

Non-local muscle fatigue effects on muscle performance and corticospinal excitability

by

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Thesis Abstract

Fatigue is defined as any decrease in the ability to produce force whether or not the task can be sustained. Inability of the central nervous system to adequately drive muscles has been termed central fatigue, while any physiological changes that occur at the neuromuscular junction or distal to it, have been classified as peripheral fatigue. Contractions of one muscle group can have direct local effects on the exercised muscle as well as more global effects, influencing non-exercised muscle groups as well. Exercise induced fatigue can produce performance decrements in non-exercised muscles, and this “non-local” effect of fatigue has been termed non-local muscle fatigue.

It is thought that decrements in performance of the non-local muscles are due to central fatigue mechanisms. Decrements in performance along with deficits in voluntary activation and no changes in the peripheral system, suggest that processes within spinal and/or supraspinal structures mediate these decrements. Measuring excitatory-inhibitory balance of these structures can provide insights into how central nervous system behaves in response to non-local muscle fatigue. However, due to methodological limitations of the studies that investigated this phenomenon so far, it is not possible to provide any suggestions on that matter. Exploring this phenomenon can provide us with further understanding of the complex central nervous system functioning, as well it can provide us with further insights into the origins of fatigue. Based on this data, important rehabilitation and athletic strategies can be employed.

Non-local muscle fatigue research literature is not expansive and there are contradictory results between studies. Different fatiguing protocols, outcome variables, and population samples, are just a few factors that can explain inconsistent findings.

Literature seems to suggest that non-local muscle fatigue might be muscle and contraction type specific. Studies have shown that dominant arms are more susceptible to non-local muscle fatigue, and that fatiguing protocols with higher intensity and higher volume are able to produce larger magnitude of non-local muscle fatigue. There are only a few studies that have investigated changes in corticospinal excitability in non-exercised muscles after non-local muscle fatigue. These measurements were usually taken during rest, which is not a good representation of what occurs during a contraction. Furthermore, all non-local muscle fatigue studies so far measured excitability of the corticospinal tract as a whole, without taking into account modulation of spinal and supraspinal excitability separately.

To further the knowledge on this topic, we investigated the effect of knee extensor fatigue on dominant elbow flexor force output, voluntary activation, corticospinal excitability and muscle contractile properties. To answer these questions, subjects were required to come for two testing sessions consisting of the same pre- and post-test measurements. At pre-test, subjects performed three maximal voluntary contractions (MVCs), where baseline values for previously described variables were obtained. A fatiguing protocol (intervention condition) that consisted of 12 MVCs with 10 sec rest period between repetitions ensued (or rest for control condition), which was followed by the post-test protocol (measurements). The fatiguing protocol consisted of 5 sets of dynamic bilateral knee extension contractions until task failure, which has been shown previously to be successful in inducing cross-over fatigue. Based on the previous literature, it has been hypothesized that knee extensor fatigue will cause dominant elbow

flexor decrements in force production, voluntary activation and corticospinal excitability, without affecting muscle contractile properties.

The results demonstrated that knee extensor fatigue was successful in eliciting non-local muscle fatigue in the dominant elbow flexors. Knee extensor fatigue lowered dominant elbow flexor force production, voluntary activation and interestingly, it caused elbow flexor peripheral fatigue. Furthermore, knee extensor fatigue modulated corticospinal excitability differently compared with control, suggesting that complex changes in corticospinal excitability might have contributed to voluntary activation decrements.

Dedication

This thesis is dedicated to my parents, Dragana and Vladislav, who have raised me to be the person who I am today. Thank you for your unconditional love, guidance and support that you have provided me throughout my life.

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List of Symbols, Nomenclature, Abbreviations

ANOVA – Analysis of variance

CMEP – Cervicomedullary Evoked Potential

CSP – Cortical Silent Period

TMES – Transmastoid Electrical Stimulation

EMG – Electromyography

ES – Effect Size

H Reflex – Hoffman Reflex

ITT – Interpolated Twitch Technique

M-Wave – Compound muscle action potential

MEP – Motor Evoked Potential

MVC – Maximal Voluntary Contraction

NLMF – Non-local Muscle Fatigue

PNS – Peripheral nerve stimulation

rms – root mean square

SD – Standard Deviation

SE – Standard Error

TMS – Transcranial Magnetic Stimulation

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1. Literature Review

1.1 Introduction

Fatigue is a universal and daily phenomenon that involves a myriad of complex mechanisms, with varying definitions depending on the research field of interest (Gandevia 2001). From a psychological standpoint, fatigue has been represented as subjective and mental variable, acting most likely as a warning sign to prevent physical damage (Ament and Vekerke 2009). In neuromuscular research, fatigue caused by exercise is defined as any decrease in the ability to apply muscle force whether or not the task can be maintained (Behm 2004). Although most exercise-related studies focus on the neuromuscular system, all of the human body systems are involved in determining physical performance (Ament and Vekerke 2009). For the purpose of this review, focus will be on exercise induced fatigue and its effects on the neuromuscular system in healthy population.

Exercise induced fatigue has been measured during maximal and submaximal muscle contractions. Maximal muscle contraction or maximal voluntary contraction (MVC) is defined as maximal contraction that a subject can produce while receiving appropriate continuous feedback of achievement (Gandevia 2001). Exercise induced fatigue affects the neuromuscular system as well as the internal environment (Ament and Vekerke 2009). During a fatiguing task, there are many internal sensations, such as a breathlessness and increased body temperature that may increase the perceived difficulty of exercise (MacIntosh et al. 2006). Factors such as mental fatigue (Marcora 2009), specific details of the task (Gruet et al. 2012), memory of the previous experiences

performing the task and the motivation at the onset of activity (Ulmer 1996), can all affect the timing and magnitude of fatigue. Unlike maximal contractions, fatigue with submaximal contractions can occur without noticeable changes in muscle force production (Behm 2004). Fatigue with this kind of contractions can be defined as an increased perceived effort to maintain the required muscle force and is characterized by a steady increase in electromyographic (EMG) activity and lower frequency spectrum of the EMG signal. Conversely, for maximal contractions, whole-muscle EMG begins failing immediately, and so do the firing frequencies of the motor units (Gardiner 2011). Changes in EMG and firing frequencies of the motor units can be explained by changes in corticospinal and muscle excitability (Gandevia 2001). Indeed, during contractions the output that these structures produce depends on their intrinsic properties, the extent of synaptic “fatigue” and also on the balance of excitatory and/or inhibitory input that they receive (Gandevia 2001; Tanaka and Watanabe 2000). However, there are different strategies that the neuromuscular system “uses” to try to maintain the required force output during submaximal contractions. This include potentiating the nervous system, changing motor control strategies, activating previously quiescent motor units, increasing or decreasing motor unit firing frequencies, including catch like properties, decreasing motor unit firing rates with a concomitant slowing of contractile speed with fatigue (muscle wisdom), phosphorylating the regulatory light chains inside the muscle fiber and increasing muscle stiffness (due to residual cross-bridge attachments) (Behm 2004).

Fatigue is specific to the type of contraction performed (Seow and Stephens 1988), the muscle fiber type proportion (Cairns et al. 2005) and also on age and gender (Baudry et al. 2007; Hunter et al. 2006). Now it is recognized that muscle itself is responsible for

much of the performance decrements, however an important part of the performance decrements is due to a gradual reduction in central nervous system (CNS) output. Since muscle contraction depends on a combination of muscle and CNS activities, the limiting sites contributing to muscle fatigue can be distinguished at two levels. Muscle fatigue can arise at the peripheral level, i.e. at or distal to neuromuscular junction or at the central level, i.e. above the neuromuscular junction (Gandevia 2001). With peripheral fatigue, the muscle is simply incapable of sustaining the initial force production, whereas with central fatigue, the nervous system is unable to provide enough input to the muscle which is still able to contract and produce force (Gandevia 2001). In most instances, central and peripheral fatigue occur simultaneously, leading to physical performance decrements (Gandevia, 2001). In a similar vein, exercise induced fatigue can be classified as having local or global effects (Cote et al. 2008). Local effects refer to exercise induced impairments in muscle force production for that particular muscle, whereas the global effect of fatigue refers to exercise induced deficits in muscle force production for muscles that have not been actively involved in the exercise (Halperin et al. 2014a). This global effect has been defined as a non-local muscle fatigue phenomenon (NLMF; Halperin et al. 2014a). In the literature, most of the studies agree that the NLMF effect of fatigue is CNS mediated (Doix et al. 2013), however NLMF can be influenced by blood mediated migration of metabolic by-products originating in distant fatiguing muscles (Nordsborg et al. 2003). Similar to NLMF effects of fatigue to other distant muscles, chronic unilateral motor training can cause motor pathway adaptations projecting to contralateral untrained homologous muscle, leading to performance increments specific to the task practiced by contralateral trained muscle (Ruddy and Carson 2013). This phenomenon is referred to as

cross education, and similar to cross-over fatigue, it is believed to be CNS mediated. These two phenomena indicate complex inter-limb connectedness, which are still not properly understood (Huang 2009).

1.2. Measuring Fatigue

When measuring fatigue, locating the site of failure is difficult due to the complex chain of events that result in muscle contraction. A history of controversy exists in regard to the role of central and peripheral factors in human muscle fatigue. In comparison to the investigation of peripheral mechanisms, there has been a notable delay in the establishment of central mechanisms involved in muscle fatigue (Gandevia 2001). Recently, with advances in technology, it became possible to gain deeper understanding of the mechanisms involved in central fatigue processes and structures involved. Techniques most often used to measure and localize fatigue are electromyography (EMG), peripheral nerve stimulation (PNS), transmastoid electrical stimulation (TMES), transmastoid magnetic stimulation, transcranial electric stimulation, transcranial magnetic stimulation (TMS), near-infrared spectroscopy, doppler ultrasound, functional magnetic resonance imaging, and electroencephalography (Gandevia 2001; Tanaka and Watanabe 2000). Review of each of these techniques and their use in fatigue research is beyond the scope of this literature review. Hence, the review will focus on techniques most often used in neuromuscular research and the ones that will be used for this thesis project: PNS, TMS and TMES.

1.2.1. Peripheral Nerve Stimulation

Peripheral nerve stimulation technique involves placing stimulating electrodes on the skin overlying the nerve trunk or placing electrodes directly inside the nerve, which then excite the nerve, producing action potentials ultimately leading to muscle excitation. A major advancement in the evaluation of different components of fatigue occurred when Merton (1954) demonstrated that nerve electrical stimulation during voluntary contraction produced involuntary twitch-like increment in force. He used the technique called interpolated twitch technique (ITT), which consists of stimulating the nerve during contraction and immediately after while the muscle is in a relaxed state (Behm et al. 1996; Behm et al. 2001). Measuring the involuntary increment in force during contraction (superimposed twitch) to the one right after the contraction (potentiated twitch), it is possible to calculate the extent of peripheral or central fatigue. The principle of twitch interpolation is to stimulate the peripheral nerve during a voluntary contraction to add, in most motor axons, an additional action potential to those produced voluntarily (Behm et al. 1996). If any individual motor unit is not activated or not firing fast enough to produce its maximal force, then this additional action potential will evoke an increment in force, indicating inability of CNS to fully activate muscles, and thus indication of central fatigue (Behm et al. 1996). The more motor units recruited and the higher the firing frequency of those motor units, the smaller the superimposed twitch. During a muscle contraction that produces maximal force, all motor units in the muscle should be recruited and firing close to their maximal firing frequencies, resulting in almost absent superimposed twitch. However, during fatigue, there is a decrease in motor unit recruitment and firing

frequency, which will result in higher superimposed twitch (Behm et al. 1996). Superimposed twitch is also dependent on peripheral fatigue, therefore when measuring voluntary activation it is important to take into account these changes at the periphery (i.e. muscle) by measuring the potentiated twitch. With fatiguing contractions, there is a decrease in potentiated twitch force, rate of twitch force development and rate of twitch force relaxation (Behm and St-Pierre 1997; Lepers et al. 2002). In addition to a purely mechanical response to peripheral nerve stimulation, there is also an electric muscle response called the compound muscle action potential or M-wave. The M-wave is a measure of action potential propagation in the muscle or muscle excitability. With fatigue, the amplitude of M-wave was found to decrease and its duration to increase suggesting lower action potential conduction velocity (Allen et al. 2008; Behm and St-Pierre 1997; Lepers et al. 2002). These measurements indicate different aspect of fatigue induced impairments, which are important to the understanding of how different types fatiguing contractions affect different aspects of muscle qualities.

When stimulating peripheral nerves, besides exciting motor axons that directly send an action potential to the neuromuscular junction, muscle afferents (Ia) also get excited. When afferents get excited, they send an action potentials back to the spinal cord which then excite a portion of the motoneuron pool monosynaptically, which then send the signal to the muscle producing muscle electric response called Hoffman reflex or H-reflex (Zehr 2002).

1.2.1.1 Hoffman Reflex (H-reflex)

H-reflex can be elicited by stimulating the peripheral nerve with an electric stimulus of a duration and intensity that preferentially excites Ia afferents (which are the largest axons in the peripheral nerve and thus most excitable; Maffiuletti et al. 2000; Zehr 2002). The amplitude of the muscle EMG response that ensues following the stimulation at monosynaptic latency is used as an estimate of the afferent excitability of the motoneuron pool. However, there are certain factors that need to be considered before interpreting the results (Knikou 2008; Zehr 2002). H-reflex amplitude depends on the intrinsic properties of spinal motoneurons, level of presynaptic inhibition and the sum of all excitatory and inhibitory influences acting on the motoneuron (Knikou 2008; Zehr 2002). With fatiguing voluntary contractions, there is usually a decrease in H-reflex amplitude, suggesting a possible decrease in excitability of the monosynaptic stretch reflex pathway (Duchateau and Hainaut 1993; McKay et al. 1995; Garland and McComas 1990). In addition, muscle fatigue induced by electrical stimulation can also cause decrease in H-reflex amplitude (Duchateau and Hainaut 1993). Thus, H-reflex amplitude decreases with fatigue contractions, whether the activation is voluntary or artificial.

The amplitude of the H-reflex varies among subjects, therefore, it is necessary to normalize this value so between-subject comparisons can be made. These amplitude variations can result from variations in skin resistance, different amounts of subcutaneous fat, and locations of the nerve relative to the stimulus (Palmieri et al. 2004). The most advocated method of H-reflex normalization is eliciting the H-reflex at a certain percentage of the maximal M-wave (M-max). This method entails finding the amplitude of the M-max first and then adjusting the stimulation intensity to produce an H-reflex

with amplitude equal to some percentage of the M-max. The ability to stimulate the same portion of motoneurons for each subject is desired to permit assessment of the motoneurons reaction to different interventions at a consistent point for all subjects. An advantage of this method is it allows the stimulating and recording conditions to be monitored by examining the M-wave. The M-wave is thought to be a fairly stable value, because it is simply due to the depolarization of the motor axons and is not influenced by spinal centers. If a constant stimulus is being delivered, then the M-wave amplitude should stay stable (Palmieri et al. 2004). However, it has been shown that M-wave amplitude can decrease with fatigue due to motor axon hyperpolarization (Vagg et al. 1998), which indicates a need to use a supramaximal stimulation. The H-reflex can be utilized to assess modulation of spinal inhibitory interneuronal circuits, but attention is needed to the factors previously discussed that affect Ia action potential transmission. The H-reflex is not hard-wired but is dramatically modulated during various motor tasks (task dependence) or during different phases of a cyclical movement (Knikou 2008).

1.2.2 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive, safe and relatively painless technique used to investigate the motor cortex (Temesi et al 2013). Magnetic stimulation is based upon the rate of change of the magnetic field emitted from a coil. The differential rate of change of the magnetic field creates virtual anodes and cathodes, areas of depolarization and hyperpolarization, respectively. The rate of change of the induced magnetic field is the means by which an electric current is induced in the tissues of the

body. This electric current, not the induced magnetic fields, if sufficiently strong, causes depolarization of cell membranes in human tissue and results in stimulation of the tissue (Temesi 2013). The strength of the magnetic field decreases rapidly as the distance from the stimulating coil increases, thus it is most effective to stimulate with the coil in direct contact with the body. The rise time, the maximal energy delivered to the coil and the spatial distribution of the magnetic field, all affect magnetic pulse characteristics. In TMS research there are three main types of coils used: circular, figure-of-eight and double-cone coils. All of them produce magnetic pulses with different spatial characteristics and for the same pulse intensity and coil position, there is a different effect on the tissues affected by this pulse. Thus, when reviewing the literature, it is important to take into account what type of coil researchers used. Unlike transcranial electric stimulation which predominately produces direct waves (D-waves) by directly exciting corticospinal tract axons at the initial portion of the upper motoneuron, TMS produces predominately indirect wave (I waves) by exciting upper motoneurons trans-synaptically (Temesi et al. 2013).

TMS can be used to investigate voluntary drive similarly to PNS. During an MVC, TMS can produce a twitch-like increment in force from the contracting muscles, which would indicate that voluntary activation (VA) is less than maximal (TMS superimposed twitch). However, the size of the increment cannot be used to generate the conventional measure of VA because the same stimulus does not produce a maximal twitch in relaxed muscle as with the stimulation of the peripheral nerve. This is due to the lack of precision of the TMS (Taylor et al. 2006) and the fact that both motor cortical and motoneuronal excitability is much lower at rest than during contraction (Di Lazzaro et al.

1998). Instead, a linear extrapolation of the relationship between SIT and voluntary force is used to estimate the size of the resting twitch that would be produced by TMS under comparable conditions of corticospinal excitability (Todd et al. 2003). In general, the presence of evoked force increments to TMS signifies suboptimal voluntary activation, but their absence does not exclude it (Gandevia 2001).

TMS can elicit both excitatory and inhibitory responses that present in the electromyogram (EMG). These include both the motor evoked potentials (MEP) and cortical silent period (CSP). MEP's are the recorded electrical responses in muscle elicited by TMS and are a direct result of the descending I and D waves. Due to the possibility for TMS to cause multiple discharges of a single motoneuron, MEP amplitude/area can exceed that of M-max. Changes in MEP amplitude/area are indicative of changes in corticospinal excitability as a whole. Conversely, the CSP is the TMS induced period of EMG near-silence after the MEP and in the evaluation of fatigue is generally measured as the duration from TMS delivery (i.e. stimulus artifact) to the resumption of continuous voluntary EMG (Taylor et al. 2000). Since the EMG interruption continues beyond the recovery of motoneuronal excitability, the latter part of the CSP is understood to be mediated through intracortical inhibitory mechanisms (Inghilleri et al.1993), although it is acknowledged that spinal mechanisms contribute to the early part (~50 ms) of this suppression (Inghilleri et al. 1993). It is suggested that this inhibition is caused by long-lasting GABA_B receptors in the brain (Zieman 2004). Intracortical inhibition is clinically important to understand because chronic pain disorders (migraines) and psychiatric disorders (post-traumatic stress disorder) have demonstrated less cortical inhibition during a voluntary contraction, while other disorders

such as Parkinson's and Alzheimer's diseases have demonstrated abnormal corticospinal excitatory circuits at rest.

Initial investigations with TMS delivered single and then paired pulses while the muscle was in the relaxed state. Unlike PNS which stimulates the lower motoneurons at their axons that are unaffected or only marginally affected by voluntary contraction intensity (Todd et al. 2003; Lee and Carroll 2005), TMS-induced motoneuronal output is greatly affected by the rapid increase in corticospinal excitability from rest to weak and moderate voluntary muscular contraction (Ugawa et al. 1995). In addition to just measuring voluntary activation, measuring MEP and cortical silent period, permits greater understanding of the supraspinal changes in response to fatigue. TMS can only identify corticospinal changes, but in conjunction with transmastoid electric stimulation (TMES), TMS can be used to partition responses and changes into supraspinal and spinal components.

1.2.3 Transmastoid Electrical Stimulation

Transmastoid electrical stimulation (TMES) involves stimulation of the corticospinal tract at the level of the mastoids, which produces a single descending volley which can be recorded at the target muscle as a cervicomedullary motor evoked potential (CMEP). CMEP's can be evoked by passing a brief high-voltage pulse between electrodes fixed near the mastoid processes or by using magnetic stimulation with a double cone coil placed at the back of the head, with the center of the coil near theinion

(TMMS). Several terms describe these forms of stimulations and they are often used interchangeably.

The responsiveness of spinal motoneurons depends on multiple descending and afferent inputs, as well as on intrinsic motoneuronal properties (Gandevia 2001). Because the stimulus is delivered sub-cortically, CMEPs are not affected by changes in cortical excitability but are sensitive to motoneuron excitability. Unlike the Ia afferent input, which evokes H-reflexes, corticospinal-motoneuronal synapses lack classical presynaptic inhibition (McNeil et al. 2013), however signal transmission over the synapse may change in response to activity (Gandevia et al. 1999; Petersen et al. 2003).

TMES is usually used in conjunction with TMS, to try to localize fatigue and changes in excitability to either spinal or supraspinal sites (McNeil et al. 2009). It is important to note that TMES activates the same corticospinal axons as TMS as judged by collision experiments (Gandevia et al. 1999; Temesi 2013). One of the disadvantages of TMES is that it is painful and also it is sometimes difficult to obtain responses of sufficient size in some subjects. Even with subjects who can tolerate stimuli within the normal range of intensities, it may not be possible to activate motoneurons via descending tract stimulation and record a valid CMEP. Sometimes the stimulus intensity required to evoke a response also activates nerve roots distal to the lower motoneuron soma. Such direct activation of the motor axon will mean that the CMEP is contaminated by a direct motor response and may not reflect motoneuron excitability accurately. The presence of nerve root stimulation can be identified in two ways: an abrupt approximately 1-2ms reduction in onset latency of the CMEP with an increase in stimulus intensity or the absence of a

large increase in CMEP size (relative to the CMEP recorded in relaxation) if a given stimulus is delivered during a weak voluntary contraction (Forman et al. 2014).

Changes in CMEP or MEP amplitude or area indicate changes in corticospinal excitability. CMEP's and MEP's should be normalized to M-max (MEP/M-max ratio and CMEP/M-max ratio), since this way any peripheral changes that might occur are accounted for. Furthermore, by expressing normalized MEP's and CMEP's in relation to each other (MEP/CMEP ratio), it is possible to directly assess corticospinal excitability.

1.3 Central and Peripheral Contributions to Fatigue

With fatigue, as previously stated, there is a decrease in the ability of the neuromuscular system to produce force. Researchers have tried to pinpoint the contributions of different structures involved in motor output by using the techniques and methods described in previous sections and by using different fatigue protocols and different combinations of these techniques. It is now accepted that both central and peripheral factors have roles in the development of fatigue. Moreover, Amann (2011) suggest they are inter-related, since motoneuronal recruitment depends on the descending drive from the supraspinal sites, and motor output from the supraspinal sites is influenced by both excitatory and inhibitory signals coming from the muscle afferents.

1.3.1 Central Fatigue

Inability to fully activate muscles or lack of voluntary activation (motor drive from CNS) has been defined as central fatigue (Gandevia 2001). Voluntary activation can be

measured by employing twitch interpolation technique (ITT; Behm et al. 1996). ITT represents a valid and reliable technique for measuring voluntary activation (Behm 2009). However there are limitations to its use (Behm 2009). ITT technique does not measure descending drive to the motoneurons or take into account the nonlinear input-output relationship of the motoneuron pool (Taylor 2009). Furthermore, validity of this method also depends on the muscle tendon length being short, single muscles acting around a joint, muscle being investigated and experience of the individuals performing the ITT (Behm 2009; Behm et al. 2002). It also cannot account for changing voluntary activation of other muscles contributing to a movement or of antagonists. Therefore, the ITT technique should be used as a general measure of motoneuronal output and the presence of superimposed twitch should be interpreted as central fatigue. To produce most valid estimates of voluntary activation, one should use predictions based on linear regression or second order polynomial equation (Behm 2009). Other methods of evaluating central fatigue include measuring changes in the ratio of maximal voluntary force to induced tetanic force (Bigland-Ritchie et al. 1978) or the ratio of root mean square (RMS) EMG to M-wave amplitude (Millet and Lepers 2004).

Research examining central fatigue is ubiquitous and has been observed during and after a variety of exercise protocols. It has been observed after intermittent (Goodall et al. 2010) and sustained (Sogaard et al. 2006) submaximal and as well as with intermittent (Temesi 2013) and sustained (Gandevia et al. 1996) maximal isometric voluntary contractions (MVCs). Central fatigue has also been observed after running (Millet et al. 2003) and cycling (Lepers et al. 2002; Sidhu et al. 2014). More recently, attempts have

been made to divide the central component of fatigue into spinal and supraspinal components.

1.3.1.1 Muscle Responses to TMS

The size of MEP in the elbow flexors increased in size with contractions of up to 50% of MVC, whereas in adductor policis MEP's increased in size with weak voluntary contractions (5%), but did not grow larger with stronger contractions (Taylor et al. 1997). Furthermore, during sustained fatiguing contractions at 30% and 100% MVC, MEP's from biceps brachii and brachioradialis increased in size (Taylor et al. 1996).

During sustained constant force submaximal isometric contractions, MEP/M-max ratio has been observed to increase in the elbow flexors (Sogaard et al. 2006; Levenez et al. 2008) indicating increased corticospinal excitability. In conjunction with a progressive increase in voluntary EMG activity, this has been interpreted as an augmentation of central drive to muscle fibers to maintain a constant force level (Sogaard et al. 2006). McNeil et al. (2011) discovered that, during 10 minute constant EMG submaximal contractions of the elbow flexors, strong (50% M-max) TMS pulses did not change MEP/M-max, however CMEP/M-max area decreased, indicating a compensatory increase in supraspinal excitability to counteract decreased spinal excitability. However, when using weak TMS pulse (15% M-max), both MEP/M-max and CMEP/M-max ratios decreased and suggest that TMS stimulus intensity may be an important factor. In contrast, Taylor et al. (1997) found that MEP's grew little with increasing stimuli intensities, however in adductor policis, stimulus intensity exerted a significant effect on

MEP size, suggesting that the effects of increasing TMS pulse might be muscle dependent. Levenez et al. (2008) found that during fatiguing submaximal elbow contractions (50% MVC) until task failure, MEP/M-max and CMEP/M-max ratios both increased, indicating increased spinal excitability.

Increased MEP/M-max ratio during a sustained MVC has been observed in the biceps brachii (Taylor et al. 1999) and first dorsal interosseous (Szubski et al. 2007). MEP/M-max ratio remained unchanged during brief MVCs interspersed throughout a series of sustained 3-min submaximal isometric contractions of the elbow flexors at 20% MVC to task failure, and it wasn't different during recovery (Yoon et al. 2012). Furthermore, there were no differences between older and younger population (Yoon et al. 2012). Sogaard et al. (2006) found that MEP/M-max ratio during sustained isometric elbow flexion at 15% MVC increased, however, when measured during short MVC's, MEP/M-max ratio did not change. Furthermore, MEP/M-max measured during recovery reverted back to baseline values within several minutes. Any delay between exercise cessation and post-exercise evaluation allows MEP to recover and masks exercise induced MEP changes as demonstrated by recovery within the initial 30 s post exercise (Taylor et al. 1999; Sogaard 2006). The effect of delayed post-exercise evaluations is much more of a concern in studies investigating dynamic contractions or any other exercise that cannot be conducted on the same testing equipment, since a delay would be necessary for subject installation. Therefore, in conducting future experiments special attention needs to be given to organizational setup in order to prevent any delay between experimental protocol and post-protocol measures.

Taylor et al. (1997) found that cortical silent period increases with increasing stimulus intensity, but was not affected by changes in contraction strength. CSP lengthens during sustained fatiguing isometric contractions (Taylor et al. 1996; Sogaard et al. 2006). The sustained level of force appears to influence CSP kinetics. During prolonged low to moderate intensity contractions, CSP gradually increases in length (Taylor et al. 1996; Sogaard et al. 2006; Levenez et al. 2008) whereas during sustained MVCs it increases rapidly over the first seconds before plateauing (Taylor et al. 1996; Todd et al. 2003). This suggests that exercise intensity is an important factor in the manifestation of intracortical inhibition. However, not all studies reported lengthening of the silent period with fatiguing contractions (Girard et al. 2013; Temesi et al. 2013). Many factors can induce CSP variability and thus confound results. These include the instructions given to the subjects (Mathis et al. 1998), transient post-silent period bursts in EMG that exceed baseline EMG levels (Chin et al. 2012), large inter-subject (Orth and Rothwell 2004) and inter-examiner variability in measuring the CSP (Saisanen et al. 2008).

1.3.1.2. Muscle Responses to TMES

Similar to TMS, voluntary activation of the target muscle has a marked effect on the size of the CMEP. When CMEP's are evoked during voluntary contraction, the latency of the signal doesn't change much, but their size increases markedly (Taylor et al. 2002). Furthermore, threshold intensity of the stimulation needed to evoke a response is decreased (Taylor et al. 2002). The amount of facilitation of the CMEP is influenced by the size of the response at rest, the level of voluntary contraction, and the target muscle

(Taylor et al. 2002). When measured during rest, 2 minute isometric MVC depressed CMEP's by approximately 50%, however it recovered over a one minute period (Taylor and Gandevia 2004). Levenez et al. (2008) found that with fatiguing submaximal elbow flexor contractions (50% MVC) there is an increase in CMEP/M-wave ratio, indicating increased spinal excitability. Conversely, in the study by Butler et al. (2003), during the sustained 2 min MVC, CMEP/M-max ratio initially increased, but in the latter half of the sustained contraction the size of the CMEP decreased to control levels, and remained on that level within 15 seconds after the fatiguing contraction. In the second session of the same study, the experimental protocol was identical to the previous one, the only difference being that after the 2min contraction, the muscles were held ischemic to see if type III and IV muscle afferents would affect recovery of CMEP responses after the fatiguing contraction. Contrary to their hypothesis, there was no change in CMEP responses during two minute recovery between conditions, thus indicating that most likely the decrease in CMEP was not caused by the increased activity of group III and IV muscle afferents associated with fatiguing contractions. In a study by Petersen et al. (2003), CMEP's measured during rest were depressed immediately after the 10 second 50-100% MVC contractions, with the depression being the greatest for the strongest contraction and least for the weakest one. Furthermore, when measured during slight voluntary contraction (5% MVC) after 10 second 100% MVC, CMEP's were depressed as well. Taken together, these results indicate that synaptic transmission in the human CNS depends on the previous and on the current activity, and changes depending on the fatiguing task (Petersen et al. 2003).

1.3.1.3. Supraspinal Contributions to Central Fatigue

Excitability of the cortical neurons declines during sustained contractions, which indicates a decrease in supraspinal function due to fatigue (Gandevia 2001). During sustained submaximal isometric elbow flexions, supraspinal fatigue can represent up to 40% of the total loss in strength (Soggard et al. 2006). The factors that contribute to supraspinal fatigue are not very well understood. One of the proposed mechanisms that can induce central fatigue is increased concentration of the neurotransmitter serotonin, which has been shown to modulate motor activity, mood and sleep (Newsholme and Blomstrand 2006). During acute exercise, serotonin levels in the different areas of the brain increase and this increase in serotonin concentration has been associated with symptoms of overtraining, lethargy and acceleration of the onset of fatigue (Boyas 2011). In contrast, low serotonin favors improved performance through the maintenance of motivation and arousal (Boyas 2011). Newsholme and Blomstrand (2006) suggest that increased entry of tryptophan into the brain is responsible for increased serotonin concentration. Since serotonin cannot cross the blood-brain barrier, the brain's neurons must thus synthesize this compound themselves from its precursor tryptophan (Newsholme and Blomstrand 2006). In the blood, unbound tryptophan competes with branched chain amino acids for the transport into the brain. When the ratio of unbound tryptophan to branched chain amino acids increases, as occurs with prolonged exercise (Boyas 2011), the brain's synthesis of serotonin also increases. This response is due to increases in blood free fatty acid concentration that normally occurs during exercise. Blood free fatty acids compete with tryptophan to bind to albumin, causing an increase in unbound free tryptophan concentration. Furthermore, during prolonged exercise, the body

progressively increases usage of branched chain amino acids for energy production, causing a further increase in tryptophan to branched chain amino acids ratio. Another factor that can influence serotonin activity is brain glycogen stores. During exercise, the brain glycogen breakdown occurs and it is hypothesized that prolonged exercise can induce depletion of brain glycogen stores, which can then influence serotonin activity by affecting its precursor 5-HT (Nybo and Secher 2004).

Klass et al. (2012) showed that by ingesting drug Rebox, which primarily increase noradrenaline brain concentration, can hinder cycling performance by inducing greater supraspinal fatigue (as showed by larger increase in TMS superimposed twitch), whereas ingesting methyl-phenidate which primarily increases dopamine concentrations, did not show any effect. Findings from this study are in accordance with previous studies (Roelands et al. 2008). Moreover, other neurotransmitters such as acetylcholine, adenosine, glutamate, and gamma-aminobutyric acid have been proposed to be involved in the generation of central fatigue (Nybo and Secher, 2004). However, a thorough understanding on how these neurotransmitters might influence central motor commands remains elusive.

It has been proposed that small-diameter muscle afferents (group III/ IV) might contribute to supraspinal fatigue (Gandevia 2001). These afferents respond to changes in noxious, mechanical and chemical changes within the muscle and provide inhibitory feedback to the regulation of central motor drive and voluntary muscle activation (Amman 2012). Gandevia et al. (1996) have shown that by arresting blood flow to and from the arm after 2-min maximal voluntary biceps brachii contraction and thus prolonging the firing of muscle afferents, central motor drive and voluntary muscle

activation remain low and do not recover until the circulation is restored. Interestingly, when the afferents feedback of these receptors was blocked (via lumbar intrathecal fentanyl), central motor drive was significantly higher compared to control group after 5km cycling trial and this higher central motor drive resulted in a substantially higher power output (Amman2012). These studies show the complex nature of central motor command generation and indicate that multitude of factors influence this process.

1.3.1.4. Spinal Contributions to Central Fatigue

With fatiguing exercise, there is decrease in motor output from spinal motor neurons and multiple mechanisms have been proposed to explain this reduction (Gandevia 2001). Spinal motoneurons receive different types of afferent feedback. Similar to supraspinal mechanisms of fatigue, it has been proposed that group III and IV muscle afferent activity inhibits spinal motoneuron motor output (Boyas 2011). Furthermore, it has been shown that group Ia and II excitatory afferent feedback progressively decreases during an isometric contraction below 30% MVC which might decrease spinal motoneuron output (Macefield et al. 1991). Moreover, with repeated contractions or stretching, the sensitivity of these receptors, and thus their discharge, can be reduced (Avela et al. 1999).

The Golgi tendon organs (group Ib afferents) at the musculo-tendinous and musculo-aponeurotic junctions provide the CNS with the feedback on the intramuscular tension (Boyas 2011). These mechanoreceptors are thought to inhibit homonymous and synergistic motoneuron activity, however their actions are difficult to derive since their

effects are modulated presynaptically and their projections include a class of spinal interneuron receiving convergent input from Ia afferents (Gandevia 2001). With sustained MVCs, the Ib afferents may fire less frequently and thus the strength of their inhibitory effects on the motoneurons is not clear (Behm 2004). Renshaw cells are another source of spinal motoneuron inhibition with their autogenic inhibition effect to homonymous and synergist motoneurons (Gandevia 2001). Renshaw cell output is dependent on descending motor system, local interneurons and peripheral reflex inputs (Gandevia 2001). During a sustained submaximal contraction lasting 10 min (20% MVC), recurrent inhibition was shown to decrease, while, paradoxically, it appeared to increase over the first 30s of a sustained MVC of ankle plantar flexors (Gandevia 2001).

Fatigue at the spinal level can be attributed to changes in the spinal motoneuron excitability. Previous studies have shown that artificial injection of current into the soma of a motoneuron through a sharp microelectrode, which mimics a net synaptic excitation reaching soma under normal physiological conditions, produces a decline in motoneuron firing rate (Gandevia, 2001). This effect in turn will create a greater need for excitatory input to spinal motoneurons in order to produce the same amount of output. Therefore, with fatiguing exercise there are changes in intrinsic motoneuronal properties and afferent feedback that they receive which reduced motoneuron responsiveness and thus, create spinal fatigue.

1.3.2 Peripheral Fatigue

Peripheral fatigue comprises of ionic and metabolic changes at the muscle fiber level that could result in impaired action potential propagation and excitation-contraction

coupling. This leads to decrease in mechanical muscle response and altered electrical activity compared with pre-fatigue (Allen et al. 2008; Enoka and Duchateau 2008). Muscle fatigue induces ionic changes, such that the electrical activity of the membrane will be impaired and the Na^+ / K^+ balance will no longer be maintained. Potassium ions (K^+) accumulate outside of the membrane and prevent Na^+ channel activation and at the same time the conduction velocity of the action potential over the membrane decreases (Allen et al. 2008). The sarcolemma and the T-tubules may no longer be capable of conducting the action potential and the membrane excitability fails with fatigue. During the excitation-contraction coupling process, there is a decrease in the amount of calcium being released from the sarcoplasmic reticulum, which has been suggested to reduce force production (Ament and Verkerke 2009; MacIntosh et al. 2006). In addition, calcium influx back into the sarcoplasmic reticulum is reduced, therefore, calcium begins to accumulate within the myoplasm, which can increase the relaxation time at the end of the contraction (Ament and Verkerke 2009; MacIntosh et al. 2006). Increasing sarcoplasm concentrations of inorganic phosphate (Pi) during exercise has been suggested to reduce the calcium efflux from the sarcoplasmic reticulum via the precipitation of calcium phosphate in the lumen of the sarcoplasmic reticulum and the phosphorylation of the calcium release channels on the membrane of the sarcoplasmic reticulum (Allen et al. 2008; Ament and Verkerke 2009). Furthermore, a reduction in muscle pH created from the accumulation of hydrogen ions can lead to impaired binding of calcium on troponin (Ament and Verkerke 2009). In addition, MacIntosh et al. (2006) stated that action potential propagation may also be hindered by higher potassium concentrations in the transverse tubules. Research is still polarized as to the exact mechanisms of peripheral

fatigue, and there is still need for investigations that will try to elucidate what are the causes of peripheral fatigue and how they interact with each other.

1.4. Cross-Education

Fatigue is a complex phenomenon with global and localized effects that negatively influence motor-task and exercise performance. Intuitively, one expects that exercising a specific muscle will have little to no effects on the other rested muscles; however, this expectation is not always realized. This is illustrated by two different phenomena: cross-education and crossover or non-local muscle fatigue (NLMF). Examining these two phenomena can provide us with further understanding on the mechanisms of exercise-induced fatigue

Cross-education or the cross-training effect is an inter-limb phenomenon, where chronic unilateral motor practice causes increase in the motor performance of the opposite untrained limb which is specific to the motor task trained (Lee and Carrol 2007). As early as 1894, Scripture and colleagues employed a simple manometer to demonstrate that unilateral strength training gives rise to enhanced performance of the same task by the untrained opposite limb (Scripture et al. 1894). This effect has been reproduced in a wealth of research investigations, encompassing both the transfer of strength and motor skill (Lee and Carroll 2007; Zhou 2000). It seems that strength gains in the opposite untrained limb are not muscle specific. Cross-education effects have been reported for first dorsal interosseous (Hortobagyi et al. 2011), wrist muscles (Hortobagyi et al. 2003), knee extensors (Zhou et al. 2002), plantar flexors (Shima et al. 2002), biceps brachii

(Magnus et al. 2010) and triceps brachii (Brown et al. 1990; Magnus et al. 2010). It also seems that cross-education effects are contraction specific. Hortobagyi et al (1997) showed that unilateral eccentric training of the knee extensors produced a greater increase in eccentric than concentric strength in the contralateral untrained limb. Also, concentric training produced a greater increase in concentric than eccentric strength in the contralateral untrained limb. Seger et al. (1998) demonstrated that cross-education may be specific to the velocity as well as to the contraction type used during training. At a training velocity of 1.57 rad/s, unilateral eccentric training of the knee extensors produced an increase in strength in the contralateral untrained limb only for the eccentric strength test at 1.57 rad/s and not for the eccentric or concentric tests at higher or lower velocities. Unilateral concentric training at 1.57rad/s, likewise, only produced increases in strength for the concentric strength test in the untrained limb at 1.57 rad/s. It seems that cross-education transfer is asymmetrical, which usually favors the dominant to non-dominant direction (Farthing et al. 2009). Cross-education is not solely produced by physical execution of muscle contractions. Imagined maximal voluntary contractions of a small hand muscle can also produce cross-education (Yue and Cole 1992). An imagined MVC of the hypothenar muscle, which was completely electrically silent during the 4-week training program, increased significantly by 10%. These improvements in MVC occurred without changes in twitch force, suggesting that adaptations occurred in the areas of the CNS that are associated with motor programming and execution. Cross-education has important clinical implications, especially in situations where one limb is injured (immobilized), whereby chronic unilateral motor practice of the contralateral healthy limb can largely offset functional decline of the injured limb and reduce the time it takes to get

back to normal daily activities (Farthing et al. 2009). However, despite long-standing interest in this phenomenon, there is, however, little consensus concerning contributing mechanisms (Ruddy and Carson 2013).

There have been many proposed mechanisms of cross-education and it is possible that there are numerous adaptations that collectively work together to produce the strength increase in untrained limb (Ruddy and Carson 2013). Studies have not found any peripheral system adaptations in the muscles that exhibited cross-education (Shima et al. 2002; Zhou 2009). Yasuda and Miyamura (1983) reported vascular changes and strength gains in the contralateral untrained forearm after six weeks of unilateral gripping training, and suggested that these vascular changes might have contributed to cross-education by increasing blood circulation. On the other hand, Madarme et al. (2008) suggested that cross education may be mediated by adaptations in systemic hormone production, particularly to increases in noradrenaline concentration. However, it seems that these latter two mechanisms unlikely contributed to cross education (Lee and Carroll 2007), and in the absence of any muscle enzymatic and morphological changes, modifications in neural control are likely to explain the phenomenon of cross education following unilateral motor practice (Lee and Carroll 2007). Adaptations leading to cross education may reside in different areas within the CNS. However, there are two main potential sites that may be classified - spinal and supraspinal structures.

Strong support exists for spinal mechanisms involved in the cross-transfer of strength, as cross-education appears to be greater with electrical muscle stimulation compared to voluntary muscle contractions (Hortobagyi et al. 1999). Skin and muscle afferents may be activated in the stimulated muscle and have possible excitatory effects

on the untrained contralateral homologous muscle (Farthing and Chilibeck 2003). Hortobagyi et al. (1999) suggests group II muscle afferent involvement with possible changes in the cross-extension reflex by strengthening excitatory synaptic connections to the contralateral limb. Toca-Herrera et al. (2008) was able to induce cross-education in the contralateral rectus femoris following only 10-minutes of surface electrical stimulation. The researchers also suggested involvement of the cross-extension reflex and changes in reflex impulses at the medullar level (Toca-Herrera et al. 2008). High force unilateral voluntary contractions are also known to affect the excitability of spinal motor pathways that project to the contralateral side. For example, Hortobagyi et al (2003) showed that the H-reflex in human wrist flexors (flexor carpi radialis) is depressed by strong unilateral flexion and extension of the contralateral wrist, but unaffected by small contractions. This depression of the flexor carpi radialis H-reflex is long-lasting and can persist for up to 30 seconds after the end of the contraction. Although the mechanisms mediating this long-lasting depression could not be identified with the methods used, the authors speculated that presynaptic inhibition of Ia afferent motoneuron synapse may be partially responsible for this contralateral effect. Additionally, spinal interneurons and propriospinal pathways may also be involved in cross-education by exerting crossed spinal effects following unilateral training (Hortobagyi 2005). Since there are no direct connections between motoneurons on either side of the spine or from group I-IV afferents, Hortobagyi (2005) strongly suggests the involvement of spinal interneurons in cross-education.

Within the supraspinal centers, there appear to be two principal theoretical models that can explain neural mechanisms underlying cross education. The first model is

derived from observations that the execution of many unilateral tasks is associated with increased excitability of both contralateral and ipsilateral cortical motor areas (Ruddy and Carson 2013) and it is named “cross-activation” model. The principal tenet of the cross-activation model is that bilateral cortical activity generated during unilateral training drives concurrent neural adaptations in both cerebral hemispheres. Accordingly, unilateral training induces task specific changes in the configuration of cortical motor networks that normally control the muscles of the opposite (quiescent) limb (Ruddy and Carson 2013). The second model, called the “bilateral access” model, holds that motor engrams elaborated during unilateral training are not specific to the control of trained limb. Rather they are encoded in a more abstract fashion, at a location that is also accessible for the control of the opposite untrained limb (Ruddy and Carson 2013). In this scheme, the degree of transfer is predicted to vary with the complexity of the training task (Farthing 2009).

It is also important to recognize that despite clear distinctions between the bilateral access and cross-activation hypotheses, they are not mutually exclusive, but instead can work together to contribute to cross education effect (Lee et al. 2010). For a more detailed review on the supraspinal mechanisms of cross education, please see Ruddy and Carson (2013).

Dominant neurophysiological mechanisms explaining this phenomenon has still yet to be discovered. Resulting in an average of 7.6% increase of strength in the untrained limb (Carroll et al. 2006), unilateral training can be an important aspect of rehabilitation, e.g. following a stroke (Ruddy and Carson 2013) or a trauma involving immobilization (Farthing et al. 2009). Evidence appears to support central rather than peripheral

mechanisms for cross-education and include neural adaptations that originate at cortical and spinal levels. Similarly to cross education, it is thought that NLMF is mediated by neural mechanisms, since the current body of knowledge does not support non-neural mechanisms. Unlike cross-education which involves chronic unilateral motor practice, NLMF represents acute performance decrements to the unexercised muscles.

1.5. Non-Local Muscle Fatigue

Investigating NLMF is important in gaining deeper understanding about the physiological determinants of fatigue. Specifically, it will provide us with the knowledge of whether fatigue is specific to the working muscles or is it more of a systemic response, and if it is, what systemic changes contribute to NLMF. Furthermore, practical insights might be gained as to the order of exercise in a training or rehabilitation program depending on the specific goals.

Similar to cross education experiments, studies investigating NLMF usually performed unilateral fatiguing contractions after which the performance of the contralateral homologous muscle was measured. In addition to this paradigm, NLMF experiments also measured performance decrements in contralateral and ipsilateral heteronymous muscles.

Research exploring NLMF appears to be equivocal. Several studies have not shown significant cross-over fatigue effects between homologous (Elmer et al. 2013; Grabiner and Owings 1999; Todd et al. 2003; Zijdwind et al. 1998) and heterologous muscles (Decorte et al. 2012; Millet and Lepers 2003; Place et al. 2004). In contrast,

significant crossover fatigue effects have been found in contralateral homologous (Doix et al. 2013; Martin and Rattey 2007; Rattey et al. 2006; Triscott et al. 2008) and heterologous muscle groups (Kennedy et al. 2013; Takahashi et al. 2011) following isolated muscle fatigue. Moreover, NLMF has been shown to affect postural control (Paillard et al. 2010). Recently, Kawamoto et al. (2014) found that unilateral dynamic knee extensor fatigue to task failure reduced MVC of the contralateral knee extensors. They have used two different intensities for their fatiguing protocol (40% and 70% of the pre-test MVC), but found no significant difference between two, but larger effect size for the 70% fatigue protocol ($ES = 0.62$ compared to $ES = 0.82$). In a similar vein, Kennedy et al. (2013) found that a maximal fatiguing protocol to task failure was more impactful on heterologous muscle performance compared to submaximal (30% of the pre-test) fatiguing protocol to task failure. Therefore, it seems that intensity of the fatiguing contractions plays an important role in NLMF. In addition to intensity, studies have shown that volume plays an equal role in the development of NLMF. Doix et al. (2013) have shown that one 100 second MVC with knee extensors was insufficient to produce NLMF, whereas two bouts of 100 second MVC was successful in creating NLMF. Interestingly, it appears that males are more susceptible to NLMF compared to females (Martin and Rattey 2007).

Evidently, there is a conflict in the literature regarding the crossover fatigue phenomenon despite studies claiming that subjects were brought to temporary exhaustion. The discrepancy in results may be related to the inconsistency of unilateral fatiguing protocols as several variables differ such as exercise intensities, volumes and types of contractions. Halperin et al. (2014a) suggested that discrepancies in the literature could be

due to the way the non-localized effects of muscle fatigue are being measured. Most studies thus far have employed either an exhaustive submaximal exercise (Johnson et al. 2013; Nordsborg et al. 2003), a single MVC or repeated MVC's with extended resting periods (Doix et al. 2013; Elmer et al. 2013; Kennedy et al. 2013), but only recently Halperin et al. (2014a) have measured repeated elbow flexor MVC output (12 MVC at post-test after the fatiguing protocol) with short rest intervals between contractions (10 seconds). In that study they showed that after dynamic bilateral knee extensor fatiguing protocol, dominant elbow flexor MVC force was only affected for the last 5 repetitions of the 12 post-test elbow flexor MVC protocol. Since the first elbow flexor MVC was measured approximately 3 minutes after the fatiguing protocol, it is possible that force generating capability recovered by the time the first elbow flexor MVC was measured. In a subsequent study coming from the same lab, Halperin et al. (2014b) showed that non-dominant knee extensors might be more susceptible to NLMF effects than non-dominant elbow flexors. While there were significant knee extensor NLMF fatigue effects, elbow flexor force and voluntary activation was not different than control after two sets of 100 second isometric MVC's of the contralateral elbow flexors or contralateral knee extensors. Disparate findings compared to the previous study by the same authors could be due to the differing protocols used (dynamic vs isometric), thus indicating that dynamic contractions could produce larger NLMF effects in the elbow flexors. Another possibility for disparate findings between these two studies could be due to use of dominant or non-dominant arm, indicating that dominant arm could be more susceptible to NLMF effects compared to non-dominant arm.

Studies have shown that in parallel to decreases in the performance of the tested non-exercised muscle, there are decreases in voluntary activation (Kennedy et al. 2013; Martin and Rattey 2007; Halperin et al. 2014a,b; Rattey et al. 2006) thus suggesting CNS fatigue contributions to NLMF. Furthermore, studies by Rattey et al. (2006), Martin and Rattey (2007) and Kennedy et al. (2013) showed that there was no change in peak twitch force and M-wave properties in the non-exercised muscles which exhibited NLMF effects, thus further supporting this hypothesis. In study by Kennedy et al. (2015), they were not able to find any significant cross-over effects fatigue effects, even in the situation where they maintained the firing of the type III and IV muscle afferents in the contralateral homologous muscle. These findings suggest that continued firing of group III and IV muscle afferents from the contralateral limb does not contribute to the cross-over effects of fatigue. Interestingly, when prolonged firing of these afferents was maintained in the antagonistic knee flexor muscles, there was a significant effect of these afferents on cross-over effects of fatigue. Similar findings were observed in their previous study where they found that antagonistic ipsilateral muscle ischemia contributed to 14% decrease in elbow flexor force. This suggests that effects of this afferents might be muscle specific.

Studies investigating corticospinal excitability projecting to the non-exercised muscles have produced mixed results. Todd et al. (2003) have not found NLMF effects in the contralateral homonymous muscles after 1min of sustained elbow flexor isometric MVC in terms of force production, but found slight decrease in voluntary activation (2.9%) and no change in biceps brachii MEP amplitude or cortical silent period between conditions. Similarly, even though Takahashi et al. (2011) did not report force changes in

the non-exercised muscles, there was facilitation of motor evoked potentials (MEP's) during the short 2 minute rest periods between the three sets of 5 minute periods of intense leg exercise in these muscles (first dorsal interosseus and the biceps brachii). Brasil-Neto et al. (1993) have showed that repetitive finger movements performed with the dominant hand produced post-exercise MEP facilitation in the corticospinal pathway supplying the contralateral homologous muscle. In a study by Samii et al. (1996), there was no change in MEP amplitude in the forearm muscles after prolonged contralateral homologous muscle contraction. In these studies spinal excitability was not assessed separately from the assessment of the whole corticospinal pathway, thus it is unclear how changes in supraspinal and/or spinal excitability may have affected MEP amplitude.

There are still conflicting results in the NLMF literature. Differences in the fatiguing protocols used, population sample and differences in timing and outcome measurements used could all explain inconsistencies in the findings. Nonetheless, research has yet to examine the effect of bilateral dynamic knee extensor fatiguing protocol on dominant elbow flexor mechanical output and corticospinal excitability changes projecting to dominant biceps brachii muscle. Based on the current body of knowledge, it is suggested that elbow flexor force will decline as a result of knee extensor fatiguing protocol and that central mechanisms will be responsible for the drop in performance as judged by decrease in voluntary activation and decreases in corticospinal excitability.

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2.Thesis Manuscript

Bilateral knee extensor fatigue modulates force and responsiveness of the corticospinal pathway in the non-fatigued, dominant limb elbow flexors

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Running Head: knee extensor fatigue modulates elbow flexor performance

2.1. Abstract

Exercise-induced fatigue affects muscle performance and modulates corticospinal excitability in non-exercised muscles. The purpose of this study was to investigate the effect of bilateral knee extensor fatigue on dominant elbow flexor (EF) maximal voluntary force production, voluntary activation (interpolated twitch technique: ITT) and corticospinal excitability. Transcranial magnetic, transmastoid electrical and brachial plexus electrical stimulation were used to investigate corticospinal, spinal, and muscle excitability of the dominant EF before and after a bilateral knee extensor fatiguing protocol or time matched rest period (control). For both sessions three stimuli were delivered every 1.5s during the three pre-test time points and during the 1st, 3rd, 6th, 9th and 12th post-test 5s EF isometric MVCs. In both conditions, EF MVC force ($p < 0.001$), ITT voluntary activation ($p=0.001$), and potentiated twitch force ($p=0.002$) decreased progressively from repetition #1 to #12 during the post-test MVC protocol. MVC force ($p < 0.001$, $ES = 0.9$, $\Delta 10.3\%$), ITT voluntary activation ($p=0.045$, $ES=2.9$, $\Delta 6.9\%$) and potentiated twitch force ($p=0.010$, $ES=0.8$, $\Delta 19\%$) decrements were more pronounced in the fatigue intervention condition. In addition, there were no significant differences between conditions for biceps brachii EMG activity ($p = 0.43$), MEP amplitude ($p=0.908$) or MEP silent period ($p=0.776$). However, the fatigue condition exhibited a lower MEP/CMEP ratio ($p=0.042$, $ES=2.5$, $\Delta 25\%$) and a trend toward higher CMEP values ($p=0.08$, $ES=0.5$, $\Delta 20.4\%$). These findings suggest that bilateral knee extensor fatigue can impair performance and modulate corticospinal excitability of the EF.

2.2. Introduction

Exercise-induced neuromuscular fatigue can reduce maximal force output and corticospinal excitability in exercised muscles (Gandevia 2001). Fatiguing contractions can also alter cortico-motor responses (Takahashi et al. 2011; Brasil-Neto et al. 1993; Sidhu et al. 2014; Todd et al. 2003) and muscle performance (Kennedy et al. 2015; Halperin et al. 2014a,b) in the non-exercised muscles. Fatigue in one muscle group, which leads to an acute drop in muscle performance in another muscle group, has been termed non-local muscle fatigue (NLMF; Zijdwind 1998). This phenomenon has been demonstrated for heteronymous (Halperin et al. 2014a,b; Kennedy et al. 2013a) and homonymous muscles (Halperin et al. 2014b; Kawamoto et al. 2014; Martin and Rattey 2007). Studies which exhibited force and voluntary activation decrements in the rested non-local muscle groups have also demonstrated an absence of peripheral fatigue (Rattey et al. 2006; Martin and Rattey 2007; Kennedy et al. 2013a), which strongly supports the hypothesis that central mechanisms contribute to the NLMF phenomenon.

Fatiguing contractions can alter the metabolic environment in the working muscles, thus leading to an increase in the discharge rate of group III and IV muscle afferents (Amman et al. 2012; Amman et al. 2013; Kennedy et al. 2015). These afferents react to increases in ATP, lactate and H⁺ concentration (Kennedy et al. 2015), which through a feedback loop provide an inhibitory input to the central nervous system (Amman et al. 2012; Amman et al. 2013). It is thought that this inhibition can cause a reduction in voluntary activation and maximal force output of the non-exercised muscles (Amman et al. 2013; Kennedy et al. 2013b, 2014; Sidhu et al. 2014). Sidhu and

colleagues (2014) found that a lower body cycling task to failure reduced elbow flexor maximal voluntary contraction (MVC) and voluntary activation and suggested that group III/IV afferent feedback was responsible for a “spill-over” of central fatigue. On the other hand, Kennedy et al. (2013a) found that maximal and submaximal handgrip contractions to task failure affected ankle plantar flexor MVC and voluntary activation. Similarly, Halperin et al. (2014b) found a significant decrease in force (8%) and voluntary activation (5.5%) of knee extensors following a unilateral elbow flexor isometric fatiguing protocol. Although these findings advocated that the exercise-induced fatigue in one extremity (e.g. knee extensors) could impair muscle performance in another extremity (e.g. elbow flexors), few studies have investigated the responsiveness of supraspinal and spinal circuitries supplying central commands to the non-fatigued elbow flexors.

Using transcranial magnetic stimulation (TMS), Sidhu et al. (2014) demonstrated a decrease in the amplitude of motor evoked potentials (MEP) recorded from the elbow flexors following lower body cycling task to failure. Takahashi et al. (2011) also demonstrated that a leg press fatiguing protocol could affect corticospinal excitability to the first dorsal interosseus and biceps brachii muscles. However, MEP amplitude used in these two studies was a measure of corticospinal excitability, therefore it is not known how spinal motoneuron excitability would contribute to corticospinal responses recorded from non-exercised limb. Indeed, evidence regarding the role of spinal motoneuron excitability in modulation of central motor drive to non-exercised muscles in the other extremity is scarce. Aboodarda et al. (2015) demonstrated that bilateral elbow flexor fatigue could increase knee extensor spinal motoneuron excitability, however these authors did not measure supraspinal excitability. Therefore, further research is required to

elucidate the changes in supraspinal and spinal excitability in the non-exercise elbow flexors following knee extensors muscle fatigue

In a previous study from our laboratory (Halperin et al. 2014a), we found that bilateral knee extensor fatigue reduced dominant elbow flexor maximal force production only in the last five MVCs of the 12 post-test MVC contractions. Since voluntary activation and corticospinal excitability of the elbow flexors were not measured in that study, it was not clear how the supraspinal and spinal structures mediated central command to the non-fatigued elbow flexors. Therefore, by employing a very similar experimental design, we aimed to investigate the muscle performance and corticospinal excitability (stimulation of both motor-cortical and subcortical areas to monitor supraspinal and spinal excitability, respectively) of the elbow flexor muscles before and after bilateral knee extensor fatigue.

2.3. Materials and Methods

Participants

Fourteen healthy male (178 ± 4 cm, 78 ± 6 kg, 24 ± 3 years) active but not specifically trained participants from the university population volunteered for the study. None of the participants had a history of musculoskeletal or neurological disease or were taking medications. Thirteen participants were right hand dominant as determined using the Edinburgh handedness inventory (Veale 2014). They were verbally informed of the procedures and provided written consent prior to participation. The procedures were conducted in accordance with declaration of Helsinki and approved by the Health

Research Ethics Authority of Memorial University of Newfoundland (#20141100-HK). Prior to study commencement, each participant completed a magnetic stimulation safety questionnaire for potential contraindications with magnetic stimulation procedures (Rossi et al. 2009) and Physical Activity Readiness Questionnaire (Canadian Society of Exercise Physiology Guidelines 2003). Participants were asked to refrain from ingesting caffeine or participating in vigorous physical activity at least 1 day before attending each experimental session.

Experimental overview

Subjects attended the laboratory on two occasions separated by at least 5 days and performed one of the two conditions in a random and counterbalanced order (Figure 1): 1) control (no intervention) and 2) intervention (bilateral knee extensor fatigue protocol). Before and after each experimental condition, elbow flexion maximal voluntary isometric contractions (MVCs) were performed and motor-cortical, spinal and muscle responses were recorded from dominant biceps brachii muscle during MVCs.

Experimental set up

At the beginning of each session, participants were equipped with surface electrodes for both stimulation and electromyographic (EMG) activity recording on the dominant arm. EMG was recorded from biceps brachii and triceps brachii (lateral head) muscles using pairs of self-adhesive Ag/AgCl electrodes (Kendall MediTrace foam electrodes, MA, USA) placed 2 cm apart (center to center) on the mid-muscle belly (Hermens et al. 1999). A ground electrode was placed on the lateral epicondyle. Before the placement of electrodes, the area of skin was shaved and abraded to remove dead skin with sandpaper and cleansed with an isopropyl alcohol swab to decrease skin resistance.

An inter-electrode impedance of < 5 kOhms was obtained prior to recording to ensure an adequate signal-to-noise ratio. All EMG signals were recorded (Biopac System Inc., DA 100: analog-digital converter MP150WSW; Holliston, MA, USA) with a sampling rate of 5000 Hz using a commercially designed software program (AcqKnowledge III, Biopac System Inc.). EMG signals were amplified ($\times 1000$, bi-polar differential amplifier, input impedance = $2M\Omega$, common mode rejection ratio > 110 dB min (50/60 Hz), noise > 5 μ V), analog-to-digital converted (12 bit), filtered with 10-500 Hz band-pass filter and stored on personal computer for further analysis.

After 5 minutes cycling on a stationary bike at a cadence of 70 rpm at 1 kp, participants were seated in the knee extension machine (Modular Leg Extension, Cybex International, Medway, MA, USA) with their upper arm supported and elbow flexed at 90° , and with the hip and knee fixed at 90° and 83° , respectively. The knee flexion angle was pre-determined by the inclined angle of the seat, which could not be adjusted. The dominant wrist and ankle were inserted into a padded straps attached by a high tension wire to a load cell (Omega Engineering Inc., LCCA 500 pounds; sensitivity = 3 mV/V, OEI, Canada) that was used to measure elbow flexion and knee extension force, respectively. To eliminate upper body involvement, a strap was placed around the waist and upper body. Following positioning on the chair, the participants performed a muscle warm-up that included 12 brief (2s 'on' and 2s 'off') dominant elbow flexor contractions at 50% of perceived MVC. Then subjects performed one 5 s isometric elbow flexion MVC with their dominant arm, which was subsequently used to determine 5% MVC during which appropriate stimulation intensities were ascertained for three motor responses recorded from dominant biceps brachii muscle via: 1) transcranial magnetic

stimulation (TMS), 2) transmastoid electric stimulation (TMES) and 3) brachial plexus electrical stimulation at Erb's point (BPES). Appropriate stimulation intensities were determined for TMS, TMES and BPES during a 5% MVC of the elbow flexors as opposed to complete rest, since excitability of the corticospinal tract increases significantly from rest to low intensity contractions (Taylor et al., 1997). Participants received real-time visual feedback regarding the intensity of the elbow flexion from a computer monitor screen.

Brachial plexus electrical stimulation (BPES). To determine the size of the maximal compound muscle action potential (M-max) of the biceps brachii, the peripheral nerve innervating elbow flexor and extensor muscles was stimulated by a single stimuli elicited at the brachial plexus area called the Erb's point. The stimulating electrodes (Ag-AgCl discs, 20mm diameter) were placed on the supraclavicular fossa (cathode) and on the acromion process (anode). BPES was performed using high-voltage percutaneous electrical stimuli (stimulator Model DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK). The stimulation intensity (200 μ s pulse duration; 400 volt square-wave) increased in incremental steps (20mA) until a plateau in compound action potential was achieved.

Transcranial magnetic stimulation (TMS). Motor evoked potential (MEP) responses of the biceps brachii muscle were elicited using a Magstim 200 stimulator (Magstim Company, UK) with a circular coil (13.5 cm outside diameter) centered at the vertex and oriented tangentially to the scalp in an anterior posterior direction. The current in the coil flowed clockwise (preferential stimulation of the right hemisphere for left-handed participant) and anticlockwise (preferential stimulation of the left hemisphere for

right-handed participants). To locate the vertex, the distance from nasion to inion and from tragus to tragus was measured and marks were placed halfway directly on the scalp for both measurements. The intersection of both halfway marks was defined as vertex. The position was marked on the scalp with ink to allow an accurate repositioning of the coil throughout the whole experiment. The TMS intensity was increased stepwise to produce MEP amplitudes of approximately 20% of M-max in the biceps muscle during brief 5% MVC contraction. The mean stimulation intensity was $56 \pm 11\%$ of maximum stimulator output. The MEP amplitude recorded at this TMS intensity could be differentiated from the background EMG, during 100% MVC contractions. This stimulation intensity was then used for the remainder of the experiment.

Transmastoid electrical stimulation (TMES). The descending corticospinal tract was stimulated at the level of cervicomedullary junction (pyramidal decussation), eliciting cervico-medullary motor evoked potentials (CMEPs). A high-voltage electrical current was passed between surface electrodes placed over the skin covering mastoid processes stimulator Model DS7AH; Digitimer, Welwyn Garden City, Hertfordshire, UK). The stimulation intensity (pulse duration: 100 μ s; 400 volt square-wave) was adjusted to produce CMEP amplitudes that matched the MEP amplitudes during a brief 5% MVC contraction.

Experimental protocol

The experimental protocol (Fig. 1) used for this study was very similar to the one used previously in our laboratory (Halperin et al. 2014a; Pearcey et al. 2014). Subjects performed 3 elbow flexor MVCs of the dominant arm with 2 minutes rest between trials. A set of three responses (MEP, CMEP and M-max) were recorded with an inter-stimulus

interval of 1.5 s during 5 s MVCs (Pearcey et al. 2014). During each contraction, MEP, CMEP and M-max were elicited at 2, 3.5 and 5 s, respectively. TMS and TMES were triggered automatically, whereas stimulation of the peripheral nerve was performed manually at 5 s. All participants were able to regain voluntary force adequately after each stimulus. The reason that MEP was evoked at 2 s was to give the participants adequate time to reach maximal force production. Furthermore, two seconds after the MVC ceased and subjects completely relaxed, they received another peripheral nerve stimulus eliciting a potentiated twitch (PT), which was used to measure evoked contractile properties of the elbow flexors. After completion of the upper-body pretest measurements, subjects performed a warm-up for the knee extensors, consisting of 12 isometric contractions at 50% of perceived knee extension MVC. This was followed by two dominant knee extension MVCs with 2 min rest between contractions. Then, subjects either performed the fatiguing protocol (intervention condition) or rested for 7 min (control condition).

The fatiguing protocol used in this study has been employed previously in our laboratory (Halperin et al. 2014a), which resulted in considerable knee extensor muscle fatigue (i.e. post-test MVC dropped by 35% compared with baseline values) and accumulation of blood lactate. The fatiguing protocol consisted of five sets of dynamic bilateral knee extensor contractions performed until task failure. The load was equivalent to the isometric knee extension MVC force in dominant leg. One-minute rest was given between sets. Failure was defined as the inability to fully extend the knee during the contractions, which was measured by touching the shin to an exercise band tied parallel to the ground at full extension, or by not keeping a constant pace of “1 s concentric and 1 s eccentric contraction” dictated by a metronome. Subjects were constantly motivated

during the protocol and were reminded to keep their upper body as relaxed as possible. Biceps brachii activity was monitored throughout the entire fatiguing protocol to ensure that EMG activity was no different than during rest. If there was evidence of activation exceeding 0.05V, the subjects were first reminded to relax their arm, and if after two warnings they were not able to relax their biceps brachii muscle, exercise was stopped. Immediately after the last set of knee extension contractions, subjects performed the post-test protocol, which included 12 isometric elbow flexion MVCs at a work to rest ratio of 5 to 10 s. During the 1st, 3rd, 6th, 9th and 12th MVC, subjects received the same set of stimuli as the pre-test MVCs. Potentiated twitches were elicited after these contractions as well. During the control session, subjects underwent the exact same pre- and post-test measurements, but instead of performing the fatiguing dynamic knee extensions, they sat on the knee extension chair for 7 min, which was the approximate time period required to complete the fatiguing protocol.

Outcome measures

Knee extensors. During the fatigue session, the number of repetitions performed at each set was measured.

Dominant elbow flexors. The following variables were measured for the three elbow flexion MVCs at pre-test, as well as the 1st, 3rd, 6th, 9th and 12th post-test MVCs. Mean muscle force production and the background EMG (root mean square (rmsEMG)) of the biceps and triceps brachii muscles were quantified over 50 ms duration prior to the point of each stimulation (TMS, TMES, BPES). Peak-to-peak amplitudes of the MEP, CMEP and M-max were measured. The duration of silent period (ms) was assessed for

MEPs as the interval from the stimulus artifact to the return of the continuous EMG by visual inspection. Because the M-max can change as a result of the voluntary activation, the MEP and CMEP were divided by the following M-max during each MVC. Normalized MEP and CMEP data made it possible to compare these values between different testing sessions. The recorded MEP from the target muscle, elicited by magnetic stimulation of the motor cortex, accesses the entire motor pathway from the motor cortex to the muscles performing the task. Stimulation of corticospinal pathway at the transmastoid level evokes CMEP from the same motor axons that are activated by TMS (Gandevia et al. 1999). Therefore, differences between the MEP and CMEP (MEP/CMEP ratio) were measured to assess whether any changes were occurring at the supraspinal or spinal levels. Maximal twitch tension evoked by BPES at rest after MVCs (potentiated twitch) was calculated before and after the fatiguing task to monitor changes in muscle contractile properties. BPES at the Erb's point stimulates multiple nerves innervating different muscles in the upper limb, including the antagonistic elbow extensors. However, muscle electric stimulation does not fully activate stimulated muscles (Maffiuletti 2010) and synergists (Allen et al. 1998), and similarly, has been shown to stimulate antagonistic muscles as well (Lampropoulou et al. 2012; Neyroud et al. 2015). We chose the BPES method as opposed to muscle electric stimulation in an attempt to decrease the number of stimulation techniques from four (TMS, TMES, BPES and muscle electric stimulation) to three, in order avoid the prolongation of MVCs (four stimuli required each MVC to be at least a 7s) and ii) to give a more detailed timeline of changes in corticospinal excitability which can change quickly following an exercise intervention (Taylor et al. 2000). Furthermore, we were interested in the relative change in muscle contractile properties

and voluntary activation (interpolated twitch technique: ITT) and not absolute values. In this study, we measured ITT voluntary activation by comparing M-max superimposed twitch with potentiated twitch using the following formula:

$$VA\% = (1 - M\text{-max SIT}/PT) * 100 \text{ (Behm et al. 1996)}$$

To account for variability in the outcome measurements between the testing days, all elbow flexor dependent variables were normalized to the average value of the three pre-test trials and as such are reported as a percentage.

Statistical Analysis

Statistical analyses were computed using SPSS software (Version 16.0, SPSS, Inc, Chicago, IL). Assumption of normality (Shapiro-Wilk test) and sphericity (Mauchly test) were tested for all of the dependent variables. If the assumption of sphericity was violated, the corrected value for non-sphericity with Greenhouse-Geisser epsilon was reported. First, intraclass correlation coefficients (ICC) were measured for mean force, EMG, superimposed twitch and potentiated twitch for the three pretests of both conditions to assess consistency of this data. Second, a two-way repeated measures analysis of variance (ANOVA) (2 conditions \times 5 MVCs) was conducted to determine differences between conditions in the following variables: normalized dominant elbow flexion MVC force, biceps brachii and triceps brachii EMG, MEP, CMEP and M-max amplitude, MEP/CMEP ratio, cortical silent period, superimposed twitch force, potentiated twitch force and ITT voluntary activation. To ensure that background EMG activity was similar before TMS and TMES, we compared EMG activity 50ms before

each stimulation. Paired sample t-tests corrected with Bonferroni were used to decompose significant interactions, and Bonferroni post hoc tests were used if main effects were found. Significance was set at 0.05. Cohen's d effects sizes (ES; Cohen 1988) were also calculated to investigate the standardized magnitude of change for all significant results according to the criterion of $d < 0.2$ was classified as "trivial", $d = 0.2 - 0.49$ was considered as "small" effect size; $d = 0.5 - 0.79$ represented a "medium" effect size; and $d > 0.8$ represented a "large" effect size (Cohen 1988). Data in the text are reported as means \pm SD, and shown in the figures as means \pm SE.

One subject was removed from M-max analysis, since his values were multiple times higher compared with other subjects, and thereby defined as an outlier.

2.4. Results

Reliability: The ICC of pre-test trials for various measurements between two testing sessions are as follows: absolute force (0.92), EMG of biceps brachii (0.87) and triceps brachii (0.94), potentiated twitch force (0.89), superimposed twitch force (0.91).

Knee extension fatigue protocol: All subjects were able to successfully complete the knee extension fatiguing protocol. The number of repetitions (mean \pm SD) decreased throughout the 5 sets: set 1 (23 ± 6), set 2 (16 ± 3), set 3 (14 ± 3), set 4 (13 ± 3) and set 5 (10 ± 3).

Elbow flexion MVC: Normalized elbow flexion MVC (Fig. 2) force showed a main effect for condition ($F_{(1, 13)} = 18.0, p < 0.001$) and repetitions ($F_{(4, 52)} = 41.77, p < 0.001$), whereas there was no significant interaction of condition \times repetitions ($F_{(4, 52)} = 0.16, p = 0.953$). The average MVC of all post-test MVCs was significantly lower in the intervention session compared with control session ($p < 0.001, ES = 0.9, \Delta = 10.3\%$) (Table 1). Furthermore, the maximal force progressively and significantly decreased from repetition #1 to repetition #12 ($p < 0.001$) (Figure2). Repetition #1 was significantly higher compared with repetition #3 ($p = 0.003, ES = 0.6, \Delta 6\%$), repetition #6 ($p < 0.001, ES = 1.2, \Delta 12.1\%$), repetition #9 ($p < 0.001, ES = 1.7, \Delta 16.2\%$) and repetition #12 ($p < 0.001, ES = 2.1, \Delta 19.6\%$).

Biceps and triceps brachii rmsEMG: Biceps brachii rmsEMG before MEP, CMEP and M-wave showed a significant main effect for repetitions ($p < 0.001$), whereas there was no significant condition ($p > 0.43$) or interaction ($p > 0.24$) effects. Within each condition, there were no significant differences in biceps brachii EMG activity before TMS and TMES ($p > 0.08$). In addition, no significant repetition ($p = 0.13$), condition ($p = 0.96$) or interaction effects ($p = 0.25$) were observed for triceps rmsEMG. Biceps brachii EMG before MEP (Fig. 2) was significantly lower at repetition #9 compared with repetition #1 ($p = 0.035, ES = 1, \Delta 18.4\%$), #3 ($p = 0.14, ES = 0.7, \Delta 12.9\%$) and #6 ($p = 0.04, ES = 0.5, \Delta 9.38\%$), whereas repetition #12 was significantly lower compared with repetition #1 ($p = 0.03, ES = 1, \Delta 19.38\%$), #3 ($p = 0.002, ES = 0.7, \Delta 13.8\%$) and #6 ($p = 0.02, ES = 0.5, \Delta 10.3\%$). Similarly, biceps brachii EMG before CMEP was significantly lower at repetitions #6 ($p = 0.009, ES = 0.6, \Delta 10.7\%$), #9 ($p = 0.02, ES = 0.6, \Delta 13.1\%$), and #12 (p

= 0.26, $ES = 0.5$, $\Delta 11\%$) compared with #1, whereas biceps brachii EMG before M-wave showed lower values at repetitions #6 ($p = 0.003$, $ES = 0.8$, $\Delta 16.7\%$), #9 ($p = 0.001$, $ES = 0.9$, $\Delta 19\%$), #12 ($p = 0.001$, $ES = 1.1$, $\Delta 24.7\%$) compared with repetition #1 and lower values at repetitions #6 ($p = 0.014$, $ES = 0.5$, $\Delta 9.6\%$), #9 ($p = 0.015$, $ES = 0.6$, $\Delta 12\%$), #12 ($p = 0.001$, $ES = 0.8$, $\Delta 17.6\%$) compared with #3.

Potentiated Twitch Force: PT force (Fig. 3) demonstrated a main effect for condition ($F_{(1, 13)} = 9.2$, $p = 0.010$) and repetitions ($F_{(4, 52)} = 8.06$, $p = 0.002$), whereas there was no significant interaction effect ($F_{(4, 52)} = 0.67$, $p = 0.497$). PT force was significantly lower in the intervention session compared with control session ($p = 0.01$, $ES = 0.8$, $\Delta 19\%$). Furthermore, PT force was significantly lower at repetition #12 compared with repetition #1 ($p < 0.005$, $ES = 0.96$, $\Delta 23.6\%$), #3 ($p < 0.005$, $ES = 1.27$, $\Delta 29.6\%$), and #6 ($p < 0.005$, $ES = 0.67$, $\Delta 17\%$), but not different compared with repetition #9 ($p > 0.05$).

Supraspinal and spinal responses: MEP peak-to-peak amplitude (Fig. 4) showed no significant condition ($F_{(1, 13)} = 0.01$, $p = 0.908$), repetitions ($F_{(4, 52)} = 0.61$, $p = 0.655$) or interaction effects ($F_{(4, 52)} = 0.90$, $p = 0.471$). Similarly, MEP cortical silent period did not show any condition ($F_{(1, 13)} = 0.08$, $p = 0.776$), repetitions ($F_{(4, 52)} = 1.11$, $p = 0.358$) or interaction effects ($F_{(4, 52)} = 0.632$, $p = 0.642$).

There was a non-significant trend toward higher CMEP amplitudes (Fig. 4) between the two intervention sessions ($F_{(1, 13)} = 3.64$, $p = 0.08$, $ES = 0.5$, $\Delta 20.4\%$), however no repetitions ($F_{(4, 52)} = 0.88$, $p = 0.481$) or interaction effect ($F_{(4, 52)} = 1.25$, $p = 0.299$) was observed for this measurement.

Although the MEP/CMEP ratio did not show a repetitions effect ($F_{(4, 52)} = 0.39$, $p = 0.810$), a significant condition effect ($F_{(1, 13)} = 5.07$, $p = 0.042$, $ES = 2.5$, $\Delta 25\%$) was evident which indicated that the MEP/CMEP ratio was lower in the intervention session (Fig. 4). Furthermore, there was no interaction effect, however there was a strong trend toward significance ($F_{(4, 52)} = 2.42$, $p = 0.06$).

Compound muscle action potential and muscle contractile property: M-max amplitude recorded during MVCs did not show main effects for condition ($F_{(1, 13)} = 1.18$, $p = 0.296$), repetitions ($F_{(4, 52)} = 1.98$, $p = 0.111$), or interaction ($F_{(4, 52)} = 0.34$, $p = 0.71$). The superimposed twitch amplitude showed a significant main effect for repetitions ($F_{(4, 48)} = 2.86$, $p = 0.033$). Post-hoc analysis indicated that only repetition #9 ($p = 0.05$, $ES = 0.49$, $\Delta 80\%$) and #12 ($p = 0.001$, $ES = 0.75$, $\Delta 125\%$) were significantly greater than repetition #1. However, there was not a main effect for condition ($F_{(1, 12)} = 0.09$, $p < 0.768$) or interaction ($F_{(4, 48)} = 1.30$, $p = 0.282$).

Voluntary activation (ITT): The intervention condition showed significantly lower voluntary activation levels (Fig. 3) compared with the control condition ($F_{(1, 12)} = 4.98$, $p = 0.045$, $ES = 2.9$, $\Delta 6.9\%$). There was also a main effect for repetition ($F_{(4, 48)} = 7.975$, $p = 0.001$), whereas no significant interaction effect ($F_{(4, 48)} = 1.056$, $p = 0.39$) was found for this measurement. Post-hoc test showed that repetition #12 was significantly lower compared with repetition #1 ($p = 0.001$, $ES = 2$, $\Delta 7.1\%$), repetition #3 ($p = 0.008$, $ES = 1.6$, $\Delta 6.5\%$), repetition #6 ($p = 0.014$, $ES = 1.2$, $\Delta 4.9\%$) and repetition #9 ($p = 0.005$, ES

= 0.9, Δ 3.6%). Furthermore, repetition #9 ($p = 0.02$, $ES = 1$, Δ 3.5 %) and #6 ($p < 0.034$, $ES = 0.78$, Δ 2.3 %) were significantly lower compared with repetition #1.

2.5. Discussion

The main findings of this study were that bilateral knee extensor neuromuscular fatigue decreased dominant elbow flexors force production and voluntary activation as assessed via the ITT. The bilateral knee extensor fatigue also resulted in a lower MEP/CMEP ratio and a trend toward higher CMEP amplitude recorded from biceps brachii, suggesting that there might be a reduction in supraspinal excitability. We also found evidence of peripheral fatigue in elbow flexor muscles (as evidenced by the reduction of the PT amplitude), which could indicate that systemic metabolic disruption caused by the bilateral knee extensor fatiguing protocol may have contributed to elbow flexor peripheral fatigue. Therefore, our results suggest that the NLMF observed in the elbow flexor muscles could have been mediated by a combination of factors, including decreases in supraspinal excitability, reduction of central motor drive (decreased ITT voluntary activation) and increased peripheral fatigue.

Exercise-induced fatigue can affect rested, non-exercised muscles and impair their performance (Martin and Rattey 2007; Kennedy et al. 2013a; Halperin et al. 2014a,b; Kawamoto et al. 2014). Data in the present study support this concept since elbow flexion MVC force was significantly lower following bilateral knee extension fatigue compared with control condition. The protocol used in our study was previously employed by Halperin et al. (2014a) where they demonstrated that the NLMF induced by knee

extensors did not affect biceps brachii EMG, but managed to decrease elbow flexors force production, which is in line with our findings. Lower force production and no change in biceps brachii EMG in the intervention condition could be due to the non-linear relationship between force and EMG, EMG insensitivity to small force changes and/or a minor shift in wrist pronation-supination, which could influence synergistic muscle contribution (Halperin et al. 2014a). Halperin et al. (2014b) found force and voluntary activation decrements only in the knee extensor muscle, but no change was observed in elbow flexor force performance after contralateral elbow flexor and knee extensor isometric fatigue protocols, suggesting that NLMF effects might be muscle specific. Most of the NLMF studies that have found decrements in force have also found voluntary activation impairments, thus suggesting central nervous system fatigue mediating this phenomenon.

NLMF effects on ITT voluntary activation have been found in several muscle groups including knee extensors (Martin and Rattey 2007; Doix et al. 2013), ankle plantar flexors (Kennedy et al. 2013a), elbow flexors (Sidhu et al. 2014; Todd et al. 2003) and first dorsal interossei (Post et al. 2008). These decrements were evident after various non-local fatiguing tasks such as cycling (Sidhu et al. 2014), dynamic (Halperin et al. 2014a) and isometric (Kennedy et al. 2013a; Martin and Rattey 2007) fatiguing protocols. In the present study, ITT voluntary activation decreased in both conditions throughout the 12 post-test MVCs, however it was more pronounced after the dynamic bilateral knee extensor fatigue. Interestingly, the difference between conditions became progressively larger from the beginning (4.8% difference at repetition #1) to the end (8.8 % difference at the repetition #12) of the post-test contractions (Figure 3). It is not clear what

contributed to ITT voluntary activation deficits, however a potential explanation could be an alteration in responsiveness of corticospinal circuitries that may contribute in inability of central nervous system to adequately drive non-exercised muscles (Millet and Lepers 2004).

In the present study, the knee extension fatigue resulted in lower MEP/CMEP ratio and a trend toward higher CMEP amplitude ($p = 0.08$), with no change in MEP amplitude. It could be speculated that the non-significant but moderate magnitude increased spinal excitability in the intervention condition compensated or balanced for a decreased supraspinal excitability (Nardone et al. 2015) and thereby prevented a drop in the excitability of the corticospinal pathway (i.e. no change in MEP). Our results are in line with Samii et al. (1996) who reported that forearm muscles did not show any changes in MEP after a contralateral homologous fatiguing protocol. Todd et al. (2003) also found no change in MEP amplitude recorded from the biceps brachii after a contralateral homologous fatiguing protocol. However both of these studies measured the effect of NLMF phenomenon on contralateral homologous muscles. To the best of our knowledge, there are only two published articles that have investigated the effect of lower extremity neuromuscular fatigue on upper limb corticospinal excitability. Takahashi et al. (2011) showed MEP facilitation in the resting first dorsal interosseus and the biceps brachii muscles during and immediately following intense leg press exercise. Contrary to their findings, Sidhu et al. (2014) reported a depression of MEP amplitude in the elbow flexors after leg cycling task to failure. The reason for the inconsistency between these finding is unclear, however differences in fatiguing and measurement protocols might explain some of the disparities. Furthermore, neither of these two studies have measured spinal

excitability separately from the measurement of the corticospinal excitability as a whole. Therefore, it was not known how changes in spinal motoneuron excitability contributed to these changes in MEP amplitude.

It has been suggested that group III/IV muscle afferents from working muscles can cause spinal and supraspinal fatigue to remote muscles (Amman et al. 2013; Sidhu et al. 2014). Indeed, Sidhu et al. (2014) found that in the presence of leg fatigue, group III/IV muscle afferents feedback might contribute to elbow flexor supraspinal fatigue. The authors suggested that decreased responsiveness of the motor cortical cells and/or increased intracortical inhibition may be secondary to the group III/IV-mediated inhibition, or disfacilitation. However, it is unlikely that intracortical inhibition contributed to NLMF effects in present study, since the duration of the cortical silent period did not change (Taylor et al.1996). Pearcey et al. (2014) suggested that the decrease in MEP could be masked by increased spinal excitability. In this study, the trend towards an increase in spinal excitability could be attributed to decreased presynaptic inhibition and/or fusimotor system facilitation (Millet and Lepers 2004). However, the impact of these corticospinal excitability changes on voluntary activation and force production is unclear. Indeed, Gandevia et al. (1996) found that muscle ischemia impaired voluntary activation while it did not have any effect on measures of corticospinal excitability. Furthermore, Goodall et al. (2012) and Sidhu et al. (2009) reported a significant decrease in voluntary activation after fatiguing exercise, but no change in MEP, suggesting that failure in central motor drive (i.e. decrease in voluntary activation) differs from an impaired corticospinal excitability.

In most of the studies that found NLMF effects, authors suggested that central fatigue was responsible for these performance decrements, without giving much attention to the peripheral system. To the best of our knowledge, this is the first study that detected peripheral fatigue in the non-exercised muscles after lower body fatigue. Contrary to our findings, some studies (Kennedy et al. 2013a; Martin and Rattey 2007; Rattey et al. 2006) that were able to find NLMF effects on performance and voluntary activation, were not able to find peripheral changes (no differences in peak-twitch force and M-wave compared with control). What these studies have in common is that they all used relatively shorter duration isometric fatiguing protocols (Martin and Rattey 2007; Rattey et al. 2006) or fatigued small muscle groups (Kennedy et al. 2013a), which might not be able to produce the same amount of systemic metabolic disturbance compared with our bilateral dynamic knee extension fatiguing protocol. Nordsburg et al. (2003) and Bangsbo et al. (1996) suggested that higher extracellular potassium concentrations might be responsible for the crossover fatigue effect. In their studies, preceding arm exercise caused shorter leg time to exhaustion, with higher potassium concentration found when leg exercise was paired with arm exercise. Indeed, extracellular accumulation of potassium has been shown to depolarize muscle cells, leading to lower excitability and subsequently causing muscle fatigue (Cairns et al. 1995). It is possible that other factors/metabolites like ATP, H⁺ and inorganic phosphates, work together to disrupt the excitation-contraction coupling and cause muscle fatigue (Allen et al. 2008; Ament and Verkerke 2009). However, due to methodological limitations, Nordsburg et al. (2003) and Bangsbo et al. (2003) did not measure peripheral fatigue specifically. Halperin et al. (2014a), who employed the same leg fatiguing protocol as the present study, found a 19%

increase in blood lactate detected at the finger, however peripheral fatigue was not directly measured. Lactate progressively increases with other metabolites (Allen et al.2008; Ament and Verkerke 2009), and therefore it is plausible to suggest that an increase in metabolic byproducts was responsible for peripheral elbow flexor fatigue. We observed a decrease in PT, however the M-max amplitude did not change, suggesting that a relatively similar membrane action potential resulted in a lower evoked force output (Kent-Braun 1997).Further NLMF research is needed to examine the effect of different types and durations of contractions and their effect on metabolic byproduct concentrations and peripheral properties.

In conclusion, our data indicate that bilateral knee extensor fatigue produced force production, voluntary activation and excitation-contraction coupling deficits in the dominant elbow flexors with no change in biceps brachii EMG activity. Furthermore, bilateral knee extensor fatigue modulated corticospinal excitability projecting to the dominant elbow flexor muscles, indicating complex interactions between central and peripheral mechanisms contributing to NLMF effects. More research is needed employing different types of fatiguing protocols that monitor peripheral muscle properties in conjunction with central system properties and performance measures. Moreover, contributions of corticospinal excitability changes to cross-over fatigue need to be investigated, where spinal excitability will be measured independently of the excitability of the whole corticospinal tract, since this will provide insights to the response of different structures to non-local muscle fatigue

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2.7. References

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2.8. Table Legends

Table 1. The mean (\pm SD) of the absolute data recorded from the non-exercised dominant elbow flexors muscles including compound muscle action potential superimposed twitch (M-max SIT), potentiated twitch (PT) force, voluntary activation, peak-to-peak amplitudes of compound muscle action potentials (M-max) and motor evoked potentials (MEP) and cervicomedullary evoked potentials normalized to M-max recorded from non-exercised biceps brachii muscle, MEP/CMEP ratio, duration of MEP silent period (SP) for two conditions (Intervention and Control) and five time points (repetition number 1, 3, 6, 9 and 12)

2.9. Figure Legends

Figure 1. Experimental design and procedures. The order in which the different types of stimulations were delivered is depicted and was kept constant for all subjects and for both sessions. Black arrow pointing down represents transcranial magnetic stimulation, grey arrow represents transmastoid electric stimulation, white arrow represent brachial plexus electrical stimulation and black arrow pointing to the right represents rest period.

Figure 2. MVC force and biceps brachii EMG. Group data are expressed as means \pm SE and normalized to pre-test. A) Arm MVC force normalized to pre-test. ‡ indicates that MVC force was significantly lower in the intervention condition compared with control condition ($p < 0.001$). * indicates that MVC force significantly and progressively decreases from repetition #1 until repetition #12 ($p < 0.001$). B) EMG activity of the biceps brachii muscle measured 50 ms before MEP. * indicates that biceps brachii EMG activity was significantly lower at repetition #9 and #12 compared with repetition #1, #3 and #6 ($p < 0.04$).

Figure 3. Muscle evoked responses and voluntary activation. Group data are expressed as means \pm SE and normalized to pre-test. A) M-max SIT normalized to pre-test. * indicates that M-max SIT was significantly higher at repetition #9 ($p = 0.05$) and #12 ($p = 0.001$) compared with repetition #1. SIT – superimposed twitch. B) Peak potentiated twitch force (PT) normalized to pre-test. * indicates that PT force was significantly smaller at repetition #12 compared with repetition #1 ($p < 0.005$), repetition #3 ($p <$

0.005) and repetition #6 ($p < 0.005$); ‡ indicates that potentiated twitch was significantly lower in the intervention condition compared with control condition ($p = 0.010$). C) Voluntary activation normalized to pre-test. ‡ indicates that intervention condition was significantly smaller compared with control condition. * indicates that repetition #12 was significantly smaller compared to all other repetitions ($p < 0.02$). † indicate that repetition #1 was significantly higher compared with repetition #6 and #9. SIP – superimposed twitch.

Figure 4. Corticospinal excitability. Group data are expressed as means \pm SE and normalized to pre-test. MEP/M-max normalized to pre-test did not show any condition, repetition or interaction effect ($p > 0.47$). There was a trend toward higher CMEP amplitude in the intervention session ($p = 0.08$), however there wasn't any repetition or interaction effect ($p > 0.29$). ‡ indicates that MEP/CMEP ratio was significantly smaller in the intervention condition compared with control condition ($p = 0.042$).

Figure 5. Evoked raw EMG responses recorded from biceps brachii muscle of a single subject in response to motor cortical (MEP), spinal (CMEP) and peripheral nerve stimulation (M-max) for intervention (dark line) and control (light line) condition. Data are presented for the pre-test (average of the three pre-test values) and for 5 post-test MVC contractions (repetition #1, #3, #6, #9, #12)

2.10. Tables

Variable	Conditions	Pre-test	Repetition #1	Repetition #3	Repetition #6	Repetition #9	Repetition #12
MVC (N)	Intervention	375.8 (59)	352.8 (64)	327.6 (53)	304.3 (49)	288.8 (45)	275.2 (49)
	Control	358.6 (46)	366.9 (50)	347.2 (49)	323.7 (30)	309.8 (33)	300.6 (40)
Biceps EMG (mV)	Intervention	0.45 (0.17)	0.50 (0.18)	0.44 (0.14)	0.42 (0.13)	0.40 (0.14)	0.37 (0.12)
	Control	0.36 (0.10)	0.37 (0.13)	0.37 (0.10)	0.36 (0.10)	0.31 (0.08)	0.31 (0.07)
M-max SIT (N)	Intervention	2.22 (1.55)	2.76 (1.90)	3.32 (1.76)	3.95 (2.72)	4.87 (3.02)	5.23 (3.52)
	Control	2.37 (1.47)	2.97 (1.73)	3.23 (2.10)	4.18 (2.45)	2.88 (1.81)	3.94 (2.28)
PT force (N)	Intervention	68.5 (21.9)	54.5 (23.1)	61.2 (26.1)	50.6 (17.4)	47.0 (13.9)	39.1 (16.2)
	Control	66.3 (27.8)	67.3 (30.4)	69.7 (30.7)	57.2 (22.9)	55.9 (22.5)	45.9 (21.1)
VA (%)	Intervention	96.3 (3.4)	92.1 (9.7)	91.9 (9.2)	89.2 (10.4)	87.5 (10.3)	83.9 (11.1)
	Control	95.3 (5.5)	95.1 (3.8)	94.6 (7.4)	93.5 (7.1)	93.7 (5.1)	90.4 (7.6)
M-max	Intervention	5.68 (1.80)	6.21 (2.04)	6.79 (2.35)	7.02 (2.25)	6.96 (2.18)	6.72 (2.70)
	Control	5.42 (1.44)	5.51 (1.81)	5.76 (1.65)	5.86 (1.28)	5.84 (1.61)	6.01 (1.34)
MEP/M-max ratio	Intervention	0.67 (0.18)	0.69 (0.19)	0.73 (0.18)	0.69 (0.21)	0.65 (0.16)	0.71 (0.23)
	Control	0.62 (0.11)	0.70 (0.22)	0.68 (0.25)	0.67 (0.21)	0.66 (0.18)	0.59 (0.22)
CMEP/M-max ratio	Intervention	0.55 (0.14)	0.56 (0.19)	0.52 (0.18)	0.71 (0.49)	0.58 (0.28)	0.67 (0.29)
	Control	0.54 (0.14)	0.50 (0.22)	0.53 (0.24)	0.52 (0.19)	0.46 (0.20)	0.40 (0.23)
MEP/CMEP ratio	Intervention	1.35 (0.45)	1.37 (0.63)	1.66 (0.90)	1.14 (0.42)	1.28 (0.58)	1.24 (0.60)
	Control	1.35 (0.30)	1.57 (0.61)	1.41 (0.52)	1.58 (1.07)	1.72 (0.74)	1.86 (0.96)
MEP SP (sec)	Intervention	0.12 (0.03)	0.12 (0.03)	0.11 (0.04)	0.11 (0.04)	0.12 (0.04)	0.11 (0.04)
	Control	0.12 (0.05)	0.12 (0.05)	0.12 (0.05)	0.12 (0.05)	0.13 (0.05)	0.12 (0.05)

Table 1. The mean (\pm SD) of the absolute data recorded from the non-exercised dominant elbow flexors muscles including compound muscle action potential, superimposed twitch (M-max SIT), potentiated twitch (PT) force, voluntary activation, peak-to-peak amplitudes of compound muscle action potentials (M-max) and motor evoked potentials (MEP) and cervicomedullary evoked potentials normalized to M-max recorded from non-exercised biceps brachii muscle, MEP/CMEP ratio, duration of MEP silent period (SP) for two conditions (Intervention and Control) and five time points (repetition number 1, 3, 6, 9 and 12)

2.11. Figures

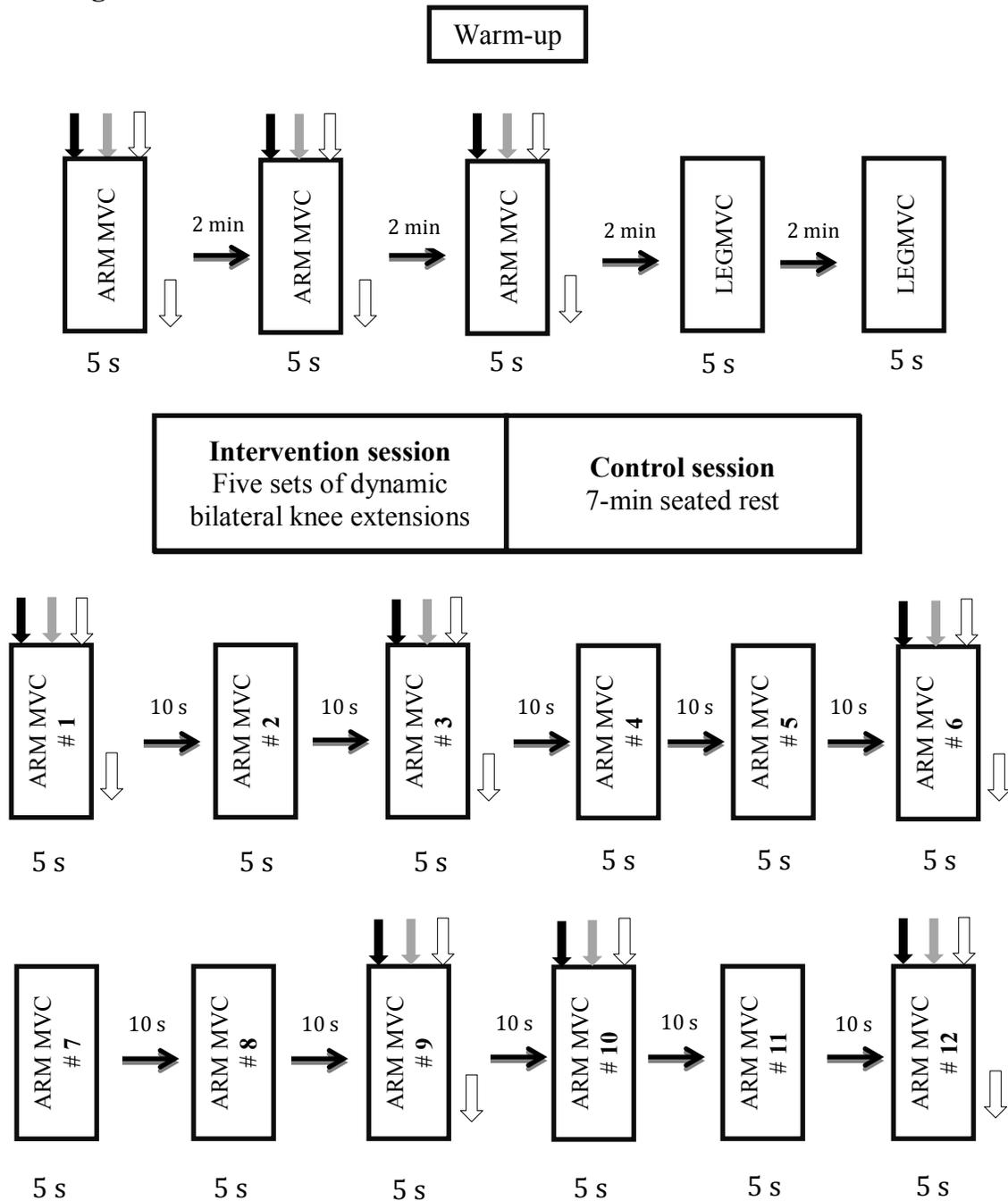


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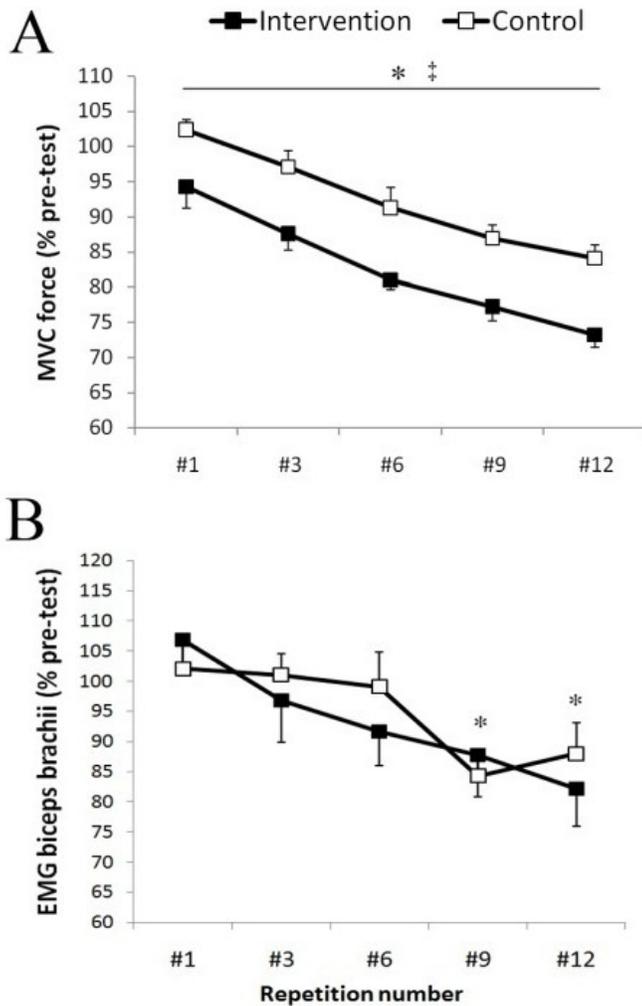


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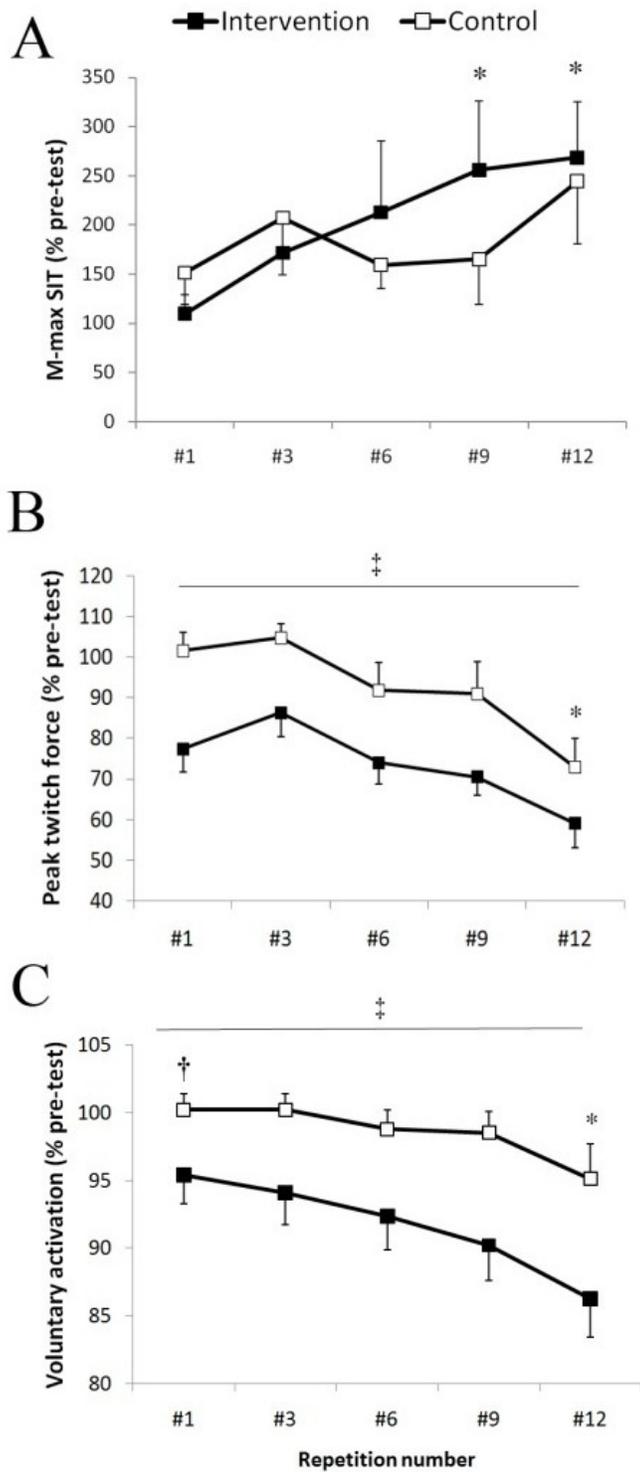


Figure 3. Muscle evoked responses and voluntary activation. Group data are expressed as means \pm SE and normalized to pre-test. A) M-max SIT normalized to pre-test. * indicates

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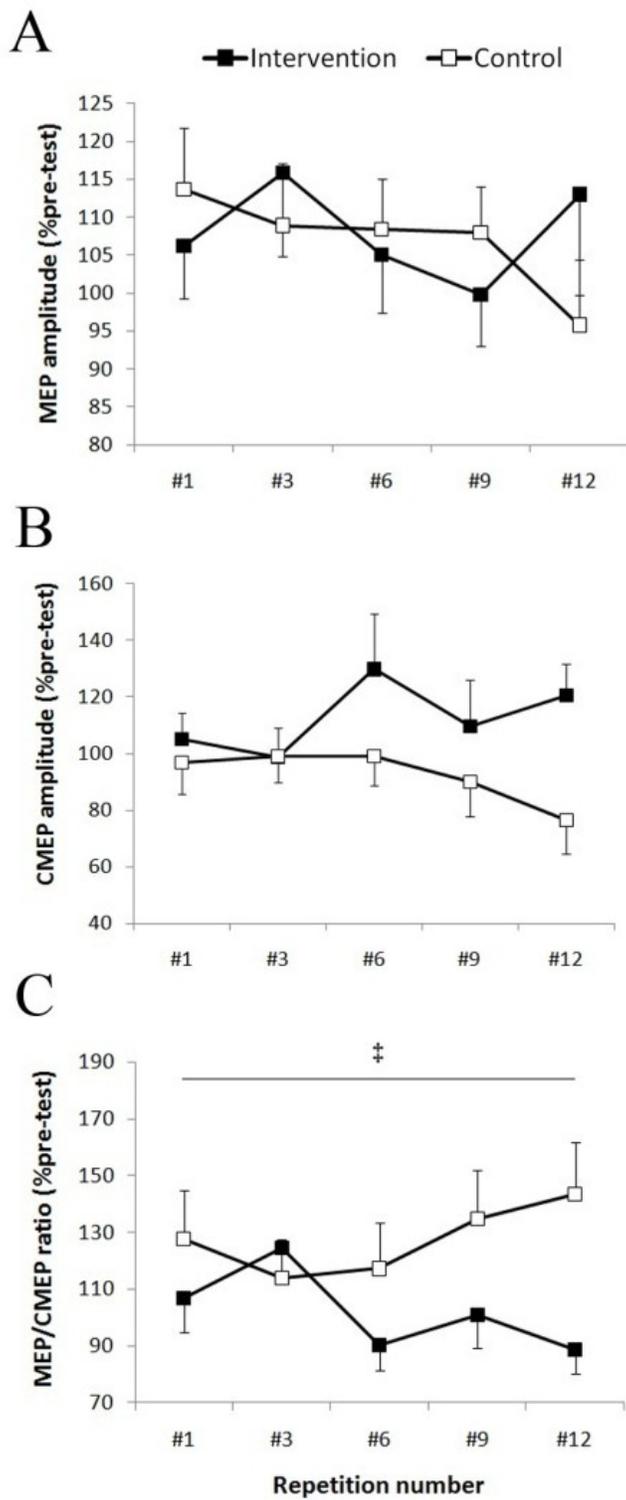


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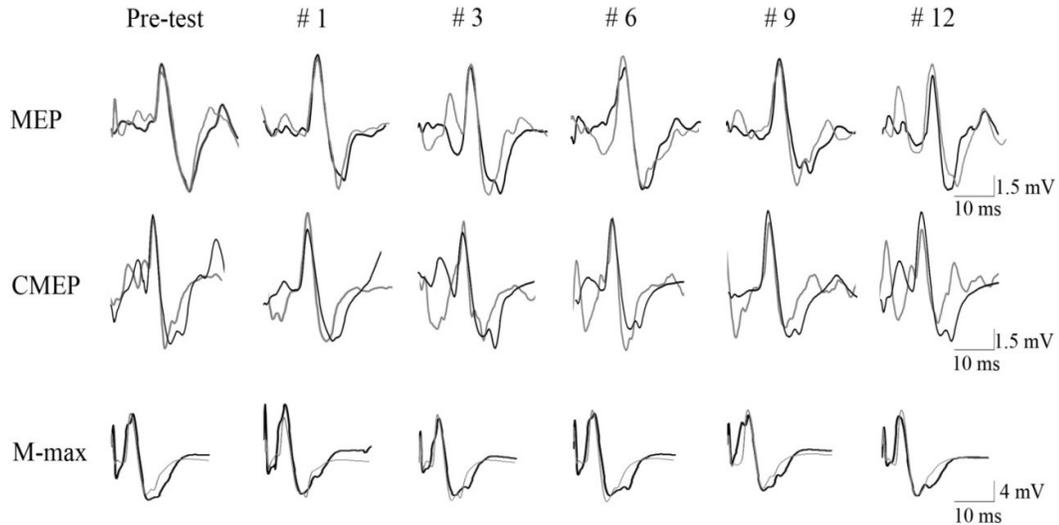


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APPENDIX A: Magnetic Stimulation safety checklist

Please answer the following questions by circling yes or no.

1. Do you suffer from epilepsy, or have you ever had an epileptic seizure? **YES/NO**
2. Does anyone in your family suffer from epilepsy? **YES/NO**
3. Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings) **YES/NO**
4. Do you have an implanted medication pump? **YES/NO**
5. Do you wear a pacemaker? **YES/NO**
6. Do you suffer any form of heart disease? **YES/NO**
7. Do you suffer from reoccurring headaches? **YES/NO**
8. Have you ever had a skull fracture or serious head injury? **YES/NO**
9. Have you ever had any head surgery? **YES/NO**
10. Are you pregnant? **YES/NO**
11. Do you take any medication? **YES/NO**
 - a. Note if taking medication, check list for contraindicated medication on next page.
12. Do you suffer from any known neurological or medical conditions? **YES/NO**
13. Are you out of 18-45 year range who is not involved in regular physical activity (at least 3-5 days per week) such as running and resistance training? **YES/NO**

Comments:

Name: _____

Signature: _____

Date:

Medications contraindicated with magnetic stimulation: 1) Tricyclic antidepressants

Name	Brand name
amitriptyline (&butriptyline)	Elavil, Endep, Tryptanol, Trepiline
desipramine	Norpramin, Pertofrane
dothiepin hydrochloride	Prothiaden, Thaden
imipramine (&dibenzepin)	Tofranil
iprindole	-
nortriptyline	Pamelor
opipramol	Opipramol-neuraxpharm, Insidon
protriptyline	Vivactil
trimipramine	Surmontil
amoxapine	Asendin, Asendis, Defanyl, Demolox, Moxadil
doxepin	Adapin, Sinequan
clomipramine	Anafranil

2) Neuroleptic or Antipsychotic drugs

A) Typical antipsychotics

• Phenothiazines: • Thioxanthenes:

- o Chlorpromazine (Thorazine) o Chlorprothixene
- o Fluphenazine (Prolixin) o Flupenthixol (Depixol and Fluanxol)
- o Perphenazine (Trilafon) o Thiothixene (Navane)
- o Prochlorperazine (Compazine) o Zuclopenthixol (Clopixol and Acuphase)
- o Thioridazine (Mellaril) • Butyrophenones:
- o Trifluoperazine (Stelazine) o Haloperidol (Haldol)
- o Mesoridazineo Droperidol
- o Promazineo Pimozide (Orap)
- o Triflupromazine (Vesprin) o Melperone
- o Levomepromazine (Nozinan)

B) Atypical antipsychotics

- Clozapine (Clozaril)
- Olanzapine (Zyprexa)
- Risperidone (Risperdal)
- Quetiapine (Seroquel)
- Ziprasidone (Geodon)
- Amisulpride (Solian)
- Paliperidone (Invega)

C) Dopamine partial agonists:

Aripiprazole (Abilify)

D) Others

Symbyax -A combination of olanzapine and fluoxetine used in the treatment of bipolar depression. Tetrabenazine (Nitoman in Canada and Xenazine in New Zealand and some parts of Europe Cannabidiol One of the main psychoactive components of cannabis.

APPENDIX B: Physical activity readiness questionnaire

PAR-Q & YOU

(A questionnaire for People Aged 15-69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with your doctor before you start.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly: check **YES** or **NO**

YES <input type="checkbox"/>	NO <input type="checkbox"/>	1. Has your doctor ever said that you have a heart condition <u>and</u> that you should only do physical activity recommended by a doctor?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	2. Do you feel pain in your chest when you do physical activity?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	3. In the past month, have you had chest pain when you were not doing physical activity?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	4. Do you lose your balance because of dizziness or do you ever lose consciousness?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	5. Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	6. Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	7. Do you have a diabetes or thyroid condition?
YES <input type="checkbox"/>	NO <input type="checkbox"/>	8. Do you know of <u>any other reason</u> why you should not do physical activity?

If you answered:

YES to one or more questions

A medical clearance form is required of all participants who answer 'yes' to any of the eight PAR-Q questions.

Note: Personal training staff reserve the right to require medical clearance from any client they feel may be at risk.

- Discuss with your personal doctor any conditions that may affect your exercise program.
- All precautions must be documented on the medical clearance form by your personal doctor.

NO to all questions

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can:

- start becoming much more physically active - begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal - this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.



DELAY BECOMING MUCH MORE ACTIVE:

- If you are not feeling well because of a temporary illness such a cold or a fever - wait until you feel better; or
- If you are or may be pregnant - talk to your doctor before you start becoming more active.

PLEASE NOTE: If your health changes so that you then answer YES to any of the above questions, tell your fitness or health professionals. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability to persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

“I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction.”

NAME _____

SIGNATURE _____

DATE _____

SIGNATURE OF PARENT _____

WITNESS _____

or GUARDIAN (for participants under the age of majority)



Note: This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Supported by:  Health Canada Santé Canada Physical Activity
Readiness Questionnaire – PAR-Q (revised 2006 by CW)