2019

Spinal Motor Neuron Excitability During Fatigue

Nathalie Cecire
ceci3030@mylaurier.ca

SPINAL MOTOR NEURON EXCITABILITY DURING FATIGUE

By

Nathalie Cecire

THESIS

Submitted to the Faculty of Kinesiology

In partial fulfilment of the requirements for

Masters of Kinesiology

Wilfrid Laurier University

ABSTRACT

Fatigue during sustained or repeated muscular contractions can be from contractile failure within the muscle or reduced excitability in the supraspinal and spinal motor neurons. However, spinal motor neurons can also compensate for fatigue. We speculate that one way the spinal motor neuron compensates for fatigue is through an increase in excitability via the activation of persistent inward currents (PIC). In other conditions where there is a reduction in descending drive, such as during spinal cord injury and aging, there are adaptions of the spinal motor neurons to have elevated PIC amplitudes or towards a greater prevalence of PIC, respectively. Although this increase may not necessarily be beneficial, it indicates that there is a compensatory increase in PIC during persistent reductions of descending drive. The purpose of this study was to explore how the motor neuron responds to fatigue by examining firing rates, PIC, and motor neuron pool excitability. Twelve participants attended two testing sessions. During one session the participants performed five three-minute sets of intermittent isometric plantarflexion contractions, during the other session the participants sat quietly. In both sessions participants performed maximal voluntary contractions and submaximal contractions for estimating PIC at the start, after each set, and again after fifteen minute of recovery. Using peripheral nerve stimulation, H-reflex and M-wave recruitment curves were elicited at the start, after the final set, and after fifteen minutes of recovery. Motor unit firing rates were assessed during the intermittent fatiguing contractions. Additional analyses were performed to assess whether the activation of the antagonist muscle group during fatigue influenced PIC, whether level of physical activity affected the initial PIC amplitude, and whether there were any sex-differences. There was no significant change in maximal voluntary force over the fatigue protocol, however there was a significant increase in muscle EMG during the fatiguing contractions. There were no significant changes in motor unit firing rates, spinal motor neuron pool excitability, or PIC. Although there was no change in PIC,

a secondary analysis was performed to assess whether there was a sex-difference in PIC over the fatigue protocol. There was a significant interaction, indicating that females had a significant increase in PIC during the fatigue protocol, but males did not. There were no other interactions between session, time, and sex for the other measures of fatigue and motor neuron behaviour. Therefore, from these results we conclude that PIC is modulated during fatigue in women, but not men. This finding may contribute to our understanding of the mechanisms leading to greater fatigue-resistance in females. Further research should examine the stability of this relationship during more vigorous fatigue protocols.

TABLE OF CONTENTS

CHAPTER 1: REVIEW OF LITERATURE

SPINAL MOTOR NEURON INTRINSIC EXCITABILITY: PIC

Motor neurons are the “final common pathway” to muscle (Charles S. Sherrington, 1911, pg 46). Their soma and dendrites integrate all sensory and descending input and, if there is adequate net excitatory input, generate action potentials that will be propagated down the axon to the muscle. Motor neuron firing rates are modifiable by ionotropic inputs and neuromodulatory inputs (Heckman, Mottram, Quinlan, Theiss, & Schuster, 2009). Ionotropic inputs are neurotransmitters released from descending motor tracts, afferents, and interneurons that bind to ionotropic receptors and result in graded hyperpolarizations (inhibitory post-synaptic potentials) or depolarizations (excitatory post-synaptic potentials) of the cell membrane (Heckman et al., 2009). The linear summation of these postsynaptic potentials was once thought to be the only control for motor neuron firing. If there were enough excitatory potentials for the membrane voltage at the axon hillock to reach a threshold value, then the neuron would fire and the firing rate would increase in proportion to the amount of synaptic input. However, this simplistic model of linear integration cannot account for the wide range of motor output (Heckman et al., 2009).

Neuromodulatory inputs can change the motor neuron intrinsic excitability, which accounts for the wide range in motor output. Rather than brief changes in membrane voltage caused by synaptic input, neuromodulatory inputs activate G-protein-coupled receptors, which have a longer-lasting effect on membrane voltage (Heckman et al., 2009). Motor neuron intrinsic excitability can be modulated through a change in afterhyperpolarization, persistent inward current, spike frequency adaptation, and spike threshold accommodation. For this project, I will be focusing on persistent inward current (PIC). PIC is activated by a diffuse release of neuromodulatory inputs, including norepinephrine and serotonin from the locus coeruleus and the caudal raphe nucleus respectively (Heckman, Gorassini, & Bennett, 2005; Heckman, Johnson,

Mottram, & Schuster, 2008). When norepinephrine and serotonin bind to the neuromodulatory receptors on the motor neuron dendrites, there is a cascade of intracellular events that lead to the opening of L-type calcium channels (CaPIC) (Heckman et al., 2005) and persistent sodium channels (NaPIC). The NaPIC is quick to activate and deactivate, but its purpose may be to prolong a brief synaptic input until the slower CaPIC are activated (Heckman et al., 2009; Lee & Heckman, 2001). The CaPIC is slower to activate, however it is also longer lasting. Once PIC is activated the membrane potential of the neuron increases, which increases the sensitivity of the membrane to excitatory synaptic input (Heckman, Lee, & Brownstone, 2003). Furthermore, the long-lasting CaPIC allows the motor neuron to continue firing once the synaptic input is removed resulting in what is known as self-sustained firing (Heckman et al., 2009). Self-sustained firing resulting from PIC may be an important contributor to postural control and other motor tasks (Heckman et al., 2009).

i. Estimating PIC in Humans

Plateau potentials and self-sustained firing in invertebrate motor neuron preparations provided the first evidence that the motor neuron played a larger role in spinal excitability than simply relaying the summation of synaptic input to muscles (Hartline, Russelut, Raper, & Graubard, 1988). Investigation then shifted from invertebrates to decerebrate cats, turtle spinal cord slices , and intact rats (Conway, Hultborn, Kiehn, & Mintz, 1988; Crone, Hultborn, Kiehn, Mazieres, & Wigstrom, 1988; Eken & O., 1989; Erim, De Luca, Mineo, & Aoki, 1996; Hounsgaard & Kiehn, 1985). In humans, self-sustained firing was first assessed by recording single motor units during isometric contractions then applying a vibration to the muscle tendon (Gorassini, Bennett, & Yang, 1998). Motor units that were silent prior to the vibration were activated and continued to fire following the removal of the vibration and motor units that

increased firing rate in response to the stimulus maintained the higher firing rates after the vibration was removed. Gorassini et al. (1998) also showed that even if the participant decreased the force of the isometric contraction (reducing synaptic input to the motor unit pool) following the removal of vibration the self-sustained firing persisted below the force level that the motor units were recruited. This method estimates the occurrence of self-sustained firing, which is a result of PIC, but does not give any measure of the amplitude of PIC. In order to assess the amplitude of PIC, the paired motor unit technique is used. Estimating the amplitude of PIC provides a means of quantifying the contribution of PIC to motor unit firing and how that may change with age and fatigue. This technique employs intramuscular electromyography (EMG) to record from two or more motor units during a triangular or trapezoidal ramp contraction. The first motor unit recruited is a control unit. The control unit provides an estimate of the synaptic input to the motor neuron pool. The next unit recruited one to two seconds after the control unit, is the test unit. The one to two second gap before the test unit is necessary to ensure that the control unit is in the secondary firing range. The paired motor unit technique provides an estimate of the test unit PIC amplitude. To assess PIC amplitude, the instantaneous firing rate for each unit is plotted against time (Figure 1). The idea is that the firing rate of the control unit reflects synaptic input to the motor neuron pool. The test unit is recruited ("turned on") by a threshold level of synaptic input that is denoted by control unit firing rate. If a PIC is activated in the test unit, then the test unit will continue to fire at a lower level of synaptic input than was required to turn it on. Thus, as force is ramped down, the test unit will be derecruited ("turned off") at a lower control unit firing rate. If the test unit does not have a PIC, the test unit will be recruited and derecruited at the same level of synaptic input. Thus, the difference in the control unit firing rate (ΔF) at the recruitment and decruitment of the test unit provides the estimate of the magnitude of PIC.

The paired motor unit technique is widely used to estimate PIC, however a recent simulation study revealed that PIC, spike frequency adaptation and spike threshold accommodation all contribute to a positive ΔF value (Revill & Fuglevand, 2011). Spike frequency adaptation is a time-dependent decrease in motor unit firing rate and spike threshold accommodation is an increase in the recruitment threshold of a motor neuron in response to a decrease in the rate of rise of the current. Based on an investigation of the contributions of each property to the ΔF value derived from the paired motor unit technique, spike frequency adaptation and spike threshold accommodation can be reduced by increasing the ramp rate of rise and decreasing the duration of the ramp (Vandenberk & Kalmar, 2014). Therefore, a triangular ramp contraction with a short time to peak results in better estimation of PIC amplitude (Vandenberk & Kalmar, 2014). The intensity of the contraction should be at a level that at least two motor units are recruited (~10% of MVC).

MOTOR NEURON POOL EXCITABILITY: H-REFLEX GAIN

The Hoffmann reflex (H-reflex) was first described by Paul Hoffmann in 1910 (Zehr, 2002). The H-reflex is the response to an externally applied electrical stimulation of a mixed nerve. It is often considered the electrical equivalent of the mechanical stretch reflex; however, the stretch reflex activates muscle spindles, which then relay the signal via the Ia afferent neurons to the motor neuron whereas the H-reflex is applied to the Ia afferents directly and bypasses the muscle spindle. The electrical stimulus depolarizes the afferent neurons and the signal is relayed to the motor neuron pool. The excitability of the motor neuron pool will then determine how many motor neurons reach threshold and relay the stimulus to muscle where the signal is recorded as an H-reflex (Figure 3). Thus, the more excitable the motor neuron pool, then the more motor neurons that become recruited by the stimulus and the greater the H-reflex response.

Electrical stimulation of the mixed nerve also stimulates efferent neurons (Figure 3). When the stimulation depolarizes the efferent neurons (spinal motor neurons) above their threshold, an action potential is propagated in the orthodromic and antidromic directions. The orthodromic action potential results in an M-wave. Maximum M-wave amplitude represents the recruitment of the entire motor unit pool. The M-wave has a shorter latency than the H-reflex because of the shorter distance that the signal must travel before reaching the muscle. Antidromic action potentials from efferent nerve stimulation collide with the orthodromic action potentials arising from afferent nerve stimulation to reduce or eliminate the H-reflex response recorded from muscle at higher simulation intensities (Figure 3) (Funase, Imanaka, & Nishihira, 1994; Zehr, 2002).

i. Measuring H-reflex

There are many methods of estimating spinal motor neuron pool excitability using the H-reflex, including comparing maximum H-reflex amplitude (H_{max}) to maximum M-wave amplitude (M_{max}), comparing submaximal H-reflex amplitude to M-max or submaximal M-wave amplitude, and comparing the slope of the H-reflex recruitment curve (H_{slp}) to the slope of the M-wave recruitment curve (M_{slp}) (Garland & Mccomas, 1990; Walton, Kuchinad, Ivanova, & Garland, 2002). For this project, we will use the comparison of the slopes of the H-reflex and M-wave recruitment curves (H_{slp}/ M_{slp}).

The method of estimating spinal excitability of the motor neuron pool using the slope of recruitment curves was first described by Funase et al. (1994). This method measures the slopes of the linear regions of the H-reflex recruitment curve (H_{slp}) and the M-wave recruitment curve (M_{slp}) (Funase et al., 1994). Recruitment curves are generated by stimulating a mixed nerve with a series of pulses of incremental intensity and recording the resultant H-reflex and M-wave responses. The peak-to-peak amplitude of the H-reflex and M-wave from the EMG recording are

plotted as a function of stimulus intensity (Figure 4). The H_{slp}/M_{slp} ratio is a measurement of spinal motor neuron pool excitability. An increase in the slope of the linear region of the H-reflex curves indicates an increase in motor neuron pool excitability (Funase et al., 1994).

There are many advantages of this method. The first is that it is theoretically more valid because the influence of the antidromic action potentials colliding with the H-reflex is reduced since each point would be affected similarly relative to the size of the M-wave (Funase et al., 1994). A second advantage is that this measurement is less susceptible to variability resulting from postural changes or changes in skin resistance (Walton, Kalmar, & Cafarelli, 2003).

POTENTIATION OF MOTOR SYSTEM THROUGH ACTIVATION

Movement is produced when the spinal alpha-motor neuron fires, signalling the muscle to contract; however, upstream from the muscle there are changes within the motor system facilitating this movement generation. When the motor system shifts from a resting to an active state there is an increase in cortical and spinal motor neuron excitability (Folland, Wakamatsu, & Fimland, 2008; Hess, Mills, & Murray, 1987; Jacobs, Martín-Cora, & Fornal, 2002; Zijdewind, Zwarts, & Kernell, 2000). The increase in spinal motor neuron excitability during a motor state is due in part to an increase in the descending drive to the motor neuron pool, an increase in facilitation of excitation and/or a reduction of inhibition through sensory afferents, and increased monoaminergic drive (Fukushima, Yamashita, & Shimada, 1982; Hess et al., 1987; Jacobs et al., 2002; Lafleur, Zytnicki, Horcholle-Bossavit, & Jami, 1992; Rasmussen, Morilak, & Jacobs, 1986; Taylor, Butler, & Gandevia, 2000; Zijdewind et al., 2000). The increase in monoaminergic drive activates PIC channels, which increase the motor neurons sensitivity to synaptic input and help facilitate motor neuron firing (Heckman et al., 2003). Facilitation of alpha-motor neuron firing in turn, leads to muscular activation.

FATIGUE

Fatigue is the reversible decline in the ability to produce or maintain force caused by sustained or repeated muscular voluntary or evoked contractions (Allen, Lamb, & Westerblad, 2008). Fatigue is also characterized by a slowing of contraction and relaxation of the muscle (Allen et al., 2008). Fatigue is typically thought of as failure of the muscle to generate force but is also be caused by failure of the central nervous system to activate the muscle (Gandevia, Allen, Butler, & Taylor, 1996; Merton, 1954; Morel et al., 2015; Todd, Petersen, Taylor, & Gandevia, 2003; Zijdewind, Zwarts, & Kernell, 1998). Peripheral fatigue can be investigated through analysis of the muscle twitch response to a maximal peripheral nerve stimulation (Baker, Kostov, Miller, & Weiner, 1993; Bigland-Ritchie, Furbush, & Woods, 1986; West, Hicks, McKelvie, & O'Brien, 1996). Peripheral fatigue causes a reduction in the twitch amplitude and reduces the rate of force development and relaxation (Bigland-Ritchie, Furbush, et al., 1986; Westerblad & Lännergren, 1994). One method of assessing central fatigue is through voluntary activation (Merton, 1954). Central fatigue causes in a greater evoked response to a maximal stimulation during a maximal voluntary contraction in relation to a potentiated twitch following the contraction (Behm & Perez, 1996; Merton, 1954).

Peripheral fatigue within the muscle is multifactorial, and there are many mechanisms that have been investigated as the source (for review see Allen, Lamb, & Westerblad, 2008). Some mechanisms that have been posited include the metabolic changes in the muscle fibers and the increase in reactive oxygen species (ROS) (Allen et al., 2008). Some of the metabolites that have been examined in their relation to fatigue include inorganic phosphate, lactate and hydrogen ions, ATP, and glycogen (Blazev & Lamb, 1999; Bruton, Wretman, Katz, & Westerblad, 1997; Chin & Allen, 1997; Dahlstedt, Katz, & Westerblad, 2001; Dutka & Lamb, 2004; Owen, Lamb, &

Stephenson, 1996; Phillips, Wiseman, Woledge, & Kushmerick, 1993; Sahlin & Ren, 1989). During fatigue the increase in inorganic phosphate has been associated with greater force reduction, potentially due to a decline in Ca^{2+} sensitivity within the sarcolemma (Bruton et al., 1997; Dahlstedt et al., 2001; Phillips et al., 1993). Reductions in available ATP during fatigue reduce ability to produce force (Blazev & Lamb, 1999; Dutka & Lamb, 2004; Owen et al., 1996) as is reduction in available glycogen (Chin & Allen, 1997). ROS have been speculated to play a role in fatigue because when ROS scavengers are present there is reduced fatiguability, however the mechanism by which they cause fatigue is still uncertain (Reid, Stokić, Koch, Khawli, & Leis, 1994; Shindoh, DiMarco, Thomas, Manubay, & Supinski, 1990). These and other cellular changes that occur during fatigue are responsible for the contractile failure of the muscle.

Failure to produce or maintain voluntary force is the result of both contractile failure of the muscle and failure to activate the muscle adequately due to reduced central drive. As mentioned, assessing voluntary activation by evoking a maximal twitch during a maximal contraction and following a maximal contraction is one common method for estimating central fatigue (Merton, 1954). There is a decline in voluntary activation during fatigue, assessed using a supramaximal peripheral nerve stimulation (Gandevia et al., 1996; Morel et al., 2015; Todd et al., 2003; Zijdewind et al., 1998). This method of estimating central drive, however, fails to differentiate between failure at the spinal motor neurons or at supraspinal levels.

When the motor cortex is stimulated through transcranial magnetic stimulation during a maximal contraction there is a progressive increase in the superimposed twitch with fatigue, indicating suboptimal motor cortex drive (Gandevia et al., 1996; Hunter, Butler, Todd, Gandevia, & Taylor, 2006; Smith, Martin, Gandevia, & Taylor, 2007; Søgaard, Gandevia, Todd, Petersen, & Taylor, 2006). The spinal motor neuron pool experiences decreased excitability, examined using

the H-reflex and cervicomedullary evoked potentials (Avela, Kyröläinen, Komi, & Rama, 1999; Butler, Taylor, & Gandevia, 2003; Duchateau & Hainaut, 1993; Duchateau, Balestra, Carpentier, & Hainaut, 2002; Garland & Mccomas, 1990; Kuchinad, Ivanova, & Garland, 2004; Martin, Smith, Butler, Gandevia, & Taylor, 2006; Nordlund, Thorstensson, & Cresswell, 2004; Walton et al., 2002). The reduction in H_{max}:M_{max} due to fatigue occurs in various muscle groups and with various types of fatigue protocols (sustained contractions, intermittent contractions, electrical stimulation or locomotor activities) (Avela, Kyröläinen, Komi, & Rama, 1999; Duchateau, Balestra, Carpentier, & Hainaut, 2002; Duchateau & Hainaut, 1993; Garland & Mccomas, 1990; Kuchinad, Ivanova, & Garland, 2004; Walton, Kuchinad, Ivanova, & Garland, 2002). In the spinal motor neuron pool, the reduction in excitability could be due to the afore mentioned reduced descending drive, a change in sensory afferent synaptic input, or a reduction in the sensitivity of the motor neuron to synaptic input (Taylor & Gandevia, 2008).

The fatigue-induced changes to PIC in spinal motor neurons has not been examined to the same extent. PIC could be a compensatory mechanism to help overcome hypoexcitability upstream, or muscle contractile failure downstream. One study found that during fatigue there was a reduction in cortical excitability and an increase in spinal excitability (Pearcey et al., 2016). This could indicate that initially during fatigue there is a compensatory mechanism within the spinal cord to overcome cortical hypoexcitability. Compared to previous work cited, indicating a decrease in H-reflex during fatigue, the extent of fatigue in this study was less, with maximal force only dropping 8.7%, compared to 25-60% (Avela et al., 1999; Duchateau & Hainaut, 1993; Duchateau et al., 2002; Garland & Mccomas, 1990; Kuchinad et al., 2004; Pearcey et al., 2016; Walton et al., 2002). In other conditions with reduced descending drive, such as during spinal cord injury and aging, the spinal motor neurons adapt to have an increased PIC (Button et al., 2008;

Harvey, Li, Li, & Bennett, 2006; Kalmar et al., 2009; Li, Gorassini, & Bennett, 2004). Therefore, PIC may also have a compensatory increase during fatigue, to increase spinal excitability to offset cortical hypoexcitability and muscle contractile failure. However, the relationship between PIC and fatigue could be more complex since PIC is also sensitive to inhibitory synaptic input, which increase with fatigue.

i. Motor Unit Firing Rates

The firing rates of motor neurons are optimally matched with the contractile properties of the muscle fibers it innervates. During fatigue, there is a change in muscle contractile properties, and in response there is a change in motor unit firing rates. The Muscle Wisdom Hypothesis describes the slowing of motor neuron firing rates that occurs concomitantly with a slowing of muscle rate of relaxation during fatigue (Garland & Gossen, 2002). When the rates of muscle fiber relaxation slow, motor unit firing rates slow as well because fused tetanus can occur at a lower firing rates, and it prevents fatigue by reducing redundant motor neuron firing (Bigland-Ritchie, Dawson, Johansson, & Lippold, 1986; Bigland-Ritchie, Johansson, Lippold, & Woods, 1983; Bigland-Ritchie, Jones, & Woods, 1979; Jones, Bigland-Ritchie, & Edwards, 1979). In contrast, if the motor neuron continued to fire at the higher frequency then a decrease in force would be evident earlier (Bigland-Ritchie et al., 1979; Jones et al., 1979). However, the type, intensity, and duration of the contraction may affect how the motor neuron firing rates adapt during fatigue.

During fatiguing sustained contractions of the hand (Bigland-Ritchie, Dawson, et al., 1986; McManus, Hu, Rymer, Lowery, & Suresh, 2015), arm (Mottram, Jakobi, Semmler, & Enoka, 2005), leg (Vila-Chã, Falla, Correia, & Farina, 2012), and foot (Kelly, Racinais, & Cresswell, 2013), motor unit firing rates decreased during or following fatigue. However, in one instance, the motor unit firing rate pattern of the adductor pollicis was to initially decrease but then increase

throughout the fatigue protocol (Mettler & Griffin, 2016). Asides from the muscle being assessed, another main difference between the studies that may explain the difference in findings is the duration of the fatigue protocol. In the studies where a decrease in motor unit firing rates is found, the fatiguing contractions lasted two to four minutes, in contrast to the protocol by Mettler and Griffin (2016), which lasted on average nine and a half minutes. Additionally, a one-minute sustained contraction lead to no change in firing rates (de Ruiter, Elzinga, Verdijk, van Mechelen, & de Haan, 2005). Therefore, the length of the sustained contraction or the level of fatigue may be influential on the motor unit firing rates.

Another factor that appears to influence the behaviour of motor unit firing during fatigue is the type of contraction. Repetitions of 50s isometric contractions caused an increase in firing rates in the vastus lateralis muscle (Adam & De Luca, 2005; Contessa, Puleo, & De Luca, 2016). Yet, repetitions of 10-second isometric contractions at 50% and 100% MVC caused a decrease in motor unit firing rates, similarly to the sustained contractions (Enoka, Robinson, & Kossev, 1989; Stock, Beck, & Defreitas, 2012). Work by Bigland-Ritchie and colleagues (1986) shows that the reduction of motor unit firing rates does not recover if the muscle remains ischemic, indicating that an increase in metabolites could be reflexively inhibiting the motor neuron and reducing the firing rate. This could explain why only high intensity intermittent contractions or sustained contractions lead to a decline in motor unit firing rates.

ii. Antagonist Activation During Acute Exercise and Fatigue

As previously mentioned, PICs are activated through a diffuse release or norepinephrine and serotonin from the brainstem (Björklund & Skagerberg 1982). However, increasing the excitability of both the agonist and antagonist muscle groups through PIC activation could increase the incidence and amount of co-activation (Heckman et al., 2008). In order to efficiently control

the muscle groups, there must be a mechanism by which PIC is turned off in antagonist motor neurons while the agonist muscle is contracting (Hyngstrom, Johnson, Miller, & Heckman, 2007). Simply reducing synaptic excitation is not sufficient to shut off PIC, since the nature of PIC is to maintain motor neuron firing in conditions of lower synaptic excitation (Gorassini et al., 1998); however, PICs are highly sensitive to inhibitory synaptic input (Hultborn, Denton, Wienecke, & Nielsen, 2003; Kuo, 2003). The reciprocal inhibition circuitry between agonist and antagonist muscle pairs allows for PIC activation only in the agonist muscle group (Heckman et al., 2005). The influence of the antagonist stretch reflex on PIC amplitude was studied by Hyngstrom and colleagues (2007) by changing the degree of rotation of the ankle. There was nearly a 60% difference in PIC amplitude between 10 degrees of flexion versus 10 degrees of extension, indicating that the PIC was highly sensitive to reciprocal inhibition (Hyngstrom et al., 2007). However, it is uncertain whether co-activation of the antagonist muscle may cause the same inhibitory response in the agonist motor neurons.

During fatiguing isometric contractions there is commonly an increase in the activation of the antagonist muscle group (Dimitrijevic et al., 1992; Levenez, 2005; Psek & Cafarelli, 1993). This could be due in part to a decrease in group Ia afferent activation of the target muscle group (Bongiovanni & Hagbarth, 1990; Macefield, Hagbartht, Gorman, Gandevia, & Burke, 1991), thus reducing the reciprocal inhibition to the antagonist muscle group. The lack of inhibition to the antagonist motor neurons may facilitate antagonist muscle activation, which would cause reciprocal inhibition to act on the target muscle group motor neurons (Morin & Pierrot-Deseilligny, 1977; Vallbo, 1971, 1974). Because PICs are sensitive to inhibitory synaptic input, this may cause a reduction of PIC.

iii. Sex Differences in Fatigability

When assessing muscle fatigue, it is important to take into consideration sex-differences that could be present. Females are generally more fatigue resistant than males, but the type of contraction, the intensity of contraction, the duration of the exercise, and the muscle group used all play a role in the presence and magnitude of that difference (for review see: Hicks, Kent-Braun, & Ditor, 2001; Hunter, 2009, 2014, 2016). During sustained and intermittent fatiguing contractions, females have a longer endurance time or have a lower decline in maximal voluntary force than males (Avin et al., 2010; Clark, Collier, Manini, & Ploutz-Snyder, 2005; Clark, Manini, Thé, Doldo, & Ploutz-Snyder, 2003; Fulco et al., 1999; Hunter, Critchlow, Shin, & Enoka, 2004; Hunter & Enoka, 2001; Hunter et al., 2006; Hunter, Griffith, Schlachter, & Kufahl, 2009; Hunter, Critchlow, & Enoka, 2004; Kent-Braun, Ng, Doyle, & Towse, 2002; Martin & Rattey, 2007; Maughan, Harmon, Leiper, Sale, & Delman, 1986; West, Hicks, Clements, & Dowling, 1995). However, the magnitude of difference between males and females is intensity-dependent such that females perform better on lower intensity tasks, and become more similar to males as the intensity increases (Hunter, 2016; Maughan et al., 1986; Yoon, Delap, Griffith, & Hunter, 2007).

The mechanisms of the sex-difference in fatigue resistance is task-dependent. During isometric contractions, one explanation for the sex-difference is that males have greater muscle mass which causes more pronounced vascular compression and build up of metabolites from anaerobic metabolism, which in turn leads to reduced central drive through group III and IV afferent activation (Amann, 2012; Kent-Braun et al., 2002; Martin & Rattey, 2007; Rotto & Kaufman, 1988; Russ & Kent-Braun, 2003; Russ, Lanza, Rothman, & Kent-Braun, 2005). Furthermore, when participants are tested under ischemic conditions, sex differences in endurance time that are present in non-ischemic conditions disappear (Clark et al., 2005). Also, when males

and females are strength-matched there is no difference in endurance time during a sustained contraction (Hunter et al., 2006; Hunter, Critchlow, Shin, & Enoka, 2004), indicating that muscle mass does effect fatigability potentially through vascular compression. However, when strength-matched males and females perform intermittent contractions females still have an advantage over males (Fulco et al., 1999; Hunter et al., 2004; Hunter et al., 2009), indicating that when perfusion is not a concern there is an additional mechanism that allows females to be more fatigue resistant than males (Hunter et al., 2009).

Another explanation for the sex differences in fatigability is the difference in skeletal muscle physiology between males and females. Females have a greater oxidative capacity, whereas males rely more heavily on the glycolytic pathway, which is due to the difference in proportion of type I and type II muscle fiber cross-sectional area (Roepstorff et al., 2006; Simoneau & Bouchard, 1989). Males and females have similar ratios of the number of type I and type II muscle fibers, however females have a greater ratio of type I muscle fiber cross-sectional area than males (Simoneau & Bouchard, 1989). This causes lower relative forces, slower rates of rise and relaxation, and greater fatigue resistance (Hunter, Butler, Todd, Gandevia, & Taylor, 2006; Wüst, Morse, De Haan, Jones, & Degens, 2008; Yoon, Doyel, Widule, & Hunter, 2015).

When considering sex differences in muscle fatigability another key consideration is the muscle group being used for the investigation. In a review, Hunter (2016) uncovers a trend that the difference in endurance time between males and females is greater during upper-limb exercises than it is during lower limb protocols. The majority of studies has focused on the elbow flexors (Avin et al., 2010; Hunter, Butler, Todd, Gandevia, & Taylor, 2006; Hunter et al., 2004; Hunter & Enoka, 2001; Hunter, Critchlow, & Enoka, 2004; Hunter, Critchlow, Shin, et al., 2004; Maughan et al., 1986; Miller, MacDougall, Tarnopolsky, & Sale, 1993), knee extensors (Clark et al., 2005;

Glace, Kremenic, & McHugh, 2013; Martin & Rattey, 2007; Maughan et al., 1986; Miller et al., 1993; Stern, Kuenze, Herman, Sauer, & Hart, 2012; Temesi et al., 2015), and hand muscles (Ditor & Hicks, 2000; Fulco et al., 1999; Hunter et al., 2006, 2009). One study that examined plantar flexors found no difference in voluntary force following 110km trail race, but there was a reduction in evoked twitch amplitude (Temesi et al., 2015). This may indicate that in the plantar flexors, males exhibit greater peripheral fatigue and females exhibit more central fatigue, however the findings may not generalize to a less extreme level of fatigue (Temesi et al., 2015).

There is a wider gap in fatigability between men and women during upper-limb fatigue than in lower limb. One explanation for this difference is that there are different mechanism leading to a sex difference in fatigability. During lower limb exercise the sex difference appears to be driven by a change in voluntary activation, such that men have a greater reduction in the neural drive to muscles than women (Hunter, 2009).

One other consideration are the biomechanical and behavioural differences between males and females. It is more common for females to wear high-heeled shoes, which results in biomechanical changes in the soleus (Farrag & Elsayed, 2016). The effect of these biomechanical differences on fatigability has not yet been examined.

TRAINING ADAPTATIONS TO SPINAL MOTOR NEURON EXCITABILITY

Physical training leads to physical adaptations to improve an individual's ability to perform the trained task. Different physical tasks lead to different adaptations. This section will focus on the adaptations within the nervous system, specifically in the spinal motor neurons, to physical training.

Level of physical activity has a positive relationship with spinal motor neuron pool excitability (Chalmers & Knutzen, 2000; Nielsen, Crone, & Hultborn, 1993). However, the relationship is dependent on the type of physical activity. Power trained athletes have lower spinal motor neuron pool excitability than endurance trained or untrained individuals, whereas endurance trained athletes have a higher spinal motor neuron pool excitability than untrained (Casabona, Polizzi, & Perciavalle, 1990; Kyröläinen & Komi, 1994; Maffiuletti et al., 2001; Nielsen et al., 1993; Rochcongar, Dassonville, & Le Bars, 1979). However when participants undergo a training program of either resistance training or power training protocol, there are no reported changes in H-reflex amplitude (Per Aagaard, Simonsen, Andersen, Magnusson, & Dyhre-Poulsen, 2002; Gruber et al., 2007; Taube et al., 2007; Vila-Cha, Falla, Correia, & Farina, 2012). Studies of endurance training protocols, however, have mostly demonstrated an increase in H-reflex excitability (Pérot, Goubel, & Mora, 1991; Vera-Ibañez, Colomer-Poveda, Romero-Arenas, Viñuela-García, & Márquez, 2017; Vila-Cha et al., 2012), with only one study resulting in no change (Behrens et al., 2015). It is hypothesized that the increase in H_{max}/M_{max} following endurance training could be due to increased slow-twitch muscle fibre ratio, since they are innervated by lower threshold motor neurons (Kyröläinen & Komi, 1994; Maffiuletti et al., 2001). The increase in the spinal motor pool excitability could be a mechanism of reducing fatiguability.

The electrophysiological properties of motor neurons demonstrate plasticity in conditions of injury, aging, disuse, and exercise (Beaumont & Gardiner, 2002, 2003; Button et al., 2008; Cormery, Beaumont, Csukly, & Gardiner, 2005; Kalmar et al., 2009; Krutki, Mrówczyński, Bączyk, Łochyński, & Celichowski, 2017; MacDonell, Button, Beaumont, Cormery, & Gardiner, 2012a). In a rat model, two studies looked at the adaptations to running, each with slightly different intensity, which each resulted in slightly different adaptations in spinal motor neurons of the

hindlimbs. When rats were allowed to run spontaneously for 12 weeks, the motor neurons of the hindlimb had more hyperpolarized spike trigger level and resting membrane potential, and a larger afterhyperpolarization (AHP) amplitude, in the slow motor neurons only (Beaumont & Gardiner, 2002). However, after 16 weeks of treadmill running, which consisted of running at a higher intensity than spontaneous running, the motor neuron of rat hindlimbs had more hyperpolarized spike trigger level and resting membrane potential, and faster antidromic spike rise times in both fast and slow motor neurons (Beaumont & Gardiner, 2003). Higher intensity exercise was necessary to elicit any adaptation in fast motor neurons, however longer duration low intensity exercise was necessary to elicit changes in AHP amplitude (Beaumont & Gardiner, 2003). In both of these studies, there were no changes in the rheobase, which would be indicative of a change in the excitability of the motor neuron (Beaumont & Gardiner, 2002, 2003).

Examination of the rhythmic firing properties show that there are intensity-dependent adaptations to exercise, however overall there is increased excitability following training (Adam et al., 1998; Krutki et al., 2017; MacDonell et al., 2012). Strength training resulted in a left-ward shift in the frequency/current (*f/I*) curve, and increases in maximum early state and steady state maximum firing frequencies in rats (Krutki et al., 2017). In contrast, endurance trained rats, although similarly had a left-ward shift in the *f/I* curve, also had a decrease in the slope of the curve, a decrease in steady state firing rates, and a decrease in spike frequency adaptation (MacDonell et al., 2012). The increased excitability caused by physical training could be attributed to an increase in PIC. Endurance training caused a decrease in spike frequency adaptation, which is negatively correlated with the estimated PIC amplitude (Button, Kalmar, Gardiner, Cahill, & Gardiner, 2007; MacDonell et al., 2012). Additionally, strength training led to a decrease in the rheobase of fast motor neurons and the overall sample of fast and slow motor neurons (Krutki et

al., 2017). Motor neurons with lower rheobase tend to have a greater incidence of PIC (Kalmar et al., 2009).

In humans, motor unit of the dominant hand show similar activity-dependent adaptations that are not present in the motor units of the non-dominant hand (Adam et al., 1998). These include lower recruitment threshold and lower initial and average firing rates (Adam et al., 1998). This provides further evidence that there is are training adaptations at the spinal motor neuron level that increase the excitability.

Furthermore, the adaptations to decreased exercised are opposite to those of increased exercise (Cormery et al., 2005). Motor neurons in rat hindlimbs that were suspended for two weeks changed towards fast motor neuron properties: higher rheobase, faster firing frequencies, and a right-ward shift of the *f/I* curve (Cormery et al., 2005). Therefore it is expected that individuals who are more physically active will show indices of more excitable motor neurons.

i. Measuring Physical Activity

Physical activity can refer to muscular activity of any sort, however quantifying a meaningful amount of physical activity can be difficult. Methods of quantifying physical activity include objective measurements of movements via accelerometer motions sensors, or heart rate using a heart rate monitor (Ainsworth et al., 2000; Freedson & Miller, 2000; Troiano et al., 2008). However, these methods require equipment, user compliance, and a longer data collection duration than the administration of a questionnaire. Although a questionnaire relies on participant recall, it is also administered at one time point and does not depend on participant compliance. There are many different physical activity questionnaires, however one measure is the International Physical Activity Questionnaire (IPAQ). The IPAQ can be advantageous over other questionnaires because

it quantifies occupation, transportation, and leisure time physical activity, as well as sedentary behaviour. There are two English print versions of the IPAQ, the short form and the long form. Both versions ask about time spent doing vigorous physical activity, moderate physical activity, and walking, however the long form indicates occupational, transportation, and leisure separately whereas the short form asks about time spent doing each level of physical activity and just cues the participant to think about the entire day. For this study, the IPAQ short form (IPAQ-s) was selected over the IPAQ long form since the time spent in physical activity did not need to be further broken down into leisure time, transportation, or occupational physical activity.

The reliability of the IPAQ-s has been assessed through correlation of test-retest scores. Vigorous physical activity has consistently strong test-retest reliability with R-values that range from 0.61 and 0.75 (Kurtze, Rangul, & Hustvedt, 2008; Lee et al., 2011; Macfarlane, Lee, Ho, Chan, & Chan, 2007; Saglam et al., 2010). Moderate physical activity has moderate-strength test-retest reliability with R-values that range from 0.30 and 0.50 (Kurtze, Rangul, & Hustvedt, 2008; Lee et al., 2011; Macfarlane, Lee, Ho, Chan, & Chan, 2007; Saglam et al., 2010). Time spent walking has moderate to strong reliability, with R-values that range from 0.42 and 0.93. Time spent sitting has strong reliability, with R-values between 0.78 and 0.97 (Kurtze, Rangul, & Hustvedt, 2008; Lee et al., 2011; Macfarlane, Lee, Ho, Chan, & Chan, 2007; Saglam et al., 2010).

The validity of the IPAQ-s has been tested against accelerometers, physical activity logs, and VO_{2max}. One study compared the IPAQ-s scores to accelerometer data, and found very weak correlations between the scores: vigorous r_s=0.16 and moderate r_s=0.09 (Lee et al., 2011). However, this study had participants wear the accelerometer for four days after the completion of the questionnaire, therefore accelerometer data and the questionnaire data were based on different days (Lee et al., 2011). Again, low validity was reported when IPAQ-s scores were compared to

accelerometer data from two separate weeks: vigorous r_s=0.05, moderate r_s=0.16, walking r_s=0.16, sitting r_s=-0.25 (Kurtze et al., 2008). However, there was a moderate correlation between vigorous physical activity and V_{O2max} (r_s=0.41) (Kurtze et al., 2008). When physical activity is measured over the same time period, there is better validity between the IPAQ-s and accelerometer data: vigorous R=0.93, moderate R=0.22, walking R=0.90 (Macfarlane et al., 2007). IPAQ-s scores compared to physical activity logs has weak to strong validity as well: vigorous R=0.59, moderate R=0.82, walking R=0.10 (Macfarlane et al., 2007).

Overall the IPAQ-s has moderate to strong reliability, and inconsistent validity. However, when the IPAQ-s scores are compared to data measured over the same time period, validity is mostly moderate to strong between IPAQ-s and accelerometer data or physical activity logs. Additionally, amount of vigorous physical activity may be a valid measure of physical fitness (Kurtze et al., 2008).

In summary, during fatigue there is a change in spinal motor neuron discharge rates, the direction of which is dependent on the muscle, intensity, and type of fatiguing activity, as well as a decrease in the spinal motor neuron pool excitability. It is speculated that the intrinsic excitability of the motor neuron increases in order to compensate for failure in the supraspinal areas or for muscle contractile failure. However, during fatigue there may be increased antagonist muscle activation, which could deactivate PIC, and its reduce its ability to compensate for fatigue. Finally, the level of habitual physical activity may play a role in the amplitude of PIC, which may alter how PIC responds to fatigue.

PURPOSE

Fatigue, or the inability to maintain optimal force output, may be due a failure of the central nervous system to drive muscle. There are several sites of adaptation between the brain and muscle at which drive to muscle may be improved or impaired during prolonged muscle activity. The spinal motor neuron is the final common pathway from the nervous system to muscle, and is therefore a likely site of adaptation. Therefore, the first purpose of this study was to determine how spinal motor neuron excitability (assessed using the H-reflex gain and paired-motor unit estimates of PIC amplitude) and spinal motor neuron output (firing rates) changed in response to an intermittent isometric fatigue protocol. Inhibitory input reduces the amplitude of PIC in animal models. One source of inhibition is reciprocal inhibition from the antagonist muscle. Accordingly, the second purpose was to determine whether level of antagonist muscle activation contributed to fatigue-induced changes in PIC. Physical training causes adaptations to spinal motor neuron electrophysiological properties and firing properties in an animal model. Additionally, PIC has long-term adaptations in other conditions, namely spinal cord injury and aging, therefore physical training may also cause a long-lasting adaption of PIC. For this reason, the final purpose was to assess the level of physical activity of participants to determine if there is a correlation between physical activity and initial PIC amplitudes.

HYPOTHESES

1. Muscular fatigue (lower force, slower rate of force generation, and slower rate of relaxation) will be associated with lower motor unit firing rates.
2. There will be a decrease in motor neuron pool excitability following fatiguing contractions.

3. There will be an initial compensatory increase in persistent inward current that correlates with contractile failure of the muscle.
4. As the fatigue protocol progresses, antagonist muscle activation will increase, which will in turn reduce the amplitude of persistent inward current.
5. People who are more physically active will have greater initial persistent inward current amplitudes.

CHAPTER 2: MANUSCRIPT

INTRODUCTION

Fatigue occurs when muscular effort is sustained or repeated and is described as the inability to maintain or produce the same level of force (Allen et al., 2008). Neuromuscular fatigue can originate anywhere along the motor path between the brain and muscle (Taylor, Amann, Duchateau, Meeusen, & Rice, 2016). However, prior to a measurable change in force, fatigue can cause disruptions within the motor system (Taylor et al., 2016). These disruptions can occur without a measurable change in force because of compensations within the motor system to prevent loss of force.

At the muscle, contractile failure can be offset by increased motor unit recruitment (Edwards & Lippold, 1956), motor unit rotation (Bawa & Murnaghan, 2009), and optimized motor unit firing rates (Garland & Gossen, 2002). Increase in muscle EMG during submaximal fatiguing contractions indicates that more motor units are being recruited to produce the same submaximal force (Edwards & Lippold, 1956). Additionally during some types of fatiguing contractions, motor unit firing rates lower in order to optimize tetanic force in the muscle fibers that are slowing due to fatigue (Bigland-Ritchie, Dawson, et al., 1986; Kelly et al., 2013; McManus et al., 2015; Mottram et al., 2005; Vila-Chã et al., 2012). This prevents redundant firing and further fatigue (Bigland-Ritchie, Dawson, et al., 1986; Bigland-Ritchie et al., 1983, 1979; Jones et al., 1979).

Along with contractile failure, during fatigue there is a decrease in H-reflex amplitude (Avela, Kyröläinen, Komi, & Rama, 1999; Duchateau, Balestra, Carpentier, & Hainaut, 2002; Duchateau & Hainaut, 1993; Garland & Mccomas, 1990; Kuchinad, Ivanova, & Garland, 2004; Walton, Kuchinad, Ivanova, & Garland, 2002) and a decrease in central drive to the motor neuron pool drive (Gandevia et al., 1996; Hunter et al., 2006; Smith et al., 2007; Søgaard et al., 2006). One potential mechanism that may prevent force loss is an increase in the persistent inward current

(PIC). PIC increases the intrinsic excitability of the motor neuron by allowing calcium to enter through the channels in the dendrites (Heckman et al., 2003). The increased excitability allows the motor neuron to fire more readily and to maintain firing during lowered or eliminated excitatory synaptic input (Heckman et al., 2003). During conditions of reduced spinal excitability, such as during spinal cord injury or aging, there is an increase in PIC (Button et al., 2008; Kalmar et al., 2009), so it is possible that during fatigue it increases to compensate for the lowered spinal motor neuron pool excitability or for contractile failure.

PIC increases the responsiveness to excitable synaptic input, however PIC is highly sensitive to inhibitory synaptic input which cause PIC to deactivate. Therefore, the activation of one muscle can cause the deactivation of PIC of the antagonist muscle through reciprocal inhibition (Heckman et al., 2008). Previous research indicates that there is an increase in antagonist muscle activation during fatiguing contractions (Dimitrijevic et al., 1992; Levenez, 2005; Psek & Cafarelli, 1993), therefore it is possible that PIC may not be able to compensate for fatigue in the agonist muscle due to increased inhibition from antagonist muscle activation.

Finally, as mentioned, PIC is persistently affected during spinal cord injury (Button et al., 2008) and aging (Kalmar et al., 2009), therefore we speculated that it may also adapt to habitual levels of physical activity. So, the last purpose of this study was to assess whether the level of habitual physical activity influences baseline PIC amplitude. Endurance training leads to an increase in H-reflex amplitude in humans (Chalmers & Knutzen, 2000; Nielsen et al., 1993), as well as increased excitability in rat spinal motor neurons measured through changes in electrical physical properties and rhythmic firing properties (Beaumont & Gardiner, 2002, 2003; MacDonell et al., 2012). Following strength training and in power-trained athletes there is a decrease in the H-reflex amplitude in humans (Casabona et al., 1990; Kyröläinen & Komi, 1994; Maffiuletti et

al., 2001; Nielsen et al., 1993; Rochcongar et al., 1979), however again in the rat model there is an increase in excitability (Krutki et al., 2017). Additionally, long-term sedentary behaviour causes a decrease in the excitability of spinal motor neurons in the rat model (Cormery et al., 2005). We speculate that alterations in excitability of the spinal motor neuron to level of physical activity could reflect changes to PIC.

Therefore, the purpose of this study was threefold. The first was to assess how motor unit firing rates, H-reflex gain, and PIC amplitude in the soleus muscle change in response to an intermittent isometric fatigue protocol. The second purpose was to assess the level of antagonist muscle activation, to measure any fatigue-related increases in the antagonist muscle activation, and if so if that has an inhibitory affect on PIC. The final purpose was to assess the level of physical activity of participants to determine if there is a correlation between physical activity and initial PIC amplitudes.

METHODS

Participants

Twelve young adults aged 22.8 (1.3) years were recruited to take part in this study through poster advertisements on Wilfrid Laurier University Waterloo Campus and through word of mouth. Participants were all free of any self-reported neurological disorder or injury to their right lower limb. Participants were non-smokers and not taking any medication that acts as a sedative, stimulant, or narcotic to prevent confounding effects on spinal motor neuron excitability. Participants were all physically able to hear the voice recording that guided the fatiguing contraction protocol and were able to see the computer monitor screen that provided visual feedback of force during the protocol. Participants were asked to abstain from vigorous physical

activity 24 hours prior to testing and were asked not to consume caffeine-containing food or beverages 12 hours prior to testing.

Participants all read and signed an informed consent that outlined the procedures and the potential risks associated with the protocol. The research procedures were approved by the Wilfrid Laurier Research Board of Ethics (REB #5409).

Experimental protocol

This study utilized a within-subject repeated-measures design. There were two experimental testing conditions, fatigue and rest, which were completed on two separate days at the Exercise and Neurophysiology Lab at Northdale Campus at Wilfrid Laurier University. During each testing session, measures were taken before, during, and after a series of fatiguing contractions (fatigue day) or during a rest period (rest day). Prior to these testing sessions, participants first attended an orientation session where they read and signed the informed consent (Appendix B), filled out a screening questionnaire (Appendix C) and the physical activity questionnaire (Appendix D). Participants also practiced maximally contracting their plantarflexors, tracing the triangular ramps on a computer monitor by gradually increasing the force of their plantarflexor contraction and then gradually decreasing the force. They were also familiarized with the peripheral nerve stimulation.

The order of the fatigue-day experimental session and a rest-day experimental session was randomized and counter-balanced. During the two testing sessions, participants were seated in a car seat fixed to a hydraulic base. The height of the seat was adjusted to position the right leg in a custom-made ankle dynamometer with the hip, knee, and ankle both flexed to approximately ninety degrees (Figure 1). To prevent unwanted movement, the participants' feet were strapped to

the footplate on the dynamometer, a brace was put in front of their lower leg to prevent forward movement, and a brace was placed above the knee to prevent the heel from excessive lifting during isometric plantarflexion contractions.

The protocols for the two experimental sessions were the same, except that participants performed sets of intermittent plantarflexion muscle contractions during the fatigue session whereas the participants sat at rest during the rest session. Prior to each testing session, participants performed submaximal contractions to warm-up their muscle, and the stimulation intensity that elicited a maximum M-wave was determined. The protocol included pre-fatigue/rest testing, fatiguing/quiet sitting protocol, immediate post-fatigue/rest testing, and fifteen minutes post-fatigue/rest testing (Figure 5). The pre-, post- and fifteen minutes-post testing consisted of maximal voluntary contractions (MVCs), triangular ramp contractions for estimating PIC using the paired motor unit technique, and H-reflex and M-wave recruitment curves to assess the excitability of the soleus motor neuron pool. During the MVC's, a supramaximal twitch (120% M_{max} stimulation intensity) was evoked during the contraction (superimposed twitch) and at rest following the contraction (potentiated twitch) to estimate voluntary activation and assess muscle contractile properties.

Fatigue Protocol

Participants all completed the same fatigue protocol, therefore the level of fatigue varied between participants. The fatigue protocol was a series of intermittent submaximal plantarflexion contractions. Submaximal contractions with intermittent rest periods were chosen to elicit greater central fatigue and not peripheral fatigue resulting from ischemia (Baker et al., 1993).

The fatigue protocol consisted of five sets of 40 contractions. During each three-minute set, participants would contract their plantar flexors to reach a target of 50% of maximal force that was displayed to the participant on a computer monitor. Each contraction lasted 3 s and was followed by 1 s of rest. Immediately following the last contraction of each set, participants performed an MVC with superimposed and potentiated twitches and three to five triangular ramp contractions before beginning the next set.

Procedures

Electromyography

Surface EMG was recorded using silver parallel-bar single-differential surface electrodes (DELSYS, Massachusetts, USA). The recording surfaces were 1 cm in length and 0.1 cm in width, the distance between the two silver bars was 1 cm. One electrode was placed over the muscle belly of the soleus and a second electrode was placed over the muscle belly of the tibialis anterior (Figure 1). In both cases, electrodes were positioned such that one silver bar was more distal than the other on the muscle. Surface EMG was amplified 1000x (Bagnoli, DELSYS, MA, USA) and sampled at 2000 Hz (CED 1401, mk3), then recorded on Spike 2 software (CED, Cambridge, UK). Surface EMG was grounded using a reusable self-adhesive ground electrode (40mm x 40mm, Lemonbest, Guangdong, China) placed on the medial condyle of the tibia. Before the recording and ground electrode were adhered to the skin, the area was shaved, abraded, and cleaned to improve the signal to noise ratio. Prior to analysis surface EMG signals were filtered using a high pass filter set at 10 Hz.

Single motor unit recordings were obtained using intramuscular EMG. Three insulated stainless steel wires of 50.8 μm diameter (California Fine Wire) were inserted into the belly of the

soleus muscle using a sterilized 25- or 26-gauge (40mm or 16mm length) hypodermic needle. The ends of the wires attached to the preamplifier had one centimetre of the insulation scraped off so that the signal could be transferred at that point through the preamplifier. The recording area of each intramuscular electrode is the cut end (50.8 µm diameter). Two of the three wires were secured with thumbscrews to a preamplifier with a gain of 300x (Motion Lab Systems, Louisiana, USA). The two wires secured to the preamplifer were randomly selected of the three, however if the two initially selected did not provide a usable signal then a different combination of two wires would be attempted. From the preamplifier, the signal was transferred to a variable gain second-stage amplifier (Neurolog, Model 106, Digitimer, Greenvale, NY, USA) for further amplification and filtering. At this stage the signal was amplified by 10 for a total of 3000x amplification and bandpass filtered at 1000 Hz to 20000 Hz. The signal was sampled at 20000 Hz (CED 1401, mk3) and was recorded using Spike2 software (CED, Cambridge, UK). The intramuscular EMG was grounded with a stainless-steel reusable ground electrode (1 $^{1}/_{5}$ inch diameter). Conductive electrode gel was placed on the ground electrode prior to securing it to a distal section of the tibia with tape. Skin was shaved, abraded, and cleaned prior to electrode placement to reduce noise in the signal. Prior to analysis intramuscular EMG was filtered using a high pass filter set at 100 Hz.

Electrical Stimulation

The tibial nerve was stimulated using monopolar electrodes. A 1 cm^2 cathode was placed over the nerve in the popliteal fossa and a dispersive anode (2.5 cm^2) was placed just superior to the patella. The surface of the electrode was covered with conductive electrode gel to improve stimulation conductance. The electrodes were secured to the leg using tape and tensor wraps. The electrodes were connected to a constant-current square-pulse stimulator (Digitimer, DS7A, Hertfordshire, UK). The pulse duration was 1000 µs for all stimulations.

Maximal Voluntary Contractions

Once the set-up was complete the participant completed at least three plantar flexion MVCs; if their maximum force produced did not plateau by the third MVC, participants did additional MVCs until the force did not continue to increase. Each participant had two minutes of rest between each contraction to prevent an inability to produce maximal forces due to fatigue. The contraction with the greatest force was considered the participants' MVC value and was used set relative submaximal contraction targets for the triangular ramp contractions and fatiguing contractions. Participants then performed an MVC before the triangular ramp contractions prior to the first fatigue or rest set, after each set, and after 15 mins of recovery.

Triangular Ramp Contractions

Participants performed triangular ramp contractions following the pre-fatigue/rest MVC, after each set, and after the 15 minutes of recovery. The participants traced the shape of a triangle that was on a transparency by gradually increasing and decreasing plantar flexion force. The triangle ramps were all 10 s in duration. During the pre-fatigue/rest contractions, ramp height was initially set at 10% of MVC but adjusted to a slightly higher or lower target if the 10% ramp height did not yield a recording of two motor units, recruited at least 1 s apart, during the ascending phase of the ramp. The two to four ramp heights that provided the best recording were used for the remainder of the triangle ramp contractions.

H-reflex and M-wave Recruitment Curves

The H-reflex and M-wave recruitment curves were generated by incrementally increasing current intensity of a series of electrical stimuli applied to the tibial nerve while the participant sat quietly, with the plantar flexors relaxed and minimal movement. Once H-reflex threshold (first

detectable appearance of the H reflex above baseline) was reached, the stimulus intensity was increased by increments of 0.1 - 0.5 mA until maximum H-reflex (H_{max}). From H_{max} to M_{max} the stimulus intensity was increased by increments of 0.5 - 10 mA. There were at least four seconds between each stimuli. The stimulus-response curve was completed when there was a plateau in the response to three incremental stimuli. To ensure it was a true plateau in response, a supramaximal stimulus equal to 120% of the stimulus that caused the greatest response was discharged to ensure the response did not increase. If the M-wave response continued to increase the curve was continued until the next plateau and the same procedures were performed.

Data analysis

Muscle Contractile Properties, Maximal voluntary force, and Voluntary Activation

The maximal muscle twitch responses to supramaximal nerve stimulations while the muscle was at rest were analyzed for peak force, peak rate of rise of the force (dF/dt), and peak rate of relaxation of the force (-Df/dt) using Spike 2 software. The twitch peak force was measured from baseline prior to the stimulation when the muscle was at rest to peak force. The peak rate of rise was the peak in the first derivative of the force channel (force over time), and the peak rate of relaxation was the negative peak in the first derivative of the force channel.

The maximal voluntary force was measured from baseline prior to muscle contractions to the peak force voluntarily produced. During the MVCs a supramaximal stimulus was discharged. The twitch response superimposed on maximal voluntary force was measured from force at the time of stimulation to the peak force following the stimulation. Voluntary activation was estimated using the equation:

$$Voluntary\ Activation\ (\%) = \left[1 - \frac{Superimposed\ Twitch}{Potentiated\ Twitch}\right] x\ 100\%$$ (Merton, 1954)

Peripheral Transmission and Level of Muscle Activation

During the supramaximal stimulations at rest, the M-wave response measured to monitor peripheral transmission. The M-wave was measured as the peak to peak amplitude of the surface EMG response following the stimulus. To measure level of muscle activation during the MVC, the RMS of the soleus surface EMG signal was measured over 500 ms prior to the supramaximal stimulation. During the submaximal fatiguing contractions, the level of muscle activation was measured over one-second three times each set: once during the first five contractions, once within the middle five contractions, and once within the final five contractions. Submaximal muscle activation was measured using the RMS of the soleus surface EMG signal over 500 ms. The 500 ms windows of EMG signal were chosen based on the smoothness of the force signal and if it met the target force.

Estimates of PIC

Individual motor unit action potentials (referred to as "spikes") within the intramuscular recording were identified and sorted based on their shape, size and inter-spike interval using Spike2 software offline. Spike2 software algorithms sort spikes into templates. Templates are created during spike sorting. The first spike becomes the first template, following spikes are compared based on their shape and size, if they are within the allowed limits of the template then it is added to it, if it is not than it becomes a new template. Templates are provisional until a certain number of spikes are matched to that template and at that point it becomes confirmed. When a provisional template has enough spikes to be confirmed, it is first assessed to ensure it is not the same as an already confirmed template, and then if not it becomes a newly confirmed template. Once the software has sorted all of the spikes, a new channel is created that has each spike sorted by separate codes (Figure 2). Some spikes may not fit into any of the templates and some templates

may include two separate spikes that are similar in shape and size. In these instances, after the spikes have been sorted, the researcher manually edited the code assignment to each spike.

Once the spikes were sorted the control unit and the test unit were identified. The control unit was selected on the basis that it had to begin firing early in the contraction and continue firing throughout the entire contraction. The test unit had to be a unit that initiated firing 1-2 s following the control unit initiation, and continue firing until the latter half of the contraction, and cessation of firing had to occur prior to cessation of control unit firing. The instantaneous firing rates of these units were plotted on a X-Y scatter plot and fit to a 4^{th} order polynomial (Figure 1). The control unit firing rate at the time of test unit onset and offset were obtained using the quadratic equation of the line to approximate the instantaneous firing rate of the control unit at that time. The estimate of PIC amplitude (ΔF) is the firing rate of the control unit at the offset of the test until subtracted from the firing rate of the control unit at the onset of the test unit. If the resulting ΔF was negative, it was corrected to zero to represent that there was no PIC.

The paired motor unit technique for estimating PIC requires three assumptions in order to accurately estimate PIC: first that PIC is fully activated in the control unit before the test unit is activated, secondly that the control unit is a sensitive indicator of synaptic input, and thirdly that the test and the control units have common synaptic input Gorassini, Yang, Siu, & Bennett, 2002; Stephenson & Maluf, 2011). In order to meet these assumptions careful considerations were made when assessing the test and the control units. The first consideration was ensuring there was at least one second between the onset of the control unit and the test unit (Powers, Nardelli, & Cope, 2008). PIC must be activated in the control unit prior to test unit activation because the increase in firing rates from the first range of firing to the secondary range firing in the control unit due to PIC activation would lead to a large overestimation of PIC in the test unit. The second assumption, that

the control unit if a sensitive indicator of net synaptic input requires that the unit has not reached firing rate saturation, or the inability to increase the firing rate any further. If the firing rate of the control until reached saturation, that would lead to a lower estimate of PIC. In order to ensure that firing rate saturation has not occurred, only motor unit pairs with a ΔF within 0.5 points per second of the control unit difference in maximum firing rate and minimum firing rate (Stephenson & Maluf, 2011). The third assumption, that the control and test units must have common synaptic input, was assessed by averaging the firing rate of both units over every 500 ms and then correlating the averaged firing rates, similar to previous work (Gorassini et al., 2004; Gorassini et al., 2002; Gorassini, Yang, Siu, & Bennett, 2002; Mottram, Suresh, Heckman, Gorassini, & Rymer, 2009). The first two averaged firing rates were not included in the correlation due to instability of motor unit firing rates at the beginning of a contraction (Kiehn & Eken, 1997), a method used in previous work (Gorassini et al., 2004; Gorassini et al., 2002). A motor unit pair had to have a correlation with an R-value of 0.70 or stronger to be accepted. If all three assumptions were met, then ΔF of that motor unit pair was accepted.

H-reflex Gain from Recruitment Curves

The peak-to-peak amplitude of the H-reflex and M-wave action potentials were measured at each stimulation intensity using Spike2 software. The peak-to-peak amplitudes of both responses were then normalized to M_{max}. Stimulation intensity was normalized to the current that elicited M_{max}. The normalized H-reflex and M-wave peak-to-peak amplitudes were plotted against the normalized stimulus intensity using SigmaPlot 14.0 (Figure 4). To estimate the gain of the H-reflex (an indirect estimate of the excitability of the spinal motor neuron pool), the slope of the H-reflex curve (H_{slp}) was compared to the slope of the M-wave curve (M_{slp}). The slope of the curve at the point representing 50% of the maximal response was estimated by fitting the curves to a 3-

parameter sigmoid function. The H-reflex curve was cut off at H_{max}, and the initial plateau of the M-wave was removed to improve the fit of the curve.

The sigmoid function used to fit the curves:

$$H(s) = \frac{Hmax}{[1+e^{\frac{(s-s_{50})}{b}}]} \quad \text{or} \quad M(s) = \frac{Mmax}{[1+e^{\frac{(s-s_{50})}{b}}]} \quad \text{(Klimstra \& Zehr, 2008)}$$

Where H_{max} or M_{max} is the upper limit of the curve, b is the slope parameter, s_{50} is the stimulus intensity at 50% H_{max} or M_{max} value, s is any given stimulus intensity, and H(s) or M(s) is the amplitude of the H-reflex or M-wave at any given stimulus intensity.

The slope at 50% of the curve was estimated using the equation:

$$Slope_{50} = \frac{Hmax}{4b} \quad \text{or} \quad Slope_{50} = \frac{Mmax}{4b} \quad \text{(Klimstra \& Zehr, 2008)}$$

This sigmoid function and estimation of slope have been used previously in similar studies (Carroll, Riek, & Carson, 2001; Devanne, Lavoie, & Capaday, 1997). Additionally, in a methods study comparing different methods of analyzing the H-reflex curve, this method was found to have the best fit (Klimstra & Zehr, 2008).

Antagonist Muscle Activation

Antagonist muscle activation was assessed using the RMS of the surface EMG signal from the tibialis anterior muscle (TA) (Kallenberg & Hermens, 2008). The average root mean square (RMS) of the TA EMG signal was measured during the triangular ramp contractions used for PIC estimates. The average RMS was measured for the entire ramp (from first control unit discharge to last control unit discharge), for the ascending portion of the ramp (from first control unit discharge to peak force), and the descending portion of the ramp (from peak force to the last control

unit discharge). The three TA sEMG measures were normalized to soleus EMG during 500ms of the pre-fatigue MVC, prior to the superimposed stimulus.

Motor Unit Firing Rates

To assess motor unit firing rates (MUFR) during the fatigue protocol, motor unit action potentials (again, "spikes") were identified and sorted using the same methods described in the previous section. For MUFR analysis, spikes were sorted during 15 separate one-second intervals throughout the fatigue protocol: once during one of the first five contractions of each set (initial MUFR), once during one of the middle five contractions of each set (middle MUFR), and once during one of the last five contractions of each set (final MUFR). For each data set, one motor unit was identified and followed throughout the entire fatigue protocol. The firing frequency within each one-second interval was measured using Spike2 software.

Level of Physical Activity

Physical activity was measured using the International Physical Activity Questionnaire Short Form (IPAQ-s). The IPAQ-s asks participants how many days a week they perform vigorous intensity physical activity, moderate intensity physical activity, and time spent walking in bouts of at least ten minutes each with a follow-up question asking how much time was usually spent on one of those days in that intensity of physical activity. This provides an estimate of minutes/week for each of the three categories, and overall time spent each week doing physical activity. There is a standardized method of converting the minutes/week into a metabolic equivalent (MET)/week value, in order to calculate a weighted average for weekly total physical activity. minutes/week spent walking were multiplied by 3.3 MET/minute, minutes/week spent in moderate intensity physical activity were multiplied by 4.0 MET/minute, and minutes/week spent in vigorous

intensity physical activity were multiplied by 8.0 MET/minute. Finally, participants were asked how many hours in a day they spend sitting. From this questionnaire I had six continuous variables: vigorous intensity physical activity (minutes/week), moderate intensity physical activity (minutes/week), walking (minutes/week), total physical activity (minutes/week), weighted total physical activity (MET/week), and time spent sitting (hours/day).

Statistical analysis

All statistical analyses were performed using Statistica 13 (TIBCO Software, CA, USA), unless otherwise specified. Missing data was filled using linear trend at point using SPSS 24 (International Business Machines Corporations, NY, USA). Missing data was filled for the assessments of differences, correlational analyses did not use filled data.

To test for an effect of fatigue over the protocol on maximal voluntary force, level of voluntary activation, level of maximal muscle EMG, twitch peak force, twitch peak rate of rise, twitch peak rate of relaxation, M-wave amplitude, and antagonist muscle activation 2x7 repeated measures ANOVAs were used. The two levels of the first factor were fatigue day vs rest day and the seven levels of the second factor were the measures made initially, after each set, and after 15 mins recovery. The sample size was twelve for each of those analysis, except for the analysis of the antagonist muscle activation during fatigue, which was eleven because one subject had no tibialis anterior EMG recording. To test the change in muscle activation during submaximal contractions, a repeated measures ANOVA with 5 levels (Sets 1-5) was used, and the sample size was twelve. To test for an effect of fatigue over the fatigue protocol on spinal motor neuron pool excitability, a 2x3 repeated measures ANOVA was used. The two levels of the first factor were fatigue day vs rest day, and the three levels of the second factor were initial vs final vs following fifteen minutes of recovery. The sample size for spinal motor neuron pool excitability was three

males and five females, data were excluded if the M-wave peak-to-peak amplitude increased during or prior to H-reflex ascension or if there was no change in the H-reflex. To assess the change in ΔF over the fatigue protocol a 2x7 repeated measures ANOVA was used. One participant did not have ΔF data for the rest day only, and therefore was excluded from the analysis. To assess the change in motor unit firing rates over the fatigue protocol and the effect of when they were measured during the fatiguing set (at the beginning, middle, or end), a 3x5 repeated measures ANOVA was used. The first factor took into account the time during the fatiguing set (beginning, middle, or end) the MUFRs were measured and the second factor took into account which set (1, 2, 3, 4, 5) during the protocol the MUFRs were measured. Nine participants had a motor unit that was active and could be followed through the entire fatigue protocol. If an any ANOVA resulted in a p-value <0.05, a Fisher's LSD post-hoc analysis was run to determine the cause of the significant finding.

The change in MUFR over the fatigue protocol was correlated to the change in maximal voluntary force, twitch peak force, twitch peak rate of rise, and twitch peak rate of relaxation using Pearson's correlations. Data was first assessed for normality using a Kolmogorov & Smirnov test and outliers that were detected using Grubb's Test were removed.

The change in estimates of PIC was correlated with the change in maximal voluntary force, level of voluntary activation, twitch peak force, twitch peak rate of rise, twitch peak rate of relaxation, and level of muscle activation during submaximal contractions using Pearson's correlations. Again, data was first assessed for normality using a Kolmogorov & Smirnov test and outliers that were detected using Grubb's Test were removed. The magnitude of change in ΔF was measured from initial values to the values after the fifth set for ten of the twelve participants. In one participant the change was measured from the end of the second set to the end of the fifth set,

and in another the change was measured from the initial value to the end of the fourth set. This was necessary because these participants had negative ΔF values, which were corrected to zero, which can not be used to calculate percent change. In these participants the magnitude of change in maximal voluntary force, twitch peak force, twitch peak rate of rise, twitch peak rate of relaxation, and muscle activation during submaximal contractions were calculated from the corresponding time points.

Initial ΔF values were correlated with minutes spent a week doing vigorous physicial activity, moderate physical activity, and time spent walking, as well as total minutes of physical activity a week, total MET expenditure a week, and time spent sitting each day using Pearson's correlations. Data was first assessed for normality using a Kolmogorov & Smirnov test and outliers that were detected using Grubb's Test were removed.

Sex differences were assessed for ΔF, maximal voluntary force, level of voluntary activation, twitch peak force, twitch peak rate of rise, and twitch peak rate of relaxation using a 2 (sex) x 2 (day) x 7 (time) repeated measures ANOVA. Sex differences were assessed for level of muscle activation during submaximal contractions using a 2 (sex) x 5 (time) repeated measures ANOVA. Sex differences for spinal motor neuron pool excitability were assessed using a 2 (sex) x 2 (day) x 3 repeated measures ANOVA. Sex differences in level of physical activity were assessed using a one-way ANOVA with sex as the categorical variable. If an any ANOVA resulted in a p-value <0.05, a Fisher's LSD post-hoc analysis was run to determine the cause of the significant finding, additionally the observed power was measured. Correlations between the change in ΔF and the change in maximal voluntary force, twitch peak force, twitch peak rate of rise, and twitch peak rate of relaxation for males and females separately were run using the same methods as when they were assessed as a group. Correlations between initial ΔF and levels of

physical activity were also made for each sex separately using the same method as when they were assessed as a group.

RESULTS

Effect of fatigue protocol on estimates of fatigue

Maximal voluntary force, maximal voluntary activation, muscle contractile failure, and muscle activation during submaximal contractions were measured to assess the extent of fatigue experienced during the fatiguing protocol (Table 1). The peak-to-peak M-wave amplitude was recorded to assess peripheral transmission of the electrical signal across the protocol. There was a significant decline in M-wave amplitude on the fatigue day ($F(6,66)=7.30$, $p<0.0001$). Because there was a significant decline in the transmission of the electrical signal, measures of muscle activation (e.g. submaximal and maximal EMG during voluntary contractions) were normalized to the M-wave.

Maximal voluntary force, maximal voluntary activation, and maximal EMG did not change across the fatigue protocol (Table 1). Similarly, the fatigue protocol did not elicit contractile failure. The significant day x time interactions for measures of twitch peak force and the peak rates of rise and relaxation were a result of potentiation of these measures on the fatigue day (Table 1).

While participants retained their ability to produce maximal force or contractile function, there was a significant increase in level of muscle activation during submaximal contractions throughout the fatigue protocol ($F(4,44)=4.89$, $p=0.002$) (Figure 6). To ensure that the change in muscle activation over the fatigue protocol was not caused by participants overshooting the target force, the submaximal force was also analyzed and there were no significant changes in the

submaximal force during each set ($F(4,44)=0.40$, $p=0.81$). This indicates that the increase in muscle activation during submaximal contractions is a result of fatigue.s

Motor neuron firing rates and fatigue

Motor unit firing rates (MUFRs) were measured at the beginning (first 5 contractions), middle (middle 5 contractions), and end (last 5 contractions) of each set. There were no differences in firing rates whether measured at the beginning, middle, or end of the set ($F(2,16)=3.13$, $p=0.07$). Therefore, only the MUFR measured in the first five contractions of each set were used for further analysis.

There were no changes in average MUFR over the fatigue protocol ($F(4,32)=1.66$, $p=0.18$). However there was a significant relationship between the magnitude of change in firing rates from set one to set five with the change (Δ) in maximal voluntary force, twitch peak force, and twitch peak rate of rise (Δ MUFR vs Δ MVC: $R= -0.75$, $p=0.02$; vs Δ peak twitch force: $R= -0.77$, $p=0.02$; vs Δ df/dt: $R= -0.70$, $p=0.03$). The relationship indicates that larger declines in maximal voluntary strength, twitch peak force, and twitch peak rate of rise are associated with increases in MUFR (Figure 7). There was a similar relationship between MUFR and the twitch peak rate of relaxation, however it did not reach significance ($R= -0.65$, $p=0.06$) (Figure 7). These results indicate that although the change in MUFR were not detectable at the group level over time, there is still a relationship between the magnitude of change in the firing rates and the change in ability to produce force and in contractile function, such that failure to produce force and contractile function are associated with higher MUFR.

Motor neuron pool excitability and ΔF estimates of PIC

The fatigue protocol had no effect on spinal motor neuron pool excitability ($F(4,14)=0.41$, $p=0.67$). Similarly, the fatigue protocol had no effect on the ΔF estimates of PIC ($F(6,60)=0.49$, $p=0.81$) (Figure 8). Furthermore, on the fatigue day, there were no relationships between the magnitude of change in PIC ($\Delta\Delta F$) for each individual and the magnitude of change in their maximal voluntary force, level of voluntary activation, twitch peak force, twitch peak rate of rise and relaxation, and level of muscle activation during submaximal contractions (Table 3).

The level of tibialis anterior muscle activity was measured to determine whether fatigue-induced changes in antagonist muscle activation were associated with changes in PIC. However, there was no significant effect of fatigue on the level of antagonist muscle activation ($F(6,54)=1.80$, $p=0.12$) (Table 1). Additionally, the individual magnitude of change in antagonist muscle activation had no relationship with the magnitude of change in estimates of PIC ($R=0.01$, $p=0.97$).

Sex differences

Given the variability in the average PIC measures, and given that there were equal numbers and males and females and it is known that men and women fatigue differently (REF), an additional analysis to assess for an interaction between day time and sex for F values was performed. Interestingly, there was a significant effect of sex on estimates of PIC during fatigue, where females had a continual increase in estimated of PIC throughout the protocol, but there was no significant change in delta F values for males (females n=5, males n=6, $F(6,54)=2.71$, $p=0.02$, observed power=0.83) (Figure 9). It should be noted that missing data was filled using linear trend and that females had a greater number of missing data points in the last set and during recovery. Accordingly, we conducted a correlational analysis between our primary measure of fatigue (the decline in maximal force producing capacity) and fatigue-associated change in PIC. This analysis

also revealed a sex-dependent relationship between change in estimates of PIC and change in maximal force from initial values to set 5, such that larger declines in maximal voluntary force were associated with larger increases in estimates of PIC for females only (n=6 females, R=-0.88, p=0.02 and or n=6 males, R=0.02, p=0.97) (Figure 10).

There was no effect of sex on measures of maximal voluntary force, voluntary activation, twitch peak force, twitch peak rate of rise and relaxation, agonist and antagonist muscle activity did not have a significant day x time x sex interaction, indicating that the sex-difference in estimates of PIC over fatigue is not driven by males and females experiencing different amounts of fatigue (Table 4).

Although there was a sex-difference in how estimates of PIC responded to fatigue, there were no sex-differences in H-reflex estimates of motor neuron pool excitability following fatigue (n=3 males, n=5 females, $F(2,12)=3.25$, $p=0.07$) or motor neuron firing rates during fatiguing contractions (n=5 males, n=4 females $F(4, 28)=1.15$, $p=0.35$) (Table 4). However, the analysis for a sex-difference in H-reflex was underpowered so this absence of a significant finding is not evidence for males and females to have the same response in spinal motor neuron pool excitability during fatigue.

Effect of physically active on initial estimates of PIC

Level of physical activity was measured to assess its effect on the initial estimates of PIC. Firstly, there were no correlations between the total amount of physical activity estimated over a week when measured in either metabolic equivalents (METs) or minutes and initial estimates of PIC (METs/week R=0.05, p=0.88, mins/week R=0.05, p=0.89). When time spent doing physical activity each week was further broken down into different intensities – walking, moderate

intensity, and vigorous intensity – there were still no correlations between levels of physical activity and estimates of PIC (time spent in vigorous intensity activity R= -0.56, p=0.09, moderate intensity activity R=0.24, p=0.50 and time spent walking R=0.11, p=0.76) . Overall this indicates there is no relationship between level of physical activity and estimates of PIC.

The levels of physical activity were not significantly different between males and females ($F(6,3)=5.83$, $p=0.09$). Furthermore, there were no statistically significant relationships between levels of physical activity and initial ΔF, however, there was a sex-difference between the relationship between amount of moderate intensity physical activity and ΔF, such that there was as strong positive relationship between the two for males, but there was no relationship for females (males: R=0.82, p=0.09, females: R=-0.07, p=0.91). This indicates that moderate physical activity could elicit different neuromuscular adaptations in men and women.

DISCUSSION

The purpose of this study was to investigate how spinal motor neurons respond to muscular fatigue by examining motor unit firing rates, estimates of PIC amplitude, and spinal motor neuron pool excitability in human soleus motor neurons. Furthermore, this study assessed antagonist muscle activation as an acute variable that may affect PIC amplitude during fatiguing muscle activity, and level of physical activity as a lifestyle variable that may have a long-term affect on baseline PIC amplitude. Fatigue develops when muscular contractions are repeated or sustained (Allen et al., 2008). Fatigue is frequently defined as the inability to produce force or power, however, fatigue can manifest before muscular force or power is reduced (Taylor et al., 2016). The loss of force is typically preceded with subjective sensations of fatigue, such as pain or an increased sense of effort, and compensation within the neuromuscular system at this stage may prevent a

loss of force (Taylor et al., 2016). Therefore, assessing fatigue requires a multifaceted method of measurement. In this study, we assessed central and peripheral fatigue by measuring voluntary activation, submaximal muscle EMG, maximal voluntary force, muscle twitch peak force, twitch force peak rate of rise, and twitch force rate of relaxation. The fatigue protocol we used did not elicit a significant drop in maximal voluntary force, voluntary activation, or twitch force, rate of rise, and rate of relaxation. However, there was an increase in the muscle EMG throughout the fatigue protocol. Despite the lack of measurable changes in ability to produce maximum force or in contractile function, the increase in muscle EMG during submaximal contractions indicates that additional motor units were recruited to compensate for fatiguing muscle fibers (Edwards & Lippold, 1956). Furthermore, fatigue can be muscle specific, with some muscle groups being more resistant to fatigue than others. The soleus muscle is fatigue resistant due to its frequent activitation and muscle fiber type composition (Burke, Levine, Zajac, Tsairis, & Engel, 1971; Johnson, Polgar, Weightman, & Appleton, 1973; Joseph & Nightingale, 1952; Kugelberg, 1973; C. S. Sherrington, 1915). Despite the lack of measurable changes in voluntary force and contractile function during the fatigue protocol, there were changes in the soleus EMG.

During fatigue, an increase in muscle EMG can be caused by either increasing the rate of motor unit firing or increasing the number of motor units firing (Taylor & Gandevia, 2008). However, an increase in muscle EMG occurs during fatigue, regardless of whether the motor unit firing rates increase, decrease, or remain the same, therefore the increase in muscle EMG is driven by increased motor unit recruitment (Adam & De Luca, 2005; Carpentier, Duchateau, & Hainaut, 2001; Contessa, De Luca, & Kline, 2016; de Ruiter et al., 2005). Similarly, in this study, there was an increase in muscle EMG while there was no change in the motor unit firing rates within each set and across the fatigue protocol. Previous fatigue studies indicate that motor unit firing slows in

correlation with the slowing of muscle fiber twitch, in accordance to the Muscle Wisdom Hypothesis (Garland & Gossen, 2002). The simultaneous slowing of muscle twitch and motor unit firing that occurs during fatigue, does not occur when muscle twitch is slowed through other manipulations (Bigland-Ritchie, Furbush, Gandevia, & Thomas, 1992). Furthermore, Bigland-Ritchie and colleagues (1986) show that the decline in motor unit firing rates is due to a peripheral reflex and not central fatigue. As such, one possible mechanism is the activation of group III/IV afferents, causing inhibition at the motor neuron (Bigland-Ritchie, Dawson, et al., 1986). Group III/IV afferents are activated during fatigue because of the build up of metabolites within the muscle fibers occurs at a faster (Garland, 1991; Rotto & Kaufman, 1988). During high-intensity or sustained contractions there is reduced blood flow due to vascular compression which causes a greater build-up of metabolites (Barcroft & Millen, 1939). It is possible that since we used an intermittent fatigue protocol, which allows for increased blood flow, there wasn't significant build up of metabolites and therefore no slowing of the muscle twitch or the motor unit firing rates.

Although motor unit firing rates typically decrease during fatigue, this is dependent on the muscle studied and the fatigue protocol used (Garland & Gossen, 2002). Fatigue-resistant postural muscles are resistant to declines in motor unit firing rates and twitch contractile speed despite significant declines in ability to produce force (Kuchinad et al., 2004; Macefield, Fuglevand, Howell, & Bigland-Ritchie, 2000). Additionally, soleus muscle motor units respond differentially to fatigue depending on the fatigue protocol. Kuchinad et al., (2004) showed that maintaining a constant force near 50% MVC caused a significant decline in twitch contractile speed and motor unit firing rate; however, three 5-minute sustained contractions at 25% MVC resulted in no change in twitch contractile speed and significant increases in motor unit firing rates. Both protocols elicited significant declines in maximal voluntary force (Kuchinad et al., 2004). Therefore, the

lack of change in motor unit firing rates in this study could be because the soleus muscle is resistant to fatigue and because the fatigue protocol was intermittent contractions rather than sustained.

Despite the lack of motor unit firing rate adaptation during the fatigue protocol, the relationships between the change in firing rates and change in maximal voluntary force, twitch peak force, twitch peak rate of rise, and twitch peak rate of relaxation were examined in order to better understand the relationship between motor unit firing rates and fatigue. Although it was expected that individuals with the greatest declines in maximal voluntary force, twitch peak force, twitch rate of rise and relaxation would have the greatest decline in motor unit firing rates, in line with the Muscle Wisdom Hypothesis (Garland & Gossen, 2002), instead, the opposite occurred. Greater fatigue-induced deficits in maximal voluntary force, twitch force, twitch rate of rise and rate of relaxation were associated with higher motor unit firing rates. There have been instances in previous studies where there are increases in motor unit firing rates during fatigue, such as in the soleus motor units in response to low-intensity fatiguing contractions (Kuchinad et al., 2004), and in the vastus lateralis motor units in response to 50s-intervals at 20% of maximum force (Adam & De Luca, 2005; Contessa, De Luca, & Kline, 2016). Based on these previous studies, it appears that the type and intensity of the contraction have an effect on the motor firing rates. Fatigue protocols with quick-cycling intermittent contractions, like the one we used in the present study, have not been used to assess motor unit firing rates over fatiguing. The soleus muscle is chronically active during posture and gait, therefore investigating how the motor neuron adapts firing rates during fatigue caused by repetitive intermittent fatiguing contractions or low-level long duration contractions may lead to more relevant depictions of motor neuron behaviours during fatigue.

Motor unit firing rates are a useful measure of the drive to muscle during motor output, since they represent the sum of all neural components influencing motor neuron output, including

descending and sensory afferents. In contrast, to assess the responsiveness of the motor neuron pool to synaptic input, the H-reflex response is a practical tool, when external variables are controlled (McNeil, Butler, Taylor, & Gandevia, 2013). We found that there was no change in the gain of the H reflex following the fatigue protocol, indicating that there was no net change to the excitability of the spinal motor neuron pool.

We measured spinal motor neuron pool excitability with H_{slp}/M_{slp} in this study because this method is more reliable (Christie, Lester, LaPierre, & Gabriel, 2004), less susceptible to changes over time due to changes in posture or skin resistance (Walton et al., 2003), and antidromic volley has less influence on H-reflex gain than it has on maximum H-reflex (Funase et al., 1994). However, in the current study there was still large inter-participant variability which may have been due to the small sample size and combination of males and females in the study. While there were no significant effects of fatigue on the H-reflex gain when male and female data were assessed separately or combined, there was a trend for males to have an increase in the H-reflex gain following fatigue, which fell back near baseline following recovery. During fatigue there is usually either no change or there is a decline in the maximum H-reflex amplitude (Avela, Kyröläinen, Komi, & Rama, 1999; Duchateau, Balestra, Carpentier, & Hainaut, 2002; Duchateau & Hainaut, 1993; Garland & Mccomas, 1990; Kuchinad, Ivanova, & Garland, 2004; Walton, Kuchinad, Ivanova, & Garland, 2002), however as the motor system transitions from a resting state to an active state, there is an increase in the excitability of the motor neuron pool (Folland et al., 2008; Hess et al., 1987; Jacobs et al., 2002; Zijdewind et al., 2000). Therefore, prior to the fatigue-induced decline in H-reflex, there is an increase in the H-reflex due to activation potentiation, which could explain the trend for males to have an increase in H-reflex response following the fatigue protocol. Trimble and Harp (1998) showed that post-activation potentiation of the H-reflex

can be highly variable, with only five out of ten participants having appreciable potentiation. Five men and five women were included in this analysis, but the authors did not report the ratio of men and women in the sub groups of those that had potentiation and those that did not. Therefore, it is uncertain in the current study whether the trend for males to have an increase in H-reflex following the fatigue protocol could be due to the natural variability in the post-activation potentiation or a difference between males and females.

To continue to understand motor neuron behaviour during fatigue, we examined PIC amplitude. As mentioned previously, PIC is a property of the motor neuron that increases the responsiveness to excitatory synaptic input and allows for continued firing of the motor neuron during reduced excitatory synaptic input (Heckman et al., 2003, 2009). It was hypothesized that there would be an increase in PIC amplitude during fatigue to compensate for contractile failure or reduction in central drive. However, there was no change in the PIC amplitude over the fatiguing protocol. To continue to test the hypothesis, the change in PIC amplitude was compared to the change in maximal voluntary force, level of voluntary activation, peak twitch force, peak twitch rate of rise and relaxation, and muscle EMG. Again, there were no significant findings, but there was a trend for a greater increase in PIC amplitude when there was a larger decline in twitch peak force. Therefore, as hypothesized, those who were experiencing greater contractile failure, indicated by a decline in twitch force, also tended to have the greatest increase in PIC amplitude. This is consistent with the idea that PIC increases to compensate for the contractile failure.

Although PIC may increase to compensate for fatigue-induced contractile failure or reduced descending drive, it is also sensitive to inhibitory synaptic input such as reciprocal inhibition (Hyngstrom et al., 2007; Kuo, 2003). Therefore, the level of antagonist muscle activation was assessed to determine whether it had a deleterious effect on the compensatory

increase in PIC estimates during fatigue. We hypothesized that greater antagonist activation would lead to lower estimates of PIC, due to the fact that increasing reciprocal inhibition, via changes in joint angle causes a deactivation of PIC (Hyngstrom et al., 2007). During fatigue there is an increase in the level of coactivation of the agonist and antagonist muscles, therefore it was expected that participants with the greatest increase in antagonist muscle activation would have the smallest compensatory increase in PIC. However, there was no change in antagonist muscle activation over the fatigue protocol. It is possible that the level of fatigue was not sufficient to elicit increases in antagonist muscle activation (Levenez, Garland, Klass, & Duchateau, 2008; Psek & Cafarelli, 1993). Alternatively, because the level of antagonist muscle activation was assessed during the low-intensity triangular ramp contractions used to estimate PIC, any fatigue-related increase in its activation during the fatiguing contractions may not have remained during these easier contractions.

It is possible that habitual physical activity may elicit changes in baseline PIC amplitude or possibly alter the magnitude of fatigue-induced changes in PIC. This is consistent with the fact that physical training can cause a persistent increase in the excitability of the motor neuron and the motor neuron pool (Adam et al., 1998; Krutki et al., 2017; MacDonell, Button, Beaumont, Cormery, & Gardiner, 2012b; Pérot et al., 1991; Vera-Ibañez et al., 2017; Vila-Cha et al., 2012). However, using the International Physical Activity Questionnaire Short Form (based on a typical week), we found that there were no associations between baseline PIC amplitude and any measure of physical activity; total time per week, total METs per week, and then subdivided into total time spent walking each week, total time spend in moderate physical activity per week, and total time spent in vigorous physical activity per week. There was a weak trend indicating that more vigorous physical activity was associated with lower baseline PIC amplitude. This is opposite to what we

expected, since physical training has been associated with an increase in spinal excitability (Adam et al., 1998; Krutki et al., 2017; MacDonell et al., 2012), in a rat model. Type of training has an affect on spinal motor neuron pool excitability, where there is an increase in excitability during weight lifting training but a decrease during endurance training (Casabona et al., 1990; Kyröläinen & Komi, 1994; Maffiuletti et al., 2001; Nielsen et al., 1993; Rochcongar et al., 1979). Therefore, it is possible that the type of training may also have an affect on intrinsic motor neuron excitability, however we were unable to further examine that possibility since the questionnaire we used for this study did not differentiate between different types of activity, only intensity.

While our initial analysis would lead us to reject our *a priori* hypothesis that PIC increases during fatigue, and it was clear that the lack of change in PIC was not due to deactivation of PIC caused by increasing reciprocal inhibition, significant variability in the PIC data led us to conduct further analyses. Although we did not set out to investigate sex as a covariate, our sample included similar numbers of males and females. Accordingly, the PIC data was reanalyzed to determine whether the effect of fatigue on PIC was sex-dependent. This secondary analysis indicated that there was a sex difference in PIC amplitude during fatigue, such that PIC amplitude increased during fatigue for females only. There were no other sex differences over the fatigue protocol in maximal voluntary force, voluntary activation, twitch peak force, twitch peak rate of rise and relaxation, or muscle EMG, indicating that the difference in PIC was a unique sex difference, and not the outcome of greater or lesser fatigue experienced by women.

It is well documented that there are sex differences in muscle fatigue, endurance, and voluntary activation (Avin et al., 2010; Clark, Collier, Manini, & Ploutz-Snyder, 2005; Clark, Manini, Thé, Doldo, & Ploutz-Snyder, 2003; Fulco et al., 1999; Hunter, Critchlow, Shin, & Enoka, 2004; Hunter & Enoka, 2001; Hunter et al., 2006; Hunter, Griffith, Schlachter, & Kufahl, 2009;

Hunter, Critchlow, & Enoka, 2004; Kent-Braun, Ng, Doyle, & Towse, 2002; Martin & Rattey, 2007; Maughan, Harmon, Leiper, Sale, & Delman, 1986; West, Hicks, Clements, & Dowling, 1995). The mechanisms that allow females to perform better on endurance tasks are not fully understood, therefore, it is possible that a difference in PIC is one mechanism allowing for these sex differences. Alternatively, the sex-difference in estimates of PIC could be due to greater inhibition of the spinal motor neuron through group III/IV afferents in males because of greater metabolite accumulation (Amann, 2012; Kent-Braun et al., 2002; Martin & Rattey, 2007; Rotto & Kaufman, 1988; Russ & Kent-Braun, 2003; Russ et al., 2005). Males have greater muscle mass and therefore greater compression of the blood vessels during contractions, which causes greater ischemia and therefore more rapid fatigue (Hicks et al., 2001). When there is excessive metabolite accumulation, group III/IV muscle afferents are activated, and they have a strong inhibitory effect on the spinal motor neuron (Garland, 1991; Rotto & Kaufman, 1988). This is consistent with findings that males have a lower voluntary activation following fatigue, this could be a result of PIC deactivation (Martin & Rattey, 2007). However, vascular compression occurs when the muscle is contracted, therefore ischemia is more likely to cause these effects when the muscle sustains a contraction, since during periods of relaxation blood flow is restored. However, females are still more fatigue-resistant during intermittent and dynamic contractions than males (Hunter et al., 2009; Maughan et al., 1986; Miller et al., 1993), therefore there must be an alternative explanation for how females have greater fatigue resistant than males. Based on the results of the current study, that mechanism could be that the motor neurons of females have a greater ability to increase excitability through an increase in PIC amplitude.

While this finding is consistent with enhanced fatigue resistance in women seen in other studies, an alternative explanation could be that the increase in PIC in women was due to a greater

number of missing data points that were filled using linear trend. Therefore, it is possible that the increase in PIC observed in women may have been inflated due to greater amount of missing data, especially occurring later in the fatigue protocol. However, there was a negative correlation between the changes in PIC over fatigue and the change in maximal voluntary force over fatigue that was observed in women and not in men. The bias caused by the data substitution has a lesser affect on this analysis, and these findings continue to support the hypothesis that females have a greater PIC compensation during fatigue than males.

Conclusion

The novel and unanticipated finding of this study was the sex difference in PIC amplitude during the fatigue protocol. Although it is well documented that women have greater fatigue resistance than males, the mechanisms are not fully understood and so this finding could guide future research towards a better understanding of the sex differences during fatigue. Additionally, this indicates that when studying motor neuron behaviour, specifically during fatigue, sex differences should be taken into consideration. Additionally, the results of this study indicate that during fatigue the soleus motor units do no act according to the Muscle Wisdom Hypothesis, since there was a negative correlation between motor unit firing rates and twitch peak rate of rise.

CHAPTER 3: FUTURE DIRECTIONS

This study assessed soleus motor neuron behaviour during fatiguing isometric intermittent contractions, however, the level of fatigue achieved by this protocol was minimal. Further research should investigate how soleus motor neurons respond to a more intense intermittent contraction fatiguing protocol. It is possible that the lack of change in motor neuron firing rates, PIC, and spinal motor neuron pool excitability is due to a lack of fatigue disrupting the motor system. Additionally, longer or more rigorous fatigue protocols could be used to test to what extent PIC can compensate for fatigue. However, given the limitations associated with maintaining a consistent intramuscular recording during intense exercise protocols, PIC could be studied during fatigue in a less fatigue resistant muscle, such as the tibialis anterior.

Motor unit firing rates were only measured in a single motor unit for each participant. This lead to a low sample size and diminished statistical power. By requiring the motor unit to be active through the entire fatigue protocol, units that rotate during fatigue were not included. More recently motor unit firing rates have been assessed using decomposition of high density EMG (Contessa, De Luca, et al., 2016; Contessa, Letizi, De Luca, & Kline, 2018; Kelly et al., 2013). This allows investigation of a larger pool of motor units and distinction between recruitment threshold and change in motor unit firing rates, since researchers have shown previously that early recruited motor units tend to decrease the firing rate, whereas later recruited units increase the firing rate (Carpentier et al., 2001). The recruitment threshold was not accounted for in the current study, which could account for some of the variability of the results.

When males and females were grouped together, there was no change in PIC during fatigue. However, we found that PIC of males and females responded differently to fatigue, although there were no differences in any other measure of fatigue. As discussed earlier, there were more data points filled using the linear trend in the female sample, therefore additional

investigations for a sex-difference in PIC during fatigue are necessary. Further investigation in this sex-difference should also include more rigorous fatigue protocols to explore if and when PIC changes in men, as well as whether a sex-difference in PIC is related to a sex-difference in fatiguability.

Although it was hypothesized that antagonist activation may inhibit PIC in the agonist muscle, there was no increase in antagonist activation throughout the fatigue protocol in the current study. Antagonist activation was measured during the EMG recordings made to estimate PIC. These recordings were during the low intensity triangular ramp contractions. It is possible that any fatigue related increase in antagonist activation that may have occurred during the fatiguing contractions does not occur during the lower intensity triangular ramps, therefore it does not affect PIC during estimates of PIC. It is also possible that protocol did not elicit an increase in antagonist activation. Future studies should continue to investigate whether reciprocal inhibition during isometric contractions is strong enough to inhibit PIC, and if so whether is reduces PIC ability to compensate for fatigue.

We hypothesized that inhibition would arise from increasing activation of the antagonist muscle group, but we did not assess the potential inhibitory affects of gastrocnemius activation. Ia afferent activation of the gastrocnemius can cause inhibition on the soleus motor neurons (Gritti & Schieppati, 1989). During fatigue there can be an increase in synergist muscle activation (Stutzig & Siebert, 2016), therefore it may be beneficial for future investigators to monitor gastrocnemius muscle activation. Additionally, previous researchers have found that during isometric plantarflexion, isometric knee extension can increase soleus and decrease gastrocnemius activity. Therefore, the activation of knee extensors is facilitating the soleus motor neurons, which could

affect our estimates of PIC (Suzuki, Chino, & Fukashiro, 2014). Therefore future research should also monitor activation of knee extensor muscle when assessing PIC in the soleus.

Previous literature would suggest that PIC should be altered according to level of physical activity, however the results of this study fail to indicate that level of physical activity has any affect of PIC. It is possible that the sample assessed lack variability in levels of physical activity, therefore unable to strongly correlate PIC with physical activity. It is also possible that the measure of physical activity did not measure necessary information concerning the plasticity of PIC and physical activity. It is possible that the type, and not just the intensity, or physical activity would have an affect on PIC. Future studies should include a measure of the type of physical activity, and a population with greater variability in levels of physical activity. PIC could also be assessed before and after a training intervention to better understand of physical training affects this property.

This study only attempted to investigate the affect of physical activity on baseline PIC amplitude, however habitual physical activity may alter how PIC compensates for fatigue, without changing baseline PIC. Further study should be done to investigate whether level of physical activity has an affect on the change in PIC during fatigue by sampling from groups of different levels of physical activity.

REFERENCES

Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *Journal of Applied Physiology*, *92*(6), 2309–2318. https://doi.org/10.1152/japplphysiol.01185.2001

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S. P., & Kjær, M. (2010). Role of the nervous system in sarcopenia and muscle atrophy with aging: Strength training as a countermeasure. *Scandinavian Journal of Medicine and Science in Sports*, *20*(1), 49–64. https://doi.org/10.1111/j.1600-0838.2009.01084.x

Adam, A., & De Luca, C. J. (2005). Firing rates of motor units in human vastus lateralis muscle during fatiguing isometric contractions. *Journal of Applied Physiology*, *99*(1), 268–280. https://doi.org/10.1152/japplphysiol.01344.2004

Adam, A., Luca, C. J. De, Erim, Z., Dideriksen, J. L., Negro, F., Enoka, R. M., … Luca, C. J. D. E. (1998). Hand Dominance and Motor Unit Firing Behavior. *Journal of Neurophysiology*, *80*, 1373–1382.

Ainsworth, B. E., Bassett, D. R., Strath, S. J., Swartz, a M., O'Brien, W. L., Thompson, R. W., … Kimsey, C. D. (2000). Comparison of three methods for measuring the time spent in physical activity. *Medicine and Science in Sports and Exercise*, *32*(9 Suppl), S457–S464. https://doi.org/10.1097/00005768-200009001-00004

Allen, D. G., Lamb, G. D., & Westerblad, H. (2008). Skeletal muscle fatigue: cellular mechanisms. *Physiological Reviews*, *88*(1), 287–332. https://doi.org/10.1152/physrev.00015.2007.

Amann, M. (2012). Significance of Group III and IV muscle afferents for the endurance exercising human. *Clinical and Experimental Pharmacology and Physiology*, *39*(9), 831–835. https://doi.org/10.1111/j.1440-1681.2012.05681.x

Avela, J., Kyröläinen, H., Komi, P. V, & Rama, D. (1999). Reduced reflex sensitivity persists several days after long-lasting stretch-shortening cycle exercise. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *86*(4), 1292–1300.

Avin, K. G., Naughton, M. R., Ford, B. W., Moore, H. E., Monitto-Webber, M. N., Stark, A. M., … Law, L. A. F. (2010). Sex differences in fatigue resistance are muscle group dependent. *Medicine and Science in Sports and Exercise*, *42*(10), 1943–1950. https://doi.org/10.1249/MSS.0b013e3181d8f8fa

Baker, A. J., Kostov, K. G., Miller, R. G., & Weiner, M. W. (1993). Slow force recovery after long-duration exercise: metabolic and activation factors in muscle fatigue. *Journal of Applied Physiology*, *74*(5), 2294–300. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8335559

Barcroft, H., & Millen, J. L. E. (1939). The blood flow through muscle during sustained contraction. *Journal of Physiology*, *97*, 17–31.

Baudry, S., Collignon, S., & Duchateau, J. (2015). Influence of age and posture on spinal and corticospinal excitability. *Experimental Gerontology*, *69*, 62–69. https://doi.org/10.1016/j.exger.2015.06.006

Bawa, P., & Murnaghan, C. (2009). Motor Unit Rotation in a Variety of Human Muscles. *Journal of Neurophysiology2*, *102*, 2265–2272. https://doi.org/10.1152/jn.00278.2009.

Beaumont, E., & Gardiner, P. (2002). Effects of daily spontaneous running on the electro-physiological properties of hindlimb motoneurones in rats. *Journal of Physiology*, *540*(1), 129–138. https://doi.org/10.1113/jphysiol.2001.013084

Beaumont, E., & Gardiner, P. F. (2003). Endurance training alters the biophysical properties of hindlimb motorneurons in rats. *Muscle and Nerve*, *27*(February), 228–236.

Behm, D. G., & Perez, D. (1996). Muscle inactivation : assessment of interpolated twitch technique. *Journal of Applied Physiology*, *81*(5), 2267–2273.

Behrens, M., Weippert, M., Wassermann, F., Bader, R., Bruhn, S., & Mau-Moeller, A. (2015). Neuromuscular function and fatigue resistance of the plantar flexors following short-term cycling endurance training. *Frontiers in Physiology*, *6*(MAY), 1–10. https://doi.org/10.3389/fphys.2015.00145

Bigland-Ritchie, B., Dawson, N. J., Johansson, R. S., & Lippold, O. C. J. (1986). Reflex origin for the slowing of motorneurone firing rates in fatigue of human voluntary contractions. *Journal of Physiology*, *379*, 451–459.

Bigland-Ritchie, B., Furbush, F., & Woods, J. J. (1986). Fatigue of intermittent submaximal voluntary contractions: central and peripheral factors. *Journal of Applied Physiology*, *61*(2), 421–429. https://doi.org/10.1152/jappl.1986.61.2.421

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. *Journal of Neurophysiology*, *50*(1), 313–24. https://doi.org/10.1152/jn.1983.50.1.313

Bigland-Ritchie, B., Jones, D. A., & Woods, J. J. (1979). Excitation-frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions. *Experimental Neurology*, *64*, 414–427. https://doi.org/10.1016/0014-4886(79)90280-2

Bigland-Ritchie, B. R., Furbush, F. H., Gandevia, S. C., & Thomas, C. K. (1992). Voluntary

discharge frequencies of human motoneurons at different muscle lengths. *Muscle & Nerve*, *15*(2), 130–137. https://doi.org/10.1002/mus.880150203

Blazev, R., & Lamb, G. D. (1999). Low [ATP] and elevated [Mg2+] reduce depolarization-induced Ca2+ release in rat skinned skeletal muscle fibres. *Journal of Physiology*, *520*(1), 203–215. https://doi.org/10.1111/j.1469-7793.1999.00203.x

Bongiovanni, L. G., & Hagbarth, K. E. (1990). Tonic vibration reflexes elicited during fatigue from maximal voluntary contractions in man. *The Journal of Physiology*, *423*(1), 1–14. https://doi.org/10.1113/jphysiol.1990.sp018007

Bruton, J. D., Wretman, C., Katz, A., & Westerblad, H. (1997). Increased tetanic force and reduced myoplasmic [P(i)] following a brief series of tetani in mouse soleus muscle. *The American Journal of Physiology*, *272*(3 Pt 1), C870-4. https://doi.org/10.1152/ajpcell.1997.272.3.C870

Burke, R. E., Levine, D. N., Zajac, F. E., Tsairis, P., & Engel, W. K. (1971). Mammalian motor units: Physiological-histochemical correlation in three types in cat gastrocnemius. *Science*, *174*, 709–712.

Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2003). Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(32), 10224–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14614080

Button, D. C., Kalmar, J. M., Gardiner, K., Cahill, F., & Gardiner, P. F. (2007). Spike frequency adaptation of rat hindlimb motoneurons. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *102*(3), 1041–1050. https://doi.org/10.1152/japplphysiol.01148.2006

Button, D. C., Kalmar, J. M., Gardiner, K., Marqueste, T., Zhong, H., Roy, R. R., … Gardiner, P. F. (2008). Does elimination of afferent input modify the changes in rat motoneurone properties that occur following chronic spinal cord transection? *The Journal of Physiology*, *586*(2), 529–44. https://doi.org/10.1113/jphysiol.2007.141499

Carpentier, A., Duchateau, J., & Hainaut, K. (2001). Motor unit behaviour and contractile changes during fatigue in the human first dorsal interosseus. *Journal of Physiology*, *534*(3), 903–912.

Carroll, T. J., Riek, S., & Carson, R. G. (2001). Reliability of the input-output properties of the cortico-spinal pathway obtained from transcranial magnetic and electrical stimulation. *Journal of Neuroscience Methods*, *112*(2), 193–202. https://doi.org/10.1016/S0165-0270(01)00468-X

Casabona, A., Polizzi, M. C., & Perciavalle, V. (1990). Differences in H-reflex between athletes trained for explosive contractions and non-trained subjects. *European Journal of Applied Physiology and Occupational Physiology*, *61*(1–2), 26–32. https://doi.org/10.1007/BF00236689

Chalmers, G. R., & Knutzen, K. M. (2000). H-Reflex Size in Young and Elderly Adults of Varying Physical Activity Levels. *Journal of Aging and Physical Activity*, *8*, 20–32.

Chin, E. R., & Allen, D. G. (1997). Effects of reduced muscle glycogen concentration on force,

Ca2+ release and contractile protein function in intact mouse skeletal muscle. *The Journal of Physiology*, *498*(1), 17–29. https://doi.org/10.1113/jphysiol.1997.sp021838

Christie, A., Lester, S., LaPierre, D., & Gabriel, D. A. (2004). Reliability of a new measure of H-reflex excitability. *Clinical Neurophysiology*, *115*(1), 116–123. https://doi.org/10.1016/S1388-2457(03)00306-7

Clark, B. C., Collier, S. R., Manini, T. M., & Ploutz-Snyder, L. L. (2005). Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. *European Journal of Applied Physiology*, *94*(1–2), 196–206. https://doi.org/10.1007/s00421-004-1293-0

Clark, B. C., Manini, T. M., Thé, D. J., Doldo, N. A., & Ploutz-Snyder, L. L. (2003). Gender differences in skeletal muscle fatigability are related to contraction type and EMG spectral compression. *Journal of Applied Physiology*, *94*(6), 2263–2272. https://doi.org/10.1152/japplphysiol.00926.2002

Contessa, P., De Luca, C. J., & Kline, J. C. (2016). The compensatory interaction between motor unit firing behavior and muscle force during fatigue. *Journal of Neurophysiology*, *116*(4), 1579–1585. https://doi.org/10.1152/jn.00347.2016

Contessa, P., Letizi, J., De Luca, G., & Kline, J. C. (2018). Contribution from motor unit firing adaptations and muscle coactivation during fatigue. *Journal of Neurophysiology*, *119*(6), 2186–2193. https://doi.org/10.1152/jn.00766.2017

Contessa, P., Puleo, A., & De Luca, C. J. (2016). Is the notion of central fatigue based on a solid foundation? *Journal of Neurophysiology*, *115*(2), 967–977. https://doi.org/10.1152/jn.00889.2015

Conway, B. Y. B. A., Hultborn, H., Kiehn, O. L. E., & Mintz, I. (1988). Plateau Potentials in Alpha-Motoneurones Induced by Intravenous Injection of L-Dopa and Clonidine in the Spinal Cat. *Journal of Physiology*, *405*, 369–384.

Cormery, B., Beaumont, E., Csukly, K., & Gardiner, P. (2005). Hindlimb unweighting for 2 weeks alters physiological properties of rat hindlimb motoneurones. *Journal of Physiology*, *568*(3), 841–850. https://doi.org/10.1113/jphysiol.2005.091835

Crone, C., Hultborn, H., Kiehn, O., Mazieres, L., & Wigstrom, H. (1988). Maintained Changes in Motoneuronal Excitability by Short-Lasting Synaptic Inputs in the Decerebrate Cat. *Journal of Physiology*, *405*, 321–343.

Dahlstedt, A. J., Katz, A., & Westerblad, H. (2001). Role of myoplasmic phosphate in contractile function of skeletal muscle: Studies on creatine kinase-deficient mice. *Journal of Physiology*, *533*(2), 379–388. https://doi.org/10.1111/j.1469-7793.2001.0379a.x

de Ruiter, C. J., Elzinga, M. J. H., Verdijk, P. W. L., van Mechelen, W., & de Haan, A. (2005). Changes in force, surface and motor unit EMG during post-exercise development of low frequency fatigue in vastus lateralis muscle. *European Journal of Applied Physiology*, *94*(5–6), 659–669. https://doi.org/10.1007/s00421-005-1356-x

Devanne, H., Lavoie, B. A., & Capaday, C. (1997). Input-output properties and gain changes in the human corticospinal pathway. *Experimental Brain Research*, *114*(2), 329–338.

https://doi.org/10.1007/PL00005641

Dimitrijevic, M. R., McKay, W. B., Sarjanovic, I., Sherwood, A. M., Svirtlit, L., & Vrbovà, G. (1992). Co-activation of ipsi- and contralateral muscle groups during contraction of ankle dorsiflexors. *Journal of the Neurological Sciences*, *109*(1), 49–55. https://doi.org/10.1016/0022-510X(92)90092-Y

Ditor, D. S., & Hicks, a L. (2000). The effect of age and gender on the relative fatigability of the human adductor pollicis muscle. *Canadian Journal of Physiology and Pharmacology*, *78*(10), 781–90. https://doi.org/10.1139/y00-061

Duchateau, J., Balestra, C., Carpentier, A., & Hainaut, K. (2002). Reflex regulation during sustained and intermittent submaximal contractions in humans. *The Journal of Physiology*, *541*(Pt 3), 959–967. https://doi.org/10.1113/jphysiol.2002.016790

Duchateau, J., & Hainaut, K. (1993). Behaviour of short and long latency reflexes in fatigued human muscles. *The Journal of Physiology*, *471*, 787–99. https://doi.org/10.1113/jphysiol.1993.sp019928

Dutka, T. L., & Lamb, G. D. (2004). Effect of low cytoplasmic [ATP] on excitation-contraction coupling in fast-twitch muscle fibres of the rat. *Journal of Physiology*, *560*(2), 451–468. https://doi.org/10.1113/jphysiol.2004.069112

Edwards, R. G., & Lippold, O. C. J. (1956). The Relation Between Force and Integrated Electrical Activity in Fatigued Muscle. *Journal of Physiology*, *132*, 677–681. Retrieved from http://www.saburchill.com/physics/chapters/0009b.html

Eken, T., & O., K. (1989). Bistable firing properties of soleus motor units in unrestrained rats. *Acta Physiologica Scandinavica*, *136*(3), 383–394. https://doi.org/10.1111/j.1748-1716.1989.tb08679.x

Enoka, R. M., Robinson, G. A., & Kossev, A. R. (1989). Task and fatigue effects on low-threshold motor units in human hand muscle. *Journal of Neurophysiology*, *62*(6), 1344–1359.

Erim, Z., De Luca, C. J., Mineo, K., & Aoki, T. (1996). Rank-ordered regulation of motor units. *Muscle and Nerve*, *19*(5), 563–573. https://doi.org/10.1002/(SICI)1097-4598(199605)19:5<563::AID-MUS3>3.0.CO;2-9

Farrag, A., & Elsayed, W. (2016). Habitual Use of High-Heeled Shoes Affects Isokinetic Soleus Strength More Than Gastrocnemius in Healthy Young Females. https://doi.org/10.1177/1071100716649172

Folland, J. P., Wakamatsu, T., & Fimland, M. S. (2008). The influence of maximal isometric activity on twitch and H-reflex potentiation, and quadriceps femoris performance. *European Journal of Applied Physiology*, *104*(4), 739–748. https://doi.org/10.1007/s00421-008-0823-6

Freedson, P. S., & Miller, K. (2000). Objective monitoring of physical activity using motion sensors and heart rate. *Res Q Exerc Sport*, *71*(2), 21–29.

Fukushima, Y., Yamashita, N., & Shimada, Y. (1982). Facilitation of H-reflex by homonymous

Ia-afferent fibers in man. *Journal of Neurophysiology*, *48*(5), 1079–1088.

Fulco, C. S., Rock, P. B., Muza, S. R., Lammi, E., Cymerman, A., Butterfield, G., … Lewis, S. F. (1999). Slower fatigue and faster recovery of the adductor pollicis muscle in women matched for strength with men. *Acta Physiologica Scandinavica*, *167*(3), 233–239. https://doi.org/10.1046/j.1365-201X.1999.00613.x

Funase, K., Imanaka, K., & Nishihira, Y. (1994). Excitability of the soleus motoneuron pool revealed by the developmental slope of the H-reflex as reflex gain. *Electromyography and Clinical Neurophysiology*, *34*(8), 477–89. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7882891

Gandevia, S. C., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal ouput from motor cortex. *Journal of Physiology*, (490), 529–536.

Garland, S. J. (1991). Role of small diameter afferents in reflex inhibition during human muscle fatigue. *Journal of Physiology*, *435*, 547–558.

Garland, S. J., & Gossen, E. R. (2002). The muscular wisdom hypothesis in human muscle fatigue. *Exercise and Sport Sciences Reviews*, *30*(1), 45–49. https://doi.org/10.1097/00003677-200201000-00009

Garland, S. J., & Mccomas, A. J. (1990a). Reflex inhibition of human soleus muscle during fatigue. *Journal of Physiology*, *429*, 17–27.

Garland, S. J., & Mccomas, A. J. (1990b). Reflex Inhibition of Human Soleus Muscle During Fatigue. *Journal of Physiology*, *429*, 17–27.

Glace, B. W., Kremenic, I. J., & McHugh, M. P. (2013). Sex differences in central and peripheral mechanisms of fatigue in cyclists. *European Journal of Applied Physiology*, *113*(4), 1091–1098. https://doi.org/10.1007/s00421-012-2516-4

Gorassini, M. A., Bennett, D. J., & Yang, J. F. (1998). Self-sustained firing of human motor units. *Neuroscience Letters*, *247*(1), 13–16. https://doi.org/10.1016/S0304-3940(98)00277-8

Gorassini, M. A., Knash, M. E., Harvey, P. J., Bennett, D. J., Yang, J. F., & Gorassini, M. (2004). Role of motoneurons in the generation of muscle spasms after spinal cord injury. *Brain*, *127*, 2247–2258. https://doi.org/10.1093/brain/awh243

Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: Possible Contribution to Motor Unit Excitation. *Journal of Neurophysiology*, *87*(4), 1850–1858. https://doi.org/10.1152/jn.00025.2001

Gorassini, M., Yang, J. F., Siu, M., & Bennett, D. J. (2002). Intrinsic activation of human motoneurons: reduction of motor unit recruitment thresholds by repeated contractions. *Journal of Neurophysiology*, *87*(4), 1859–1866. https://doi.org/10.1152/jn.00025.2001

Gritti, B. Y. I., & Schieppati, M. (1989). Short-latency inhibition of soleus motoneurones by impluses in Ia afferents from the gastrocnemius muscle in humans. *Journal of Physiology*, *416*, 469–484.

Gruber, M., Taube, W., Gollhofer, A., Beck, S. C., Amtage, F., & Schubert, M. (2007). Training-specific adaptations of H-and stretch reflexes in human soleus muscle. *Journal of Motor Behavior*, *39*(1), 68–78. https://doi.org/10.3200/JMBR.39.1.68-78

Hartline, D. K., Russelut, D. F., Raper, J. A., & Graubard, K. (1988). Special Cellular and Synaptic Mechanisms in Motor Pattern Generation. *Russell The Journal Of The Bertrand Russell Archives*, *9*(1), 115–131.

Harvey, P. J., Li, X., Li, Y., & Bennett, D. J. (2006). 5-HT 2 Receptor Activation Facilitates a Persistent Sodium Current and Repetitive Firing in Spinal Motoneurons of Rats With and Without Chronic Spinal Cord Injury. *J Neurophysiol*, *96*, 1158–1170. https://doi.org/10.1152/jn.01088.2005.

Hayashi, R., Tako, K., Tokuda, T., & Yanagisawa, N. (1992). Comparison of amplitude of human soleus H-reflex during sitting and standing. *Neuroscience Research*, *13*(3), 227–233. https://doi.org/10.1016/0168-0102(92)90062-H

Heckman, C. J., Gorassini, M. A., & Bennett, D. J. (2005). Persistent inward currents in motoneuron dendrites: Implications for motor output. *Muscle and Nerve*, *31*(2), 135–156. https://doi.org/10.1002/mus.20261

Heckman, C. J., Johnson, M., Mottram, C., & Schuster, J. (2008). Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. *Neuroscientist*, *14*(3), 264–275. https://doi.org/Doi 10.1177/1073858408314986

Heckman, C. J., Lee, R. H., & Brownstone, R. M. (2003). Hyperexcitable dendrites in motoneurons and their neuromodulatory control during motor behavior. *Trends in Neurosciences*, *26*(12), 688–695. https://doi.org/10.1016/j.tins.2003.10.002

Heckman, C. J., Mottram, C., Quinlan, K., Theiss, R., & Schuster, J. (2009). Motoneuron excitability: The importance of neuromodulatory inputs. *Clinical Neurophysiology*, *120*(12), 2040–2054. https://doi.org/10.1016/j.clinph.2009.08.009

Hess, C. W., Mills, K. R., & Murray, N. M. (1987). Responses in small hand muscles from magnetic stimulation of the human brain. *The Journal of Physiology*, *388*(1), 397–419. https://doi.org/10.1113/jphysiol.1987.sp016621

Hicks, A. L., Kent-Braun, J., & Ditor, D. S. (2001). Sex differences in human skeletal muscle fatigue. *Exercise and Sport Sciences Reviews*, *29*(3), 109–112. https://doi.org/10.1097/00003677-200107000-00004

Hötting, K., & Röder, B. (2013). Beneficial effects of physical exercise on neuroplasticity and cognition. *Neuroscience and Biobehavioral Reviews*, *37*(9), 2243–2257. https://doi.org/10.1016/j.neubiorev.2013.04.005

Hounsgaard, J., & Kiehn, O. (1985). Ca ++ dependent bistability induced by serotonin in spinal motoneurons. *Exp Brain Res*, *57*, 422–425.

Hultborn, H., Denton, M. E., Wienecke, J., & Nielsen, J. B. (2003). Variable amplification of synaptic input to cat spinal motoneurones by dendritic persistent inward current. *The Journal of Physiology*, *552*(Pt 3), 945–952. https://doi.org/10.1113/jphysiol.2003.050971

Hunter, S. K. (2009). Sex Differences and Mechanisms of Task-Specific Muscle Fatigue. *Exercise and Sports Science Reviews*, *37*(3), 113 – 122. https://doi.org/10.1097/JES.0b013e3181aa63e2.Sex

Hunter, S. K. (2014). Sex differences in human fatigability: Mechanisms and insight to physiological responses. *Acta Physiologica*, *210*(4), 768–789. https://doi.org/10.1111/apha.12234

Hunter, S. K. (2016). The relevance of sex differences in performance fatigability. *Medicine and Science in Sports and Exercise*, *48*(11), 2247–2256. https://doi.org/10.1249/MSS.0000000000000928

Hunter, S. K., Butler, J. E., Todd, G., Gandevia, S. C., & Taylor, J. L. (2006). Supraspinal fatigue does not explain the sex difference in muscle fatigue of maximal contractions. *Journal of Applied Physiology*, *101*(4), 1036–1044. https://doi.org/10.1152/japplphysiol.00103.2006

Hunter, S. K., Critchlow, A., & Enoka, R. M. (2004). Influence of aging on sex differences in muscle fatigability. *Journal of Applied Biomechanics*, *53201*(97), 1723–1732. https://doi.org/10.1152/japplphysiol.00460.2004.

Hunter, S. K., Critchlow, A., Shin, I.-S., & Enoka, R. M. (2004). Fatigability of the elbow flexor muscles for a sustained submaximal contraction is similar in men and women matched for strength. *Journal of Applied Physiology*, *96*(1), 195–202. https://doi.org/10.1152/japplphysiol.00893.2003

Hunter, S. K., Critchlow, A., Shin, I.-S., & Enoka, R. M. (2004). Men are more fatigable than strength-matched women when performing intermittent submaximal contractions. *Journal of Applied Physiology*, *96*(6), 2125–2132. https://doi.org/10.1152/japplphysiol.01342.2003

Hunter, S. K., & Enoka, R. M. (2001). Sex differences in the fatigability of arm muscles depends on absolute force during isometric contractions. *Journal of Applied Physiology*, *91*(6), 2686–2694. https://doi.org/10.1152/jappl.2001.91.6.2686

Hunter, S. K., Griffith, E. E., Schlachter, K. M., & Kufahl, T. D. (2009). Sex differences in time to task failure and blood flow for an intermittent isometric fatiguing contraction. *Muscle and Nerve*, *39*(1), 42–53. https://doi.org/10.1002/mus.21203

Hunter, S. K., Schletty, J. M., Schlachter, K. M., Griffith, E. E., Polichnowski, A. J., & Ng, A. V. (2006). Active hyperemia and vascular conductance differ between men and women for an isometric fatiguing contraction. *Journal of Applied Physiology*, *101*(1), 140–150. https://doi.org/10.1152/japplphysiol.01567.2005

Hyngstrom, A. S., Johnson, M. D., Miller, J. F., & Heckman, C. J. (2007). Intrinsic electrical properties of spinal motoneurons vary with joint angle. *Nature Neuroscience*, *10*(3), 363–369. https://doi.org/10.1038/nn1852

Jacobs, B. L., Martín-Cora, F. J., & Fornal, C. A. (2002). Activity of medullary serotonergic neurons in freely moving animals. *Brain Research Reviews*, *40*(1–3), 45–52. https://doi.org/10.1016/S0165-0173(02)00187-X

Johnson, M. A., Polgar, J., Weightman, D., & Appleton, D. (1973). Data on the distribution of

fibre types in thirty-six Human Muscles: An autopsy study. *Journal of the Neurological Sciences*, *18*, 111–129.

Jones, D. A., Bigland-Ritchie, B., & Edwards, R. H. T. (1979). Excitation frequency and muscle fatigue: Mechanical responses during voluntary and stimulated contractions. *Experimental Neurology*, *64*(2), 401–413. https://doi.org/10.1016/0014-4886(79)90279-6

Joseph, J., & Nightingale, A. (1952). Electromyography of muscles of posture: Leg muscles in males. *Journal of Physiology1*, *117*, 484–491.

Kallenberg, L. A. C., & Hermens, H. J. (2008). Behaviour of a surface EMG based measure for motor control : Motor unit action potential rate in relation to force and muscle fatigue. *Journal of Electromyograpy and Kinesiology*, *18*, 780–788. https://doi.org/10.1016/j.jelekin.2007.02.011

Kalmar, J. M., Button, D. C., Gardiner, K., Cahill, F., Gardiner, P. F., Jm, K., … Gardiner, P. F. (2009). Caloric Restriction Does Not Offset Age-Associated Changes in the Biophysical Properties of Motoneurons. *Journal of Neurophysiology*, *101*, 548–557. https://doi.org/10.1152/jn.90617.2008.

Kamen, G., Sullivan, R., Rubinstein, S., & Christie, A. (2006). Evidence of self-sustained motoneuron firing in young and older adults. *Journal of Electromyography and Kinesiology*, *16*(1), 25–31. https://doi.org/10.1016/j.jelekin.2005.06.008

Kelly, L. A., Racinais, S., & Cresswell, A. G. (2013). Discharge properties of abductor hallucis before, during, and after an isometric fatigue task. *Journal of Neurophysiology*, *110*(4), 891–898. https://doi.org/10.1152/jn.00944.2012

Kent-Braun, J. A., Ng, A. V., Doyle, J. W., & Towse, T. F. (2002). Human skeletal muscle responses vary with age and gender during fatigue due to incremental isometric exercise. *Journal of Applied Physiology*, *93*(5), 1813–1823. https://doi.org/10.1152/japplphysiol.00091.2002

Kiehn, O., & Eken, T. (1997). Prolonged firing in motor units: evidence of plateau potentials in human motoneurons? *Journal of Neurophysiology*, *78*(6), 3061–3068. Retrieved from http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=9405525&retmode=ref&cmd=prlinks%5Cnpapers3://publication/uuid/7F9C9A73-0595-4577-B450-3BBE15CEE9F1

Kim, K. M., Hart, J. M., & Hertel, J. (2013). Influence of body position on fibularis longus and soleus Hoffmann reflexes. *Gait and Posture*, *37*(1), 138–140. https://doi.org/10.1016/j.gaitpost.2012.06.009

Klimstra, M., & Zehr, E. P. (2008). A sigmoid function is the best fit for the ascending limb of the Hoffmann reflex recruitment curve. *Experimental Brain Research*, *186*(1), 93–105. https://doi.org/10.1007/s00221-007-1207-6

Knaepen, K., Goekint, M., Heyman, E. M., & Meeusen, R. (2010). Neuroplasticity -- Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor: A Systematic Review of Experimental Studies in Human Subjects. *Sports Medicine*, *40*(9), 765–801. Retrieved from

http://search.ebscohost.com/login.aspx?direct=true&db=cin20&AN=2010862583&site=ehost-live

Krutki, P., Mrówczyński, W., Bączyk, M., Łochyński, D., & Celichowski, J. (2017). Adaptations of motoneuron properties after weight-lifting training in rats. *Journal of Applied Physiology*, jap.00121.2017. https://doi.org/10.1152/japplphysiol.00121.2017

Kuchinad, R. A., Ivanova, T. D., & Garland, S. J. (2004). Modulation of motor unit discharge rate and H-reflex amplitude during submaximal fatigue of the human soleus muscle. *Experimental Brain Research*, *158*(3), 345–355. https://doi.org/10.1007/s00221-004-1907-0

Kugelberg, E. (1973). Histochemical composition, contraction speed and fatiguability of rat soleus motor units. *Journal of the Neurological Sciences*, *20*(2), 177–198. https://doi.org/10.1016/0022-510X(73)90029-4

Kuo, J. J. (2003). Active Dendritic Integration of Inhibitory Synaptic Inputs In Vivo. *Journal of Neurophysiology*, *90*(6), 3617–3624. https://doi.org/10.1152/jn.00521.2003

Kurtze, N., Rangul, V., & Hustvedt, B.-E. (2008). Reliability and validity of the international physical activity questionnaire in the Nord-Trøndelag health study (HUNT) population of men. *BMC Medical Research Methodology*, *8*, 63. https://doi.org/10.1186/1471-2288-8-63

Kyröläinen, H., & Komi, P. V. (1994). Neuromuscular performance of lower limbs during voluntary and reflex activity in power- and endurance-trained athletes. *European Journal of Applied Physiology and Occupational Physiology*, *69*(3), 233–239. https://doi.org/10.1007/BF01094794

Lafleur, J., Zytnicki, D., Horcholle-Bossavit, G., & Jami, L. (1992). Depolarization of Ib afferent axons in the cat spinal cord during homonymous muscle contraction. *1Journal of Physiology*, *445*, 345–354.

Lee, P. H., Yu, Y. Y., Mcdowell, I., Leung, G. M., Lam, T. H., & Stewart, S. M. (2011). Performance of the international physical activity questionnaire (short form) in subgroups of the Hong Kong chinese population, *1998*, 1–10.

Lee, R. H., & Heckman, C. J. (2001). Essential role of a fast persistent inward current in action potential initiation and control of rhythmic firing. *Journal of Neurophysiology*, *85*(1), 472–475.

Levenez, M. (2005). Spinal reflexes and coactivation of ankle muscles during a submaximal fatiguing contraction. *Journal of Applied Physiology*, *99*(3), 1182–1188. https://doi.org/10.1152/japplphysiol.00284.2005

Levenez, M., Garland, S. J., Klass, M., & Duchateau, J. (2008). Cortical and Spinal Modulation of Antagonist Coactivation During a Submaximal Fatiguing Contraction in Humans. *Journal of Neurophysiology*, *99*(2), 554–563. https://doi.org/10.1152/jn.00963.2007

Li, Y., Gorassini, M. A., & Bennett, D. J. (2004). Role of Persistent Sodium and Calcium Currents in Motoneuron Firing and Spasticity in Chronic Spinal Rats. *Journal of Neurophysiology*, *91*(2), 767–783. https://doi.org/10.1152/jn.00788.2003

MacDonell, C. W., Button, D. C., Beaumont, E., Cormery, B., & Gardiner, P. F. (2012a).

Plasticity of rat motoneuron rhythmic firing properties with varying levels of afferent and descending inputs. *Journal of Neurophysiology*, *107*(1), 265–72. https://doi.org/10.1152/jn.00122.2011

MacDonell, C. W., Button, D. C., Beaumont, E., Cormery, B., & Gardiner, P. F. (2012b). Plasticity of rat motoneuron rhythmic firing properties with varying levels of afferent and descending inputs. *Journal of Neurophysiology*, *107*(1), 265–272. https://doi.org/10.1152/jn.00122.2011

Macefield, B. Y. G., Hagbartht, K., Gorman, R., Gandevia, S. C., & Burke, D. (1991). Decline in spindle support to alpha-motoneurons during sustained voluntary contractions. *Journal of Physiology*, *440*, 497–512.

Macefield, V. G., Fuglevand, A. J., Howell, J. N., & Bigland-Ritchie, B. (2000). Discharge behaviour of single motor units during maximal voluntary contractions of a human toe extensor. *The Journal of Physiology*, *528 Pt 1*, 227–34. https://doi.org/10.1111/j.1469-7793.2000.00227.x

Macfarlane, D. J., Lee, C. C. Y., Ho, E. Y. K., Chan, K. L., & Chan, D. T. S. (2007). Reliability and validity of the Chinese version of IPAQ (short, last 7 days). *Journal of Science and Medicine in Sport*, *10*(1), 45–51. https://doi.org/10.1016/j.jsams.2006.05.003

Maffiuletti, N. A., Martin, A., Babault, N., Pensini, M., Schieppati, M., Lucas, B., … Schiep-, M. (2001). Electrical and mechanical H max -to-M max ratio in power- and endurance-trained athletes Electrical and mechanical H max -to-M max ratio in power- and endurance-trained athletes. *J Appl Physiol*, 3–9.

Martin, P. G., & Rattey, J. (2007). Central fatigue explains sex differences in muscle fatigue and contralateral cross-over effects of maximal contractions. *Pflugers Archiv European Journal of Physiology*, *454*(6), 957–969. https://doi.org/10.1007/s00424-007-0243-1

Martin, P. G., Smith, J. L., Butler, J. E., Gandevia, S. C., & Taylor, J. L. (2006). Fatigue-Sensitive Afferents Inhibit Extensor but Not Flexor Motoneurons in Humans. *Journal of Neuroscience*, *26*(18), 4796–4802. https://doi.org/10.1523/JNEUROSCI.5487-05.2006

Maughan, R. J., Harmon, M., Leiper, J. B., Sale, D., & Delman, A. (1986). Endurance capacity of untrained males and females in isometric and dynamic muscular contractions. *European Journal of Applied Physiology and Occupational Physiology*, *55*(4), 395–400. https://doi.org/10.1007/BF00422739

McManus, L., Hu, X., Rymer, W. Z., Lowery, M. M., & Suresh, N. L. (2015). Changes in motor unit behavior following isometric fatigue of the first dorsal interosseous muscle. *Journal of Neurophysiology*, *113*(9), 3186–3196. https://doi.org/10.1152/jn.00146.2015

McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Frontiers in Human Neuroscience*, *7*(April), 1–9. https://doi.org/10.3389/fnhum.2013.00152

Merton, P. A. (1954). Voluntary Strength and Fatigue. *Journal of Physiology*, *123*, 553–564.

Mettler, J. A., & Griffin, L. (2016). Muscular endurance training and motor unit firing patterns during fatigue. *Experimental Brain Research*, *234*(1), 267–276.

https://doi.org/10.1007/s00221-015-4455-x

Miller, A. E. J., MacDougall, J. D., Tarnopolsky, M. A., & Sale, D. G. (1993). Gender differences in strength and muscle fiber characteristics. *European Journal of Applied Physiology and Occupational Physiology*, *66*, 254–262.

Morel, B., Clémençon, M., Rota, S., Millet, G. Y., Bishop, D. J., Brosseau, O., … Hautier, C. A. (2015). Contraction velocity influence the magnitude and etiology of neuromuscular fatigue during repeated maximal contractions. *Scandinavian Journal of Medicine and Science in Sports*, *25*(5), e432–e441. https://doi.org/10.1111/sms.12358

Morin, C., & Pierrot-Deseilligny, E. (1977). Role of Ia afferents in the soleus motoneurones inhibition during a tibialis anterior voluntary contraction in man. *Experimental Brain Research*, *27*(5), 509–522. https://doi.org/10.1007/BF00239040

Mottram, C. J., Jakobi, J. M., Semmler, J. G., & Enoka, R. M. (2005). Motor-Unit Activity Differs With Load Type During a Fatiguing Contraction. *Journal of Neurophysiology*, *93*(3), 1381–1392. https://doi.org/10.1152/jn.00837.2004

Mottram, C. J., Suresh, N. L., Heckman, C. J., Gorassini, M. A., & Rymer, W. Z. (2009). Origins of Abnormal Excitability in Biceps Brachii Motoneurons of Spastic-Paretic Stroke Survivors. *Journal of Neurophysiology*, *102*(4), 2026–2038. https://doi.org/10.1152/jn.00151.2009

Nielsen, J., Crone, C., & Hultborn, H. (1993). H-reflexes are smaller in dancers from The Royal Danish Ballet than in well-trained athletes. *European Journal of Applied Physiology and Occupational Physiology*, *66*(2), 116–121. https://doi.org/10.1007/BF01427051

Nordlund, M. M., Thorstensson, A., & Cresswell, A. G. (2004). Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *96*(September 2003), 218–225. https://doi.org/10.1152/japplphysiol.00650.2003

Owen, V. J., Lamb, G. D., & Stephenson, D. G. (1996). Effect of low [ATP] on depolarization-induced Ca 2+ release in skeletal muscle fibres of the toad. *Journal of Physiology*, *493*(2), 309–315.

Pearcey, G. E. P., Bradbury-Squires, D. J., Monks, M., Philpott, D., Power, K. E., & Button, D. C. (2016). Arm-cycling sprints induce neuromuscular fatigue of the elbow flexors and alter corticospinal excitability of the biceps brachii. *Applied Physiology, Nutrition, and Metabolism = Physiologie Appliquée, Nutrition et Métabolisme*, *41*(2), 199–209. https://doi.org/10.1139/apnm-2015-0438

Pérot, C., Goubel, F., & Mora, I. (1991). Quantification of T- and H-responses before and after a period of endurance training. *European Journal of Applied Physiology and Occupational Physiology*, *63*(5), 368–375. https://doi.org/10.1007/BF00364464

Phillips, S. K., Wiseman, R. W., Woledge, R. C., & Kushmerick, M. J. (1993). The effect of metabolic fuel on force production and resting inorganic phosphate levels in mouse skeletal muscle. *J Physiol*, *462*, 135–146. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8331580

Powers, R. K., Nardelli, P., & Cope, T. C. (2008). Estimation of the contribution of intrinsic currents to motoneuron firing based on paired motoneuron discharge records in the decerebrate cat. *Journal of Neurophysiology*, *100*(1), 292–303. https://doi.org/10.1152/jn.90296.2008

Psek, J. A., & Cafarelli, E. (1993a). Behavior of coactive muscles during fatigue. *Journal of Applied Physiology*, *74*(1), 170–175. https://doi.org/10.1152/jappl.1993.74.1.170

Psek, J. A., & Cafarelli, E. (1993b). Behavior of coactive muscles during fatigue. *Journal of Applied Physiology*, *74*(1), 170–175. https://doi.org/10.1152/jappl.1993.74.1.170

Rasmussen, K., Morilak, D. A., & Jacobs, B. L. (1986). Single unit activity of locus coeruleus neurons in the freely moving cat. I. During naturalistic behaviors and in response to simple and complex stimuli. *Brain Research*, *371*(2), 324–334. https://doi.org/10.1016/0006-8993(86)90370-7

Reid, M. B., Stokić, D. S., Koch, S. M., Khawli, F. A., & Leis, A. A. (1994). N-acetylcysteine inhibits muscle fatigue in humans. *Journal of Clinical Investigation*, *94*(6), 2468–2474. https://doi.org/10.1172/JCI117615

Revill, A. L., & Fuglevand, A. J. (2011). Effects of persistent inward currents, accommodation, and adaptation on motor unit behavior: a simulation study. *Journal of Neurophysiology*, *106*(June), 1467–1479. https://doi.org/10.1152/jn.00419.2011

Rochcongar, P., Dassonville, J., & Le Bars, R. (1979). Modification of the Hoffman Reflex in Function of Athletic Training. *European Journal of Applied Physiology and Occupational Physiology*, *40*(3), 165–170. https://doi.org/10.1007/BF00426939

Roepstorff, C., Thiele, M., Hillig, T., Pilegaard, H., Richter, E. A., Wojtaszewski, J. F. P., & Kiens, B. (2006). Higher skeletal muscle α2AMPK activation and lower energy charge and fat oxidation in men than in women during submaximal exercise. *Journal of Physiology*, *574*(1), 125–138. https://doi.org/10.1113/jphysiol.2006.108720

Rotto, D. M., & Kaufman, M. P. (1988). Effect of metabolic products of muscular contraction on discharge of group III and IV afferents. *Journal of Applied Physiology*, *64*, 2306–2313.

Russ, D. W., & Kent-Braun, J. A. (2003). Sex differences in human skeletal muscle fatigue are eliminated under ischemic conditions. *Journal of Applied Physiology*, *94*(6), 2414–2422. https://doi.org/10.1152/japplphysiol.01145.2002

Russ, D. W., Lanza, I. R., Rothman, D., & Kent-Braun, J. A. (2005). Sex differences in glycolysis during brief, intense isometric contractions. *Muscle and Nerve*, *32*(5), 647–655. https://doi.org/10.1002/mus.20396

Saglam, M., Arikan, H., Savci, S., Inal-Ince, D., Bosnak-Guclu, M., Karabulut, E., & Tokgozoglu, L. (2010). International Physical Activity Questionnaire: Reliability and Validity of the Turkish Version. *Perceptual and Motor Skills*, *111*(1), 278–284. https://doi.org/10.2466/06.08.PMS.111.4.278-284

Sahlin, K., & Ren, J. M. (1989). Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *Journal of Applied Physiology (Bethesda, Md. : 1985)*, *67*(2), 648–654. https://doi.org/10.1152/jappl.1989.67.2.648

Sherrington, C. S. (1911). *The integrative action of the nervous system.* New Haven: Yale University Press. https://doi.org/10.1037/13798-000

Sherrington, C. S. (1915). Postural Activity of Muscle and Nerve, *38*.

Shindoh, C., DiMarco, A., Thomas, A., Manubay, P., & Supinski, G. (1990). Effect of N-acetylcysteine on diaphragm fatigue. *Journal of Applied Physiology*, *68*(5), 2107–2113. https://doi.org/10.1152/jappl.1990.68.5.2107

Simoneau, J., & Bouchard, C. (1989). Human variation in skeletal muscle fiber-type proportion and enzyme activities. *American Journal of Physiology- …*, *257*, 567–572. https://doi.org/10.1152/AJPENDO.1989.257.4.E567

Smith, J. L., Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2007). Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *Journal of Applied Physiology*, *103*(2), 560–568. https://doi.org/10.1152/japplphysiol.00220.2007

Søgaard, K., Gandevia, S. C., Todd, G., Petersen, N. T., & Taylor, J. L. (2006). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *Journal of Physiology*, *573*(2), 511–523. https://doi.org/10.1113/jphysiol.2005.103598

Stephenson, J. L., & Maluf, K. S. (2011). Dependence of the paired motor unit analysis on motor unit discharge characteristics in the human tibialis anterior muscle. *Journal of Neuroscience Methods*, *198*(1), 84–92. https://doi.org/10.1016/j.jneumeth.2011.03.018

Stern, A., Kuenze, C., Herman, D., Sauer, L. D., & Hart, J. M. (2012). A Gender Comparison of Central and Peripheral Neuromuscular Function after Exercise. *Journal of Sport Rehabilitation*, *21*(3), 209–217. https://doi.org/10.1123/jsr.21.3.209

Stock, M. S., Beck, T. W., & Defreitas, J. M. (2012). Effects of fatigue on motor unit firing rate versus recruitment threshold relationships. *Muscle and Nerve*, *45*(1), 100–109. https://doi.org/10.1002/mus.22266

Stutzig, N., & Siebert, T. (2016). Assessment of the H-reflex at two contraction levels before and after fatigue. *Scandinavian Journal of Medicine and Science in Sports*, 1–9. https://doi.org/10.1111/sms.12663

Suzuki, T., Chino, K., & Fukashiro, S. (2014). Human Movement Science Gastrocnemius and soleus are selectively activated when adding knee extensor activity to plantar flexion. *Human Movement Science*, *36*, 35–45. https://doi.org/10.1016/j.humov.2014.04.009

Taube, W., Kullmann, N., Leukel, C., Kurz, O., Amtage, F., & Gollhofer, A. (2007). Differential reflex adaptations following sensorimotor and strength training in young elite athletes. *International Journal of Sports Medicine*, *28*(12), 999–1005. https://doi.org/10.1055/s-2007-964996

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: From the brain to the muscle and back again. *Medicine and Science in Sports and Exercise*, *48*(11), 2294–2306. https://doi.org/10.1249/MSS.0000000000000923

Taylor, J. L., Butler, J. E., & Gandevia, S. C. (2000). Changes in muscle afferents , motoneurons and motor drive during muscle fatigue. *European Journal of Applied Physiology*, *83*, 106–

115.

Taylor, J. L., & Gandevia, S. C. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. *Journal of Applied Physiology*, *104*, 542–550. https://doi.org/10.1152/japplphysiol.01053.2007.

Temesi, J., Arnal, P. J., Rupp, T., Feasson, L., Cartier, R., Gergel??, L., … Millet, G. Y. (2015). Are females more resistant to extreme neuromuscular fatigue? *Medicine and Science in Sports and Exercise*, *47*(7), 1372–1382. https://doi.org/10.1249/MSS.0000000000000540

Todd, G., Petersen, N. T., Taylor, J. L., & Gandevia, S. C. (2003). The effect of a contralateral contraction on maximal voluntary activation and central fatigue in elbow flexor muscles. *Experimental Brain Research*, *150*(3), 308–313. https://doi.org/10.1007/s00221-003-1379-7

Trimble, M. H., & Harp, S. S. (1998). Postexercise potentiation of the H-reflex in humans. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1097/00005768-199806000-00024

Troiano, R., Berrigan, D., Dodd, K., Mâsse, L., Tilert, T., & Mcdowell, M. (2008). Physical activity in the United States measured by accelerometer. *Med Sci Sport Exer*, *40*(1), 181–188. https://doi.org/10.1249/mss.0b013e31815a51b3

Vallbo, A. B. (1971). Muscle spindle response at the onset of isometric voluntary contractions in man. Time difference between fusimotor and skeletomotor effects. *Journal of Physiology*, *318*, 405–431.

Vallbo, A. B. (1974). Human Muscle Spindle Discharge during Isometric Voluntary Contractions. Amplitude Relations between Spindle Frequency and Torque. *Acta Physiologica Scandinavica*, *90*(2), 319–336. https://doi.org/10.1111/j.1748-1716.1974.tb05594.x

Vandenberk, M. S., & Kalmar, J. M. (2014). An evaluation of paired motor unit estimates of persistent inward current in human motoneurons. *Journal of Neurophysiology*, *111*(9), 1877–84. https://doi.org/10.1152/jn.00469.2013

Vera-Ibañez, A., Colomer-Poveda, D., Romero-Arenas, S., Viñuela-García, M., & Márquez, G. (2017). Neural adaptations after short-term wingate-based high-intensity interval training. *Journal of Musculoskeletal Neuronal Interactions*, *17*(4), 275–282.

Vila-Chã, C., Falla, D., Correia, M. V., & Farina, D. (2012). Adjustments in motor unit properties during fatiguing contractions after training. *Medicine and Science in Sports and Exercise*, *44*(4), 616–624. https://doi.org/10.1249/MSS.0b013e318235d81d

Vila-Cha, C., Falla, D., Correia, M. V., & Farina, D. (2012). Changes in H reflex and V wave following short-term endurance and strength training. *Journal of Applied Physiology*, *112*(1), 54–63. https://doi.org/10.1152/japplphysiol.00802.2011

Walton, C., Kalmar, J., & Cafarelli, E. (2003). Caffeine increases spinal excitability in humans. *Muscle and Nerve*, *28*(3), 359–364. https://doi.org/10.1002/mus.10457

Walton, D. M., Kuchinad, R. A., Ivanova, T. D., & Garland, S. J. (2002). Reflex inhibition during muscle fatigue in endurance-trained and sedentary individuals. *European Journal of*

Applied Physiology, *87*(4–5), 462–468. https://doi.org/10.1007/s00421-002-0670-9

West, W., Hicks, A., Clements, L., & Dowling, J. (1995). The relationship between voluntary electromyogram, endurance time and intensity of effort in isometric handgrip exercise. *European Journal of Applied Physiology and Occupational Physiology*, *71*(4), 301–305. https://doi.org/10.1007/BF00240408

West, W., Hicks, A., McKelvie, R., & O'Brien, J. (1996). The relationship between plasma potassium, muscle membrane excitability and force following quadriceps fatigue. *Pflugers Archiv European Journal of Physiology*, *432*(1), 43–49. https://doi.org/10.1007/s004240050103

Westerblad, H., & Lännergren, J. (1994). Changes of the force-velocity relation, isometric tension and relaxation rate during fatigue in intact, single fibres of Xenopus skeletal muscle. *Journal of Muscle Research and Cell Motility*, *15*(3), 287–298. https://doi.org/10.1007/BF00123481

Wüst, R. C. I., Morse, C. I., De Haan, A., Jones, D. A., & Degens, H. (2008). Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Experimental Physiology*, *93*(7), 843–850. https://doi.org/10.1113/expphysiol.2007.041764

Yoon, T., Delap, B. S., Griffith, E. E., & Hunter, S. K. (2007). Mechanisms of fatigue differ after low- and high-force fatiguing contractions in men and women. *Muscle and Nerve*, *36*(4), 515–524. https://doi.org/10.1002/mus.20844

Yoon, T., Doyel, R., Widule, C., & Hunter, S. K. (2015). Sex differences with aging in the fatigability of dynamic contractions. *Experimental Gerontology*, *70*, 1–10. https://doi.org/10.1016/j.exger.2015.07.001

Zehr, E. P. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *European Journal of Applied Physiology*, *86*(6), 455–468. https://doi.org/10.1007/s00421-002-0577-5

Zijdewind, I., Zwarts, M. J., & Kernell, D. (1998). Influence of a voluntary fatigue test on the contralateral homologous muscle in humans? *Neuroscience Letters*, *253*(1), 41–44. https://doi.org/10.1016/S0304-3940(98)00609-0

Zijdewind, I., Zwarts, M. J., & Kernell, D. (2000). Potentiating and fatiguing cortical reactions in a voluntary fatigue test of a human hand muscle. *Experimental Brain Research*, *130*(4), 529–532. https://doi.org/10.1007/s002210050055

FIGURES AND TABLES

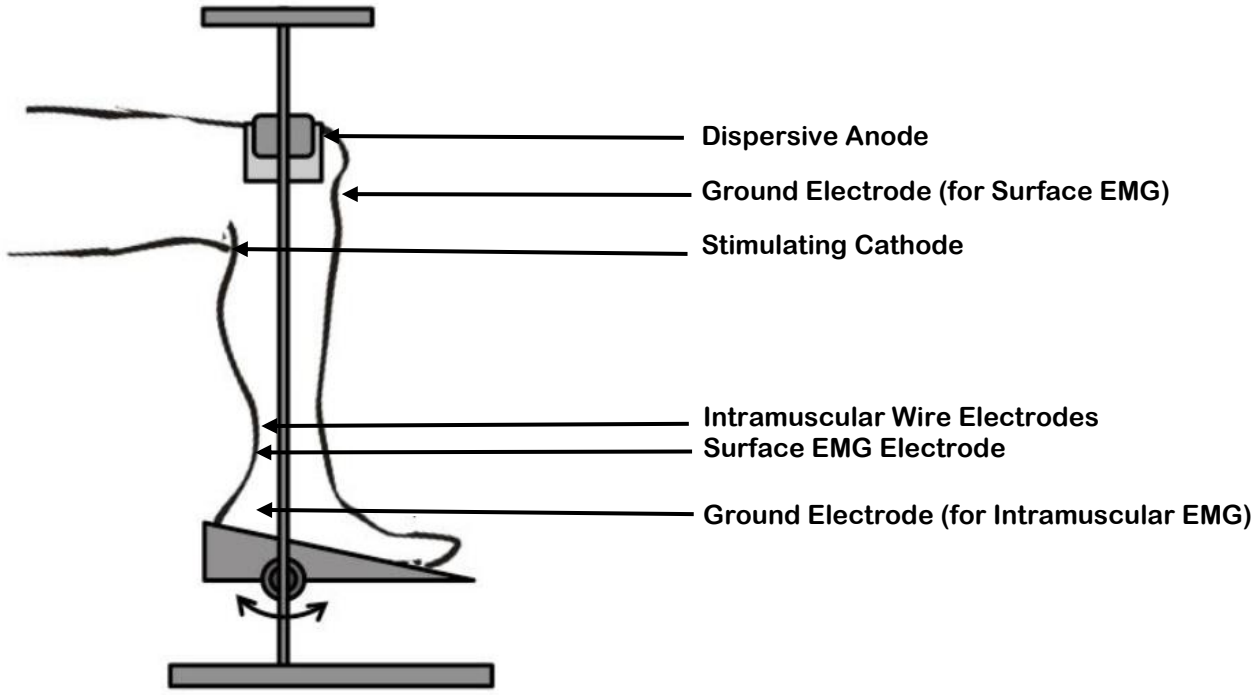

FIGURE 1: MCCOMA'S BOOT AND ELECTRODE PLACEMENT
McComa's boot has a force transducer under the foot plate to measure plantarflexion and dorsiflexion force. Knee is held down with an adjustable brace to prevent it from rising during plantarflexion. Ankle joint angle is adjustable, but will be secured at 90. Not shown in figure is a brace in front of the lower leg to prevent forward movement of the lower leg. Electrode placements depicted are the general locations of each electrode. Figure adapted from Ryan Foley "Estimates of persistent inward current in human motor neurons during postural sway" Master's Thesis, 2015.

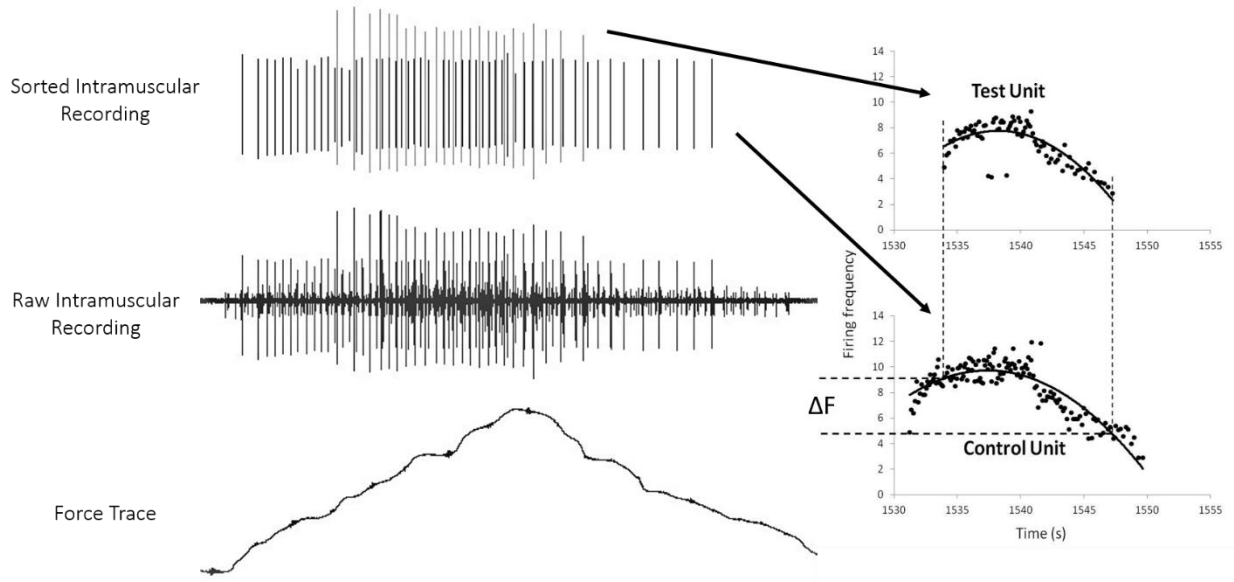

FIGURE 2: **PAIRED MOTOR UNIT TECHNIQUE**

Example of the test motor unit and control motor unit recordings used for the paired motor unit technique. Firing frequencies of each unit are plotted and fitted to a fourth-order polynomial. The difference in the firing frequency of the control unit at the onset and offset of the test unit is denoted by ΔF.

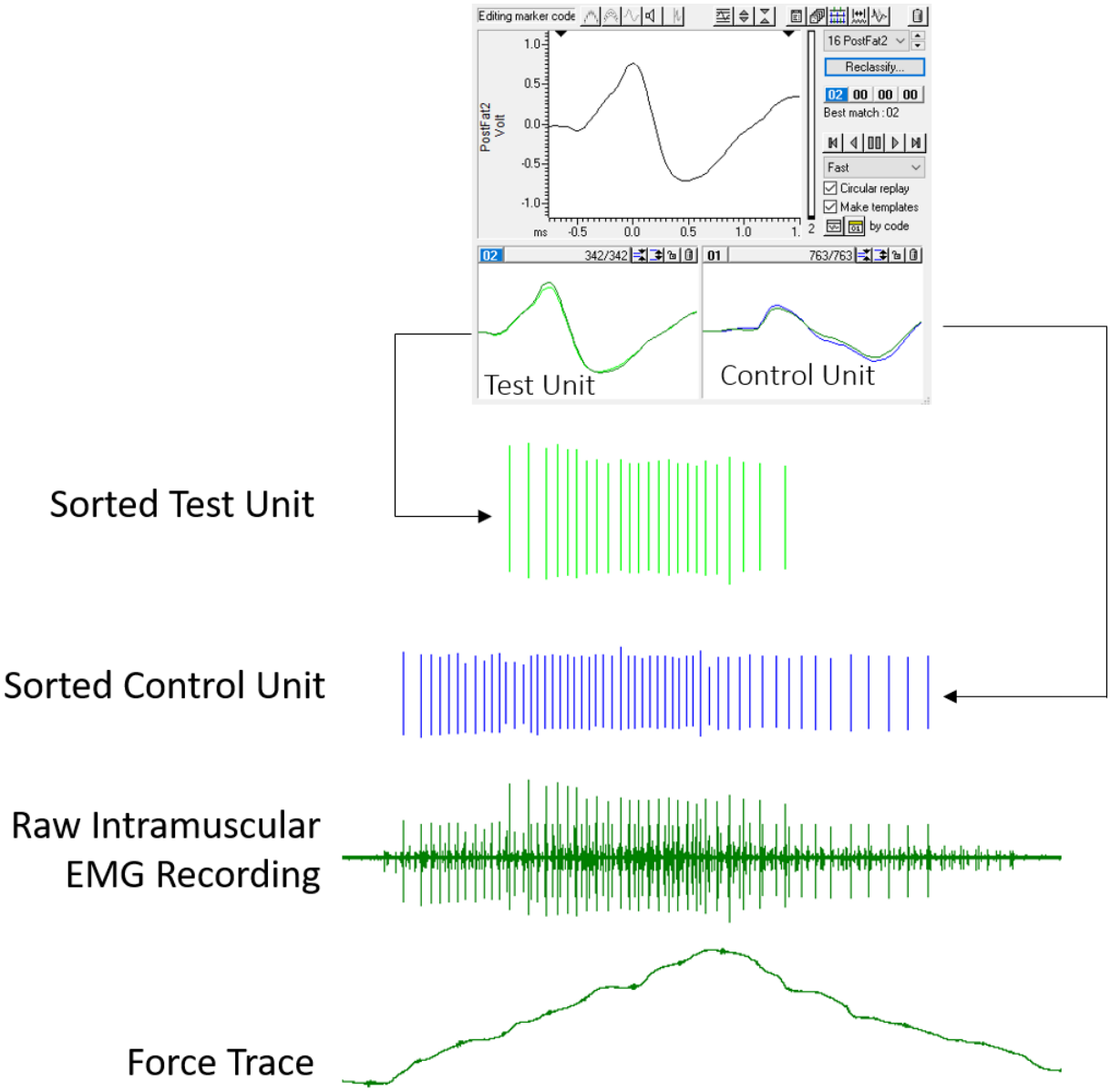

FIGURE 3: INTRAMUSCULAR RECORDING
Example of how the intramuscular EMG recordings are sorted into spike templates based on shape and amplitude in order to measure the

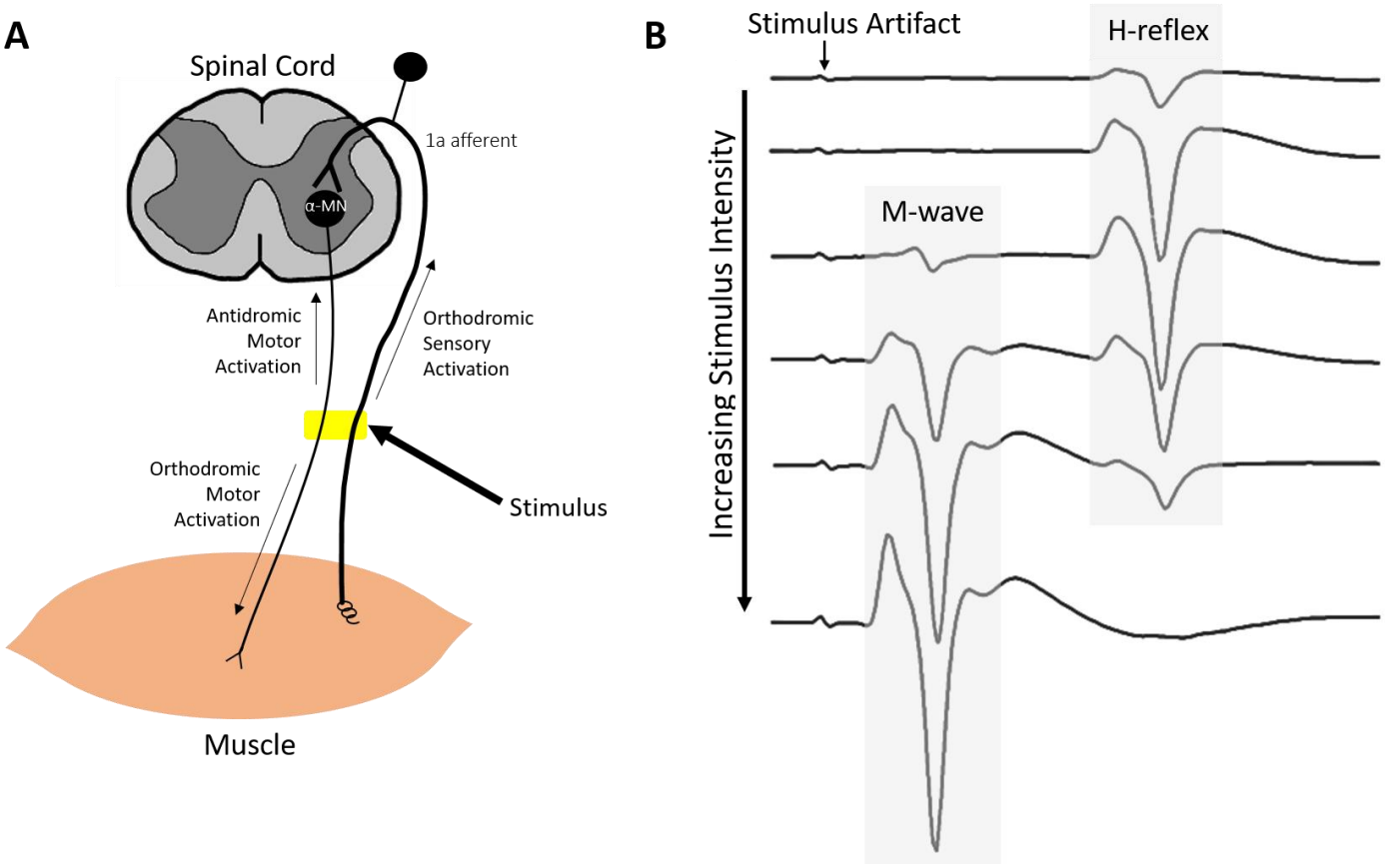

FIGURE 4: H-REFLEX AND M-WAVE RESPONSE PATHWAY AND TIMING
A. Diagram of response pathways to peripheral nerve stimulation. Stimulus causes a response in the afferent and efferent motor neurons. The afferent nerve is thicker and more easily excitable, therefore causing a response at a lower stimulus intensity. The orthodromic sensory activation excites the motor neuron pool which will cause a long-latency response, the H-reflex. The efferent fiber will become excited at a higher threshold, and will cause antidromic motor activation, which collides with the orthodromic sensory activation reducing the size of the H-reflex. The orthodromic motor activation leads to a short-latency response, the M-wave. B. H-reflex and M-wave timing. An example of H-reflex and M-wave responses with increasing stimulus intensity.

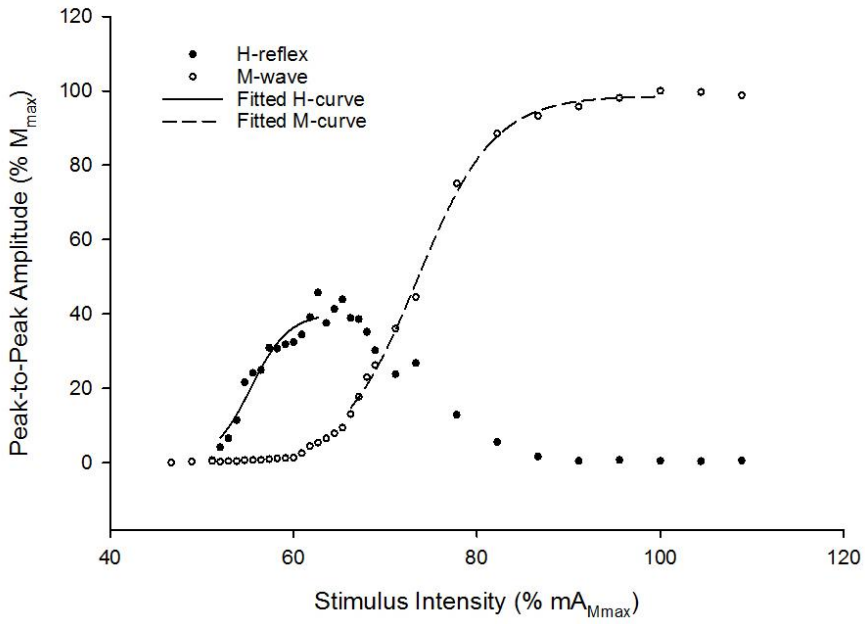

FIGURE 5: EXAMPLE OF AN H-REFLEX AND M-WAVE RECRUITMENT CURVE
This is an example of how the peak-to-peak amplitude of the H-reflex and the M-wave responses are plotted to create recruitment curves. The H-reflex and M-wave are first normalized to the maximum M-wave peak-to-peak amplitude and the stimulus intensity is normalized to the intensity that elicits M_{max}. The normalized H-reflex and M-wave peak-to-peak amplitudes (y-axis) are then plotted against the normalized stimulus intensity (x-axis). The curves are then fitted to a three-parameter sigmoidal curve and the slope at the midpoint of the H-curve is normalized to the slope at the midpoint of the M-curve.

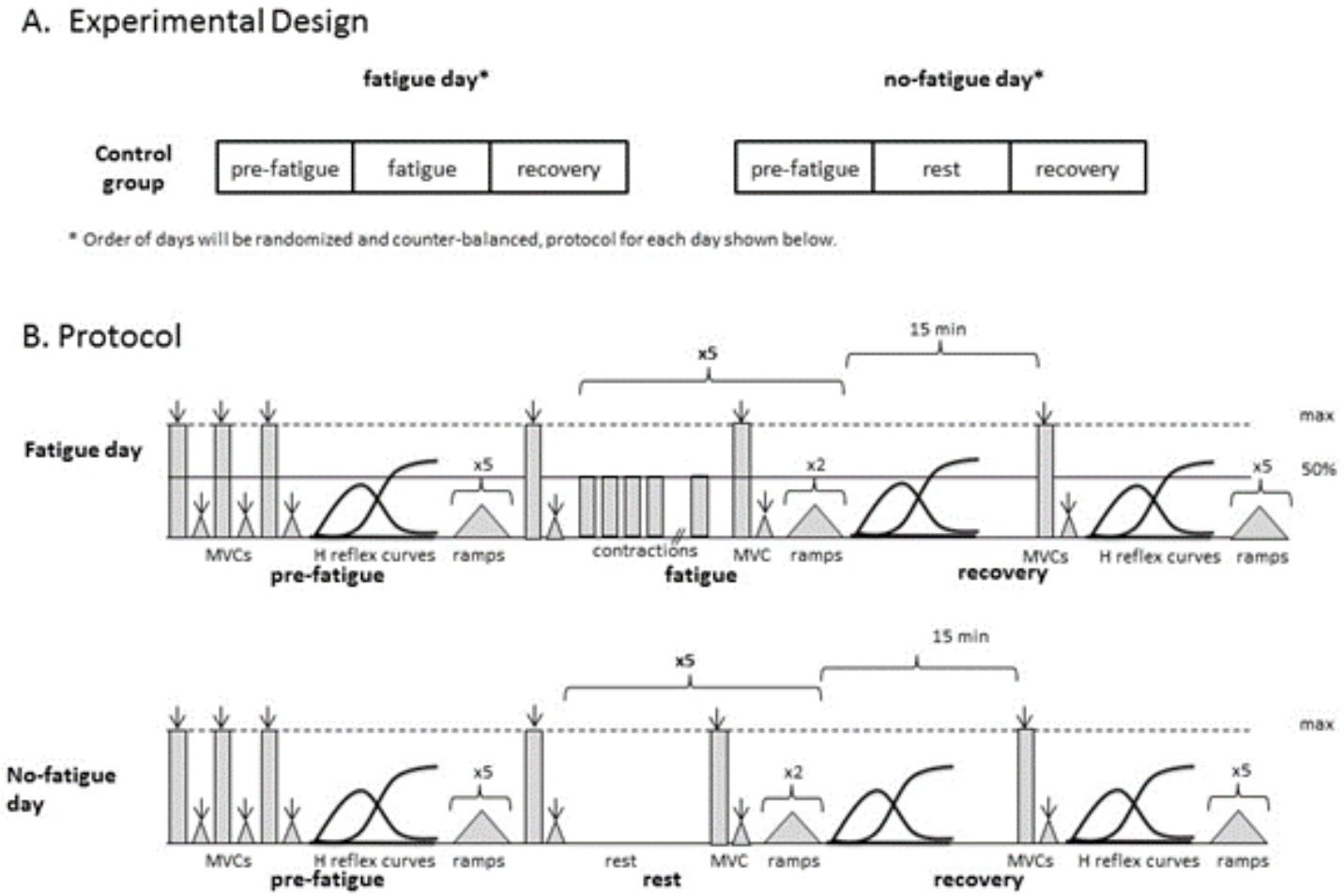

FIGURE 6: EXPERIMENTAL PROTOCOL

A. Experimental Design: an overview of the experimental sessions. Each group will attend two experimental sessions in a randomized and counter-balanced order. B. Protocol: An in-depth view of the procedures within each testing section. Downward arrows indicate supramaximal stimulations.

TABLE 1

Averages for maximum voluntary force, maximum muscle EMG, maximum voluntary activation, twitch peak force, twitch peak rate of rise, peak twitch rate of relaxation, M-wave size, and level of antagonist muscle activation during the fatigue day (bold font) and rest day. Results for day x time repeated measures ANOVA for each measure provided, * indicates where Fisher's LSD post-hoc analysis results in a significant difference between fatigue day and rest day.

								Day x time Interaction			
	Initial	Set 1	Set 2	Set 3	Set 4	Set 5	Recovery	n	df	F	p
Maximal Contractions:											
Max Force (N)	**530.6±151.9**	**511.7±146.0**	**508.0±134.7**	**498.6±133.6**	**497.5±143.7**	**488.0±143.4**	**503.1±150.9**	12	6,66	1.15	0.34
	548.0±153.0	534.6±143.3	532.2±134.0	542.8±134.2	541.5±149.1	537.0±164.2	529.2±142.9				
Max EMG RMS (%M-wave)	**3.9±3.6**	**3.7±2.7**	**3.8±3.1**	**3.7±2.9**	**3.8±2.8**	**3.6±2.7**	**3.7±3.2**	12	6,66	0.72	0.64
	4.0±2.0	3.4±1.4	3.5±1.6	3.6±1.5	3.6±1.5	3.5±1.6	3.5±1.2				
Max Voluntary Activation (%)	**97.1±4.8**	**97.9±3.2**	**97.8±4.5**	**98.2±3.3**	**98.4±3.1**	**96.7±8.1**	**96.9±3.9**	12	6,66	0.56	0.76
	98.2±3.3	97.8±5.5	98.1±4.5	98.5±2.8	98.3±3.5	98.1±3.0	98.7±1.3				
Contractile Properties:											
	*	*	*	*		*					
Twitch peak Force (N)	**108.4±50.7**	**114.4±57.9**	**113.7±53.1**	**111.9±53.0**	**108.6±57.3**	**109.6±55.0**	**90.2±46.5**	12	6,66	2.78	0.02
	102.3±49.6	103.8±51.3	103.0±49.7	102.3±46.5	104.0±48.3	100.7±46.9	92.9±40.4				
			*	*	*	*		12	6,66	3.17	0.01
Twitch peak rate of rise (N/s)	**1280±689**	**1373±814**	**1405±738**	**1362±719**	**1359±787**	**1375±767**	**1104±621**				
	1394±770	1249±690	1263±691	1178±670	1195±653	1156±653	1121±563				
	*						*				
Twitch peak rate of relaxation (N/s)	**-1888±1227**	**-1959±1382**	**-2010±1345**	**-2011±1337**	**-1974±1387**	**-1996±1367**	**-1590±1019**	12	6,66	2.35	0.04
	-2171±1365	-2028±1331	-2039±1283	-1982±1302	-1973±1283	-1937±1271	-1753±1027				
Peripheral Transmission:											
	*			*	*	*	*				
M-wave (mV) *	**3.0±1.2**	**2.9±1.3**	**2.9±1.3**	**2.8±1.3**	**2.7±1.3**	**2.7±1.3**	**2.8±1.1**	12	6,66	7.30	<0.001
	2.8±1.0	2.9±1.0	2.9±1.0	3.0±1.1	2.9±1.0	3.0±1.0	3.0±1.1				
Antagonist Activation											
TA EMG (%MVC EMG)	**4.74±3.94**	**4.78±3.94**	**6.02±6.57**	**5.77±6.08**	**5.69±5.50**	**5.39±5.26**	**6.01±5.22**	11	6,54	1.80	0.12
	9.89±11.71	10.11±13.16	6.63±11.12	9.20±11.27	9.31±11.90	9.57±11.76	8.90±10.93				

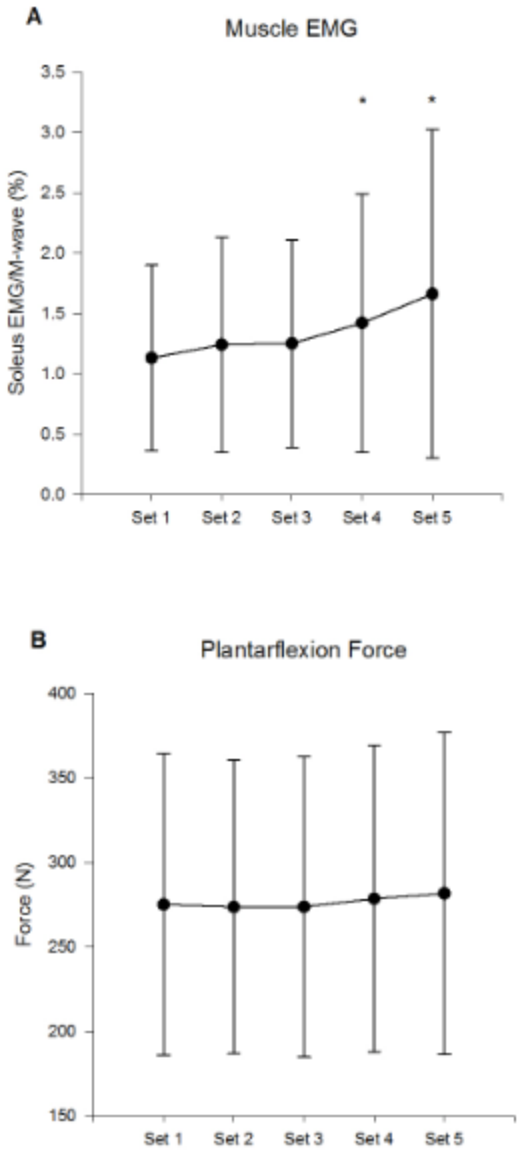

FIGURE 7: MUSCLE EMG AND FORCE DURING SUBMAXIMAL CONTRACTIONS
(A) Muscle EMG during submaximal contractions, normalized to the M-wave peak-to-peak amplitude. (B) Plantarflexion force during the measures voluntary muscle activation. Significantly greater muscle activation occurred during the fourth and fifth set ($p=0.002$) without any change in the force produced ($p=0.28$). Measures where made during the one of the initial five contractions of each set. * indicates sets with significantly greater muscle EMG than set 1.

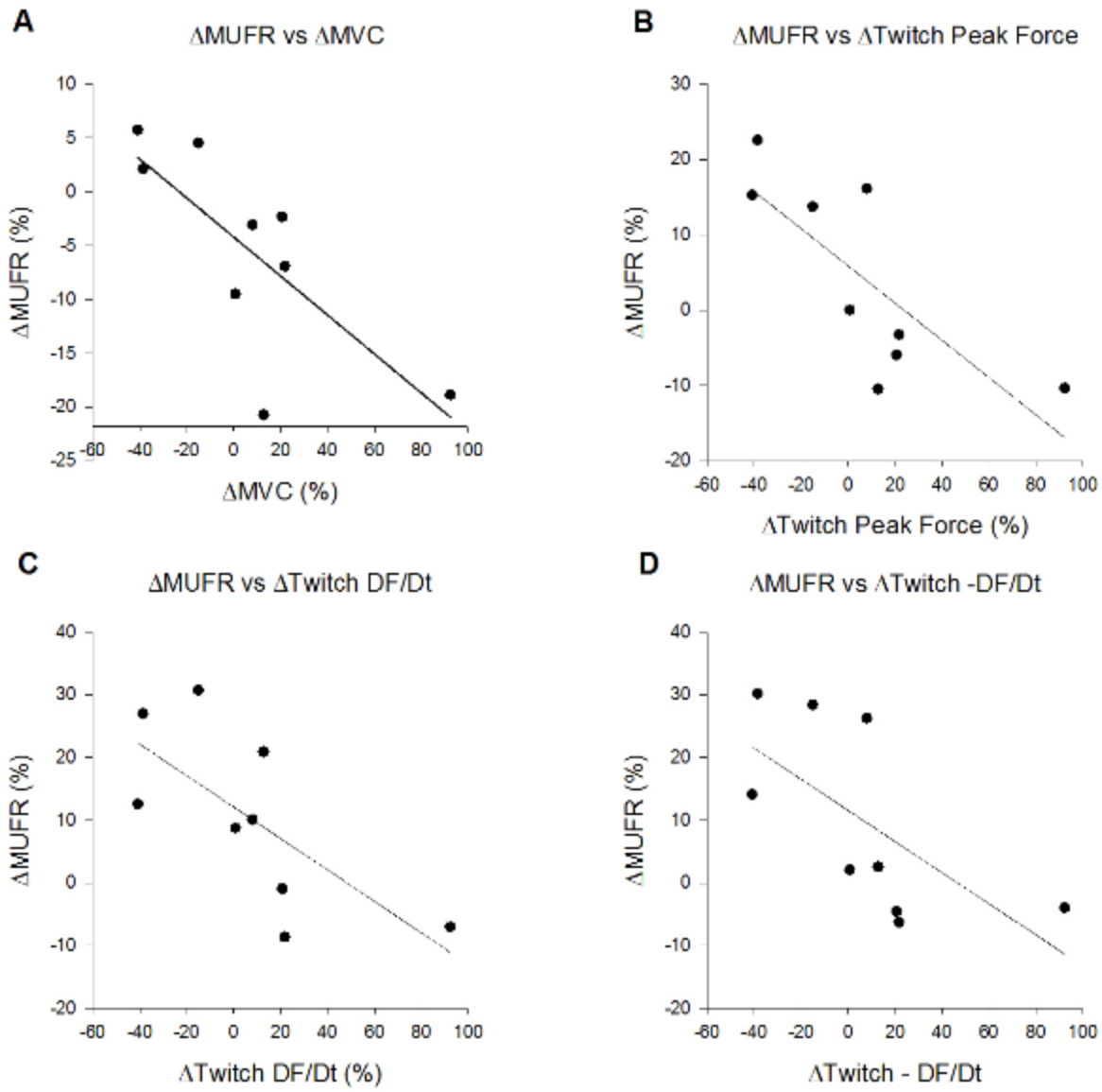

FIGURE 8: ΔMUFR VS ΔMUSCLE CONTRACTILE PROPERTIES

Pearson's correlations between the percentage change in motor unit firing rates from the initial set to the fifth set compared to the percentage change in (A) maximal voluntary force (R=-0.75, p=0.02), (B) twitch peak force (R=-0.77, p=0.02), (C) twitch force peak rate of rise (R=-0.70, p=0.03), and (D) twitch force peak rate of relaxation (R=-0.65, p=0.06) from the initial measure to after set 5.

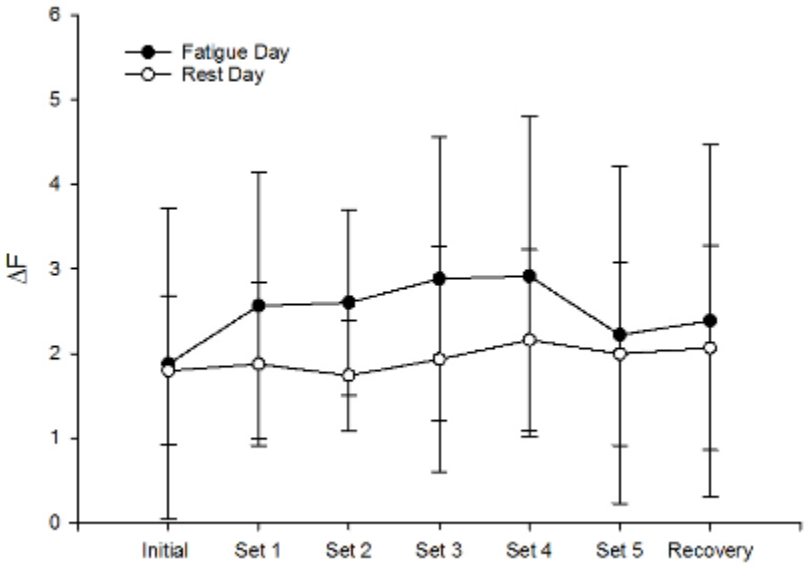

FIGURE 9: AVERAGE ΔF VALUES ON THE FATIGUE DAY AND REST DAY.
Repeated measures ANOVA indicates that there was no significant day x time interaction for ΔF values over the fatigue protocol (p=0.81).

TABLE 2

Pearson's correlations for assessing the relationship between change in ΔF from initial to fifth set of the fatigue protocol and changes in maximal voluntary force, voluntary activation, twitch peak force, twitch force peak rate of rise, twitch force peak rate of relaxation, and submaximal muscle EMG.

	R	p	n
Maximum Voluntary Force	-0.14	0.69	11
Voluntary Activation	-0.54	0.11	10
Twitch Peak Force	-0.59	0.06	11
Twitch Peak Rate of Rise	-0.10	0.79	10
Twitch Peak Rate of Relaxation	-0.50	0.11	11
Submaximal Muscle EMG	-0.07	0.85	11

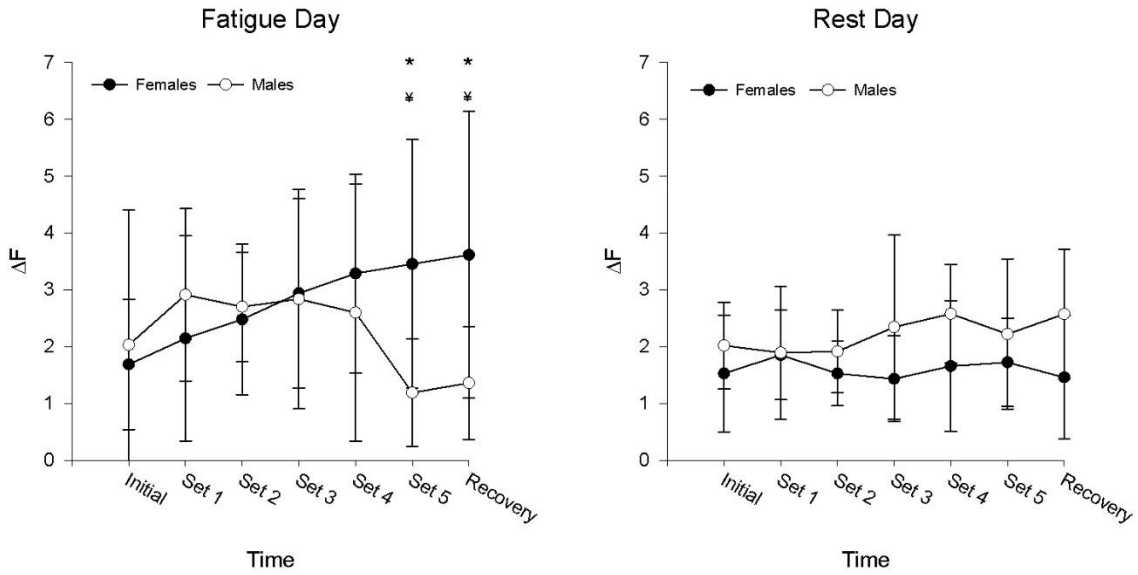

FIGURE 10: SEX-DIFFERENCES IN ΔF VALUES ON FATIGUE AND REST DAYS
Repeated measures ANOVA indicates that there was a significant interaction between sex, day and time ($p=0.02$). * indicates were females had a significantly greater ΔF than males ($p<0.05$). ¥ indicates when ΔF becomes significantly greater than initial ΔF for females.

TABLE 3

Pearson's correlations for assessing the relationship between change in ΔF from initial to fifth set of the fatigue protocol and changes in maximal voluntary force, voluntary activation, twitch peak force, twitch force peak rate of rise, twitch force peak rate of relaxation, and submaximal muscle EMG for males and females separately.

	Males			Females		
	R	p	n	R	p	n
Maximum Voluntary Force	-0.02	0.97	6	-0.88	0.02	6
Voluntary Activation	-0.63	0.25	5	-0.75	0.14	5
Twitch Peak Force	-0.20	0.70	6	-0.67	0.15	6
Twitch Peak Rate of Rise	-0.06	0.92	6	0.25	0.68	5
Twitch Peak Rate of Relaxation	-0.04	0.93	6	-0.45	0.37	6
Submaximal Muscle EMG	-0.11	0.83	6	-0.38	0.53	5

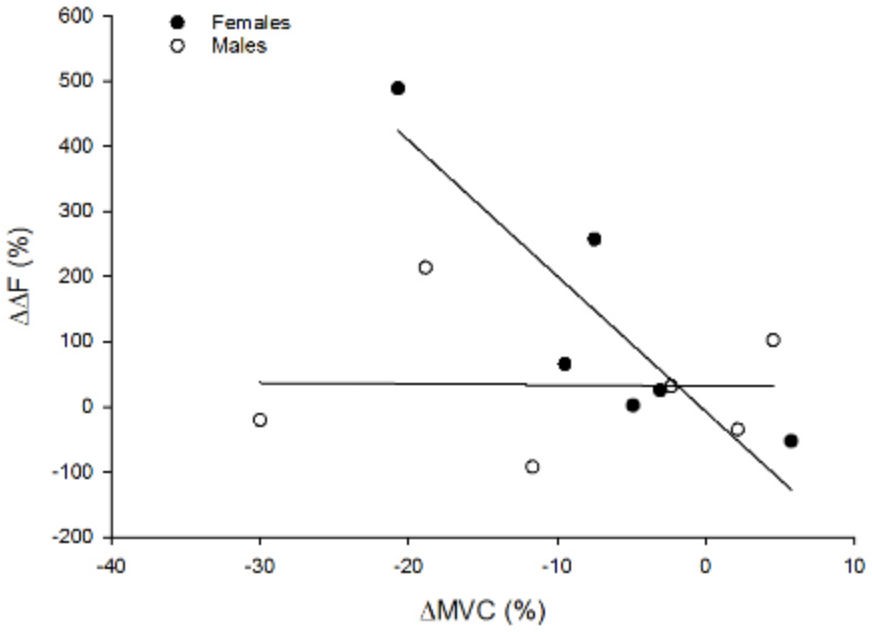

FIGURE 11: ΔΔF VS ΔMAXIMUM VOLUNTARY FORCE FOR MALES AND FEMALES

The change in ΔF over the fatigue protocol compared to the change in maximal voluntary force for males and females separately. Pearson's correlations show that there is a significant relationship between the decline in maximal voluntary force and the increase in ΔF over the fatigue day for females (R=-0.88 p=0.02), but not males (R=-0.02, p=0.97).

TABLE 4

Summary of sex differences for variables measured over the fatigue protocol. Data is presented by the average female value as a percentage of average male value for each variable. Fatigue day is in bold font. Repeated measures ANOVAs were used to assess sex differences (regardless of day or time) and either sex x day x time interactions or sex x time interactions.

	Initial	Set 1	Set 2	Set 3	Set 4	Set 5	Recovery	n (males, females)	Sex difference			Day x Time x Sex Interaction		
									df	F	p	Df	F	p
Maximal Contractions:														
Max Force	**96.7**	**97.0**	**100.0**	**103.3**	**98.2**	**98.7**	**93.8**	6,6	10, 1	0.00	0.95	6, 60	0.67	0.68
	102.0	101.8	104.1	105.8	102.3	102.1	107.8							
Max EMG RMS	**57.1**	**57.2**	**51.2**	**55.1**	**50.6**	**56.9**	**52.9**	6,6	10, 1	0.28	0.61	6,60	1.28	0.28
	114.9	110.7	94.4	129.3	103.6	100.3	131.8							
Voluntary Activation	**101.1**	**99.6**	**101.3**	**101.5**	**101.4**	**106.0**	**99.1**	6,6	10, 1	0.30	0.60	6, 60	1.56	0.17
	97.1	96.3	96.6	97.6	96.8	96.4	98.2							
Contractile Properties:														
Twitch peak Force	**67.7**	**60.8**	**63.8**	**63.1**	**60.1**	**61.6**	**64.2**	6,6	10, 1	2.88	0.12	6, 60	0.66	0.68
	67.7	64.7	68.2	71.9	68.5	68.0	73.2							
Twitch Peak Rate of Rise	**72.3**	**67.8**	**71.5**	**68.0**	**68.2**	**67.9**	**69.5**	6,6	10, 1	1.60	0.24	6, 60	0.27	0.95
	81.8	65.0	71.1	60.9	63.0	59.1	74.8							
Twitch Peak Rate of Relaxation	**44.2**	**37.6**	**40.5**	**40.9**	**36.6**	**37.5**	**39.4**	6,6	10, 1	5.17	0.05	6, 60	0.45	0.85
	62.2	54.3	59.5	57.8	58.2	55.1	62.5							
Peripheral Transmission:														
M-wave	**96.9**	**111.5**	**114.1**	**115.4**	**118.7**	**121.4**	**104.7**	6,6	10, 1	0.04	0.85	6, 60	2.41	0.04
	81.0	82.7	83.8	83.7	83.9	84.3	82.1							
Antagonist Activation														
TA EMG (%MVC EMG)	**108.3**	**109.6**	**66.9**	**73.4**	**88.5**	**93.1**	**126.4**	5,5	1, 8	3.24	0.11	6, 48	1.17	0.34
	685.2	752.8	544.9	657.1	731.3	588.6	600.3							
Spinal Excitability:														
Estimates of PIC	**83.1**	**73.7**	**91.7**	**103.6**	**126.5**	**290.8**	**265.9**	5,6	1, 9	0.00	0.96	6, 54	2.71	0.02
	75.7	98.0	79.8	61.2	64.3	77.6	56.7							
H-reflex Gain	**118.3**					**57.1**	**73.9**	3,5	1, 6	0.68	0.44	2, 12	3.25	0.07
	62.3					79.5	73.1							
												Time x Sex Interaction		
												Df	F	p
Submax Muscle EMG:		**55.3**	**61.1**	**53.5**	**55.6**	**51.0**		6,6	1, 10	2.25	0.16	4, 40	1.08	0.38
Motor Unit Firing Rates:		**129.9**	**98.5**	**129.6**	**100.7**	**100.8**		5,4	1, 7	0.50	0.50	4, 28	1.15	0.35

APPENDICES

APPENDIX A: DATA TABLES

Table A1: Maximal voluntary force (N)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	563.4	567.8	537.1	540.3	543.8	546.1	546.5
F2	663.7	615.6	618.5	598.5	578.5	572.2	562.0
F3	275.4	250.1	312.2	293.7	241.7	218.4	225.3
F4	481.3	494.2	479.9	523.6	490.2	509.0	472.8
F5	600.1	564.7	580.4	568.2	583.1	543.2	578.9
F6	547.0	531.4	520.3	514.9	520.4	520.2	535.6
M1	312.2	275.3	282.9	283.7	268.0	253.3	286.0
M2	669.7	674.1	650.9	649.0	655.8	654.2	675.6
M3	621.2	624.5	627.3	640.9	623.5	634.6	643.8
M4	529.5	596.8	585.5	555.8	591.0	553.5	589.1
M5	759.4	620.3	599.1	525.0	570.6	531.6	622.8
M6	344.1	326.0	302.0	289.0	304.0	320.3	298.1
Average	**530.6**	**511.7**	**508.0**	**498.6**	**497.5**	**488.0**	**503.1**
SD	**151.9**	**146.0**	**134.7**	**133.6**	**143.7**	**143.4**	**150.9**
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	583.9	557.4	562.9	520.6	537.5	533.1	541.6
F2	616.8	600.6	601.0	611.9	576.9	567.2	606.0
F3	440.5	432.5	456.5	510.1	440.2	449.6	441.3
F4	500.6	485.4	465.3	521.5	538.2	524.3	537.1
F5	591.2	567.1	584.1	594.1	599.6	599.9	577.4
F6	586.8	593.0	587.3	590.1	593.2	582.1	590.5
M1	270.1	267.6	273.2	274.2	280.7	267.1	278.3
M2	715.3	683.5	666.8	663.6	630.3	643.2	638.7
M3	677.8	697.5	690.5	687.3	678.7	682.7	671.9
M4	530.8	524.3	538.4	549.8	524.0	433.6	462.1
M5	767.3	705.0	653.8	688.0	806.2	865.3	729.9
M6	295.1	300.8	306.9	302.5	292.7	296.2	274.8
Average	**548.0**	**534.6**	**532.2**	**542.8**	**541.5**	**537.0**	**529.2**
SD	**153.0**	**143.3**	**134.0**	**134.2**	**149.1**	**164.2**	**142.9**

Table A2: Level of Voluntary Activation (%)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	98.1	99.9	97.4	96.0	98.5	99.0	92.3
F2	98.7	99.4	99.9	99.6	99.9	99.0	98.5
F3	91.2	89.9	99.7	99.5	100.1	100.0	98.6
F4	99.6	99.4	94.6	100.0	96.6	99.8	91.6
F5	99.8	97.9	99.3	99.5	99.7	100.0	100.0
F6	98.2	99.6	99.7	99.1	99.8	99.4	98.0
M1	99.9	100.0	99.9	99.0	98.4	98.8	100.0
M2	99.9	99.0	99.2	98.8	99.9	99.7	99.9
M3	99.4	98.9	99.4	99.6	98.9	99.5	99.7
M4	96.7	98.2	100.0	99.7	100.0	93.7	95.1
M5	83.9	92.6	84.5	88.3	89.0	71.6	89.2
M6	99.7	100.0	100.0	99.7	100.0	100.0	100.0
Average	**97.1**	**97.9**	**97.8**	**98.2**	**98.4**	**96.7**	**96.9**
SD	**4.8**	**3.2**	**4.5**	**3.3**	**3.1**	**8.1**	**3.9**
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	97.2	98.7	99.4	98.7	97.2	99.5	96.2
F2	100.0	99.7	99.6	95.2	100.0	93.0	96.7
F3	94.8	98.1	97.7	100.0	97.9	97.9	97.4
F4	88.9	80.5	84.2	90.6	87.6	91.4	98.0
F5	99.8	100.0	99.9	100.0	97.5	99.4	99.6
F6	99.8	98.3	97.5	99.6	100.0	96.5	99.1
M1	100.0	99.4	99.9	99.4	99.9	100.0	100.0
M2	100.0	99.9	100.0	99.9	99.9	99.7	99.8
M3	98.4	99.6	100.0	99.7	99.7	100.0	99.7
M4	99.2	99.8	99.8	99.7	100.0	99.9	99.0
M5	100.1	99.1	98.9	99.5	100.0	100.0	100.0
M6	100.0	99.8	100.0	100.0	100.0	99.5	99.3
Average	**98.2**	**97.8**	**98.1**	**98.5**	**98.3**	**98.1**	**98.7**
SD	**3.3**	**5.5**	**4.5**	**2.8**	**3.5**	**3.0**	**1.3**

Table A3: Twitch Peak Force (N)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	111.6	141.7	123.7	123.7	129.7	129.7	99.6
F2	105.6	57.5	93.6	81.5	57.5	63.5	45.4
F3	57.5	63.5	63.5	57.5	51.5	51.5	45.4
F4	39.4	45.4	39.4	51.5	39.4	45.4	33.4
F5	171.8	171.8	171.8	165.8	171.8	171.8	165.8
F6	39.4	39.4	39.4	39.4	39.4	39.4	33.4
M1	123.1	119.9	123.1	119.9	113.5	110.4	97.6
M2	201.9	220.0	201.9	195.9	189.9	189.9	165.8
M3	159.8	183.9	183.9	195.9	201.9	195.9	129.7
M4	87.6	99.6	99.6	99.6	99.6	99.6	81.5
M5	105.6	129.7	129.7	123.7	123.7	123.7	99.6
M6	97.6	100.8	94.4	88.1	84.9	94.4	84.9
Average	**108.4**	**114.4**	**113.7**	**111.9**	**108.6**	**109.6**	**90.2**
SD	**50.7**	**57.9**	**53.1**	**53.0**	**57.3**	**55.0**	**46.5**
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	87.6	93.6	93.6	93.6	87.6	87.6	87.6
F2	87.6	69.5	75.5	81.5	81.5	81.5	81.5
F3	75.5	81.5	81.5	81.5	81.5	81.5	75.5
F4	51.5	45.4	45.4	51.5	51.5	51.5	45.4
F5	153.8	159.8	165.8	165.8	159.8	147.8	141.7
F6	39.4	39.4	39.4	39.4	45.4	39.4	39.4
M1	75.3	78.5	78.5	81.7	81.7	72.1	75.3
M2	195.9	183.9	177.8	159.8	171.8	165.8	147.8
M3	159.8	159.8	159.8	159.8	159.8	171.8	135.7
M4	147.8	171.8	159.8	159.8	171.8	153.8	147.8
M5	93.6	99.6	99.6	93.6	99.6	99.6	87.6
M6	59.4	62.6	59.4	59.4	56.2	56.2	49.8
Average	**102.3**	**103.8**	**103.0**	**102.3**	**104.0**	**100.7**	**92.9**
SD	**49.6**	**51.3**	**49.7**	**46.5**	**48.3**	**46.9**	**40.4**

Table A4: Twitch force peak rate of rise (N/s)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	1807.8	2299.2	1981.2	1849.6	2078.8	1990.1	1472.6
F2	985.9	540.5	1008.9	797.7	509.8	517.6	462.5
F3	1228.8	1172.0	1258.7	1221.8	1200.8	***1142.3***	981.2
F4	2679.1	2888.9	2769.8	2675.7	***2670.2***	2653.7	2172.3
F5	1872.1	2285.4	2305.6	2395.6	2340.5	2377.0	1724.0
F6	889.5	1027.3	1085.8	1137.0	1185.6	1162.5	865.7
M1	605.2	677.0	807.3	722.8	770.1	731.5	671.7
M2	463.9	526.2	425.7	543.5	460.7	522.2	352.3
M3	1183.4	1361.9	1434.6	1410.0	1402.9	1527.7	1259.5
M4	1063.1	1081.9	981.2	893.7	900.5	971.5	813.7
M5	2076.7	2069.7	2184.6	2051.2	2256.3	2257.8	2014.8
M6	508.7	545.8	620.5	650.7	541.6	655.3	458.2
Average	**1280.4**	**1373.0**	**1405.3**	**1362.4**	**1359.8**	**1375.8**	**1104.1**
SD	**689.9**	**814.5**	**738.9**	**719.9**	**787.2**	**767.3**	**621.7**
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	2198.1	1519.9	1779.5	740.5	889.5	875.0	1615.0
F2	799.4	663.6	692.1	744.1	762.9	791.7	808.7
F3	804.6	863.9	816.0	825.6	815.8	797.4	775.1
F4	2840.7	2579.2	2473.6	2366.6	2375.4	2434.4	2097.1
F5	2184.4	2177.4	2177.7	2129.3	2181.8	2206.4	1848.7
F6	1772.0	1728.9	1757.3	1842.9	1808.4	1576.4	1467.0
M1	1753.3	903.6	907.4	889.5	894.1	878.0	840.4
M2	517.6	483.9	476.7	532.6	562.6	489.7	461.9
M3	988.0	1053.8	1018.2	1008.9	1042.3	1097.5	978.4
M4	614.3	683.9	615.5	619.6	573.0	608.1	534.5
M5	1639.3	1780.5	1809.4	1867.5	1778.5	1605.8	1531.5
M6	621.4	559.5	637.0	578.6	657.3	512.6	500.1
Average	**1394.4**	**1249.8**	**1263.4**	**1178.8**	**1195.1**	**1156.1**	**1121.5**
SD	**770.8**	**690.6**	**691.1**	**670.0**	**653.5**	**653.4**	**563.6**

Table A5: Twitch peak rate of relaxation (N/s)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	-827.8	-1094.1	-897.8	-896.2	-1016.0	-1045.0	-758.9
F2	-1929.8	-983.9	-1879.3	-1544.1	-1020.6	-1089.5	-859.9
F3	-1052.8	-1089.5	-1089.5	-1158.4	-1158.4	***-1080.0***	-997.7
F4	-717.6	-846.2	-690.0	-938.0	***-749.0***	-818.6	-593.6
F5	-1567.0	-1562.5	-1548.7	-1546.7	-1584.3	-1599.2	-1423.2
F6	-846.2	-850.7	-846.2	-919.6	-814.0	-901.3	-763.5
M1	-2083.0	-1954.3	-2070.9	-2068.5	-2032.0	-2000.4	-1728.3
M2	-4836.3	-5056.7	-4850.1	-4620.5	-4606.7	-4615.9	-3899.6
M3	-3688.4	-4386.3	-4427.7	-4739.9	-4822.5	-4799.6	-3187.9
M4	-1553.3	-1663.5	-1828.8	-1934.4	-2053.8	-1994.1	-1557.9
M5	-1972.0	-2441.1	-2526.7	-2448.6	-2476.2	-2531.3	-1998.7
M6	-1584.9	-1580.0	-1470.7	-1320.0	-1354.0	-1485.3	-1317.6
Average	**-1888.3**	**-1959.1**	**-2010.5**	**-2011.2**	**-1974.0**	**-1996.7**	**-1590.6**
SD	**1227.1**	**1382.7**	**1345.6**	**1337.6**	**1387.7**	**1367.8**	**1019.2**
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	-1346.6	-909.1	-1154.6	-421.2	-515.5	-520.9	-873.7
F2	-1557.9	-1259.4	-1305.3	-1410.9	-1443.1	-1539.5	-1493.6
F3	-2072.1	-1374.2	-1374.2	-1401.7	-1470.6	-1401.7	-1181.3
F4	-832.4	-781.9	-795.6	-869.1	-855.3	-809.4	-791.1
F5	-3279.7	-3376.2	-3587.4	-3702.2	-3536.9	-3155.8	-2940.0
F6	-905.8	-864.5	-910.4	-915.0	-887.5	-832.4	-809.4
M1	-1349.2	-1407.5	-1414.8	-1424.5	-1422.1	-1303.0	-1276.3
M2	-5033.8	-4478.2	-4299.1	-3940.9	-4069.5	-4120.0	-3527.7
M3	-3936.3	-3973.1	-3885.8	-3775.6	-3881.3	-4037.4	-3201.7
M4	-3183.3	-3169.5	-3059.3	-3293.5	-2967.5	-2788.4	-2609.4
M5	-1622.1	-1686.4	-1677.2	-1691.0	-1764.5	-1815.0	-1571.6
M6	-940.9	-1064.9	-1006.6	-948.2	-868.0	-921.5	-763.5
Average	**-2171.7**	**-2028.7**	**-2039.2**	**-1982.8**	**-1973.5**	**-1937.1**	**-1753.3**
SD	**1365.6**	**1331.9**	**1283.7**	**1302.9**	**1283.6**	**1271.3**	**1027.5**

Table A6: M-wave peak-to-peak amplitude (V)

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	2.01	1.92	1.92	1.84	1.78	1.79	1.95
F2	3.84	4.29	4.27	4.23	4.31	4.31	3.96
F3	4.11	4.30	4.25	4.15	4.20	***4.21***	3.94
F4	3.09	3.35	3.29	3.19	***3.19***	3.13	2.92
F5	3.21	3.30	3.29	3.02	3.06	3.05	2.97
F6	1.36	1.40	1.26	1.25	1.15	1.09	1.35
M1	1.04	0.83	0.86	0.87	0.81	0.76	0.88
M2	3.66	3.34	3.17	2.92	2.81	2.67	3.35
M3	3.35	3.36	3.26	3.12	3.01	3.00	3.24
M4	2.26	1.87	1.74	1.75	1.65	1.71	1.97
M5	2.78	2.37	2.00	1.75	1.68	1.56	2.48
M6	5.09	4.89	4.99	4.90	4.94	4.79	4.39
Average	2.98	2.94	2.86	2.75	2.72	2.67	2.78
SD	1.17	1.26	1.30	1.27	1.32	1.32	1.09
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	3.08	2.99	3.02	3.11	2.94	2.96	3.14
F2	3.89	3.96	3.94	3.96	3.93	3.94	3.84
F3	2.21	3.16	3.26	3.34	3.35	3.36	3.38
F4	2.18	2.16	2.17	2.26	2.25	2.26	2.20
F5	3.09	3.01	2.97	2.97	2.99	3.14	3.09
F6	0.58	0.58	0.63	0.51	0.58	0.60	0.53
M1	1.91	2.12	2.16	2.11	2.12	2.21	2.23
M2	3.80	4.09	4.11	4.14	3.97	4.10	4.28
M3	3.63	3.62	3.64	3.63	3.66	3.74	3.82
M4	3.63	3.83	3.65	3.72	3.79	3.72	3.84
M5	1.94	1.94	1.98	1.97	1.91	1.92	1.99
M6	3.65	3.58	3.56	3.72	3.67	3.59	3.55
Average	2.80	2.92	2.92	2.95	2.93	2.96	2.99
SD	1.03	1.04	1.01	1.06	1.03	1.03	1.07

Table A7: Tibialis Anterior Muscle Activation (normalized to maximum Soleus EMG).
Note: Data from participant F1 was excluded from fatigue day average because the rest day data is missing for this participant.

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	0.12	***0.12***	***0.12***	***0.12***	0.11	0.14	***0.12***
F2	2.14	2.05	***1.99***	1.87	1.87	1.86	1.76
F3	2.31	2.53	2.03	2.73	4.82	***4.45***	4.97
F4	6.59	6.47	6.74	***6.94***	7.16	***7.26***	7.42
F5	10.18	10.02	9.41	9.22	8.96	8.74	8.62
F6	3.44	3.91	3.97	3.65	3.9	3.68	10.77
M1	1.23	1.22	1.21	1.21	***1.20***	***1.20***	1.19
M2	2.51	2.82	2.74	2.74	2.71	2.61	2.55
M3	4.69	4.13	7.74	6.44	5.11	3.91	3.69
M4	12.79	13.07	22.86	21.33	19.64	18.66	17.57
M5							
M6	1.56	1.55	1.54	1.55	1.53	1.53	1.53
Average	4.74	4.78	6.02	5.77	5.69	5.39	6.01
SD	3.94	3.94	6.57	6.08	5.50	5.26	5.22
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	3.08	2.99	3.02	3.11	2.94	2.96	3.14
F2	3.89	3.96	3.94	3.96	3.93	3.94	3.84
F3	2.21	3.16	3.26	3.34	3.35	3.36	3.38
F4	2.18	2.16	2.17	2.26	2.25	2.26	2.20
F5	3.09	3.01	2.97	2.97	2.99	3.14	3.09
F6	0.58	0.58	0.63	0.51	0.58	0.60	0.53
M1	1.91	2.12	2.16	2.11	2.12	2.21	2.23
M2	3.80	4.09	4.11	4.14	3.97	4.10	4.28
M3	3.63	3.62	3.64	3.63	3.66	3.74	3.82
M4	3.63	3.83	3.65	3.72	3.79	3.72	3.84
M5	1.94	1.94	1.98	1.97	1.91	1.92	1.99
M6	3.65	3.58	3.56	3.72	3.67	3.59	3.55
Average	2.80	2.92	2.92	2.95	2.93	2.96	2.99
SD	1.03	1.04	1.01	1.06	1.03	1.03	1.07

Table A8: Soleus EMG (normalized to M-wave) and plantarflexion force (N) during submaximal fatiguing contractions.

	FATIGUE DAY				
Subject #	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**
F1	0.73	0.83	0.76	0.85	0.77
F2	0.94	0.83	0.90	1.03	0.81
F3	0.76	0.90	0.88	1.31	2.02
F4	0.60	0.63	0.68	0.62	0.64
F5	0.57	0.56	0.66	0.58	0.50
F6	1.31	1.33	1.63	1.62	1.88
M1	2.90	3.08	3.03	3.08	3.43
M2	0.84	0.99	0.97	1.07	1.34
M3	0.64	0.68	0.72	0.74	0.78
M4	1.61	1.91	1.91	2.25	2.38
M5	2.27	2.78	2.61	3.69	4.88
M6	0.33	0.35	0.29	0.22	0.46
Average	**1.13**	**1.24**	**1.25**	**1.42**	**1.66**
SD	**0.77**	**0.89**	**0.86**	**1.07**	**1.36**
	REST DAY				
Subject #	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**
F1	286.8	288.9	287.5	286.5	289.2
F2	335.7	334.4	338.3	341.0	334.4
F3	155.8	151.3	155.8	155.3	153.7
F4	358.4	349.6	353.9	355.3	354.7
F5	313.3	316.5	310.6	324.9	391.6
F6	305.2	307.3	298.5	302.8	302.7
M1	154.2	155.9	155.7	158.9	156.4
M2	239.0	226.4	230.5	232.0	223.9
M3	435.9	423.8	434.1	442.6	435.5
M4	141.8	148.2	140.4	146.1	144.2
M5	302.2	307.9	307.2	319.9	319.8
M6	271.5	272.1	270.8	274.9	272.3
Average	**275.0**	**273.5**	**273.6**	**278.4**	**281.5**
SD	**89.3**	**87.0**	**89.0**	**90.7**	**95.4**

Table A9: Motor unit firing rates (Hz)

	Beginning of Set					Middle of Set					End of Set				
Subject #	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
F1	11.8	12.0	12.7	12.8	12.8	12.2	11.5	12.2	12.9	13.2	11.5	11.5	11.8	12.1	12.9
F2															
F3	10.9	12.3	13.2	13.0	12.3	11.8	13.5	13.5	8.3	9.2	11.8	11.2	12.5	11.2	12.0
F4	9.6	5.3	6.8	7.5	5.7	8.6	6.3	6.2	6.8	6.1	8.3	3.9	8.5	4.5	6.3
F5	6.9	6.1	10.3	11.5	6.9	6.5	7.1	9.4	8.5	8.3	6.1	5.6	8.3	8.1	8.9
F6															
M1	6.6	6.5	5.5	7.7	12.7	4.6	12.0	5.0	11.9	11.8	5.9	3.5	4.6	10.4	9.7
M2	7.3	6.1	7.2	7.0	8.9	6.8	7.1	6.6	7.7	7.1	5.7	6.5	6.7	6.3	7.0
M3	9.5	6.4	7.6	11.5	5.9	7.3	10.5	7.3	7.4	8.2	10.2	10.9	9.5	6.0	7.2
M4	12.2	11.6	12.3	12.7	10.4	10.2	10.4	10.9	9.7	9.0	9.2	10.1	9.9	10.9	9.5
M5															
M6	7.8	9.3	9.4	9.3	9.4	8.8	8.7	10.0	8.5	9.4	8.6	9.2	9.1	8.5	9.8
Average	**9.2**	**8.4**	**9.4**	**10.4**	**9.4**	**8.5**	**9.7**	**9.0**	**9.1**	**9.1**	**8.6**	**8.0**	**9.0**	**8.7**	**9.2**
SD	**2.1**	**2.9**	**2.8**	**2.5**	**2.8**	**2.5**	**2.5**	**2.9**	**2.1**	**2.2**	**2.3**	**3.2**	**2.4**	**2.7**	**2.2**

Table A10: ΔF Estimates of PIC

Note: Data from participant F1 was excluded from fatigue day average because the rest day data is missing for this participant.

	FATIGUE DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1	3.83	***3.78***	***3.88***	***3.98***	3.32	4.8	***4.29***
F2	***0***	0	***0.70***	1.50	1.80	***2.50***	***3.20***
F3	1.13	2.2	4.08	4.94	5.05	***6.65***	***7.71***
F4	1.92	0.95	1.63	***1.21***	1.06	***0.91***	***0.77***
F5	2.7	4.67	3.16	4.34	4.08	4.47	3.45
F6	2.69	2.91	2.82	2.7	4.45	2.75	2.95
M1	0.37	2.35	2.21	0.68	***1.22***	***1.16***	0.95
M2	0.8	1.06	1.13	0.64	0	1.05	1.2
M3	1.28	1.91	3.17	2.72	5.01	0.83	1.32
M4	1.43	3.93	2.48	5.37	4.17	2.89	1.66
M5	1.51	***2.93***	3.74	4.46	4.68	1.2	3.03
M6	***6.8***	***5.3***	3.48	3.15	0.52	***0***	***0***
Average	1.88	2.56	2.60	2.88	2.91	2.22	2.39
SD	1.84	1.62	1.09	1.72	1.98	1.94	2.10
	REST DAY						
Subject #	**Initial**	**Set 1**	**Set 2**	**Set 3**	**Set 4**	**Set 5**	**Recovery**
F1							
F2	0.00	0.94	1.11	0.68	0.51	1.08	0.68
F3	1.42	1.11	0.99	1.01	1.42	2.19	2.40
F4	2.36	2.07	1.70	2.39	2.46	1.54	1.76
F5	2.58	2.52	2.42	2.09	3.20	2.82	2.46
F6	1.28	2.63	1.42	1.00	0.70	0.99	0.00
M1	3.02	2.68	1.91	4.43	3.64	3.49	3.63
M2	1.07	0.00	1.10	0.00	1.61	0.09	1.18
M3	1.57	1.87	2.24	1.78	2.78	2.06	2.24
M4	1.48	1.29	1.83	1.42	1.45	1.46	1.47
M5	2.67	3.37	1.28	2.69	2.85	2.67	2.92
M6	***2.30***	2.15	3.13	3.75	3.14	3.56	***4.00***
Average	**1.80**	**1.88**	**1.74**	**1.93**	**2.16**	**2.00**	**2.07**
SD	**0.88**	**0.96**	**0.66**	**1.33**	**1.07**	**1.09**	**1.21**

Table A11: H-reflex gain

	FATIGUE DAY			REST DAY		
Subject #	**Initial**	**Final**	**Recovery**	**Initial**	**Final**	**Recovery**
F1	3.36	1.67	2.25	1.42	1.31	1.15
F2	0.95	1.30	1.19	0.93	1.27	1.06
F3	1.00	***0.92***	0.83	1.89	1.50	0.90
F4	1.66	2.06	1.70	1.37	2.74	1.83
F5						
F6	3.37	3.72	3.12	3.23	3.44	4.45
M1	0.37	2.31	0.23	1.29	1.03	1.08
M2	3.20	3.16	2.16	3.71	4.08	2.85
M3						
M4						
M5	1.69	4.69	4.98	3.51	2.63	3.77
M6						
Average	**1.95**	**2.48**	**2.06**	**2.17**	**2.25**	**2.14**
SD	**1.20**	**1.29**	**1.49**	**1.13**	**1.14**	**1.38**

Table A12: Levels of physical activity.

Subject #	Total Mins/Wk	Total METs/Wk	Vigorous Mins/Wk	Moderate Mins/Wk	Walking Mins/Wk	Sitting Hrs/Day
F1	525	1986	45	60	420	10
F2	1140	7146	720	0	420	7
F3	310	1373	70	30	210	5
F4	3030	11436	270	240	2520	6
F5	295	1350	60	135	100	7
F6	390	1865	180	120	90	10
M1	390	2979	360	0	30	6
M2	470	2453	180	80	210	9
M3	540	3093	270	60	210	12
M4	810	4026	270	120	420	6
M5	360	1533	60	90	210	15
M6	340	1812	120	180	40	10
Average	**717**	**3421**	**217**	**93**	**407**	**9**
SD	**768**	**2998**	**189**	**71**	**680**	**3**

APPENDIX B: INFORMED CONSENT FORM

WILFRID LAURIER UNIVERSITY
INFORMED CONSENT STATEMENT
Fatigue-Induced Changes in Spinal Motoneuron Excitability in Older Adults

You are invited to participate in this research study at Wilfrid Laurier University. The purpose of this study is to assess the fatigue-induced changes in spinal motoneuron excitability in older adults compared to younger adults. This project is approved by Wilfrid Laurier University Research Ethics Board (REB #5409)

Student Investigator:
Name: Nathalie Cecire
Institution: Wilfrid Laurier University
Phone: (519) 884-0710 ext. 3334
Email: ceci3030@mylaurier.ca

Student Investigator:
Name: Christopher Compton
Institution: Wilfrid Laurier University
Phone: (519) 884-0710 ext. 3334
Email: comp9000@mylaurier.ca

Supervisor:
Name: Professor Jayne Kalmar, PhD
Institution: Wilfrid Laurier University
Phone: (519) 884-0710 ext. 2033
Email: jkalmar@wlu.ca

INFORMATION

30 participants will take part in this study. The overall aim of this research study is to assess the age-associated changes in motor neuron excitability during a muscle fatigue protocol and the effect of level of physical activity. The study will take place in the Exercise Neurophysiology Lab (N119) in the Northdale Campus at Wilfrid Laurier University. You will attend three sessions in the lab: an orientation session, a fatigue day testing session, and a no-fatigue control day testing session. The orientation day will last approximately 30 minutes. During the orientation session you will fill out a screening questionnaire and a physical activity questionnaire. If you do not meet the inclusion criteria the questionnaires you filled out will be destroyed. If you do meet the inclusion criteria (no history of neurophysiological disorders, peripheral vascular disease, lower limb injury, non-smoker, not on any sedative, stimulant, narcotic, or anti-coagulant medications and within the age range of 18-28 or 55-70), you will get the chance to see all of the equipment and practice making contractions to target levels displayed on a monitor in front of you. Two Intramuscular EMG wire electrodes will be inserted into your lower leg using a hypodermic needle. Wires are surgical grade and intended for this purpose. Wires are single-use only and sterilized prior to use. Surface EMG electrodes will be attached to your leg using double-sided tape and ground electrodes will be placed near the knee and ankle using tape. The area where the surface and ground electrodes are placed will be shaved. The stimulating surface electrodes will be placed on the front and back of your knee and held in place using tape and reusable bandage. The brief stimulation (1.0 ms pulse) will cause an involuntary twitch contraction of the muscle. The stimulation you will experience is similar to what you would experience if you ever received electrical stimulation therapy for physical therapy. If we are able to obtain the necessary waveforms and if you are comfortable with the procedures and wish to take part in the study, we will schedule the two testing sessions at the end of the orientation.

Initials: _____

If you wish to proceed with the study, there will be two testing sessions, a fatigue-session and a control-session, the order will be randomly assigned. Both sessions have the same testing procedures and timing, but during the fatigue session you will perform five sets of 3s contractions at 50% of your maximum torque with 1s of rest repeated for three minutes. Because this is a fatigue protocol, you will not be able to stop and rest once you begin, but you may cease participation at any time and withdraw from the study.

Before, during, and after the fatigue protocol, you will be asked to perform maximal and submaximal contractions of your right calf muscles. A nerve in your leg will be stimulated at gradually increasing intensities while you are at rest and once during and after each maximal contraction. The entire experimental protocol should take between 1½-2 hours. You will be seated in a modified automobile chair throughout the experiment.

Your date of birth, height, and weight will be recorded for demographic information. If you do not know your height or weight it will be measured for you.

RISKS

Physical risks: Surface electromyography and electrical nerve stimulation are non-invasive procedures that do not cause any tissue damage. Redness or irritation of the skin where the electrodes are placed may occur, this is normal and should not persist more than 1-2 days. This is similar to the redness you may see after you remove an adhesive bandage (a Bandaid). Electrical nerve stimulation using a constant current stimulator feels like a "flick" at the point of stimulation (behind the knee) and causes the muscle to contract involuntarily, this may be uncomfortable for some people. You will have to opportunity to try this during the orientation and do not have to continue if you find it unpleasant.

Performing maximal voluntary contractions and fatiguing muscle contractions may result in immediate or delayed muscle soreness, similar to what would be experienced following an acute bout of exercise. Soreness should not persist more than 3 days.

Intramuscular electromyography requires the insertion of the wire electrodes into the muscle using a needle. Initially there may be a stinging sensation due the alcohol used to clean your skin and the prick of the needle. Localized bruising is possible (<0.5cm diameter) around the insertion site of the electrode, similar to that experienced following a blood test. The bruising generally subsides within 48 hours and is not typically associated with any discomfort. There is a small risk of infection due to the insertion, however we take several precautions to reduce this risk: needles and electrodes are sterilized using an autoclave, the skin is cleaned with alcohol, and the researcher will be wearing non-latex gloves during the needle insertion. Needles and wire electrodes are never reused. This is a commonly used experimental procedure and Professor Kalmar has used this technique for 20 years without any incidence of infection.

Emotional risks: Electrical stimulation may cause anxiety and emotional stress. Stimulation intensity will increase gradually to allow you to become accustomed with the sensation of the stimulation. The experimenter will always let you know when your nerve will be stimulated and you may stop at any time.

Initials: _____

BENEFITS

You will not receive direct benefit from this study, however the information will help us understand aging and how it affects the excitability of motor neurons during fatigue. These findings may indirectly lead to improvements in rehabilitation protocols for age-associated neuromuscular deficits.

EXCLUSION CRITERIA

Exclusion criteria include right ankle or knee injury or arthritis, lumbar spine injury, lack of sensation in the right leg, or the diagnosis of any neurological disorder.

CONFIDENTIALITY

All data collected in this study will be stored in NC119 and will only be accessible to the investigators. All data will be coded and will only be identified using a participant code. Anonymous data will be maintained indefinitely and may be reanalyzed as a second project.

Group results will be submitted for publishing in a research journal and presented to faculty and at a conference. Individual results will remain completely confidential and not published to ensure your privacy.

CONTACT

If you have questions at any time about the study or the procedures, (or you experience adverse effects as a result of participating in this study) you may contact the researcher, Nathalie Cecire, at ceci3030@mylaurier.ca and (519) 884-0710 ext. 3334 or the supervisor at jkalmar@wlu.ca and (519) 884-0710 ext. 2033 . This project has been reviewed and approved by the University Research Ethics Board (which receives funding from the Research Support Fund). If you feel you have not been treated according to the descriptions in this form, or your rights as a participant in research have been violated during the course of this project, you may contact Dr. Robert Basso, Chair, University Research Ethics Board, Wilfrid Laurier University, (519) 884-0710 x4994 or rbasso@wlu.ca

PARTICIPATION

You may withdraw from this study at any point for any reason without consequence. Participation is strictly voluntary. Your results will be omitted from further analysis if you decide so after data collection is completed prior to publication.

Initials: _____

FEEDBACK AND PUBLICATION

During and following completion of this study results will be published as a master's thesis document and as a peer-reviewed article in an academic journal. Data might be made available through Open Access resources. Any questions following the completion of this study or if you wish to receive feedback upon completion of the study you may contact the principal investigator at ceci3030@mylaurier.ca. If you would like to receive notification of the studies results please indicate and provide an email below.

I would like to receive an email notification of this study's results upon its completion:
[ ] email _____

CONSENT

I have read and understand the above information. I have received a copy of this form. I agree to participate in this study.

Participant's signature_____ Date _____

Investigator's signature_____ Date _____

APPENDIX C: PARTICIPANT SCREENING QUESTIONNAIRE

Screening Questionnaires

Have you been diagnosed with a neurophysiological disorder?

YES NO

Do you have an injury or arthritis in your right knee or ankle joint?

YES NO

Do you have peripheral vascular disease?

YES NO

Do you smoke?

YES NO

Are you on any medications?

YES NO

If yes, what are you currently taking?

_____

_____

_____

Have you ever fainted giving blood or having blood drawn in the past?

YES NO

APPENDIX D: PHYSICAL ACTIVITY QUESTIONNAIRE

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRES

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health-related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken in 12 countries (14 sites) across 6 continents during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages. IPAQ is suitable for use in regional, national and international monitoring and surveillance systems and for use in research projects and public health program planning and evaluation. International collaboration on IPAQ is on-going and an international prevalence study is under development.

Using IPAQ

Worldwide use of the IPAQ instruments for monitoring and research purposes is encouraged.

It is strongly recommended, to ensure data quality and comparability and to facilitate the development of an international database on health-related physical activity, that

- no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments,
- if additional questions on physical activity are needed they should follow the IPAQ items,
- translations are undertaken using the prescribed back translation methods (see website)
- new translated versions of IPAQ be made available to others via the web site to avoid duplication of effort and different versions in the same language.
- a copy of IPAQ data from representative samples at national, state or regional level be provided to the IPAQ data storage center for future collaborative use (with permission) by those who contribute.

More Information

Two scientific publications presenting the methods and the pooled results from the IPAQ reliability and validity study are due out in 2002.

More detailed information on the IPAQ process, the research methods used in the development of the IPAQ instruments, the use of IPAQ, the published papers and abstracts and the on-going international collaboration is available on the IPAQ web-site.

www.ipaq.ki.se

This is the final SHORT LAST 7 DAYS SELF-ADMINISTERED version of IPAQ from the 2000/01 Reliability and Validity Study. Completed May 2001.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

IPAQ: SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS

NOTE: EXAMPLES OF ACTIVITIES MAY BE REPLACED BY CULTURALLY RELEVANT EXAMPLES WITH THE SAME METS VALUES (SEE AINSWORTH *ET AL*., 2000).

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. This is part of a large study being conducted in many countries around the world. Your answers will help us to understand how active we are compared with people in other countries.

The questions are about the time you spent being physically active in the last 7 days. They include questions about activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Your answers are important.

Please answer each question even if you do not consider yourself to be an active person.

THANK YOU FOR PARTICIPATING.

In answering the following questions,

- **vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder that normal.

- **moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder that normal.

1a. During the last 7 days, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling,?

Think about *only* those physical activities that you did for at least 10 minutes at a time.

_____ **days per week** ⇨

1b. How much time in total did you usually spend on one of those days doing vigorous physical activities?

_____ **hours** _____ **minutes**

or

☐ **none**

2a. Again, think *only* about those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

_____ **days per week** ⇨

2b. How much time in total did you usually spend on one of those days doing moderate physical activities?

_____ **hours** _____ **minutes**

or

☐ **none**

3a. During the last 7 days, on how many days did you **walk** for at least 10 minutes at a time? This includes walking at work and at home, walking to travel from place to place, and any other walking that you did solely for recreation, sport, exercise or leisure.

_____ **days per week** ⇨

3b. How much time in total did you usually spend walking on one of those days?

_____ **hours** _____ **minutes**

or

☐ **none**

The last question is about the time you spent sitting on weekdays while at work, at home, while doing course work and during leisure time. This includes time spent sitting at a desk, visiting friends, reading traveling on a bus or sitting or lying down to watch television.

4. During the last 7 days, how much time in total did you usually spend *sitting* on a **week day?**

____ **hours** _____ **minutes**

This is the end of questionnaire, thank you for participating.

APPENDIX E: THE EVOLUTION OF THE PURPOSE OF THIS STUDY

We originally designed this study to assess the age-related changes in PIC and H-reflex gain during fatigue, and whether physical activity had a protective effect against age-related changes. Previous work shows an increase in the prevalence of PIC in an aging animal model (Kalmar et al., 2009) and in humans (Kamen, Sullivan, Rubinstein, & Christie, 2006), whereas the change in H-reflex with aging are inconsistent. There are reports of a decreased H_{max}/M_{max}, however it appears to be posture dependent (Baudry, Collignon, & Duchateau, 2015; Hayashi, Tako, Tokuda, & Yanagisawa, 1992; Kim, Hart, & Hertel, 2013), therefore we measured the H-reflex using the H-reflex gain to attempt to gain a better understanding of the age-related changes to spinal motor neuron pool excitability in older adults. We purposed assessing physical activity, because it may help to prevent age-related physical decline, and may be an important contributor to maintenance of neuromuscular function in old age. For example, resistance training can help to offset muscle atrophy, maintain and improve strength while aging (P. Aagaard, Suetta, Caserotti, Magnusson, & Kjær, 2010) and aerobic exercise is associated with increased neuroplasticity in aged rats and humans, likely resulting from release of brain derived neurotrophic factor (Hötting & Röder, 2013; for review, see Knaepen, Goekint, Heyman, & Meeusen, 2010). Therefore, physical activity may prevent or reduce any age-related changes in the H-reflex gain and PIC amplitude.

The target population for this study was young adults aged 18-29 and older adults aged 55-70 years. Unfortunately, despite recruitment efforts we were unable to appeal to enough older adults that met our recruitment criteria. There was interest from a handful of older participants, however only two of those who expressed interest met the criteria and followed through with

data collection. Some of the difficulties in recruitment were due to the age range selected in this study. Recruitment posters were placed in around the city of Waterloo, including at the Waterloo Memorial Recreational Complex, Albert McCormick Community Center, Adult Recreation Center, Wing 404 RCAFA, Waterloo Public Library, Stork Family YMCA, and seven different churches throughout the city. I was able to present my project to recruit participants at the Adult Recreation Center, Wing 404 RCAFA, and at Mount Zion Lutheran Church. The first obstacle I encountered was that those who were most interested were well above my target age range. The age range was selected in order to prevent the inclusion of participants with health conditions or taking medications that may confound the results. The second obstacle was simply a lack of interest by individuals meeting my recruitment criteria, many cited not being comfortable with the intramuscular recordings or the peripheral nerve stimulation, and most people asked about how the results would tangibly affect them and other older adults. After six months of recruiting and receiving very low interest, it was necessary to decide whether I would be able to recruit, collect, and analyze enough older adult participants in a reasonable amount of time or whether I would have to shift the focus of my thesis which would include additional data analysis (tibialis anterior activity, motor unit firing rates, correlational analyses).

Future investigators interested in studying PIC during adult aging, should consider their affiliations with potential participants and ability to recruit their target population. Additionally, they could consider the inclusion of a wider age range, and rather than excluding certain health conditions, take them into consideration when analyzing the data. Additionally, future investigators could reduce the commitment necessary from participants by assessing only PIC, and not including the H-reflex, and therefore also assessing PIC in a more fatigable muscle to shorten the fatigue protocol.