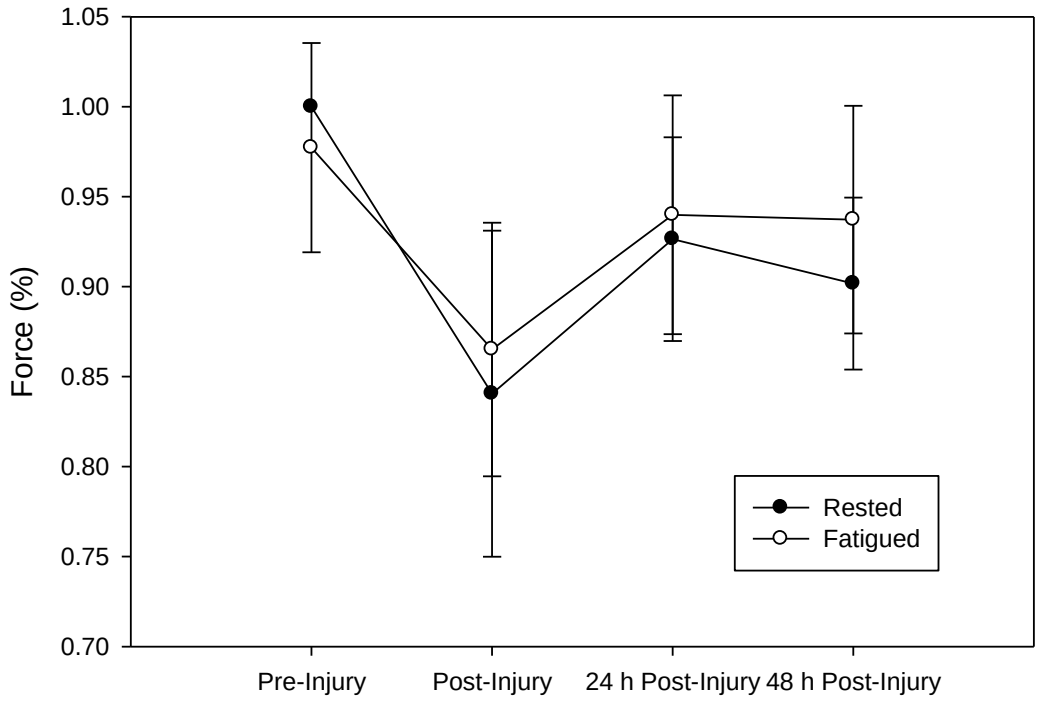

Figure 2.7. Knee extensor electrically stimulated strength between the rested and fatigued state in the control group in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Subjects (n = 6) performed the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run before and after a fatiguing protocol. Strength was normalized to non-fatigued pre-injury values. Knee extensor electrically stimulated strength was found to be significantly affected by time (P = 0.004), but post-hoc testing failed to find any differences (P > 0.125). Values are reported as mean ± SEM.

In the injury group, knee extensor electrically simulated strength was compared between the rested and fatigued states. Values were normalized to the rested pre-injury measurements. Overall, there was a 2.5 ± 6.7% change in electrically stimulated strength following the fatigue protocol, but this change was not found to be significant (P = 0.721). Significant changes in electrically stimulated strength occurred over time (P = 0.004), but with no significant interaction (P = 0.841). Overall, the twitch strength declined significantly immediately post-injury 43.2 ± 5.6%. Twitch strength partially recovered at 24 h to a 26.2 ± 6.8% and remained only partially recovered at 48 h. The changes in knee extensor electrically stimulated force following a fatiguing protocol are depicted below in Figure 2.8.

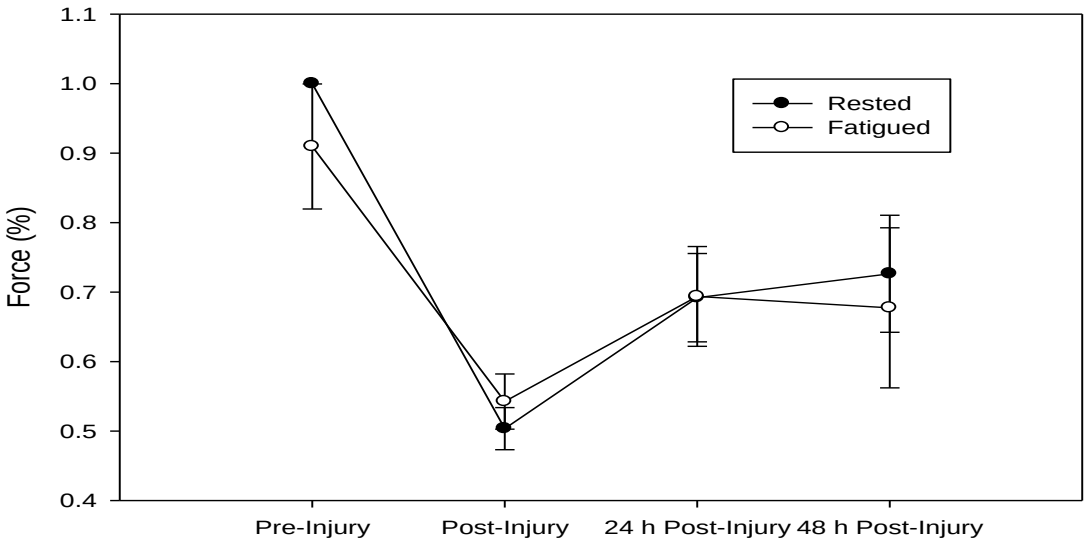

Figure 2.8. Knee extensor electrically stimulated force between the rested and fatigued state in the injury group in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Subjects (n = 5) performed the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run before and after a fatiguing protocol. Strength was normalized to non-fatigued pre-injury values. Knee extensor electrically stimulated force was found to be significantly reduced overall immediately at all time points after the injury (P < 0.030). Significant between group effects are denoted with (*). Values are reported as mean ± SEM.

Voluntary Activation of the Knee Extensor Muscles

Knee extensor voluntary activation measured prior to a fatiguing protocol was found to have a significant interaction (P = 0.047) but post-hoc testing failed to detect any significant differences. One subject was excluded from this analysis due to an inability to tolerate current stimulations above 200 mA. The knee extensor activation prior to a fatiguing protocol over time is depicted below in Figure 2.9.

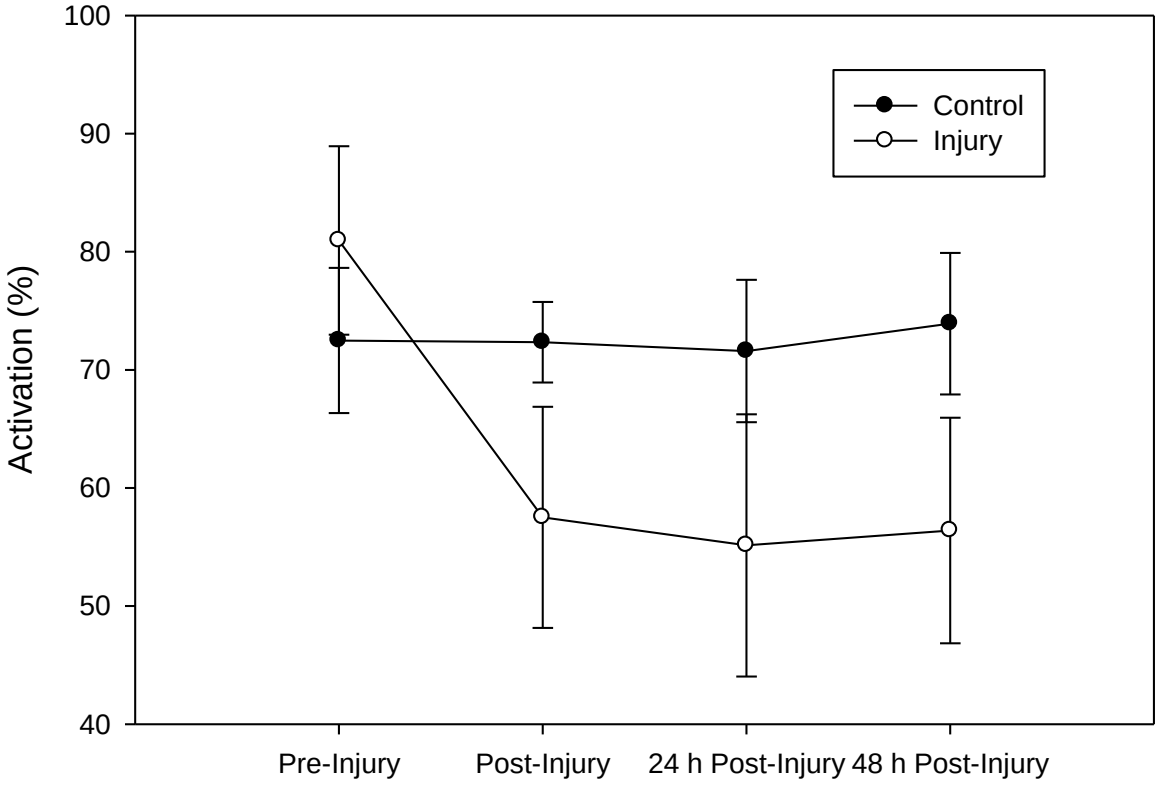

Figure 2.9. Knee extensor activation in relation to an injury inducing downhill run. Subjects (n = 11) performed the interpolated twitch technique to measure voluntary activation at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Knee extensor activation was found to be significantly reduced overall based on time (P = 0.049), but post-hoc analysis failed to detect any differences. Values are reported as mean ± SEM.

In the control group, knee extensor voluntary activation was compared between the rested and fatigued states. Overall, there was a significant 13.7 ± 4.9% change in electrically stimulated strength following the fatigue protocol (P = 0.019). No significant changes occurred in activation over time (P = 0.986). The changes in knee extensor electrically stimulated force following a fatiguing protocol are depicted below in Figure 2.10.

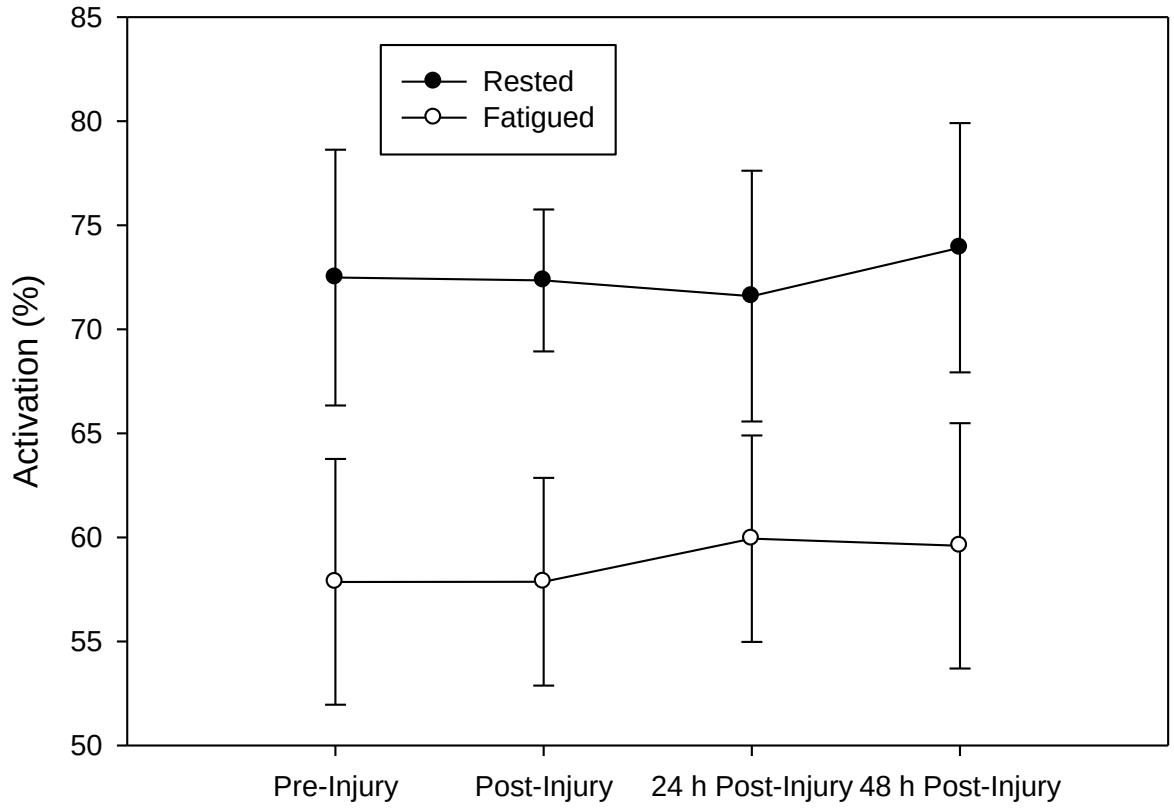

Figure 2.10. Knee extensor activation between the rested and fatigued state in the control group relation to an injury inducing downhill run. Subjects (n = 6) performed the interpolated twitch technique to measure voluntary activation at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Knee extensor activation was found to be significantly reduced overall based between the rested and fatigued states (P = 0.019), but no changes occurred over time (P = 0.986). Values are reported as mean ± SEM.

Knee extensor voluntary activation was compared between the rested and fatigued states in the injury group. Overall, there was a 13.2 ± 11.5% change in activation following the fatigue protocol, but this was not found to be significant (P = 0.285). Significant changes occurred in activation over time (P = 0.003), but there was no significant interaction (P = 0.648). The changes in knee extensor electrically stimulated force following a fatiguing protocol are depicted below in Figure 2.10.

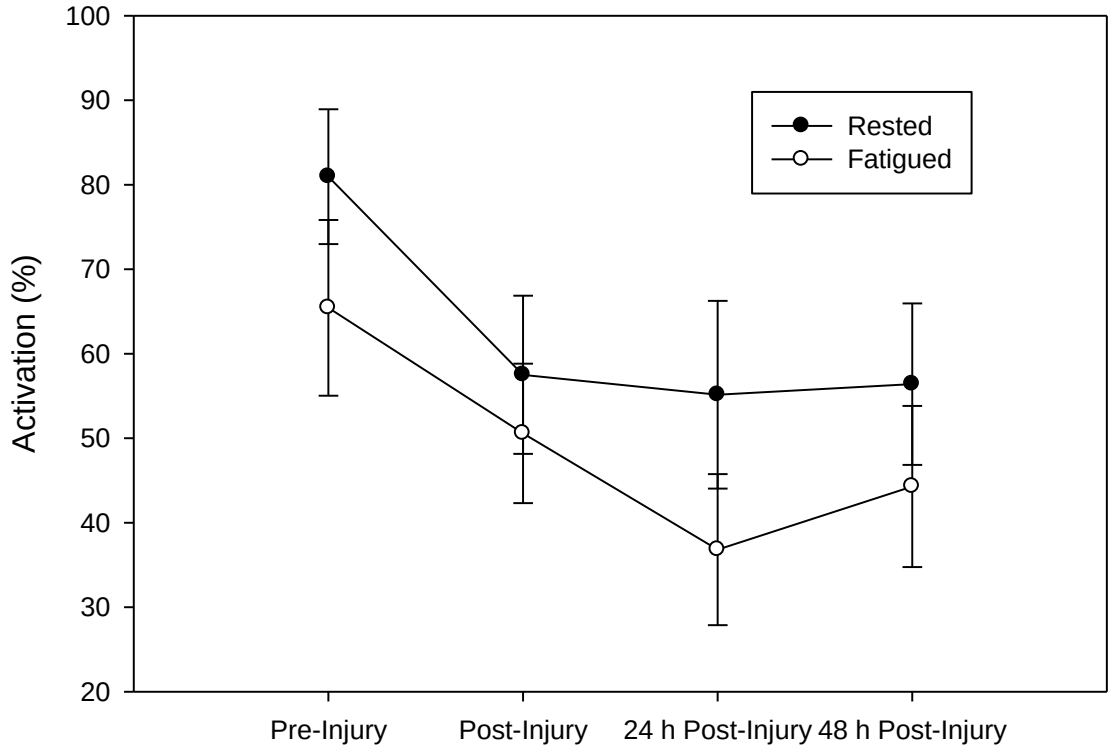

Figure 2.11. Knee extensor activation between the rested and fatigued state in the injury group in relation to an injury inducing downhill run. Subjects (n = 5) performed the interpolated twitch technique to measure voluntary activation at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Knee extensor activation was found to be significantly reduced immediately post-injury (P = 0.023). Significant effects are denoted with (*). Values are reported as mean ± SEM.

MVC Strength of the Elbow Flexor Muscles

Prior to the downhill run there was no significant difference in resting elbow flexor strength between the injury and control groups (P = 0.715). There were no significant changes from pre-injury strength in the control group, but the injury group was significantly reduced immediately, and 24 h post-injury. For a summary of results, see Figure 2.12.

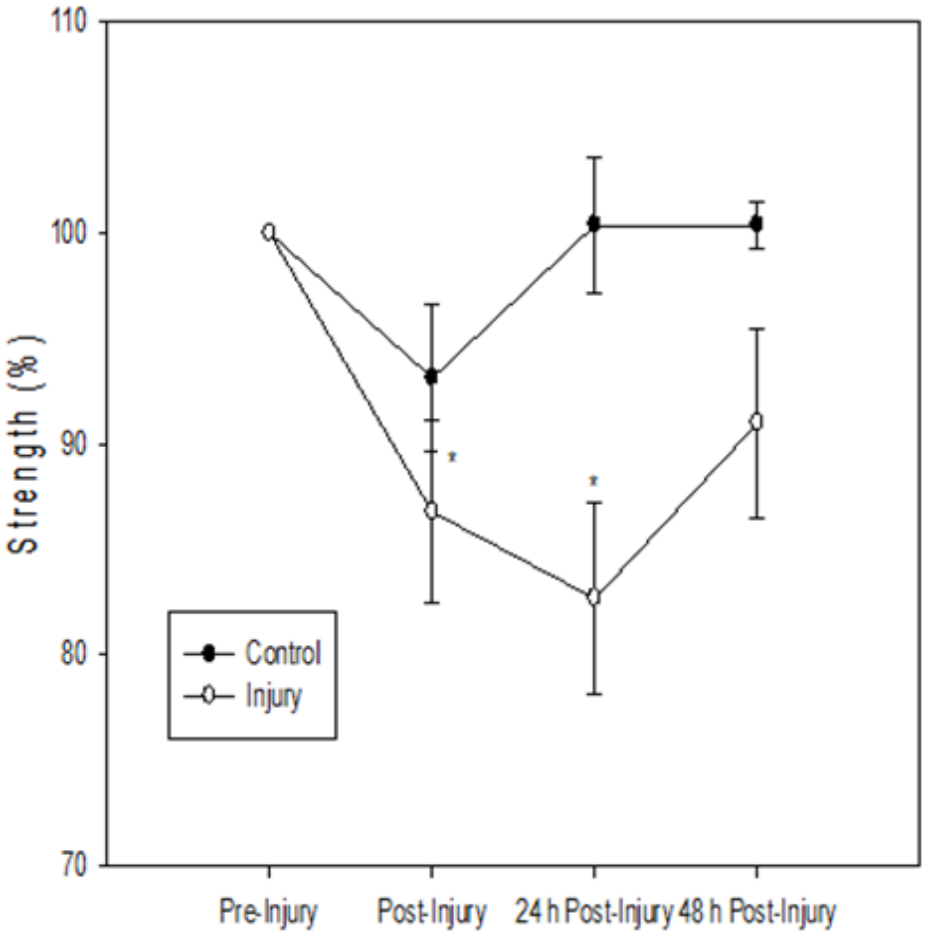

Figure 2.12. Elbow flexor force normalized to pre-injury values in relation to an injury inducing downhill run. Subjects (n = 12) performed MVCs at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to pre-injury values. Elbow flexor force was found to be significantly reduced in the injury group immediately, and 24 h post-injury. Significant between group effects are denoted with (*). Values are reported as mean ± SEM.

In the control group, elbow flexor MVC strength was compared between the rested and fatigued states. Overall, there was a significant 27.4 ± 4.1% reduction in strength following the fatigue protocol ($P < 0.001$). No significant changes occurred in strength over time ($P = 0.451$). The changes in elbow flexor MVC strength are depicted below in Figure 2.13.

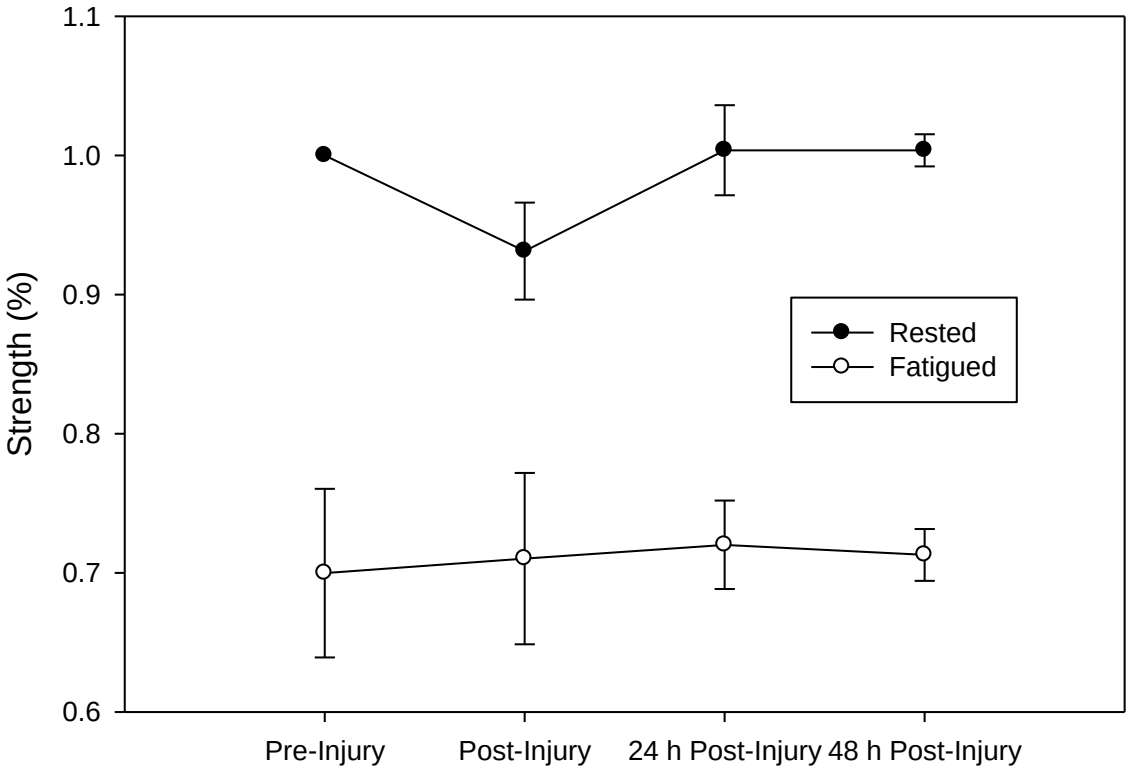

Figure 2.13. Elbow flexor force normalized to non-fatigued pre-injury values between rested and fatigued states in the control group in relation to an injury inducing downhill run. Subjects (n = 6) performed MVCs immediately after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to non-fatigued pre-injury values. Elbow flexor force was significantly lower in the fatigued state compared to the rested state ($P < 0.001$), but no significant changes occurred over time ($P = 0.451$). Values are reported as mean ± SEM.

Elbow flexor muscle MVC strength was compared between the rested and fatigued states in the injury group. Overall, there was a significant 23.7 ± 4.2% reduction in strength following the fatigue protocol ($P < 0.001$). No significant changes occurred in strength over time ($P = 0.154$). The changes in elbow flexor MVC strength are depicted below in Figure 2.14.

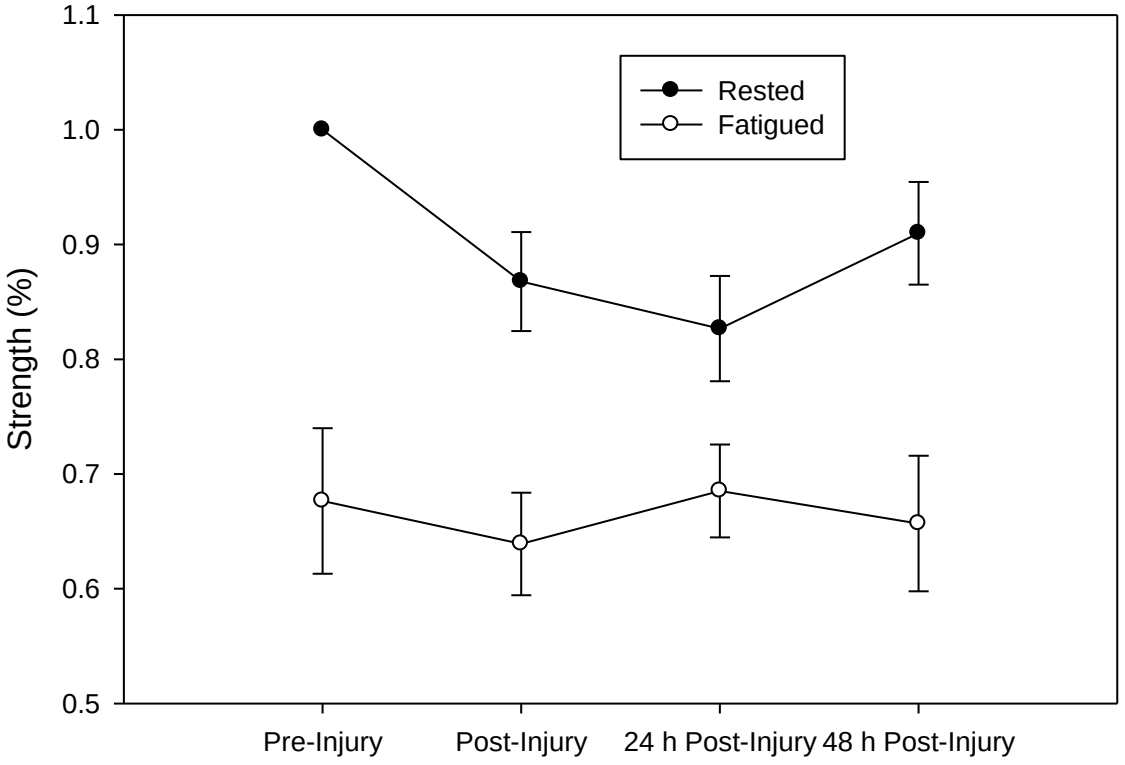

Figure 2.14. Elbow flexor force normalized to non-fatigued pre-injury values between rested and fatigued states in the injury group in relation to an injury inducing downhill run. Subjects (n =12) performed MVCs immediately after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Strength was normalized to non-fatigued pre-injury values. Elbow flexor force was significantly lower after the fatiguing protocol ($P < 0.001$), but no significant changes occurred over time ($P = 0.154$). Values are reported as mean ± SEM.

Elbow Flexor Electrically Stimulated Force

Prior to the downhill run, there was no difference in electrically stimulated force measured prior to a fatigue protocol between the control and injury groups ($P = 0.820$). No significant changes occurred in elbow flexor electrically stimulated force relative to pre-injury values over time ($P = 0.539$). No changes were observed in electrically stimulated force between the injury and control groups ($P = 0.187$). The elbow flexor electrically stimulated force at rest data is depicted below in Figure 2.15.

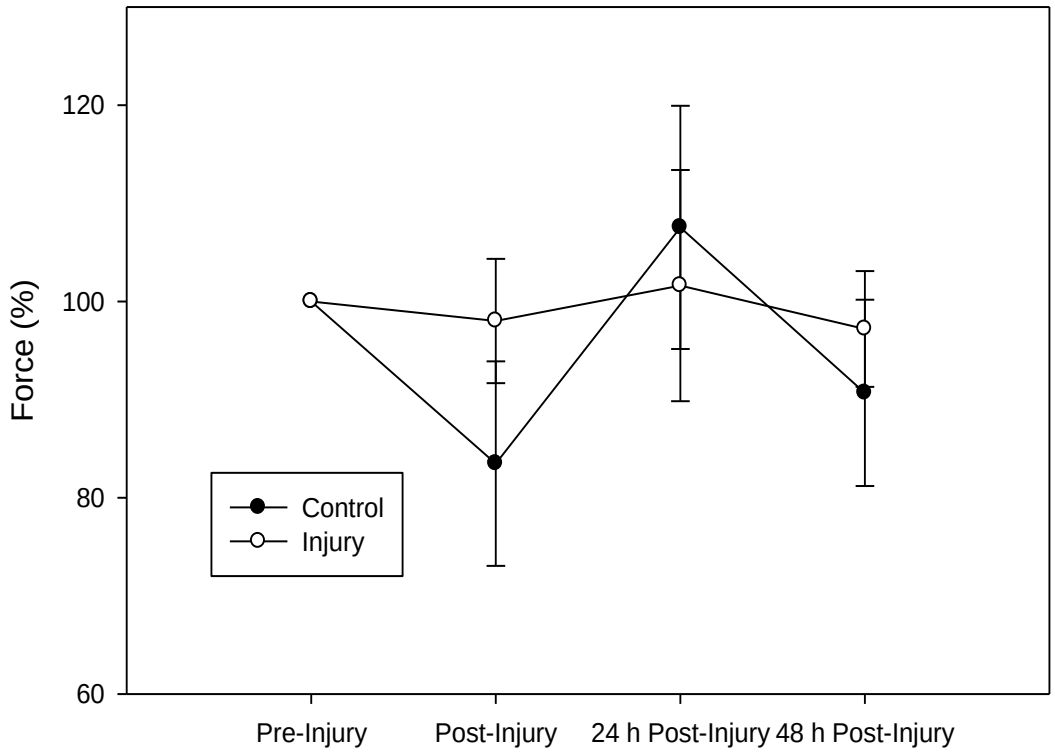

Figure 2.15. Elbow flexor electrically stimulated force at rest in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Subjects (n = 12) performed the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run to measure elbow flexor electrically stimulated force immediately prior to a fatiguing protocol. Elbow flexor electrically stimulated force was not found to be significantly different at any time point ($P \geq 0.268$). Values are reported as mean ± SEM.

In the control group, elbow flexor muscle electrically simulated strength was compared between the rested and fatigued states. Overall, there was a 15.4 ± 9.4% change in electrically stimulated strength following the fatigue protocol, but this change was not found to be significant (P = 0.131). No significant changes in electrically stimulated strength occurred over time (P = 0.095), and there was not a significant interaction (P = 0.771). The changes in elbow flexor muscle electrically stimulated strength in the control group are depicted below in Figure 2.16.

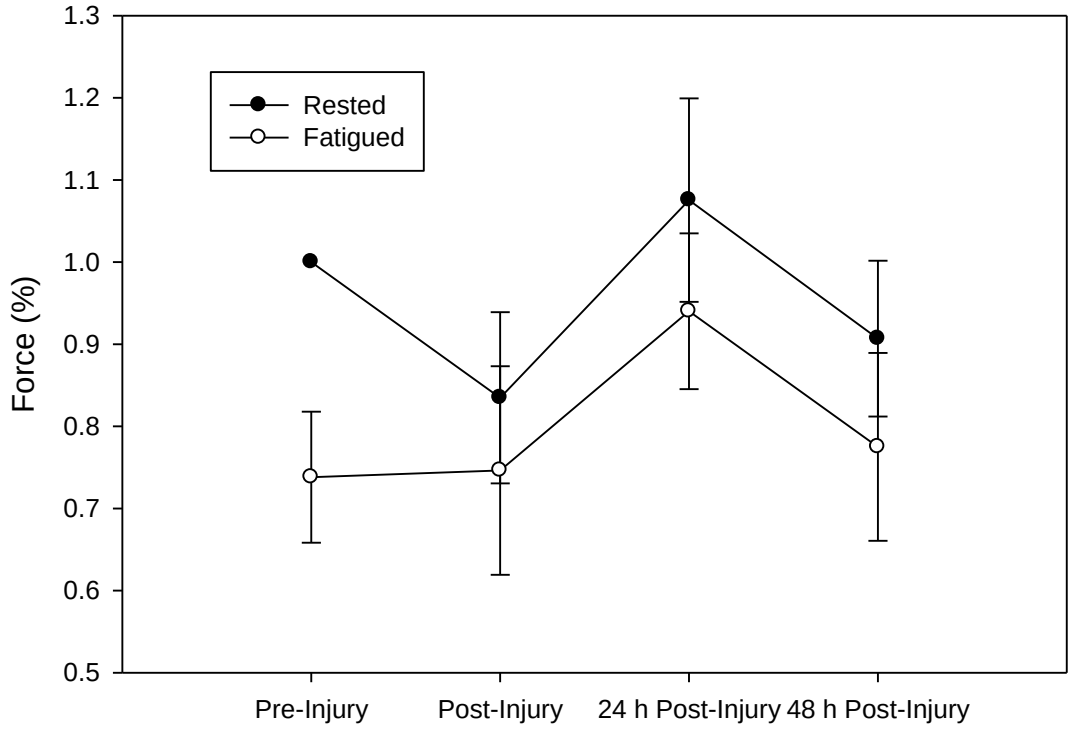

Figure 2.16. Elbow flexor electrically stimulated force between the rested and fatigued states in the control group in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Subjects (n = 6) performed the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run to measure elbow flexor electrically stimulated force immediately prior to a fatiguing protocol. Elbow flexor electrically stimulated force was not found to be significantly different at any time point (P ≥ 0.095). Values are reported as mean ± SEM.

Elbow flexor muscle electrically simulated strength was compared between the rested and fatigued states in the injury group. Overall, there was a significant 24.4 ± 7.2% change in electrically stimulated strength following the fatigue protocol (P = 0.007). No significant changes in electrically stimulated strength occurred over time (P = 0.798), and there was not a significant interaction (P = 0.704). The changes in elbow flexor muscle electrically stimulated strength in the injury group are depicted below in Figure 2.17.

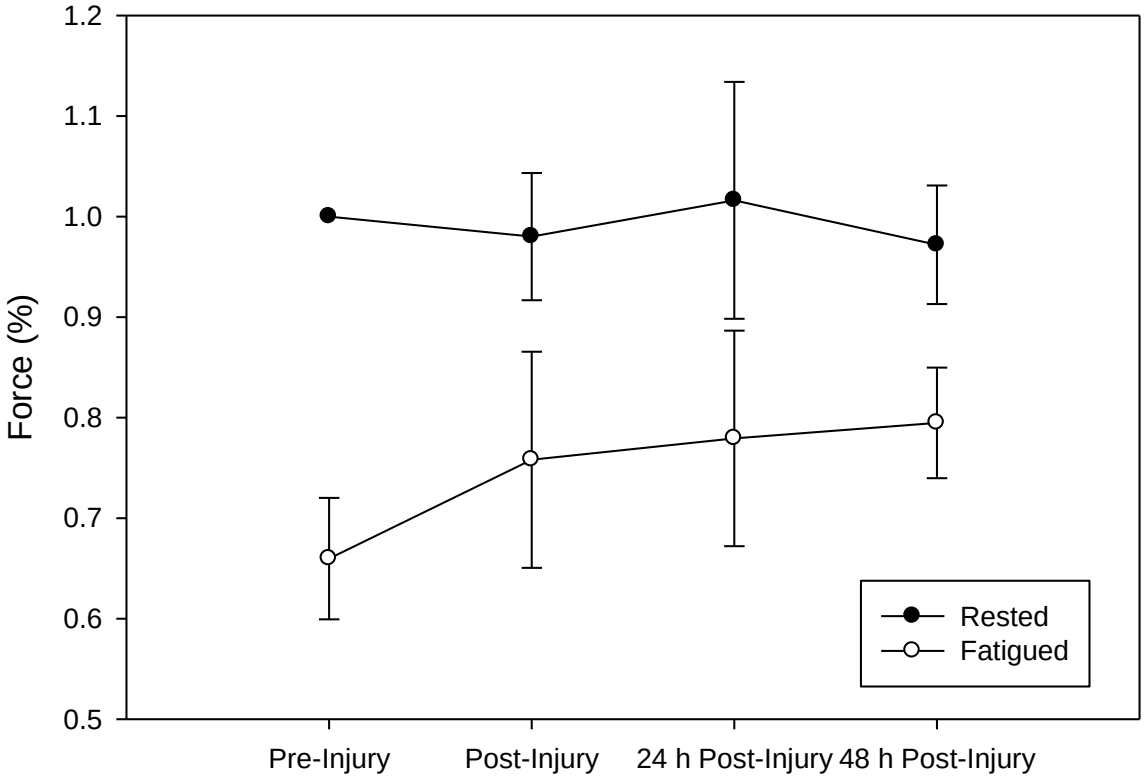

Figure 2.17. Elbow flexor electrically stimulated force between the rested and fatigued states in the injury group in relation to an injury inducing downhill run normalized to pre-injury twitch force at rest. Subjects (n = 6) performed the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run to measure elbow flexor electrically stimulated force immediately prior to a fatiguing protocol. Elbow flexor electrically stimulated strength was overall reduced in the fatigue measurements (P = 0.007). Values are reported as mean ± SEM.

Elbow Flexor Activation

Voluntary activation in the elbow flexors prior to a fatiguing protocol was not different between the control and injury groups prior to the downhill run (P = 0.682). Activation did not significantly change over time in the control group. Immediately post-injury there was a 16.2 ± 5.1% change in voluntary activation in the elbow flexors, but this was not found to be significant. By 24 h the elbow activation had declined to 20.9 ± 6.7%, and remained significantly reduced at 48 h post-injury when compared to controls. The changes in voluntary activation over time by group are depicted below in Figure 2.18.

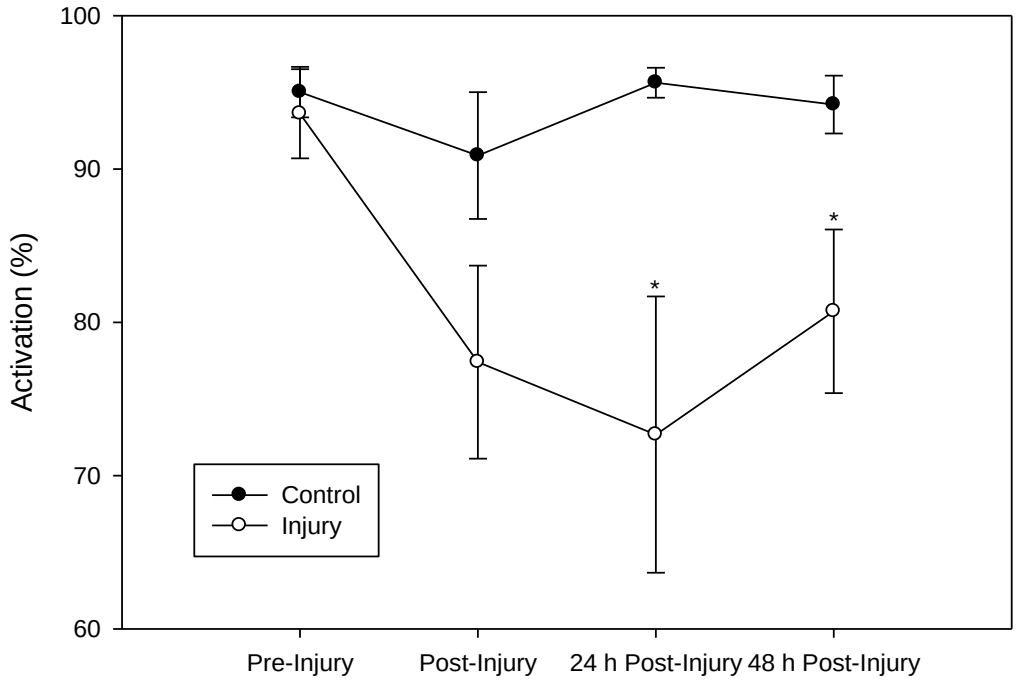

Figure 2.18. Elbow flexor activation in relation to an injury inducing downhill run. Elbow flexor voluntary activation was measured using the interpolated twitch technique at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Elbow flexor activation was found to be significantly reduced 24 h (P = 0.030) and 48 h (P = 0.039) post-injury when compared to control. Significant effects are denoted with (*). Values are reported as mean ± SEM.

Voluntary activation of the elbow flexor muscles was compared between the rested and fatigued states in the control group. Overall, there was a significant 10.8 ± 3.3% reduction between rested and fatigued states (P = 0.009). However, there was not found to be significant time (P = 0.551) or interaction effects (P = 0.143). Voluntary activation of the elbow flexors in a fatigued state is depicted below in Figure 2.19.

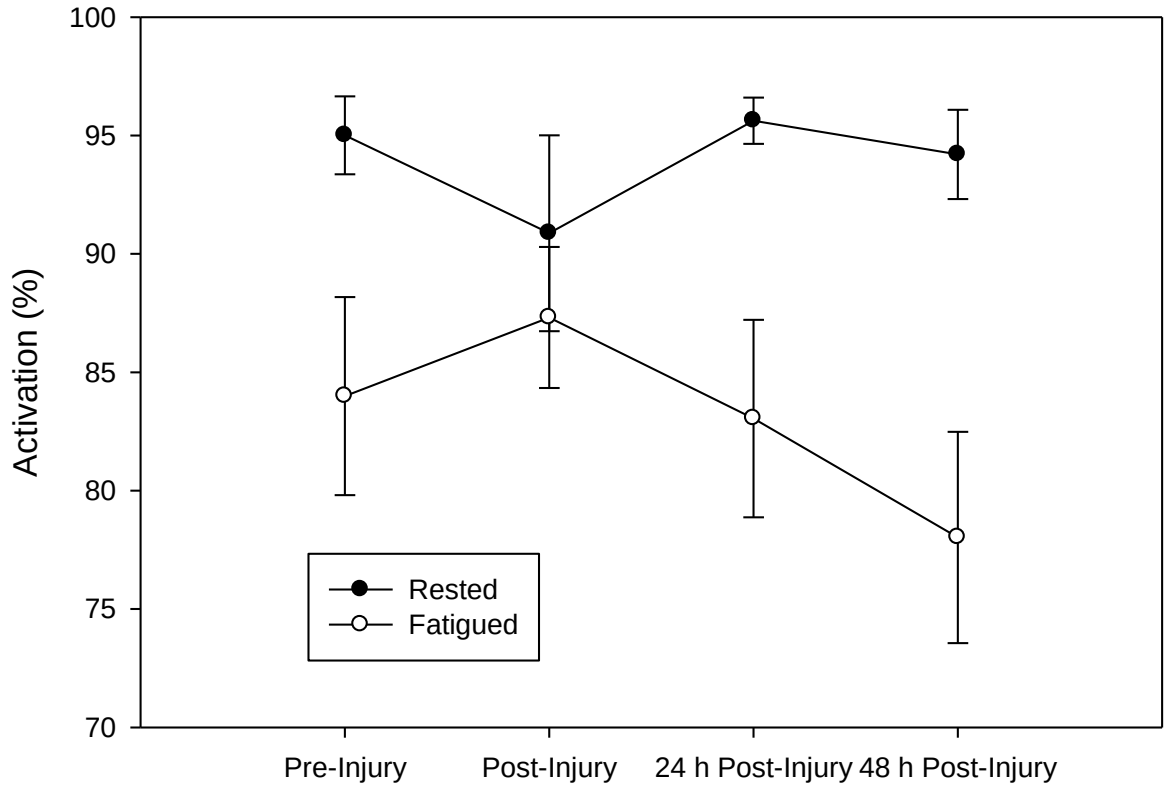

Figure 2.19. Elbow flexor activation between rested and fatigued states in the control group in relation to an injury inducing downhill run. Subjects (n = 12) performed the interpolated twitch technique to measure elbow flexor activation before and after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Elbow flexor activation was found to be significantly reduced in the fatigued state compared to the rested state (P = 0.009). Values are reported as mean ± SEM.

Voluntary activation of the elbow flexor muscles was compared between the rested and fatigued states in the injury group. Overall, there was a significant 13.6 ± 9.5% change in activation between rested and fatigued states, but this was not found to be significant (P = 0.184). There was not found to be significant time (P = 0.291) or interaction effects (P = 0.092). Voluntary activation of the elbow flexors in a fatigued state is depicted below in Figure 2.20.

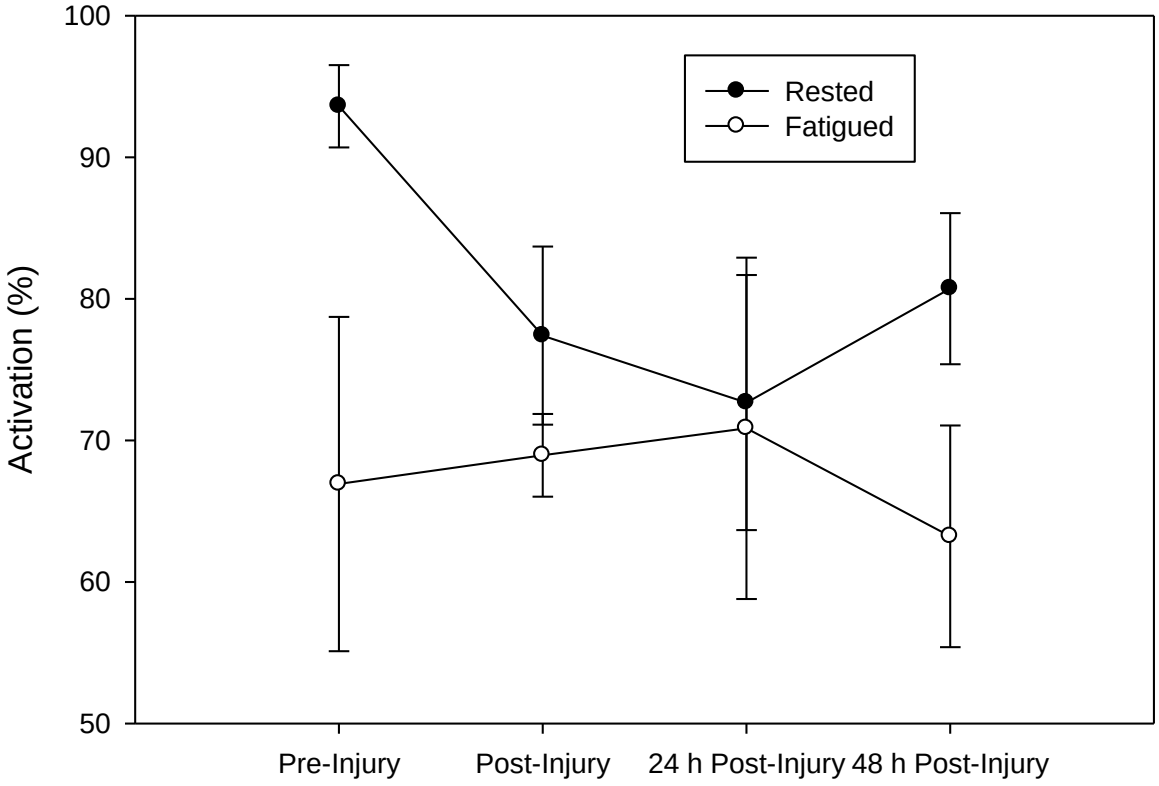

Figure 2.20. Elbow flexor activation between rested and fatigued states in the injury group in relation to an injury inducing downhill run. Subjects (n = 12) performed the interpolated twitch technique to measure elbow flexor activation before and after a fatiguing protocol at the indicated time points in relation to a 1 h lower body injury inducing downhill run. Elbow flexor activation was not found to be significantly reduced in the fatigued state compared to the rested state (P = 0.184). Values are reported as mean ± SEM.

Inflammatory Cytokines

Plasma IL-1β concentrations remained unchanged for both groups at all time point observed ($P > 0.235$). For the majority of subjects tested, concentrations remained below the threshold of detection (.3 pg/ml). The reliability of the assay was examined using a bivariate correlation between the duplicate measures ($R = 0.999$, $P < 0.001$). Only 9 subjects were included in this analysis due to availability of useable samples. Urine samples were not analyzed due to a lack of funding. Plasma IL-1β concentrations over time are depicted below in Figure 2.21.

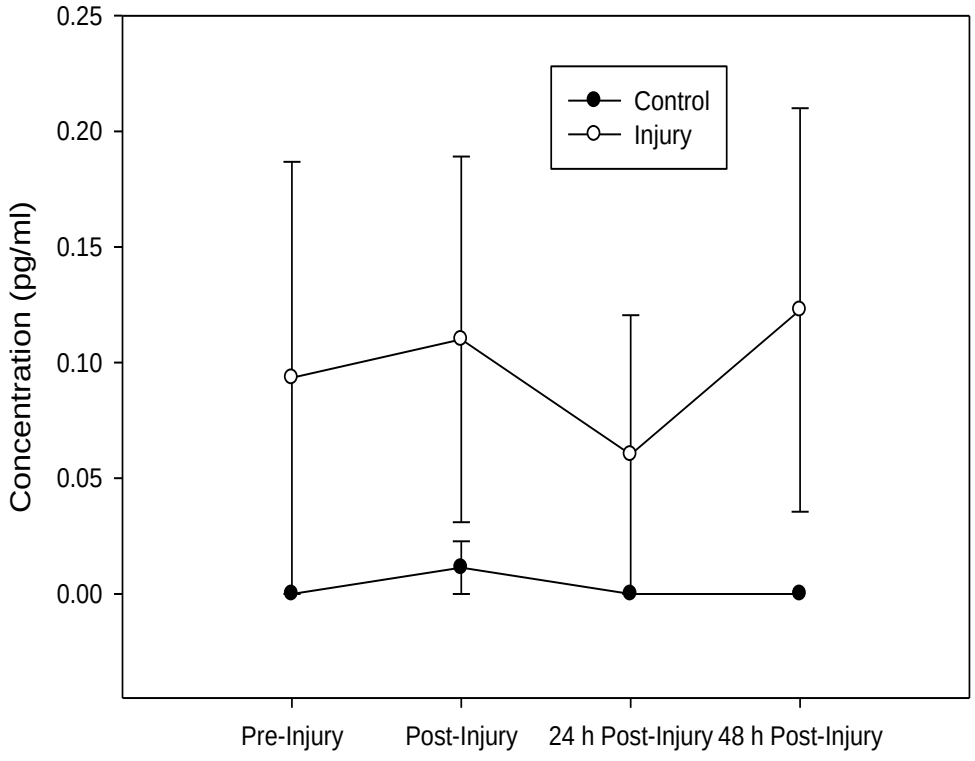

Figure 2.21. Plasma IL-1β concentrations in relation to an injury inducing downhill run. Subjects (n = 9) had blood drawn and measured using an ultra-sensitive ELISA kit at indicated time points in relation to a 1 h lower body injury inducing downhill run. IL-1β was not found to be significantly different between groups at any time points measured ($P > 0.235$). Values are reported as mean ± SEM.

There was no significant difference in plasma TNF-α concentrations between the control and injury groups at any time point tested (P = 0.456). Compared with the pre-injury levels, TNF-α concentrations were found to be significantly reduced in both groups immediately (P = 0.002) and 24 h post-injury (P = 0.048). Concentrations returned to baseline by 48 h (P = 0.702). The reliability of the assay was examined using a bivariate correlation between the duplicate measures (R = 0.991, P < 0.001). Only 5 subjects were included in this analysis due to availability of useable samples. Urine samples were not analyzed due to a lack of funding. TNF-α concentrations over time are depicted below in Figure 2.22.

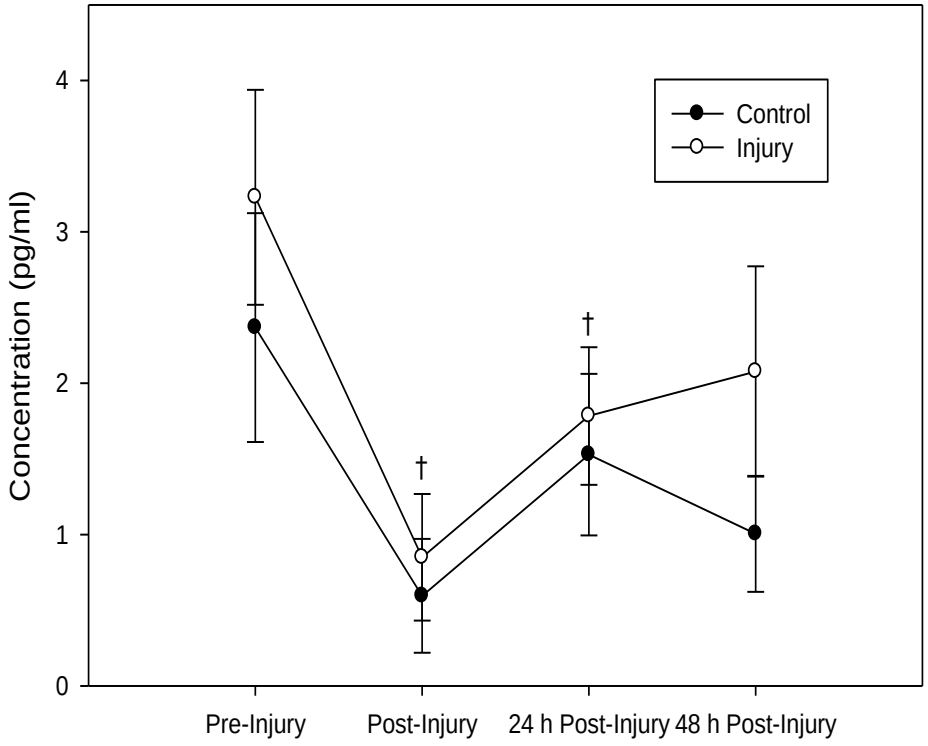

Figure 2.22. Plasma TNF-α concentrations in relation to an injury inducing downhill run. Subjects (n = 5) had blood drawn and measured using an ultra-sensitive ELISA kit at indicated time points in relation to a 1 h lower body injury inducing downhill run. TNF-α was found to be significantly lower overall immediately (P = 0.002) and 24 h post-injury (P = 0.046) compared to the pre-injury concentrations. Significant overall effects are denoted with (†). Values are reported as mean ± SEM.

Limb Pain

Thigh pain was not found to be different between the groups prior to the downhill run (P = 0.217). After the downhill run, thigh pain was significantly elevated in the injury group at all time points measured ($P \leq 0.001$). Only 10 subjects were included in this analysis due to missing data. The thigh pain between groups over time is depicted below in Figure 2.23.

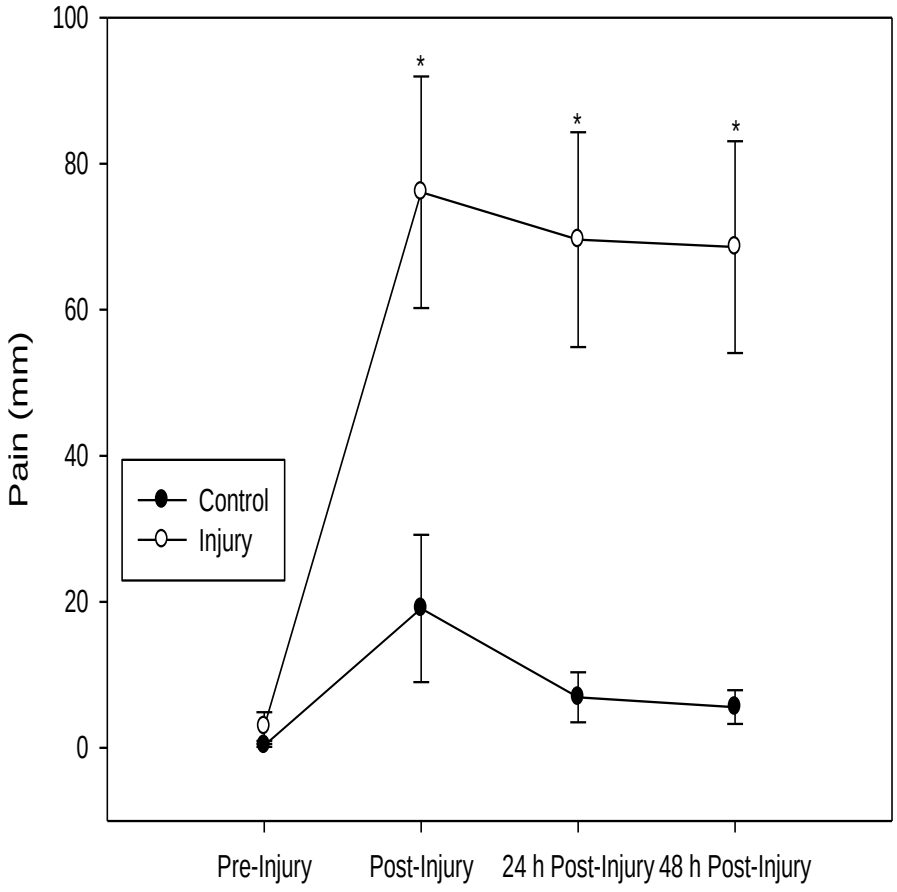

Figure 2.23. Thigh pain in relation to an injury inducing downhill run. Subjects (n = 10) estimated their thigh pain using a visual analog scale at indicated time points in relation to a 1 h lower body injury inducing downhill run. Thigh pain was found to be significantly elevated in the injury group at all time point ($P < 0.001$) when compared to control. Significant between group effects are denoted with (*). Values are reported as mean ± SEM.

Arm pain was not significantly different between the injury and control groups at any time point ($P \geq 0.092$). There was a significant time effect on Pain at 24 h ($P = 0.049$), but not at other time points ($P \geq 0.077$). Only 10 subjects were included in this analysis due to missing data. The upper arm pain between groups over time is depicted below in Figure 2.24.

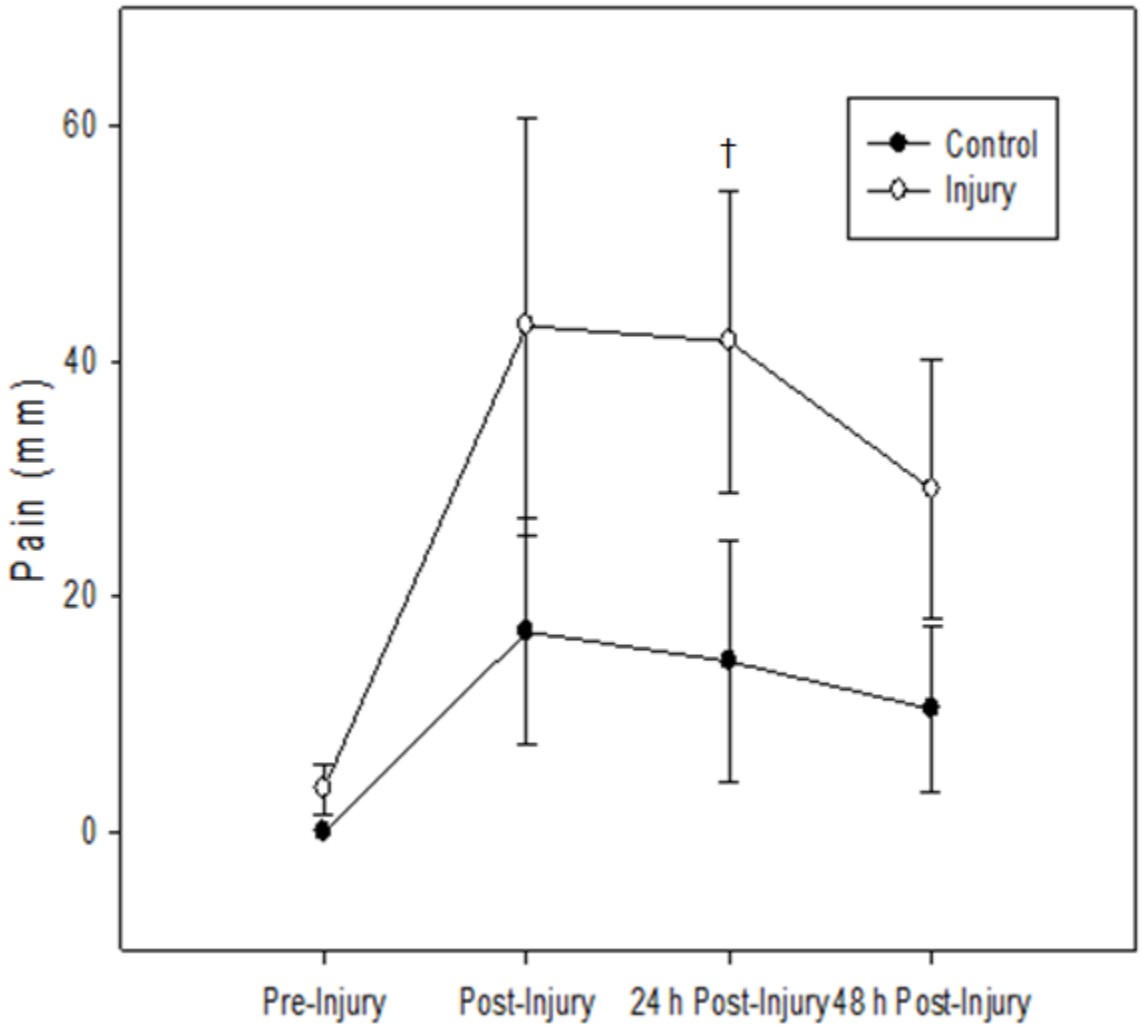

Figure 2.24. Upper arm pain in relation to an injury inducing downhill run. Subjects (n = 10) rated their upper arm pain using a visual analog scale at indicated time points in relation to a 1 h lower body injury inducing downhill run. Upper arm pain was found to be significantly elevated overall at 24 h post-injury ($P = 0.049$), with no differences between injury and control groups ($P > 0.05$). Significant overall effects are denoted with (†). Values are reported as mean ± SEM.

Limb Circumference

Thigh circumference was significantly larger in the injury group at all time points compared with the control group (P = 0.041). However, there were no changes in thigh circumference over time (P = 0.407). The thigh circumference data is depicted in Figure 2.25.

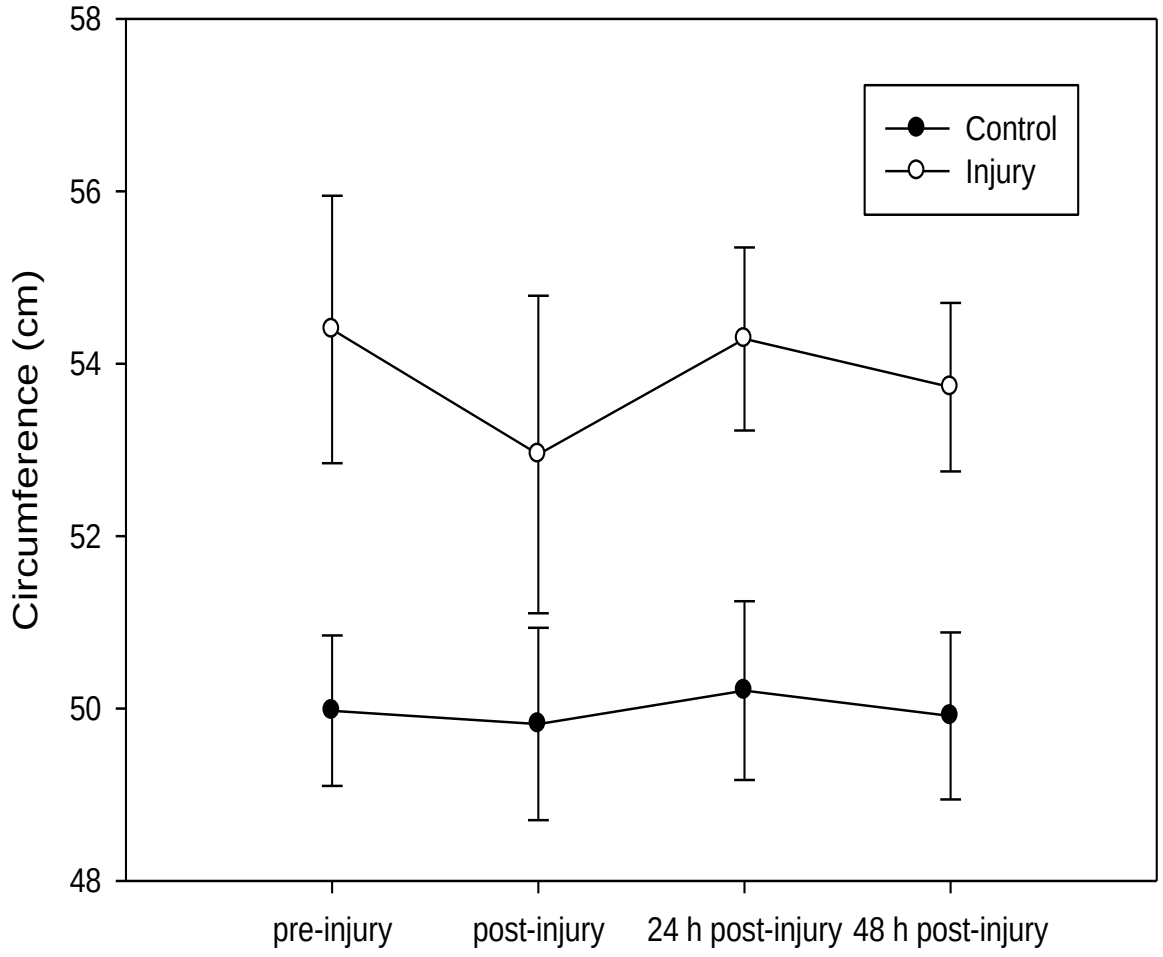

Figure 2.25. Thigh circumference in relation to an injury inducing downhill run. Subject (n = 10) thigh circumference was measured at indicated time points in relation to a 1 h lower body injury inducing downhill run. Thigh circumference was not found to be significantly different at any time point (P > 0.05).

Arm circumference was not significantly different between the injury and control groups (P = 0.590), and did not change over time (P = 0.331). The upper arm circumference data is depicted in Figure 2.26.

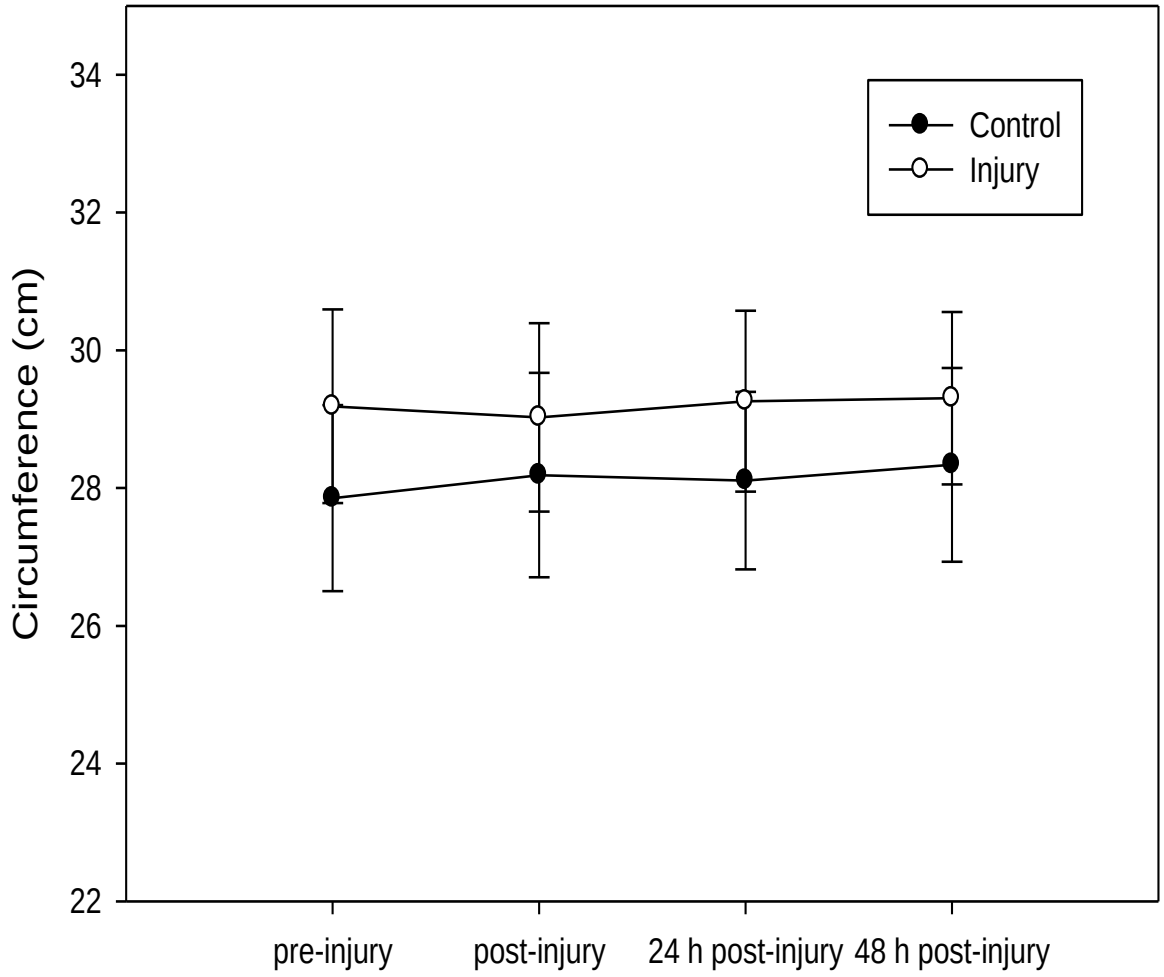

Figure 2.26. Upper arm circumference in relation to an injury inducing downhill run. Subject (n = 10) upper arm circumference was measured at indicated time points in relation to a 1 h lower body injury inducing downhill run. Upper arm circumference was not found to be significantly different at any time point (P > 0.05).

DISCUSSION

Running downhill for one hour resulted in injury to the knee extensor muscles of recreationally active individuals, impairing function for up to 48 h. This leg muscle injury was associated with a reduction in elbow flexor strength immediately, and 24 h post injury ($P = 0.010$). These impairments were not due to intrinsic changes of the elbow flexor muscles since no changes occurred in electrically stimulated force ($P = 0.539$), but can be attributed to changes in elbow flexor activation beginning 24h ($P = 0.030$) after the injury, with significant deficits in activation lasting up to 48 h ($P = 0.039$). No changes were found in plasma IL-1β ($P = 0.235$) or plasma TNF-α ($P = 0.456$) when compared with the control group, which suggests that the impairment of function was not mediated by an inflammatory response.

Knee Extensor Muscle Strength

In the downhill running group, strength of the knee extensors was reduced by 46.49 ± 6.55% immediately post-injury, and failed to significantly recover at 24 h or 48 h ($p < 0.05$). This reduction is larger than what other studies have shown in quadriceps strength following a bout of downhill running (Martin, Millet, Martin, Deley, & Lattier, 2004; Rowlands, Eston, & Tilzey, 2001) which showed a 14 – 17% and 21 – 27% decline in force, respectively. The larger force loss observed in this study is likely indicative of the longer duration of downhill running, 1 h in the current study vs 15 min in Martin et al. 2004, and 30 min in Rowlands et al. 2001. Some of the force loss observed in the initial post injury measurement may be due to fatigue from the metabolic disruption due to the downhill run. The persistent loss of force observed in the injury group at 24 h and 48 h is confirmation that the subjects were significantly injured by the downhill run. In support of this, previous studies have shown that downhill running injures the knee extensor muscles (Byrnes et al., 1985; R. Eston, Lemmey, McHugh,

Byrne, & Walsh, 2000; R. G. Eston, Mickleborough, & Baltzopoulos, 1995; Malm et al., 2004). The absence of significant strength loss in the knee extensors for the control group ($P > 0.05$) provides evidence that the strength assessment procedures did not significantly injure the KE muscles.

Measurements of knee extensor muscle strength in the control group following a fatigue protocol were 17.3 ± 9.8% lower than rested measures, but this change was not significant ($P = 0.108$). In the injury group, the fatigue protocol only led to a loss of 9.7 ± 4.9% which was also not significant ($P = 0.075$). It was originally hypothesized that a bout of fatiguing contractions would lead to a further reduction in strength, however, the 46.49 ± 6.55% reduction in strength following the downhill run may have made it impractical to produce enough force with the knee extensor muscles to achieve fatigue. Even when the resistance of the contraction was quite low (55% of 53.5% of original strength) the subjects required considerable help to perform the 50 contractions.

Knee Extensor Twitch Strength

The knee extensors electrically stimulated strength measured prior to the fatiguing protocol in the injury group was reduced by 49.66 ± 6.79% immediately post-injury, and 30.81 ± 14.24% 24 h post-injury. By 48 h post-injury the electrically stimulated force had recovered to the point that there was no longer a significant difference between the control and injury group. This would suggest that changes within the muscle account for most of the force loss immediately after injury, but other factors may become more important throughout recovery.

Twitch strength in the knee extensor muscles following the fatigue protocol was not significantly affected. In the control group there was only an overall 1.3 ± 7.7% reduction, and in the injury group there was only an overall reduction of twitch strength of 2.5 ± 6.7% compared to rested measures. It appears that the fatigue protocol was not of sufficient intensity to induce peripheral muscle fatigue within the knee extensor muscles.

Voluntary Activation of the Knee Extensor Muscles

This study found a significant interaction effect on knee extensor voluntary activation prior to the fatiguing protocol ($P = 0.047$), but post-hoc analysis did not detect any significant differences, suggesting that this study was underpowered for the voluntary activation measures in the knee extensor muscles. This might make sense because subject number projection was based on the effect size for the elbow flexor muscle MVC. Knee extensor MVC and voluntary activation have been found to be reduced after a 30-km running race (Millet, Martin, Lattier, & Ballay, 2003), and a 24 h treadmill run (Gimenez, Kerhervé, Messonnier, Féasson, & Millet, 2013). This failure to detect differences reflects the low power, which may be due to the large amount of variation in knee extensor voluntary activation in this study. Two of the injury group subjects experienced < 10% decline in activation at 24 and 48 h post injury, while the remaining subjects had a mean decline in voluntary activation of 44% and 48% respectively over the same period. Despite this weakness, when taken in context with the electrically evoked force of knee extensors, it appears most of the force loss after injury may be attributed to changes in the skeletal muscle.

Measurements after the fatiguing protocol showed a significant 13.7 ± 4.9% change in voluntary activation for the control group, without any significant changes in voluntary activation over time (P = 0.986). In the injury group, there was an overall 13.2 ± 11.5% change in measurements after the fatiguing protocol, but these changes were not significant (P = 0.285), however, activation did change over time in the injury group (P = 0.003). While the fatigue protocol was able to reduce voluntary activation in the control group, it appears the overall decline in activation from the downhill run prevented the fatigue protocol from lowering activation further in the injury group. The low amount of work performed following the injury protocol, and the fact that the control group did not suffer this reduction make interpreting these results difficult.

Strength of the Elbow Flexor Muscles

Elbow flexor MVC strength in the injury group was found to be significantly reduced in the injury group after 1 h of downhill running, immediately post-injury, and 24 h post-injury, but not in the control group. The magnitude of the decline was 13.2 ± 3.9% immediately post-injury, and 17.3 ± 4.0% 24 h post-injury. At 48 h post injury, there was a 9.0 ± 3.3% change, but this was no longer significant. These changes suggest that the downhill run led to a decline in elbow flexor MVC strength.

The fatigue protocol significantly reduced strength by 27.4 ± 4.1% in the control group and 23.7 ± 4.2% in the injury group. In both cases, there were no significant changes over time. While the amount of strength lost due to the protocol seems relatively constant across time points in both the control and injury groups, this result is difficult to interpret because subjects in the

injury group required considerable help to complete the 50 contractions. If this study were performed again, it would be interesting to record the subjects' rating of perceived exertion during the fatigue protocol to examine whether the perception of effort changes as a result of the downhill run.

Currently, there do not appear to be any published studies demonstrating that exercise-induced injury to the leg muscles alters MVC strength in the elbow flexors. Some evidence suggests that fatiguing the legs produces a small (6.8%) reduction in strength of the elbow flexors due to inhibitory signals from the group III/IV afferent nerves (Sidhu et al., 2014). This is in contrast to findings from another study that showed no effect of single leg high intensity cycling on the uninvolved limb (Elmer, Amann, McDaniel, Martin, & Martin, 2013). The mean MVC strength loss in the elbow flexors at 24 h in this study was 2.5 times higher than that previously observed in leg fatigue protocols (Sidhu et al., 2014).

Twitch Force of the Elbow Flexor Muscles

No significant changes occurred in twitch force of the elbow flexors when comparing the control and injury groups over time ($P > 0.187$). This suggests that the elbow flexor muscles were not significantly injured by the downhill run. The fatigue protocol led to an overall reduction of $15.4 \pm 9.4\%$ in the control group, which was not significant. The injury group experienced a significant $24.4 \pm 7.2\%$ reduction in twitch force of the elbow flexors.

Elbow Flexor Voluntary Activation

Elbow flexor activation was found to be significantly reduced in the injury group at 24 h ($P = 0.030$) and 48 h ($P = 0.039$) compared with controls. The magnitude of the reductions at 24 h (22.96 ± 9.096) are larger than the force reductions observed in the elbow flexors ($17.7 \pm 5.6\%$). Since there was no change in the electrically stimulated strength in the elbow flexors ($P > 0.05$) at any time point, all the force loss observed can be attributed to changes in voluntary activation. This suggests that the central nervous system's ability to activate peripheral muscle was reduced, but due to limitations in the study design, the exact site (brain or spinal cord) of the dysfunction cannot be determined. It may be possible to examine specific sites of the CNS to determine where the dysfunction occurs by testing the motor cortex silent period, and h-reflex (Fuhr, Agostino, & Hallett, 1991; Triggs et al., 1993).

Measurements of elbow flexor activation taken after the fatiguing protocol showed no differences in activation. This suggests that immediately after a set of fatiguing contractions the deficit in elbow flexor strength are eliminated when peripheral fatigue is present. However, it is also possible that the large variation present in the elbow flexor voluntary activation measurements (see Figure 2.12) after the fatiguing contraction may have obscured any effect present, making it difficult to identify whether peripheral muscle fatigue is altering voluntary activation in the elbow flexors.

One previous study has shown that fatiguing the knee extensors led to a small decline in force of the elbow flexors. An intrathecal injection of fentanyl prevented this loss of force from the elbow flexor muscles suggesting the effect was due to the activation of group III/IV afferent

nerves, but the changes observed in the current study exceed the 6.86% change observed previously (Sidhu et al., 2014) suggesting other mechanisms may have played a role. Previous evidence suggests that the pro-inflammatory cytokines retained within the muscle are capable of sensitizing the group III/IV afferent nerves (Hoheisel, Unger, & Mense, 2005). Whether pro-inflammatory cytokines are present in the muscle after 1 h of downhill running would need to be examined through muscle biopsies. One possible explanation for the greater force loss observed in this study is that afferent nerve activation was much greater than previously observed due to the sensitization by pro-inflammatory cytokines. To test for this, future studies should examine force loss in the elbow flexors using a similar injury protocol to this study with the additional application of intrathecal fentanyl to determine the role of afferent nerves in the force loss observed.

Since there are significant differences between the control group elbow flexor muscle activation and the injury group in activation, but not in twitch force, it follows that the force losses observed were likely due to a decline in activation, not in peripheral changes within the muscle. This excludes the possibility that significant injury or fatigue occurred at 24 h in the elbow flexors due to the downhill run. This reduction in elbow flexor strength was mostly recovered by 48 h post-injury based on the lack of significant differences in elbow strength between the control and injury groups at the 48 h time point.

Pro-inflammatory Cytokines

Prior to conducting this experiment, it was hypothesized that 1 h of downhill running would significantly elevate TNF-α and IL-1β. This study did not find any evidence that pro-

inflammatory cytokines were elevated after 1 h of downhill running. IL-1β levels remained below the detectable limit (0.3 pg/ml) for most subjects. TNF-α was detected, but the concentrations in blood declined post-injury, and remained reduced for at least 24 h post-injury for both groups (see Figure 2.16). The measurements of pro-inflammatory cytokines should be interpreted with caution since the overall number of usable samples obtained for testing was low for IL-1β (n =9) and TNF-α (n = 5).

Measuring IL-1β has been a recurring challenge, as noted throughout the literature, with some studies showing muscle injury increased IL-1β (Evans et al., 1986; Haahr et al., 1991; Ostrowski, Rohde, Asp, & Pedersen, 1998; Pournot et al., 2011; Scott et al., 2013; Sprenger et al., 1992), and other showing no change (Smith et al., 2007; Van de Vyver, 2013). The physiological concentrations in blood are very low, femtomolar to picomolar concentration, making its detection difficult (Moldoveanu, Shephard, & Shek, 2001). It is also possible that something in the circulation interferes with the test measurement. The results from TNF-α testing are more interpretable. While changes were observed in TNF-α over time, these changes occurred uniformly across groups. This likely means that TNF-α in the blood played no role in the communication of the injury of the knee extensors to the central nervous system, as previously described. However, the manufacturer of the ELISA kits used in this study reported that soluble inhibitors or anything bound to the cytokines would likely interfere with the test. It is also possible that elevations of the cytokines in question occur outside the measurement time points of this experiment.

The decline in TNF-α across both groups in the post measurements (see Fig 2.16) suggests that the exercise associated with the fatiguing protocol and the interpolated twitch procedure led to a decrease in measurable plasma TNF-α. This decrease could have occurred either due to increased metabolism of the cytokine, the uptake TNF-α by some other tissue, or increased secretion of endogenous soluble TNF inhibitors. Moderate exercise has been shown to increase glomerular filtration in the kidneys leading to increased urinary protein after exercise (Bellinghieri, Savica, & Santoro, 2008; Poortmans, 1985). Previous research suggests that exercise increases the urinary excretion of lipoxin A 4, another cytokine (Gangemi et al., 2003). If funding becomes available, the urine samples should be used to confirm whether urinary excretion of TNF-α or IL-1β was elevated after exercise.

Role of the Central Nervous System in MVC Strength Loss after Muscle Injury

This study demonstrated that a 1 h downhill run at 75% VO2max can reduce both knee extensor and elbow flexor strength for up to 48 hours. Strength loss in the knee extensors results primarily from the injury associated with eccentric contractions as described by previous reviewers (R. G. Eston et al., 1995). Without an external load being applied to the elbow flexors, it is unlikely that injury occurred in these muscles. In support of this, no changes were found in elbow flexor electrically stimulated strength. Changes in strength were observed in the immediate post measurements for both control and injury groups that suggest immediate changes may be due to fatigue from the testing procedures. The time frame associated with these measurements was approximately 5 h, and may have contributed to a loss in strength. However, significant reductions in MVC strength were also observed at 24 h post injury, after MVC strength reductions were resolved in the control group. Some mechanism(s) likely exist to

transmit the information of muscle injury from the injured muscle to the central nervous system to achieve this effect. Based on the evidence obtained in this study, that mechanism is likely not circulating pro-inflammatory cytokines.

CNS Dysfunction Possibly Due to the Perception of Pain

This study found that downhill running compared with control significantly elevated pain in the knee extensors ($P < 0.05$). In the injury group, there was a change in the elbow flexors of 47.2 ± 15.0 mm immediately post-injury, 45.6 ± 12.7 mm 24 h post-injury, and 30.5 ± 9.5 mm 48 h post-injury but this change was not significant ($p > 0.05$). It is possible that this measurement was under powered due to a small sample size ($n = 10$). It is unclear why there would be any change in pain in the elbow flexor muscles since the twitch force measurements suggest there was no injury present in the elbow flexor muscles. The timing of the pain is uncharacteristic of an exercise induce muscle injury, which typically results in significant widespread pain 24 h following the injury (Cheung, Hume, & Maxwell, 2003). Instead, there is an immediate 87.8 ± 9.3 mm increase in pain following the downhill run that failed to increase further at 24 h post-injury.

Previous studies suggest that experimentally inducing pain can have varied effects at submaximal intensities, with some studies showing increased muscle activity (Del Santo, Gelli, Spidalieri, & Rossi, 2007; Svensson, Houe, & Arendt-Nielsen, 1997), others showing decreased muscle activity (Del Santo et al., 2007; Farina, Arendt-Nielsen, & Graven-Nielsen, 2005) or no change (Farina, Arendt-Nielsen, Merletti, & Graven-Nielsen, 2004). The differences in these studies may be due to the intensity at which force is measured. Maximal force and voluntary

activation have been shown to be reduced by experimentally induced pain (Graven-Nielsen, Svensson, & Arendt-Nielsen, 1997; Graven-Nielsen, Lund, Arendt-Nielsen, Danneskiold-Samsøe, & Bliddal, 2002). This suggests that some of the force loss may be attributed to pain, but pain would have to be experimental manipulated to confirm this possibility.

CNS Dysfunction and the Inflammatory Response

First, the inflammatory particles TNF-α and brain derived neurotrophic factor, retained within the injured muscles have been shown to sensitize group IV afferent nerves making them discharge at lower force thresholds (Hoheisel et al., 2005). If the group IV afferents were affected in this manner, it would likely reduce maximal force production, since the spinal cord would likely reduce motor unit recruitment once they are activated (Amann, 2011; Amann & Dempsey, 2008; Amann et al., 2008). Future studies should take muscle biopsies to determine whether pro-inflammatory cytokines from the injured muscle can cross into uninjured muscles, since an attenuation of inflammatory pathways has been noted in response to eccentric exercise in both the injured and uninjured muscles (Hubal, Chen, Thompson, & Clarkson, 2008).

Second, the pro-inflammatory cytokines secreted by the injured muscle could cross the blood brain barrier as demonstrated in other studies (Carmichael et al., 2010; Gabay, Lamacchia, & Palmer, 2010; Lee, Liu, Dickson, Brosnan, & Berman, 1993) and alter the function of the substantia nigra (Brydon, Harrison, Walker, Steptoe, & Critchley, 2008; Ferrari et al., 2006; Herrera, Tomas-Camardiel, Venero, Cano, & Machado, 2005), which leads to a reduced ability to initiate/activate muscle for a physical task (Brydon et al., 2008; Godoy, Tarelli, Ferrari, Sarchi, & Pitossi, 2008). The results from this study do not support a role for circulating IL-1β

and TNF-α following 1 h of downhill running. The search may need to broadened in the future to include other circulating proteins since other circulating proteins could contribute to brain dysfunction as recently demonstrated in models of Parkinson's disease (Mulak & Bonaz, 2015).

Practical Application of Findings

The demonstration that muscle injury can affect neuromuscular function of an uninjured muscle group suggesting that the central nervous system senses the injury through some signaling mechanism and reduces force generation of skeletal muscle by lowering activation. Other muscle groups would need to be tested to determine whether this effect is global, or particular to the knee extensors and elbow flexors. It must be understood that the injury model used may determine whether such a widespread effect would occur.

The benefits to this mechanism are three-fold. First, from an evolutionary perspective, activity following a muscle injury would be significantly lower reducing exposure to possible predators during the recovery period. Second, the reduction in overall activity preserves energy that can be used to recover from the injury. Third, having an overall reduction instead of targeted reduction in voluntary activation would simplify the circuitry in the brain, lowering the energy needed to interpret signals of peripheral energy.

From a functional standpoint, the loss of strength in the uninjured muscle should be taken into account on days following muscle injury, and may affect joint stability, as previous studies

have shown that loss of force due to fatigue can impair joint stability (Gribble, Hertel, Denegar, & Buckley, 2004; Johnston 3rd, Howard, Cawley, & Losse, 1998; Yaggie & McGregor, 2002). This decreased joint stability may increase the probability of injury, and it may be necessary to develop recommendations for exercise that limit high risk activities in the uninjured muscles following an injury to another muscle group. In the present study, 1 h of downhill running in untrained subjects was sufficient to reduce force in the uninvolved elbow flexor muscles.

It is possible that more severe injury models, such as occur from surgical procedures could also produce widespread neuromuscular dysfunction, which is seen in ICU acquired weakness (Haas & Herridge, 2015; Na & Koh, 2011) where suppressed neuromuscular function can lead to life-threatening conditions. From a methodological standpoint, researchers should be aware that injury may affect neuromuscular function at locations distant from the injury, and not assume that a given injury model only affects the targeted muscles.

Conclusion

In summary, 1 h of downhill running was sufficient to induce injury in the knee extensors, reducing strength for up to 48 h. The injury of the leg muscles resulted in reduced strength and activation of the elbow flexors 24 h after the downhill run. This demonstrates that muscle injury can affect strength in other muscles distant from the site of injury by impairing voluntary activation of the uninjured muscle. The mechanism behind this reduced voluntary activation remains undetermined.

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APPENDICES

APPENDIX A: INSTITUTIONAL REVIEW BOARD APPLICATION

Study Application (Version 1.8)

1.0 General Information
* Please enter the full title of your study:
The effects of skeletal muscle injury on function of uninjured muscle
* Please enter a short title for your own personal reference.
The effects of skeletal muscle injury on function of uninjured muscle
* This field allows you to enter an abbreviated version of the Study Title to quickly identify this study.
2.0 Add Department(s)
2.1 Your department is listed below; click "add" to add an additional department, or select the check box next to the department and select "remove" to remove it. PLEASE DO NOT LEAVE "GSU - Georgia State University" AS YOUR PRIMARY DEPARTMENT.

Primary Dept?	Department Name
⊙	GSU - Kinesiology & Health

3.0 Assign Study Personnel
3.1 * Please add a Principal Investigator for the study:
James Doyle
3.2 If applicable, please select the Research Staff personnel (If you are adding a GSU student or faculty member and their name does not appear in the list of personnel, ask that person to log-in to iRIS with his/her campus ID and password which will populate their name in the list. If you are adding personnel from outside GSU and their name does not appear in the link they can be added with the form available at http://ursa.research.gsu.edu/working-with-individuals-outside-of-gsu/.):
A) Additional Investigators
Kyle Brandenberger
Student PI
Christopher Ingalls
Co-Investigator
Jeffrey Otis
Co-Investigator
Gordon Warren
Co-Investigator
B) Research Support Staff
Elisa J Lee
Support Staff
Noah J Robins
Support Staff

3.3 Please add a Study Contact:

Kyle Brandenberger

James Doyle

The Study Contact(s) will receive all important system notifications along with the Principal Investigator. (e.g. The project contact(s) are typically either the Study Coordinator or the Principal Investigator themselves).

3.4 Please select the Designated Department Approval(s). You must select the appropriate department sign-off for the study. If you do not know who this is, please contact your department head or chair. For the initial submission of the study, the submission must be routed to the designated department sign-off for endorsement.(YOU MUST ADD A DEPARTMENT SIGN-OFF HERE.)

Mark Geil

Department Chair

Add the name of the individual authorized to approve and sign off on this protocol from your Department (e.g. the Department Chair or Dean).

3.5 If applicable, please select the Administrative Assistant(s)

Administrative Assistant Note

4.0 Please answer the questions below regarding research personnel:

4.1 Human Subjects Training is a requirement for approval. Have you and your research team members completed Human Subjects Training? For step-by-step directions on checking research team members' training, please click here.

◉ Yes ○ No

4.2 Below is the PI you selected. Please confirm that the PI listed on the study is a current Georgia State University faculty member. Students or people outside of the University cannot serve as the PI on the study.

James Doyle

Is the PI listed a faculty member at Georgia State University?

◉ Yes ○ No

5.0 Information about your research

5.1 * Describe in lay terms the purpose of the research including the research question and what you hope to gain.

When a skeletal muscle is injured the immune system is activated, resulting in inflammation. Inflammation is the result of a number of different signaling molecules that broadly activate processes throughout the body. When someone is injured, these inflammatory signaling molecules result in pain, swelling and loss of function. It was originally thought that these signaling molecules do not readily cross the blood brain barrier. However, recent evidence suggests that inflammation is communicated across the blood brain barrier. Research in mice suggests that this communication of inflammation into the brain can result in reductions of exercise capacity. It currently remains unclear whether these measurable changes occur in humans. Since muscle injury from downhill running typically results in inflammation the injury may affect strength in unrelated muscle groups. The primary question of this study is whether skeletal muscle injury can affect strength in an uninjured muscle through changes in the central nervous system? By answering this question, we hope to better understand the role of the central nervous system in force losses after muscle injury. This information might be useful in understanding how skeletal muscle injury and the resultant inflammation affect strength throughout the body.

5.2 * Describe how human subjects will be involved. If there is to be any intervention or interaction with subjects, describe fully what researchers and subjects will do. Describe any procedures being performed already for diagnostic or treatment purposes.

Potential subjects will be screened using a health assessment form (see attachment) to ensure that they possess no contraindications to the performance of high intensity exercise. Potential subjects will also be screened to ensure that they have not had a major musculoskeletal injury of the lower limbs or elbow flexors in the last year, and that they are not performing regular exercises that may attenuate the inflammatory response (>10 miles of running per week or regular strength and plyometric training would be disqualifying).

Overview of testing procedure timeline: After providing their informed consent, will fill out a health history form. The subjects will then be asked to perform a familiarization of the twitch interpolation procedures outlined below followed by a graded exercise test. The subjects will then be scheduled for the injury protocol and subsequent twitch interpolation procedures. On the second visit, which will occur at least 48 hours after the screening trial, the subjects will be asked to provide a urine sample followed by a blood sample. Then they will be asked to perform the twitch interpolation procedures prior to the injury protocol. The subjects will then perform the downhill running injury protocol, followed by a second blood sample and the twitch interpolation procedures. Two hours after the downhill run, the subjects will provide a second urine sample. At 24 hours, and 48 hours post injury, the subjects will be asked to return to the lab to provide additional urine and blood samples, and perform additional sets of the twitch interpolation procedure to examine how the function of the elbow flexors and knee extensors are affected during the recovery process. An additional set of subjects will be recruited as a control group. The control group will do everything the experimental group does except the downhill run.

A timeline of the subject's involvement is as follows:

Day 1
1) Subject reports for the initial screening trial and provides informed consent prior to any screening.
2) Subject fills out the health history questionnaire and is screened for suitability to continue the study.
3) Subject performs a familiarization trial for the arm and leg strength assessments.
4) If selected for participation, subject performs a VO2max test.
5) Subject is scheduled for the remaining 3 data collection sessions, which will occur 1-2 weeks following the screening trial.

Day 2
1) Urine samples will be collected and venous blood will be drawn to determine the levels of inflammatory markers in the blood and urine.
2) Subject reports for the data collection and the downhill run.
3) Subject performs the leg strength assessment.
4) Next, subject will perform 5 sets of 10 maximal concentric contractions of the knee extensor muscles. The purpose of doing these 50 contractions is to induce fatigue in the muscles. Immediately upon completion, the knee extensor muscles strength will be re-assessed.
5) Subject performs the arm strength assessment.
6) Next, subject will perform 5 sets of 10 maximal concentric contractions of the elbow flexor muscles. The purpose of doing these 50 contractions is to induce fatigue in the muscles. Immediately upon completion, elbow flexor muscle strength will be re-assessed.
7) Subject performs the downhill running protocol.
8) The subject's blood will be drawn for determination of inflammatory markers.
8) Subject performs the leg strength assessment.
9) Next, subject will perform the 50 fatiguing contractions of the knee extensors. Immediately upon completion, knee extensor muscle strength will be re-assessed.
10) The subject performs the arm strength assessment.
11) Next, subject will perform the 50 fatiguing contractions of the elbow flexors. Immediately upon completion, elbow flexor muscles strength will be re-assessed.
12) 2 hours post injury, urine samples will be collected to determine the levels of inflammatory markers in the urine.

Day 3
1) Subject reports for the data collection.
2) Urine samples will be collected and venous blood will be drawn to determine the levels of inflammatory markers in the blood.
3) Subject performs the leg strength assessment.
4) Next, subject will perform the 50 fatiguing contractions of the knee extensors. Immediately upon completion, knee extensor muscle strength will be re-assessed.
5) Subject performs the arm strength assessment.
6) Next, subject will perform the 50 fatiguing contractions of the elbow flexors. Immediately upon completion, elbow flexor muscles strength will be re-assessed.

Day 4
1) Subject reports for the data collection.
2) Urine samples will be collected and venous blood will be drawn to determine the levels of inflammatory markers in the blood.
3) Subject performs the leg strength assessment.
4) Next, subject will perform the 50 fatiguing contractions of the knee extensors. Immediately upon completion, knee extensor muscle strength will be re-assessed.
5) Subject performs the arm strength assessment.
6) Next, subject will perform the 50 fatiguing contractions of the elbow flexors. Immediately upon completion, elbow flexor muscles strength will be re-assessed.

The strength assessments and fatiguing contractions will take place in Kell Hall, room 711. All other procedures will occur in Room G18 of the Sports Arena.

VO2max testing:
After being weighed without shoes on, the subjects will be asked to perform a graded exercise test. The test will begin at 6.3 miles per hour and increase in speed every two minutes until the subjects are unable to maintain their velocity. Gas exchange variables will be measured throughout the test using a metabolic cart (True Max 2400 Metabolic Measurement System). The highest 15 second average will be used as their VO2peak. Subjects will then be scheduled for an injury-inducing run. The injury protocol will take place within 1-2 weeks of the subject's graded exercise test, but at least 48 hours after the screening trial to prevent the screening trial from affecting measurements of torque, activation or inflammation.

Strength Assessments:
Isometric contraction strength of the knee extensor muscles and elbow flexor muscles will be measured before the downhill run, 15 minutes after the downhill run, 24 hours after the downhill run and 48 hours after the downhill run. Additionally, the subjects will perform 1 familiarization trail during the screening trial for selection purposes and to reduce variation in the data. Strength will be measured using a KinCom III dynamometer that is instrumented used to measure force under static (isometric) conditions. Initially, the subject will perform a warm-up consisting of 5 submaximal isometric contractions, each lasting 3 seconds; the subject will then be asked to increase the contraction intensity during each successive contraction. Strength will then be measured during a maximal voluntary effort, a brief electrical stimulation, and a combination of the two. This technique is commonly called the interpolated twitch technique. Initially, the subject will be asked to perform a 3-second maximal effort contraction of his/her knee extensor/elbow flexor muscles. At 2.5 seconds into the contraction, the subject's muscles will be stimulated using a computer-controlled Digitimer DS7AH stimulator. The stimulation will be very brief (, i.e., 10 milliseconds long). Peak force will be recorded both prior to and during the stimulation. At 2 and 4 seconds after the end of the maximal effort contraction (i.e., while the muscles are relaxed), the subject's muscles will be stimulated again and the peak forces recorded and averaged together. The stimulations will be done using two 3"x4" adhesive electrodes: one each placed on the skin overlying the proximal vastus lateralis and distal vastus medialis muscles for the knee extensors, and over the proximal belly and the distal tendon of the biceps for the elbow flexor measurements. The strength of the electrical stimulation is controlled by the stimulator current setting. The current setting will be adjusted in a preliminary series of "electrical stimulation only" contractions to the minimum needed to elicit peak strength. Because of the short duration of the electrical stimulations, they are well tolerated by most people. The purpose for using the interpolated twitch technique is that it allows a strength reduction to be partitioned into two components, one being a fatigue intrinsic to the muscle tissue itself and the other being a fatigue due a decreased motivation/drive to exert a maximal effort. If a subject is truly activating his/her muscles maximally during the so-called maximal effort contraction, then muscle force should not go up when the muscle is stimulated at 2.5 seconds into the contraction. During a given strength assessment, the interpolated twitch technique protocol will be performed a total of 6 times with 1 minute each between the protocols.

Urine Samples:
Urine samples will be collected immediately at the start of the 2nd session, 2 hours post, 24 h post, and 48 h post. The time frame of the urine samples was chosen based on previous research showing that IL-1β has a delayed appearance in the urine of 3 hours after the start of exercise (Sprenger et al., 1992). Immediately after collection, the urine samples will be centrifuged at 10,000g for 10 min at 4 C to remove leukocytes and debris (de Reijke, de Boer, Kurth, & Schamhart, 1996; Thomas, Sexton, Benson, Sutphen, & Koomen, 2010). The samples will then be frozen and stored at -75 C for later analysis.

Venipuncture:
Venous blood (3 ml) will be withdrawn once during each strength assessment trial. Blood will be withdrawn from the antecubital vein after cleaning the skin overlying the vein with an alcohol pad. Blood will be drawn by phlebotomists trained and approved by the Licensed Clinical Laboratory Director (license # 99026R) of the Applied Physiology Laboratory. Blood will be processed for collection of serum and stored in a -80 degrees Celsius freezer in the Muscle Biology Lab (Sports Arena, Room G19). The serum samples will be assayed inflammatory particle levels. These assays will be carried out in the Muscle Biology lab by Kyle Brandenberger. Kyle Brandenberger has completed GSU's online bloodborne pathogen training. Biohazardous waste will be disposed of according to GSU Biosafety guidelines.

Downhill Running Protocol:
Subjects will be asked to run downhill on a treadmill for one hour to injure the knee extensor muscles. A downhill run at 10% grade for 60 minutes at a speed equivalent to 75% of VO2peak at 0% grade was selected to induce injury because this protocol both produced a large inflammatory response, and could be employed with untrained subjects (McKune et al., 2006; L. L. Smith et al., 2007). Since the IL-1beta response appears to be dependent on both the length and the intensity of the protocol, shorter or less intense protocols might not illicit the immune response necessary to affect force production in an uninjured muscle. Subjects will be allowed one 5 minute break, as untrained subjects may not be able to complete the entire 60 minutes of running without a break.

5.3 * State who will be conducting each of the procedures detailed above. If there are multiple procedures or populations, be sure to state who will be conducting each procedure or working with each population

Only the individuals list on this application will be interacting with the subjects. Each person on this application has received the proper training in dealing with human subjects and has received training in dealing with medical waste. The primary student investigator will be the person primarily collecting data, conducting exercise testing,

injury protocols, and urine samples. Other students listed on this application may assist the student PI on an as need basis.

5.4 * Will the research be funded?

○ Yes ◉ No

5.5 * Is this study or any part of this study contributing to a dissertation or thesis?

◉ Yes ○ No

5.6 * Will this study be submitted to another IRB for review and approval?

○ Yes ◉ No

5.7 * Will the study be conducted outside of the United States?

○ Yes ◉ No

5.8 * Will the study involve interaction with human subjects?

◉ Yes ○ No

5.9 Does your study involve the use of Protected Health Information (PHI), as such term is defined by HIPAA, obtained from a Covered Entity? For more information on the definitions of PHI and Covered Entity or other terms related to HIPAA, please click here.

○ Yes ◉ No

5.10 * Will the study involve the use or possible exposure to infectious or potentially infectious material? (e.g. blood, bodily fluids, mucosal swabs, tissue samples, etc.)

◉ Yes ○ No

5.11 * Is there a research location located outside of Georgia State University?

○ Yes ◉ No

5.12 * Will your study involve data from student education records (e.g. class work, grades, attendance records, communications, projects, classroom tests, standardized tests, journals, SAT/ACT scores, etc.)? This list is not exhaustive. Please see section 1.6 of the IRB manual for more information on FERPA records.

○ Yes ◉ No

6.0 Please upload the dissertation or thesis prospectus.

6.1 Please be sure to upload the thesis, dissertation, or prospectus in the study document section at the END of the submission packet.

7.0 Human Subjects

7.1 * Will the study involve the use of FDA approved drugs? Please note: GSU's IRB can only review studies that use FDA approved drugs for approved uses. Please contact the IRB office if you are using a drug not approved by the FDA.

○ Yes ◉ No

7.2 * Will the study involve Investigational New Devices? Please note: GSU's IRB can only review studies that use FDA approved devices for approved uses. Please contact the IRB office if you are using a device not approved by the FDA.

○ Yes ◉ No

7.3 * Will the study involve Radiation?

○ Yes ◉ No

7.4 * Will the study involve deception or concealment of any information?

○ Yes ◉ No

7.5 * If you are using a survey that will be administered at Georgia State University, does it need to go through the Survey Coordinating Committee? This committee is independent of the IRB. Information on the committee can be found on their website.

○ Yes
○ No
◉ N/A

7.6 If any research personnel must have special certifications, training, or have qualifications to conduct the research procedures, the person's name and qualifications should be listed along with any certification or licensure number and dates of qualification. This includes studies that utilize venipuncture, EKGs, direct patient care, CPR, EEGs, and studies involving clinical psychologists, physicians, nurses, physical therapists, and others.

◉ Yes
○ No
○ N/A

This study involves the administration of a graded exercise test. As such, all the individuals involved in data collection need to have a current CPR qualifcation. Blood will be drawn by the following individuals, Elisa Lee Lic# RN225480, and Kyle J. Brandenberger (trained and approved by the Clinical Laboratory Director (License # 99026R)). All researchers involved in analysis of bodily fluids (blood and urine) will be trained in biohazard safety.

8.0 Vulnerable Populations

8.1 * If you are including women, are you recruiting pregnant women because they are pregnant or are you including any procedures that could be more than minimal risk for a pregnant woman or fetus?

○ Yes
○ No, I am including women of childbearing age, but the study includes no procedures that are more than minimal risk for the participant or fetus.
○ No, I am excluding women of childbearing age (a study specific justification must be provided elsewhere in the application).
◉ No, I am excluding pregnant women (a study specific justification and procedures for the exclusion must be included in the application)

8.2 * Are you including any students or trainees in your research?

◉ Yes, participants are the students or trainees of a researcher.
○ Yes, participants are students or trainees, but they are not the students or trainees of anyone on the research team.
○ No, I am not including any participants that are students or trainees.

8.3 * Are you including any employees or subordinates?

○ Yes, participants are the employees or subordinates of someone on the research team.
○ Yes, participants are employees or subordinates, but they are not the employees or subordinates of anyone on the research team.
◉ No, I am not including any participants that are employees or subordinates.

8.4 * Are you using any patients in your research?

○ Yes

◉ No
8.5 * Are you using prisoners in your study?
○ Yes ◉ No
8.6 * Are you using children (ages 0-17 in Georgia) in your research?
○ Yes ◉ No
8.7 * Are you including any individuals that may be cognitively or decisionally impaired?
○ Yes ◉ No

9.0 Students or trainees
9.1 * Will participants be recruited by or involved in research with their own (current) supervisor or teacher? If so, how will perceived coercion be handled? (Please see the IRB website for more information on using one's students in research.)
○ Yes ◉ No Current students of the researchers will be excluded because of the potential for coercion.
9.2 * Describe steps that will be taken to ensure additional protection of the rights and welfare of the students or trainees.
Current students of the researchers will be excluded because of the potential for coercion. The term student in this section of the application will refer only to students who are not current students of the researchers. Any student that volunteers for this study will be treated the same as non-student volunteers. Every subject will be thoroughly informed of all procedures prior to participation. Each subject will be asked to provide their informed consent. The privacy of subjects will be maintained throughout the study. The students may cease being in the study at any time they choose.

10.0 Population Data
10.1 * Will enrollment be limited to a specific ethnic, social, or gender group? If so, describe and justify.
○ Yes ◉ No
10.2 * Total Number of Subjects
20
10.3 * Total number of subjects per year.
20.00
10.4 * Justification for the number of subjects
20; This sample size is slightly greater than the minimum number of subjects (i.e., 14) needed to detect a small (0.2 SD) decline in strength with a repeated measures ANOVA on biceps MVC peak torque with a 2 group (control vs downhill running) design with an experiment-wise alpha level of 0.05 and assuming a correlation between repeated trials of 0.9. The need for five additional subjects is in anticipation of drop-outs.
10.5 * Please list the anticipated age range of subjects to be enrolled on this study.

18-44

10.6 * What is the time commitment for each participant? If you are using multiple populations, provide the time commitment required for a participant in each population. (e.g., "Participation will take 2 hours of time, one day a week, for 9 weeks for a total of 18 hours over 9 weeks.")

Participation will take 7 hours. The first session will take 1 hour. The second downhill running session will take 4 hours, and the following 2 sessions will take an 1 hour each.

10.7 * Describe where the procedures will take place. If you are conducting multiple procedures or using multiple populations, be sure to describe where each interaction will take place. Describe the location including how privacy will be maintained while conducting procedures. Please Note: If research is to be conducted off site and not at a public location, you MUST submit the approval letter from the site stating that the research may be conducted there.

Testing will take place in the applied physiology lab (G18) and Kell Hall Room 711. No one except the researchers listed on this application will be allowed in the room during testing with the subject.

10.8 * Federal regulations require that you include minors (e.g. participants aged 0 - 17) in your research unless you can justify their exclusion. Are you including minors? If not, check the appropriate box and provide a justification specific to this study in the text box.

- (●) No, inappropriate due to lack of safety data in studies conducted in adults
- ( ) No, inappropriate with respect to the purpose of the research
- ( ) Other
- ( ) Yes, minors are included

Please provide justification for not including minors in your study if applicable.

It is unclear whether development would predispose youths to injury of the growth plates during a downhill run. While downhill running is intended to injure the skeletal muscle, such injuries are typically low in severity and recover quickly.

10.9 * Federal regulations require that you include minorities (i.e. minority ethnic, racial, gender groups, etc.) in your research unless you can justify their exclusion. Are you including minorities? If not, describe and provide a justification specific to this study

- ( ) No, minorities are not included
- (●) Yes, minorities are included

Please justify your reasoning for excluding minorities if applicable.

11.0 Recruitment

11.1 * Describe in detail the recruitment plan. Who will be recruited and how (i.e. will the study use a subject pool, announcements, recruitment ads, word of mouth, email, etc.?) If materials such as flyers, emails, advertisements, screen shots from websites, or any other recruitment material is used, it must be uploaded with this application.

University students between the ages of 18-44 years old will be recruited for this study. This study is being restricted to students due to ease of access of the subject population. Subjects will be recruited via posted ads (attached to application) and by asking participants to provide the flyer to their friends and acquaintances. The researchers may also contact known associates and classmates via personal communication for recruitment purposes. Assignment of subject number will be assigned via a random number generator in excel. The group assignment will be done with a stack of shuffled cards red cards will be downhill running and black cards will be control group.

11.2 * Describe the inclusion and exclusion criteria.

Current students of the researchers will be excluded to prevent any possible coercion. Subjects between the ages of 18-44 years old will be the target population for this study. After providing their informed consent, subjects will fill out a health history form (attached to this application) which includes a section asking the subjects to provide the types and quantities of activity they perform in a typical week. Participants are required to indicate on the form

that they complete regular physical activity as defined by either 30 minutes a day of moderate activity (this is usually walking) or 15 minutes a day of vigorous activity as per ACSM guidelines. The researcher will visually ensure the participant is wearing running shoes. This study will be limited to individuals that do not have a diagnosed disease limiting exercise capacity, and that have no more than one risk factor for cardiovascular disease as described in the ACSM risk stratification procedures. This will ensure subjects are low risk according to the American College of Sports Medicine risk stratification process. Subjects who indicate kidney problems on their health history form will be excluded due to the risk of rhabdomyolysis. Following risk stratification, the subjects will perform a practice session of the strength test for the elbow flexor and knee extensor muscles. If the subject is unable to demonstrate an adequate plateau of force, or cannot tolerate the electrical stimulation they will be excluded from further testing. Following the strength assessments, the subjects will perform a graded exercise test. If the subjects experience any signs or symptoms such as: exertional breathlessness, chest pain, headache, blurred vision, fainting, vomiting, nausea, extreme fatigue or swelling in the legs, ankles or abdomen then they will be excluded from further testing.

11.3 * Will subjects be compensated or incur any costs for their participation? If so, provide details of the compensation (i.e. what the compensation is, the total amount, etc.). Compensation might include money, gifts, food, class credit, or extra credit provided for participation. Any costs to the subjects that may result from participation in the research should also be described. Detail what compensation participants will be given if they do not complete the study. If extra credit is given, describe the assignment of equal difficulty and length that will be provided for the same amount of credit if students wish to not participate in the research. If a lottery or drawing will be used, specific information must be provided to ensure it meets requirements in GSU policy and state law.

○ Yes ◉ No

12.0 IBC

12.1 * Describe any infectious or possibly infectious materials that will be used (e.g. blood, bodily fluids, mucosal swabs, tissue samples, etc.)

Urine samples, and blood samples will be collected.

12.2 * Has the Institutional Biosafety Committee (IBC) reviewed and approved this research?

◉ Yes
○ No
○ N/A

If yes, please enter the name of the person who holds the approved IBC protocol and the IBC Registration Number

Dr. J. Andrew Doyle

13.0 Benefits & Risks

13.1 * Describe the benefits, if any, to the subjects and to society from the proposed research. Please note: Compensation is not a benefit of participating in research

The subjects may not benefit directly from the results of this study. However, society as a whole will likely benefit from the knowledge of whether muscle injury can impact strength in uninjured limbs. Such an effect could have clinical relevance when assessing the relative risk of injuring healthy structures in the body acutely after skeletal muscle injury occurs. Additionally, the results of this study may apply to post-operative recovery.

13.2 * Describe the risks or discomforts, if any, to the subjects, whether physical, psychological, or social, and the means proposed to minimize them. If subjects may become upset or require medical or psychological attention as a result of the research procedures, a means of addressing attention to these concerns should be described in this section. A subject is at risk in research if he or she may be exposed to a possibility of harm that is greater than that ordinarily encountered in daily life or during routine examinations or tests. Each investigator should make a conscientious assessment of possible harms and disclose them to the IRB. It is the IRB's responsibility, as well, to examine each human subject protocol and make its own determination of the research risks and benefits.

Maximal Graded Exercise Test: During maximal graded exercise testing there is a risk of acute myocardial infarction, cardiac arrest, cardiac arrhythmia, stroke, and musculoskeletal injury. However, the risks of these events is very small in an apparently healthy population that does not have elevated risk of heart disease. In

order to ensure subjects are at a low risk for these adverse incidents, the subjects' ages have been restricted to 18-44, and the subjects will undergo a risk assessment according to the procedure outlined by the American College of Sports Medicine. Briefly, subjects will provide relevant health history information on a health history form. If the subjects have indicated more than 1 risk factor for cardiovascular disease, or have an Illness/injury/condition that limits exercise capacity then the subjects will not be allowed to proceed with further testing. The subjects will be visually monitored throughout the test and asked to signal the researchers with a thumbs down if they have any breathlessness, chest pain, nausea, blurred vision, or swelling. In the event of any signs or symptoms mentioned above, the treadmill will be stopped and testing terminated. There is an additional risk that subjects may fall off the treadmill during the test. To minimize this risk, a spotter will stand behind the treadmill to catch subjects that fall. Subjects will be visually monitored throughout the test and researchers will stop the treadmill immediately in the event that a subject appears to or actually falls.

Downhill Running: Downhill running typically results in excessive force on the knee extensors, causing mild injury to the muscle. The downhill running protocol in this study is designed to injure the muscle and will result in physical pain. This pain should be no greater than what someone would experience from a novel bout of exercise. To limit the injury, only physically active individuals will be recruited for this study. The subjects will be visually monitored throughout the test and asked every 5 minutes whether they have any breathlessness, chest pain, nausea, blurred vision, or swelling. In the event of any signs or symptoms mentioned above, the treadmill will be stopped and testing terminated. There is an additional risk that subjects may fall off the treadmill during the test. To minimize this risk, a spotter will stand behind the treadmill to catch subjects that fall. Subjects will be visually monitored throughout the test and researchers will stop the treadmill immediately in the event that a subject appears to or actually falls.

Electrical stimulation during strength measurement: Some subjects will perceive the electrical stimulation procedure as uncomfortable. This is because sensory neurons are being stimulated at the same time as the motor neurons and the brain is unsure as how to process the sensory neuron stimulation. There is most certainly no harm being done to any tissues by the stimulation. Any person perceiving the stimulation as intolerable will be identified during the familiarization session for strength assessment and it will be recommended to the subject that he/she terminate his/her participation in the study.

Performance of fatiguing bout of 50 concentric contractions: Some subjects will perceive the bout of exercise as uncomfortable, though the sensation will be shortlived. The degree of muscle strength loss immediately after the bout will be 20-25%. Muscle strength will recover within 2-3 hours.

Venipuncture: This procedure is associated with momentary pain from the needle prick. There is also a chance of bruising and swelling surround the puncture site, and a remote chance of infection resulting.

14.0 Subject Data

14.1 * Will the data contain any information that could personally link the subject to the research?

(•) Yes ( ) No

If **Yes**, describe what identifying information will be collected. If participants' identifying information will be kept separately from the data using a key or code sheet, describe the means of protecting these.

Subjects are required to fill out a health history for screening purposes. This information will be stored in a locked filing cabinet in room G20 of the sports arena. A separate code sheet will be stored separately from data in a locked office G20 of the sports arena.
This code sheet will be destroyed upon the completion of data collection.

14.2 * Will audio recordings or videotaping be used?

( ) Yes (•) No

If **Yes**, describe and provide information how any special precautions used to protect photographs, audio or video recordings.

14.3 * Describe the means by which the confidentiality of data will be maintained.

Identifiable data should only be released if participants explicitly agree. Describe where and how that data will be stored, who will have access, and how it will be disposed of or kept after the study. Describe protections for the storing or sharing of any electronic data. A Certificate of Confidentiality from the Federal Government may be necessary to protect research against being compelled to disclose the identity of subjects in legal proceedings.

Copies of health screening forms will be kept on file in a locked filing cabinet in room G20 of the sports arena. All other data will be de-identified using a random subject number and stored on a password protected computer in a separate room from identifiable information.

15.0 Review Categories

15.1 Review Categories

Select Category	Description
☑ Full Board Review	More than minimal risk/does not meet other categories' requirements
☐ Exempt - Category 1	Research conducted in established or commonly accepted educational settings, involving normal educational practices, such as (i) research on regular and special education instructional strategies, or (ii) research on the effectiveness of or the comparison among instructional techniques, curricula, or classroom management methods.
☐ Exempt - Category 2	Research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures or observation of public behavior, unless: (i) information obtained is recorded in such a manner that human subjects can be identified, directly or through identifiers linked to the subjects; and (ii) any disclosure of the human subjects responses outside the research could reasonably place the subjects at risk of criminal or civil liability or be damaging to the subjects financial standing, employability, or reputation.
☐ Exempt - Category 3	Research involving the use of educational tests (cognitive, diagnostic, aptitude, achievement), survey procedures, interview procedures, or observation of public behavior that is not exempt under paragraph (b)(2) of this section, if: (i) the human subjects are elected or appointed public officials or candidates for public office; or (ii) Federal statute(s) require(s) without exception that the confidentiality of the personally identifiable information will be maintained throughout the research and thereafter.
☐ Exempt - Category 4	Research involving the collection or study of existing data, documents, records, pathological specimens, or diagnostic specimens, if these sources are publicly available or if the information is recorded by the investigator in such a manner that subjects cannot be identified, directly or through identifiers linked to the subjects.
☐ Exempt - Category 5	Research and demonstration projects which are conducted by or subject to the approval of Department or Agency heads, and which are designed to study, evaluate, or otherwise examine: (i) Public benefit or service programs; (ii) procedures for obtaining benefits or services under those programs; (iii) possible changes in or alternatives to those programs or procedures; or (iv) possible changes in methods or levels of payment for benefits or services under those programs.
☐ Exempt - Category 6	Taste and food quality evaluation and consumer acceptance studies, (i) if wholesome foods without additives are consumed or (ii) if a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture.
☐ Expedited - Category 1	

	Clinical studies of drugs and medical devices only when condition (a) or (b) is met. (a) Research on drugs for which an investigational new drug application (21 CFR Part 312) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.) (b) Research on medical devices for which (i) an investigational device exemption application (21 CFR Part 812) is not required; or (ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.
☐ Expedited - Category 2	Collection of blood samples by finger stick, heel stick, ear stick, or venipuncture as follows: (a) from healthy, nonpregnant adults who weigh at least 110 pounds. For these subjects, the amounts drawn may not exceed 550 ml in an 8 week period and collection may not occur more frequently than 2 times per week; or (b) from other adults and children, considering the age, weight, and health of the subjects, the collection procedure, the amount of blood to be collected, and the frequency with which it will be collected. For these subjects, the amount drawn may not exceed the lesser of 50 ml or 3 ml per kg in an 8 week period and collection may not occur more frequently than 2 times per week.
☐ Expedited - Category 3	Collection of biological specimens by noninvasive means. Examples are: (a) hair and nail clipplings; (b) teeth routinely shed or extracted; (c) excreta and external secretions; (d) uncannulated saliva; (e) placenta removed after delivery; (f) amniotic fluid collected in accordance with accepted prophylactic techniques; (h) mucosal or skin cells collected by scraping, skin swab, or mouth washing; (i) sputum collected after saline mist nebulization;
☐ Expedited - Category 4	Collection of data through noninvasive procedures (not involving general anesthesia or sedation) routinely employed in clinical practice, excluding procedures involving x-rays or microwaves. Where medical devices are employed, they must be cleared/approved for marketing. (Studies intended to evaluate the safety and effectiveness of the medical device are not generally eligible for expedited review, including studies of cleared medical devices for new indications.)Examples: (a) physical sensors that are applied either to the surface of the body or at a distance and do not involve input of significant amounts of energy into the subject or an invasion of the subject's privacy; (b) weighing or testing sensory acuity; (c) magnetic resonance imaging; (d) electrocardiography, electroencephalography, thermography, detection of naturally occurring radioactivity, electroretinography, ultrasound, diagnostic infrared imaging, doppler blood flow, and echocardiography; (e) moderate exercise, muscular strength testing, body composition assessment, and flexibility testing where appropriate given the age, weight, and health of the individual.
☐ Expedited - Category 5	Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis). (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101(b)(4). This listing refers only to research that is not exempt.)
☐ Expedited - Category 6	Collection of data from voice, video, digital, or image recordings made for research purposes.
☐ Expedited - Category 7	

	Research on individual or group characteristics or behavior (including, but not limited to, research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices, and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies. (NOTE: Some research in this category may be exempt from the HHS regulations for the protection of human subjects. 45 CFR 46.101 (b)(2) and (b)(3). This listing refers only to research that is not exempt.)

16.0 Informed Consent

16.1 Directions: Check all applicable consent procedures. These procedures must be approved by the IRB.

Name	Description
☑ Signed Consent Required	Signed consent will be sought from the subject or the subject's legally authorized representative.
☐ Waiver of Consent or Waiver/Alteration of the required elements of consent	Per 45CFR46.116 (d) an IRB may approve a consent procedure which does not include, or which alters, some or all of the elements of informed consent set forth in this section, or waive the requirements to obtain informed consent provided the IRB finds and documents that: (1) the research involves no more than minimal risk to the subjects; (2) the waiver or alteration will not adversely affect the rights and welfare of the subjects; (3) the research could not practicably be carried out without the waiver or alteration; (4) whenever appropriate, the subjects will be provided with additional pertinent information; and (5) the research is not FDA-regulated OR Waiver of Consent Process-Demonstration Project (1)The research is conducted by or subject to the approval of state or local government officials (2)The research or demonstration protocol is designed to study, evaluate, or otherwise examine: - Public benefit or service programs. - Procedures for obtaining benefits or services under those programs. - Possible changes in or alternatives to those programs or procedures. - Possible changes in methods or levels of payment for benefits or services under those programs. (3)The research cannot practicably be carried out without the waiver or alteration. (4)The research is not FDA-regulated
☐ Waiver of Documentation of Consent	Per 45CFR46.117(c) an IRB may waive the requirement for the investigator to obtain a signed consent form for some or all subjects if it finds either: (1) That the only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern; or (2) That the research presents no more than minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

16.2 If subjects are unable to give consent (i.e., children or decisionally impaired adults) describe how and by whom permission or consent will be granted. For children, permission must be obtained from the child's parent or legal guardain unless a waiver of consent is approved to waive the parental permission.

Not applicable due to the subject population.

16.3 Provide a description of the informed consent procedures. Include who will obtain consent, where, when, and how. Include steps taken to minimize the possibility of coercion or undue influence, the language that will be used by those obtaining consent, how you will ensure the language is understood by the prospective participant or the legally authorized representative, and any information that will be communicated to the prospective participant or the legally authorized representative. Also state if there will be any waiting period between informing the prospective participant and obtaining consent.

The research personnel listed on this application will provide a written consent form to the subjects in a private location. The potential subjects will be allowed to read the form in its entirety. The research personnel will then ask the potential subject if they have any questions regarding the study and its procedures. When all questions have been satisfactorily answered, the potential subject will be allowed to sign the informed consent document if they choose.

16.4 What is the estimated lowest reading level of each population?

The estimated lowest reading level is 12th grade.

16.5 What is the reading level of your informed consent document? The reading level of the consent form must be at the lowest estimated reading level for the population. Keep in mind that half of all adult Americans read at or below the 8th grade reading level. To check the readability of your consent form please see Obtaining Grade Level Information.

9.8

16.6 If your population includes participants that are non-English speaking, a translated consent form must be provided. The translation must be completed by a certified translator (provide documentation) or a translation and aback translation must be provided. A researcher cannot complete his/her own translation. In addition to the translated documents, the consent form must be uploaded to the application in English. The IRB may request changes to the consent; therefore we recommend that you can indicate a translated consent form will be uploaded through an amendment after the study is approved.

N/A

17.0 COI

17.1 * Does the PI, Co-Investigators, or other research staff including their spouse and dependents have a significant financial conflict of interest defined as: - An equity interest that, when aggregated for the Investigator or research staff and their spouse or dependents meets all of the following tests: Exceed $5,000 in value as determined through reference to public prices or other reasonable measures of fair market value, represents more than a 5% ownership interest in any single entity, and value is affected by the outcome of the research; or - Salary, royalties or other payments that, when aggregated for the Investigator or research staff and their spouse and dependents over the next 12 months, are expected to exceed $5,000 and value is affected by the outcome of the research.

○ Yes ◉ No

17.2 * Does the PI, Co-Investigators, or other research staff including their spouse and dependents have: - A board or executive relationship related to the research regardless of compensation. - Proprietary interest related to the research including by not limited to a patent, trademark, copyright, or licensing agreement.

○ Yes ◉ No

18.0 Endorsement

18.1 * Please affirm the following endorsement statements: I will not begin this research study before receiving a formal letter of IRB approval; I will document informed consent according to my approved procedure; I will report to the IRB in a timely manner any unanticipated events to subjects; I will renew my IRB application before expiration or submit a study closure form; I will gain IRB approval before altering the research study and/or consent forms; I will notify the IRB if there are any changes in my contact information.

◉ I agree

APPENDIX B: INSTITUIONAL REVIEW BOARD LETTER OF APPROVAL

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999
Phone: 404/413-3500
Fax: 404/413-3504

In Person: Dahlberg Hall
30 Courtland St, Suite 217

October 10, 2016

Principal Investigator: James Doyle

Key Personnel: Brandenberger, Kyle; Doyle, James; Geil, Mark; Ingalls, Christopher; Lee, Elisa J; Otis, Jeffrey; Robins, Noah J; Warren, Gordon

Study Department: GSU - Kinesiology & Health

Study Title: The effects of skeletal muscle injury on function of uninjured muscle

Submission Type: Submission Response for Initial Review Submission Form

Review Type: Full Board Review

IRB Number: H17067

Reference Number: 339360

The above referenced study was reviewed and given pending approval under the Full board review process by the Georgia State University Institutional Review Board (IRB) on **09/15/2016**. This approval **became effective on 10/10/2016** after all pending issues were addressed and is **valid through 09/14/2017** in accordance with 45 CFR 46.111. The IRB has reviewed and approved the research protocol and any informed consent forms, recruitment materials, and other research materials that are marked as approved in the application. The approval period is listed above. Research that has been approved by the IRB may be subject to further appropriate review and approval or disapproval by officials of the Institution.

Federal regulations require researchers to follow specific procedures in a timely manner. For the protection of all concerned, the IRB calls your attention to the following obligations that you have as Principal Investigator of this study.

1. For any changes to the study (except to protect the safety of participants), an Amendment Application must be submitted to the IRB. The Amendment Application must be reviewed and approved before any changes can take place

2. Any unanticipated/adverse events or problems occurring as a result of participation in this study must be reported immediately to the IRB using the Unanticipated/Adverse Event Form.

3. Principal investigators are responsible for ensuring that informed consent is properly documented in accordance with 45 CFR 46.116.
   - The Informed Consent Form (ICF) used must be the one reviewed and approved by the IRB with the approval dates stamped on each page.

4. For any research that is conducted beyond the approval period, a Renewal Application must be submitted at least 30 days prior to the expiration date. The Renewal Application must be approved by the IRB before the expiration date else automatic termination of this study will occur. If the study expires, all research activities associated with the study must cease and a new application must be approved before any work can continue.

5. When the study is completed, a Study Closure Report must be submitted to the IRB.

All of the above referenced forms are available online at http://protocol.gsu.edu. Please do not hesitate to contact the Office of Research Integrity (404-413-3500) if you have any questions or concerns.

Sincerely,

Susan K. Laury

Susan Laury, IRB Chair

Federal Wide Assurance Number: 00000129

APPENDIX C: INSTITUTIONAL REVIEW BOARD AMENDMENT ONE LETTER OF APPROVAL

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999
Phone: 404/413-3500
Fax: 404/413-3504

In Person: Dahlberg Hall
30 Courtland St, Suite 217

January 17, 2017

Principal Investigator: James Doyle

Key Personnel: Brandenberger, Kyle; Doyle, James; Geil, Mark; Ingalls, Christopher; Lee, Elisa J; Otis, Jeffrey; Robins, Noah J; Warren, Gordon

Study Department: GSU - Kinesiology & Health

Study Title: The effects of skeletal muscle injury on function of uninjured muscle

Review Type: Expedited Amendment

IRB Number: H17067

Reference Number: 342263

Approval Date: 09/15/2016

Expiration Date: 09/14/2017

Amendment Effective Date: 01/17/2017

The Georgia State University Institutional Review Board reviewed and **approved** the amendment to your above referenced Study.
This amendment is approved for the following modifications:

- Updates who will complete the blood collection procedures
- The IBC approval is being added to the study.

The amendment does not alter the approval period which is listed above and the study must be renewed at least 30 days before the expiration date if research is to continue beyond that time frame. Any unanticipated/adverse events or problems resulting from this investigation must be reported immediately to the University Institutional Review Board.

For more information visit our website at www.gsu.edu/irb.

Sincerely,
Susan K. Laury
Susan Laury, IRB Chair

Federal Wide Assurance Number: 00000129

APPENDIX D: INSTITUTIONAL REVIEW BOARD AMENDMENT TWO LETTER OF APPROVAL

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999
Phone: 404/413-3500
Fax: 404/413-3504

In Person: Dahlberg Hall
30 Courtland St, Suite 217

January 20, 2017

Principal Investigator: James Doyle

Key Personnel: Brandenberger, Kyle; Doyle, James; Geil, Mark; Ingalls, Christopher; Otis, Jeffrey; Robins, Noah J; Warren, Gordon

Study Department: GSU - Kinesiology & Health

Study Title: The effects of skeletal muscle injury on function of uninjured muscle

Review Type: Expedited Amendment

IRB Number: H17067

Reference Number: 342703

Approval Date: 09/15/2016

Expiration Date: 09/14/2017

Amendment Effective Date: 01/20/2017

The Georgia State University Institutional Review Board reviewed and **approved** the amendment to your above referenced Study.
This amendment is approved for the following modifications:

- We are requested that Elisa Lee be removed from the study personnel.

The amendment does not alter the approval period which is listed above and the study must be renewed at least 30 days before the expiration date if research is to continue beyond that time frame. Any unanticipated/adverse events or problems resulting from this investigation must be reported immediately to the University Institutional Review Board.

For more information visit our website at www.gsu.edu/irb.

Sincerely,

Yan Ki Wai, IRB Member

Federal Wide Assurance Number: 00000129

APPENDIX E: INSTITUTIONAL REVIEW BOARD AMENDMENT THREE LETTER OF APPROVAL

INSTITUTIONAL REVIEW BOARD

Mail: P.O. Box 3999
Atlanta, Georgia 30302-3999
Phone: 404/413-3500
Fax: 404/413-3504

In Person: Dahlberg Hall
30 Courtland St, Suite 217

February 20, 2017

Principal Investigator: James Doyle

Key Personnel: Brandenberger, Kyle; Doyle, James; Geil, Mark; Ingalls, Christopher; Otis, Jeffrey; Robins, Noah J; Thomas, Jason W; Warren, Gordon

Study Department: GSU - Kinesiology & Health

Study Title: The effects of skeletal muscle injury on function of uninjured muscle

Review Type: Expedited Amendment

IRB Number: H17067

Reference Number: 343006

Approval Date: 09/15/2016

Expiration Date: 09/14/2017

Amendment Effective Date: 02/20/2017

The Georgia State University Institutional Review Board reviewed and **approved** the amendment to your above referenced Study.
This amendment is approved for the following modifications:

- Adding Jason Thomas to the support staff.

The amendment does not alter the approval period which is listed above and the study must be renewed at least 30 days before the expiration date if research is to continue beyond that time frame. Any unanticipated/adverse events or problems resulting from this investigation must be reported immediately to the University Institutional Review Board.

For more information visit our website at www.gsu.edu/irb.

Sincerely,

Yan Ki Wai, IRB Member

Federal Wide Assurance Number: 00000129

APPENDIX F: SUBJECT RECRUITMENT FLYER

Have you ever wondered whether inflammation can cause muscle fatigue? Researchers at Georgia State University, department of Kinesiology are attempting to answer this question and would like you to volunteer! Participation in this study involves having your strength tested, and running downhill on a treadmill. The study may cause moderate to severe muscle soreness and other injuries associated with running downhill. This study will require 7 hours of your time over 5 separate days. During those 5 days, we will ask you not to do any exercise other than the exercise for the research. You must also have properly fitting running shoes.

Reasons to Volunteer:

- You are a University Student between the ages of 18-44.
- Get first-hand experience in research as a subject.
- Learn about the field of exercise physiology.

Signup today by contacting Kyle Brandenberger Today!
Kbrandenberger1@gsu.edu , The Sport Arena G01.

APPENDIX G: CONTROL GROUP INFORMED CONSENT FORM

Georgia State University
Department of Kinesiology and Health
Informed Consent

Title: THE EFFECTS OF SKELETAL MUSCLE INJURY ON FUNCTION OF UNINJURED MUSCLE

Principal Investigator: Dr. J. Andrew Doyle
Student Principal Investigator: Kyle J. Brandenberger

I. Purpose:

You are invited to participate in a research study. The purpose of this study is to investigate if injuring leg muscles with downhill running can affect strength in arm muscles. You are invited to participate because you are physically active, and between the ages of 18 – 44. A total of 20 participants will be recruited for this study. Participation will require 7 hours of your time over 4 sessions.

II. Procedures:

If you decide to participate, the study will include 4 sessions over a two-week period during which we will ask you to refrain from exercise other than the exercise done in the study. The first day you will be asked to complete a health history form. If you have fewer than 2 risk factors for heart disease then you will be asked to continue with the study.

After these forms, you will have the strength of your front thigh (quadriceps), and right front upper arm (biceps) muscles measured. Your strength will be measured using an exercise machine that is able to measure force during conditions in which your leg and arm does not move even when you are applying pressure against the machine. Your strength will be measured during a maximal voluntary effort, a brief electrical stimulation, and a combination of the two. Initially, you will do a 3-second maximal effort contraction of your front thigh muscles. At 2.5 seconds into the contraction, your muscles will be stimulated using an electrical stimulator. The stimulation will be very short, only one-hundredth of a second long. At 2 and 4 seconds after the end of your maximal effort contraction, your relaxed muscles will be stimulated again. The stimulations will be done using two adhesive electrodes, one each placed on the skin overlying your upper and lower thigh muscles. The strength of the electrical stimulations that you will receive will be adjusted in a preliminary series of electrically-stimulated contractions to the minimum needed to elicit peak strength. During the strength assessment, you will do this sequence of contractions (maximal effort contraction followed by the electrical stimulations) six times with a minute between attempts. The results from your three best attempts will be used in the data analysis.

Version Date:

At the start of the second session you will be asked to provide a urine sample and a very small blood sample will be taken from one of your forearm veins. Then you will repeat strength tests for the biceps and quadriceps explained above. Next, you will perform a fatigue exercise for the front thigh muscles. You will be asked to perform 50 maximal effort concentric contractions of your front thigh muscles. This will require you to kick out as hard as you can against a bar on the exercise machine until the bar stops moving. The intent of these contractions is to induce fatigue in your muscles. Following the 50 concentric contractions, the strength of your front thigh muscles will be measured again as described above. Next, you will perform 50 maximal effort concentric contractions of your front upper arm muscles. This will require you to pull as hard as you can against a bar on the exercise machine until the bar stops moving. The intent of these contractions is to induce fatigue in your arm muscles. Following the 50 concentric contractions, the strength of your front upper arm muscles will be measured again as described above.

You will then be asked to return to the lab in 1 hour. This will be followed by a very small blood sample taken from one of your forearm veins. Then you will be asked to perform the front thigh muscle strength test followed by the fatigue exercise for the front thigh muscles. After fatiguing your front thigh muscles, you will perform another round of the strength test for the front thigh muscles. Then you will be asked to perform the front upper arm muscle strength test followed by the fatigue exercise for the front upper arm muscles. After fatiguing your arm muscles, you will perform another round of the strength test for the front upper arm muscles. You will then be asked to provide a urine sample 2 hours after the end of the downhill run.

You will be asked to return to the lab the following 2 days. You will be asked to provide a urine sample and a very small blood sample will be taken from one of your forearm veins on each of these days. Then you will be asked to perform the front thigh muscle strength test followed by the fatigue exercise for the front thigh muscles. After fatiguing your front thigh muscles, you will perform another round of the strength test for the front thigh muscles. Then you will be asked to perform the front upper arm muscle strength test followed by the fatigue exercise for the front upper arm muscles. After fatiguing your arm muscles, you will perform another round of the strength test for the front upper arm muscles.

III. Risks:

There is the possibility that participation in this study may cause you some temporary muscle weakness after doing the strength testing and the bout of 50 concentric contractions. Your muscles should however feel normal within a few hours. There is also the possibility that the electrical stimulation of your thigh muscles may feel uncomfortable. Despite this, no injury is

Version Date:

occurring to your leg. When your muscles are being stimulated, the nerves that sense pressure, temperature, position, and pain are all being stimulated and your brain does not know how to process the information from those nerves. If you find the stimulations too uncomfortable, you should withdraw from the study. When you have your blood drawn, you will most likely experience momentary pain from the needle prick. There is also a chance of bruising and swelling surrounding the puncture site and a remote chance of infection associated with the procedure. If you have any question about this study, or believe you have suffered any injury because of participation in the study, you may contact Dr. J. Andrew Doyle and Kyle Brandenberger at kbrandenberger1@student.gsu.edu or at (404) 413-8050. Georgia State University, however, has not set aside funds to pay for health care or to compensate you if something should occur.

IV. Benefits:

Participation in this study may notbenefit you personally. Overall, we hope to gain information about whether inflammation can affect susceptibility to fatigue.

VI. Voluntary Participation and Withdrawal:

Participation in research is voluntary. You do not have to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. You may stop participating at any time. Whatever you decide, you will not lose any benefits to which you are otherwise entitled.

VII. Confidentiality:

We will keep your records private to the extent allowed by law.Dr. J. Andrew Doyle, and Kyle Brandenberger will have access to the information you provide. Information may also be shared with those who make sure the study is done correctly (GSU Institutional Review Board, the Office for Human Research Protection (OHRP), and/or the Food and Drug Administration (FDA).We will use a randomly assigned subject numberrather than your name on study records. A code sheet linking your name and subject number will kept until all data collection is complete, at which time the code sheet will be destroyed. The information you provide will be stored. The information you provide will be stored on computers that are protected by passwords. Any paper copies of your information will be stored in a locked filing cabinet in room G20 of the sports arena. Your name and other facts that might point to you will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

VIII. Contact Persons:

ContactDr. J. Andrew Doyle and Kyle Brandenberger at kbrandenberger1@gsu.edu or at (404) 413-8050.If you have questions, concerns, or complaints about this study. You can also call if you think you have been harmed by the study. Call Susan Vogtner in the Georgia State University Office of Research Integrity at 404-413-3513 or svogtner1@gsu.edu if you want to talk to someone who is not part of the study team. You can talk about questions, concerns, offer

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input, obtain information, or suggestions about the study. You can also call Susan Vogtner if you have questions or concerns about your rights in this study.

IX. Copy of Consent Form to Participant:
We will give you a copy of this consent form to keep.

If you are willing to volunteer for this research, please sign below.

_____	_____
Participant	Date
_____	_____
Principal Investigator or Researcher Obtaining Consent	Date

Version Date:

APPENDIX H: DOWNHILL RUNNING GROUP INFORMED CONSENT FORM

Georgia State University
Department of Kinesiology and Health
Informed Consent

Title: THE EFFECTS OF SKELETAL MUSCLE INJURY ON FUNCTION OF UNINJURED MUSCLE

Principal Investigator: Dr. J. Andrew Doyle
Student Principal Investigator: Kyle J. Brandenberger

I. Purpose:

You are invited to participate in a research study. The purpose of this study is to investigate if injuring leg muscles with downhill running can affect strength in arm muscles. You are invited to participate because you are physically active, and between the ages of 18 – 44. A total of 20 participants will be recruited for this study. Participation will require 7 hours of your time over 4 sessions.

II. Procedures:

If you decide to participate, The study will include 4 sessions over a two-week period during which we will ask you to refrain from exercise other than the exercise done in the study. During The first day you will be asked to complete a health history form and your blood pressure will be measured. If you have fewer than 2 risk factors for heart disease then you will be asked to continue with the study.

You will have the strength of your front thigh (quadriceps), and right front upper arm (biceps) muscles measured. Your strength will be measured using an exercise machine that is able to measure force during conditions in which your leg and arm does not move even when you are applying pressure against the machine. Your strength will be measured during a maximal voluntary effort, a brief electrical stimulation, and a combination of the two. Initially, you will do a 3-second maximal effort contraction of your front thigh muscles. At 2.5 seconds into the contraction, your muscles will be stimulated using an electrical stimulator. The stimulation will be very short, only one-hundredth of a second long. At 2 and 4 seconds after the end of your maximal effort contraction, your relaxed muscles will be stimulated again. The stimulations will be done using two adhesive electrodes, one each placed on the skin overlying your upper and lower thigh muscles. The strength of the electrical stimulations that you will receive will be adjusted in a preliminary series of electrically-stimulated contractions to the minimum needed to elicit peak strength. During the strength assessment, you will do this sequence of contractions (maximal effort contraction followed by the electrical stimulations) six times with a minute between attempts. The results from your three best attempts will be used in the data analysis.

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If you perform well on the strength tests, you will then be asked to perform a graded exercise test. You will be asked to run on a treadmill with a starting speed of 5 miles per hour. The speed will be increased every 2 minutes until you cannot continue. During the test you will wear a head gear with a mouth piece. Your breaths will be measuredduring the test with the mouth piece to look at how much oxygen you use. This test will be used to select a speed for the downhill running session.

At the start of the second session you will be asked to provide a urine sample and a very small blood sample will be taken from one of your forearm veins. Then you will repeat strength tests for the biceps and quadriceps explained above. Next, you will perform a fatigue exercise for the front thigh muscles. You will be asked to perform 50 maximal effort concentric contractions of your front thigh muscles. This will require you to kick out as hard as you can against a bar on the exercise machine until the bar stops moving. The intent of these contractions is to induce fatigue in your muscles. Following the 50 concentric contractions, the strength of your front thigh muscles will be measured again as described above. Next, you will perform 50 maximal effort concentric contractions of your front upper arm muscles. This will require you to pull as hard as you can against a bar on the exercise machine until the bar stops moving. The intent of these contractions is to induce fatigue in your arm muscles. Following the 50 concentric contractions, the strength of your front upper arm muscles will be measured again as described above.

You will then be asked to run downhill on a treadmill at -10% grade for 1 hour to injure front thigh muscles. This will be followed by a very small blood sample taken from one of your forearm veins. Then you will be asked to perform the front thigh muscle strength test followed by the fatigue exercise for the front thigh muscles. After fatiguing your front thigh muscles, you will perform another round of the strength test for the front thigh muscles. Then you will be asked to perform the front upper arm muscle strength test followed by the fatigue exercise for the front upper arm muscles. After fatiguing your arm muscles, you will perform another round of the strength test for the front upper arm muscles. You will then be asked to provide a urine sample 2 hours after the end of the downhill run.

You will be asked to return to the lab the following 2 days. You will be asked to provide a urine sample and a very small blood sample will be taken from one of your forearm veins on each of these days. Then you will be asked to perform the front thigh muscle strength test followed by the fatigue exercise for the front thigh muscles. After fatiguing your front thigh muscles, you will perform another round of the strength test for the front thigh muscles. Then you will be asked to perform the front upper arm muscle strength test followed by the fatigue exercise for the front upper arm muscles. After fatiguing your arm muscles, you will perform another round of the strength test for the front upper arm muscles.

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III. Risks:

There is the possibility that participation in this study may cause you moderate to severemuscle soreness and temporary muscle weakness after doing the strength testing and the bout of 50 concentric contractions. Your muscles should however feel normal within a few hours. There is also the possibility that the electrical stimulation of your thigh muscles may feel uncomfortable. Despite this, no injury is occurring to your leg. When your muscles are being stimulated, the nerves that sense pressure, temperature, position, and pain are all being stimulated and your brain does not know how to process the information from those nerves. If you find the stimulations too uncomfortable, you should withdraw from the study. When you have your blood drawn, you will most likely experience momentary pain from the needle prick. There is also a chance of bruising and swelling surrounding the puncture site and a remote chance of infection associated with the procedure. There is the possibility that the graded exercise test may cause you to have a heart attack, stroke, or feel dizzy, lightheaded, tired, or have an upset stomach,or muscle cramps.There is a possibility of knee injuries and other injuries associated with downhill running including runner's knee and Iliotibal Band Syndrome. These risks are small. To reduce these risks, trained personnel will supervise the test. If you feeldizzy, light-headed, short of breath, or have anycramping, wheezing, chest pain or changes in skin color during the exercise tests, please tell us so that wecan end the test and either have you cool down and rest, or contact emergency medical personnel ifnecessary.There is a risk of falling off the treadmill during the graded exercise test or downhill running. To reduce this risk, you will be supervised during all treadmill running, and a person will stand behind to catch you if you fall. There is a possibility that the downhill run may cause muscle soreness, muscle weakness, and kidney dysfunction. Muscle soreness and muscle weakness are a normal part of the healing process from mild muscle injury, and any pain, stiffness or weakness should be temporary. To reduce the chance of kidney problems, you will be provided instructions on how to keep hydrated. If you have difficulty urinating or painful urination, you will be referred to a physician. If you experience swelling in the legs or ankles, bruising, discoloration, inability to urinate, chest pain, chest tightness, or unusual shortness of breath, please seek immediate medical attention from your healthcare provider.If you have any question about this study, or believe you have suffered any injury because of participation in the study, you may contact Dr. J. Andrew Doyle and Kyle Brandenberger at kbrandenberger1@student.gsu.edu or at (404) 413-8050. Georgia State University, however, has not set aside funds to pay for health care or to compensate you if something should occur.

IV. Benefits:

Participation in this study may notbenefit you personally. Overall, we hope to gain information about whether inflammation can affect susceptibility to fatigue.

VI. Voluntary Participation and Withdrawal:

Version Date:

Participation in research is voluntary. You do not have to be in this study. If you decide to be in the study and change your mind, you have the right to drop out at any time. You may stop participating at any time. Whatever you decide, you will not lose any benefits to which you are otherwise entitled.

VII. Confidentiality:

We will keep your records private to the extent allowed by law.Dr. J. Andrew Doyle, and Kyle Brandenberger will have access to the information you provide. Information may also be shared with those who make sure the study is done correctly (GSU Institutional Review Board, the Office for Human Research Protection (OHRP), and/or the Food and Drug Administration (FDA).We will use a randomly assigned subject numberrather than your name on study records. A code sheet linking your name and subject number will kept until all data collection is complete, at which time the code sheet will be destroyed. The information you provide will be stored. The information you provide will be stored on computers that are protected by passwords. Any paper copies of your information will be stored in a locked filing cabinet in room G20 of the sports arena. Your name and other facts that might point to you will not appear when we present this study or publish its results. The findings will be summarized and reported in group form. You will not be identified personally.

VIII. Contact Persons:

ContactDr. J. Andrew Doyle and Kyle Brandenberger at kbrandenberger1@gsu.edu or at (404) 413-8050.If you have questions, concerns, or complaints about this study. You can also call if you think you have been harmed by the study. Call Susan Vogtner in the Georgia State University Office of Research Integrity at 404-413-3513 or svogtner1@gsu.edu if you want to talk to someone who is not part of the study team. You can talk about questions, concerns, offer input, obtain information, or suggestions about the study. You can also call Susan Vogtner if you have questions or concerns about your rights in this study.

IX. Copy of Consent Form to Participant:

We will give you a copy of this consent form to keep.

If you are willing to volunteer for this research, please sign below.

_____	_____
Participant	Date
_____	_____
Principal Investigator or Researcher Obtaining Consent	Date

Version Date:

APPENDIX I: HEALTH HISTORY QUESTIONNAIRE

Applied Physiology Laboratory
Department of Kinesiology and Health
Georgia State University

Health History

All information given is personal and confidential. The information will enable us to better understand you and your health and fitness habits.

Name_____ _____Date_____

Address_____Home Phone_____

City/State_____Zip Code_____

E-mail_____

Occupation_____Other Phone_____

Birth Date_____Gender_____Height_____Weight_____Ethnicity_____

****

I. Signs and Symptoms

****

Have you ever experienced any of the following:
(please circle yes or no)

yes no 1. Pain, discomfort, tightness or numbness in the chest, neck, jaw or arms.

yes no 2. Shortness of breath at rest or with mild exertion.

yes no 3. Dizziness or fainting.

yes no 4. Difficult, labored or painful breathing during the day or at night.

yes no 5. Ankle swelling.

yes no 6. Rapid pulse or heart rate.

yes no 7. Intermittent cramping.

yes no 8. Known heart murmur.

yes no 9. Unusual shortness of breath or fatigue with usual activities.

If you answered yes to any of the above—

How often do you experience the symptom?_____

Have you ever discussed the symptom with a doctor?_____

Explain the symptom in more detail:_____

_____

****

II. Major Risk Factors

****

yes no 1. Do you have a body mass index ≥ 30 or a waist girth >100 cm?

yes no 2. Have you had a fasting glucose of ≥ 110 mg/dl confirmed by measurements on at least 2 separate occasions.

yes no 3. Has your father or brother experienced a heart attack before the age of 55? Or has your mother or sister experienced a heart attack before the age of 65?

yes no 4. Do you currently smoke or quit within the past 6 months?

yes no 5. Has your doctor ever told you that you have high blood pressure?

yes no 6. Do you have high cholesterol?
Total cholesterol:_____HDL:_____Date tested:_____

yes no 7. Do you have a sedentary lifestyle? (sitting most of the day in your job with no regular physical activity)

****

III. Medical Diagnoses

****

Have you ever had any of the following? Circle all that apply:

heart attack	angioplasty	heart surgery	coronary artery disease
angina	hypertension	heart murmur	heart clicks
asthma	emphysema	bronchitis	stroke
anemia	phlebitis	emboli	cancer
osteoporosis	emotional disorders	eating disorders	

Any special problems not listed above:_____

If any of the above are circled, please give details and explain:_____

****

IV. General

****

yes no 1. Are you pregnant?

yes no 2. Do you have arthritis or any bone or joint problem?
If yes, please explain:_____

_____

yes no 3. Do you currently exercise?
If yes, how long have you been exercising?_____

What do you do and how often?_____

yes no 4. Are you taking any medication, vitamins or supplements?
Name them and their dosage (list both prescribed and over-the-counter medications)
Drug name and dosage / purpose of drug / prescribed or over-the-counter

_____

_____

yes no 5. Has your physician ever told you that you have reduced kidney function? Have you ever been diagnosed or treated for kidney stones?

****

My signature certifies that all of the above is true, to the best of my knowledge.

Signature:_____Date:_____

****

STAFF USE ONLY

****

Comments:_____

Stratification(circle one): Low Risk Moderate Risk High Risk

Resting blood pressure:_____Resting heart rate:_____

yes no Do meds affect BP or HR?

Date:_____Initials:_____

APPENDIX J: TREADMILL VO2MAX PROTOCOL

Georgia State University
Department of Kinesiology and Health
Applied Physiology Laboratory

Name: [redacted] Test Date: _____

ID Number: _____ Gender: M F

Date of Birth: _____ Age: _____

Predicted HRmax: _____ bpm Risk Stratification: LR MR HR

Target Heart Rate: _____ % _____ bpm Medications: _____

Resting HR: _____ bpm Standing HR: _____ bpm

Resting BP: _____ mmHg Standing BP: _____ mmHg

Treadmill Protocol								
Stage	**Duration (min)**	**Time (min)**	**Speed (mph)**		**Grade (%)**	**Heart Rate**	**VO2**	
1		1	**5**		0			
1		2	**5**		0			
2		3	**6**		0			
2		4	**6**		0			
3		5	**7**		0			
3		6	**7**		0			
4		7	**8**		0			
4		8	**8**		0			
5		9	**9**		0			
5		10	**9**		0			
6		11	**10**		0			
6		12	**10**		0			
7		13	**11**		0			
7		14	**11**		0			
8		15	**12**		0			
Recovery								

Total Exercise Time: _____ $\dot{V}O_{2\ max}$: _____ ml/kg/min

Maximal Heart Rate: _____ _____ L/min

Test Termination: _____ Tech: _____

Comments: _____

APPENDIX K: DATA COLLECTION FORM

Subject ID _____ Trial_____

Thigh Circumference

Upper Arm Circumference

Knee Extensor Force Curve

100 mA	120 mA	140 mA	160 mA	180 mA	200 mA	220 mA	240 mA	260 mA	280 mA	300 mA	320 mA	340 mA	360 mA	380 mA	400 mA	420 mA	440 mA	460 mA	480 mA	500 mA

Resting Knee Extensor: Peak Torque Prior to Twitch

Resting Knee Extensor: Activation

Fatiguing Knee Extensor: Peak Torque Prior to Twitch

Fatiguing Knee Extensor: Activation

Elbow Flexor Force Curve

60 mA	80 mA	100 mA	120 mA	140 mA	160 mA	180 mA	200 mA	220 mA	240 mA	260 mA	280 mA	300 mA	320 mA	340 mA	360 mA	380 mA	400 mA	420 mA	440 mA	460 mA

Resting Elbow Flexors: Peak Torque Prior to Twitch

Resting Elbow Flexors: Activation

Fatiguing Elbow Flexors: Peak Torque Prior to Twitch

Fatiguing Elbow Flexors: Activation

Rating of Exercise Induced – Muscle Soreness Visual Analogue Scale: 100 mm

Subject #
Date
Time
Trial/Step # (1-4)
Soreness Score (mm)

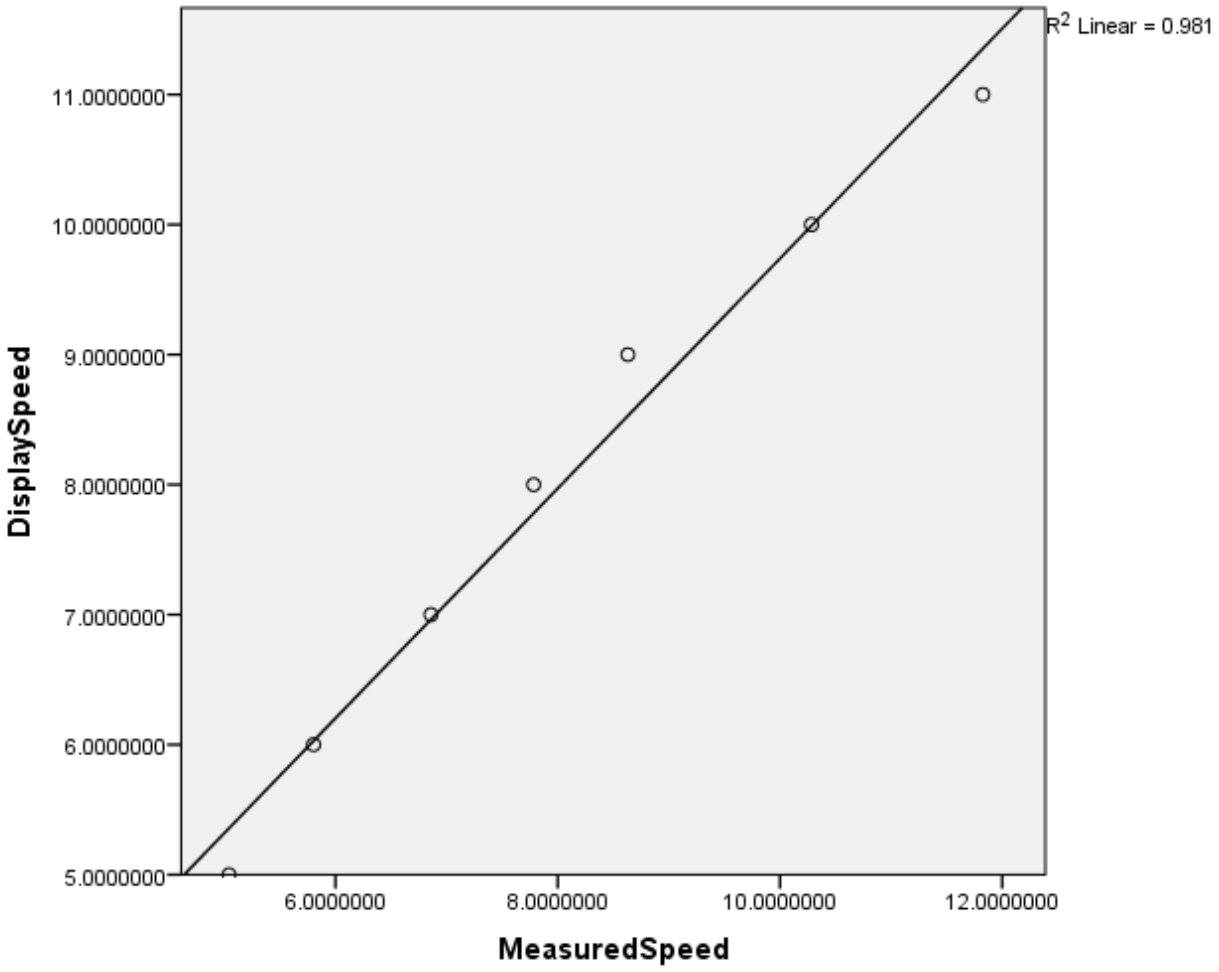
0 No Soreness

100 Very, Very Sore

APPENDIX M: TREADMILL SPEED VALIDATION

Displayed Speed (mph)	Mean Measured Speed (mph)
5	5.041699
6	5.8024802
7	6.8587684
8	7.7832249
9	8.630705
10	10.2839551
11	11.8254628

Speeds were measured in duplicate using a standard stopwatch. The mean of these two measurements is shown above.



APPENDIX N: KNEE EXTENSOR STRENGTH DATA

KNEE EXTENSOR STRENGTH AT REST FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	1.00	0.95	1.08	1.05
2	1.00	1.44	1.30	1.39
3	1.00	0.53	0.80	0.78
4	1.00	1.07	0.98	1.02
5	1.00	0.94	0.88	0.84
6	1.00	0.80	1.13	1.14
7	1.00	0.70	0.38	0.30
8	1.00	0.34	0.33	0.43
9	1.00	0.48	0.60	0.70
10	1.00	0.24	0.57	0.56
11	1.00	0.52	0.78	0.77
12	1.00	0.51	0.58	0.57
Mean	1.00	0.71	0.78	0.80
SD	0.00	0.34	0.30	0.32

KNEE EXTENSOR STRENGTH AFTER A FATIGUING PROTOCOL FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	0.82	0.89	0.94	0.85
2	0.92	1.17	1.12	1.28
3	0.90	0.55	0.61	0.49
4	0.66	0.77	0.65	0.89
5	0.96	0.85	0.82	0.98
6	0.49	0.73	0.75	0.87
7	0.80	0.66	0.42	0.37
8	0.72	0.34	0.27	0.37
9	0.95	0.35	0.49	0.58
10	0.83	0.25	0.35	0.49
11	0.74	0.68	0.49	0.72
12	0.92	0.43	0.42	0.38
Mean	0.81	0.64	0.61	0.69
SD	0.14	0.27	0.26	0.29

APPENDIX O: KNEE EXTENSOR VOLUNTARY ACTIVATION DATA

KNEE EXTENSOR VOLUNTARY ACTIVATION AT REST FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	79.92	77.15	73.90	83.82
2	47.58	84.39	51.72	59.03
3	84.07	71.66	69.33	55.56
4	67.19	71.44	74.22	88.16
5	67.30	59.08	63.90	69.07
6	88.83	70.35	96.45	87.84
7	86.84	38.48	23.78	37.89
8	97.89	75.53	50.24	58.77
9	68.17	50.12	55.25	56.53
10	56.72	39.61	53.17	38.47
11	95.13	83.78	93.27	90.36
12	-	-	-	-
Mean	76.33	65.60	64.11	65.95
SD	16.13	16.44	20.72	19.33

KNEE EXTENSOR VOLUNTARY ACTIVATION AFTER A FATIGUING PROTOCOL FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	65.87	70.81	79.78	69.50
2	51.97	70.67	48.33	48.10
3	82.27	47.89	58.24	36.31
4	40.32	44.31	48.23	69.60
5	54.69	48.69	57.15	61.51
6	52.02	64.80	67.88	72.53
7	55.11	38.99	12.91	16.22
8	98.60	62.28	33.84	44.99
9	57.80	35.11	39.83	45.24
10	38.05	39.09	29.69	39.04
11	77.63	77.39	67.80	75.94
12	42.10	45.00	25.36	26.07
Mean	59.70	53.75	47.42	50.42
SD	18.35	14.58	19.84	19.42

APPENDIX P: KNEE EXTENSOR ELECTICALLY STIMULATED STRENGTH DATA

KNEE EXTENSOR ELECTRICALLY STIMULATED STRENGTH AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	1.00	0.50	0.68	0.70
2	1.00	0.95	1.02	0.96
3	1.00	0.61	0.92	0.87
4	1.00	0.99	0.97	0.93
5	1.00	0.96	0.89	0.90
6	1.00	1.02	1.07	1.05
7	1.00	0.54	0.56	0.47
8	1.00	0.50	0.68	0.70
9	1.00	0.59	0.87	0.99
10	1.00	0.41	0.80	0.79
11	1.00	0.47	0.55	0.68
12				
Mean	1.00	0.69	0.82	0.82
SD	0.00	0.24	0.18	0.17

KNEE EXTENSOR ELECTRICALLY STIMULATED STRENGTH AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	0.73	0.54	0.64	0.71
2	1.16	0.92	1.10	1.09
3	0.97	0.82	0.95	0.79
4	0.97	0.97	0.95	0.96
5	1.04	0.98	0.93	1.01
6	0.98	0.97	1.07	1.07
7	0.66	0.61	0.74	0.29
8	0.73	0.54	0.64	0.71
9	1.06	0.65	0.90	0.98
10	0.99	0.44	0.73	0.81
11	1.11	0.48	0.46	0.60
12	-	-	-	-
Mean	0.95	0.72	0.83	0.82
SD	0.17	0.22	0.20	0.24

APPENDIX Q: ELBOW FLEXOR STRENGTH DATA

ELBOW FLEXOR STRENGTH AT REST FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	1.00	0.86	1.00	0.99
2	1.00	0.92	0.97	1.00
3	1.00	1.10	1.16	1.03
4	1.00	0.88	0.99	1.01
5	1.00	0.93	0.99	1.03
6	1.00	0.90	0.92	0.96
7	1.00	0.87	0.86	0.89
8	1.00	1.00	0.70	0.92
9	1.00	0.92	0.95	1.10
10	1.00	0.71	0.71	0.87
11	1.00	0.93	0.95	0.91
12	1.00	0.79	0.79	0.77
Mean	1.00	0.90	0.92	0.96
SD	0.00	0.10	0.13	0.09

ELBOW FLEXOR STRENGTH AFTER A FATIGUING PROTOCOL FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	0.50	0.41	0.64	0.68
2	0.93	0.74	0.76	0.72
3	0.81	0.81	0.85	0.78
4	0.66	0.75	0.64	0.74
5	0.65	0.81	0.73	0.65
6	0.65	0.73	0.69	0.71
7	0.78	0.81	0.86	0.81
8	0.67	0.62	0.66	0.65
9	0.89	0.69	0.60	0.40
10	0.46	0.53	0.64	0.67
11	0.56	0.52	0.74	0.77
12	0.70	0.67	0.62	0.64
Mean	0.69	0.67	0.70	0.68
SD	0.15	0.13	0.09	0.11

APPENDIX R: ELBOW FLEXOR ACTIVATION DATA

ELBOW FLEXOR VOLUNTARY ACTIVATION AT REST FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	96.42	95.34	97.54	92.99
2	96.51	75.64	91.27	98.45
3	94.93	99.54	96.62	91.90
4	87.81	81.10	96.08	95.49
5	94.38	93.62	94.65	86.95
6	100.00	100.00	97.60	99.44
7	81.63	64.17	52.27	69.62
8	93.48	96.05	90.29	82.00
9	89.26	77.30	93.30	97.56
10	98.22	60.38	51.91	73.75
11	99.01	95.65	94.73	94.80
12	100.00	70.85	53.52	66.57
Mean	94.30	84.14	84.15	87.46
SD	5.57	14.29	19.18	11.71

ELBOW FLEXOR VOLUNTARY ACTIVATION AFTER A FATIGUING PROTOCOL FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	94.95	92.52	94.78	84.37
2	88.05	73.27	65.19	70.41
3	95.25	87.88	90.15	91.65
4	74.00	93.60	80.17	81.69
5	72.03	87.85	82.61	60.74
6	79.69	88.76	85.38	79.29
7	74.17	77.54	68.12	64.26
8	74.24	74.53	87.72	64.22
9	88.33	69.13	82.92	88.42
10	10.69	57.46	13.89	34.02
11	88.22	64.85	96.32	77.39
12	65.85	70.16	76.13	51.05
Mean	75.46	78.13	76.95	70.63
SD	22.52	11.81	22.00	16.77

APPENDIX S: ELBOW FLEXOR ELECTICALLY STIMULATED STRENGTH DATA

ELBOW FLEXOR ELECTRICALLY STIMULATED STRENGTH AT REST FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	1.00	1.00	1.57	1.15
2	1.00	0.93	0.85	1.15
3	1.00	0.74	0.96	0.71
4	1.00	0.67	0.71	0.86
5	1.00	0.48	1.15	0.58
6	1.00	1.19	1.21	0.99
7	1.00	0.96	0.96	0.77
8	1.00	1.00	1.57	1.15
9	1.00	0.97	0.93	0.91
10	1.00	1.26	0.71	1.12
11	1.00	0.81	0.97	0.90
12	1.00	0.88	0.96	0.97
Mean	1.00	0.91	1.05	0.94
SD	0.00	0.22	0.28	0.19

ELBOW FLEXOR ELECTRICALLY STIMULATED STRENGTH AFTER A FATIGUING PROTOCOL FOR EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	0.60	0.65	1.22	0.98
2	1.04	0.98	0.95	1.10
3	0.47	0.77	0.72	0.55
4	0.83	0.56	0.61	0.88
5	0.76	0.31	1.08	0.35
6	0.73	1.19	1.06	0.78
7	0.91	1.10	0.83	0.78
8	0.60	0.65	1.22	0.98
9	0.66	0.65	0.78	0.75
10	0.52	1.00	0.50	0.76
11	0.53	0.37	0.52	0.59
12	0.75	0.78	0.82	0.90
Mean	0.70	0.75	0.86	0.78
SD	0.17	0.27	0.25	0.21

APPENDIX T: SERUM IL-1B DATA

SERUM IL-1β AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	-	-	-	-
3	0.00	0.00	0.00	0.00
4	0.00	0.07	0.00	0.00
5	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00
7	-	-	-	-
8	0.56	0.48	0.36	0.21
9	0.00	0.01	0.00	0.00
10	0.00	0.00	0.00	0.00
11	0.00	0.00	0.00	0.52
12	0.00	0.18	0.00	0.00
Mean	0.06	0.08	0.04	0.08
SD	0.19	0.16	0.12	0.18

APPENDIX U: SERUM TNF-A DATA

SERUM TNF-α AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	-	-	-	-
3	3.64	1.81	3.11	2.23
4	3.79	-	1.47	1.93
5	3.08	-	2.60	0.96
6	3.70	1.77	2.00	0.90
7	-	-	-	-
8	5.28	2.49	3.46	3.27
9	3.21	-	1.97	1.16
10	3.57	1.36	1.97	1.71
11	3.75	1.26	1.89	4.81
12	3.57	-	1.42	1.52
Mean	3.73	1.73	2.21	2.06
SD	0.63	0.48	0.70	1.27

APPENDIX V: UPPER ARM PAIN DATA

UPPER ARM PAIN AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	0.00	11.50	65.50	44.50
3	0.00	63.00	6.00	5.00
4	0.00	3.00	6.00	2.00
5	0.00	5.50	6.50	0.00
6	0.00	19.00	3.00	11.00
7	0.00	97.00	85.50	15.00
8	-	-	-	-
9	13.00	16.00	31.50	41.00
10	0.00	36.00	64.00	49.50
11	7.00	97.00	52.00	66.00
12	2.00	12.00	17.00	3.00
Mean	2.20	36.00	33.70	23.70
SD	4.39	36.60	30.64	24.12

APPENDIX W: THIGH PAIN DATA

THIGH PAIN AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	0.00	0.00	23.00	7.50
3	0.00	58.00	2.00	12.00
4	1.00	9.00	5.00	2.00
5	0.00	5.50	8.50	0.00
6	1.00	42.00	3.00	12.00
7	0.00	98.00	82.50	64.00
8	-	-	-	-
9	12.50	88.00	90.50	80.00
10	1.00	100.00	100.00	100.00
11	3.00	71.50	79.50	85.50
12	1.00	99.00	65.00	82.00
Mean	1.95	57.10	45.90	44.50
SD	3.82	40.68	40.98	40.93

APPENDIX X: UPPER ARM CIRCUMFERENCE DATA

UPPER ARM CIRCUMFERENCE AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	22.83	22.80	23.27	23.23
3	28.53	28.20	28.50	28.33
4	31.00	31.87	30.97	31.77
5	28.17	29.17	28.53	29.53
6	28.73	28.90	29.27	28.83
7	29.40	29.80	29.97	30.03
8	-	-	-	-
9	31.40	30.20	30.50	30.70
10	28.20	28.40	29.20	28.73
11	32.50	32.50	32.23	32.23
12	24.43	24.23	24.40	24.83
Mean	28.52	28.61	28.68	28.82
SD	2.99	3.04	2.81	2.85

APPENDIX Y: THIGH CIRCUMFERENCE DATA

THIGH CIRCUMFERENCE AT EACH MEASURED TIME POINT

Subject	Immediate Pre-Injury	Immediate Post-Injury	24 h Post-Injury	48 h Post Injury
1	-	-	-	-
2	49.97	49.63	48.77	48.33
3	49.90	50.20	51.20	50.87
4	53.13	53.77	52.97	53.27
5	47.90	47.20	47.03	48.97
6	48.97	48.30	51.07	48.13
7	56.37	56.70	56.50	54.00
8	-	-	-	-
9	59.57	57.73	56.57	56.17
10	52.37	49.70	54.23	52.67
11	52.17	52.07	53.20	55.20
12	51.50	48.53	50.93	50.60
Mean	52.19	51.38	52.25	51.82
SD	3.53	3.61	3.09	2.87