Understanding corticospinal excitability to the biceps brachii during maximal repeated arm-cycling sprints

By:

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Thesis Abstract

Studies investigating neuromuscular fatigue (NMF) induced by repeated sprint exercise primarily use a tonic contraction to assess corticospinal excitability (CSE). There is a lack of information about how CSE transiently changes with rapidly changing fatigue levels. Previous research has shown that CSE is dependent on a variety of factors, such as the targeted muscle, mode of exercise, hand position, cadence, cycling direction, and level of fatigue. No study has assessed CSE during repeated maximal arm-cycling sprints. The current study circumvents the limitations of a tonic contraction to assess CSE. We performed maximal repeated arm-cycling sprints using a custom-built cycle ergometer. Transcranial magnetic stimulation (TMS), transmastoid electrical stimulation (TMES), and brachial plexus stimulation (Erb's point) were given during each sprint to determine how CSE to the biceps brachii was modulated during 5, approximately 20 second repeated sprints. The sprint protocol induced NMF as evidenced by mean power ($p < 0.0001$) and total work ($p < 0.0001$) dropping by 36.8% from sprint one to five respectively. There was a 4.57% decrease in TMES intensity required for the active motor threshold (AMT) from pre- (134.00 ± 31.52 mA) to post sprinting (124.17 ± 30.08 mA), $t(12) = 3.445$, $p = 0.0055$. Our findings suggest that our protocol increased spinal and peripheral excitability from pre to post sprinting, but not cortical because of the absence in change in MEP amplitudes from pre to post sprinting Changes at the spinal motoneuron and post-activation potentiation are likely mechanisms associated with the modulations.

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List of Abbreviations

AMT – active motor threshold

CMEP – cervicomedullary motor evoked potential

CNS – central nervous system

EMG – electromyography

H-relflex – Hoffman reflex

ITT – interpolated twitch technique

MEP – motor evoked potential

Mmax – maximum amplitude of the compound muscle action potential

MVC – maximum voluntary contraction

M-wave – compound muscle action potential

mV – millivolt

μV – microvolt

PNS – peripheral nervous system

RPE – rate of perceived exertion

RPM – revolutions per minute

SD – standard deviation

TMS – transcranial magnetic stimulation

TMES – transmastoid electrical stimulation

CHAPTER 1: INTRODUCTION

1.1 Background of the study

Fatigue is the most significant limiting factor in exercise performance and is often a significant symptom of illness and disease. The central and peripheral nervous systems are influenced by various factors that contribute to neuromuscular fatigue (NMF). Intense exercise over a prolonged period is a simple method for inducing NMF. It is crucial to understand the etiology of how the brain, spinal cord, and periphery modulates corticospinal excitability (CSE) in the presence of fatigue induced by intense exercise. The basic neurophysiological mechanisms provide insights to reveal methods to improve and mitigate fatigue.

The majority of research on repeated sprints was done using the lower body in athletic or chronically resistance trained individuals (Billaut et al., 2006; Girard et al., 2013; Mendez-Villanueva et al., 2008; Monks et al., 2016; Pearcey et al., 2015) . Therefore, it is prudent to understand the physiological differences between the upper and lower limbs prior to performing research on repeated arm-cycling sprints. There are notable physiological differences between the upper and lower body, such as higher oxygen (O_2) extraction in the legs than the arms due to a longer mean transit time, larger diffusing area in the legs, and a smaller diffusing area in the arms (Calbet et al., 2005). Furthermore, the most significant physiological difference between the legs and arms is muscle fiber composition (Koppo et al., 2002). The legs have a higher proportion of type 1 muscle fibers, and the arms have a higher proportion of type 2 muscle fibers. However, research is limited on the upper body regarding sprint-related tasks. Therefore, it merits further investigation. Current methodologies for assessing CSE during fatiguing exercise are provide inconsistent results because changes in supraspinal and spinal excitability in response to fatigue are task-dependent (Carroll et al., 2006; Taylor & Gandevia, 2008) and the neural control of voluntary motor output depends both on the mode of the task (Forman et al., 2014) and the muscle

type (flexor versus extensors or fast versus slow twitch muscle fibers) being examined (Giesebrecht et al., 2010; Hoffman et al., 2009). To our knowledge, there are no studies that have assessed CSE during maximal repeated sprints in the upper or lower body. Thus, in theory, the correct method to truly understand how CSE changes temporally during a fatiguing protocol is to assess it during the task, so it is task-specific. Previous research has shown that arm-cycling produces higher CSE to the biceps brachii than an intensity matched tonic contraction (Forman et al., 2014; Forman et al., 2019). Various physiological factors account for such changes, including increased activity of the central pattern generators (Zehr & Duysens, 2004) and excitatory synaptic input to the motor pool (Forman et al., 2015). The utilization of transcranial magnetic stimulation (TMS) (supraspinal), transmastoid electrical stimulation (TMES) (spinal), and Erb's point (periphery) provide a segmented picture of the corticospinal pathway. Incorporating these techniques in a study allows us to confine changes to each segment and consequently provide insights to the mechanisms that account for observed differences.

Any CSE modulations observed during a fatiguing protocol involving human participants are predominantly inferred assumptions. It is unsuitable to do invasive experimental research during dynamic motor outputs such as maximal repeated arm-cycling sprints. Therefore, this study intends to provide a better explanation for the changes in CSE during intense fatiguing exercise.

1.2 Purpose of the study

To investigate how CSE of the biceps brachii is modulated during maximal repeated arm-cycling sprints using chronically resistance-trained individuals. The research objectives of this thesis are 1) to successfully measure CSE of the biceps brachii during repeated maximal arm-cycling sprints and 2) to determine if CSE of the biceps brachii is altered during an exhaustive upper-body locomotor activity (i.e., arm-cycling sprints).

1.3 Significance of the study

This study will help us understand how CSE in the upper body is modulated with increasing fatigue levels during dynamic rhythmic exercise. This study is both methodologically and physiologically significant because it involves using a novel technique for assessing CSE. While this study will inevitably provide insight for chronically resistance trained populations, findings may also apply to non-resistance trained individuals and even provide information for rehabilitative purposes.

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CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Corticospinal excitability (CSE) has been measured intermittently during a maximal locomotive task via a submaximal isometric voluntary contraction (MVC) (Pearcey et al., 2016)). CSE has also been measured frequently during arm cycling (Forman et al., 2019; Forman et al., 2015; Lockyer et al., 2018; Spence et al., 2016). However, these studies did not measure CSE during the sprints. Therefore, the neural adjustments that might occur during repeated maximal arm-cycling sprints are unknown. Supraspinal and spinal modulations in response to fatigue are exercise task-dependent (Carroll et al., 2006; Hoffman et al., 2009), and the neural control of voluntary motor output depends both on the mode of task (Forman et al., 2014) and muscle type (Giesebrecht et al., 2010; Hoffman et al., 2009). Accordingly, experimental protocols should not use a tonic contraction to assess CSE for arm-cycling sprints. Instead, experimental protocols should assess CSE during the sprint. However, there is no evidence of this in the literature. The purpose of this literature review is to discuss past and current research related to the development of neuromuscular fatigue (NMF) during repeated sprinting, including mechanisms underlying this fatigue and the methods used to determine this fatigue.

2.2 Neuromuscular Fatigue

NMF occurs when there is an impairment in the skeletal muscle's ability to sustain force output (Gandevia, 2001; Gandevia et al., 1999). Peripheral and central nervous system factors contribute to the development of NMF (Collins et al., 2018). In consideration of rhythmic exercise such as arm-cycling, a typical observation of NMF includes a decrease in power output, whereby power is a function of revolutions per minute and resistance applied by the mechanical brake of the cycle ergometer (Pearcey et al., 2016). During tonic contractions, a typical observation of NMF includes a decrease in force output measured by a load cell (Cadigan et al., 2017). While NMF

occurs during both modes of exercise, the development of fatigue is different, including the time course and the resultant cortical, spinal, and peripheral modulations (Collins et al., 2018).

2.3 Peripheral Fatigue

Peripheral fatigue may result in impairments of all or any combination of the following factors: neuromuscular junction, sarcolemmal membrane, excitation-contraction coupling, accumulation of metabolites, or depletion of fuels (Kirkendall, 1990). Essentially, any impairment distal to the motoneuron can be classified as peripheral fatigue (Kirkendall, 1990). Peripheral fatigue is generally studied using electromyography (EMG) and muscle twitch properties as there are no in vivo methods to study it directly (Kirkendall, 1990). The interpolated twitch technique measures the central nervous system (CNS)'s capacity to completely activate contracting muscle (Shield & Zhou, 2004). Pearcey et al. (2016) utilized the interpolated twitch method to assess voluntary activation with increasing levels of fatigue intermittently between sprints. They found that as fatigue increased, voluntary activation decreased. Changes with the potentiated twitch help understand contractile properties of the muscle by means of measuring the peak twitch, time to peak twitch, and half-relaxation time (Pearcey et al., 2015).

A muscle twitch is the basic contractile property of a motor unit. The time to peak twitch force primarily depends on the rate that Ca^{2+} is released from the sarcoplasmic reticulum (Enoka, 2008). Long or fast rates to peak force are correlated to slow or fast-twitch motor units. The peak twitch is known to reflect excitation-contraction coupling (Robbins et al., 2010). A reduction in twitch force indicates an impairment of the excitation-contraction coupling process of the muscle (Pearcey et al., 2015). Furthermore, half-relaxation time implies reuptake of Ca^{2+} at the sarcoplasmic reticulum. Factors such as impaired release and restoration of intracellular calcium from the sarcoplasmic reticulum, and lower sensitivity between calcium and the contractile

proteins. Inferences from interpreting EMG using a frequency-force relationship have alluded to limited acetylcholine and decreased postsynaptic excitation as potential mechanisms for peripheral fatigue at the neuromuscular junction (Maclaren et al., 1989). EMG median frequency tends to portray changes at the sarcolemma, indicative of muscle fiber fatigue during exercise (Gerdle & Fugl-Meyer, 1992). Furthermore, fatiguing repeated sprint exercise has shown to decrease EMG median frequency – a decrease in frequency of the EMG signal, suggesting that muscle fiber activation may adjust to maintain force output when fatigued (Billaut et al., 2006). A decrease in muscle conduction velocity is also a likely contributing factor to fatigue induced by repeated sprinting (Billaut et al., 2006).

A reduction in muscle fiber conduction velocity may also be related to dysfunction along the sarcolemma (Juel, 1988a). A decrease in muscle pH may cause this dysfunction due to increased concentrations of metabolites such as muscle lactate, pyruvate, H^+, K^+, or P_i (De Luca, 1997; Gerdle & Fugl-Meyer, 1992; Juel, 1988b; Taylor et al., 1997).

Understanding muscle fiber conduction velocity and how recruitment pattern shifts from slow twitch to fast-twitch motor units and vice-versa during repeated sprinting should provide insight into how voluntary activation, hence central motor drive, is modulated during fatiguing exercise. (Billaut et al., 2006). It appears that as the number of sprints increases, type II muscle fibers fatigue more than type I. There is a shift to type I muscle fibers to compensate for a decrement in force (Billaut & Bishop, 2009). Thus, the median frequency decreases during fatiguing exercise. Interestingly, Bishop (2012) suggests that peripheral mechanisms are the primary cause of fatigue during intermittent-sprint exercise.

2.4 Neural Response to Peripheral Fatigue

Peripheral fatigue occurs quickly preceding central fatigue most of the time (Hureau et al., 2016; Pearcey et al., 2016; Weavil & Amann, 2018). Peripheral fatigue seems to reach a "critical threshold" which limits the functional capacity of a muscle in an attempt to prevent damage or failure of the periphery (Amann, 2011) and frequently occurs before the onset of central fatigue (Decorte et al., 2012; Monks et al., 2016; Pearcey et al., 2015). Taylor et al. (2016) detailed that small muscle afferents such as type III and IV are responsible for a feedback loop that results in continuous peripheral nervous system interactions with the CNS and vice-versa. This loop can be seen in Amann (2011) (Figure 1). Therefore, it is intuitive to think that the CNS mitigates the critical threshold of peripheral fatigue. Hence, the reason why central fatigue often develops later during exhaustive exercise.

Simply put, metabolites produced from intensive exercise causes afferent activity to increase, which exert inhibitory feedback on the CNS, decreasing the magnitude of central motor drive and, consequently, reducing muscle power output – portrayed as central fatigue (Amann, 2011). This process is a proposed mechanism for central fatigue during maximal repeated arm-cycling sprints (Pearcey et al., 2016). Furthermore, the rate of perceived exertion by a person is thought to be related to the perception of pain during exercise (Pearcey et al., 2016). Pearcey et al. (2016) showed that as participants performed more sprints, their rate of perceived exertion increased, and from the midway to the end of the sprint protocol, central fatigue increased (Pearcey et al., 2016). Though it cannot be confirmed, the rate of perceived exertion suggests that the participants felt more pain which activates the inhibitory feedback loop shown in (Amann, 2011) (Figure 1). Borg et al. (1985) have demonstrated a similar relationship between blood lactate levels, ratings of perceived exertion, and increasing levels of exercise intensity during cycling.

Other peripheral changes can occur, such as exercise-induced hypoglycemia and external environment-induced hyperthermia, decreasing cortical drive and resulting in central fatigue (Nybo & Secher, 2004). As Nybo and Secher (2004) stated, central and peripheral fatigue mutually affects one another, limiting exercise by a combination of factors.

Commonly, the brain is considered the major component of central fatigue, when in fact, the spinal cord contains neural pathways that are susceptible to inhibition or excitation. Thus, the spine is also a likely component for mitigating peripheral fatigue, like the factors listed above. An example of neural inhibition within the spine is peripheral reflex inhibition of the alpha motoneuron pool (Garland & McComas, 1990). The Hoffman Reflex (H-reflex) is primarily used to assess post-synaptic inhibition of Ia afferent terminals on spinal motoneurons. This technique is performed by electric stimulation, whereby the stimulus is low enough to activate the sensory Ia afferents (Palmieri et al., 2004). The action potentials elicited travel to the spinal cord and elicit excitatory post-synaptic potentials, triggering actions potentials down the alpha motor to the muscle. The resultant signal is recorded in the muscle as an H-reflex. Garland and McComas (1990); (Palmieri et al., 2004) inferred that fatigued muscles might have receptors that project inhibitory afferents to motoneurons, consequently responsible for impairments observed via the H-reflex during fatiguing exercise. Yet, the possibility exists that the descending drive from the brain may still contribute to decreased alpha motoneuron excitability as well.

2.5 Motoneuron

Spinal excitability may decrease during some forms of fatiguing exercise. Opposite to Pearcey et al. (2016) who showed increased spinal excitability due to fatiguing arm-cycling sprints, Weavil et al. (2016) concluded that fatiguing leg cycling exercise diminished spinal excitability. Both studies utilized cervicomedullary motor evoked potential (CMEP)s to assess spinal

excitability. However, there are a few key differences to note. Pearcey et al. (2016) studied the upper limbs using repeated arm-cycling sprints and measured spinal excitability during a submaximal tonic elbow MVC. Weavil et al. (2016) studied the lower limbs during steady-state leg cycling exercise and measured spinal excitability during the exercise. CSE is task-, phase-, intensity-, direction-, and muscle-dependent (Collins et al., 2018), which could account for the differences between the two studies.

It is crucial to understand the physiological changes at the motoneuronal level when understanding alterations in spinal excitability. The following factors are in consideration of decreased spinal excitability due to fatiguing exercise. Some mechanisms for reduced excitability of the motoneuronal pool may include a lack of neurotransmitters due to insufficient release or depletion due to continuous activation (D'Amico et al., 2017). Serotonin, or 5-HT, is a powerful neurotransmitter that affects supraspinal and spinal excitability. Serotonin levels elevate with increased exercise intensity and duration due to the fatty acids released, causing a release of tryptophan – the precursor for serotonin synthesis (Taylor et al., 2016). Serotonin's actions in the central nervous system may be inhibitory or excitatory. During non-fatiguing exercise, serotonin released from the raphe-spinal pathway increases motoneuron excitability and facilitates the degree of persistent inward current activation (Cotel et al., 2013). Persistent inward currents are voltage-dependent currents that help regulate neuronal excitability and motor output (Heckman et al., 2008).

Conversely, there is a serotonin spill-over effect during prolonged activity whereby serotonin reaches the axon initial segment (Cotel et al., 2013). There, it activates 5-HT_{1A} receptors causing the inhibition of action potentials in motoneurons (Cotel et al., 2013). Accordingly, this

inhibits muscle contractions and is a cellular mechanism for central fatigue, specifically spinal fatigue.

While the high serotonergic drive to the motoneurons can cause depression in motoneuron excitability due to the activation of 5-HT_{1A} receptors (Cotel et al., 2013; D'Amico et al., 2017), changes in the motoneuron membrane potential such as increased after-hyperpolarization may also depress motoneuron excitability (Matthews, 1999).

Motoneuron activation may have a very different physiological mechanism than motoneuron inhibition. Activation of the monoaminergic system may cause an optimal dose of serotonin to fuel central pattern generator outputs by increasing neuronal activation (Fornal et al., 1996), and rhythmic outputs such as arm cycling utilize the central pattern generator (Zehr & Duysens, 2004). Moreover, persistent inward currents increase motoneuron excitability (Button et al., 2006). Non-inactivating or slowly inactivating voltage-gated Na+ and Ca2+ channels create depolarizing currents (Lee & Heckman, 1999). Thus, motoneurons can maintain self-sustained rhythmic firing (Button et al., 2006), hence, a more excitable motoneuron pool. Another mechanism for motoneuron excitability is decreased voltage threshold for action potential initiation and decreased spike rise time (Beaumont & Gardiner, 2003; Power et al., 2010). Both of which result in faster action potentials and increase the likelihood for an action potential. Thus, this might account for increased during spinal excitability with repeated arm-cycling sprints (Pearcey et al., 2016)

2.6 Corticospinal Tract

The corticospinal tract is the primary descending pathway from the cortex to the spinal motoneuron for the voluntary control of human movement (Chouinard & Paus, 2006). There is a consensus that a large portion of the corticospinal pathway is from the primary motor cortex

(Martin, 2005). After descending through the brainstem, the axons of the corticospinal tract decussate to the contralateral aspect of the brainstem and extend down to the motoneuron (Nathan & Smith, 1955).

As the name implies, CSE is the excitability from the site of cortical neuronal depolarization to spinal motoneuron depolarization and also to the locomotor muscle (Lockyer et al., 2021). Consequently, the effectiveness of transmitting neural signals from the brain to the locomotor muscle defines CSE (Weavil & Amann, 2018). Exercise can either increase or decrease CSE, and this change is primarily dictated by whether the exercise is fatiguing or not (Collins et al., 2018). Deprivations in excitability require more synaptic input from either or both the motor cortex and spinal motoneurons to maintain the force to sustain the motor task at hand. Hence, this increase in central motor drive, the ability for the brain to deliver force output, should increase corticospinal activation. However, if it is not possible to initiate the central motor drive, such as during fatigue, the resulting decrease in CSE usually causes lower recruitment of motor units, thus, lower muscle activation (Collins et al., 2018). This phenomenon results in central fatigue (Martin et al., 2006; Taylor et al., 2016). While this phenomenon commonly occurs with single joint MVCs, this isn't always the case with fatiguing bouts of exercise. Since CSE comprises supraspinal, spinal, and peripheral excitability, a decrease in corticospinal excitability does not mean that the entire tract is impaired. Instead, reductions in supraspinal excitation may result in compensation via increases in spinal excitation. For example, Pearcey et al. (2016) showed a decrease in supraspinal excitability from pre sprint 1 to post sprint 5, while spinal excitability increased.

2.7 Stimulation Techniques

Stimulation of the brain and the peripheral nerve rely on the same physiological principle of depolarizing neuronal membranes to elicit an action potential (Rossini et al., 2015). The significant difference between stimulating the brain and the periphery is the challenge of delivering a stimulus across the high resistance barrier of the scalp, skull, meninges, and cerebrospinal fluid (Rossini et al., 2015).

2.7.1 TMS

The early approach for measuring CSE involved direct stimulation to the scalp via high-voltage stimuli through two direct-contact electrodes (Merton & Morton, 1980). TMS became a much less invasive or less painful and more practical technique (Barker et al., 1985). TMS uses electromagnetic induction to create a suprathreshold current in the brain to generate an action potential in the cerebral neurons (Rossini et al., 2015). Generally, TMS stimulates the trans-synaptic pyramidal neurons of the corticospinal tract, eliciting indirect waves followed by direct waves (Lazzaro et al., 1998). Furthermore, TMS primarily stimulates large-diameter myelinated axons of neurons (Burke et al., 2000). The action potentials travel down the motoneuron pool in the spinal cord and eventually reach the muscle via the most distal pathway, the alpha motoneuron, which synapses with the target muscle. At the muscle, the surface EMG records the signal as a MEP. A figure-of-eight TMS coil delivers a more focused stimulus to the brain than a single TMS coil (Rossini et al., 2015). However, during intense exercise, a single TMS coil is more appropriate because slight variations in movement may impact the stimulation area and consequently, alter the target area to be assessed (Cadigan et al., 2017; Forman et al., 2015; Pearcey et al., 2016; Weavil et al., 2015). Stimulation of the motor cortex is favored in neurophysiology because alterations in

motor activation and excitability are assessed easily by recording MEPS via EMG (Hallett, 2000; Rossini et al., 2015).

Measuring CSE is necessary to monitor gross changes in the central and peripheral nervous systems. Changes in MEPs indicate a change in the corticospinal pathway (i.e., from cortex to motoneuron), peripheral nerve, and muscle fibers (Pearcey et al., 2016). Thus, MEPs are almost always compared to maximal compound muscle action potential (M_{max}) to confine the changes to the corticospinal pathway (Todd et al., 2003). Usually, amplitude, area, latency, and cortical spinal silent period are analyzed to make inferences about CSE (Aboodarda et al., 2015; Forman et al., 2014; Forman et al., 2016; Pearcey et al., 2016).

Changes in amplitude include mechanisms associated with temporal dispersion and changes within the spine (Bestmann & Krakauer, 2015). The size of a MEP is dependent on the stimulus intensity and excitability of the cortical neurons and motoneuron pool. (Chen et al., 1999). The MEP amplitude provides a measure of the amount of CSE, and the MEP area when compared to the area of maximal EMG identifies the amount of the motor unit pool recruited by TMS (Chen et al., 1999). The size of a MEP increases when corticospinal neurons and motoneurons become more excitable. Thus, contracting muscle during exercise increases MEP size (Chen et al., 1999). A plateau in MEP amplitude during higher force output is the result of a decrease in motoneuron output in response to excitatory input (Todd et al., 2003). Also, a plateau in MEP area at higher forces is due to an inability of the cortical stimulus to excite the motoneuron during the beginning of a recovery cycle (Matthews, 1999).

MEP latency assesses the conduction time for neural impulses from the cortex to reach the muscles of the periphery and provides evidence of the direct pathways to the muscle (Kallioniemi et al., 2015). MEP latency is affected the TMS coil location and direction. Muscle contraction

decreases the threshold for neuronal excitation, which reduces MEP latency. This result may be due to the recruitment of motor units with a higher excitation threshold and the multiple firing of motor units (Kallioniemi et al., 2015).

Supraspinal and spinal factors influence the corticospinal silent period. It provides information about the inhibitory functions within the brain and spine (Hupfeld et al., 2020). The early part of the silent period appears to be mediated by the spine, while the late part seems to be related to motor cortex excitability (Chen et al., 1999). The silent period isanalyzed by a change in length. Specifically, an increase in the silent period is thought to indicate an increase in cortical inhibition during fatiguing exercise (Benwell et al., 2007). Furthermore, it is thought that the cortical spinal silent period is mediated by $GABA_B$ inhibitory networks. However, the exact mechanisms contributing to the silent period length are not well understood (Škarabot et al., 2019).

2.7.2 TMES

TMES utilizes two electrodes placed over the mastoid processes (Taylor, 2006). TMES delivers high voltage electrical pulses across the spinal cord between the electrodes, stimulating the fast-descending axons at the level of the pyramidal decussation of cervicomedullary junction. The resultant action potential travels down the axons of the motoneuron pool in the spinal cord where they synapse with alpha motoneurons. From there, the action potentials travel to the targeted muscle to evoke a short-latency excitatory response in the designated muscle. The electrical signal is recorded via surface EMG as a CMEP. CMEPs help to provide more insight into the corticospinal pathway by alluding to inhibition or excitation in the motoneuron pool. Hence, a greater CMEP suggests excitation and vice-versa. TMES activates many of the same descending axons as TMS, which infers that that they are triggering the same pathway (Taylor, 2006). If the CMEP and MEP amplitudes are matched, it represents the same motoneuron pool activated by

MEPs; however, they are unaltered by excitability at the cortical level (Taylor, 2006). Thus, CMEPs provide an unequivocal representation of synaptic input of motoneurons and neuron excitability while also allowing for a better interpretation of MEPs (Taylor, 2006).

2.7.3 Brachial Plexus (Erb's Point) Stimulation

Stimulation of Erb's Point involves passing a high voltage pulse between two electrodes located on the subclavicular fossa and the acromion process (Cadigan et al., 2017; Forman et al., 2015; Lockyer et al., 2018; Pearcey et al., 2016). If the stimulation of a peripheral nerve is high enough, the resultant response is a maximum compound muscle action potential (M_{max}). The resultant evoked potential can be measured at the target muscle using surface EMG as an M_{max} or frequently known as an M-wave (Lockyer et al., 2021). M_{max} is used for normalization purposes and to measure peripheral excitability during rhythmic motor output (Lockyer et al., 2021). Normalizing MEPs and CMEPs to M_{max} allows for delineating changes that may occur in the brain, spine, or periphery. Given that the electrical stimulus for an M_{max} is located outside of the central nervous system, it provides a neutral basis to normalize MEP and CMEP responses because any changes within the CNS are assumed to be proximal to the site of Erb's Point stimulation (Lockyer et al., 2021).

2.7.4 Active Motor Threshold

When measuring CSE during a dynamic task, setting the correct TMS intensity is a primary limitation. A standard method is to gradually increase the stimulation intensity until clearly discernable MEP with an amplitude of ≥ 50 microvolts (μV) is found 50% of the time (4 out of 8 stimulations) (Forman et al., 2015; Lockyer et al., 2018). Finding the motor threshold for a stimulation technique when the target muscle is active is known as the active motor threshold (AMT). The AMT is closely related to descending volleys in the fast-conducting neurons of the

cortical spinal tract (Rossini et al., 2015). First recruited descending volleys aid in discharging spinal motoneurons near the firing threshold in an active condition (Rossini et al., 2015). Afterward, we increase the stimulation intensity by a percentage (ex., 20%) to ensure that MEPs are measurable during exercise (Forman et al., 2015; Lockyer et al., 2018). The utilization of this method during maximal repeated sprints of any kind remains untested.

2.8 The Assessment of Corticospinal Excitability

CSE is the excitation of the supraspinal spinal, and peripheral neural pathways in conjunction (Ruotsalainen et al., 2014). Changes in CSE may indicate changes in either site are critical to understanding the etiology of NMF development, as demonstrated by Pearcey et al. (2016). The methods for measuring CSE are primarily TMS, TMES, and brachial plexus stimulation (Erb's point stimulation) (Aboodarda et al., 2015; Forman et al., 2018; Pearcey et al., 2014; Power & Copithorne, 2013). they provide a "big picture" rather than a scope into the basic biological mechanisms.

2.9 Corticospinal Excitability and Fatigue

Using MEPs and CMEPs for measuring CSE is well established and provides mechanisms when interpreting NMF. Supraspinal and spinal modulations in response to fatigue are exercise task-dependent (Carroll et al., 2006; Taylor & Gandevia, 2008), and the neural control of voluntary motor output depends both on the mode of task (Forman et al., 2014) and muscle type (Giesebrecht et al., 2010; Hoffman et al., 2009). After repeated maximal arm-cycling sprints, the induction of NMF caused a decrease in MEP amplitudes and an increase in CMEP amplitudes (Pearcey et al., 2016). Considering that these changes occurred concomitantly, the spine seemed to compensate for a decrease in the neural drive. It may be evidence that the NMF may work in an orderly fashion to prevent harm from occurring. For example, with repeated maximal arm-cycling sprints, the

orderly manner of NMF appears to happen in order from peripheral fatigue, supraspinal fatigue, and then spinal fatigue (Pearcey et al., 2016). The contribution of the central pattern generator (CPG) may be a reason for the simultaneous changes in central fatigue because intensity-sensitive serotonergic neurons are activated up to 5-fold during CPG motor-driven outputs (Fornal et al., 1996). The flooding of serotonin is thought to be a mechanism of inhibition, which would cause fatigue in the spinal cord. However, a significant question remains is the CPG even active during high-intensity sprints? Lockyer et al. (2018) found varying results with various cycling cadences and power outputs and the resultant MEP and CMEP amplitudes which furthers the notion that muscle and task dependency plays a significant role in supraspinal and spinal excitation. For example, when cycling at the 12 o'clock position, MEPs increased by ~26.8% with an increase in cadence, while CMEPs decreased by ~29.7% (Lockyer et al., 2018). Weavil et al. (2016) conducted a study using constant load cycling to fatigue with MEP and CMEP measurements taken throughout the task. Contradictory to a decrease in supraspinal excitability and an increase in spinal excitability found by Pearcey et al. (2016), the researchers found no change in MEP or CMEP from pre-fatigue to post-fatigue, which asserts that fatigue did not affect the excitability of the corticospinal pathway (Weavil et al., 2016). To further the complexity of NMF, typical studies in the past utilized isometric MVCs and have shown that spinal and supraspinal excitability decreased with the onset of fatigue (Gandevia et al., 1999; McNeil et al., 2011). According to Gandevia (2001), it is impossible to specify all the sites within the CNS that contribute to central fatigue. Any change in the network may or may not alter force production because of the compensatory nature of the system (Gandevia, 2001). Therefore, it is difficult to assume what will occur with NMF during any mode of exercise.

2.10 Repeated Sprint Exercise

Previous research suggests that all repeated sprint exercise induces NMF. However, the etiology of the development of NMF is dependent on the mode of exercise. For example, Tomazin et al. (2017) compared fatiguing repeated sprints in both running (treadmill) and leg cycling (cycle ergometer). They used voluntary activation via the interpolated twitch technique and M-Wave to measure central fatigue and peripheral fatigue, respectively. While peripheral fatigue was evident with both modes of exercise, evidence of central fatigue evidenced by a decrease in voluntary activation occurred only post-running. Unfortunately, Tomazin et al. (2017) did not use TMS or TMES to differentiate changes at the supraspinal or spinal levels. Weavil et al. (2016) utilized fatiguing constant-load leg cycling on a cycle ergometer to induce fatigue on a more comparable level. There was no change in corticospinal excitability measured using TMS and TMES, despite an increase of nearly 50% in muscle activation.

In contrast, a study by Pageaux et al. (2015) fatigued a single limb during high-intensity one-leg dynamic exercise using a dynamometer. Unlike Weavil et al. (2016), Pageaux et al. (2015) found significant decreases in spinal excitability while supraspinal excitability increased. Coversely, Pearcey et al. (2016) utilized intermittent tonic contractions between maximal arm-cycling sprints to examine CSE to the biceps brachii. They found that supraspinal excitability decreased and spinal excitability increased with the development of NMF. Therefore, not only is the development of NMF and the corticospinal modulations different for modes of repeated sprint exercise, but it is also incomparable with other fatiguing modes of exercise.

2.11 Upper body Versus Lower Body Differences

NMF and CSE are somewhat incomparable between different exercise modes in the lower limbs. Furthermore, NMF and CSE may be vastly different in the upper limbs compared to the

lower limbs during fatiguing exercise. There is limited research on repeated sprint exercise in the upper body. Therefore, understanding the differences in the upper body versus the lower body is pertinent for designing an upper body sprint study. The upper body is different physiologically and anatomically from the lower body. The upper body contains less muscle mass, which includes a higher ratio of fast-twitch to slow-twitch muscle fibers (Koppo et al., 2002; Sanchis-Moysi et al., 2010).

Additionally, upper body exercise elicits less vascular reactivity (Richardson et al., 2006), less mean transit time for blood, and greater diffusion distance with a lesser diffusion area (Calbet et al., 2005; Stöggl & Karlöf, 2013). The inherent nature of assessing CSE in the upper body versus the lower body will also provide challenges for direct comparison. The use of TMS provides a top-down method, whereby a magnetic pulse is delivered at the top of the skull. The nerve conduction time or latency will vary, depending on the muscle of interest (Khan et al., 2019). Hence, a reading from the biceps brachii will have a shorter latency than the tibialis anterior. Thus, it may be challenging to compare CSE during the fatiguing exercise of the upper limbs versus the lower limbs. However, the differences may be minimal.

2.12 Fatigue Differences During Cycling Exercise

The modulation of CSE can occur with or without fatigue. However, changes in CSE during cycling exercise appear to be heavily dependent on whether fatigue occurs or not. Neva et al. (2017) performed an acute bout of leg cycling to avoid fatigue. CSE did not change in either the upper or lower body. Similarly, Weavil et al. (2016) also did not find a change in CSE during fatiguing leg cycling. Yet, they found a 40% increase in CSE during non-fatiguing cycling during the same study. This finding is conflicting with what Neva et al. (2017) found. The answer to the conflicting studies above may be associated with exercise intensity. Increasing muscle activation

by altering leg cycling intensity during non-fatiguing exercise increases CSE (Weavil et al., 2015). However, there was a plateau during the testing, which indicated that CSE is likely intensity specific.

Furthermore, the muscle of interest likely plays a significant role despite the intensity. While Weavil et al. (2015) found that the Vastus Lateralis muscle plateaued, the rectus femoris muscle did not plateau during the same protocol. Evidently, for comparisons about CSE to be made across studies, researchers should abide by the fact that CSE is task-, phase-, intensity-, direction-, and muscle-dependent (Collins et al., 2018).

2.13 Sex Differences in Fatiguability

Fatigue-related sex differences induced by exercise are under-studied and widely unconsidered. Most studies utilize male participants during repeated cycling protocols (Billaut et al., 2006; Pearcey et al., 2016; Tomazin et al., 2017; Weavil et al., 2016). While there is a lack of explanation for why male participants are usually selected, some minor physiological characteristics may differentiate the two sexes. Unfortunately, few studies exist with females and NMF, especially for repeated sprint exercise (Billaut & Bishop, 2009). The purpose of reviewing sex differences in fatiguability is grounded within our intentions to include both males and females in our study without research bias.

In general, men have greater absolute muscle strength and power output than women (Batterham & Birch, 1996; Cramer et al., 2002). However, women have more resistance to fatigue and faster recovery from fatigue (Hunter & Enoka, 2001; Miller et al., 1993). The CNS factors that may account for fatiguability sex differences are likely unknown. However, Ditor and Hicks (2000) found that males were more prone to transmission failure at the neuromuscular junction or decreases in muscle membrane excitability. Billaut and Bishop (2009) assert that understanding

the neuromuscular activation patterns in males and females is difficult because task characteristics underpin it. Nevertheless, body composition, muscle metabolism, muscular characteristics, and motor discharge rate may account for many known differences (Billaut & Bishop, 2009).

Despite various known physiological and fatiguability differences between males and females, the question about restricting one sex to a study parameter may be unethical. Unless the study intends to learn about sex differences during a protocol, the difference in values should not matter because the data is normalized and averaged to eliminate abnormalities.

2.14 Active Motor Threshold and Chronic Resistance Training

Maeo et al. (2021) determined a significant difference in active motor threshold when comparing chronically resistance-trained individuals to untrained individuals. Chronically resistance-trained individuals require less corticospinal activation and a lower stimulation intensity to perform an assigned task (Maeo et al., 2021), which may alter how the modulation of the active motor threshold occurs despite an increase in stimulation intensity during intense exercise. Longer chronically resistance-trained participants may need a lower increase to elicit a MEP during intense, fatiguing exercise, while lesser trained individuals may require a more significant increase in stimulator intensity. Chronically resistance-trained individuals demonstrate increased corticomotor drive to the exercised muscle because of decreased short-interval intracortical inhibition (Lahouti et al., 2019). Hence, Lahouti et al. (2019) and Maeo et al. (2021) found similar results. Thus, this limits our participant selection for the study to focus on chronically resistance-trained individuals because the evoked responses are likely more comparable among the group.

2.15 Arm Cycling Considerations

With very little to no information on CSE measurement during arm-cycling sprints, it is pertinent to understand the neural control during arm cycling. As already stated, arm-cycling

sprinting is a high-intensity locomotor activity that differs from low-intensity arm cycling. Comparing the two may be a false analogy; however, it is the most relevant information until the completion of further experimentation.

Assessing CSE during arm-cycling is a technical challenge. Many studies have assessed CSE with tonic contractions in the past (Aboodarda et al., 2015; Cadigan et al., 2017; Todd et al., 2003); However, CSE during tonic contractions in comparison to arm cycling is an entirely different entity. Thus, the basis for comparing the two requires matching the EMG of a designated muscle for each task to represent the same motoneuron pool (Forman et al., 2014).

Compared to a tonic contraction, arm cycling results in greater CSE of the biceps brachii (Forman et al., 2014). Furthermore, CSE to the biceps brachii is modulated differently between rhythmic arm cycling and tonic elbow flexion (Forman et al., 2016). Spence et al. (2016) showed that supraspinal and spinal excitability was greater during the flexion phase than the extension phase of arm cycling, suggesting that CSE changes during arm cycling are phase-dependent. These phases are visible in a schematic overview by Lockyer et al. (2021) (Figure 2). Forman et al. (2019) assessed CSE through stimulus-response curves and found similar results. It is intuitive to think that there would be greater CSE of the biceps brachii at the initiation of elbow flexion during arm cycling, considering that the biceps brachii is a primary elbow flexor. But this does not explain why CSE is less for a tonic contraction. Accordingly, CSE during arm cycling is also task-dependent. At the 12 o'clock crank position (relative to a clock face) during arm cycling, there is less CSE to the biceps brachii than a tonic contraction in the same position (Forman et al., 2019). Simply changing the hand grip from a pronated to a neutral grip resulted in greater CSE for arm cycling and a tonic contraction.

Additionally, CSE during forward and backward arm cycling is both phase and direction-dependent (Nippard et al., 2019). All the studies mentioned above also indicated that CSE to the triceps brachii is unlike the biceps brachii. Thus, CSE during arm cycling is also muscle dependent (Forman et al., 2014; Forman et al., 2019; Forman et al., 2016; Nippard et al., 2019; Spence et al., 2016). To complicate matters even more, CSE during arm cycling is intensity-dependent. Cycling cadence influences CSE to the biceps brachii, whereby a faster cadence result in greater CSE; However, this was also phase-dependent for spinal excitability (Forman et al., 2015; Lockyer et al., 2018). Lockyer et al. (2018) also found that increased power output during arm cycling increased CSE, which was phase-dependent. Many factors attribute to such differences during arm cycling, and designing a study to measure CSE during repeated maximal arm-cycling sprints is likely even more challenging. Understanding that cadence is nonlinear during maximal sprinting exercise will influence CSE in addition to the mode of sprinting, and the muscles being analyzed.

2.16 Void in the Literature

The available methodologies to measure CSE are limited. Measuring voluntary activation using the interpolated twitch technique (ITT), as first demonstrated by Merton (1954), is another consideration; However, Cadigan et al. (2017) found the ITT via TMS to be unreliable with increasing levels of NMF. In addition, Mira et al. (2017) discovered that voluntary activation estimated by TMS resulted in a time delay with isometric contractions. This evidence suggests that TMS is not a reliable modality for measuring voluntary activation, especially with fatigue.

Having considered the mechanisms of CSE and how it is often analyzed and interpreted, there are noticeable gaps in the literature that merits further investigation. Firstly, after an exhaustive search, it seems that there are no studies that consider NMF of the quadriceps assessed by MEPs and CMEPs during or intermittently via leg-cycling sprints. This notion is analogous to

the study by Pearcey et al. (2016), which measured CSE intermittently throughout the protocol. However, the central void is the methodology for assessing CSE during a maximal sprinting task. Moreover, no studies assessed CSE during a task such as arm-cycling sprinting or leg-cycling sprinting using TMS or TMES. In the past, CSE is well-documented during fatiguing MVCs (Cadigan et al., 2017; Nemanja et al., 2016; Todd et al., 2003). All previous maximal sprinting studies that analyzed CSE used a setup that measured CSE during a tonic contraction. As aforementioned, CSE is dependent on many factors such as exercise task, joint kinematics, muscle type, and exercise intensity. Consequently, analyzing CSE during fatiguing, tonic contractions following repeated sprints is likely, not ideal for illustrating if or how CSE is altered during maximal repeated arm cycling sprints.

Additionally, switching from the cycle ergometer to a device used to measure CSE results in a time delay – this can be viewed as recovery time (Collins et al., 2018). Aboodarda et al. (2019) surmise that the recovery time course for NMF and CSE is transient and occurs at different paces. Therefore, the assessment of CSE should occur immediately following cessation of exercise (Aboodarda et al., 2019). Failure to do so may lead to an underestimation in the modulation of CSE.

Perhaps the void in using TMS and TMES during cycling sprints is because of its complicated nature. Measuring MEPs and CMEPs in a relatively controlled environment provides fewer challenges than bodily oscillations elicited during a maximal locomotor activity. Recognizably, maintaining a TMS paddle in the appropriate position during such a task is not easy. Additional technicalities include the timing of TMS and TMES stimulations during sprinting. Setting the stimulators to initiate exactly when the hands are at a designated crank position is difficult. Factors such as the time it takes for the stimulation to travel from the stimulator to the

paddle, the brain, and the intended muscle elicits a time delay. Furthermore, the greater the cadence a person pedals will also increase the time delay relative to the crank position.

2.17 Conclusion

After closely examining the research about the etiology of both upper body and lower body NMF during exhaustive rhythmic and non-rhythmic exercise, it is reasonable to hypothesize how CSE is altered during maximal repeated arm-cycling sprints. However, generating a hypothesis is complicated for maximal rhythmic exercise. Firstly, it appears that no one has ever measured CSE during exhaustive rhythmic exercise using the non-invasive stimulation techniques such as TMS and TMES. This acknowledgment is concerning and interesting because it may or may not be possible due to the above factors. Secondly, many factors influence CSE, such as task dependency (Carroll, Baldwin, Collins, & Zehr, 2006; Taylor & Gandevia, 2008). The neural control of voluntary motor output depends on the mode of task (Forman, Raj, Button, & Power, 2014) and muscle type (Giesebrecht, Martin, Gandevia, & Taylor, 2010; Hoffman, Oya, Carroll, & Cresswell, 2009). Regardless, studying CSE to the biceps brachii during maximal repeated arm-cycling sprints is likely a viable endeavor. However, the consistency and reliability of the results are yet to be understood.

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CHAPTER 3: STATEMENT OF CONTRIBUTIONS

3.1 Co-authorship Statement

This study is an elongation of Dr. Button's tireless effort to understand the etiology of neuromuscular fatigue during exercise. Past methodologies for assessing CSE during fatiguing sprint exercise did not align with the most recent literature's findings. After an intensive review of the literature, we examined CSE to the biceps brachii during maximal repeated arm-cycling sprints. Dr. Button, Evan Lockyer, and I wrote the project outline and planned the experiment together.

Evan Lockyer, Shahab Alizadeh, and I carried out the experimental protocol. Also, Evan Lockyer assisted with data analysis and the interpretation of the study results.

Dr. Button and I discussed the findings, and I wrote the thesis under his supervision.

CHAPTER 4: THESIS MANUSCRIPT

Title: Understanding corticospinal excitability to the biceps brachii during maximal repeated arm-cycling sprints

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4.1 Abstract

The purpose of this study was to assess corticospinal excitability (CSE) of the biceps brachii during maximal repeated arm-cycling sprints. Twelve chronically resistance-trained participants completed a familiarization session and an experimental session at least 48 hours later. The participants cycled at 60 revolutions per minute (RPM) while completing pre-and post- active motor threshold (AMT) testing to determine transcranial magnetic stimulation (TMS), transmastoid electrical stimulation (TMES), and Erb's point stimulation intensities. Pre and post-AMT were intermediated by five, 17-22 second maximal sprints. A mechanical brake applied a 5% of bodyweight torque factor following a 20 second period to ramp up to 100 RPM. Each sprint consisted of three TMS, TMES, and Erb's point stimulations. Both mean power and total work dropped by 36.8% from sprint one to five ($p < 0.0001$). There were no supraspinal excitability, spinal, or peripheral excitability changes throughout the sprint protocol. However, there was a 4.57% decrease in TMES intensity from pre-AMT (134.0 ± 31.5 mA,) to post-AMT (124.17 30.08 ± mA), $t(12) = 3.445$, $p = 0.0055$. There was also a significant difference found between pre (12.73 ± 3.973 mA) and post (14.27 ± 4.402 mA), $t(11) = 4.261$, $p = 0.0013$ maximum compound muscle action potential (M_{max}) amplitudes. Our findings suggest 1) no change in nervous system excitation during sprinting and 2) the sprint protocol reduced the amount of excitation required to activate the motoneuron pool as evidenced by a reduction in the AMT for CMEP and an increased M_{max} from pre- to post-sprinting while recorded during submaximal cycling. Hence, the protocol resulted in enhanced spinal excitability.

4.2 Keywords

Motoneuron, neuromuscular fatigue, corticospinal excitability, supraspinal excitability, spinal excitability, peripheral excitability.

4.3 Introduction

Neuromuscular fatigue (NMF) develops because of an inability to activate the target muscle, thus, resulting in the diminishment in capacity to sustain force output due to exercise (Gandevia, 2001; Gandevia et al., 1999). Peripheral nervous system (PNS) and central nervous system (CNS) factors contribute to NMF (Collins et al., 2018). NMF has been shown to develop in various forms of high-intensity sprinting exercise (Billaut et al., 2006; Bishop, 2012; Mendez-Villanueva et al., 2008; Monks et al., 2016; Pearcey et al., 2016; Pearcey et al., 2015; Tomazin et al., 2017; Zinner et al., 2016). NMF results in modulations in corticospinal excitability (CSE), which may occur at the supraspinal or spinal level (Collins et al., 2018). Supraspinal and spinal modulations in response to fatigue are exercise task-dependent (Carroll, Baldwin, Collins, & Zehr, 2006; Taylor & Gandevia, 2008), and the neural control of voluntary motor output depends both on the mode of exercise (Forman, Raj, Button, & Power, 2014) and muscle type (Giesebrecht, Martin, Gandevia, & Taylor, 2010; Hoffman, Oya, Carroll, & Cresswell, 2009). However, very little is known about how CSE is modulated during sprinting.

Peripheral fatigue is influenced by a multitude of factors such as increased concentrations of metabolites (De Luca, 1997; Gerdle & Fugl-Meyer, 1992; Juel, 1988b; Taylor et al., 1997), reduced muscle fiber conduction velocity (Juel, 1988a), presynaptic inhibition, limited acetylcholine, and decreased postsynaptic excitation (Maclaren et al., 1989) contribute to peripheral fatigue. Furthermore, changes within the muscle, such as adjustments in cross-bridge formation and function and depolarization of the tubular membrane, contribute to peripheral fatigue (Cè et al., 2020). Peripheral fatigue generally occurs early during fatiguing exercise until a "critical threshold" is reached (Amann, 2011). This critical threshold may prevent further damage

within the muscle and may be an innate function of the CNS because once it is reached, central fatigue generally occurs soon after (Decorte et al., 2012; Monks et al., 2016; Pearcey et al., 2015).

Central fatigue is comprised of supraspinal and spinal factors. While the contributing factors of central fatigue are mainly unknown, several studies have assessed CSE within a sprinting protocol by transcranial magnetic stimulation (TMS), which elicits motor evoked potentials (MEPs) (Goodall et al., 2015; Suruagy et al., 2017; Tomazin et al., 2017; Zinner et al., 2016). Furthermore, the use of transmastoid electrical stimulation (TMES) to elicit cervicomedullary motor evoked potentials (CMEPS) is commonly used to assess spinal excitability during an intense cycling exercise (Pearcey et al., 2016; Weavil et al., 2015; Weavil et al., 2016). Pearcey et al. (2016) analyzed CSE using TMS and TMES to understand and delineate between changes that were supraspinal, spinal, and peripheral. While MEPs and CMEPs were elicited during a tonic contraction, Pearcey et al. (2016)demonstrated that repeated sprints decreased supraspinal excitability decreased and spinal excitability increased with increasing levels of NMF. Despite this, the CSE modulations are poorly understood in response to NMF because of the current methods for assessing CSE. Current and past studies assessing CSE throughout a sprinting protocol show some inconsistencies. Most studies measure CSE during an isometric contraction between pre and post-sprinting (Girard et al., 2013; Goodall et al., 2015; Pearcey et al., 2016; Suruagy et al., 2017; Temesi et al., 2014). However, CSE is higher during arm-cycling than an intensity-matched tonic contraction (Forman et al., 2014), but not necessarily during high-intensity cycling (Lockyer et al., 2018). This finding asserts the difference between assessing CSE with differing exercises. Hence, it is prudent only to assess CSE during the targeted movement. For example, if the protocol includes repeated arm-cycling sprints, CSE should be measured during the sprint.

The purpose of this study is to determine how CSE of the biceps brachii is modulated during repeated maximal arm-cycling sprints. The intent is to generate increasing levels of fatigue and interpret CSE changes in real-time. Considering that the biceps brachii is generally the muscle of focus in arm-cycling studies (Forman et al., 2014; Forman et al., 2018; Forman et al., 2016; Forman et al., 2015; Pearcey et al., 2016; Power & Copithorne, 2013), we will specifically be targeting changes within the biceps brachii. We assessed CSE through TMS, TMES, and M_{max} to delineate supraspinal, spinal, and peripheral excitability changes. Based on conventional methodologies from past studies, we hypothesized that supraspinal excitability would decrease, and spinal excitability would increase, over the duration of the sprint protocol.

4.4 Methods

4.4.1 Participants

Based on prior research (Taylor et al., 2000), a statistical power analysis determined that six participants were necessary to achieve an alpha of 0.05 with a power of 0.8. Ten male and two female recreationally active (~10 hours of activity/week) participants (177.3 ± 10.2cm, 81.9 ± 12.1 kg, 26.4 ± 5.8 years) were recruited from the university population. All participants completed the magnetic stimulation safety checklist (Rossi, 2009) and Physical Activity Readiness Questionnaire and were instructed to refrain from heavy exercise 24 hours before testing and to follow the Canadian Society for Exercise Physiology preliminary instructions (no eating, drinking caffeine, smoking, or drinking alcohol for 2, 2, 2, or 6 hours, respectively) before the start of testing. All participants were also told they would complete 5 maximal intensity arm-cycling sprints of between 17 and 22 seconds each. All participants were verbally informed of all procedures and read and signed a written consent form. The Memorial University of Newfoundland Interdisciplinary Committee on Ethics in Human Research approved the study (#20192592-HK)

and was in accordance with the Tri-Council guidelines in Canada with full disclosure of potential risks to participants.

4.4.2 Repeated sprint protocol

The primary sprint protocol began with 20 seconds of moderate cycling preceding each sprint at a workload of 0.5 kg of resistance. This portion was considered a ramp-up phase whereby the participant was instructed to increase to and maintain ~100 revolutions per minute (RPM). A tracer line guided this entire process on a screen placed directly in front of the participant. Immediately following the 20 seconds of moderate cycling, the participants began the sprint phase where the mechanical brake applied a 5% torque factor (i.e., the participant pedaled against a resistance that was equal to 5% of their body weight), which gives the highest mean power output over 30 seconds in trained individuals and participants were then given verbal encouragement to cycle as fast as they could for 17-22 seconds followed by 90 seconds of rest. The 17-22 second timeframe was necessary because as fatigue increased and cadence decreased during sprinting, it took longer for the pedal to reach the crank location where the stimulus was triggered. Thus, there was some variance for each participant during each sprint. The participants repeated this process 5 times. All power output data were recorded using Monark Wingate software and stored on a computer. The mean and peak power (Watts) were measured during each sprint. Additionally, mean RPM, anaerobic capacity, anaerobic power, fatigue index, and total work was recorded for each sprint. At the end of each sprint, participants were shown a Borg scale and questioned about their perceived exertion rate (RPE) (Figure 1).

4.4.3 Electromyography

Electromyography (EMG) signals were recorded from the biceps brachii, brachioradialis, and the triceps brachii of the dominant arm using pairs of disposable Ag-AgCl surface electrodes

(MeditraceTM 130 ECG conductive adhesive electrodes). With a bipolar configuration, electrodes were positioned ~2 cm apart (center to center) over the midline of the biceps brachii and brachioradialis and over the midline of the lateral head of the triceps brachii. A ground electrode was positioned on the lateral epicondyle of the dominant arm. Preceding the electrode placement, the skin was thoroughly prepared by removing the hair (via a handheld razor) and dead epithelial cells (via abrasive paper), followed by sanitization using isopropyl alcohol swabs. This was done to reduce the impedance for EMG recordings. An interelectrode impedance of < 5 kO was obtained via a standard multimeter before recording to ensure an adequate signal-to-noise ratio. The raw EMG signals were DC bias removed and bandpass filtered using a 3-Pole Butterworth filter with cut-off frequencies ranging from 10 to 1,000 Hz (Common mode rejection (at x100 gain) 80 dB at 50 Hz). The signals were amplified (gain of 300) and sampled at a rate of 5 kHz using a 16-bit CED 1902 interface and the associated Signal 5 (Cambridge Electronic Desi, Cambridge, UK) software.

4.4.4 Experimental Protocol

4.4.5.1 Familiarization session

Participants completed a familiarization and an experimental session, which was separated by at least 48 hours. During the familiarization session, participants were subjected to TMS, TMES, and Erb's Point stimulation. Several stimulations were given with each form of stimulation at similar intensities that participants encountered during the experimental day. A brief cycling warm-up was done, followed by several short sprints (5-10 seconds in duration).

4.4.5.2 Experimental Protocol

Participants were prepped for the stimulation conditions and EMG during the experimental session and began the session with a five-minute warm-up on the custom cycle-ergometer at an

intensity of ~60 RPM and a resistance of 0.5 kg. The arm-cycle ergometer was set at 0.5 kg of resistance. Participants were positioned so that the center of the crank was in line horizontally with the acromion. The elbows were at a 90-degree angle when at the six o'clock position of the crank. Immediately before and after the primary sprint protocol, AMT was determined for TMS and TMES as well as Erb's point stimulation. AMT was generally completed over a five-minute period, acting as a warm-up and cooldown for the main sprint protocol. AMT for TMS and TMES was determined by finding a discernable MEP and CMEP of ≥ 50 μV respectively from the background EMG 50% of the time (4/8 or 5/10 discernable MEPs or CMEPs). Once AMT was found, the stimulator intensity was increased by 30% to ensure an appropriate stimulus was delivered during the sprint protocol. M_{max} was also found during the warm-up by gradually increasing the stimulator electrical current until the M-wave (i.e., M_{max}) of the biceps brachii no longer increased. A supramaximal stimulation current (i.e., 30% higher than that required to elicit M_{max}) was used for the remainder of the experiment. Participants completed five consecutive arm-cycling sprints of ~17-22 seconds in duration interspersed with 90 seconds of rest. Three stimulation intervals were delivered during each sprint, which followed the same pattern of MEP, CMEP, and $M_{max,}$ respectively. There were blank sequences between each interval. Stimulations were recorded when the dominant arm initiated elbow flexion (i.e., 3 o'clock made relative to a clock face) via two magnetic sensors, whereby one was attached to the sprocket and the other was attached to the crank shaft. Although biceps brachii activation was higher during other phases such as the 6 o'clock position, triggering a stimulus at the 3 o'clock position when the biceps activation was initiated allowed for an increased "ceiling" during the flexion phase to observe changes in the evoked response. Participants provided their rate of perceived exertion on a Borg scale of 6-20 with 20 being a maximum effort was taken following each sprint.

4.4.6 Stimulation

4.4.6.1 Brachial Plexus (Erb's Point) Stimulation

Stimulation of the brachial plexus (i.e., Erb's point) was used to induce a maximal compound muscle action potential (M_{max}). All stimulations were obtained under the same pedaling conditions and acted as a warm-up. Erb's point was electrically stimulated via adhesive Ag-AgCl electrodes (diameter 10 mm) fixed to the skin over the supraclavicular fossa (cathode) and the acromion process (anode). Current pulses were delivered as a singlet (200 μs duration, 175-250 mA) via a constant current stimulator.

4.4.6.2 Transcranial Magnetic Stimulation

MEP responses of the biceps brachii were elicited via TMS (49-92% maximal stimulator output) over the motor cortex in the left hemisphere using a circular coil (13.5 cm outside diameter) attached to a Magstim 200 stimulator (Magstim, Dyfed, UK). Electrical currents flow in an anticlockwise direction through the circular coil. The coil was placed horizontally over the vertex so that the direction of the current flow in the coil preferentially activated the left motor cortex. Vertex was determined by marking the intersection of the measured halfway points from nasion to inion and from tragus to tragus.

4.4.6.3 Transmastoid Electrical Stimulation

CMEP responses of the biceps brachii were elicited via adhesive Ag-AgCl electrodes fixed to the skin over the mastoid processes and the current was passed between them (100 μs duration, 150–250 mA; model DS7AH, Digitimer Ltd, Welwyn Garden City, UK) with the anode on the right side and cathode on the left side.

4.4.6.4 Stimulation Sequence

Stimulations for the sprint protocol were all completed during the 17-22 second sprint timeframe. Three intervals interspersed with periods of no stimulations or blanks created the stimulation sequence. Each stimulation period followed the same pattern of MEP, CMEP, and $M_{max,}$ respectively. The timing of each stimulus and blank interval was one second each. Accordingly, the sequence was blank, stimulus interval, two blanks, stimulus interval, two blanks, and stimulus interval. This stimulus sequence allowed for the analysis of supraspinal, spinal, and peripheral excitability during the sprint.

4.5 Data Analysis

All data were stored and analyzed offline using Signal 5.08 data collection software (CED). The averaged peak-to-peak amplitudes of MEPs, CMEPs, and M_{max} were measured from the biceps brachii of the dominant arm from the initial deflection of the voltage trace to the return of the trace to baseline background EMG levels. Additionally, latencies of all evoked responses were examined and monitored. To account for changes in peripheral neuromuscular propagation during exercise (Taylor, 2006), averaged MEP and CMEP amplitudes were normalized to the averaged M_{max} evoked during the same trial. Prestimulus EMG, defined as a window of the mean rectified EMG immediately prior to the stimulation artifact, was measured from the rectified traces. The length of the window was 50 ms prior to the stimulus artifact. Sprint performance measures including mean and peak power output, and mean and peak RPM were recorded using the Velotron Wingate Version 1.0.1 software to understand the development of NMF. Using the same software anaerobic performance measures including anaerobic capacity and power, fatigue index, and total

work were recorded to understand the energy usage during each sprint and to understand the physical effort exerted for each sprint.

4.6 Statistical Analysis

All statistics were performed using GraphPad Prism version 9.2.0. Assumptions of sphericity were tested using Mauchly's test, and if violated, the appropriate correction was made to the degrees of freedom using the Greenhouse-Geisser or the Hyundt-Felt method (Field 2013; Park et al. 2009). Separate one-way repeated ANOVA's were done for measures of sprint performance (mean power, mean RPM, RPE, and anaerobic power) and for corticospinal excitability measures (MEPs, CMEPs, and M_{max}). The Bonferroni post hoc comparisons test was used to determine where significant differences may exist for each measure only if main effects were found. Effect sizes using Cohen's d were used to provide the standardized magnitude of the effect. Paired T-Tests were used to compare stimulation intensities to elicit AMT for TMS, TMES, and M_{max} amplitudes pre and post-sprinting. All statistics were performed on group data and reported as mean ± SD in text, tables, and figures. Significance levels were set at $p \leq 0.05$.

4.7 Results

4.7.1 EMG Recordings at the Biceps Brachii

Raw data values about recordings from the biceps brachii are shown in Table 2.

Corticospinal excitability to the biceps brachii. MEP amplitude and stimulation intensity. A one-way ANOVA with repeated measures showed a significant main effect for sprint 1 MEPs [$F_{(1.806, 18.96)} = 3.842$, $p = 0.0434$]. Further investigation using pairwise comparisons revealed that there was no significant difference for sprint 1 MEP amplitudes ($p > 0.05$ for all comparisons). There

was no significant main effect for MEP amplitudes during sprint 1 [$F_{(1.624, 17.87)}$= 3.023, p = 0.0826], sprint 2 [$F_{(1.573, 17.30)}$= 3.678, p = 0.0557], sprint 3[$F_{(1.888, 20.77)}$= 0.5517, p = 0.5744], and sprint 5 [$F_{(1.514, 16.65)}$= 0.7456, p = 0.4541]. Since there were no changes in MEP amplitudes within each sprint, the MEPs were averaged within a sprint and the average MEP was compared across sprints. There was no significant main effect for the average MEP amplitude across sprints [$F_{(1.811, 19.92)}$= 1.620, p = 0.2235] (Figure 2A). There was also no significant difference between pre- and post-AMT TMS intensities (p > 0.05) (Figure 3A).

Spinal excitability to the biceps brachii. CMEP amplitude and stimulation intensities. There was no significant main effect for CMEP amplitudes during sprint 1 [$F_{(1.612, 16.93)}$= 1.752, p = 0.2055], sprint 2 [$F_{(1.398, 15.38)}$= 2.772, p = 0.1070], sprint 4 [$F_{(1.286, 14.15)}$= 1.564, p = 0.2380], and sprint 5 [$F_{(1.786, 19.64)}$= 0.9269, p = 0.4024]. However, there was a significant main effect for sprint 3 CMEP amplitudes [$F_{(1.440, 15.84)}$= 5.135, p = 0.0274]. Pairwise comparison revealed that there was a significant difference between CMEP #2 and CMEP #3 (p = 0.0019) during sprint 3. There was no significant main effect for the average CMEP amplitude across sprints [$F_{(2.467, 27.14)}$= 1.365, p = 0.2744] (Figure 2B). There was a 4.57% decrease in TMES stimulation intensity from pre to post AMT, which resulted in a significant difference between pre-sprint TMES intensities during AMT (134.00 ± 31.52 mA) testing and post-sprint TMES intensities during AMT testing (124. ± 30.08 mA), t (12) = 3.445, p = 0.0055 (Figure 3B).

Peripheral excitability to the biceps brachii. M_{max} amplitude. There was a significant main effect for M_{max} amplitudes during sprint 3 [$F_{(1.274, 14.01)}$= 5.109, p = 0.0333]. Pairwise comparisons revealed that there was significant difference between M_{max} #1 and M_{max} #2 amplitudes (p = 0.0294). There was a significant main effect for M_{max} amplitudes during sprint 4 [$F_{(1.337, 13.37)}$= 6.440, p = 0.0180]. Pairwise comparisons revealed that there was a significant difference between

M_{max} #2 and M_{max} #3 amplitudes (p = 0.0050). There were no significant main effects for M_{max} amplitudes during sprint 1 [$F_{(1.256, 10.68)}$= 3.054, p = 0.1038], sprint 2 [$F_{(1.423, 15.65)}$= 3.688, p = 0.0612], and sprint 5 [$F_{(1.645, 18.09)}$= 2.831, p = 0.0932]. There was no significant main effect for the average M_{max} amplitude across all sprints [$F_{(1.582, 16.22)}$= 3.148, p = 0.0793] (Figure 2C). There was a significant difference found between pre-sprint M_{max} amplitudes during AMT (12.73 ± 3.973 mV) testing and post-sprint M_{max} amplitudes during AMT testing (14.27 ± 4.402 mV), t (11) = 4.261, p = 0.0013 (Figure 3C).

Background EMG

There was no significant main effect for the average background EMG for the biceps brachii [$F_{(4, 55)}$= 1.510, p = 0.2120] (Figure 2D), triceps brachii [$F_{(4, 55)}$= 1.957, p = 0.1140] or brachioradialis [$F_{(4, 55)}$= 0.6861, p = 0.6046].

4.7.2 Sprint Performance Measures

All sprint performance values are shown in Table 1.

Mean power output. There was a significant main effect for mean power output from sprints one to five [$F_{(4, 55)}$= 9.247, p < 0.0001]. Pairwise comparisons revealed that there was a 36.8% decrease in mean power output from sprint 1 to 5. Furthermore, there were significant differences for sprints 1 vs. 3 (p = 0.0036), 1 vs. 4 (p = 0.0001), and 1 vs. 5 (p < 0.0001), and 2 vs. 5 (p = 0.0289).

Peak power output. There was a significant main effect for mean power output from sprints one to five [$F_{(2.459, 27.05)}$= 23.53, p < 0.0001]. Pairwise comparisons revealed that there was a 23.5% decrease in peak power output from sprint 1 to 5. Furthermore, there were significant differences

between sprints 1 vs. 3 (p = 0.0087), 1 vs. 4 (p = 0.0016), 1 vs. 5 (p = 0.0003), 2 vs. 4 (p = 0.0004), and 2 vs. 5 (p = 0.0004).

Mean RPM. There was a significant main effect for mean RPM from sprints one to five [$F_{(1.427, 15.70)}$= 39.93, p < 0.0001]. Pairwise comparisons revealed a 27.3% decrease in mean RPM output from sprint 1 to 5. Thus, there were significant differences for all sprint comparisons except for sprint 4 vs. 5 (p = 0.2901).

Peak RPM. There was a significant main effect for peak RPM from sprints one to five [$F_{(4, 55)}$= 18.02, P < 0.0001]. Pairwise comparisons revealed that there was a 23.2% decrease in peak RPM from sprint 1 to 5. Thus, there were significant differences for sprints 1 vs. 2 (p = 0.0271), 1 vs. 3 (p < 0.0001), 1 vs. 4 (p < 0.0001), 1 vs. 5 (p < 0.0001), 2 vs. 4 (p = 0.0076), and 2 vs. 5 (p = 0.0022).

4.7.3 Anaerobic Performance Measures

All anaerobic performance values are shown in Table 1.

Anaerobic capacity. There was a significant main effect for anaerobic capacity from sprints one to five [$F_{(4, 55)}$= 20.16, p < 0.0001]. Pairwise comparisons revealed that there was a 36.8% decrease in anaerobic capacity from sprint 1 to 5. Thus, there were significant differences for sprints 1 vs. 2 (p = 0.0320), 1 vs. 3 (p <0.0001), 1 vs. 4 (p <0.0001), 1 vs. 5 (p <0.0001), 2 vs. 4 (p = 0.0016), and 2 vs. 5 (p = 0.0003).

Anaerobic power. There was a significant main effect for anaerobic power from sprints one to five [$F_{(4, 55)}$= 10.11, p < 0.0001]. Pairwise comparisons revealed that there was a 23.1% decrease in

anaerobic power from sprint 1 to 5. Thus, there were significant differences for sprints 1 vs. 3 (p = 0.0025), 1 vs. 4 (p = 0.0002), 1 vs. 5 (p < 0.0001), 2 vs. 4 (p = 0.0284), and 2 vs. 5. (p = 0.0031).

Fatigue Index

There was no significant main effect found for fatigue index [$F_{(4, 55)}$= 0.8924, p = 0.4747].

Total Work

There was a significant main effect for total work from sprints one to five [$F_{(4, 55)}$= 9.165, p < 0.0001]. Pairwise comparisons revealed that there was a 36.8% decrease in total work from sprint 1 to 5. Thus, there were significant differences for sprints 1 vs. 3 (p = 0.0038), 1 vs. 4 (p = 0.0001), 1 vs. 5 (p < 0.0001), and 2 vs. 5. (p = 0.0318).

4.7.4 RPE (Borg Scale)

There was a significant main effect for rate of perceived exertion (RPE) from sprints one to five [$F_{(4, 55)}$= 17.15, p < 0.0001]. Pairwise comparisons revealed that there was a 32.8% increase in RPE from sprint 1 to 5. Thus, there were significant differences for sprints 1 vs. 2 (p = 0.0439) 1 vs. 3 (p < 0.0001), 1 vs. 4 (p = 0.0001), 1 vs. 5 (p < 0.0001), 2 vs. 4 (p = 0.0143), and 2 vs. 5. (p = 0.0005).

4.8 Discussion

To our knowledge, this is the first study to assess CSE of the biceps brachii during repeated maximal sprints. We demonstrated that CSE could be assessed during repeated maximal arm-cycling sprints by successfully recording MEP, CMEP, and M_{max} responses multiple times per sprint. The repeated sprint protocol was fatiguing, as evidenced by the reduction in sprint

performance throughout the sprints. Interestingly, in contrast to our initial hypothesis, no changes in measures of CSE (i.e., MEP, CMEP) were observed during the sprinting protocol, suggesting neither supraspinal nor spinal excitability of the biceps brachii were altered during the performance of the fatiguing sprint exercise. These findings suggest that CSE of the biceps brachii is not directly linked to the development of neuromuscular fatigue during repeated sprints. Interestingly, at post-sprinting, while cycling at a cadence of 60 RPM, it appeared as though spinal and peripheral excitability may have increased as evidenced by the reduced stimulation intensity required to elicit AMT for CMEPs and the increased M_{max} amplitude observed at post-sprinting, respectively. Thus, the fatiguing protocol led to a nervous system potentiation of the biceps brachii post-sprinting while cycling at a submaximal intensity.

4.8.1 Supraspinal Excitability of the Biceps Brachii Throughout the Arm-Cycling Sprints

When assessed during a slight tonic contraction, supraspinal excitability has been shown to change during and following repeated arm-cycling sprints (Pearcey et al., 2016). We did not see any changes in supraspinal excitability during the sprinting protocol. In the Pearcey et al. (2016) study, CSE was measured between sprints using a tonic contraction. Hence, it is not surprising that our results differed because arm-cycling produces higher CSE to the biceps brachii than an intensity matched tonic contraction (Forman et al., 2014; Forman et al., 2019). Weavil et al. (2016) found similar results during exhaustive leg cycling exercise, but contrary to our study, they did not perform repeated sprints. However, they did assert that a lack of change in the corticospinal pathway is likely due to the facilitating effects of muscle activation hindered by the inhibitory influences on spinal motoneurons.

While our findings differed from our hypothesis, the results showed how CSE remained unaltered during maximal repeated arm-cycling sprints. To our knowledge, we are the first to study CSE *during* maximal repeated sprinting exercise. Supraspinal and spinal modulations in response to fatigue are exercise task-dependent (Taylor & Gandevia, 2008), and the neural control of voluntary motor output depends both on the mode of the task (Forman et al., 2014) and the muscle type being examined (Giesebrecht et al., 2010; Hoffman et al., 2009). We utilized a novel approach for assessing CSE during sprinting. A primary concern when developing the protocol was determining the most appropriate stimulation intensity. As aforementioned, CSE is dependent on many factors. Therefore, the most appropriate way to assess it is by setting the stimulus intensity during sprinting. Instead, we set the intensity during a 60 RPM cycling pace and increased the intensity by 30%. Accordingly, we may have set the stimulus intensity too high or too low, preventing us from seeing CSE modulations. In an ideal world, perhaps the most appropriate way to set the stimulus intensity would be via stimulus-response curves during sprinting or normalizing MEPs and CMEPs to M_{max}. Stimulus-response curves consist of a broad range of stimulus intensities, which results in a sigmoidal curve (Forman et al., 2019). The most appropriate stimulus would be somewhere before the peak of the curve so that we could observe changes in the response. Both techniques would be technically challenging, though, and would likely involve setting stimulations on a separate day to avoid fatigue before commencing a fatigue protocol.

The difficulty with setting the stimulus intensity during sprinting is that the sprinting itself would induce fatigue before the fatiguing protocol commenced. Stimulus-response curves require a significant amount of time and would prove to be very difficult to perform during sprinting. The high number of sprints needed to produce a stimulus-response curve for each stimulus type (MEPs, CMEPs, and M_{max}) would inevitably induce fatigue. CSE is highly influenced by fatigue (Cadigan

et al., 2017; Collins et al., 2018; Pearcey et al., 2016; Taylor et al., 2016; Weavil et al., 2015). Thus, the problem lies within the timing of the protocol. Furthermore, using stimulation response curves for each stimulus on separate days and performing the sprint protocol on another day would also be methodologically challenging. Factors such as sleep quality and quantity could affect CSE (Bertini et al., 2004), which would be a complex variable to control if stimulus thresholds were set on a different day than the experimental protocol.

4.8.2 Spinal Excitability of the Biceps Brachii Throughout the Arm-Cycling Sprints

The modulation of spinal excitability during fatiguing exercise is task-dependent. Pearcey et al. (2016) found an increase in spinal excitability following arm cycling sprinting, while Weavil et al. (2016) found the opposite (i.e., reduced spinal excitability) following fatiguing leg cycling exercise. While CMEP amplitudes did not change during the repeated sprints, there was a significant reduction in stimulation intensity required to evoke a discernable CMEP (i.e., AMT) at post-sprinting. This finding suggested that spinal excitability may have been increased at post-sprinting in the presence of fatigue when assessed during submaximal arm cycling at 60 RPM. While the exact mechanisms underlying this proposed increase in spinal excitability are unknown, heightened feedback from group III/IV afferent fibers caused by the sprinting may be partially responsible. Alternatively, there were changes to the motoneurons that were easiest to recruit. Furthermore, the electrodes may have moved, or the sweat and altered hydration could have altered the volume conduction of the stimulation, which could better activate the axons of the nerves.

During the sprinting, RPE increased concomitantly with fatigue. Pain induced by intense cycling exercise and subsequent RPE values are moderately correlated (Borg et al., 1985; Cook et al., 1997). Group III and IV afferents are highly associated with pain (Martin et al., 2008). In

comparison, Pearcey et al. (2016) found an increase in CMEP amplitude and attributed the change to enhanced motoneuron pool excitability. Martin et al. (2008) noted activity in group III and IV afferents facilitated CMEP amplitude in the biceps brachii during elbow flexion, which supports our findings. Conversely, group III and IV afferents are thought to promote endurance performance as peripheral fatigue increases (Amann et al., 2020). However, group III and IV afferents restrict motoneuronal output and locomotor muscle activation by increasing central fatigue (Amann et al., 2020). The variance with increased or decreased CSE during intense exercise may manifest in the level of exhaustion and effort exerted during the protocol.

Some mechanisms for increased excitability of the motoneuronal pool may include increased activation of the monoaminergic system. Greater activation of the monoaminergic system may cause an optimal dose of serotonin to fuel central pattern generator outputs (Fornal et al., 1996), and rhythmic outputs such as arm cycling utilize the central pattern generator (Zehr & Duysens, 2004). Considering that spinal excitability is in part facilitated by the central pattern generator (Zehr & Duysens, 2004), increased peripheral excitability might have influenced the autonomy associated with the central pattern generator, consequently increasing spinal excitability. This would be opposed to a negative feedback loop, whereby metabolites induced from intensive exercise affect small afferents such as group III and IV afferents, which would exert inhibitory feedback on the CNS and decrease CNS excitability (Amann, 2011).

4.8.3 Peripheral Excitability of the Biceps Brachii Throughout the Arm-Cycling Sprints

M_{max} is a measure of neuromuscular transmission (i.e., sarcolemma excitability) (Collins et al., 2017). We observed an increase in M_{max} amplitudes from pre- to post-sprinting, which suggests an increase in sarcolemma excitability. Skeletal muscle activation post-sprinting may be

interpreted as potentiation of the muscle, known as post-activation potentiation (Kilduff et al., 2007). While M_{max} is not a direct interpretation of post-activation potentiation, it implies that post-activation potentiation is likely. It has been shown that potentiation and fatigue can coexist concomitantly (Behm et al., 2004; Rassier & Macintosh, 2000). Without performing H-reflex or muscle twitches, we cannot confine where changes may have occurred in the sarcolemma to enhance peripheral excitability. By performing these tests, we could have differentiated between alpha motoneuron excitability versus peripheral excitability (i.e., the muscle). We demonstrated that potentiation and fatigue existed concomitantly during a cycling motion at 60 RPM but not during sprinting. The sprinting likely acted to potentiate the M-wave, which was evidenced during passive cycling at 60 RPM post-sprinting. Unfortunately, we could not measure force from Erb's point stimulation during cycling because there were no force measures, unlike isometric contractions.

Despite no significant difference for M_{max} amplitudes throughout the sprints, except for sprint 3, there was a slight, ~6% increase in M_{max} amplitudes from sprint 1 to 5. However, at post-sprinting, there was a significant increase in the amplitude of the M_{max} compared to at pre-sprinting. Notably, M_{max} was evoked at pre-and post-sprint protocol during submaximal arm cycling at 60 RPM. As mentioned above, the increase in M_{max} is likely an indication of muscle potentiation, which occurred during fatigue. Two opposing processes within the muscle cells happen during repetitive stimulation: i) muscle performance enhancement and ii) muscle performance decrement (Rassier & Macintosh, 2000). While it is not fully understood how these two mechanisms can coexist, low Ca2+ concentrations can induce fatigue, and increased regulatory light chains of myosin phosphorylation can create activity-dependent potentiation. This

mechanism is possible considering that M_{max} infers that changes are likely occurring within the sarcolemma (Collins et al., 2017).

4.8.4 Sprint Performance Measures

The sprint protocol used in the present study was successful in inducing NMF. NMF occurs when skeletal muscle can no longer maintain or produce force output (Gandevia, 2001; Gandevia et al., 1999) or when power output drops during repeated sprint exercise (Billaut et al., 2006; Girard et al., 2011; Hureau et al., 2016; Lockyer et al., 2021; Monks et al., 2016; Pearcey et al., 2016). Similar to previous repeated sprint studies, as the number of sprints performed increased, the power output produced decreased (Billaut et al., 2006; Billaut & Bishop, 2009; Girard et al., 2011; Goodall et al., 2015; Hureau et al., 2016; Mendez-Villanueva et al., 2008; Monks et al., 2016; Pearcey et al., 2016; Weavil et al., 2016). In the current study, NMF was evident as early as sprint 2 and persisted throughout the sprint protocol. However, mean and peak power elicited significant changes from sprints 1 to 3. In the typical fashion of sprint studies, the most significant change in power output occurred during the early part of the sprint protocol (Girard et al., 2013; Mendez-Villanueva et al., 2008; Pearcey et al., 2016).

4.8.5 Methodological Considerations

This was a methodologically challenging study. Several factors must be considered when interpreting the present results.

A stimulus-response curve during sprinting on a separate day from the main protocol may have been a better method to determine CSE because it would allow for precise stimulus intensity and avoid potential miss of the true evoked response. Secondly, the length of each sprint is different from the most comparable study completed by Pearcey et al. (2016), being nearly double in length. Testing MEPs, CMEPs, and M_{max} on separate days would shorten sprints. Our pilot studies found

that participants could not complete 10 ~20-second sprints because of the intense amount of exhaustion. Hence, 10, 10-second sprints would be more appropriate, but this short period would not allow for an appropriate number of stimuli to be measured. Thirdly, delivering MEPs, CMEPs, and M_{max} stimulations during sprinting is inherently difficult. Despite controlling head movement with a restraining device, the head still moves during the sprint. Accordingly, the TMS paddle may not always be directly over the marked vertex. In addition, the electrodes that were used to record EMG and induce stimulation may have moved during fatiguing sprints due to sweat and rapid bodily movements. It was common for experimenters to have to reinforce the electrodes mid-protocol. Inevitably, minor changes can alter the physiological responses being recorded.

Finally, 90 seconds of rest time between each sprint may not be enough time due to the aerobic nature of the repeated sprints. Some participants were unable to recover enough to provide the intended explosive effort. Thus, some participants may have been limited by their aerobic capacity instead of their anaerobic capacity.

4.9 Conclusion

Five maximal intensity bouts of arm-cycling sprints interspersed with 90 seconds of recovery elicited NMF, resulting in modulation within the CNS and PNS. There were no changes in supraspinal or spinal excitability during the repeated sprint protocol. However, at post-sprinting, spinal excitability was enhanced (as evidenced by reduced stimulation intensity at AMT). Peripheral excitability was also increased during and following the sprint protocol. Further investigation is needed to understand the exact mechanism(s) underlying the power loss during repeated sprinting. Finally, there are methodological considerations to consider when developing future studies to determine how CSE may be altered during repeated sprinting.

4.10 References

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4.11 Table Legends

Table 1 Participant averages for MEP amplitude, CMEP amplitude, M_{max} amplitude, and average bEMG from sprint 1 to 5. All data shown as mean.

Table 2 Participant average data for repeated sprint performance recorded during each sprint in the pronated forearm position. All data shown as mean ± SD.

4.12 Figure Legends

Figure 1 Schematic diagram of the experimental setup and protocol. TMS, TMES, brachial plexus stimulation (Erb's Point), and the Biceps Brachii EMG locations are delineated on the participant. The directions of the arrows demonstrate the direction that all participants cycled during the experimental protocol.

Figure 2 Box and whisker plots for stimulation responses and pre-stimulus EMG (mV). Data points represent means ± SD. **(A)** MEP amplitudes were averaged within a sprint and the average MEP was compared across sprints. There was no significant main effect for the average MEP amplitude across sprints [$F(1.811, 19.92)= 1.620$, $p = 0.2235$]. **(B)** CMEP amplitudes were averaged within a sprint and the average CMEP was compared across sprints. There was no significant main effect for the average CMEP amplitude across sprints [$F_{(2.467, 27.14)}= 1.365$, $p = 0.2744$]. **(C)** M_{max} amplitudes were averaged within a sprint and the average M_{max} was compared across sprints. There was no significant main effect for the average M_{max} amplitude across all sprints [$F(1.582, 16.22)= 3.148$, $p = 0.0793$]. **(D)** Average background pre-stimulus EMG was compared across sprints. There was no significant main effect for the average background EMG for the biceps brachii [$F(4, 55)= 1.510$, $p = 0.2120$].

Figure 3 Box and whisker plots representing stimulation intensities for TMS (%MSO) and TMES (mA), and M_{max} amplitudes for pre and post AMT. Data points represent means ± SD and asterisks represents statistical significance of $p < 0.05$. **(A)** There was no significant difference between pre- and post-AMT TMS intensities ($p > 0.05$). **(B)** There was a 4.57% decrease in TMES intensity from pre to post AMT, which resulted in a significant difference between pre-sprint TMES intensities during AMT (134.00 ± 31.52 mA) testing and post-sprint TMES intensities during AMT testing (124. ± 30.08 mA), t (12) = 3.445, $p = 0.0055$. **(C)** There was a significant difference found between pre-sprint M_{max} amplitudes during AMT (12.73 mV ± 3.973 mV) testing and post-sprint M_{max} amplitudes during AMT testing (14.27 ± 4.402 mV), t (11) = 4.261, $p = 0.0013$.

4.13 Tables

Table 1: Average raw data for TMS, TMES, Erb's Point stimulation, and background EMG during each sprint shown as mean only.

TABLE 1. Raw data values for the bicep brachii

SPRINT	MEP Amplitude (mV)	CMEP Amplitude (mV)	Mmax Amplitude (mV)	Average bEMG (mV)
1	9.19546	9.33505	11.51565	0.43985
2	9.33505	8.16781	11.54791	0.37744
3	9.98175	8.23322	12.09795	0.27406
4	10.09049	7.37176	12.47356	0.24712
5	9.93661	7.70972	12.23170	0.24034
% CHANGE				
SPRINTS 1-5	↑ 8.06	↓ 17.41	↑ 6.16	↓ 45.36

Table 2: Average raw data for repeated sprint performance and anaerobic performance recorded during each sprint shown as mean ± SD.

TABLE 2. Sprint performance for each sprint

SPRINT	Mean Power (W)	Peak Power (W)	Mean RPM	Peak RPM	Anaerobic Capacity (W/kg)	Anaerobic Power (W/kg)	Fatigue Index (W/s)	Total Work (Joules)	Borg Scale
1	380.2 ± 91.2	457.3 ± 104.0	133.0 ± 18.0	143.42 ± 20.4	4.6 ± 0.8	5.6 ± 0.8	12.3 ± 4.3	6989.4 ± 1674.5	14.5 ± 2.4
2	324.6 ± 69.8	428.3 ± 88.0	120.5 ± 12.5	128.67 ± 11.3	3.9 ± 0.6	5.2 ± 0.7	10.6 ± 2.7	5955.9 ± 1290.5	16.4 ± 1.8
3	277.1 ± 58.9	381.6 ± 84.2	106.5 ± 10.2	115.08 ± 7.7	3.4 ± 0.4	4.6 ± 0.4	10.0 ± 3.4	5099.4 ± 1084.9	17.8 ± 1.4
4	249.2 ± 55.1	364.1 ± 66.8	99.2 ± 10.6	111.92 ± 5.9	3.0 ± 0.5	4.5 ± 0.5	10.8 ± 2.3	4583.4 ± 1015.1	18.6 ± 1.0
5	240.2 ± 48.0	350.1 ± 68.9	96.7 ± 9.6	110.08 ± 4.8	2.9 ± 0.4	4.3 ± 0.4	10.3 ± 3.3	4417.7 ± 884.0	19.3 ± 0.8
% CHANGE									
SPRINTS 1-5	↓ 36.8	↓ 23.5	↓ 27.3	↓ 23.2	↓ 36.8	↓ 23.1	↓ 16.5	↓ 36.8	↑ 32.8

4.14 Figures

Figure 1

Note*: *The image shown does not include the head restraint that was used during the actual protocol. Also, EMG leads for triceps brachii and brachioradialis are not shown, which were also recorded during the protocol.*

Figure 2

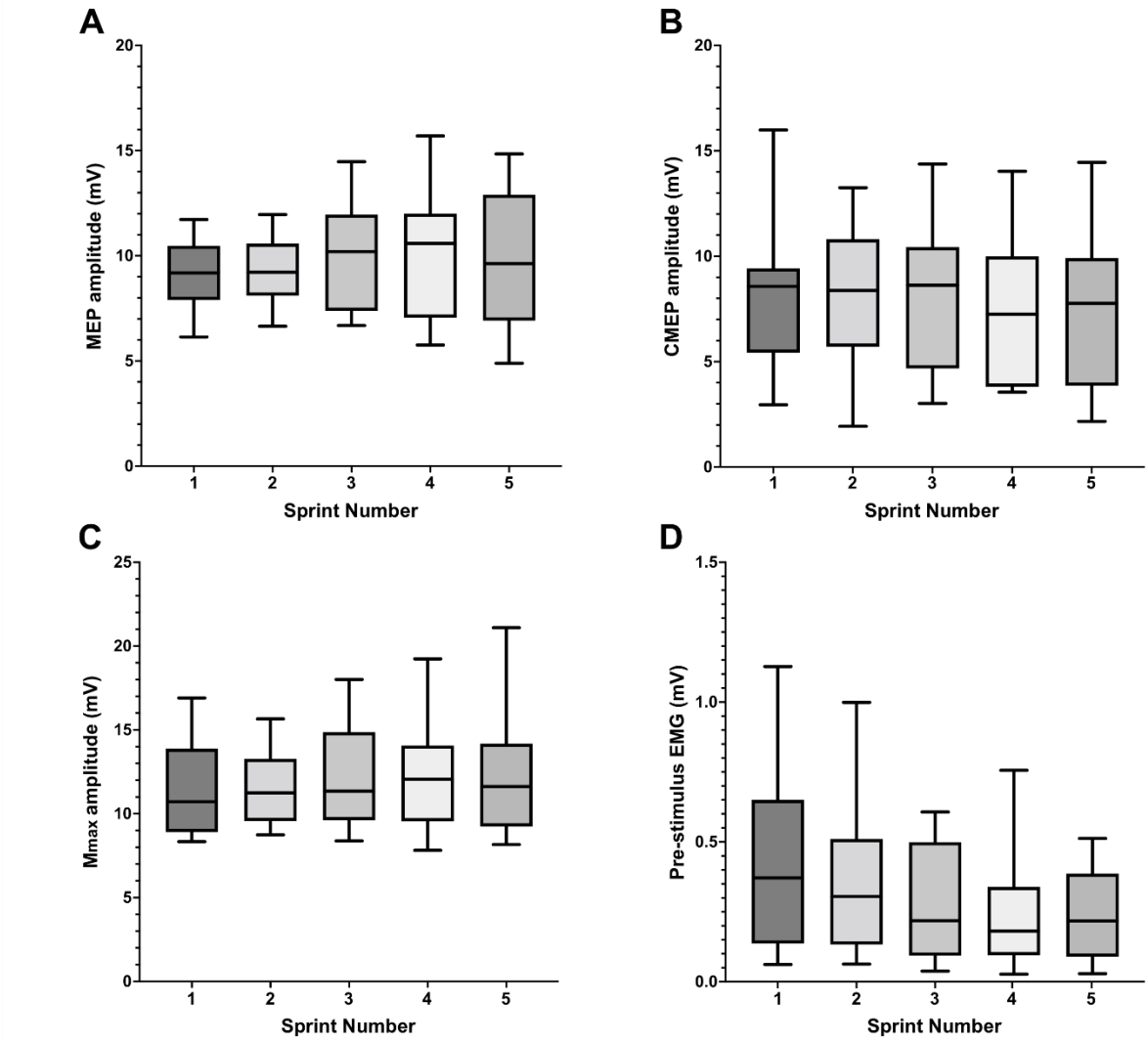
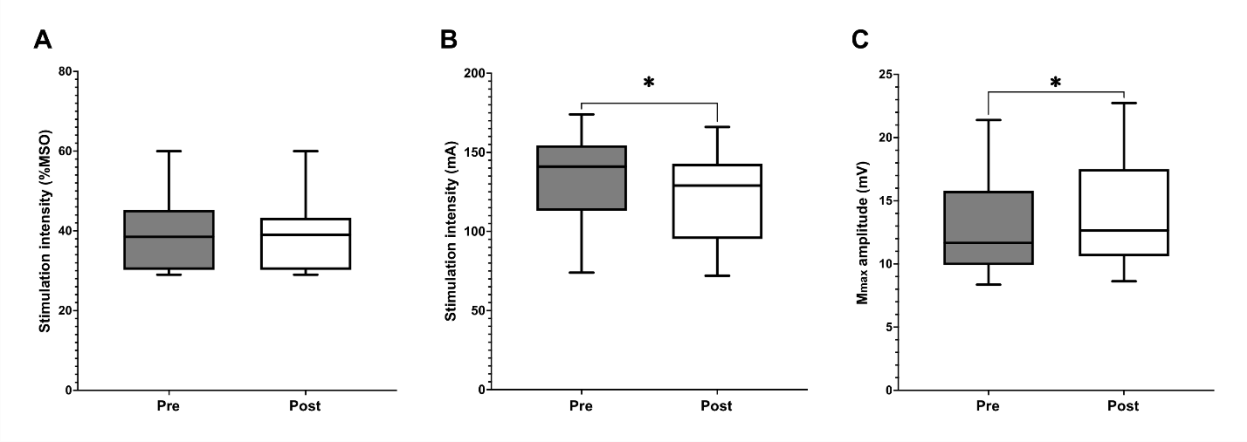


Figure 3



Appendix 1: TMS Safety Checklist

Magnetic Stimulation Safety Checklist

Please read the checklist below. If the answer to any of the questions is yes please indicate that you are ineligible to participate in the study.

You are NOT required to circle a response nor are you required to provide any further information. This checklist is for safety screening only.

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**

If you are using any of the medications listed in the table below you are ineligible to participate in this study.

1) Tricyclic Antidepressants

2) Neuroleptic or Antipsychotic drugs

    a) Typical antipsychotics
        - Phenothiazines
        - Thioxanthenes
            - Chlorpromazine (Thorazine)
            - Chlorprothixene
            - Fluphenazine (Prolixin)
            - Flupenthixol (Depixol and Fluanxol)
            - Perphenazine (Trilafon)
            - Thiothixene (Navane)
            - Prochlorperazine (Compazine)
            - Zuclopenthixol (Clopixol and Acuphase)
            - Thioridazine (Mellaril)
            - Butyrophenones
            - Trifluoperazine (Stelazine)
            - Haloperidol (Haldol)
            - Mesoridazine
            - Droperidol
            - Promazine
            - Pimozide (Orap)
            - Triflupromazine (Vesprin)
            - Melperone

- 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 2: Ethics Approval

MEMORIAL UNIVERSITY

Interdisciplinary Committee on Ethics in Human Research (ICEHR)

St. John's, NL Canada A1C 5S7 Tel: 709 864-2561
icehr@mun.ca

www.mun.ca/research/ethics/humans/icehr

ICEHR Number:	**20192592-HK**
Approval Period:	April 3, 2019 – April 30, 2020
Funding Source:	NSERC (RGCS:20181886; PI: Button)
Responsible Faculty:	Dr. Duane Button School of Human Kinetics and Recreation
Title of Project:	*Understanding changes in corticospinal excitability of neuromuscular fatigue induced by maximal arm cycling sprints*

April 3, 2019

Mr. Garreth Kippenhuck
School of Human Kinetics
and RecreationMemorial
University of
Newfoundland

Dear Mr. Kippenhuck:

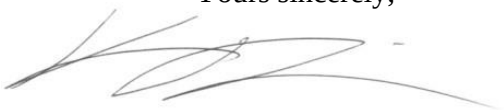
Thank you for your correspondence of April 1, 2019 addressing the issues raised by

the Interdisciplinary Committee on Ethics in Human Research (ICEHR) concerning the above-named research project. ICEHR has re-examined the proposal with the clarification and revisions submitted, and is satisfied that the concerns raised by the Committee have been adequately addressed. In accordance with the *Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans (TCPS2)*, the project has been granted *full ethics clearance* to April 30, 2020. ICEHR approval applies to the ethical acceptability of the research, as per Article 6.3 of the *TCPS2*.Researchers are responsible for adherence to any other relevant University policies and/or funded or non-funded agreements that may be associated with the project.

The *TCPS2* **requires** that you submit an Annual Update to ICEHR before April 30, 2020. If you planto continue the project, you need to request renewal of your ethics clearance and include a brief summary on the progress of your research. When the project no longer involves contact with human participants, is completed and/or terminated, you are required to provide an annual update with a brief final summary and your file will be closed. If you need to make changes during the project which may raise ethical concerns, you must submit an Amendment Request with a description of these changes for the Committee's consideration prior to implementation. If funding is obtained subsequent to approval, you must submit a Funding and/or Partner Change Request to ICEHR beforethis clearance can be linked to your award.

All post-approval event forms noted above can be submitted from your Researcher Portal account by clicking the *Applications: Post-Review* link on your Portal homepage. We wish you success with your research.

Yours sincerely,



Kelly Blidook, Ph.D.
Vice-Chair,
Interdisciplinary
Committee onEthics in
Human Research

KB/lw

cc: Supervisor – Dr. Duane Button, School of Human Kinetics and RecreationDirector, Research Grant and Contract Services

Appendix 3: Informed Consent Form

Informed Consent Form

Title: Understanding changes in corticospinal excitability of neuromuscular fatigue induced by maximal arm-cycling.

Researcher(s):

Mr. Garreth Kippenhuck

Masters Student

School of Human Kinetics and Recreation

Memorial University

Email: gtk121@mun.ca

Dr. Duane Button
Associate Professor
School of Human Kinetics and Recreation
Memorial University
Email: dbutton@mun.ca

Dr. Kevin Power

Associate Professor

School of Human Kinetics and Recreation

Memorial University

Email: kevin.power@mun.ca

Mr. Shahab Alizadeh

Masters Student

School of Human Kinetics and Recreation

Memorial University

Email: shahab.a91@gmail.com

Mr. Evan Lockyer

PhD Student

School of Human Kinetics and Recreation

Memorial University

Email: ejl006@mun.ca

Ms. Erika Lynn Noel
Masters Student
School of Human Kinetics and Recreation
Memorial University
Email: elnoel@mun.ca

You are invited to take part in a research project entitled "Understanding changes in corticospinal excitability of neuromuscular fatigue induced by maximal arm-cycling." This project is the MSc thesis for Mr. Garreth Kippenhuck which is supervised by Dr. Duane Button.

This form is part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. **It also describes your right to withdraw from the study at any time**. In order to decide whether you wish to participate in this research study, you

should understand enough about its risks and benefits to be able to make an informed decision. This is the informed consent process. Take time to read this carefully and to understand the information given to you. Please contact the lead researcher, Mr. Garreth Kippenhuck, if you have any questions about the study or would like more information before you consent.

It is entirely up to you to decide whether to take part in this research study. If you choose not to take part in this research or if you decide to withdraw from the research once it has started, there will be no negative consequences for you, now or in the future. Furthermore, potential participants must be resistance trained (trained on average of greater than or equal to 3 sessions a week for approximately an hour each session for at least 1 year). Physical health will be deemed ready for exercise based on the Physical Activity Readiness Questionnaire for Everyone as per CSEP guidelines. Students in classes currently being taught by either of the investigators cannot participate in the study.

Introduction:

This research is being conducted by Mr. Garreth Kippenhuck, a Master's Student in the School of Human Kinetics and Recreation at Memorial University of Newfoundland, to investigate the contribution of brain and spinal mechanisms in the neural control of increasing levels of neuromuscular fatigue in biceps brachii muscle during arm-cycling sprints. In other words, we are examining how the central nervous system will change as fatigue increases during arm-cycling sprints. Such research will provide invaluable information regarding the modulation of supraspinal (brain) and spinal (spine) excitability with neuromuscular fatigue during an exhaustive upper-body locomotion activity.

Purpose of study:

The purpose of this study is to examine the modulation of brain and spine excitability during arm-cycling sprints with increasing levels of neuromuscular fatigue.

What you will do in this study:

<u>Familiarization Day:</u>

At least one day prior to the main experiment, you will be subjected to transcranial magnetic stimulation (TMS) to magnetically stimulate the brain and transmastoid electrical simulation (TMES) to stimulate the back of the neck (spinal cord). Furthermore, you will be stimulated just above the collar bone (Erb's Point). A couple stimulations will be given with each at similar intensities that will be done during the experimental day. This is to ensure that you are fine with the sensation of the stimulations come the experimental day. A brief cycling warm-up will also be done followed by several short sprints

(5-10 seconds in duration) to ensure that you are capable and willing to engage in the exhaustive cycling protocol during the experimental day.

Experimental Day:

We will use a combination of TMS and TMES to assess the excitability of the biceps brachii during arm-cycling sprints. Prior to the test, we will shave and place electrodes electrodes on your Biceps and Triceps Brachii muscles, the side of the elbow, above your collar bone, and on your shoulder. This is for the usage of Electromyography (EMG) which records the electrical response of the muscles. Furthermore, electrodes will be placed on the back of your neck as this is where TMES is elicited to. Afterwards, you will be positioned on a custom chair. After positioning, you will perform some light cycling to become accustomed to the testing procedure and to warm up your muscles. While lightly cycling, a stimulus to the neck will elicit a response in the arm muscles. You will be asked to perform several bouts of light cycling. During cycling, we will deliver TMS and TMES, and Erb's point stimulation (the area just above the collar bone) to determine the amount of stimulus to use while sprinting. The experimental protocol will consist of 10 repeated maximal effort sprints, each sprint being 10-20 seconds in duration, which will be interspersed with 2-3 minutes of rest. A 20-second window will be given for you to maintain around 100 revolutions per minute prior to sprinting. At the 20 second mark, a load equivalent to 5% of your body mass will be automatically applied to the arm cycle. TMS and TMES will be used during each sprint. You will be verbally encouraged to maintain maximal exertion for the duration of each sprint. Furthermore, you can stop at any time if unforeseen circumstances were to impede your ability to perform.

SPECIFIC DESCRIPTION OF STIMULATION CONDITIONS

The stimulation technique, TMS, will occur over the brain. The stimulation will be delivered via a circular coil to the brain tissue and responses will be recorded from muscle. This method is widely used to test brain excitability. By the comparison of the size of the response recorded from the muscle during arm-cycling sprints, useful information about the differences of neural activity will be obtained. TMES is an electrical stimulation directly to the back of the neck. It provides a measure of the excitability of neurons which runs throughout the spinal cord. Also, an electrical stimulation will be delivered via an electrode located above the collar bone and on the shoulder to record a response in the biceps brachii. The values of the responses will be used to determine how the brain and spinal cord react to fatigue. These stimulations are designed for human research. They are completely safe and have been used extensively by Drs. Power and Button. Skin preparation will be undertaken for all electrodes, including shaving hair off the desired area followed by cleansing with an isopropyl alcohol swab. The electrodes do contain an adhesive that allows them to stick to the skin.

I will gladly answer any questions or concerns you may have regarding any portion of the study if the procedures are not completely clear.

Length of time:

Participation in this study will require you to come to a lab located in the School of Human Kinetics and Recreation at Memorial for two sessions. The first being about an hour and the second about two hours for a total participation time of three hours.

Withdrawal from the study:

You will be free to withdraw from this study at any point up until the end of the testing session. To do so you simply need to inform the researchers and you will be free to leave. Any data collected up to that point will not be used in the study and will be destroyed. In addition, you may request for the removal of your data at any time up to one year later. If you are a student, your participation in and/or withdrawal from this study will not in any way, now or ever, negatively impact either your grade in a course, performance in a lab, reference letter recommendations and/or thesis evaluation.

Possible benefits:

The benefit of participating in my study is that you will learn about the functioning of your nervous system during repeated, arm-cycling sprints. Also, your participation will definitely help us to understand the mechanisms of impaired muscle function due to fatigue. It also helps us provide insight into arm-cycling performance and potential mechanisms to improve fatigue, which may have positive impact in rehabilitation after injury and athletic training. The findings of this research may be used for guiding rehabilitation strategies and exercise interventions for clinical and non-clinical populations.

Possible risks:

There are several minor risks associated with participating in this study:

1) You will have electrodes placed on the front and back of your arm. These electrodes have an adhesive that has a tendency to cause redness and minor irritation of the skin. This mark is temporary (usually fades within 1-2 days) and is not generally associated with any discomfort or itching.

2) As mentioned above, electrical stimulation will be delivered to the brachial plexus before the main experiment to record M-wave. This will be used to analyze the response and adjust the stimulus intensity. As mentioned, this stimulation protocol will be performed prior to start the test, and will not repeat during the test procedure. The electrical stimulations will cause twitching of the the muscles. The sensation will give you a sharp pain and discomfort, yet, will be very brief (less

than a second) and will in no way result in any harm to either muscles or skin in a short or long-term period.

3) TMS is used to assess brain excitability and is applied at the surface of the top of the skull. This will cause activation of the brain resulting in small muscle contraction. The stimulation is not painfull and most individuals do not experience any discomfort.

4) TMES is used to assess spinal excitability and is applied to the mastoid processes located posteriorly on the skull. Stimulations may be of slight discomfort in the form of sharp pain and discomfort that normally lasts less than a second and will in no way result in any harm to either muscles or skin in a short or long-term period.

5) Post experiment muscle soreness, simlilar to that following an acute bout of exercise will be experienced by some participants.

6) Psychological risks such as nervousness or anxiety may be experienced due to the various stimulation techniques used (top of head and transmastoid). You will be given the opportunity to ask any questions you have.

7) The use of stimulations has other small risks associated with it. The most immediate of these risks is the rare occurrence of fainting and the even rarer occurrences of induced seizures. Precautions are taken to avoid such occurrences by completing a magnetic stimulation safety checklist, a medication screening, and a Physical Activity Readiness Questioner for Everyone.

Each investigator is first aid certified and has access to emergency services in the unlikely event that you require medical assistance. The following address is for the University Counselling Centre should you feel the need to avail of their services.

University Counselling Centre

5th Floor University Centre, UC-5000

Memorial University of Newfoundland

St. John's NL A1C 5S7

Tel: (709) 864-8874

Fax: (709) 864-3011

Director/Associate Professor: Peter Cornish, Ph.D.

NOTE: The stimulators used for the experiment are designed for human research, are completely safe and have been used extensively by Dr. Button.

Confidentiality:

The ethical duty of confidentiality includes safeguarding participants' identities, personal information, and data from unauthorized access, use, or disclosure.

Your identity will be guarded by maintaining data in a confidential manner and in protecting anonymity in the presentation of results (see below).

Results of this study will be reported in written (scientific article) and spoken (local and national conferences and lectures) forms. For both forms of communication only group average data will be presented. In cases where individual data needs to be communicated it will be done in such a manner that your confidentiality will be protected (i.e. data will be presented as coming from a representative subject).

Anonymity:

Anonymity refers to protecting participants' identifying characteristics, such as name or description of physical appearance. Only the researchers will be aware of your participation. In addition to Drs. Duane Button and Kevin Power, the other researchers, all graduate students, required to assist with data collection are:

1. Garreth Kippenhuck
2. Shawn Wiseman
3. Evan Lockyer
4. Shahab Alizadeh
5. Erika Lynn Noel

Every reasonable effort will be made to ensure anonymity; and you will not be identified in publications without explicit permission.

Recording of Data:

There will be no video or audio or photographic recordings made during testing.

Storage of Data:

The only individuals who will access to this data are the researchers involved in this study. Data will be retained for a minimum of five years, as per Memorial University policy on Integrity in Scholarly Research after which time it will be destroyed. All data will be kept in a secured location: paper-based records will be kept in a locked cabinet in the office of Dr. Button while computer based records will be stored on a password protected computer in the office of Dr. Button. The data collected as a result of your participation can be withdrawn from the study at your request up until the point at which the results of the study have been accepted for publication (~1-year post study). During this period, participants' data will be removed from the study by using participant codes.

Reporting of Results:

Results of this study will be reported in written (scientific article) and spoken (local and national conferences and lectures) formats. Generally speaking, all results will be presented as group averages. In cases where individual data needs to be communicated it will be done in such a manner that your confidentiality will be protected (i.e. data will be presented as coming from a representative participant). The master's thesis will be publically available at the QEII Library site (http://collections.mun.ca/cdm/search/collection/theses.) upon completion.

Sharing of Results with Participants:

Following completion of this study please feel free to ask any specific questions you may have about the activities you were just asked to partake in. Also, if you wish to receive a brief summary of the results then please indicate this when asked at the end of the form and provide us with your contact information, including name and Email address.

Questions:

You are welcome to ask questions at any time before, during, or after your participation in this research. If you would like more information about this study, please contact Garreth Kippenhuck (gtk121@mun.ca) or Dr. Duane Button (dbutton@mun.ca).

The proposal for this research has been reviewed by the Interdisciplinary Committee on Ethics in Human Research and found to be in compliance with Memorial University's ethics policy. If you have ethical concerns about the research, such as the way you have been treated or your rights as a participant, you may contact the Chairperson of the ICEHR at icehr@mun.ca or by telephone at 709-864-2861.

Magnetic Stimulation Safety Checklist

Please read the checklist below. If the answer to any of the questions is yes please indicate that you are ineligible to participate in the study.

You are NOT required to circle a response nor are you required to provide any further information. This checklist is for safety screening only.

13. Do you suffer from epilepsy, or have you ever had an epileptic seizure? **YES/NO**

14. Does anyone in your family suffer from epilepsy? **YES/NO**

15. Do you have any metal implant(s) in any part of your body or head? (Excluding tooth fillings) **YES/NO**

16. Do you have an implanted medication pump? **YES/NO**

17. Do you wear a pacemaker? **YES/NO**

18. Do you suffer any form of heart disease? **YES/NO**

19. Do you suffer from reoccurring headaches? **YES/NO**

20. Have you ever had a skull fracture or serious head injury? **YES/NO**

21. Have you ever had any head surgery? **YES/NO**

22. Are you pregnant? **YES/NO**

23. Do you take any medication? **YES/NO**
    a. Note if taking medication, check list for contraindicated medication on next page.

24. Do you suffer from any known neurological or medical conditions? **YES/NO**

If you are using any of the medications listed in the table below you are ineligible to participate in this study.

3) Tricyclic Antidepressants

4) Neuroleptic or Antipsychotic drugs

e) Typical antipsychotics
- Phenothiazines
- Thioxanthenes
  - Chlorpromazine (Thorazine)
  - Chlorprothixene
  - Fluphenazine (Prolixin)
  - Flupenthixol (Depixol and Fluanxol)
  - Perphenazine (Trilafon)
  - Thiothixene (Navane)
  - Prochlorperazine (Compazine)
  - Zuclopenthixol (Clopixol and Acuphase)
  - Thioridazine (Mellaril)
  - Butyrophenones
  - Trifluoperazine (Stelazine)
  - Haloperidol (Haldol)
  - Mesoridazine
  - Droperidol
  - Promazine
  - Pimozide (Orap)
  - Triflupromazine (Vesprin)
  - Melperone

- Levomepromazine (Nozinan)

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

g) Dopamine partial agonists:
    - Aripiprazole (Abilify)

h) 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.

Consent:

Your signature on this form means that:

- You have read the information about the research.
- You have been able to ask questions about this study.
- You are satisfied with the answers to all your questions.
- You understand what the study is about and what you will be doing.
- You understand that you are free to withdraw participation in the study without having to give a reason, and that doing so will not affect you now or in the future.
- You understand that if you choose to end participation **during** data collection, any data collected from you up to that point will destroyed.
- You understand that if you choose to withdraw **after** data collection has ended, your data can be removed from the study up to one year after the conclusion of data collection.

By signing this form, you do not give up your legal rights and do not release the researchers from their professional responsibilities.

Your signature confirms:

☐ I have read what this study is about and understood the risks and benefits. I have had adequate time to think about this and had the opportunity to ask questions and my questions have been answered.

☐ I agree to participate in the research project understanding the risks and contributions of my participation, that my participation is voluntary, and that I may end my participation.

☐ A copy of this Informed Consent Form has been given to me for my records.

☐ I would like to receive a summary of the results of the study. (If you check this box, please provide us with your Email address and/or Mail address)

_____ _____

Signature of participant Date

Researcher's Signature:

I have explained this study to the best of my ability. I invited questions and gave answers. I believe that the participant fully understands what is involved in being in the study, any potential risks of the study and that he or she has freely chosen to be in the study.

_____ _____

Signature of Principal Investigator Date