CORTICOSPINAL CONTRIBUTIONS TO NEUROMUSCULAR FATIGUE FOLLOWING EXAUSTHIVE STRETCH-SHORTENING CYCLE ACTIONS

Jacopo Bascherini

Master`s Thesis
Faculty of Sport and Health Sciences
University of Jyväskylä
Spring 2024

ABSTRACT

Neuromuscular fatigue refers to any exercise-induced decline in force generation capacity. It may stem from disturbances in processes at or distal to the neuromuscular junction, referred to as peripheral fatigue, as well as proximal to it, referred to as central fatigue. Central fatigue can be further distinguished into spinal or supraspinal fatigue. No studies have assessed central fatigue or the degree of supraspinal fatigue after exhaustive stretch-shortening cycle (SSC) exercise. Therefore, the purpose of the present study was to investigate the acute corticospinal contribution to neuromuscular fatigue following exhaustive SSC exercise. Ten healthy active individuals were assigned to the fatigue group (FAT) and completed the SSC fatigue protocol. Ten different individuals did not engage in any exercise, serving as control group (CON). Maximal voluntary contraction (MVC) and tibial nerve electrical, as well as primary motor cortex magnetic stimulation evoked force and surface EMG (M-wave and MEP) responses, were recorded before and immediately after SSC exercise to assess voluntary activation ratio and corticospinal excitability. To assess the magnitude of acute exercise-induced fatigue, assessments of neuromuscular and corticospinal functions were completed within 3.5 minutes after exercise cessation. Ankle plantar flexor MVC for FAT decreased by ~18.12% ($p < 0.001$; $d = 0.89$) after exhaustive SSC exercise. Cortical voluntary activation ratio for FAT declined from 90.3 ± 10.1% at baseline to 77.4 ± 15.4% after SSC exercise ($p = 0.001$, $r = 0.79$). Voluntary activation ratio measured via motor nerve stimulation declined from 92.7 ± 6.8% at baseline to 82.3 ± 12.9% after SSC exercise ($p = 0.037$; $r = 0.66$). Resting twitch amplitude declined by ~ 9.2% ($p = 0.03$; $d = 0.34$). Silent period duration lengthened by ~13.5% ($p = 0.01$; $d = 1.38$), while MEPs remained unchanged. Thus, exhaustive SSC exercise induced considerable central fatigue and caused an impairment in the capacity of the motor cortex to drive the ankle plantar flexors along with increased level of intracortical inhibition. As a result, maximum force-generation capacity was significantly reduced by central fatigue as well as by peripheral mechanisms following SSC exercise.

ABBREVIATIONS

aMT	Active Motor Threshold
CNS	Central Nervous System
CON	Control Group
CSE	Corticospinal Excitability
CV	Coefficient of Variation
DJ	Drop Jump
EMG	Electromyography
ERT	Estimated Resting Twitch
FAT	Fatigue Group
GABA	Gamma-Aminobutyric Acid
GCT	Ground Contact Time
GRF	Ground Reaction Force
ICC	Intraclass Correlation Coefficient
ITT	Twitch Interpolation Technique
JH	Jump Height
LICI	Long-Interval Cortical Inhibition
*M*max	Maximal M-wave
MVC	Maximal Voluntary Contraction
MU	Motor Unit
NMFA	Neuromuscular Fatigue Assessment
M1	Primary Motor Cortex
MEP	Motor Evoked Potential
$Q_{tw,pot}$	Potentiated Twitch
PA	Posterior-Anterior
PNS	Motor Nerve Stimulation
PT	Passive Twitch
POT	Potentiation Effect
SD	Standard Deviation

SICI	Short-Interval Cortical Inhibition
SIT	Superimposed Twitch
SMN	Spinal Motor Neurons
SP	Silent Period
SSC	Stretch-Shortening Cycle
TE	Typical Error
TES	Transcranial Electrical Stimulation
TMS	Transcranial Magnetic Stimulation
VA	Voluntary Activation
5-HT	Serotonin
αMN	Alpha-motoneuron
γMN	Gamma-motoneuron

CONTENTS

1. INTRODUCTION

Fatigue is not a unitary phenomenon and cannot be defined as such. As suggested by Kluger et al. (2013), a more thorough comprehension of the concept of fatigue in the context of human performance should not only acknowledge but also distinguish between perceptions of fatigue and fatigability. In healthy adults, perceptions of fatigue and fatigability are predictable and transient phenomena typically induced by prolonged exercise that diminishes with rest and does not interfere with regular daily activities. Perception of fatigue refers to the increase in the subjective perception of fatigue emerging during a motor task that can affect motor task performance. It is often defined as a transient sensation of tiredness, weariness, or exhaustion. Recently, it has also been defined as a sensation of the need for rest or a mismatch between effort expended and actual performance (Skau et al., 2021). The nature and extent of perceived fatigue depend on the psychological state of the individual and psychological factors, such as perceptions of effort, expectations, familiarity, motivation, arousal, and mood, that influence the perceptual and cognitive processes during exercise. Some evidence suggests that these factors, particularly perceptions of effort, may be the primary factors limiting prolonged performance for many motor and cognitive tasks in healthy adults (Kluger et al., 2013; Skau et al., 2021). Fatigability, also known as neuromuscular fatigue (Place & Millet., 2020), constitutes a multifaceted phenomenon depending on the contractile capabilities of the involved muscles and the capacity of the nervous system to provide a sufficient activation signal derived from descending commands and afferent feedback for the prescribed task (Kluger et al., 2013; Enoka & Duchateau., 2016). The term neuromuscular fatigue has been used to refer to any exercise-induced reduction in the ability to exert muscle force or power, regardless of whether or not the task can be sustained, with rest reversing its course (Gandevia, S. C. 2001). It is important to emphasize that the decline in maximal functional capacities should not be confounded with task failure, as the underlying mechanisms can differ. Furthermore, the maximal force-generating capacity of muscles starts to decline once exercise commences so that fatigue really begins almost at the onset of the exercise and develops progressively before the muscles fail to perform the required task. A development of the definition was proposed by Kluger et al. (2013), defining neuromuscular fatigue in more comprehensive terms as the magnitude or rate of change in a performance criterion relative to a reference value over a given

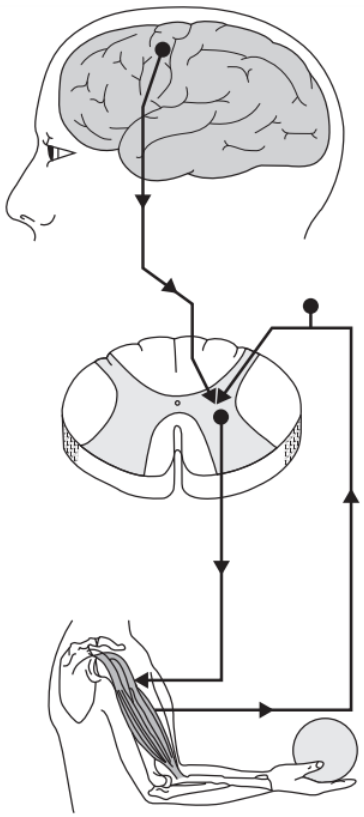
task of performance or measure of mechanical output. Throughout the rest of this document, we will consistently refer to the initial definition as it is the most employed in the existing literature. Yet even when considering the loss of maximal function capacities, or transient loss of work or force generating capacities, two main issues can be highlighted. Firstly, neuromuscular fatigue is often quantified, albeit in a potentially oversimplified manner, by the pre-exercise-to-post-exercise reduction in the maximal voluntary contraction (MVC). However, it is possible to observe no decrease in MVC despite the presence of some level of fatigue. Specifically, low-frequency fatigue, also referred to as prolonged low-frequency force depression (Burton et al., 2008), may persist even when MVC force, characterized by high motor units discharge rate, does not exhibit a change or experience a full recovery. Secondly, when neuromuscular fatigue is only measured at exhaustion, where the decision to terminate it is a voluntary act and thus can be influenced by cognitive processes, one must assume that the motivation of the participants is optimal. The scope of the present study is limited to a more in-depth exploration of the neuromuscular fatigue phenomenon and its multifaceted nature. However, it is crucial to acknowledge that neuromuscular fatigue and perceived fatigue are interconnected phenomena since most voluntary actions performed by humans involve significant interactions between the two domains.

The exercise-induced decline in force-generating capacity may arise because of peripheral changes at the level of the contractile machinery, but also because the central nervous system (CNS) fails to drive the motoneurons adequately. As mentioned above, neuromuscular fatigue can be addressed by various processes along the motor pathway that are broadly split into central and peripheral origins. Peripheral or distal fatigue can be attributed to the alterations of processes at or distal the neuromuscular junction, such as the failure of the propagation of action potential, disruption of excitation-contraction coupling processes, or the incorrect functioning of the contractile machinery leading to an attenuated response to neural excitation (Bigland-Ritchie et al., 1978). Central fatigue refers to a disturbance of processes occurring proximal to the neuromuscular junction, further discernable in spinal and supraspinal origins, leading to a reduction in the capacity of activate the muscle voluntarily. Therefore, the ability of a muscle to be fully activated depends on the net effect of excitatory and inhibitory influences of spinal and supraspinal neural circuits (Tanaka & Watanabe., 2012). Such neural circuits, however, receive excitatory and inhibitory inputs from both skeletal muscles and various sites within the CNS itself (Taylor & Gandevia., 2008). In this regard, voluntary activation refers to the level of “drive” to the motoneurons, modulating the recruitment and firing rate of these latter, and

muscle fibers, which rely on muscle fibers to translate the motoneuron firing into force (Gandevia., 2001). It is crucial to avoid compartmentalizing these two categories of fatigue as distinct entities since peripheral-generated fatigue signals result in the modulation of central commands, and descending inputs from the cortical level can similarly impact events at the periphery. The extent to which peripheral and central processes contribute to overall neuromuscular fatigue is strongly related to the nature of the exercise task. It has been shown that a higher degree of peripheral fatigue is appreciable after short-duration, high-intensity locomotor exercise (~6 min), whereas central fatigue is exacerbated after long-duration, low-intensity exercise (~30 min) (Thomas et al. 2015).

2. MOTOR SYSTEM

Prior to proceeding into a more comprehensive exploration of the multifaceted neuromuscular fatigue phenomena, it is essential to provide a structural and organizational overview of the motor system. The motor system encompasses neural and musculoskeletal components involved in movement production (Enoka. 2008, 171) (Figure 2.1). From a comprehensive standpoint, activation signals originate from neural components, while musculoskeletal elements translate these signals into force production and, subsequently, movement. The nervous system comprises two main parts: the central nervous system (CNS) and the peripheral nervous system (Figure 2.1). Within the motor system, the CNS is composed of two primary parts – namely, the brain and the spinal cord (Enoka. 2008, 288). The peripheral nervous system includes afferent and efferent neurons located outside the CNS, conveying respectively sensory information from the surroundings to the spinal cord, and output signals oppositely to the effector organ (Fig 1). In the context of muscle contraction and movement, skeletal muscles serve as the ultimate effectors, innervated by efferent neurons commonly referred to as



motoneurons (Enoka. 2008, 250). The following section will provide a brief introduction of the key features of the above-mentioned structures within the motor system.

FIGURE 2.1. Schematic representation of the motor system (From Komi. 2011).

2.1 Central Nervous System

The central nervous system consists of the brain and the spinal cord. The brain can then be further divided into three parts - the cerebrum, brainstem, and cerebellum. In this complex arrangement, the brain stem, cerebellum and spinal cord mediate reflexes and automatic behaviors, while cortical motor centers are responsible for initiating and controlling voluntary actions (Enoka, 2008, 288), primarily through the primary motor cortex (M1) in the cerebrum.

2.1.1 Motor Cortex

The cerebral cortex, the outermost layer of the cerebrum, comprises tightly arranged neurons. Within this intricate structure, the motor cortex performs a series of motor functions, encompassing the planning, organization, and control of complex movements, as well as projecting neural pathways to the spinal cord (Kidgell & Pearce. 2020, 11). The motor cortex is further discernable into three distinct areas - the primary motor cortex, the premotor cortex, and the supplementary motor area - with the primary motor cortex playing a main role in the execution of voluntary actions. The primary motor cortex differentiates from other regions of the cerebral cortex due to its thickness. Within the primary motor cortex, six distinct layers of neurons (layer I to VI) showcase a structural organization where the main output cells (corticospinal neurons) consist of large pyramidal cells in layer V and smaller cells in layer III (Kidgell & Pearce, 2020, 12) (Figure 2.2). Furthermore, within the M1, these corticospinal neurons exhibit a functional organization to project to motoneurons responsible for controlling specific muscle groups, thereby demonstrating a precise somatotopic organization. The ‘final common pathway’ is represented by the spinal motoneurons (SMNs) of the spinal cord, establishing direct or indirect connections with higher centers and pyramidal cells via various descending tracts. The corticospinal pathway, originating from pyramidal cells in lamina V, is the only descending motor pathway known for establishing monosynaptic connections with

SMNs (Figure 2.2). Cortical motor neurons form synapses with spinal motor neurons, creating a reciprocal relationship where each spinal motor neuron receives inputs from multiple cortical motor neurons (upper motoneurons). The cortico-motor neuronal system undergoes excitatory and inhibitory modulation due to the action of inhibitory interneurons, predominantly found in Layers III and V, represented by stellate and basket cells. Additionally, the axon terminals of small corticocortical pyramidal neurons in layers II and III form inhibitory synapses on gamma-aminobutyric acid (GABA) cortical motor neurons. Corticospinal neurons that arise within the M1 descend through the brainstem and at the medullary spinal junction, approximately 85-90% of the corticospinal neurons cross the midline to form the motor pyramidal decussation, where they continue descending to converge onto contralateral spinal motoneurons within the ventral horn of the spinal cord that innervate limb muscles (Alawieh et al. 2017). The remaining uncrossed corticospinal tract fibers primarily project to ipsilateral spinal motoneurons, where they could alter the excitability of ipsilateral pathways (Carson. 2005).

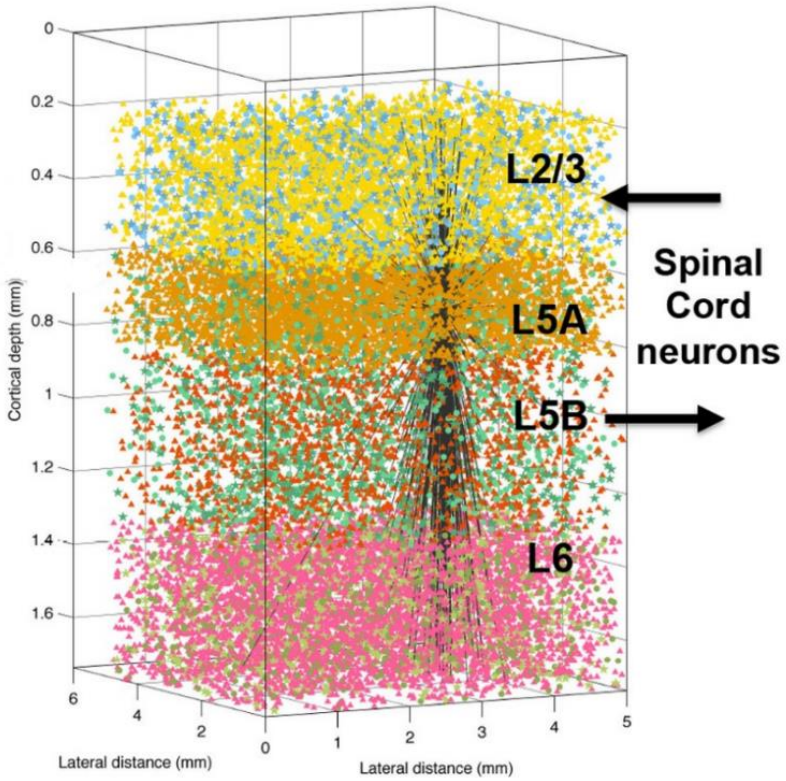


FIGURE 2.2. Virtual model of primary motor cortex. Cell types are shown with different colors. Pyramidal tract-projecting neurons in layer 5 were connected to a descending spinal cord neural population, which provided excitation to the muscles of a realistic virtual musculoskeletal arm. (From Dura-Bernal et al. 2015).

2.1.2 Spinal Cord & Motoneurons

The spinal cord, an extension of the CNS, is a cylindrical longitudinal cord of nerve tissue enclosed within the spinal canal formed by 33 vertebrae. It serves as both a pathway for motor commands from the brain and sensory information to the brain and a center for coordinating and operating reflex actions independently of the brain. Anatomically, the spinal cord originates at the base of the brain stem (medulla oblongata), corresponding to the C1 vertebra, and extends longitudinally to approximately the level of the L1 vertebra. The spinal cord is composed of gray and white matter, appearing in a cross-section as H-shaped gray matter surrounded by white matter. The gray matter consists of the cell bodies of motor and sensory neurons and interneurons. Conversely, the white matter is composed of interconnecting fiber tracts, predominantly consisting of myelinated sensory and motor axons. The grey matter presents two horns on each side, termed the dorsal and ventral horns (Harrow-Mortelliti et al. 2019). The dorsal horns serve as an input pathway for sensory information from peripheral receptors. The cell bodies of the receptors are located in the dorsal root ganglion, a nodule-like structure found on the posterior roots of each spinal nerve, which contains the cell bodies of the afferent sensory nerves. These cell bodies have T-shaped axons where their distal branches travel to sensory endings located in the periphery, and their proximal branches enter the spinal cord via the dorsal horn. The axons of many different peripheral receptors (e.g., muscle spindles, Golgi tendon organs) form a dorsal root and enter the spinal cord through the same dorsal horn (Kidgell & Pearce. 2020, 16). In contrast, the ventral horns are the major output pathway of neural signals to peripheral structures, in particular, muscles via the axons of α-motoneurons and the muscle spindles via the axons of γ-motoneurons. The axons of these neurons form the ventral roots. Most of the spinal neurons are not motor neurons, rather they are interneurons. These interneurons receive information from both afferent and efferent fibers and axons of neurons CNS, to generate action potentials transmitted to other interneurons or motoneurons. The nervous system, through several neural circuits at the spinal level, enables the integration of

sensory information, leading to an appropriate motor response typically initiated in the spinal cord via reflexes. More complex processing will eventually extend to the brainstem and cerebral cortex, whereby complex and fine motor skills are performed. Each ventral horn of the spinal grey matter contains several thousand specialized neurons called anterior motoneurons. From anterior motoneurons originate the nerve fibers that exit the spinal cord via the anterior roots to directly innervate skeletal muscles. Anterior motoneurons can be further distinguished into α-motoneurons and γ-motoneurons (Kidgell & Pearce. 2020, 17). α-motoneurons are large-diameter type of anterior motor neurons that exclusively innervate extrafusal muscle fibers and plays a key role in muscle contraction (Stifani. 2014). They also have an important role in the spinal reflex circuitry by receiving monosynaptic innervation directly from sensory neurons thus minimizing the delay of the response (Stifani. 2014). A single α-motoneuron and the muscle fibers it innervates form the motor functional unit of the neuromuscular system, known as the motor unit (See Section 3.1). Transmission of nerve impulses into skeletal muscles occurs by activating motor units, which in turn depolarize muscle cells, resulting in muscle force production. In contrast, γ-motoneurons are a smaller type of anterior motor neuron that exclusively innervates intrafusal fibers, controlling the sensitivity of muscle spindles (Kidgell & Pearce. 2020, 18). Their firing increases the tension of intrafusal muscle fibers to mimic the stretch of the muscle. Gamma-motoneurons receive only indirect sensory inputs and exhibit no motor function. Therefore, γ-motoneurons do not directly participate in spinal reflexes but instead contribute to the modulation of muscle contraction (Stifani. 2014). The contribution and response of α- and γ-motoneurons to neuromuscular fatigue will be further described in the following sections.

2.1.3 Motor Unit & Firing Rate

The motor unit (MU) is the functional unit of the neuromuscular system involved in motor output. Motor units consist of a cell body, a α-motoneuron, and all the muscle fibers innervated by the α-motoneuron (Enoka & Fuglevand. 2001). Motor units' function is to convert synaptic input received by α-motoneurons into mechanical output by the muscle. The number of muscle fibers per motor unit varies and contributes to determining the motor performance of the muscle. The number of muscle fibers per motor unit can vary from as little as four for ocular muscles, 100 for small muscles involved in fine motor performance, to as many as a 1000 or more for larger muscles involved in gross motor patterns (Duchateau et al. 2006). The group of

spinal motoneurons innervating a single muscle is referred to as a motor unit pool. The motor unit population that forms a motor pool is heterogeneous with respect to the properties of both the motor neurons and the muscle fibers that they innervate (Duchateau et al. 2006). A α-motoneuron is typically characterized by its structure, excitability and distribution of synaptic input, whilst muscles fibers are classified based upon their contractile speed, force generating capacity, and resistance to fatigue. As initially proposed by Henneman (1957), the recruitment of motor units is governed by the "size principle" according to which the smallest α-motoneuron (and motor units) are recruited first, and orderly recruitment, relative to size, occurs thereafter. Although there is a degree of variability in the recruitment order of motor units with similar thresholds (Feiereisen et al. 1997), the fundamental recruitment order of MUs remains consistent with the size principle for isometric and dynamic contractions, encompassing shortening, lengthening and ballistic contractions (Semmler et al. 2002; Søgaard et al. 1996; Stotz & Bawa. 2001; Desmedt & Godaux. 1977; Desmedt & Godaux. 1978). The absolute force at which a motor unit is recruited is not fixed and varies with the speed and type of contraction. When gradually increasing force production, most muscles completely recruit all available motor units between 50 and 85% of maximum force production. During fast or ballistic contractions, the level of force required to recruit a specific motor unit is lowered. Stated another way, larger (the so-called high-threshold) motor units are recruited at a lower force level. As evidenced by Desmedt & Godaux (1977), the recruitment thresholds of MUs in the tibialis anterior decrease progressively with an increase in the rate of force development. Due to this adjustment, during rapid contractions, motor units are activated earlier, and compared to slow-ramp contractions, approximately three times as many motor units are recruited to produce a given peak force during a rapid contraction (Desmedt & Godaux, 1977). In most muscles, the upper limit of motor unit recruitment is ~85% of the maximal force (De Luca et al. 1982; Kukulka & Clamann. 1981). In some hand muscles, however, the upper limit of motor unit recruitment is ~60% of maximum (De Luca et al. 1982; Moritz et al. 2005). The increase in muscle force beyond the upper limit of motor unit recruitment is accomplished entirely by rate coding (also known as discharge rate, firing frequency, or rate of motor unit firing), which fundamentally indicates how often an electrical discharge passes along the α -motoneuron (i.e., motor unit action potential). Therefore, the rate at which the motoneuron discharges action potential significantly impacts the force exerted by the motor unit. In this regard, a sigmoidal relation between average firing rate and muscle force has been shown (Fuglevand et al. 1999; Macefield et al. 1996; Moritz et al. 2005). When first recruited during repetitive voluntary contraction, the minimal rate at which most MUs discharge action potentials is 5-8 Hz (Cutsem

et al. 1997; Heckman & Enoka. 2012). Whereas, during brief isometric voluntary contraction, ranging from submaximal to maximal, average MU firing rates of 30-50 Hz have been recorded from different muscles (Enoka & Fuglevand. 2001). Nevertheless, in certain tasks, MUs have been reported to initially fire doublets (2 action potentials at 100 Hz or more with reduced interpulse interval) to considerably increase the torque output (up to 20%) and rate of torque development (up to 50%) without relying on a sustained increase in the average MU rate coding (Binder-Macleod & Kesar. 2005; Christie & Kamen. 2006). This can be explained by the fact that the force a MU exerts can also be significantly impacted by the discharge pattern. In particular, brief intervals (5-55 ms) between successive action potentials, referred to as double discharges or doublets, can increase the rate of force production during a rapid contraction (Van Cutsem et al. 1998; Thomas et al. 1999). Furthermore, the use of such an activation pattern during a fatiguing contraction can reduce the decline in force (Binder-Macleod et al. 1998; Bigland-Ritchie et al. 2000). During ballistic contraction, instantaneous discharge frequencies of 100-200 Hz have also been reported for single MUs without relying on any discharge pattern (Desmedt & Godaux. 1977; Van Cutsem et al. 2005).

2.2 Reflex Pathways

In order to facilitate the ongoing control of movement and the processing of incoming information within the spinal cord, interneurons are present throughout all regions of the spinal cord. Interneurons are small, highly excitable neurons that establish extensive interconnections with one another and, with many of them forming direct synapses onto anterior motoneurons, enabling the integrative function of the spinal cord to occur. This integrative process occurs via spinal reflex activity. Spinal reflexes are fast responses that involve an afferent signal into the spinal cord and an efferent signal out to the skeletal muscle. The network between the afferent and efferent axons can include from one to several synapses, respectively referred to as monosynaptic and polysynaptic reflexes (Enoka. 2008, 509). All reflexes commence with a stimulus that is of sufficient intensity to activate a sensory receptor. Upon activation, the sensory receptor will discharge action potentials through sensory afferent neurons to the CNS. The CNS, comprising the brain and spinal cord, is the principal integrative center responsible for processing all incoming afferent information and organizing an appropriate response. The orchestrated response results in a series of action potentials in efferent neurons synapsing onto

an effector organ, such as a skeletal muscle, to cause a response. This whole process is referred to as the reflex arc (Kidgell & Pearce. 2020, 18).

2.2.1 Muscle Spindles & Stretch Reflex

Muscle spindles serve as stretch-sensitive receptors, projecting afferents to the spinal cord to provide information to the CNS about muscle length and the changes in muscle length occurring during movement. Each muscle spindle is enclosed in a connective tissue capsule that is infolded by a small group of muscle fibers known as intrafusal fibers (Enoka. 2008, 513). As mentioned earlier, intrafusal fibers receive innervation from efferent axons originating from γ-motoneurons. The neuromuscular junction, established by the axons of γ-motoneurons, occurs at both ends of the intrafusal fibers, where the contractile proteins are located (Figure 2.3). When a γ-motoneuron neuron discharges an action potential, the net effect is a shortening of the intrafusal fiber at its ends and a stretch of the central region (Enoka. 2008, 515). Conversely, the central region is enveloped by sensory nerve endings (sensory Ia afferent) whose responsiveness to any change in length is determined by the amount of stretch imposed on the middle of the intrafusal fibers by the γ-motoneurons (Kidgell & Pearce. 2020, 20). Besides dynamic contraction, during isometric contraction but even at rest with tendon tap, the central region of the muscle spindle is stretched enough to activate the sensory Ia afferent, whose in turn discharge action potentials that arrive at the spinal cord and synapse directly on α-motoneurons innervating the muscle in which the muscle spindle lies, producing a monosynaptic reflex known as stretch-reflex (Figure 2.3). When α-motoneurons discharge in response to muscle spindle input, the skeletal muscle will contract, thus relieving the stiffness on the capsule of the muscle spindle. By varying γ-motoneurons activity, the CNS can alter the amount of sensory information it receives from muscle spindles, therefore dictating the level of activation of α-motoneurons via monosynaptic spinal reflexes (Enoka 2008, 517; Kidgell & Pearce. 2020, 20). The typical mode of operation involves the simultaneous activation of α -

and γ-motoneurons. Discharge from the central regions of muscle spindles leads to concurrent activation of α-motoneurons (via the monosynaptic stretch reflex pathway) and γ-motoneurons, to sustain muscle spindles activity, via a process referred to as alpha-gamma coactivation (Enoka. 2008, 517).

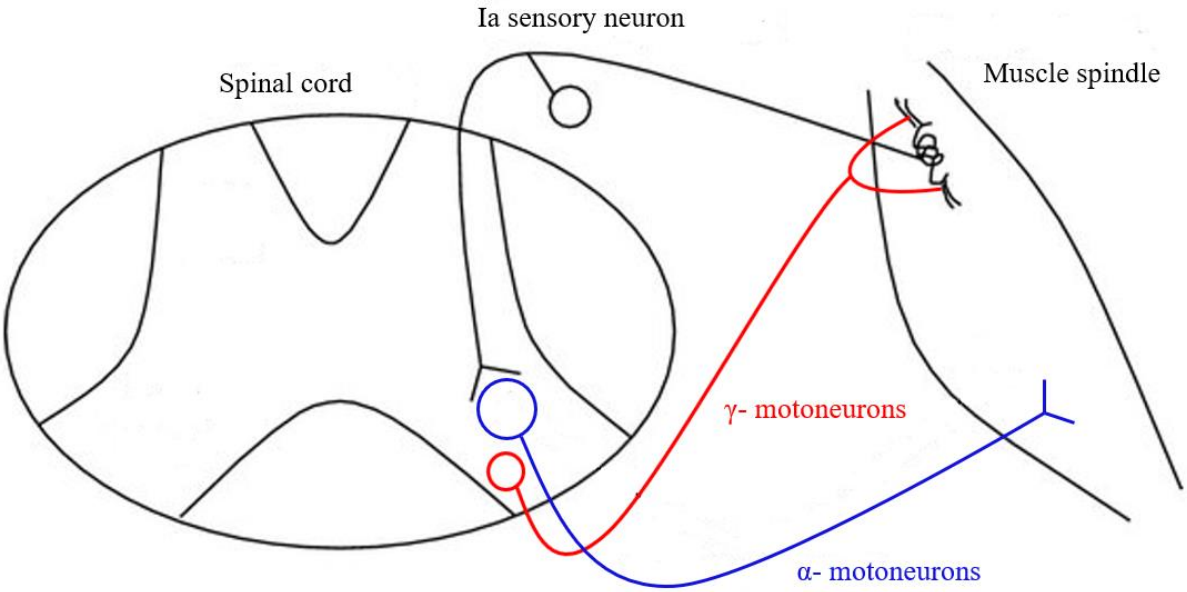


FIGURE 2.3. Schematic illustration of the monosynaptic stretch reflex and induced gamma loop (Adapted from Bhimani & Anderson. 2014).

The Ia afferents, located in the capsular region of muscle spindles, primarily establish monosynaptic excitatory connections with α-motoneurons that innervate the same muscle from where the sensory afferent originated, as well as comparatively weaker monosynaptic and/or polysynaptic excitatory connections with α-motoneurons innervating other muscles (Enoka. 2008, 520). This secondary excitatory pathway comprises Ia afferents establishing connections with interneurons, known as Ia inhibitory interneurons, which upon activation, evoke inhibitory postsynaptic potentials in the motor neurons innervating antagonist muscles. This pathway is referred to as reciprocal Ia inhibition (Enoka. 2008, 520). Because all peripheral and descending inputs to motor neurons also project to Ia inhibitory interneurons, reciprocal Ia inhibition can be facilitated by peripheral sensory feedback and by input from descending pathways. Activation of the reciprocal Ia inhibition pathway can be demonstrated as a decrease in H-reflex

- monosynaptic muscle response evoked by electrical stimulation of the Ia afferents in a peripheral nerve -amplitude in one muscle after electrical stimulation of the nerve innervating an antagonist muscle. The reciprocal Ia inhibition pathway is activated before and during voluntary action. As shown by Crone et al. (1987), the increase in inhibition to the antagonist muscle via reciprocal Ia inhibition pathway could be seen ~50 ms before the onset of contraction during ramp-and-hold dorsiflexion actions, as indicated by a transient depression of the H-reflex amplitude in the soleus muscle. The increase in inhibition before and at the very beginning of the contraction cannot be due to sensory feedback during the contraction but must depend on supraspinal control, whereas the continued depression of the H-reflex during the voluntary contraction is attributed to afferent feedback from the dorsiflexors to the Ia inhibitory interneuron. The function of reciprocal Ia inhibition is to link the inhibition of an antagonist muscle to the activation of an agonist muscle during movements that involve flexion and extension about a joint (Enoka. 2008, 537). However, tasks that involve concurrent intentional activation of both the agonist and the antagonist muscles, referred to as co-contraction (Perez et al. 2007), require depression of the reciprocal Ia inhibition pathways (Enoka. 2008, 538).

The capsular region of the muscle spindle typically also contains Group II afferents originating from minor secondary spindle endings. A single muscle group II afferents can extend to multiple motor nuclei, forming both monosynaptic and polysynaptic connections with homonymous motoneurons (Enoka. 2008, 544). The major effect of group II afferent on α-motoneurons, however, is mediated through interneurons that receive input from ipsilateral and contralateral group II afferents, Ia and Ib afferents, along with descending tracts. Small diameter group II afferent fibers play a significant role in eliciting flexor reflex effects (Schieppati & Nardone. 1999). Group II afferent fibers fire action potentials in response to muscle stretch and Ia afferents action, experiencing similar gamma and descending effects. Upon discharge, group II interneurons commonly produce excitation of α-motoneurons that innervate flexor muscles and concurrent inhibition of those supplying extensor muscles. Additionally, group II afferents provide strong excitation to γ-motoneurons, with most γ-motoneurons receiving input from Group II afferents from several muscles (Schieppati & Nardone. 1999; Enoka. 2008, 544).

2.2.2 Golgi Tendon Organ & Group Ib Pathway

Compared to muscle spindles, the Golgi tendon organ (GTO) is a relatively simple yet specialized sensory receptor that comprises a single afferent (Ib afferent) and no efferent connections (Enoka. 2008, 520). The GTO lies adjacent to the myotendinous junction and is described as being in series with skeletal muscle fibers. GTOs exhibit remarkable insensitivity to passive muscles stretch, responding only to extreme levels of stretch. Instead, due to their location, GTOs respond to muscle tension and increase in active force in discrete steps, each of which reflecting the recruitment of an additional muscle fiber (Goodman & Bensmaia. 2020). For this reason, GTOs are able to detect the amount of motor unit activity within a muscle (Kidgell & Pearce. 2020, 20). Activation of GTOs, as a result of active muscle tension, leads to the propagation of action potentials to the spinal cord by Ib afferent neurons. The Ib afferents establish connections onto α-motorneurons through pathways that involve one or two interneurons, referred to as Ib interneurons (Enoka. 2008, 543). Ib afferents from many muscles often terminate on the same Ib interneurons and mutually facilitate one another. The pathway involving a single interneuron evokes inhibitory postsynaptic potentials in agonist homonymous α-motorneurons, leading to the relaxation of the contracted muscle. The pathway involving two interneurons evokes excitatory postsynaptic potentials in α-motorneurons that innervate antagonist muscles, resulting in their activation (Enoka. 2008, 543). Therefore, the GTO reflex pathway is a safety mechanism that, through the activation of the antagonist muscles via reflex pathways, prevents the agonist muscle and attached tendon from suffering any damage due to excessive tension. The effects of the Ib afferent are widespread and subject to modulation by monosynaptic inhibitory and excitatory descending inputs from the brainstem to Ib interneurons, as well as peripheral input, including direct connections from Ib and Ia afferents (Enoka. 2008, 543). The pathway conveying inhibitory input to homonymous α-motoneurons from both Ia and Ib afferents is referred to as the nonreciprocal group I inhibitory pathway (Enoka. 2008, 554). The Ib input to the interneurons can be modulated by presynaptic inhibition arising from the Ib afferents themselves and descending pathways. The presynaptic inhibition of Ib afferents themselves contributes to the decline in inhibitory postsynaptic potentials in α-motoneurons during the course of a muscle contraction. Presynaptic inhibition refers to the suppression of neurotransmitter release from axon terminals. The central terminals of group Ia, Ib, and group II afferents receive abundant presynaptic contacts capable of mediating presynaptic inhibition through release of GABA acting to inhibit neurotransmitter release by blocking or reducing the amplitude of excitatory postsynaptic potentials (Gandevia. 2001).

3. ASSESSMENT OF MOTOR SYSTEM EXCITABILITY & FATIGUE

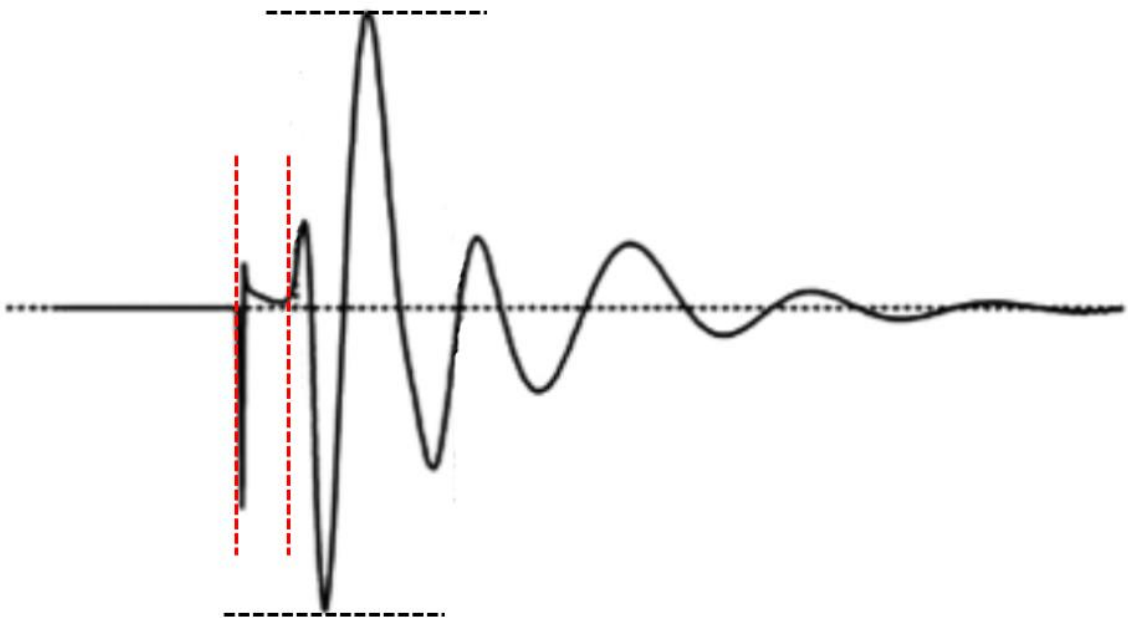
The central and peripheral components of the intricate neuromuscular fatigue phenomena are mutually dependent and inherently interconnected. Motoneuron recruitment relies on descending drive from supraspinal sites, and the central drive is modulated through a combination of excitatory and inhibitory reflex inputs from the periphery (Millet et al. 2011). By employing concomitant neurostimulation techniques and applying stimulation to different sites along the motor pathway, it becomes possible to infer the site along the motor pathway where exercise-related fatigue changes occur. All the described techniques involve the recording of electromyographic (EMG) responses elicited in skeletal muscle, regardless of the nature of the stimulation. For instance, single-pulse transcranial magnetic stimulation (TMS) reveals changes in motor-evoked potential (MEP) characteristics during and after a fatiguing exercise. MEP changes can originate at different levels of the motor pathway, and appropriate normalization to spinal and peripheral indices is needed to determine the contributions of each. Therefore, the use of various stimulation techniques is essential in the attempt to identify the contributions of different sites to these induced modifications.

3.1 Stimulation of the Motor Cortex

Transcranial magnetic stimulation (TMS) enables stimulation of the human motor cortex through the intact scalp and skull in conscious alert subjects in a painless fashion. TMS on the primary motor cortex results in a brief, relatively synchronous, and measurable muscle response known as the motor evoked potentials (MEP) (Avela & Gruber. 2010). Characteristics of the motor evoked response elicited by TMS are illustrated in Figure 3.2. The MEP latency, representing the delay of the evoked response in the EMG recording from the time of stimulation, is a measure of central motor conduction, which directly reflects the velocity at which the neural signal is propagated from the motor cortex to the muscle (Goodall et al. 2014). Conversely, the MEP amplitude, defined as the sum of absolute values for maximum and

minimum points of the biphasic evoked response, provides a measure of the magnitude of corticospinal excitability (Goodall et al. 2014). At the threshold, TMS with a posterior-anterior (PA) induced current in the primary motor cortex elicits a single descending volley that exhibits a 1-1.4 longer latency than the volley elicited by transcranial electrical stimulation (TES) - alternative noninvasive brain stimulation method that employs direct electrical discharging over the scalp – (Di Lazzaro et al. 2003). The earliest volley produced by TES, referred to as D-wave, has been proposed to arise from the direct stimulation of corticospinal tract axons. The later volley elicited by TMS, known as I-wave, is thought to result from the synaptic activation of the same corticospinal tract neurons (Di Lazzaro et al. 2004). The I-wave increases in size and is followed by later valleys (I-waves) as the intensity of stimulation is increased. I-waves are numbered according to their latency and are referred to as I1, I2, I3, and I4. Although it has been shown that TMS mainly elicits I-waves, very high stimulation intensity (180–200% of active motor threshold, aMT) can lead to detectable D-waves (Di Lazzaro et al. 2004; Hallett. 2007). The periodicity of the I-wave input, although never fully understood, may be partially influenced by reverberating activity in cortical synaptic networks or by the membrane characteristics of the pyramidal tract neuron, which lead it to fire repeatedly in response to a significant synchronous depolarizing input (Di Lazzaro et al. 2004). As mentioned earlier, cortical stimulation can activate both excitatory and inhibitory interneurons, and consequently, the motor cortical output is determined by the net effect (Weber et al., 2002). Additionally, the direction of the induced current also plays a significant role in influencing the amplitude of the cortical output. The largest MEPs are generated when the current in the brain is directed in the posterior-anterior direction (ideally at an angle perpendicular to the central sulcus), and the first wave typically generated is the I1-wave, according to comparisons between the responses from rotating the magnetic coil at various angles (at about a 1.5 ms interval from the D-wave). MEPs are also greater and occur earlier during baseline contractions as opposed to during rest. This is caused primarily by the motor neuron pool increased excitability (Hallett., 2007). When single-pulse TMS is delivered to the motor cortex during a voluntary contraction, the large excitatory response is followed by a brief period of EMG near-silence typically lasting 100-250 ms and referred to as silent period (SP). It is generally accepted that both spinal and cortical mechanisms contribute to the SP. Typically, the early portion (0-50 ms) of the SP is attributed to spinal mechanisms, including recurrent inhibition and motoneuron after-hyperpolarization (Inghilleri et al. 1993). The cortical mechanisms responsible for the later portion (50-200 ms) of EMG suppression are likely to be related to the activity of inhibitory cortical GABAergic interneurons activated upon cortical stimulation (McDonnell et al. 2006). The resulting cortical

inhibition involves the cessation of voluntary cortical output, and hence withdrawal of excitatory input to the SMNs and a cessation of their firing. The silent period is believed to provide a concrete measure of intracortical inhibition, given the predominant contribution of cortical over spinal mechanisms. The duration of the SP has been demonstrated to lengthen



during fatiguing contractions, along with an increase in the time needed for recovery with increasing task duration (Søgaard et al. 2006; Smith et al. 2007). Therefore, the prolongation of the SP following the completion of a fatiguing task due to increased intracortical inhibition indicates the presence of accumulated central fatigue and alterations of supraspinal mechanisms

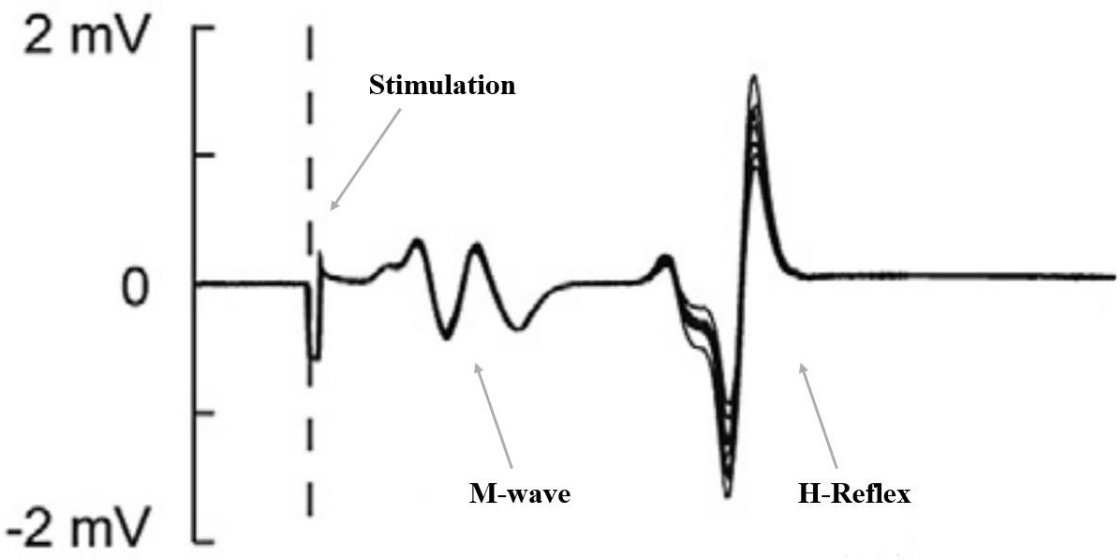
FIGURE 3.2. MEP characteristics illustration. The first red dotted line indicates the time of stimulation while the second one the onset of the evoked response. The latency is represented as the time interval between these two events. The two black dotter lines indicate the maximum and minimum points of the biphasic evoked response. The MEP amplitude is calculated as the distance between these two points (Adapted from Goodall et al. 2014).

3.2 Motor Nerve Stimulation

Electrical stimulation of a motor nerve often termed peripheral nerve stimulation (PNS), is capable of inducing muscle contraction by depolarizing the axons beneath the stimulating electrodes. The depolarization of motor axons produces contractions by signals traveling from

the stimulation location to the muscle (peripheral pathway), with no involvement of the central nervous system (CNS). Motor units recruited through this pathway discharge relatively synchronously resulting in a compound muscle action potential referred to as M-wave (Figure 3.3). The size of the M-wave is proportional to the sum of the action potentials of all the individual fibers excited (Milner-Brown & Miller, 1986). When an electrical stimulus of adequate intensity to activate the largest number of motor units by depolarizing their motor axons is applied, the resulting largest M-wave is termed the maximal M-wave or *M*max. Due to its nature, the M-wave provides a measure of neuromuscular propagation, also aiding in the investigation of alterations in neurotransmission occurring with fatigue (Rodriguez-Falces & Place. 2018). Widely utilized as a normalization factor for EMG activity, it also serves as a reference value for comparing the size of other evoked responses (i.e., MEPs) (Rodriguez-Falces & Place. 2018). In addition, the Mmax implies the activation of every α-motoneuron supplying the muscle of interest, thus serving as a measure of the overall activation of the motoneuron pool (Palmieri et al. 2004). Similar to motor axons, electrical stimulation also depolarizes sensory axons, eliciting sensory volleys directed to the spinal cord. This resulting sensory volley comprises signals from muscle spindles, Golgi tendon organs, and cutaneous receptors. In terms of synchronization, the sensory volleys generated by electrical stimulation are transmitted to the CNS in a similar manner to the sensory feedback observed during voluntary contractions. Signals evoked by electrical stimulation of the tibial nerve in the popliteal fossa have been observed to reach the motoneuron in approximately 15 ms via fast-conducting Ia afferents, with the slowest signals exhibiting an additional latency of 6-10 ms (Burke et al. 1983). With smaller dispersion at more proximal sites, the temporal dispersion of the sensory volley is dependent on the distance between the stimulating electrodes and the spinal cord. The sensory volleys induced by electrical stimulation directed towards the CNS can additionally contribute to the electrically evoked contraction through the synaptic recruitment of neurons in the spinal cord. The muscle response, known as H-reflex and monosynaptic in nature, arises from the activation of motoneurons by Ia afferents volleys elicited by peripheral nerve stimulation, which perhaps represents the most evident contribution of induced sensory volleys to evoked contraction (See Fig 3.3, 3.4). Due to a longer pathway through the spinal cord than the M-wave, motor units recruited by H-reflex pathways discharge rather synchronously, but at a longer delay, as seen in the EMG traces. The M-wave and H-reflex can appear simultaneously when the threshold for each respective type of fiber is reached. The H-reflex response begins to emerge on the EMG at low levels of stimulation. As the stimulus intensity increases, reaching the depolarization threshold for the motor fibers, the M-

wave appears in the EMG simultaneously with the H-reflex. Further increase of stimulus intensity eventually leads to the H-reflex reaching its peak and then disappearing from the EMG recording, while the M-wave reaches its maximum and remains stable. The H-reflex disappears due to the phenomenon known as antidromic collision. The high stimulation intensity evoking the M-wave also induced a current traveling toward the spinal cord (antidromic M-wave



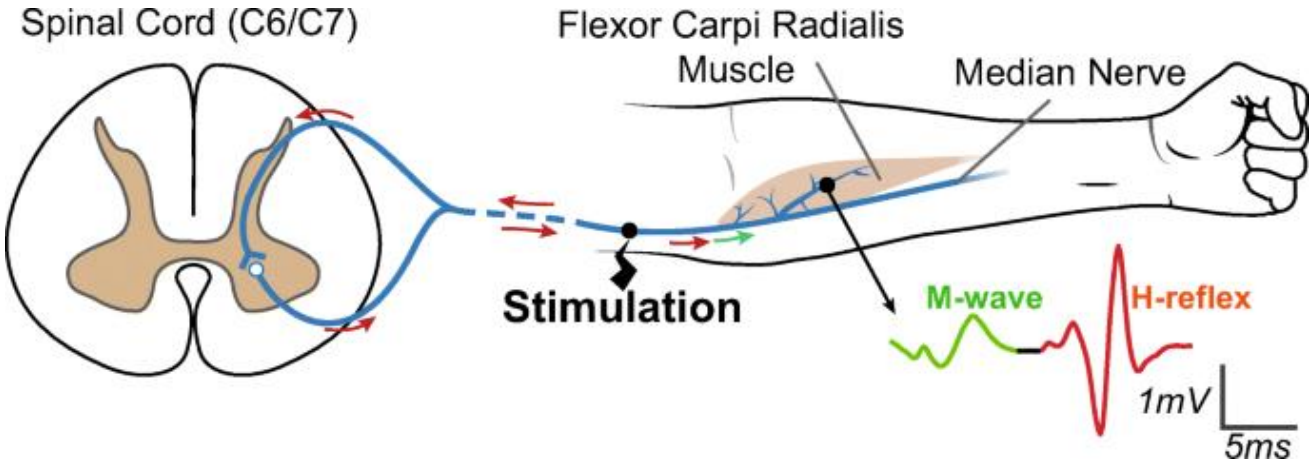
component). The collision between the descending H-wave and ascending M-wave component reduces the amplitude of the H-reflex, suppressing it at higher stimulation intensities. A recruitment curve can be obtained by gradually increasing the stimulus intensity from zero to an intensity that would elicit the maximum amplitude of the M-wave (Palmieri et al. 2004).

FIGURE 3.3. Illustration of M-wave and H-reflex (From Tseng & Shields. 2012).

FIGURE 3.4. H-reflex and M-wave pathways for the wrist flexor muscle flexor carpi radialis (From Eftekhar et al. 2018).

3.3 Twitch Interpolation Technique

The extent to which the brain can drive muscles during maximum voluntary contraction is a key issue in the study of neuromuscular fatigue. In order to investigate this query, it is necessary to determine if the motoneuron pool has been adequately excited by volition to elicit the maximum amount of force that the respective muscle can produce. In this regard, voluntary



activation (VA) refers to the level of "drive" to the motoneurons, modulating the recruitment and firing rate of these latter, which consequently rely on muscle fibers to translate the motoneuron firing into force (Gandevia. 2001). The twitch interpolation technique (ITT), initially proposed by Merton (1954), has emerged as a widely employed method for assessing the level of voluntary activation, serving as a suitable feature for characterizing central fatigue. This technique consists of superimposing a supramaximal peripheral nerve stimulation (PNS) or transcranial magnetic stimulation (TMS) during a voluntary contraction, which eventually results in a twitch-like force increment referred to as superimposed twitch (SIT). The twitch-force-like increment induced by the supramaximal stimulus arises from the unrecruited MUs or MUs firing at submaximal rate, along with motoneurons not in a refractory state. Merton (1954) observed a linear decrease in the amplitude of the SIT produced by a supramaximal stimulus to

the ulnar nerve innervating the adductor pollicis as the voluntary force increased and no additional force generation at maximal voluntary effort. Therefore, the greater the SIT relative to a reference stimulation on a relaxed muscle, the lower the VA and vice versa. When, in a maximal voluntary contraction, a subject manages to completely occlude the interpolated twitch, the level of excitation of motoneurons must have been sufficient to achieve maximal force production by the respective muscle (Herbert & Gandevia. 1999). Usually, however, subjects cannot completely occlude the twitch.

3.3.1 PNS & ITT

The extent of activation can be quantified by expressing the interpolated twitch as a percentage of the twitch evoked in relaxed muscle, known as interpolated twitch ratio. Accordingly, voluntary activation of the stimulated muscles is frequently quantified with the following linear equation.

VA (%) = [1 - (superimposed twitch / resting twitch)] *100 (From Allen et al. 1995)

Where the superimposed twitch is the force increment evoked by the supramaximal stimulation during a maximal voluntary contraction and the resting twitch is that evoked by an additional supramaximal stimulation in the relaxed muscle following the completion of the MVC (Figure 3.5). The control twitch is typically evoked 1.5-5 seconds after MVC, and it is assumed that the superimposed twitch and the resting twitch are equally potentiated by the voluntary effort. Variability and reliability of the twitch interpolation technique with motor nerve stimulation in assessing VA must be taken into account when this technique is employed for longitudinal studies and/or comparison purposes between different time- and data points. Allen et al. (1995) reported a coefficient of variation across subjects of 1.40% ± 1.13% in voluntary activation during a series of elbow flexors isometric maximal voluntary contractions. This indicates that voluntary activation assessed with the twitch interpolation technique is a reproducible measure within an individual from day to day, even if the degree of variability in voluntary activation differs between subjects. Place et al. (2007) investigated both intraday and interday (3-5 days interval) reliability of voluntary activation levels assessed with the twitch interpolation technique during knee extensor maximal voluntary contraction prior to and after a fatiguing task. The results showed the voluntary activation based on the twitch interpolation was highly

consistent before fatigue, with a coefficient of variation and a typical error lower than 5%. The occurrence of fatigue resulted in a slight alteration of the variability, with the coefficient of variation at < 8%. The analysis of limits of agreement revealed that random error (i.e., biological or mechanical variation) could account for the vast majority of the observed variance for the voluntary activation levels, as systematic bias, referring to a general trend for measurements to be different in a particular direction between repeated tests, was not significantly different from 0. In the same fashion, from the test-retest analysis, all the differences were within the 95% limits of agreement calculated for those differences.

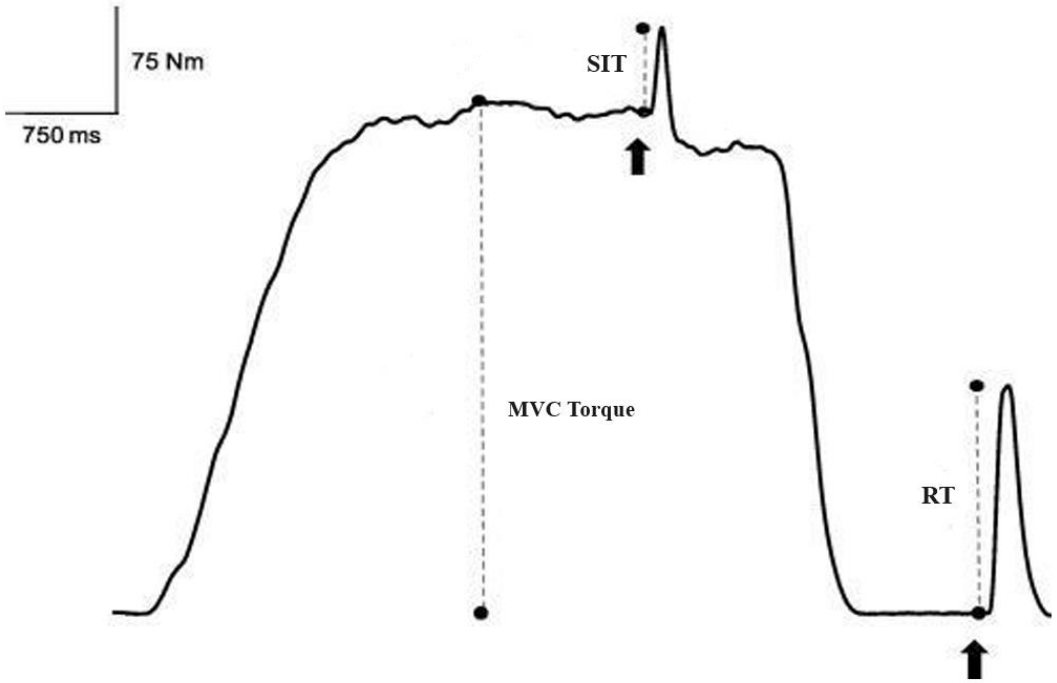


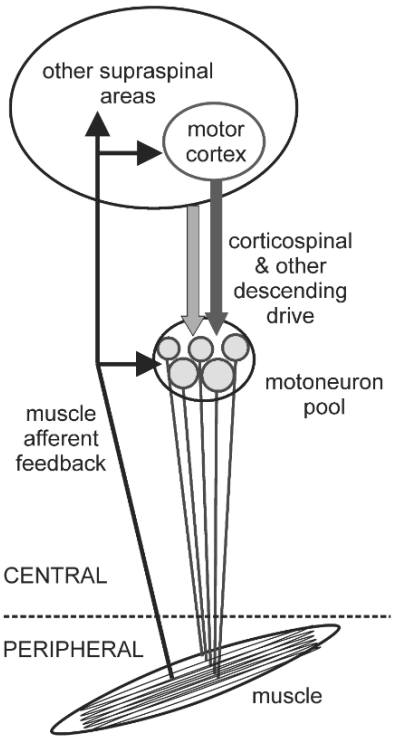
FIGURE 3.5. The arrows indicate the supramaximal stimuli delivered during and immediately after knee extensors MVC. The quantification of MVC torque, superimposed torque, and resting doublet-evoked torque is illustrated by black circles and vertical dashed lines. (Adapted from Maffiuletti et al. 2016).

3.3.2 TMS & ITT

The assessment of voluntary activation via motor cortical stimulation does not inherently correspond to that assessed by motor nerve stimulation. For instance, with pathology of the corticospinal neurons or motoneurons, a subject may be unable to drive the muscle fully. In such a case, motor nerve stimulation distal to the pathology would reveal impaired voluntary activation, whereas motor cortical stimulation would not. Hence, comparison of the two measures proves valuable in narrowing down the specific sites responsible for the impairment in voluntary drive. When using TMS to assess voluntary activation it is inappropriate to normalize the SIT evoked during a voluntary contraction to the twitch evoked at rest, as conventionally performed in the twitch interpolation technique with motor nerve stimulation. This is because motor cortical and motoneuronal excitability increase with activity. As a consequence, the same magnetic stimulus would evoke less cortical output, and therefore recruit fewer motor units at rest than during voluntary activity (Lee et al. 2008). Todd et al. (2003) devised a method to estimate the resting motor cortical output that would be produced if background excitability was the same as during MVC. The esitamted resting twitch (ERT) could then be used to quantify voluntary activation with the conventional equation. According to Todd et al. (2003), a linear correlation exists between the amplitude of the superimposed twitch evoked by motor cortical stimulation between 50 and 100% of maximal voluntary force (MVC). Therefore, to assess the amplitude of the resting twitch evoked by motor cortical stimulation, a linear regression of the superimposed twitch amplitude against voluntary force is conducted for forces between 50% and 100% of maximum. The y-intercept in this regression is then considered as the amplitude of the resting twitch (Todd et al. 2003). The reliability and variability of the twitch interpolation technique with motor cortical stimulation for assessing the level of voluntary activation have not been extensively investigated. However, Todd et al. (2004) reported a low intrasession average variability of voluntary activation for the elbow flexors (CV = 2.8 ± 2.0%).

4. CENTRAL FATIGUE

Central fatigue attributes the progressive failure to drive the motoneurons voluntarily leading to the decline in force-generating capacity, to processes residing within the central nervous system (CNS). As described in the previous section, the ITT, along with neurostimulation



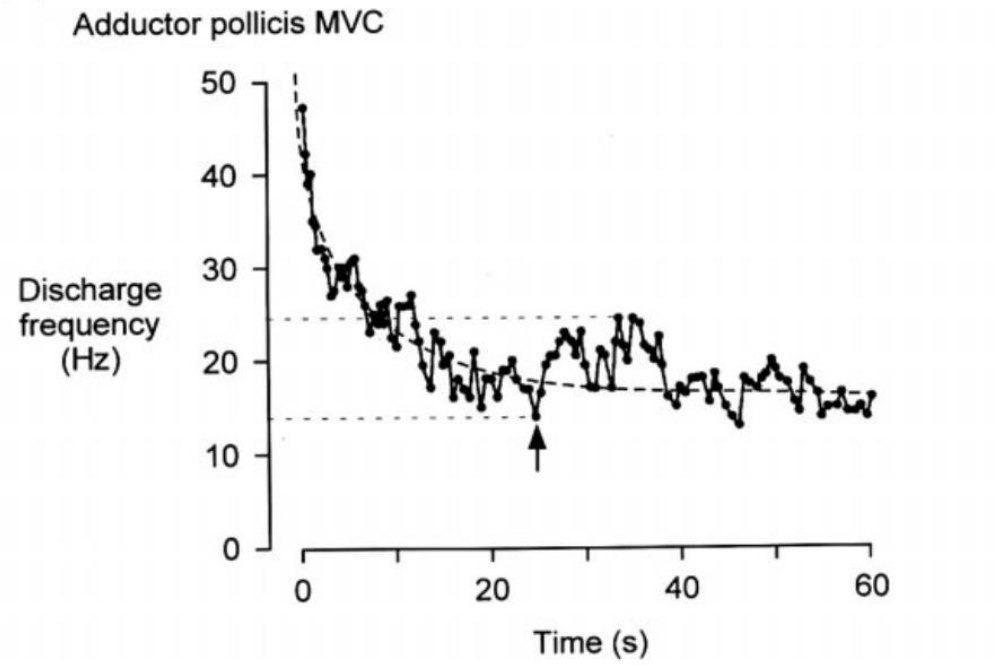
techniques, prove valuable in assessing voluntary activation and, ultimately, the occurrence of central fatigue. Any decrease in VA with exercise denotes the presence of central fatigue. While direct comparison of the level of voluntary activation assessed with both PNS and TMS is inappropriate, these two stimulation techniques, along with the ITT, prove valuable in identifying distinct failure sites proximal to the neuromuscular junction, involving spinal and supraspinal factors. Among various spinal and supraspinal factors, intrinsic characteristics and behaviors of motoneurons, afferent inputs impacting both alpha (α) and gamma (γ) motoneurons along with their presynaptic modulation, additional neuromodulatory influences on motoneurons and spinal circuitry, as well as the descending output, including corticospinal pathways to motoneurons, and the factors governing this output, collectively contribute to the inability to voluntarily drive motor neurons and decline in motor unit firing rate during fatigue (Gandevia, S. C. 2001) (Figure 4.1). The contribution of these various factors will vary during the course of fatiguing exercise.

FIGURE 4.1. Schematic illustration of spinal and supraspinal contribution to neuromuscular fatigue in single joint exercise (From Taylor et al. 2016).

4.1 Development of Central Fatigue

Voluntary activation in brief non-fatiguing maximal efforts might not be optimal and is somewhat dependent on which muscle is activated. Behm et al. (2002) reported that, during brief maximal voluntary contractions (MVC), the knee extensors, ankle plantar flexors, ankle dorsi flexors, and elbow flexors did not exhibit maximal voluntary activation, with the only exception of the elbow flexors showing minimal inactivation (1.3%). Furthermore, they observed a higher level of inactivation for the knee extensors (15.5%) compared to the ankle plantar- and dorsi flexors (5%) and the elbow flexors (1.3%), thereby showcasing a disparity in the level of VA across different muscles during maximal efforts. A key question is how VA changes during sustained or repeated contractions. During a sustained isometric MVC, the force starts to decline almost immediately, accompanied by an increase in the superimposed twitch response elicited through motor nerve stimulation, therefore indicating a greater failure of voluntary activation due to a larger portion of motor units not recruited by volition or not firing at optimal rates (Taylor & Gandevia. 2008). In a study conducted by Gandevia et al. (2008), it was observed that during the course of a 3-minute elbow flexion maximal effort, as the

contraction proceeded, voluntary force declined to 25 ± 8.6% of initial MVC. This decline in force-generating capacity was accompanied by an increase in the amplitude of the



superimposed twitch. On average, subjects initially exhibited near-maximal levels of voluntary activation (~ 99%) of biceps brachii, which dropped to about 90 % by the end of a 3-minute maximal effort protocol, hence, indicating the presence of central fatigue despite the initial high level of VA. Although the underlying mechanisms of central fatigue are complex, slowing of MU firing rates have been well documented for a range of upper and lower limb muscles (Bigland-Ritchie et al. 1983; Gandevia et al. 1990; Peters & Fuglevand. 1999; Woods et al. 1987). Sustained or repeated MVCs have been shown to result in a decline in motor unit firing rate, perhaps by as much as 50% compared with initial values regardless of their starting firing rates (Figure 4.2). This decline occurs initially acutely, then eventually reaching a plateau after approximately 30s. In addition, some motor units probably stop firing altogether in spite of the continued maximal effort. As a consequence, voluntary activation becomes progressively lower regardless of whether the initial level of voluntary activation is low or high. Bigland-Ritchie et al. (1983) were able to record the firing rate of 20-100 MUs from the adductor pollicis during a maximal contraction sustained for 40-120 seconds. Despite the maintained maximum effort, both the force developed and the MUs firing rate declined with time, with mean firing rates going from about 27 to 15 Hz (i.e., by nearly 50 %) (Figure 4.2).

FIGURE 4.2. example illustrating the decline in firing rate of a single motor unit in the adductor pollicis during a 1-minute MVC. Points were plotted at the end of the 10-spike period. A curve has been fitted to the decline in rate. The highest initial peak firing rate was 150 Hz. Arrow and horizontal dashed lines mark a period when discharge rate almost doubled (From Gandevia. 2001).

However, not all MUs appeared to behave similarly. By dividing the pooled data into time bins, they showed a marked decline in the higher frequencies, suggesting that the MUs with the highest initial firing rates tend to change most rapidly. Additionally, since no decline was seen in the muscle mass action potential evoked by single maximal stimulation of the ulnar nerve, the reduction in motor unitpotential frequencies was attributed to a corresponding reduction in mean discharge rate from the spinal motoneuron pool rather than to progressively increasing neuromuscular block. In a similar way, Rubinstein & Kamen (2005) isolated 39 firing MUs for a group of young adults (18-30 years) and 31 firing MUs for a group of older adults (>69 years) and conducted a direct comparison of force and MUs firing rate responses during a fatigue protocol consisting of 15 intermittent MVC, each requiring a 30-s dorsiflexion contraction followed by a 10-s rest period. As expected, the baseline muscular force was significantly greater in young adults than in older adults, possibly attributed to a predominant presence of fast-twitch motor units innervating fast-twitch fibers, thus allowing the production of large twitch forces. Both groups exhibited a substantial reduction in maximal dorsiflexion force over the course of 15 contractions. However, older adults demonstrated a significantly smaller decline in their normalized MVC (20.4%) compared to younger adults (33.8%) throughout the 15 contractions. Under non-fatiguing conditions, older adults exhibited a significantly slower MU firing rate (22.3 ± 4.8 imp/s) than did the young adults (28.1 ± 5.8 imp/s). Both age groups experienced significant reductions in MU firing rate between contractions 1 and 15. Following the fatiguing exercise, the decrease in MU firing rate was more pronounced for the young adults (34.9%) than for the older adults (22.0%), although statistical analysis revealed that MU firing rates between the two subject groups were not significantly different at the fifteenth contraction ($p > 0.05$). Additionally, there was a substantial difference in the MU firing rate response of the two groups, with the young subjects demonstrating a somewhat greater decline in firing rates during the fatiguing exercise than the older adults. The reduction in motor unit firing rate appears to occur consistently across various age ranges and appears to mirror the decline in force production. However, the mechanisms responsible for the lower level of fatigability in older adults remain not fully understood.

While some authors have observed a decrease in MU firing rates during submaximal efforts, similar to the pronounced decline seen in MU firing rates during maximal fatiguing tasks (Gatev et al. 1986; Maton & Gamet. 1989; Garland et al. 1997), MUs exhibit extremely variable responses during submaximal fatiguing tasks. Garland et al. (1994) investigated MU firing rate patterns during a submaximal fatiguing protocol involving the biceps brachii. In this experiment, participants were instructed to maintain a sustained voluntary contraction, averaging 6.9 ± 6.4% (11 to 64% MVC) of the previously determined force required for MU recruitment, until reaching a subjectively determined endurance limit, defined as a modification in the strategy for maintaining force (i.e., by changing body position) or the insurgence of substantial tremor of the arm. The fatiguing protocol resulted in an average immediate post-fatigue decline in MVC of 37.1%. Forty-five MUs were identified and monitored during the course of the submaximal fatiguing protocol. Notably, ten of these MUs were not initially firing at the onset of the fatiguing task but were recruited only later during the sustained contraction. Three distinct discharge patterns emerged during the fatiguing contraction. Thirty-two MUs showed a steady decrease in average firing rate, while seven MUs maintained a relatively constant firing rate, and six MUs displayed an increase in average firing rate. Of the ten MUs recruited throughout the sustained contraction, two MUs exhibited a decline in average firing rate, four MUs displayed an increase in firing rate, and four MUs maintained a constant firing rate. Hence, most of the units recruited during the fatigue task either discharged at a constant or increasing rate during the remainder of the contraction. This is in contrast to the decrease in firing rate evident in 30 of 35 MUs that were active throughout the fatigue task. Among the motor units (MUs) that demonstrated a decline in firing rate (n=32) during the fatigue task, various profiles were observed. Nine MUs exhibited initial declines in firing rate that subsequently returned to initial values, nine MUs displayed initial declines in firing rate that remained constant, yet lower, for the rest of the experiment. Additionally, five MUs showed a constant firing rate that decreased only at the end of the experiment, and nine MUs exhibited a steady, gradual slowing of firing rate throughout the entire experiment. According to the authors, the firing pattern of the MUs, however, did not relate to the duration of the fatigue task. Of particular note, no motor units (MUs) with a high initial discharge rate (i.e., > 20 Hz) displayed an increase in firing rate during the fatiguing contraction. Meanwhile, the MUs that exhibited an increased firing rate during the fatigue task were among those with low initial firing rates (i.e., < 10 Hz). One potential explanation is that, given the prevalent decline in average firing rate among most motor units (MUs) and the inevitable occurrence of fatigue in some MUs, the maintenance of a constant, albeit submaximal, force level was achieved, in part,

through the recruitment of additional initially silent MUs and the modulation of firing rates in other MUs, which initially discharged at a low rate, thereby either maintaining a steady rate or exhibiting an increase. Another study focusing on the biceps brachii reported a 'triphasic' adaptation of motor units during a series of sustained isometric contractions at submaximal levels. In the experiment conducted by Dorfman et al. (1990), subjects were instructed to maintain a steady isometric contraction at 20% MVC for a total of 45 minutes, with a 1-minute rest interposed every five minutes to allow for the slow and gradual evolution of fatigue. The researchers successfully identified three distinct phases in the behavioral response of the motor unit population to sustained submaximal activity. The first is a short-term accommodative decline in the average MU firing rate, peaking within the initial minute of activity and most likely reflecting adaptation at the level of the individual motoneuron. With further sustained activity, there was a steady and progressive increase in the mean MU firing rate, primarily attributed to increased drive on the pool of low-threshold motor units, which was evident after only 15 minutes of exercise when there was as yet little or no measurable fatigue. Subsequently, as fatigue develops, a progressive increase in mean firing rate after 30 and 45 minutes of continuing exercise was seen, with the mean MU firing rate rising from 13.4 ± 1.7 Hz at baseline to 16 ± 1.9 Hz at the 45-minute mark. The authors dismissed several hypotheses (e.g., selective dropout of MU with low firing rate or selective recruitment of fast firing MUs) to address the progressive rise in mean firing rate, ultimately concluding that the increase in mean MU firing rate with exercise reflects a progressive increase in firing rate of those MUs already active. With still further activity, during the latter part of the exercise period, there was de novo recruitment of previously inactive high-threshold motor units, in agreement with the so-called size principle of motoneuron activation. According to the authors, the increase in mean MU firing rate and the late recruitment of these MUs serve to maintain the target force output despite the presence of fatigue and are proposed to account jointly for the increase in total EMG activity seen during fatigue. Many factors including whether sustained or intermittent tasks, muscles of upper limb or lower limb, proximal or distal muscles, the composition and architecture of the muscle in terms of muscle fiber types and MU numbers, and training status among others can all be reasonably speculated to variably influence firing rate changes with submaximal fatiguing contractions. Furthermore, unlike at higher contractile intensities, during fatiguing submaximal tasks there is a greater potential for alterations in the recruitment and de-recruitment of MUs, possibly influencing discharge rates.

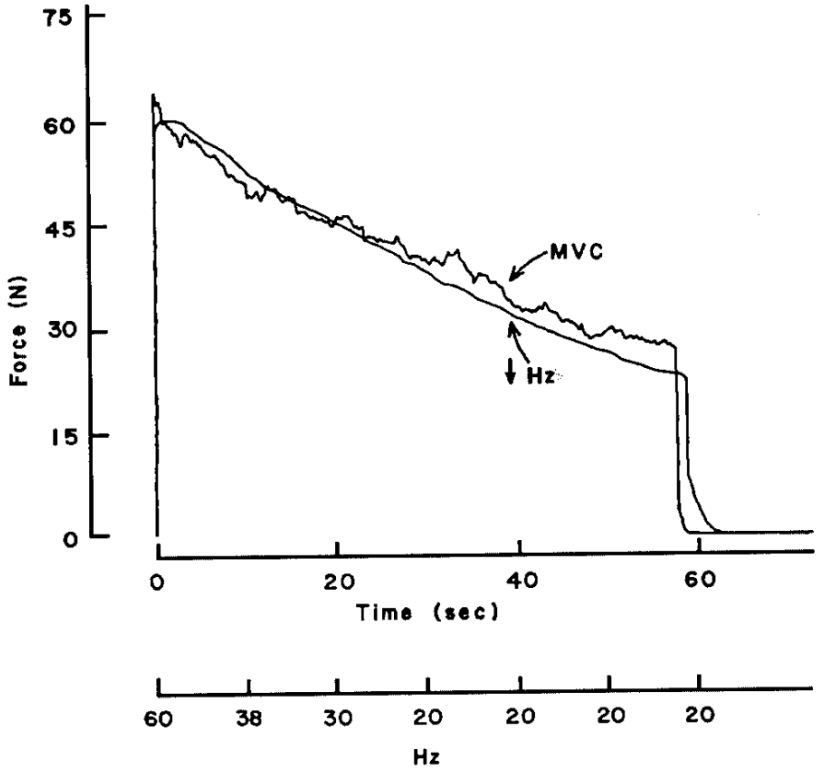
4.2 Muscular Wisdom Hypothesis

During prolonged and sustained voluntary muscle contractions, a loss in force-generating capacity ensues, and the ability to maintain a given force declines. As muscle fatigue progresses, adaptations in the way the muscle is activated by the central nervous system occur, particularly with fatigue-related changes in the motor unit discharge rate. A decline by 50-70% in the rectified EMG is generally observed during a maximal voluntary contraction sustained for 60 seconds (Bigland-Ritchie et al. 1983). In the absence of neuromuscular block, this decline may be attributed to a reduction in motor drive from the central nervous system. However, as suggested by Bigland-Ritchie et al. (1983), if the declining excitatory drive fails to maintain full muscle activation and is responsible for the loss of force, then some of the lost force should be restored by maximal tetanic stimulation of the motor nerve. No such restoration of force has generally been observed during sustained contractions by a well-motivated subject. Thus, all motor units appear to remain fully activated and continue to respond with a fully fused tetanus despite the reduced motor drive. It is known that the frequency at which twitches summate depends on the twitch contraction and relaxation time. In addition to force loss, muscle fatigue is also accompanied by a slowing of the muscle contractile properties, primarily due to a prolongation of relaxation time, that results in increased twitch summation and fusion in response to lower excitation rates. Marsden et al. (1983) formulated the 'muscular wisdom hypothesis,' suggesting that the matching of the motor unit discharge rate with the fatigue-related contractile properties of the muscle serves to optimize the production and regulation of muscle force and to minimize fatigue. They suggested that, during fatiguing MVCs, the slowing of the motor unit discharge rate would result in a fully activated muscle because of the reduced fusion frequencies associated with the prolonged relaxation times. The muscular wisdom hypothesis was inspired by earlier research employing motor nerve stimulation to simulate the force decline typically observed during MVCs. Jones et al. (1979) found that no single frequency of stimulation could reproduce the pattern of force loss observed during a 1-min

MVC of adductor pollicis muscle. As observed by the authors, the pattern of force loss during an MVC was fairly linear over time (Figure 4.3).

FIGURE 4.3. A. Force produced by an MVC and electrical stimulation (60 Hz to 20 Hz) (From Jones et al. 1979).

The force produced by an initially high frequency of stimulation that was progressively reduced to lower frequencies (60 Hz to 20 Hz) imitated the force loss of a sustained MVC (Figure 4.3). The application of 80 Hz stimulation was necessary to elicit force levels comparable to those observed at the beginning of the MVC. However, constant stimulation at this rate led to a more rapid decline in force compared to the MVC. Conversely, a constant 20 Hz stimulation resulted in an increase in force during the 1 min but failed to produce the high forces evident at the



beginning of the MVC. Thus, the data acquired through electrical stimulation suggested that there would be an advantage in progressively reducing the motor unit discharge rate during a sustained MVC for maintaining high levels of force production. The core premise of the muscular wisdom hypothesis is the matching between MUs firing rate with the fatigue-related contractile properties of the muscle. The muscle wisdom-associated changes in MU firing rate

with sustained activation are believed to result from a combination of afferent feedback from peripheral sources, intrinsic adaptation in the firing rate of spinal interneurons and α-motoneurons, and alterations in descending commands from supraspinal centers. However, whether these changes occur by design, through reflex action, or fortuitously under certain conditions (such as isometric contractions) remains not clearly understood. A series of independent studies by Bigland-Ritchie et al. (1986) and subsequently by Garland et al. (1988) suggested that peripheral reflexes arising from sensory afferents are, at least partially, responsible for the decline in motoneuron firing rates associated with the muscular wisdom hypothesis in response to fatigue-induced changes within the muscle. Bigland-Ritchie et al. (1986) and Woods et al. (1986) observed a decline in MUs mean firing rate during MVCs of the biceps brachii, quadriceps, and adductor pollicis. The firing rate did not recover during 3 minutes of rest while the arm was maintained ischemic but did recover, returning up to 95 ± 10% of their initial values at the onset of the first MVC within 3 min after resumption of blood flow. The reduced MUs firing rate following a 3-minute ischemic rest could be attributed to a progressive failure of neuromuscular transmission. Nevertheless, percutaneous stimulation of the ulnar nerve ruled out the possibility that a loss of excitability at the neuromuscular junction or sarcolemma was responsible for the observed behavior in MUs' firing rate because the M*max* remained consistent throughout the contraction protocol, despite depression in voluntary EMG. Therefore, it has been suggested that peripheral reflexes from the fatigued muscle are also involved, as any central effect was shown to recover within 3 minutes. This firing rate modulatory reflexes are likely to arise from receptors that are sensitive either to the changes in the muscle contractile properties or to the metabolic state of the muscle, which might include muscle spindles or Golgi tendon organs, as well as small diameter group III and IV afferents that also respond to changes in the muscle contractile properties and have a strong inhibitory input onto interneurons, γ-motoneurons and modulatory effect on Renshaw cells (Bigland-Ritchie et al. 1986; Gandevia. 2001). Subsequently, findings by Garland et al. (1988) supported the hypothesis of sensory afferents contributing to muscle wisdom-associated changes in MUs firing rate. After ischemic ankle dorsiflexor muscles had been fatigued solely through repetitive stimulation of the peroneal nerve at 15 Hz, a persistent reduction in voluntary EMG activity was observed as long as the arterial cuff remained inflated. This frequency of stimulation was previously found to be similar to the MU firing rates of 8-20 Hz in fatigued anterior tibial muscles. The observed reduction in voluntary EMG activity could not have been due to the loss of excitability at the neuromuscular junctions or muscle fiber membranes, as evidenced by only a modest reduction in M-waves evoked by peroneal nerve stimulation. Furthermore, the loss of

motoneuron excitability could not have been due to the failure of descending motor pathways, as these pathways were not involved in producing fatigue. Therefore, according to the authors, these observations are consistent with the view that the reduction in motor unit firing rate resulted from reflex inhibition of motoneurons by afferents from the fatigued muscle.

4.3 Spinal Contribution to Central Fatigue

4.3.1 Muscle Spindles Input & Presynaptic Inhibition

Muscle spindles exert an excitatory influence on α-motoneurons. Muscle spindle endings are recruited by γ-motoneurons, whose firing constitutes an important component in the response of muscle spindle afferents during exercise tasks. In a non-fatigued muscle, the mean muscle spindle firing rate increases with a rise in voluntary drive and the strength of the voluntary contraction. Overall, during sustained maximal or submaximal contractions the decline and irregularity in spindles firing seem to parallel that of motor units. Macefield et al. (1991) observed a progressive decline in 72% of the identified spindle endings, whereas the remaining percentage of spindle endings (32%) exhibited no decline and maintained a constant discharge frequency during sustained voluntary contractions of human ankle dorsiflexors lasting a minute at submaximal intensities (~30% MVC). Similar to MUs, muscle spindle firing rate experiences an initial rapid decline, becoming statistically significant by 10 seconds (Macefield et al. 1991). Subsequently, a slower phase of decline ensues, with a greater degree of reduction observed in spindle endings with higher initial firing rates. Despite a certain degree of reduction in discharge rate, the afferents would still be capable of firing at higher rates when subjected to local stretch, given their particular responsiveness to local length changes (Gandevia et al. 2001). Muscle spindles have the potential to facilitate α-motoneurons during sustained maximal and submaximal isometric contractions and fatigue. Hagbarth et al. (1995) reported a decrease in motor units' firing rate and an increase in the irregularity of excitatory discharges when the motor nerve was subjected to partial block induced by local anesthetic, which preferentially targeted small fibers, including the γ-motoneurons axons innervating the spindle endings, also underpinning the important role played by the γ-motoneurons in providing excitatory support to α-motoneurons during sustained effort. Bongiovanni & Hagbarth (1990) examined the effect of high-frequency (150 Hz) muscle vibration during dorsiflexors MVCs. They observed that the fatigue-induced decline in motor output during sustained MVcs was temporarily

counteracted during the initial phase of superimposed high-frequency (150 Hz) muscle vibration. The authors suggested that the decline in motor unit firing rates is influenced by a reduction in γ-motoneurons driven Ia afferent inflow from the spindles. Gandevia et al. (1990) observed that after a complete block of the ulnar nerve, the mean discharge frequencies of single motor axons were significantly lower than those of normally innervated motor units during attempted maximal voluntary efforts. These findings should not be utilized to claim that decreased muscle spindle facilitation alone is the only factor contributing to the fall in maximum motor unit firing rates during sustained MVCs. Instead, the presented evidence suggests that Ia afferents exert a net facilitatory influence on α-motoneurons, and augmentation of muscle spindle firing facilitates α-motoneuron discharge when voluntary activation has declined during fatigue. If, as seems likely, human muscle spindle discharge declines during fatiguing isometric contraction, then this may progressively disfacilitate motoneurons (Gandevia. 2001). It is likely that during sustained MVCs, the muscle spindle primary endings, which are preferentially sensitive to small local high-frequency regular length changes due to muscle contraction, would provide more effective spindle-mediated facilitation to the motoneuron pool early in a sustained contraction when the internal length changes produced by motor units are small (fatigue not occurring yet), rather than later when due to central fatigue contractions become uneven and irregular (Gandevia. 2001).

At a spinal level, the central terminals of especially group Ia afferents receive abundant presynaptic contacts. Presynaptic inhibition of Ia afferents could originate from GABA-mediated primary afferent depolarization or homosynaptic postactivation depression, acting to inhibit neurotransmitter release by reducing the amplitude of the relative terminal excitatory postsynaptic potential (Nordlund et al. 2004). The control of primary afferent depolarization appears to involve both afferent and descending projections from motor centers. In contrast, homosynaptic postactivation depression is thought to result from intrinsic mechanisms within the Ia terminal itself manifesting only when the muscle spindle is currently or has recently been discharging. Presynaptic inhibition generally has a long duration, on the order of 500 milliseconds for primary afferent depolarization and up to 15 seconds for homosynaptic postactivation depression (Nordlund et al. 2004). It is crucial to differentiate between two presynaptic mechanisms in order to understand whether muscle spindle excitation increases or decreases during fatiguing exercise. A reduction in homosynaptic postactivation depression is likely indicative of decreased muscle spindle firing. Conversely, a decrease in primary afferent depolarization mediated inhibition, coupled with sustained muscle spindle firing, suggests an

enhanced excitation from Ia afferents onto the motoneuron pool (Nordlund et al. 2004). Hultborn et al. (1987) observed that at the onset of muscle contraction, the degree of presynaptic inhibition decreases then eventually increases when the contraction is maintained involving a gradual reduction in the motoneuron excitation, even during ramp contractions (Meunier et al. 1989). Grosprêtre et al. (2018) investigated the changes in presynaptic following two distinct neuromuscular electrical stimulation protocols (20 Hz vs 100 Hz) by conditioning H-reflexes from the tricep surae with prior stimulations of the tibialis anterior Ia afferents projecting on the primary afferent depolarization interneuorns. The use of a conditioning-test interval of ~21 milliseconds selectively influence the Ia-Mnα transmission, enabling precise monitoring of modulations of the presynaptic pathway (Achache et al. 2010). Presynaptic inhibition for the soleus, analyzed by the ratio of conditioned H-reflex over the unconditioned H-reflex, exhibited an increment following both protocols (+22 ± 5.8% at 100 Hz vs. +8 ± 3.7% at 20 Hz, $P < 0.001$), but to a greater extent following the 100 Hz session. Presynaptic inhibition in the medial gastrocnemius and lateral gastrocnemius was also altered, but to a similar extent after both 20 Hz and 100 Hz protocols. Thus, it is likely that reduced facilitation from Ia afferents via presynaptic inhibition could be sufficiently significant to affect the net excitatory input to the motoneuron pool and thereby contribute to lowering the level of voluntary activation. (Nordlund et al. 2004).

4.3.2 Small-Diameter Muscle Afferents

Sensory neurons innervating skeletal muscle are activated by muscle contractions through various receptors. Group III and IV muscle afferents innervate the plentiful free nerve endings distributed throughout the muscle. These afferents respond to various stimuli, including but not limited to mechanical stimuli, biochemical stimuli, muscle stretch, hypoxemia, and muscle ischemia. However, they are generally either silent or maintain low background discharge rates (<1 Hz) (Gandevia et al. 2001). Muscle contractions induce changes in the discharge frequency of these sensory afferents, thereby increasing the amount of sensory feedback transmitted to the central nervous system at both spinal and supraspinal levels. Among the factors contributing to their discharge, impaired muscle perfusion during sustained contractions and fatigue lead to an

increase in their activity. During fatiguing exercise, feedback from group III/IV muscle afferents directly or indirectly impairs the output from spinal motoneurons. This interference may compromise voluntary muscle activation and subsequently impact exercise performance. Gandevia et al. (1996) observed a decline in maximal voluntary force (to 25.9 ± 8.6% of initial MVC) and voluntary activation (from 99% to ~90%) for the biceps brachii during 3-minute MVCs, suggesting suboptimal output from the motoneuron pool. By inflating a sphygmomanometer cuff around the upper arm, the brachioradialis was kept ischemic at the end of the sustained MVC. Maximal voluntary force and voluntary activation did not recover during ischemic conditions following the sustained MVC, and only returned to baseline levels after the cuff was deflated and circulation restored. However, fatigue-induced changes in EMG responses to magnetic cortical stimulation recovered rapidly despite maintained ischemia. As suggested by the authors, the suboptimal output from the motoneuron pool may involve fatigue-related firing of group III and IV muscle afferents, impairing the output of spinal α-motoneurons. Group III and IV muscle afferents have been shown to exert inhibitory influences on SPNs output, even during whole-body exercise. Amann et al. (2009) investigated the role of somatosensory feedback from locomotor muscles on central motor drive during a series of 5 km time trials. They used an opioid analgesic (i.e., fentanyl) to selectively block the activity in sensory pathways without affecting motor nerve activity. In particular, lumbar intrathecal fentanyl increases the subject's tolerance for pain in the lower extremities by binding to spinal opiate receptors and attenuating the activity of group III and IV muscle afferents. The central motor drive was estimated as the mean EMG amplitude recorded from the quadriceps muscle. As reported by the authors, selective blockade of the above-mentioned sensory afferents led to a significant increase in EMG in the quadriceps (+8 ± 3%) and presumably central motor drive, as well as higher power output than in normal conditions. In a similar way, Amann et al. (2011) in a different study investigated the influence of group III/IV muscle afferents on central motor drive during constant-load cycling exercise to exhaustion under placebo conditions and with selective opioid blockade of group III/IV muscle afferents. Despite an initially similar level between the two trials, the mean EMG amplitude was 9 ± 3% higher at the end of exercise with fentanyl compared to placebo ($P < 0.05$), although the time to exhaustion was shorter during the group III and IV blockade trial. Taken together, these findings suggest that sensory feedback from group III and IV muscle afferents exerts an inhibitory effect on spinal motoneurons, contributing to the observed decline in motor unit firing rate during fatigue. However, these sensory afferents also appear to play a role in minimizing the development of locomotor muscle fatigue by stimulating appropriate ventilatory and circulatory responses to exercise.

Furthermore, Gandevia et al. (2001) proposed three possible mechanisms through which feedback from group III and IV muscle afferents affects the firing rate of α-motoneurons during fatigue. These mechanisms include: 1) Reflex facilitation of α-motoneurons through the action of group III and IV afferents on the γMN-muscle spindle system. 2) Direct reflex inhibition of motoneurons by group III and IV afferents. 3) Group III and IV muscle afferents acting via spinal and supraspinal drives, involving both presynaptic and polysynaptic actions.

4.3.3 Motoneuron Intrinsic Properties

Motoneurons are recruited upon reaching the threshold membrane potential required for initiating an action potential. Once the voltage threshold for spike initiation is exceeded, action potentials ensue due to the influx of sodium into the motoneuron. Following recruitment, the firing rate of motoneurons increases as function of increasing depolarizing synaptic input. In humans, the motoneuron action potential is followed by a prolonged afterhyperpolarization lasting 50-200 ms (Sawczuk et al. 1995). The magnitude and duration of the afterhyperpolarization vary based on motoneuron size and motor unit type, with slow-fatiguing motoneurons exhibiting larger and longer afterhyperpolarizations compared to fast-fatiguing motoneurons. Repetitive discharge occurs if the injected or synaptic current exceeds the threshold required for eliciting a single action potential. It has been demonstrated that motoneurons exhibit a rapid increase in instantaneous firing rate following intracellular current injection (Sawczuk et al. 1995). This elevated firing rate experiences an initial sharp decline within the first few interspike intervals (<1s), followed by a subsequent, more gradual decrease persisting throughout the entire firing duration. This slowing of the firing rate has been referred to as spike frequency adaptation and is considered to ensure the production of high firing rates during intermittent repeated contractions (Sawczuk et al. 1995; Gandevia. 2001). The initial and acute phase of spike frequency adaptation has been attributed to an increase in afterhyperpolarization amplitude and duration, along with an increase in membrane voltage threshold for action potential initiation. Moreover, alterations in the accommodative properties of the axon initial segment – the segment responsible for generating and shaping the action potential before propagation along the axon – resulting from changes in sodium channels within the initial segment, may be accountable for variations in firing rate during repetitive discharge. Concurrently, the gradual decrease in motoneuron firing rate observed during late adaptation may reflect a progressive increase in outward currents, an incremental reduction in inward

currents, or a combination of both mechanisms (Sawczuk et al. 1995). Overall, spike frequency adaptation would result in a gradual rise in the motoneuron threshold, which in turn lengthen interspike intervals, as a specific rate of membrane depolarization would require more time to exceed the spike threshold. Hence, unless there is an augmentation in the net driving current, the firing rate of motoneurons is expected to decrease during both short and prolonged sustained efforts. Eventually, motoneurons may cease firing altogether.

Descending neuromodulatory systems are likely contributors to both motoneuron intrinsic properties and central fatigue-related changes in motoneuron firing (Taylor et al. 2016). In particular, an increased level of the monoamine serotonin (5-HT) in the CNS appears to contribute to the insurgence and time course of central fatigue. Serotonin is produced by brainstem cells in the raphe nucleus, and descending serotonergic neurons synapse onto the dendrites of spinal motor neurons, where serotonin acts by binding to specific metabotropic receptors. Several lines of evidence suggest that serotonin exerts excitatory actions on SMNs. The activation of 5-HT_{1A} receptors contributes to this excitatory effect by inhibiting leak conductances (Perrier et al. 2003) and calcium-activated potassium conductances (Grunnet et al. 2004), resulting in depolarization and increased input resistance in SMNs, which ultimately increases the responsiveness of motoneurons to excitatory signals. While the excitatory action facilitated by 5-HT is expected to enhance motor output and muscle contraction by partially counteracting changes in intrinsic properties that typically lead to reduced excitability with repetitive firing of motoneurons, questions arise regarding how 5-HT can both increase motor neuron activity and induce central fatigue simultaneously. Indeed, low levels of serotonin release during periods of low activity enhance the excitability of motoneurons. However, during prolonged activity with elevated levels of release, 5-HT spills over to reach extrasynaptic receptor sites in the axon initial segment, actively inhibiting the generation of action potentials in motoneurons, and consequently, muscle contraction (Cotel et al. 2013). Therefore, according to the authors, the increased extracellular concentration of 5-HT in the motoneuron pool contributes to the decline in motoneuron firing rate. Ultimately, the descending serotonergic system has the potential to reduce motoneuron excitability, contributing to central fatigue. This could occur through withdrawal of its facilitatory actions at the dendrites, as seen in prolonged exercise, or via spill over and activation of its inhibitory extrasynaptic receptors during strong contractions (Taylor et al. 2016).

4.4. Supraspinal Contribution to Central Fatigue

Despite its limitations, stimulation of the motor cortex by transcranial magnetic or electrical stimulation and the respective excitatory short-latency responses (MEPs) allows the investigation of the supraspinal contribution to central fatigue. A change in the amplitude of the MEP yields information about the excitability of the cortical cells and spinal cord, whereas the silent period (SP) yields information about the degree of inhibition presents at the cortical level. As previously described in section 3.1, stimulation of the motor cortex with a single TMS pulse during a brief MVC provides a method to assess the maximality of the output from the brain in humans. Although the final output from the motoneuron pool is determined by various factors, a twitch-like increment response in the force signal indicates suboptimal brain output, preventing the activation of the MUs at their full capacity to produce maximal force despite the subject's maximal effort. This observation may not unequivocally imply a reduction in motor cortical output or excitability since the same descending drive could become less effective due to reduced motoneuron excitability. Nevertheless, the persistent untapped output despite voluntary effort suggests a failure to fully employ all available resources in generating output from the motor cortex. Therefore, an exercise-related increase of the superimposed twitch elicited by cortical stimulation during an MVC is considered to be a marker of supraspinal fatigue (Taylor & Gandevia. 2008). The increase in SIT, however, may not provide comprehensive insights when exploring the origin and mechanisms underlying supraspinal fatigue and suboptimal descending drive to the motoneuron pool. MEPs exhibit increased size when evoked during voluntary contraction compared to the relaxed muscle, thus reflecting increased cortical and spinal excitability. However, Brasil-Neto et al. (1993) demonstrated that immediately following a wrist-flexion and -extension exercise to volitional fatigue, the amplitude of MEPs evoked by TMS immediately decreased progressively over a train of eight test stimuli. The decrease occurred in the absence of any marked changes in the amplitudes of the M-wave or H-reflexes, suggesting unaltered α-motoneuron excitability, as well as unaffected nerve conduction, neuromuscular transmission, and muscle membrane excitability. Taylor et al. (1996) observed an increase in MEP size evoked by TMS for the biceps brachii (156 ± 51%) and brachioradialis (178 ± 68%) during a 2-minute fatiguing sustained contraction at 30%, 64%, and 100% MVC. Simultaneously, after 30 seconds into the sustained contraction, they noted a lengthening of the silent periods following the MEPs for both the biceps brachii (53 ± 28 ms) and brachioradialis (45 ± 25 ms). However, despite the continued contraction, this lengthening of the silent period was maintained without further increase. Although the elbow

flexors were maintained ischemic at the end of the sustained MVC, the EMG responses to TMS rapidly returned to control values, thereby ruling out the contribution of group III and IV small afferents in these fatigue responses. Vibration of the biceps brachii tendon was also employed to enhance Ia afferent input in the later stages of a sustained MVC. The last four cortical stimuli during the sustained contraction comprised the earlier two without vibration and the latter two with vibration. However, no significant differences were observed in the MEPs area. Moreover, vibration demonstrated no impact on the MEP area or SP duration during control contractions and at the end of a sustained MVC. This observation suggests that alterations in responses to TMS were not mediated by reduced muscle spindle inputs. Ultimately, no increase was seen in the size of muscle action potentials elicited in the brachioradialis via electrical stimulation at the cervicomedullary junction during sustained MVCs, indicating unaltered motoneuron excitability. Although the silent period following cervicomedullary stimulation lengthened, it remained considerably shorter than the cortically evoked silent period. Altogether, the alterations in EMG response to TMS during and following fatigue imply both increased excitation and inhibition within the motor cortex. As these changes were unaffected by manipulation of afferent input they presumably arise from intrinsic cortical processes and/or altered voluntary drive to the motor cortex. In a separate study, Taylor et al. (2000) observed similar responses during intermittent maximal voluntary contractions of the human elbow flexors. TMS stimuli were administered during MVCs in four fatigue protocols with varying duty cycles. The MEPs area increased by more than 50% in all protocols, while the silent period lengthened by 20-75 ms. Evidence from studies using paired-pulse TMS of the motor cortex indicates fatigue-related decreases in inhibition attributed to both $GABA_A$ and $GABA_B$ receptor activation, which contrasts with the aforementioned evidence supporting the association of fatigue development with an increase in corticomotor excitability and long-lasting cortical inhibition. Benwell et al. (2006) investigated the modulation of short-interval cortical inhibition (SICI) during fatiguing exercise. Single and paired-pulse TMS was employed to measure MEP amplitude and SICI in the first dorsal interosseous and abductor digiti minimi muscles of the hand during a 10-minute intermittent maximal voluntary abduction of the index finger, both during and for 20 minutes after the exercise. SICI can be investigated with paired-pulse TMS with short inter-stimulus intervals (~3ms) using a subthreshold conditioning stimulus that attenuates the amplitude of a test stimulus (Kujirai et al. 1993). This attenuation is thought to be due to transynaptic activation of $GABA_A$-ergic inhibitory cortical interneurons by the conditioning stimulus, and therefore the degree of attenuation can be used as a measure of the level of SICI. They observed an increase in MEP amplitude for both the first dorsal interosseous

and abductor digiti minimi above control values during the exercise, followed by a subsequent decrease below control values during the recovery period. The index of SICI increased at the onset of exercise and then decreased throughout the course of the fatiguing task. At the beginning of recovery, SICI again increased and remained elevated for the 20-minute recovery period. Paired-pulse TMS at long interstimulus intervals can also be used to derive an index of long-interval cortical inhibition (LICI), which may be related to at least the late portion of the cortical SP, both principally mediated by $GABA_B$ receptors. In a separate study, Benwell et al. (2006) used single and paired-pulse TMS to measure MEP amplitude, LICI, and SP duration during a 10-minute intermittent maximal fatiguing exercise of the index finger, and throughout a 10-minute recovery period following the completion of the task. Single-pulse MEP amplitude and SP duration increased during the fatiguing exercise for the first dorsal interosseous muscle. Following the exercise, MEPs declined and remained suppressed below the baseline level during the recovery period, while the SP returned to baseline by the end of the recovery period. In contrast, the index of LICI decreased compared to baseline during the fatiguing task and subsequently returned to baseline after the completion of the protocol. Collectively, these observations indicate that central fatigue is associated with a progressive increase in corticomotor excitability, concomitant with a reduction in both short and long-interval intra-cortical inhibition. This suggests an increased central motor drive achieved through modulation of both excitatory and inhibitory networks. The alterations in LICI, SICI, and MEP amplitude are consistent with an elevated M1 output during fatigue, potentially compensating for a diminishing central motor drive.

5. STRETCH-SHORTENING CYCLE FATIGUE

A considerable number of movements in both daily and athletic activities involve a characteristic muscle action, where the muscle undergoes a lengthening phase (eccentric) immediately preceding the shortening phase (concentric) (Figure 5.1). This muscle function is referred to as the stretch-shortening cycle (SSC). As previously described, several mechanisms can potentially contribute to fatigue events. However, the vast majority of the notions known about muscle fatigue have primarily derived from studies utilizing isolated forms of muscle action (e.g., isometric, concentric, eccentric). As a consequence, when referring to neuromuscular fatigue in SSC type of muscle action, many of these fundamental fatigue

mechanisms may not be directly transferable to the SSC without taking into account the unique and distinctive features of the SSC and the associated loading mechanisms. The stretch-shortening cycle involves an eccentric component by definition, but its loading form differs from the traditionally accepted eccentric action. In the active braking phase of SSC, the impact loads and nature of stretches are generally very fast, brief, and regulated simultaneously by reflex and central neural pathways (e.g., muscle pre-activation). It has been demonstrated that the rapid coupling of eccentric and concentric actions, compared to a pure concentric action, during a non-fatiguing SSC exercise, results in a significant performance enhancement with increased force and work output at a given shortening velocity (Komi. 2000; Nicol et al. 2006). The concentric action enhancement typical of the SSC has been attributed by numerous researchers to the pre-stretch inducing the storage and subsequent release of elastic energy by the muscle-tendon complex as well as to reflex input acting predominantly at the spinal level but with a certain degree of modulation by the supraspinal centers. The short-latency stretch-reflex component, elicited by the imposed stretch and originating from muscle spindle Ia afferents, significantly contributes to increasing force generation during the eccentric phase of the stretch-shortening cycle (Komi. 2000). Both the level of fatigue and the increased stretch-load can affect the amplitude of the stretch reflex and the ensuing force enhancement. An important aspect characterizing SSC and related to modifications induced by occurring neuromuscular fatigue is the variation of the degree of muscle pre-activation typical of SSC-type muscle action prior to the stretch. This variation and more precisely downfall in the level of pre-activation is responsible for alterations in the mechanical behavior of the muscle-tendon unit. This change is most evident during the phase of muscle damage, a common feature in intensive and exhaustive SSC exercises (Nicol et al. 2006).

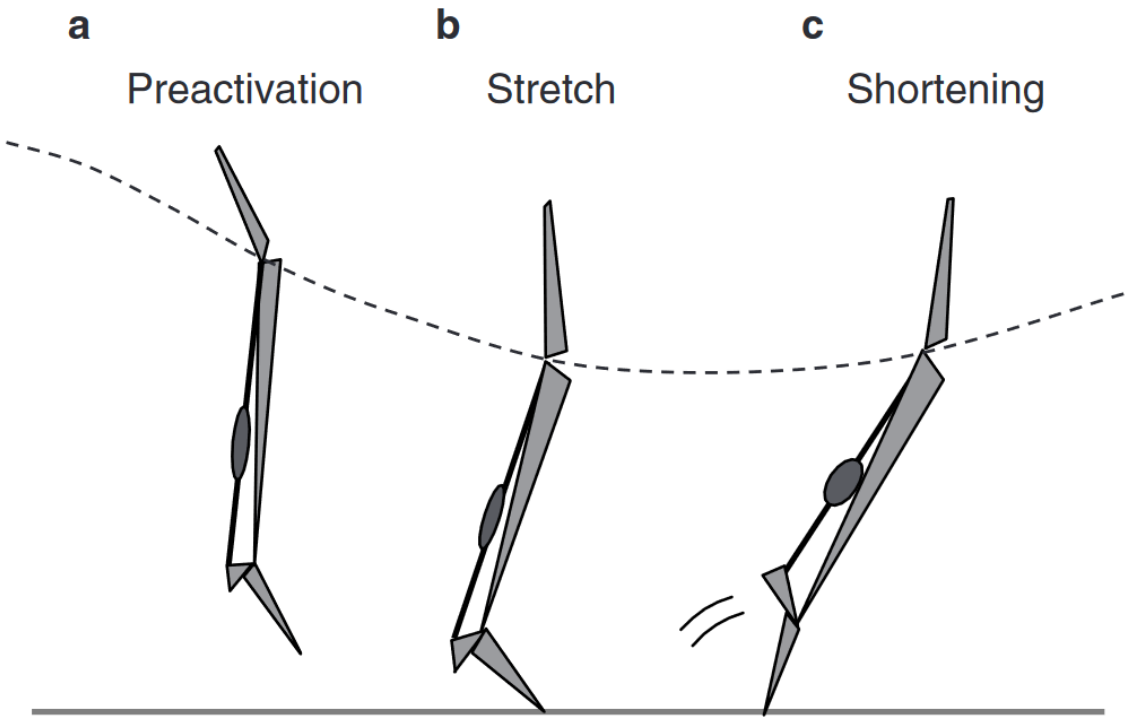
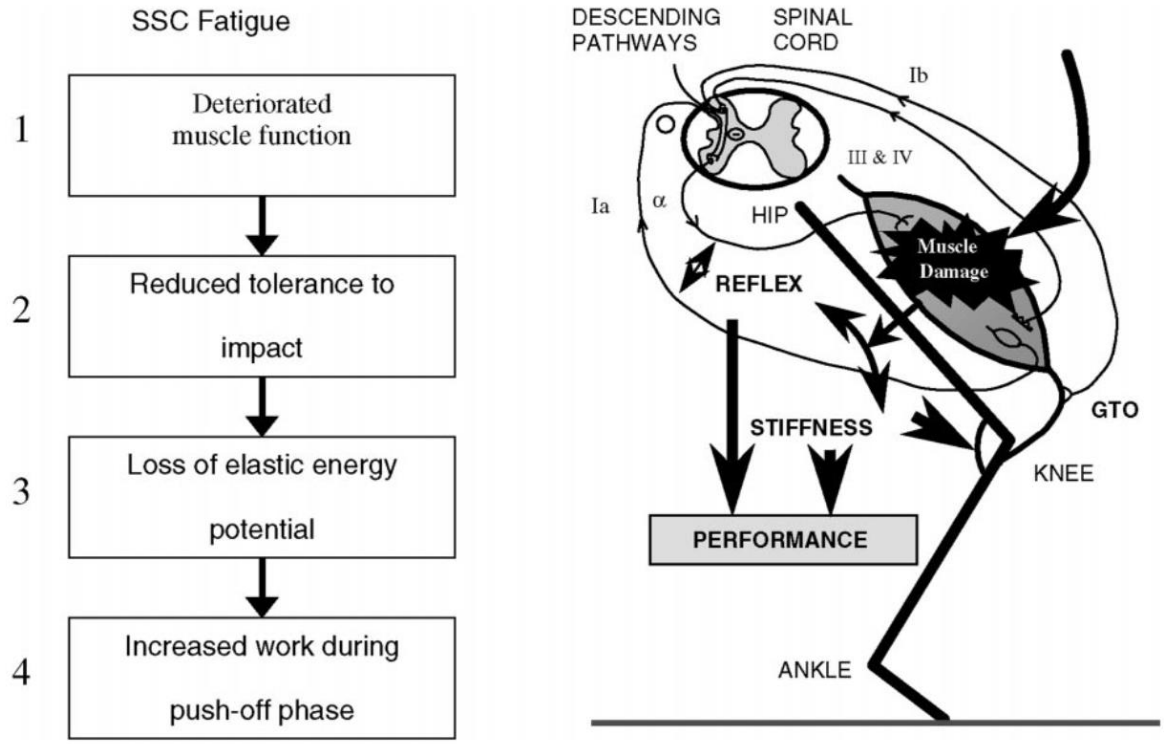


FIGURE 5.1. Schematic illustration of the three main phases characteristic of the SSC: a) muscle pre-activation prior to ground contact, b) stretching or lengthening of the muscles due to ground contact, and c) shortening of the muscles, leading to the consequent release of stored elastic energy, resulting in takeoff (From Nicol et al. 2006).

The mechanical behavior and neural pathways can undergo substantial stress during SSC fatigue, resulting in notable alterations in the contribution of these mechanisms to SSC performance. In SSC-induced fatigue, the recurrent high-impact loads associated with SSC muscle function over a specific exercise duration contribute to the taxing of major elements involved in movement production and control, including metabolic, mechanical, and neural components. Intensive and/or unaccustomed fatiguing SSC exercises usually result in reversible muscle damage, typically associated with significant and acute changes in muscle mechanics, activation, and reflex activity ultimately leading to major consequences on joint and muscle stiffness regulation, that may persist for several days. These fatigue-induced performance deteriorations and subsequent long-term recovery typically occur in a bimodal fashion. In this bimodality, the initial acute metabolically driven drop is followed by a brief short-term recovery which, in turn, is followed by a secondary and subsequent reduction with more durable recovery. The tendency towards bimodality may not always be observable, especially when

SSC fatigue has not been sufficiently exhaustive. The SSC-induced fatigue response, especially that neural in nature, is very individual and thus protocols using fixed exercise duration may not reveal true effects of fatigue. Some authors reported facilitation of performance following submaximal SSC fatigue tests, whereas performance deterioration appears to be better appreciable when involving maximal or near maximal intensity exhaustive tests (Nicol et al. 2006). According to a comprehensive review by Nicol et al. (2006), the majority of SSC fatiguing studies (24 of 26) reported an immediate reduction in MVC (22 ± 11%) following the fatiguing task. Additionally, 8 out of 12 studies observed an acute 8-70% reduction in peak twitch torque, electrically evoked in resting conditions, upon completion of the fatiguing task. In a similar way, the voluntary activation, measured as whole-muscle EMG amplitude, and the short-latency stretch-reflex component amplitude, in either passive or active stretching conditions, have been observed to significantly decrease with SSC fatigue immediately post-exercise (Nicol et al. 2006).

FIGURE 5.2. Schematic illustrations depicting the interaction between muscle damage, reduced



stretch-reflex sensitivity, reduced stiffness regulation, and the subsequent deterioration of SSC performance (From Komi. 2000).

In both maximal and submaximal SSC tasks, changes have been observed in centrally pre-programmed activation prior to ground contact, along with changes in the active stretch-reflex response. Moreover, meaningful relationships have been shown between the fatigue-induced modifications in pre-activation and the subsequent postlanding stiffness. These induced changes, in turn, contribute to weakened muscle performance due to impaired utilization of elastic energy (Avela & Komi. 1998). The mechanisms contributing to fatigue at spinal and supraspinal levels, discussed in sections 4.3 and 4.4, are expected to also occur during and following fatiguing SSC exercise (Figure 5.2). The fatigue-induced neural changes are believed to compensate for the contractile failure, as proposed with the muscular wisdom hypothesis, where the slowing of MUs firing rate serves to match the slowing of muscle contractile properties. Moreover, neural adjustments during SSC fatigue are believed to induce contractile failure via inadequate neural drive to the spinal motoneuron pool. It is not fully understood why the neural drive itself becomes a limiting factor for force output. However, in a fatiguing exercise where muscle damage occurs, inadequate neural drive might serve as a protective mechanism employed by the neuromuscular system to prevent the muscle-tendon unit from additional irreversible damage (Nicol et al. 2006). Any reduction in neural drive to the SMNs, regardless of the purpose, ensuing during or following an SSC fatigue task indicates the presence of central fatigue. Motor cortex stimulation (TMS), described in section 3.1, has not been extensively utilized to investigate the supraspinal contribution to SSC fatigue. Nonetheless, the involvement of higher centers in SSC fatigue cannot be ruled out, particularly given the significant reductions in maximal neural activation reported immediately after prolonged SSC exercises. As previously described in section 2.3, the monosynaptic reflex loop originating from muscle spindles is closely controlled by γ-motoneurons. Considering that the eccentric phase of the SSC muscle action imposes a substantial load on the muscle spindles, prolonged SSC loading can alter the gamma-loop system. The reduction in motor unit firing rate, supported by Ia-afferents, is therefore considered to exert a disfacilitatory effect on α-motoneurons, contributing to the observed decrease in force output during and after SSC fatigue tasks. Due to related technical difficulties, the mechanism responsible for the reduced Ia afferent input has not yet been thoroughly explained. It has been suggested that the withdrawal of γ-motoneurons support to the muscle spindles, intrafusal fiber fatigue itself, mechanical unloading of the muscle spindle, or a combination of these factors may be responsible for the observed reduction in Ia afferent input during SSC fatigue (Komi. 2000; Nicol et al. 2006). Overall, the combination of muscle damage, reduced stretch-reflex sensitivity, alterations in input from Ia afferents, and group III and IV nerve endings ultimately lead to changes in

stiffness regulation and reduction in SSC performance (Figure 5.2). The sequence of events is thought to unfold as follows: muscle damage leads to a decrease in stretch-reflex sensitivity, causing disruption in the regulation of muscle (and joint) stiffness, ultimately diminishing the efficiency of SSC function and resulting in a decrease in performance (Komi. 2000) (Figure 5.2).

6. PURPOSE OF THE STUDY

Neuromuscular fatigue has been extensively explored for decades. Evidence from numerous studies has provided a wealth of information, and several mechanisms have been proposed and proven to explain the various fatigue events. However, the vast majority of evidence has primarily stemmed from studies utilizing isolated forms of muscle action in both human and animal models. Hence, many of the proposed fundamental fatigue mechanisms may not directly transfer to SSC-induced neuromuscular fatigue. Some studies have investigated SSC-induced fatigue central and peripheral changes in submaximal (Strojnik & Komi. 2000) and maximal (Strojnik & Komi. 1998) fatigue protocols. However, in these investigations central fatigue has been assessed only utilizing motor nerve electrical stimulation, which can only in part provide insights about the occurring fatigue at the spinal and supraspinal level. In the past decade, TMS has been progressively utilized more in neuromuscular fatigue research settings and emerged as a promising tool in investigating supraspinal fatigue. However, TMS has primarily been employed to demonstrate fatigue occurrence at the spinal and supraspinal levels in upper limb muscles (e.g., elbow flexors) (Søgaard et al., 2006; Todd et al., 2004; Taylor et al., 2006). Yet, it has not been utilized to investigate spinal and supraspinal fatigue in lower limb muscles following exhaustive and prolonged stretch-shortening cycle actions. Therefore, the purpose of the current investigation is twofold:

1.) To investigate the mechanisms of central and peripheral fatigue induced by prolonged maximal and submaximal stretch-shortening cycle exercises performed to exhaustion.

2.) To investigate the sites of induced central fatigue by measuring the voluntary activation ratio via motor nerve and cortical stimulation following exhaustive stretch-shortening cycle exercise.

We hypothesize that prolonged and exhaustive stretch-shortening cycle exercise will result in both central and peripheral fatigue, ultimately impairing force-generating capacity, as observed in different whole-body locomotor exercise paradigms (Ross et al. 2010; Thomas et al., 2015). The resulting central fatigue would cause the impairment of the neural drive from the motor cortex to the working muscles, leading to a reduction in voluntary activation, primarily as a result of suboptimal output from the motor cortex, increased level of intracortical inhibition, and/or suboptimal transmission of the neural signal from the higher centers to the spinal motoneurons due to decreased corticospinal excitability (Sidhu et al. 2009; Sidhu et al. 2018).

7. METHODS

7.1 Participants

Twenty healthy active males with age ranging between 18 and 35 years old volunteered to participate in the study. Participants were randomly assigned to either the fatigue group (FAT) (n = 10; age: 23.3 ± 4.4; body mass: 74.8 ± 8.5; height: 177.9 ± 6.1; BFP%: 12.5 ± 4.7) or the control group (CON) (n = 10; age: 25.4 ± 2.6; body mass: 78.9 ± 4.2; height: 179.1 ± 6.5; BFP%: 15.4 ± 5.5). Endurance athletes or individuals with recent endurance-based training backgrounds were not included in the investigation. Their familiarity with exerting maximal effort towards or at the end of performance, often in the presence of existing neuromuscular fatigue, could significantly increase the risk of compromising the validity and reliability of the results. None of the participants had a history of cardiovascular or neuromuscular disease, musculoskeletal injuries of the lower limbs in the six months prior to the experiment or were taking any medications that affect the nervous system. Participants were informed about the procedures and possible risks associated with the experiment and gave their informed consent prior to the initiation of the experiment. Participants were asked to refrain from physical activity, and ingestion of caffeine and alcohol at least 24 hours prior to the measurement. Participants were allowed to withdraw from the measurements at any point at will. The procedures were approved by the local Ethics Committee and performed in accordance with the Helsinki Declaration.

7.2 Experimental Design

Each participant visited the laboratory for two sessions 1.) familiarization session. 2.) SSC fatigue protocol, and pre- and immediately post-fatigue protocol neuromuscular function assessment (NMFA). The FAT group performed 1.) Structured 15-minute warm-up. 2.) Baseline NMFA. 3.) SSC-fatigue protocol. 4.) Post-fatigue protocol NMFA. Conversely, the CON group performed 1.) A structured 15-minute warm-up. 2.) Baseline NMFA. 3.) Rest period followed by an additional 15-minute warm-up. 4.) Second baseline NMFA. The FAT group was measured first to determine the average duration of the fatigue protocol, which lasted 38.34 ± 6.33 minutes. As a result, for the control group, the time interval between the first and second baseline NMFA measurements was set at 40 minutes. During this interval, participants rested

for the initial 25 minutes and then repeated the 15-minute warm-up prior to the second baseline NMFA. Approximately one week prior to the fatigue session, participants visited the laboratory for a familiarization session where they were introduced to TMS and motor nerve stimulation and shown how to perform and practice MVCs. Participants assigned to the FAT group were also familiarized with the sledge apparatus, which was used to perform the SSC-fatigue protocol.

7.2.1 Preliminary Procedures

The procedures for determining the hotspot and active motor threshold (aMT) for TMS, as well as the optimal electrode placement for peripheral nerve stimulation (PNS) and identification of *M*max, are described in the materials section.

Participants performed a standardized, moderate-intensity warm-up prior to the baseline NMFA. The warm-up was structured as follows: 1.) 10 minutes of self-paced cycling on a cycle ergometer. 2.) 20 submaximal bilateral drop jumps (DJ) from a height of 20 cm. 3.) 20 low-intensity continuous rebounds on the sledge ergometer.

The determination of the optimal dropping height from where maximal DJs were performed in the SCC-fatigue protocol was conducted after completing the baseline NMFA and prior to commencing the fatigue protocol in order to prevent any potentiation phenomena that could affect the validity and reliability of the baseline NMFA. Participants were instructed to keep the arms folded across their chest to limit upward propulsion of the upper body as well as to allow their knees to passively flex freely during the rebound airborne phase to reduce fatigue of the hip and knee extensors and to limit their knee angle to a maximum of 90° during ground contact. According to Avela et al. (2006), to determine the optimal dropping height participants were instructed to rebound jump as high and as fast as they could and dropped from 10 cm intervals with the rebound jump height recorded for every trial. Dropping heights were increased until the rebound height no longer improved, and the dropping height with the corresponding highest rebound height was determined to be the optimal dropping height and marked on the side of the sledge apparatus.

7.2.2 Neuromuscular Function Assessment

Measures of neuromuscular function for the assessment of central and peripheral fatigue were evaluated before and after the SSC-fatigue protocol using both TMS of the motor cortex and electrical stimulation of the femoral nerve, with evoked responses recorded with surface EMG. In order to effectively investigate the SSC exercise-induced fatigue, the post-SSC fatigue neuromuscular function assessment was completed within 3.5 minutes of exercise cessation. The rapid nature of this procedure was necessary to capture the magnitude of the acute fatigue responses to exercise before they dissipated. Prior to the baseline NMFA, aMT and *M*max were determined in order to identify the final stimulation intensity for TMS and PNS respectively.

Each neuromuscular function assessment consisted of (Figure 7.1):

i) Two ankle plantar flexors MVC with 1 min of rest in between. Whenever the difference in force production between the two MVCs was above 5%, an additional MVC was performed. At the completion of the SSC fatigue protocol, only 1 ankle plantar flexors MVC was performed.

ii) One supramaximal (125% *M*max) PNS of the tibial nerve upon ankle plantar flexors MVC as explained in the respective section.

iii) TMS at 100% stimulator output upon 100%, 75%, and 50% MVC ankle plantar flexors MVC with 5 seconds rest in between contractions. TMS upon 100% MVC was performed first, followed by stimulation at 50 and 75% MVC in a randomized order.

iv) Ten single pulse TMS at 120% aMT upon 10% MVC, with randomized interstimulus interval between 8 and 10.5 seconds.

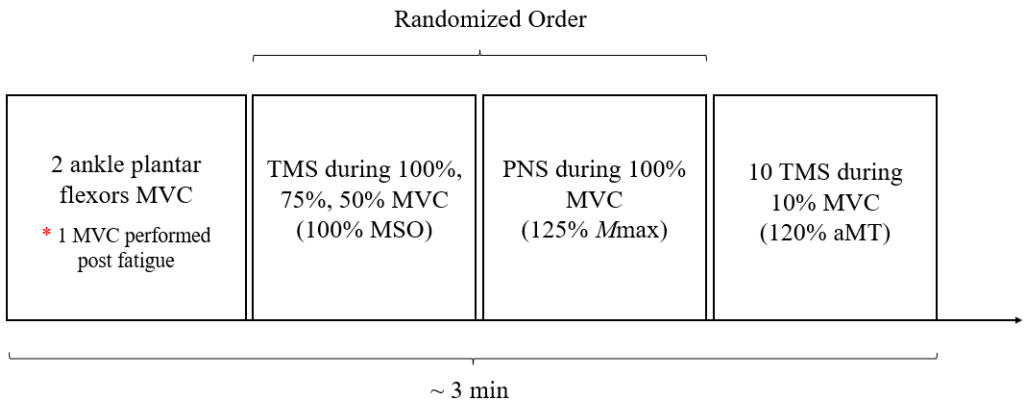


FIGURE 7.1. Schematic illustration of neuromuscular function assessment.

7.2.3 SSC-Fatigue Protocol

In the SSC-fatigue protocol participants performed 100 successive maximal bilateral DJs (one DJ every 5 s) from the optimal dropping height, split into 5 sets of 20 with 2 min rest given between sets (Kositsky et al., 2019) (Figure 7.2). A researcher grasped the sledge at the end of the jump and repositioned it to the optimal dropping height between each maximal DJ. Immediately after the completion of the maximal DJs, the subjects will begin an incremental continuous submaximal rebounding starting at a height corresponding to 50% of the initial maximal rebound height for a duration of 5 minutes. At the completion of the 5 minutes of continuous submaximal rebounding, the target rebound height was increased to 60% of the initial maximal rebound height for an additional 5 minutes. Eventually, at the completion of this step, the target rebound height was increased to 70% of the initial maximal rebound height for an additional 3 minutes. From this point onwards, the progression consisted of stepwise 10% increments (e.g., 70%, 80%, 90%) of the target rebound height up to 90% of the initial maximum rebound height, with each step lasting 3 minutes (Figure 7.2). If participants reached the 90% stage, then the target rebound height was maintained constant at 90% for 3-minute stages. Before every step increment, a maximal DJ test from the optimal dropping height was performed. Rebound jumping continued until either 50% of maximal rebound height could not be attained or volitional fatigue occurred. After ceasing the submaximal rebounding, an additional 3 maximal DJs were performed from the optimal dropping height to assess the changes in pre- to post-fatigue performance responses.

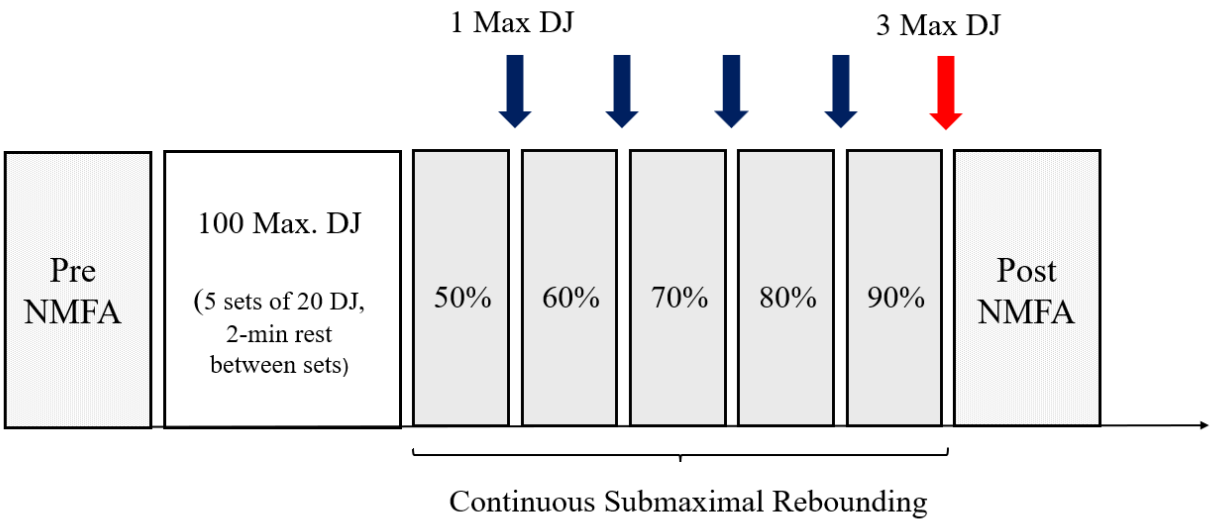


FIGURE 7.2. Schematic illustration of the SSC-fatigue protocol.

The first and last 3 maximal DJs were recorded via high-speed camera (100 fps) for further analysis. Visual feedback was provided to the participants throughout the entire SSC-fatigue protocol via an additional camera positioned on the side of the sledge apparatus, with the images displayed on a screen positioned in front of the sledge apparatus. Verbal encouragement and feedback were given to each participant by the researchers.

7.3 Materials

Force Recordings. Isometric force produced by the right limb ankle plantarflexors during voluntary and evoked contractions was measured through a custom-made ankle dynamometer (Neuromuscular Research Centre, University of Jyväskylä, Jyväskylä, Finland), consisting in a vertically mounted footplate, and an adjustable car seat, at the opposite ends. Participants were seated with the hip, knee, and ankle joints respectively at 110°, full extension, and 90°. This specific knee-ankle joint position was selected to maximize triceps surae force development (Cresswell et al. 1995) and enhance neural recruitment (Kennedy et al., 2001) as well as to leverage the anatomical features (Kawakami et al., 1998) of the two-joint gastrocnemius muscles during isometric plantarflexion. The seat position was adjusted individually for each participant to ensure the specific knee-ankle joint position was respected and tightened to prevent any potential backward slack of the hip during contractions. Additionally, the tested leg was strapped above the knee to prevent any knee and shank movement. A support was positioned below the tested leg at the ankle to prevent fatigue from occurring due to maintaining the position. Force signals from the footplate were sampled at 2000 Hz using a 16-bit A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK), and recorded using Spike2 software (version 6.10, Cambridge Electronics Design).

Sledge Apparatus. The SSC fatigue protocol was performed on a custom-made sledge apparatus (Figure 7.3) originally proposed by Komi et al. (1987). As shown in Fig. 7.3 the apparatus consists of a sledge (m = 33kg) to which the subject is fixed in a sitting position, running along an inclined (20.3° from the horizontal) low friction aluminum track. To maximize the activation of the plantar-flexors, heels were kept off the landing footplate by adding a supplementary and smaller footplate, thereby forcing a larger forefoot landing. The jump height for the maximal bilateral drop jump test was calculated by subtracting the highest position reached by the sledge's top roller and the participant's relative standing height on the sledge. A height marker

was fixed on the side along the entire length of the aluminum runway. The different jump percentages (See section 7.2.3) for the continuous submaximal jump protocol were tagged with reflective markers on the height marker and were readily identifiable. Participants received real-time feedback on jump height during the continuous submaximal jump protocol from an additional camera positioned on the side of the sledge apparatus, with the images viewed on a screen placed in front of the sledge apparatus. Force signals from the footplate were sampled at 2000 Hz using a 16-bit A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK), and recorded using Spike2 software (version 6.10, Cambridge Electronics Design).

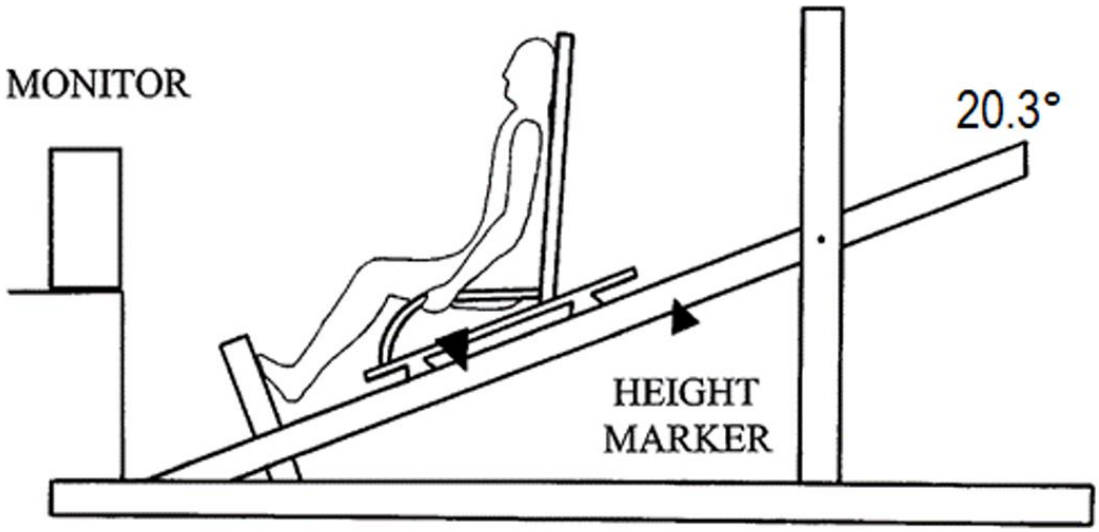


FIGURE 7.3. Schematic illustration of the sledge apparatus (Adapted from Komi et al. 1987).

Kinematics recording. To investigate the impact of SSC exercise-induced fatigue on lower limbs kinematics, the first three maximal DJs at the beginning of the fatigue protocol and the last three maximal DJs after completion of the fatigue protocol were recorded using a high-speed camera (DSC-RX10 Mark IV, Sony, Japan) at 100 frame per second. Reflective markers were positioned over the greater trochanter, lateral femoral condyle, lateral malleolus, heel, and base of the fifth metatarsal of the right lower limb, with optimal placement determined by palpation of the respective area.

Electromyography. Surface EMG activity of the medial gastrocnemius (MG), soleus (SOL), and tibialis anterior (TA) was recorded using self-adhesive electrodes (Blue Sensor N, Ag/AgCl, Ambu A/S, Ballerup, Denmark) arranged in a bipolar fashion and with an inter-electrode

distance of 20mm. A single ground electrode (Blue Sensor N, Ag/AgCl, Ambu A/S, Ballerup, Denmark) was positioned distally over the lateral malleolus. Electrodes placement followed SENIAM guidelines, with slight adjustment if individual-specific muscle morphologies required. To maintain low inter-electrode impedance (<5 kΩ), the skin area was dried, shaved, abraded with sandpaper, and cleaned with alcohol. The inter-electrode impedance was then tested, and the procedure was repeated if necessary. The raw EMG signals were amplified (x100) by a preamplifier (NL824, Digitimer Ltd., Welwyn Garden City, Hertfordshire, UK). Surface EMG signals were sampled at 2000 Hz using a 16-bit A/D converter (CED 1401, Cambridge Electronics Design, Cambridge, UK), and recorded using Spike2 software (version 6.10, Cambridge Electronics Design).

Peripheral Nerve Stimulation. Square pulses with 1 ms pulse duration were delivered to the right tibial nerve via surface electrodes (V Trode, Mettler Electronics Corp., Anaheim, USA) using a voltage constant current stimulator (Model DS7AH, Digitimer Ltd., Welwyn Garden City, UK).The cathode (32 mm diameter round electrode) was placed over the tibial nerve in the popliteal fossa, while the anode (50 mm diameter round electrode) was placed superior to the patella. The optimal position for the stimulating electrode was determined using a custom-made hand-held cathode (approximately 10 mm in diameter) as the site eliciting the highest amplitude of evoked responses in the MG with minimal current intensity. To determine *M*max, single stimuli were delivered in 5-mA stepwise increments from 5 mA until a plateau in M-wave was observed. To ensure that *M*max was attained, a supramaximal single stimulus was delivered. If a further increase in M-wave amplitude was observed, starting from that stimulating intensity, single stimuli were delivered in 5-mA stepwise increments until a plateau in the M-wave was observed. According to the twitch interpolation technique (ITT) proposed by Merton (1954), to assess voluntary activation (VA), double-pulse supramaximal stimulation (1 ms pulse duration and 10 ms interstimulus interval) was delivered upon MVC when the force plateau was attained, and approximately 2-3 seconds after the completion of the MVC while at rest ($Q_{tw,pot}$). To ensure a supramaximal stimulus, the final intensity was further increased by 25% (125% Mmax stimulation intensity). When performing the ITT, an additional double-pulse supramaximal stimulation was delivered (PT) approximately 2-3 seconds prior to commencing the MVC to investigate the impact of exercise-induce fatigue on the potentiation effect of MVC on the post-MVC evoked twitch.

Transcranial Magnetic Stimulation. Single-pulse (1 ms) TMS was delivered to the left motor cortex via a concave double-cone coil, powered by two connected monophasic Magstim 200^2 magnetic stimulator units (BiStim^2, The Magstim Company Ltd., Whitland, United Kingdom). Navigated stimulation was employed using a navigation system for TMS (Localite TMS navigator, Localite, Bonn-Buel, Germany) in order to increase stimulation accuracy and intrasession repeatability. The coil was held and tilted laterally to the vertex to ensure a posteroanterior (PA) intracranial current over the primary motor cortex. The hotspot for stimulating the ankle plantar flexors was identified by delivering single stimuli over the area of the primary motor cortex corresponding to the leg area near the vertex while the participant held a contraction at 10% MVC. The coil position eliciting a large MEP in the medial gastrocnemius and soleus, and a concurrent small MEP in the tibialis anterior was taken as a hotspot and saved by the TMS navigator to ensure consistent placement in repeated trials. Active motor threshold (aMT) was defined as the minimum intensity needed to evoke MEPs of >200 μV in 5 out of 10 consecutive stimuli with the participant holding a contraction at 10% MVC. Starting at subthreshold intensity (35% of stimulator output), single-pulse TMS was delivered over the optimal site of stimulation in 5% increments until the peak-to-peak amplitude of the evoked MEP consistently exceeded 200 μV. Subsequently, the stimulus intensity was reduced by 1% decrements until MEP response was >200 μV in 5 out of 10 consecutive stimuli. To assess corticospinal excitability, during the neuromuscular function assessment, 10 TMS stimuli were delivered at 120% aMT to ensure a supra threshold stimulus. To assess voluntary activation, single-pulse TMS at 100% stimulator output was delivered during contractions at 100%, 75%, and 50% MVC, with no stimulation delivered at rest after the MVC. When utilizing TMS to assess voluntary activation, normalizing the superimposed twitch (SIT) evoked during a voluntary contraction to that evoked at rest by TMS is inappropriate, as both cortical and motoneuronal excitability increase during voluntary contraction (Lee et al., 2008). Hence, it is necessary to estimate, rather than directly measure, the amplitude of the resting twitch in response to M1 stimulation. The amplitude of the estimated resting twitch (ERT) was calculated as the y-intercept of the linear regression between the amplitude of the superimposed twitches evoked by TMS at 100%, 75%, and 50% MVC and voluntary force (Todd et al. 2003) (See Fig. 7.4).

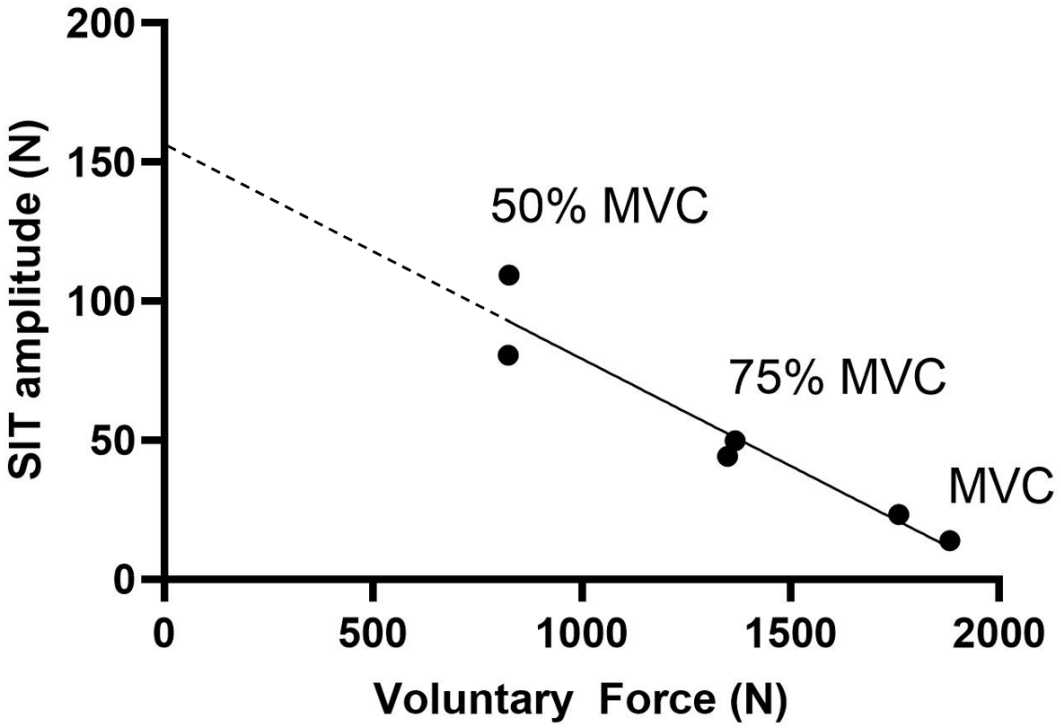


FIGURE 7.4. Single subject data displaying the linear correlation between the SIT amplitude evoked by cortical stimulation during 50, 75 and 100 % of maximal voluntary force (MVC). The linear regression was extrapolated to the y-axis and the y-intercept was taken as the estimated amplitude of the resting twitch by cortical stimulation.

7.4. Data Analysis

Force Recordings. MVC, evoked contractions, and ground reaction force (GRF) were analyzed using embedded tools within Spike2. Following adjustment for the offset between the zero-level and the pre-contraction baseline force value, force production during MVC and evoked contraction was quantified as the peak force value attained during these contractions. For the pre-fatigue neuromuscular function assessment, the best MVC trial was considered. GRF calculation for the first three maximal DJs at the beginning of the fatigue protocol and the last three maximal DJs after completion of the fatigue protocol, followed the same procedure. For each DJ, the peak force value attained following the initial force spike, corresponding to the impact phase, was considered. Subsequently, the pre-fatigue and post-fatigue GRF values were respectively averaged together. The ground contact time (GCT) for the same DJs was also calculated using the force signals. GCT was determined as the time elapsed from the initial

impact with the force plate until toe-off was completed. The pre-fatigue and post-fatigue GRF values were respectively averaged together. The twitch-like increment (SIT) following TMS or PNS was initially visually inspected for any inflection resembling a twitch within a opportune latency (< 50 ms) from the time of stimulation. If such an inflection was observed, the amplitude of the SIT was calculated from the inflection point to the maximum amplitude. The MVC potentiation effect on the potentiated twitch ($Q_{tw,pot}$) evoked by supramaximal electrical stimulation at rest after completion of the MVC, was assessed as the difference between $Q_{tw,pot}$ and the PT.

Voluntary Activation. Voluntary activation assessed with PNS (VA_{PNS}) was calculated using the linear equation VA_{PNS} (%) = [1 − (SIT/ $Q_{tw,pot}$)] × 100, where SIT represents the amplitude of the superimposed twitch and $Q_{tw,pot}$ represents the amplitude of the potentiated twitch, evoked by supramaximal electrical stimulation during MVC and at rest after the MVC, respectively. Voluntary activation assessed with TMS (VA_{TMS}) was calculated using a modified linear equation: VA_{TMS} (%) = [1 − (SIT/ERT)] × 100, where ERT represents the amplitude of the estimated resting twitch. ERT was determined as the y-intercept of the linear regression between the amplitude of the superimposed twitches evoked by TMS during contractions at 100%, 75%, and 50% of MVC and voluntary force (Todd et al., 2003).

Surface EMG Responses. Surface EMG responses evoked by TMS and PNS were quantified using MatLab (MathWorks, Natick, MA, USA). The peak-to-peak amplitude and area of MEP and *M*max were calculated from the initial deflection of the EMG signal from baseline to the second crossing of the horizontal zero-axis. The peak-to-peak amplitude was measured as the absolute difference between the maximum and minimum points of the biphasic M-wave or MEP (Thomas et al. 2015). The area was calculated as the integral of the reflected value of the entire M-wave or MEP (Thomas et al. 2015). To quantify corticospinal excitability (CSE) during contraction, the peak-to-peak amplitudes and areas of MEPs evoked during MVC were normalized to the corresponding peak-to-peak amplitudes and areas of *M*max evoked during MVC (MEP/*M*max). To quantify resting CSE, the MEP sizes from a train of 10 TMS stimuli were first averaged and then normalized to the corresponding *M*max size (MEP/*M*max). The MEP latency and silent period duration (SP) were only calculated for the train of 10 TMS stimuli administered during 10% MVC. The ten evoked MEPs were then averaged together. MEP latency was quantified as the time elapsed between the time of stimulation and the MEP onset. Whereas SP was quantified as the time elapsed between the MEP onset until the post-

stimulus EMG exceeded ± 2 SD of the pre-stimulus EMG for a period over 100 ms (Goodall et al., 2010) (Figure 7.5).

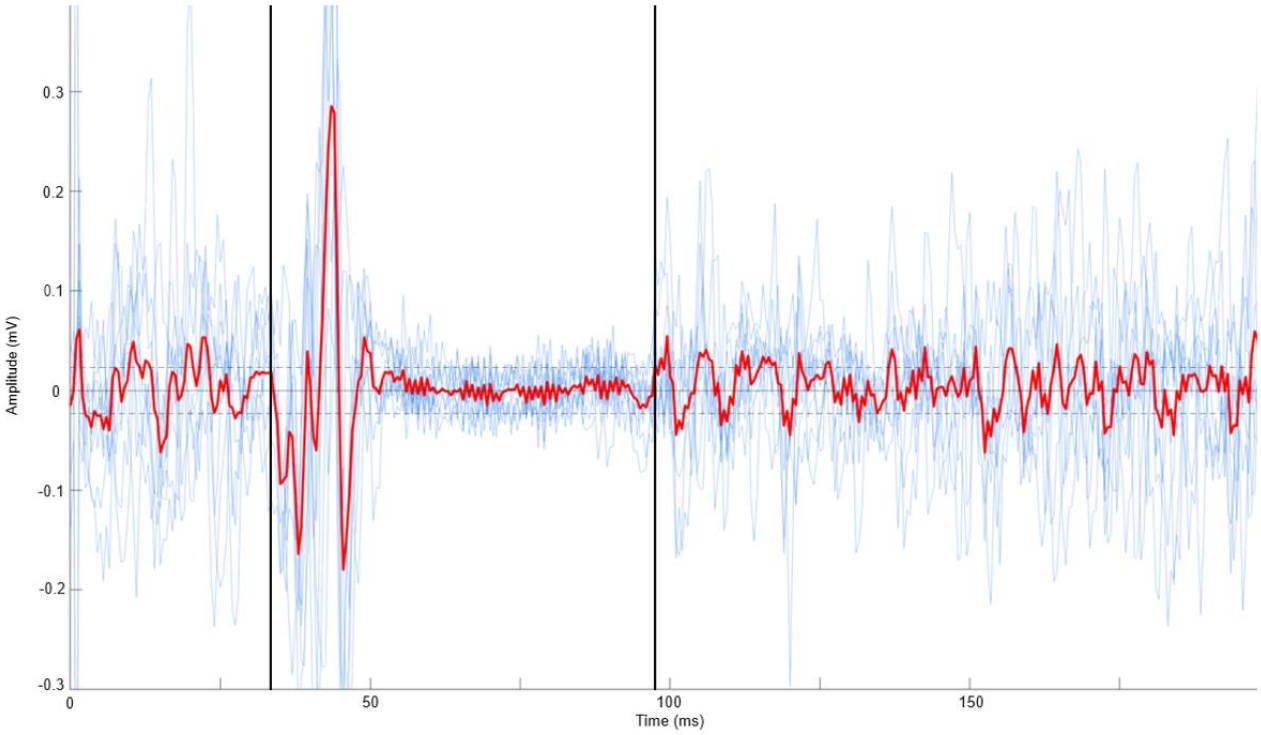


FIGURE 7.5. Example of silent period calculation. The first vertical black solid line indicates the MEP onset. The second vertical black solid line indicates the point where EMG exceeded ± 2 SD of the pre-stimulus EMG. The two horizontal dotted lines indicate ± 2 SD of the pre-stimulus EMG.

Drop Jumps. For the DJs considered for analysis, jump height (JH) was determined by subtracting the highest position reached by the sledge's top roller and the participant's relative standing height on the sledge.

Kinematics. Kinovea software (version 0.9.5) was utilized to analyze the kinematics, specifically the knee angle, from the recorded DJs. Reflective markers were manually located, and segments were defined for calculating the knee angle. For each DJ, only the lowest knee angle during the ground contact phase was considered. The knee angle relative to the first three DJs and the last three DJs were then averaged together, respectively.

7.5. Statistical Analysis

Data are presented as mean ± SD. All statistical analyses were conducted using SPSS software (version 26.0, SPSS Inc., Chicago, USA). Within-session reliability was assessed for the control group for each dependent variable across the first and second NMFA. Intraclass correlation coefficient (ICC), coefficient of variation (CV), and typical error (TE) were calculated to measure the reproducibility of the outcome measures of interest. ICC 95% confidence interval (CI) was also calculated. ICC values less than 0.5 indicate poor reliability, while values between 0.5 and 0.75 suggest moderate reliability. Values between 0.75 and 0.9 indicate good reliability, and values greater than 0.90 indicate excellent reliability (Koo & Li. 2016). CV and TE were taken as the average of the individual CVs and TEs, respectively. Normality for each dependent variable was assessed using the Shapiro-Wilk Test and further confirmed by a z-score, with an acceptance level set at ±2 (e.g., skewness score/skewness score $_{SE}$ and kurtosis score/ kurtosis score $_{SE}$) and Q-Q plots for visual inspection (Gomez-Guerrero et al., 2024). Data violating the normality assumption were Log10 transformed, which then fulfilled the requirements for normality. A Linear Mixed Model was used to assess to analyze all outcome variables (Wilkinson et al., 2023), except for exercise responses, considering both fixed and random effects simultaneously. The model included time (Pre and Post) and group (FAT and CON) as main effect and the interaction between time and group as the random effect within the model. Bonferroni adjustments were used when significant main effects were observed. A two-tailed paired-sample t-test was used to analyze the effect of the SSC-fatigue protocol on exercise responses. Effect sizes for paired sample t-test and post-hoc comparison was computed as Hedge's *g*, with $g < 0.3$, 0.3 - 0.8, > 0.8 corresponding to small, medium, and large effect sizes, respectively. For all statistical analyses, the level of statistical significance was set at an alpha level of 0.05 ($p < 0.05$).

8. RESULTS

8.1. Global Measure of Fatigue

The SSC-fatigue protocol resulted in a significant reduction in maximum force-generating capacity. For MVC, main effects for Time ($F(1,18) = 47.66$; $p < 0.01$) and a Time*Group interaction ($F(1,18) = 33,67$; $p < 0.01$) were observed. Post-hoc comparison showed that MVC for the FAT group significantly decreased post-fatigue ($p < 0.01$; $g = 0.89$) (Figure 8.1). No main effect was observed between groups at baseline ($F(1,18) = 1,08$; $p = 0.312$).

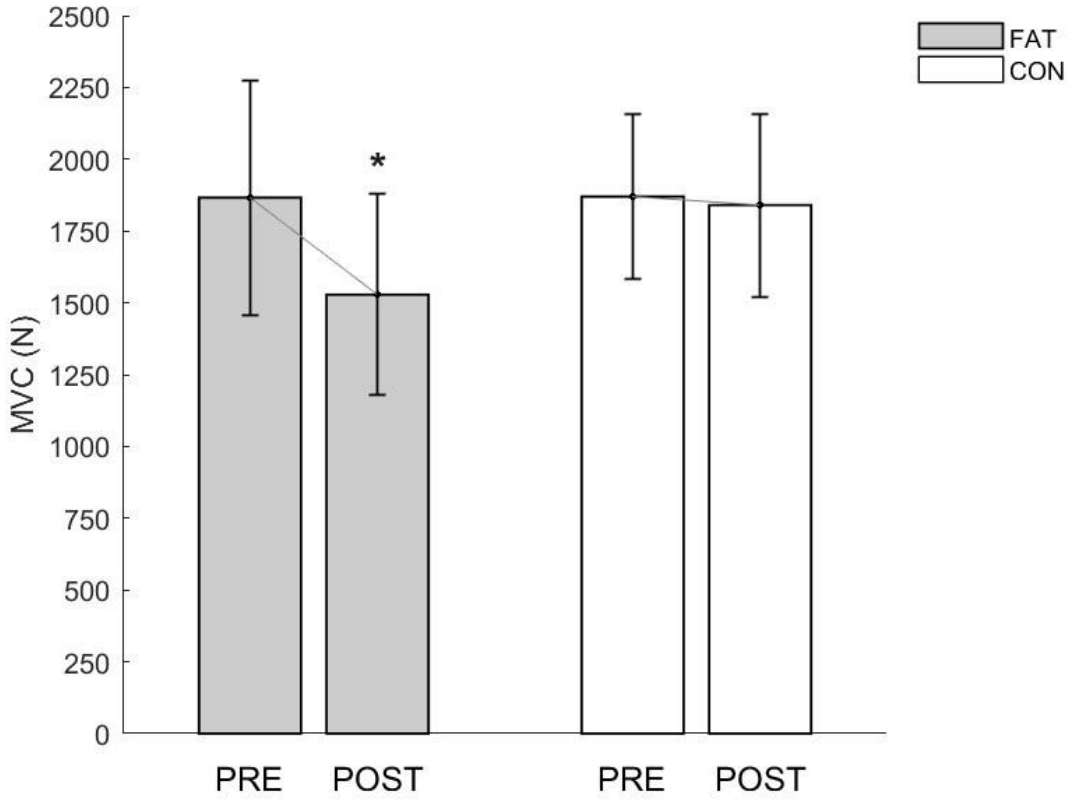


FIGURE 8.1. Pre- and post-fatigue MVC values for FAT and CON. Values are represented as mean ± SD. *Indicates a statistically significant difference between pre- to post-fatigue MVC ($p < 0.01$).

8.2. Pre- to Post-Fatigue Central Responses

Central responses to the SSC-fatigue protocol for both FAT and CON are summarized in Table 1. Significant main effects for Time ($F(1,18) = 5.26$; $p = 0.034$) and Time*Group ($F(1,18) = 9.77$; $p < 0.01$) were found for VA_{TMS}. Similarly, VA_{PNS} demonstrated a significant main effect for Time ($F(1,18) = 4.79$; $p = 0.042$) and Time*Group ($F(1,18) = 7.99$; $p = 0.01$). Post-hoc analysis revealed that the SSC-fatigue protocol resulted in significant reductions in both ($p < 0.01$; $g = 0.98$) and VAPNS ($p < 0.01$; $g = 1.01$) (Figure 8.2). For CON, no differences were observed in VATMA and VAPNS across NMFAs.

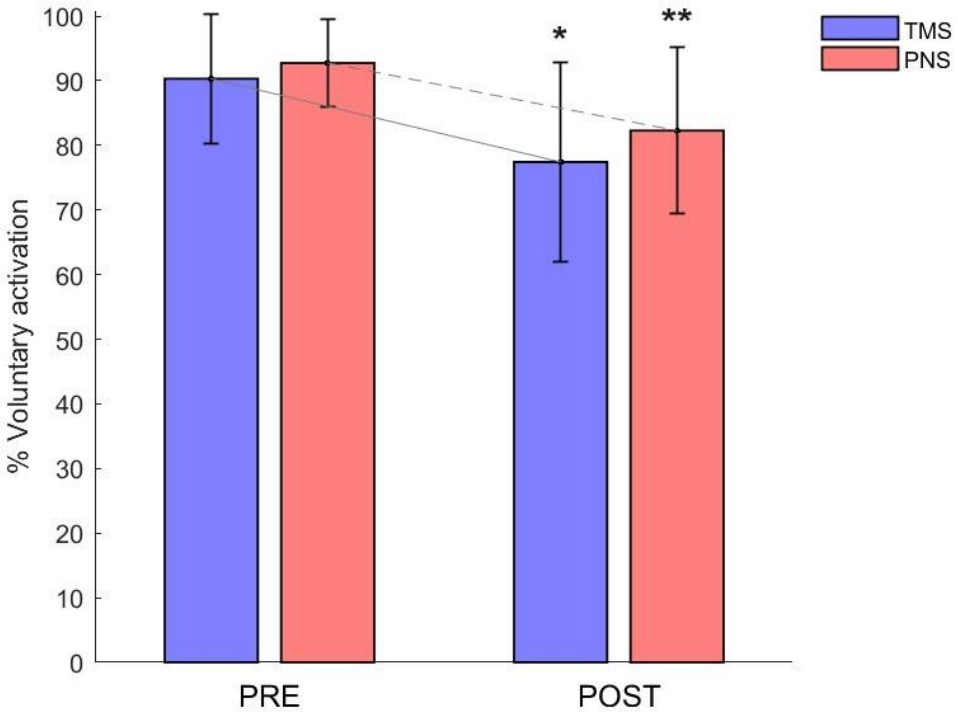


FIGURE 8.2. Pre to post-fatigue changes in VA_{TMS} and VA_{PNS} for the fatigue group. Values are represented as mean ± SD. *Indicates a statistically significant difference between pre- to post-fatigue VA_{TMS} ($p < 0.01$). **Indicates a statistically significant difference between pre- to post-fatigue VA_{PNS} ($p < 0.05$).

SP demonstrated a significant main effect for Time ($F(1,18) = 6$; $p = 0.025$) and Time*Group ($F(1,18) = 8.48$; $p < 0.01$). Post-hoc analysis showed that SP duration significantly increased post-fatigue for the FAT group ($p < 0.01$; $g = 1.38$), while no changes were observed for the CON group across NMFAs. No main effects were observed for MEP amplitude and MEP area during 10% MVC or MVC.

Measures of corticospinal excitability (CSE) for both FAT and CON groups during 10% MVC and MVC are summarized in Table 2. The SSC-fatigue protocol did not yield any significant changes for the FAT group. No main effects were observed for MEP amplitude, MEP area, or MEP amplitude or area normalized to Mmax amplitude or area (MEP/*M*max) at any contraction level.

TABLE 1. Central and peripheral responses to SSC exercise-induced fatigue.

	FAT		**CON**	
	Pre	Post	Pre	Post
Global Responses				
MVC (N)	1866.3 ± 409.5	1528.3 ± 350.8*	1869.8 ± 287.6	1866.3 ± 409.5
Central Responses				
VA_{TMS} (%)	90.3 ± 10.1	77.4 ± 15.5 *	91.1 ± 5.7	90.4 ± 7.6
VA_{PNS} (%)	92.7 ± 6.8	82.3 ± 12.9 *	92.5 ± 2.8	94.1 ± 4.7
SP (ms)	163.1 ± 13.2	185.2 ± 18.3 *	168.2 ± 40.5	166.3 ± 41.4
LAT (ms)	34 ± 5.6	33.7 ± 5.7	32.8 ± 3.1	33.1 ± 3.8
Peripheral Responses				
$Q_{tw,pot}$ (N)	159.5 ± 37.7	144.9 ± 46.3 *	172.6 ± 20.4	167.1 ± 19.7
PT (N)	129.3 ± 35.2	122.3 ± 41.4 *	145.6 ± 20.1	142.5 ± 23.5
POT (N)	28.8 ± 22.4	24.6 ± 17.1	27 ± 15.7	24.6 ± 16.7

Values are presented as mean ± SD, $n = 10$.
* Indicates a statistically significant difference from pre-fatigue values, $P < 0.05$.

8.3. Pre- to Post-Fatigue Peripheral Responses

Peripheral responses to the SSC-fatigue protocol for both FAT and CON groups are summarized in Table 1. No main effect was detected between the groups at baseline (F (1,18) = 1,51; p = 0,235). A significant main effect for Time was observed for $Q_{tw,pot}$ (F (1,18) = 8.41; p = 0.01). In the FAT group, a significant reduction in $Q_{tw,pot}$ amplitude occurred at the cessation of the SSC exercise (p < 0.01; g = 0.35). $Q_{tw,pot}$ remained unchanged for CON across NMFAs. Measures of membrane excitability for both FAT and CON groups at rest and during MVC are

summarized in Table 2. No main effects were observed for Mmax amplitude and Mmax area at any contraction level.

TABLE 2. Measures of corticospinal excitability and membrane excitability at rest and during MVC.

	Fatigue		**Control**	
	Pre	Post	Pre	Post
Resting Responses				
MEP amplitude (mV)	0.95 ± 0.24	1.14 ± 0.53	0.80 ± 0.27	0.90 ± 0.35
MEP area ($\mu V \cdot s^{-1}$)	14.8 ± 4.8	17.5 ± 9.1	12.9 ± 6.2	14.7 ± 6.8
M_{max} Amplitude (mv)	5.19 ± 3	4.73 ± 2.2	4.77 ± 1.4	5.05 ± 1.7
M_{max} Area ($\mu V \cdot s^{-1}$)	34.9 ± 7.1	29.4 ± 6.7	33.3 ± 11.8	32.1 ± 10.4
MEP/M_{max} amplitude (%)	23.3 ± 15	23.4 ± 10	18.5 ± 9	19.4 ± 9
MEP/M_{max} area (%)	54.1 ± 27	51.2 ± 25	32.4 ± 15	35.4 ± 17
During MVC				
MEP amplitude (mV)	1.37 ± 0.33	1.43 ± 0.48	1.28 ± 0.41	1.18 ± 0.31
MEP area ($\mu V \cdot s^{-1}$)	22.4 ± 4.3	20.7 ± 3.3	20.3 ± 5.9	18.1 ± 4.6
M_{max} Amplitude (mv)	6.37 ± 2.4	6.13 ± 2.9	5.55 ± 4.2	5.05 ± 2.6
M_{max} Area ($\mu V \cdot s^{-1}$)	34.9 ± 7.1	29.4 ± 6.7 **	33.3 ± 11.8	32.1 ± 10.4
MEP/M_{max} amplitude (%)	23.9 ± 9	28.6 ± 16	29.7 ± 12	25 ± 12
MEP/M_{max} area (%)	66.2 ± 15	73.8 ± 20	65.8 ± 26	60 ± 21

Values are presented as mean ± SD, $n = 10$.
** Indicates almost a statistically significant difference from pre-fatigue values, $P < 0.08$.

8.4. SSC Exercise Induced Responses

The SSC-fatigue protocol resulted in a significant decrease in GRF from the first three DJs (2177 ± 422 N) to the last three DJs (1862 ± 212 N) ($p = 0.016$, $g = 0.94$) and a parallel significant increase in GCT for the respective jumps (555 ± 115 ms vs 644 ± 100 ms; $p = 0.011$, $g = 0.83$) (Figure 8.6). As a result, JH decreased from 245 ± 12 cm to 239 ± 12 cm ($p = 0.042$, $g = 0.5$) by the conclusion of the SSC fatigue protocol (Figure 8.6). Conversely, knee angle did not exhibit any significant changes between the beginning (69.6 ± 11.5°) and end (63.1 ± 8.3°) ($p = 0.110$) of the fatigue protocol.

8.5. Within-Session Reliability

Within-session reliability was only assessed for the control group. Values of reliability for MVC, and measures of central and peripheral responses to SSC exercise-induced fatigue, are summarized in Table 3. Values of reliability for measures of corticospinal and membrane excitability are summarized in Table 4. In particular MVC and $Q_{tw,pot}$ exhibited good reliability while voluntary activation assessed via both cortical and motor nerve stimulation exhibited low to moderate level of reliability. Additionally, good level of reliability were observed for silent period duration, TMS-evoked responses and corticospinal excitability measures.

TABLE 3. Within-session reliability for MVC, and measures of central and peripheral responses for the control group.

	ICC (95% CI)	**CV (%)**	**TE**
MVC	0.918 (0.716 – 0.979)	16.3	95.8
Central Responses			
VA_{TMS} (N)	0.252 (-0.492 – 0.751)	7.3	2.1
VA_{PNS} (N)	0.545 (-0.02 – 0.859)	4	1.2
SP (ms)	0.940 (0.779 – 0.985)	24.4	12.9
Lat (ms)	0.818 (0.427 – 0.951)	10.4	1.1
Peripheral Responses			
$Q_{tw,pot}$ (N)	0.790 (0.389 – 0.942)	11.8	6.3
PT (N)	0.889 (0.634 – 0.971)	15.1	6.8
POT (N)	0.790 (0.371 – 0.943)	63.1	5.1

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval; CV = Coefficient of Variation; TE = Typical error.

TABLE 4. Within-session reliability for measures of corticospinal and membrane excitability for the control group.

	ICC (95% CI)	CV (%)	TE
Resting Responses			
MEP amplitude (mV)	0.598 (0.036 – 0.880)	72.2	0.09
MEP area ($\mu V \cdot s^{-1}$)	0.662 (0.132 – 0.902)	47.3	2.06
M_{max} Amplitude (mv)	0.683 (0.142 – 0.911)	32.2	0.50
M_{max} Area ($\mu V \cdot s^{-1}$)	0.907 (0.669 – 0.976)	47.5	6.87
MEP/M_{max} amplitude	0.422 (-0.299 – 0.821)	48.1	0.03
MEP/M_{max} area	0.456 (-0.230 – 0.832)	46.7	0.05
During MVC			
MEP amplitude (mV)	0.362 (-0.320 – 0.739)	29.1	0.11
MEP area ($\mu V \cdot s^{-1}$)	0.345 (-0.279 – 0.779)	27.3	1.66
M_{max} Amplitude (mv)	0.826 (0.432 – 0.954)	61.4	1.07
M_{max} Area ($\mu V \cdot s^{-1}$)	0.831 (0.465 – 0.955)	33.8	3.51
MEP/M_{max} amplitude	0.616 (0.074 – 0.886)	45.5	0.04
MEP/M_{max} area	0.733 (0.261 – 0.925)	37.6	0.07

ICC = Intraclass Correlation Coefficient; CI = Confidence Interval; CV = Coefficient of Variation; TE = Typical error.

9. DISCUSSION

The present study aimed to assess the acute central and peripheral contribution underlying neuromuscular fatigue following exhaustive stretch-shortening cycle exercise involving the ankle plantar flexors. Among our main findings, we observed that maximum force-generating capacity declined to 82% of baseline values after exhaustive SSC exercise, reflecting the typical decline in force-generating capacity induced by strenuous exercise and indicating that the fatiguing exercise induced considerable neuromuscular fatigue. Central fatigue ensuing during the SSC exercise considerably contributed to the decline in MVC force production since voluntary activation measured both by cortical and motor nerve stimulation was impaired following exhaustive SSC exercise. In particular, the diminished levels of cortical voluntary activation imply the contribution of spinal and supraspinal factors to the reduction in force-generating capacity stemming from exhaustive SSC exercise. Taken together, these findings suggest suboptimal output from both the primary motor cortex and the motoneurons. The decline in force-generating capacity was also associated with a decline in the amplitude of the resting and potentiated twitch evoked by motor nerve stimulation, indicating the presence of peripheral fatigue and impairment of muscle contractility. However, the current investigation failed to reveal meaningful alterations in corticospinal excitability following the exhaustive SSC exercise, despite the clear presence of spinal and supraspinal fatigue, also exacerbated by significantly lengthened silent period duration underpinning increased levels of intracortical inhibition.

9.1 Voluntary Activation at Baseline

As described in previous sections, voluntary activation refers to the nervous system's ability to drive motoneurons and the muscle fibers to translate the motoneurons firing into mechanical work, ultimately enabling the generation of maximum muscle force. The concept of voluntary activation relies on the inverse near-linear relationship between voluntary force and the amplitude of the superimposed twitch elicited by supramaximal motor nerve or cortical stimulation. Therefore, the size of the SIT serves as an indicator of the failure of the neural drive to generate maximal muscle force. Following stimulation during MVC, if no twitch-like force increments are observed, voluntary activation is generally considered to be maximal (100%). However, voluntary activation levels in healthy individuals are rarely maximal and

somewhat based on the muscle group activated (Behm et al. 2002). Submaximal voluntary activation levels (< 100%), as indicated by the presence of a SIT, suggest that at the time of the stimulation, the individual tested either failed to fully recruit the motoneuron pool voluntarily or their motoneurons were discharging at subtetanic rates. In the present study, VA assessed via motor nerve stimulation at baseline was found to be submaximal, albeit comparable between FAT (92.7 ± 6.8) and CON (92.5 ± 2.6). These findings are consistent with previous existing literature. A recent meta-analysis conducted by Rozand et al. (2020) collected measures of VA assessed with ITT via motor nerve stimulation for the ankle plantar flexors from twelve different studies, reporting a range of VA values from 88.5 ± 10.8 to 98.7 ± 1.4. Similarly, VA assessed via TMS was observed to be submaximal but consistent across FAT (90.3 ± 10.1) and CON (91.1 ± 5.7). To our knowledge, there have been no studies investigating the level of VA in ankle plantar flexors using TMS. Hence, it is not possible to determine whether these VA values fall within the normal range. Several factors could account for the observed suboptimal levels of VA assessed by either TMS or motor nerve stimulation. Although the knee-ankle joint position used in this experiment has been demonstrated to optimize force development and neural recruitment (Cresswell et al. 1995; Kennedy et al. 2001), it was unfamiliar to participants. As a result, they may not have been able to exert their true maximal effort due to their lack of familiarity with the specific knee-ankle joint position. Moreover, to prevent any movement induced by contraction, the tested leg was firmly strapped, potentially further limiting natural contractions due to perceived pressure on the leg. Another limiting factor could be represented by the discomfort induced by either TMS or electrical stimulation. Most participants were unfamiliar with these stimulation techniques, and the pain induced by such noxious stimuli is typically classified as moderate (Miller et al. 2006). Therefore, participants may have been psychologically biased, despite being instructed and verbally encouraged to exert their true maximal effort. However, it is worth noting that the superimposed stimuli were delivered only when the force trace exhibited a clear plateau and when the force exceeded 95% of a previously determined MVC (Shield & Zhou. 2004). This suggests that psychological factors might have only a minor influence on the submaximal levels of VA observed. Despite the submaximal levels of voluntary activation, which may suggest less-than-maximal effort by participants, the absence of significant fluctuations in activation levels across trials for CON, suggest that the observed levels of inactivation may be attributed to the actual inability of the subjects to fully drive their working muscles.

9.2 Central Fatigue & SSC Exercise-Induced Changes

It is now well established that besides muscle fatigue stemming from changes occurring distal to the neuromuscular junction, fatigue processes also occur at or proximal to the neuromuscular junction and reside within the central nervous system, referred to as central fatigue. Central fatigue essentially arises when the muscle's maximum force-generating capacity is reduced due to insufficient motor unit recruitment and/or suboptimal motor unit firing rate. While central mechanisms of fatigue are thoroughly understood in the context of single-joint exercises and isometric type of muscle actions (Gandevia et al. 1996; Taylor et al. 2000; Gandevia. 2001), less research has been conducted focusing on locomotor exercises involving multiple limb muscles, particularly those employing specific muscle functions such as the stretch-shortening cycle. The decrease in voluntary activation observed in the current investigation following exhaustive SSC exercise suggests suboptimal cortical and motoneuronal output, further indicating that the SSC exercise bout has clearly induced a significant level of central fatigue. Cortical voluntary activation decreased to ~77% (Δ 12.9), while voluntary activation measured via motor nerve stimulation declined to ~82% (Δ 10.4) of preintervention levels upon completion of the SSC fatigue protocol. To the best of our knowledge, no previous studies have investigated both spinal and supraspinal fatigue, particularly the alterations in the level of voluntary activation, induced by exhaustive SSC exercise. Consequently, direct comparisons remain unfeasible. However, few studies have investigated spinal and supraspinal fatigue following cycling (Sidhu et al., 2009; Thomas et al., 2015) and running (Ross et al., 2010; Temesi et al., 2014) exercises, and the reduction in voluntary activation observed in the present study appear to align with the behavior observed in those studies. After completing the SSC exercise, cortical voluntary activation decreased to a greater extent than voluntary activation measured via motor nerve stimulation despite starting from not significantly different pre-intervention levels (Δ 2.4%, $p = 0.684$). While direct quantitative comparison of voluntary activation measured via cortical and motor nerve stimulation poses challenges for various reasons (Todd et al., 2003), such as differences in the linearity of the relationship between voluntary force and the amplitude of the superimposed twitch - resulting in smaller changes in activation for a given change in voluntary force at high force levels for motor nerve stimulation - the findings of the present study suggest that a substantial proportion of the decline in voluntary drive following lower limb SSC exercise originates from supraspinal mechanisms and results from suboptimal output from the motor cortex. As suggested by Sidhu et al. (2009), the linearity between voluntary force and VA measured via cortical stimulation (Todd et al.,

2003; Sidhu et al., 2009) enables the estimation of the contribution of supraspinal fatigue to the total force loss. Ankle plantar flexor MVC decreased to ~82% of baseline after SSC exercise, while voluntary activation measured via motor cortical stimulation decreased from ~90% to ~77% (Δ13%). The 10% decrease in VA should have reduced MVC by 13% in the absence of peripheral fatigue (i.e., 87% of baseline MVC). For this reason, 56% of the total force decline (i.e., to 82% of initial MVC) can be attributed to supraspinal fatigue. Hence, exhaustive lower limb SSC exercise induces a considerable level of central fatigue, particularly exacerbating the supraspinal component. Evidence arising from a series of studies (Amann et al. 2007; Amann et al. 2008) suggests that the degree of central motor drive during exhaustive locomotor exercise is inversely related to the level of muscle fatigue. Interpretations of the proposed relationship (Amann et al., 2008; Sidhu et al., 2009) suggest that the central nervous system regulates the level of drive to the motoneurons to mitigate the risk of excessive peripheral fatigue beyond tolerable limits, thereby preventing tissue damage. This could be particularly true because SSC muscle function involves high-impact activities. Moreover, in the present experiment, the initial maximal phase of the fatigue protocol precisely aimed to induce muscle damage. Therefore, the proposition that the central motor drive is diminished to prevent the further development of peripheral fatigue could serve as a plausible explanation for the observed suboptimal cortical output. In support of this idea, evidence suggests that the firing of group III and IV afferents, sensitive to fatigue metabolites and known to project at both cortical and subcortical levels, may exert an inhibitory influence at the supraspinal level, impairing voluntary activation during prolonged and fatiguing MVC (Gandevia et al. 1996; Butler et al. 2003). The same regulatory mechanism may also function during locomotor exercise to exacerbate the levels of central and particularly supraspinal fatigue, aiming to diminish cortical output and thereby prevent tissue damage, as previously proposed. However, further work is warranted to better understand the potential existence of this regulatory mechanism.

As discussed in previous sections, cortical stimulation can activate both excitatory and inhibitory interneurons. Consequently, the motor-evoked potentials (MEPs) elicited by TMS are determined by the net effect of all excitatory and inhibitory influences on corticospinal neurons, as well as the excitability of the motoneuron pool (Weber et al., 2002). Without a concomitant measure of motoneuron excitability via stimulation at the cervicomedullary junction (i.e., CMEP), which directly activates corticospinal axons without cortical circuitry involvement, it is not feasible to discern between exercise-induced changes in cortical or motoneuron excitability (Sidhu et al. 2013). However, in the present investigation, no

significant changes in MEP amplitude were observed following exhaustive SSC exercise, both during MVC ($p = 0.628$) and at low levels of contraction ($p = 0.193$). Similarly, MEP normalized to muscle action potential obtained via supramaximal motor nerve stimulation (i.e. Mmax) to account for muscle-dependent changes, exhibited no significant changes following SSC exercise. The present findings thereby suggest that corticospinal excitability remained unaltered following SSC exercise and align with previous evidence reporting no changes in corticospinal excitability (MEPs) at volitional fatigue in cycling exercise tasks (Sidhu et al. 2009; Sidhu et al. 2012; Weavil et al. 2016). The TMS-evoked silent period (SP), which refers to the period of near-silence in EMG activity following cortical stimulation via TMS, is thought to arise from both spinal and cortical processes. The latter part of the silent period (after 100 ms) has been generally considered to be mediated by intracortical inhibition via $GABA_B$ receptors and intracortical inhibitory interneurons (Inghilleri et al. 1993; McDonnell et al. 2006). In the present study, SSC exercise resulted in a significant lengthening of the SP (~13.5%, $p = 0.01$), potentially suggesting an increased level of intracortical inhibition. Without a concomitant measure of the SP evoked by cervicomedullary stimulation, it is however not possible to conclusively infer whether the lengthening of the SP depends solely on increased intracortical inhibition or also on increased spinal inhibitory contribution (Sidhu et al. 2013). Although new evidence has suggested that the spinal component of the SP may extend up to 150 ms (Yacyshyn et al. 2016), in the present study, the average SP was already larger than 150 ms at the pre-intervention level (i.e., 163 ms) and increased to 185 ms following exhaustive SSC exercise. This supports the argument that the observed lengthening of the SP is likely of cortical origin (Sidhu et al. 2018), reflecting a greater level of intracortical inhibition. To the best of our knowledge, investigations on changes in SP duration following exhaustive locomotor exercise have been limited. The significant lengthening of SP observed in the present study contrasts with findings from Sidhu et al. (2009), where no alterations in SP duration were observed following volitional fatigue induced by cycling exercise. A possible explanation for the lengthened SP may be found in a study conducted by Sidhu et al. (2018). Using a paired TMS pulse technique with an inter-stimulus interval of 100 ms following an exhaustive cycling task, they observed a decrease in long-interval intracortical inhibition, a change documented to reflect increased excitability of $GABA_B$ receptor-sensitive inhibitory interneurons. Since long-interval intracortical inhibition remained unchanged and the SP reduced with fentanyl blockade of muscle afferents, it was postulated that the cortical depression during fatiguing locomotor exercise is, at least in part, determined by the facilitating effect of group III/IV muscle afferents on $GABA_B$-mediated inhibitory cortical neurons. While the present study lacks this specific

data, we can speculate that the observed increase in SP duration may have also been mediated by the facilitatory effect of group III/IV muscle afferents. Taken together, our current findings related to central excitability suggest that the excitability of the corticospinal cells (reflected by MEPs) remains unaltered, while the excitability of intracortical inhibitory interneurons (reflected by SP) is enhanced. Particularly, the output from the motor cortex appears insufficient for complete muscle activation, given the considerable reduction in cortical voluntary activation and increased level of intracortical inhibition. This implies that, in the current experiment, the physiological drivers of central fatigue responsible for suboptimal cortical output act upstream of the motor cortical outputs.

9.3 Peripheral Fatigue & SSC Exercise-Induced Changes

In the context of neuromuscular fatigue, peripheral fatigue broadly refers to any significant decline in force-generation capacity stemming from factors at or distal to the neuromuscular junction, leading to an attenuated response to neural excitation and often-involving failure of the contractile machinery. Among electrochemical factors, the major mechanisms affected by the development of peripheral fatigue include alterations of action potential synaptic transmission, sarcolemmal action potential propagation properties, Ca^{2+} release from the sarcoplasmic reticulum, and re-uptake as well as excitation-contraction coupling, cross-bridge kinetics. Among mechanical factors, peripheral fatigue appears to impair force transmission from skeletal muscle to tendon insertion points by altering the mechanical properties of the muscle-tendon complex, such as viscoelasticity and stiffness. Disturbances in both electrochemical and mechanical processes concurrently contribute to the generation of inhibitory feedback to the spinal cord, ultimately modulating motoneuron firing and final force output. As previously described, the maximum force-generating capacity declined to ~82% ($p < 0.01$) of baseline by the end of the fatigue protocol, indicating that the SSC exercise-induced considerable fatigue. Alongside the decrease in MVC force, a notable reduction (~9%, $p = 0.03$) in the amplitude of potentiated twitch ($Q_{tw,pot}$) evoked by supramaximal motor nerve stimulation confirms the presence of peripheral fatigue. While central and especially supraspinal fatigue were prominent in the current study, it is evident that peripheral fatigue also considerably contributed to the overall decline in maximum force-generation capacity. Ankle plantar flexor MVC force decreased by ~18%, while the reduction in $Q_{tw,pot}$ was ~9%. Therefore, peripheral fatigue accounted for approximately 50% of the overall reduction

observed in maximum force-generation capacity. To date, no other study has reported measures of $Q_{tw,pot}$ following exhaustive SSC exercise. However, the decline pattern observed in our study mirrors findings from other studies. Similar decreases were shown following exhaustive cycling exercise (Decorte et al. 2012; Sidhu et al., 2013), as well as after 5-, 20-, and 40-km time trials (Thomas et al., 2015), and fatiguing single-leg knee extensor exercise (Amann et al., 2013; Goodall et al. 2018). In the present study, exhaustive SSC exercise resulted in no significant changes in Mmax amplitude and area, which are thought to serve as measures of sarcolemma excitability and sarcolemmal action potential propagation, respectively (Sidhu et al., 2013). Since alteration of membrane excitability can be discounted as a significant contributor to peripheral fatigue, the most likely explanation for the observed reduction in resting twitch amplitude is an impairment in the excitation-contraction coupling process. This impairment may result from reductions in Ca^{2+} release from the sarcoplasmic reticulum (Westerblad et al. 1993) and a diminished capacity to form strong cross-bridge bonds between the contractile proteins (Fitts. 1994) due to metabolite accumulation. It is also reasonable to believe that, given the high-impact nature of the SSC exercise performed, muscle damage must have occurred, thereby altering the mechanical properties of the muscle-tendon complex and ultimately impairing force transmission. This argument is further supported by the fact that, at the end of the fatigue protocol, during the final three maximal drop jumps performed, although ground contact time significantly lengthened by ~16 ($p = 0.01$) allowing for addiotnal time to generate force, force production was still suboptimal compared to pre-intervention levels (Δ15%, $p = 0.02$).

9.4 Limitations and Future Directions

In our experimental approach, the nature of our study, time constraints and the techniques used reported some methodological drawbacks that must be taken into consideration when interpreting the results. To begin with, perturbation of underlying spinal and supraspinal mechanisms contributing to central fatigue has been documented to exhibit a short time course of recovery subsequent to maximal and submaximal sustained contractions, frequently estimated to occur within 1-2 min following exercise cessation, thus emphasizing the transient nature of these mechanisms (Carroll et al. 2017). Therefore, despite completing all neuromuscular function assessments within 3.5 minutes post-exercise cessation and observing a significant level of supraspinal fatigue in the present investigation, it is conceivable that some

of the exercise-induced central fatigue had already dissipated by the time the neuromuscular function assessments were completed. Consequently, voluntary activation measured by both cortical and motor nerve stimulation may have been slightly underestimated. This could be particularly relevant given the current experimental setup, where the interpolated twitch technique was not directly performed on the sledge ergometer but rather on a custom-made ankle dynamometer positioned adjacent to it. Naturally, some time was required for transitioning between machines. Therefore, for future investigations, it would be interesting to conduct measures of voluntary activation directly on the sledge ergometer to possibly complete the assessment of VA within 30 sec upon exercise cessation, and potentially explore the short-term time course of recovery of these mechanisms. Secondly, in the current experimental design, TMS-evoked responses during the interpolated twitch technique were elicited using 100% of the maximal stimulator output, diverging from the approach seen in previous literature, where stimulation intensities typically ranged between 120% to 130% of aMT during the ITT to assess cortical voluntary activation. This approach aimed to ensure the maximality of the delivered stimulus, thereby exciting the largest possible number of underlying cortical neurons and maintaining consistency in stimuli delivery before and after the fatigue protocol. This decision was influenced by the aforementioned time constraints, which prevented us from testing the aMT upon exercise cessation. For future investigations, it would be valuable to employ a stimulus-response curve to ensure that the stimulation intensity utilized elicits the maximal response, since utilizing 100% of maximal stimulator output does not necessarily guarantee the delivery of stimuli that evoke the largest response. Hence, optimizing stimulation intensity through such an approach would be beneficial.

For future research directions, although though challenging due to time constraints associated with fatigue-related responses, combining different stimulation techniques could yield valuable insights into the fatigue mechanisms occurring at both spinal and supraspinal levels during stretch-shortening cycle exercises. For instance, stimulation at the cervicomedullary junction would enable to discern between exercise-induced changes in cortical versus motoneuron excitability. Furthermore, considering the significant level of intracortical inhibition observed in the present investigation, as evidenced by the increased silent period duration, employing the paired-pulse TMS technique to explore short- and long-interval cortical inhibition, as well as intracortical facilitation, could provide a deeper understanding of the inhibitory mechanisms contributing to the reduction in cortical output typically observed during sustained and exhaustive exercise. Often intensive and/or unaccustomed SSC-type exercise results in

reversible proprioceptive and neuromuscular impairments that may last for several days. After SSC exercises, these neuromuscular perturbations are typically associated with large and acute changes in muscle mechanics and activation that result in major consequences on joint and muscle stiffness regulation, especially in SSC-type performances. These fatigue-induced performance deteriorations and subsequent long-term recovery typically occur in a bimodal fashion (Nicol et al. 2006). In this bimodality, the initial acute metabolically driven drop is followed by a brief short-term recovery which, in turn, is followed by a secondary and subsequent reduction with more durable recovery. Ultimately, it would be interesting to investigate the short- and medium-term recovery of SSC exercise-induced changes at both spinal and supraspinal levels, to ascertain if they align with the bimodal recovery pattern observed in previous literature.

9.5 Conclusions

In conclusion, the findings of the present study show that SSC exercise induces fatigue in the ankle plantar flexor muscles of both central and peripheral origin. In addition to peripheral fatigue, likely stemming from disruptions in the excitation-contraction coupling process, central fatigue, especially in its supraspinal component was estimated to account for ~50% of the total reduction in maximum force-generation capacity. Specifically, the output from the motor cortex appears insufficient to optimally drive the ankle plantar flexors motoneurons for maximum force generation, as evidenced by the considerable reduction in cortical voluntary activation alongside with increased level of intracortical inhibition. However, the SSC exercise did not induce any significant changes in corticospinal excitability during MVC or low levels of contraction. Taken together, this evidence suggests that in the current experiment, the physiological drivers of central fatigue responsible for suboptimal cortical output act upstream of the motor cortical outputs.

REFERENCES

Achache, V., Roche, N., Lamy, J. C., Boakye, M., Lackmy, A., Gastal, A., & Katz, R. (2010). Transmission within several spinal pathways in adults with cerebral palsy. Brain, 133(5), 1470-1483.

Alawieh, A., Tomlinson, S., Adkins, D., Kautz, S., & Feng, W. (2017). Preclinical and clinical evidence on ipsilateral corticospinal projections: implication for motor recovery. Translational stroke research, 8, 529-540.

Allen, G. M., Gandevia, S. C., & McKenzie, D. K. (1995). Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 18(6), 593-600.

Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempsey JA. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. J Physiol 581: 389 – 403, 2007.

Amann M, Dempsey JA. Locomotor muscle fatigue modifies central motor drive in healthy humans and imposes a limitation to exercise performance. J Physiol 586: 161–173, 2008.

Amann, M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2009). Opioid-mediated muscle afferents inhibit central motor drive and limit peripheral muscle fatigue development in humans. The Journal of physiology, 587(1), 271-283.

Amann, M., Blain, G. M., Proctor, L. T., Sebranek, J. J., Pegelow, D. F., & Dempsey, J. A. (2011). Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans. The Journal of physiology, 589(21), 5299-5309.

Amann, M., Venturelli, M., Ives, S. J., McDaniel, J., Layec, G., Rossman, M. J., & Richardson, R. S. (2013). Peripheral fatigue limits endurance exercise via a sensory feedback-mediated reduction in spinal motoneuronal output. Journal of applied physiology, 115(3), 355-364.

Avela, J., & Komi, P. V. (1998). Reduced stretch reflex sensitivity and muscle stiffness after long-lasting stretch-shortening cycle exercise in humans. European journal of applied physiology and occupational physiology, 78, 403-410.

Avela, J., Finni, J., & Komi, P. V. (2006). Excitability of the soleus reflex arc during intensive stretch–shortening cycle exercise in two power-trained athlete groups. European journal of applied physiology, 97, 486-493.

Avela, J., & Gruber, M. (2010). Transcranial magnetic stimulation as a tool to study the role of the motor cortex in human muscle function.

Behm, D. G., Whittle, J., Button, D., & Power, K. (2002). Intermuscle differences in activation. Muscle & nerve, 25(2), 236-243.

Benwell, N. M., Sacco, P., Hammond, G. R., Byrnes, M. L., Mastaglia, F. L., & Thickbroom, G. W. (2006). Short-interval cortical inhibition and corticomotor excitability with fatiguing hand exercise: a central adaptation to fatigue?. Experimental Brain Research, 170, 191-198.

Benwell, N. M., Mastaglia, F. L., & Thickbroom, G. W. (2007). Differential changes in long-interval intracortical inhibition and silent period duration during fatiguing hand exercise. Experimental brain research, 179, 255-262.

Bhimani, R., & Anderson, L. (2014). Clinical understanding of spasticity: implications for practice. Rehabilitation research and practice, 2014.

Bigland-Ritchie, B., Jones, D. A., Hosking, G. P., & Edwards, R. H. T. (1978). Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. Clinical science and molecular medicine, 54(6), 609-614.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., Smith, S., & Woods, J. J. (1983). Changes in motoneurone firing rates during sustained maximal voluntary contractions. The Journal of physiology, 340(1), 335-346.

Bigland-Ritchie, B., Johansson, R., Lippold, O. C., & Woods, J. J. (1983). Contractile speed and EMG changes during fatigue of sustained maximal voluntary contractions. Journal of neurophysiology, 50(1), 313-324.

Bigland-Ritchie, B. R., Dawson, N. J., Johansson, R. S., & Lippold, O. C. (1986). Reflex origin for the slowing of motoneurone firing rates in fatigue of human voluntary contractions. The Journal of physiology, 379(1), 451-459.

Bigland-Ritchie, B., Zijdewind, I., & Thomas, C. K. (2000). Muscle fatigue induced by stimulation with and without doublets. Muscle & nerve, 23(9), 1348-1355.

Binder-Macleod, S. A., Lee, S. C., Russ, D. W., & Kucharski, L. J. (1998). Effects of activation pattern on human skeletal muscle fatigue. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 21(9), 1145-1152.

Binder-Macleod, S., & Kesar, T. (2005). Catchlike property of skeletal muscle: recent findings and clinical implications. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 31(6), 681-693.

Bongiovanni, L. G., & Hagbarth, K. E. (1990). Tonic vibration reflexes elicited during fatigue from maximal voluntary contractions in man. The Journal of physiology, 423(1), 1-14.

Brasil-Neto, J. P., Pascual-Leone, A., Valls-Solé, J., Cammarota, A., Cohen, L. G., & Hallett, M. (1993). Postexercise depression of motor evoked potentials: a measure of central nervous system fatigue. Experimental Brain Research, 93, 181-184.

Bruton, J. D., Place, N., Yamada, T., Silva, J. P., Andrade, F. H., Dahlstedt, A. J., ... & Westerblad, H. (2008). Reactive oxygen species and fatigue-induced prolonged low-frequency force depression in skeletal muscle fibres of rats, mice and SOD2 overexpressing mice. The Journal of physiology, 586(1), 175-184.

Burke, D., Gandevia, S. C., & McKeon, B. (1983). The afferent volleys responsible for spinal proprioceptive reflexes in man. The Journal of physiology, 339(1), 535-552.

Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2003). Responses of human motoneurons to corticospinal stimulation during maximal voluntary contractions and ischemia. Journal of Neuroscience, 23(32), 10224-10230.

Carroll, T. J., Taylor, J. L., & Gandevia, S. C. (2017). Recovery of central and peripheral neuromuscular fatigue after exercise. Journal of Applied Physiology, 122(5), 1068-1076.

Carson, R. G. (2005). Neural pathways mediating bilateral interactions between the upper limbs. Brain Research Reviews, 49(3), 641-662.

CD, M. (1983). Muscular wisdom that minimizes fatigue during prolonged effort in man: peaks rates of motoneuron discharge and slowing of discharge during fatigue. Motor control mechanisms in health and disease, 169-211.

Christie, A., & Kamen, G. (2006). Doublet discharges in motoneurons of young and older adults. Journal of neurophysiology, 95(5), 2787-2795.

Cotel, F., Exley, R., Cragg, S. J., & Perrier, J. F. (2013). Serotonin spillover onto the axon initial segment of motoneurons induces central fatigue by inhibiting action potential initiation. Proceedings of the National Academy of Sciences, 110(12), 4774-4779.

Cresswell, A. G., Löscher, W. N., & Thorstensson, A. (1995). Influence of gastrocnemius muscle length on triceps surae torque development and electromyographic activity in man. Experimental brain research, 105, 283-290.

Crone, C., Hultborn, H., Jespersen, B., & Nielsen, J. (1987). Reciprocal Ia inhibition between ankle flexors and extensors in man. The Journal of physiology, 389(1), 163-185.

Cutsem, M. V., Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Mechanical properties and behaviour of motor units in the tibialis anterior during voluntary contractions. Canadian journal of applied physiology, 22(6), 585-597.

Decorte, N., Lafaix, P. A., Millet, G. Y., Wuyam, B., & Verges, S. (2012). Central and peripheral fatigue kinetics during exhaustive constant-load cycling. Scandinavian journal of medicine & science in sports, 22(3), 381-391.

De Luca, C. J., LeFever, R. S., McCue, M. P., & Xenakis, A. P. (1982). Behaviour of human motor units in different muscles during linearly varying contractions. The Journal of physiology, 329(1), 113-128.

Desmedt, J. E., & Godaux, E. (1977). Ballistic contractions in man: characteristic recruitment pattern of single motor units of the tibialis anterior muscle. The Journal of physiology, 264(3), 673-693.

Desmedt, J. E., & Godaux, E. (1978). Ballistic contractions in fast or slow human muscles; discharge patterns of single motor units. The Journal of physiology, 285(1), 185-196.

Di Lazzaro, V., Oliviero, A., Pilato, F., Mazzone, P., Insola, A., Ranieri, F., & Tonali, P. A. (2003). Corticospinal volleys evoked by transcranial stimulation of the brain in conscious humans. Neurological research, 25(2), 143-150.

Di Lazzaro, V., Oliviero, A., Pilato, F., Saturno, E., Dileone, M., Mazzone, P., ... & Rothwell, J. C. (2004). The physiological basis of transcranial motor cortex stimulation in conscious humans. Clinical neurophysiology, 115(2), 255-266.

Dorfman, L. J., Howard, J. E., & McGill, K. C. (1990). Triphasic behavioral response of motor units to submaximal fatiguing exercise. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 13(7), 621-628.

Duchateau, J., Semmler, J. G., & Enoka, R. M. (2006). Training adaptations in the behavior of human motor units. Journal of applied physiology, 101(6), 1766-1775.

Dura-Bernal, S., Kerr, C. C., Neymotin, S. A., Suter, B. A., Shepherd, G. M., Francis, J. T., & Lytton, W. W. (2015). Large-scale M1 microcircuit model with plastic input connections from biological PMd neurons used for prosthetic arm control. BMC Neuroscience, 16, 1-2.

Eftekhar, A., Norton, J. J., McDonough, C. M., & Wolpaw, J. R. (2018). Retraining reflexes: clinical translation of spinal reflex operant conditioning. Neurotherapeutics, 15(3), 669-683.

Enoka, R. M., & Fuglevand, A. J. (2001). Motor unit physiology: some unresolved issues. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 24(1), 4-17.

Enoka, R. M. (2008). Neuromechanics of human movement. Human kinetics.

Enoka, R. M., & Duchateau, J. (2016). Translating fatigue to human performance. Medicine and science in sports and exercise, 48(11), 2228.

Fuglevand, A. J., Macefield, V. G., & Bigland-Ritchie, B. (1999). Force-frequency and fatigue properties of motor units in muscles that control digits of the human hand. Journal of neurophysiology, 81(4), 1718-1729.

Feiereisen, P., Duchateau, J., & Hainaut, K. (1997). Motor unit recruitment order during voluntary and electrically induced contractions in the tibialis anterior. Experimental brain research, 114, 117-123.

Fitts, R. H. (1994). Cellular mechanisms of muscle fatigue. Physiological reviews, 74(1), 49-94.

Fritz, C. O., Morris, P. E., & Richler, J. J. (2012). Effect size estimates: current use, calculations, and interpretation. Journal of experimental psychology: General, 141(1), 2.

Gamer, M. (2010). irr: Various coefficients of interrater reliability and agreement. http://cran. r-project. org/web/packages/irr/irr. pdf.

Gandevia, S. C., Macefield, G., Burke, D., & McKenzie, D. K. (1990). Voluntary activation of human motor axons in the absence of muscle afferent feedback: the control of the deafferented hand. Brain, 113(5), 1563-1581.

Gandevia, S. C., Allen, G. M., Butler, J. E., & Taylor, J. L. (1996). Supraspinal factors in human muscle fatigue: evidence for suboptimal output from the motor cortex. The Journal of physiology, 490(2), 529-536.

Gandevia, S. C. (2001). Spinal and supraspinal factors in human muscle fatigue. Physiological reviews.

Garland, S. J., Garner, S. H., & McComas, A. J. (1988). Reduced voluntary electromyographic activity after fatiguing stimulation of human muscle. The Journal of Physiology, 401(1), 547-556.

Garland, S. J., Enoka, R. M., Serrano, L. P., & Robinson, G. A. (1994). Behavior of motor units in human biceps brachii during a submaximal fatiguing contraction. Journal of Applied Physiology, 76(6), 2411-2419.

Garland, S. J., Griffin, L., & Ivanova, T. (1997). Motor unit discharge rate is not associated with muscle relaxation time in sustained submaximal contractions in humans. Neuroscience letters, 239(1), 25-28.

Gatev, P., Ivanova, T., & Gantchev, G. N. (1986). Changes in the firing pattern of high-threshold motor units due to fatigue. Electromyography and clinical neurophysiology, 26(2), 83-93.

Gomez-Guerrero, G., Avela, J., Jussila, I., Pihlajamäki, E., Deng, F. Y., Kidgell, D. J., ... & Walker, S. (2024). Cortical and spinal responses to short-term strength training and detraining in young and older adults in rectus femoris muscle. European Journal of Applied Physiology, 1-15.

Goodall, S., Ross, E. Z., & Romer, L. M. (2010). Effect of graded hypoxia on supraspinal contributions to fatigue with unilateral knee-extensor contractions. Journal of Applied Physiology, 109(6), 1842-1851.

Goodall, S., Howatson, G., Romer, L., & Ross, E. (2014). Transcranial magnetic stimulation in sport science: a commentary. European journal of sport science, 14(sup1), S332-S340.

Goodall, S., Howatson, G., & Thomas, K. (2018). Modulation of specific inhibitory networks in fatigued locomotor muscles of healthy males. Experimental Brain Research, 236, 463-473.

Goodman, J. M., & Bensmaia, S. J. (2020). The neural mechanisms of touch and proprioception at the somatosensory periphery.

Gruet, M., Temesi, J., Rupp, T., Levy, P., Millet, G. Y., & Verges, S. (2013). Stimulation of the motor cortex and corticospinal tract to assess human muscle fatigue. Neuroscience, 231, 384-399.

Grunnet, M., Jespersen, T., & Perrier, J. F. (2004). 5-HT1A receptors modulate small-conductance Ca2+-activated K+ channels. Journal of neuroscience research, 78(6), 845-854.

Hagbarth, K. E., Bongiovanni, L. G., & Nordin, M. (1995). Reduced servo-control of fatigued human finger extensor and flexor muscles. The Journal of physiology, 485(3), 865-872.

Hallett, M. (2007). Transcranial magnetic stimulation: a primer. Neuron, 55(2), 187-199.

Harrow-Mortelliti, M., Reddy, V., & Jimsheleishvili, G. (2019). Physiology, spinal cord.

Heckman, C. J., & Enoka, R. M. (2012). Motor unit. Compr Physiol 2: 2629–2682.

Henneman, E. (1957). Relation between size of neurons and their susceptibility to discharge. Science, 126(3287), 1345-1347.

Herbert, R. D., & Gandevia, S. C. (1999). Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. Journal of neurophysiology, 82(5), 2271-2283.

Hultborn, H., Meunier, S., Pierrot-Deseilligny, E., & Shindo, M. (1987). Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. The Journal of physiology, 389(1), 757-772.

Inghilleri, M., Berardelli, A., Cruccu, G., & Manfredi, M. (1993). Silent period evoked by transcranial stimulation of the human cortex and cervicomedullary junction. The Journal of physiology, 466(1), 521-534.

Jones, D. A., Bigland-Ritchie, B., & Edwards, R. H. T. (1979). Excitation frequency and muscle fatigue: mechanical responses during voluntary and stimulated contractions. Experimental neurology, 64(2), 401-413.

Kawakami, Y., Ichinose, Y., & Fukunaga, T. (1998). Architectural and functional features of human triceps surae muscles during contraction. Journal of applied physiology, 85(2), 398-404.

Kennedy, P. M., & Cresswell, A. G. (2001). The effect of muscle length on motor-unit recruitment during isometric plantar flexion in humans. Experimental brain research, 137, 58-64.

Kidgell, D. J., & Pearce, A. J. (Eds.). (2020). Principles of Exercise Neuroscience. Cambridge Scholars Publishing.

Kluger, B. M., Krupp, L. B., & Enoka, R. M. (2013). Fatigue and fatigability in neurologic illnesses: proposal for a unified taxonomy. Neurology, 80(4), 409-416.

Komi, P. V., Kaneko, M., & Aura, O. (1987). EMG activity of the leg extensor muscles with special reference to mechanical efficiency in concentric and eccentric exercise. International journal of sports medicine, 8(S 1), S22-S29.

Komi, P. V. (2000). Stretch-shortening cycle: a powerful model to study normal and fatigued muscle. Journal of biomechanics, 33(10), 1197-1206.

Komi, P. V. (2010). From isolated actions to true muscle function. Neuromuscular Aspects of Sport Performance, 17, 1-14.

Koo, T. K., & Li, M. Y. (2016). A guideline of selecting and reporting intraclass correlation coefficients for reliability research. Journal of chiropractic medicine, 15(2), 155-163.

Kositsky, A., & Kidgell, D. J. (2019). Medial gastrocnemius muscle architecture is altered after exhaustive stretch-shortening cycle exercise. Frontiers in Physiology, 10, 498287.

Kujirai, T., Caramia, M. D., Rothwell, J. C., Day, B. L., Thompson, P. D., Ferbert, A., ... & Marsden, C. D. (1993). Corticocortical inhibition in human motor cortex. The Journal of physiology, 471(1), 501-519.

Kukulka, C. G., & Clamann, H. P. (1981). Comparison of the recruitment and discharge properties of motor units in human brachial biceps and adductor pollicis during isometric contractions. Brain research, 219(1), 45-55.

Lee, M., Gandevia, S. C., & Carroll, T. J. (2008). Cortical voluntary activation can be reliably measured in human wrist extensors using transcranial magnetic stimulation. Clinical Neurophysiology, 119(5), 1130-1138.

Macefield, G., Hagbarth, K. E., Gorman, R., Gandevia, S. C., & Burke, D. (1991). Decline in spindle support to alpha-motoneurones during sustained voluntary contractions. The Journal of physiology, 440(1), 497-512.

Macefield, V. G., Fuglevand, A. J., & Bigland-Ritchie, B. (1996). Contractile properties of single motor units in human toe extensors assessed by intraneural motor axon stimulation. Journal of neurophysiology, 75(6), 2509-2519.

Maffiuletti, N. A., Barbero, M., Cescon, C., Clijsen, R., Beretta-Piccoli, M., Schneebeli, A., ... & Togninalli, D. (2016). Validity of the twitch interpolation technique for the assessment of quadriceps neuromuscular asymmetries. Journal of Electromyography and Kinesiology, 28, 31-36.

Maton, B., & Gamet, D. (1989). The fatigability of two agonistic muscles in human isometric voluntary submaximal contraction: an EMG study: II. Motor unit firing rate and recruitment. European journal of applied physiology and occupational physiology, 58, 369-374.

McDonnell, M. N., Orekhov, Y., & Ziemann, U. (2006). The role of GABA B receptors in intracortical inhibition in the human motor cortex. Experimental brain research, 173, 86-93.

Merton, P. A. (1954). Voluntary strength and fatigue. The Journal of physiology, 123(3), 553.

Meunier, S., & Pierrot-Deseilligny, E. (1989). Gating of the afferent volley of the monosynaptic stretch reflex during movement in man. The Journal of Physiology, 419(1), 753-763.

Millet, G. Y., Martin, V., Martin, A., & Vergès, S. (2011). Electrical stimulation for testing neuromuscular function: from sport to pathology. European journal of applied physiology, 111, 2489-2500.

Miller, M., Flansbjer, U. B., Downham, D., & Lexell, J. (2006). Superimposed electrical stimulation: assessment of voluntary activation and perceived discomfort in healthy, moderately active older and younger women and men. American journal of physical medicine & rehabilitation, 85(12), 945-950.

Milner-Brown, H. S., & Miller, R. G. (1986). Muscle membrane excitation and impulse propagation velocity are reduced during muscle fatigue. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 9(4), 367-374.

Moritz, C. T., Barry, B. K., Pascoe, M. A., & Enoka, R. M. (2005). Discharge rate variability influences the variation in force fluctuations across the working range of a hand muscle. Journal of neurophysiology, 93(5), 2449-2459.

Nicol, C., Avela, J., & Komi, P. V. (2006). The stretch-shortening cycle: a model to study naturally occurring neuromuscular fatigue. Sports medicine, 36, 977-999.

Nordlund, M. M., Thorstensson, A., & Cresswell, A. G. (2004). Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. Journal of applied physiology, 96(1), 218-225.

Palmieri, R. M., Ingersoll, C. D., & Hoffman, M. A. (2004). The Hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. Journal of athletic training, 39(3), 268.

Perez, M. A., Lundbye-Jensen, J., & Nielsen, J. B. (2007). Task-specific depression of the soleus H-reflex after cocontraction training of antagonistic ankle muscles. Journal of neurophysiology, 98(6), 3677-3687.

Perrier, J. F., Alaburda, A., & Hounsgaard, J. (2003). 5-HT1A receptors increase excitability of spinal motoneurons by inhibiting a TASK-1-like K+ current in the adult turtle. The Journal of physiology, 548(2), 485-492.

Peters, E. J., & Fuglevand, A. J. (1999). Cessation of human motor unit discharge during sustained maximal voluntary contraction. Neuroscience letters, 274(1), 66-70.

Place, N., Maffiuletti, N. A., Martin, A., & Lepers, R. (2007). Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 35(4), 486-495.

Place, N., & Millet, G. Y. (2020). Quantification of neuromuscular fatigue: what do we do wrong and why?. Sports medicine, 50(3), 439-447.

Rodriguez-Falces, J., & Place, N. (2018). Determinants, analysis and interpretation of the muscle compound action potential (M wave) in humans: implications for the study of muscle fatigue. European journal of applied physiology, 118, 501-521.

Ross, E. Z., Goodall, S., Stevens, A., & Harris, I. (2010). Time course of neuromuscular changes during running in well-trained subjects. Medicine and Science in Sports and Exercise, 42(6), 1184-1190.

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., & Safety of TMS Consensus Group. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. Clinical neurophysiology, 120(12), 2008-2039.

Rozand, V., Sundberg, C. W., Hunter, S. K., & Smith, A. E. (2020). Age-related deficits in voluntary activation: a systematic review and meta-analysis. Medicine and science in sports and exercise, 52(3), 549.

Rubinstein, S., & Kamen, G. (2005). Decreases in motor unit firing rate during sustained maximal-effort contractions in young and older adults. Journal of Electromyography and Kinesiology, 15(6), 536-543.

Sawczuk, A., Powers, R. K., & Binder, M. D. (1995). Intrinsic properties of motoneurons: implications for muscle fatigue. Fatigue: Neural and Muscular Mechanisms, 123-134.

Sawczuk, A., Powers, R. K., & Binder, M. D. (1995). Spike frequency adaptation studied in hypoglossal motoneurons of the rat. Journal of neurophysiology, 73(5), 1799-1810.

Schieppati, M., & Nardone, A. (1999). Group II spindle afferent fibers in humans: their possible role in the reflex control of stance. Progress in brain research, 123, 461-472.

Semmler, J. G., Kornatz, K. W., Dinenno, D. V., Zhou, S., & Enoka, R. M. (2002). Motor unit synchronisation is enhanced during slow lengthening contractions of a hand muscle. The Journal of physiology, 545(2), 681-695.

Sidhu, S. K., Bentley, D. J., & Carroll, T. J. (2009). Locomotor exercise induces long-lasting impairments in the capacity of the human motor cortex to voluntarily activate knee extensor muscles. Journal of Applied Physiology, 106(2), 556-565.

Sidhu, S. K., Bentley, D. J., & Carroll, T. J. (2009). Cortical voluntary activation of the human knee extensors can be reliably estimated using transcranial magnetic stimulation. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine, 39(2), 186-196.

Sidhu, S. K., Cresswell, A. G., & Carroll, T. J. (2012). Motor cortex excitability does not increase during sustained cycling exercise to volitional exhaustion. Journal of applied physiology, 113(3), 401-409.

Sidhu, S. K., Cresswell, A. G., & Carroll, T. J. (2013). Corticospinal responses to sustained locomotor exercises: moving beyond single-joint studies of central fatigue. Sports Medicine, 43, 437-449.

Sidhu, S. K., Weavil, J. C., Thurston, T. S., Rosenberger, D., Jessop, J. E., Wang, E., ... & Amann, M. (2018). Fatigue-related group III/IV muscle afferent feedback facilitates intracortical inhibition during locomotor exercise. The Journal of physiology, 596(19), 4789-4801.

Shield, A., & Zhou, S. (2004). Assessing voluntary muscle activation with the twitch interpolation technique. Sports medicine, 34, 253-267.

Skau, S., Sundberg, K., & Kuhn, H. G. (2021). A proposal for a unifying set of definitions of fatigue. Frontiers in Psychology, 12, 739764.

Smith, J. L., Martin, P. G., Gandevia, S. C., & Taylor, J. L. (2007). Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. Journal of Applied Physiology, 103(2), 560-568.

Søgaard, K., Christensen, H., Jensen, B. R., Finsen, L., & Sjøgaard, G. (1996). Motor control and kinetics during low level concentric and eccentric contractions in man. Electroencephalography and Clinical Neurophysiology/Electromyography and Motor Control, 101(5), 453-460.

Søgaard, K., Gandevia, S. C., Todd, G., Petersen, N. T., & Taylor, J. L. (2006). The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. The Journal of physiology, 573(2), 511-523.

Stifani, N. (2014). Motor neurons and the generation of spinal motor neuron diversity. Frontiers in cellular neuroscience, 8, 293.

Stotz, P. J., & Bawa, P. (2001). Motor unit recruitment during lengthening contractions of human wrist flexors. Muscle & nerve, 24(11), 1535-1541.

Strojnik, V., & Komi, P. V. (1998). Neuromuscular fatigue after maximal stretch-shortening cycle exercise. Journal of Applied Physiology, 84(1), 344-350.

Strojnik, V., & Komi, P. V. (2000). Fatigue after submaximal intensive stretch-shortening cycle exercise. Medicine and science in sports and exercise, 32(7), 1314-1319.

Tanaka, M., & Watanabe, Y. (2012). Supraspinal regulation of physical fatigue. Neuroscience & Biobehavioral Reviews, 36(1), 727-734.

Taylor, J. L., Butler, J. E., Allen, G. M., & Gandevia, S. C. (1996). Changes in motor cortical excitability during human muscle fatigue. The Journal of physiology, 490(2), 519-528.

Taylor, J. L., Allen, G. M., Butler, J. E., & Gandevia, S. C. (2000). Supraspinal fatigue during intermittent maximal voluntary contractions of the human elbow flexors. Journal of Applied Physiology, 89(1), 305-313.

Taylor, J. L., Todd, G., & Gandevia, S. C. (2006). Evidence for a supraspinal contribution to human muscle fatigue. Clinical and experimental pharmacology and physiology, 33(4), 400-405.

Taylor, J. L., & Gandevia, S. C. (2008). A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. Journal of applied physiology, 104(2), 542-550.

Taylor, J. L., Amann, M., Duchateau, J., Meeusen, R., & Rice, C. L. (2016). Neural contributions to muscle fatigue: from the brain to the muscle and back again. Medicine and science in sports and exercise, 48(11), 2294.

Temesi, J., Rupp, T., Martin, V., Arnal, P. J., Féasson, L., Verges, S., & Millet, G. Y. (2014). Central fatigue assessed by transcranial magnetic stimulation in ultratrail running. Med Sci Sports Exerc, 46(6), 1166-75.

Thomas, C. K., Johansson, R. S., & Bigland-Ritchie, B. (1999). Pattern of pulses that maximize force output from single human thenar motor units. Journal of neurophysiology, 82(6), 3188-3195.

Thomas, K., Goodall, S., Stone, M., Howatson, G., Gibson, A. S. C., & Ansley, L. (2015). Central and peripheral fatigue in male cyclists after 4-, 20-, and 40-km time trials. Medicine & Science in Sports & Exercise, 47(3), 537-546.

Todd, G., Taylor, J. L., & Gandevia, S. (2003). Measurement of voluntary activation of fresh and fatigued human muscles using transcranial magnetic stimulation. The Journal of physiology, 551(2), 661-671.

Todd, G., Taylor, J. L., & Gandevia, S. C. (2004). Reproducible measurement of voluntary activation of human elbow flexors with motor cortical stimulation. Journal of applied physiology, 97(1), 236-242.

Torchiano, M. (2022). Effsize-a package for efficient effect size computation. zenodo. 2016.

Tseng, S. C., & Shields, R. K. (2012). Limb segment load inhibits post activation depression of soleus H-reflex in humans. Clinical neurophysiology, 123(9), 1836-1845.

Van Cutsem, M., Duchateau, J., & Hainaut, K. (1998). Changes in single motor unit behaviour contribute to the increase in contraction speed after dynamic training in humans. The Journal of physiology, 513(1), 295-305.

Van Cutsem, M., & Duchateau, J. (2005). Preceding muscle activity influences motor unit discharge and rate of torque development during ballistic contractions in humans. The Journal of physiology, 562(2), 635-644.

Weavil, J. C., Sidhu, S. K., Mangum, T. S., Richardson, R. S., & Amann, M. (2016). Fatigue diminishes motoneuronal excitability during cycling exercise. Journal of Neurophysiology, 116(4), 1743-1751.

Weber, M., Eisen, A. A., & AAEM. (2002). Magnetic stimulation of the central and peripheral nervous systems. Muscle & nerve, 25(2), 160-175.

Westerblad, H., Duty, S., & Allen, D. G. (1993). Intracellular calcium concentration during low-frequency fatigue in isolated single fibers of mouse skeletal muscle. Journal of Applied Physiology, 75(1), 382-388.

Wilkinson, R. D., Mazzo, M. R., & Feeney, D. F. (2023). Rethinking the statistical analysis of neuromechanical data. Exercise and sport sciences reviews, 51(1), 43-50.

Woods, J. J., Furbush, F., & Bigland-Ritchie, B. (1987). Evidence for a fatigue-induced reflex inhibition of motoneuron firing rates. Journal of neurophysiology, 58(1), 125-137.

Yacyshyn, A. F., Woo, E. J., Price, M. C., & McNeil, C. J. (2016). Motoneuron responsiveness to corticospinal tract stimulation during the silent period induced by transcranial magnetic stimulation. Experimental brain research, 234, 3457-3463.

UNIVERSITY OF JYVÄSKYLÄ

NAME OF UNIT

Date 15.11.2023

RESEARCH NOTIFICATION

1. **"Corticomotor and corticospinal excitability contributions to neuromuscular fatigue following exhaustive stretch-shortening cycle actions and associated recovery pattern" and invitation to participate in research**

We ask you to participate in "Corticomotor and corticospinal excitability contributions to neuromuscular fatigue following exhaustive stretch-shortening cycle actions and associated recovery pattern", which investigates: 1) corticomotor and corticospinal contributions to neuromuscular fatigue following exhaustive stretch-shortening cycle actions and the recovery of potential SSC exercise-induced changes at the spinal and supraspinal levels, 2.) the relationship between peripheral and central fatigue following exhaustive SSC actions and the recovery pattern of both central and peripheral fatigue index and 3.) comparison of voluntary activation assessed either by TMS or percutaneous nerve stimulation.

You are invited to the study because you are a young, healthy, and active man with the age ranging between 18 and 35 years old and you don't have a history of cardiovascular or neuromuscular disease, and any musculoskeletal injuries of the lower limbs preventing them from performing the maximal and submaximal SSC fatigue protocol. Prior to the initiation of the measurements, participants will have to complete a TMS safety checklist and a Physical Activity Readiness Questionnaire in order to be cleared to participate in the experimentation.

Endurance athletes or individuals with a recent endurance-based training background will not be admitted into the investigation because these subjects are more accustomed to exerting maximal effort towards or at the end of the performance in presence of an already consistent level of fatigue, thus exponentially increasing the risk of disrupting the validity and reliability of the results.

Participants will be asked to refrain from physical activity, ingestion of caffeine and alcohol at least 24 hours prior to the first official measurement and throughout the duration of the experiment.

This research notification describes the study and related participation. The attachment provides information on the processing of your personal data.

The study will involve in total of 15 subjects.

This is a single study, and you will not be contacted again later.

2. **Voluntariness**

Participation in this study is voluntary. You can refuse to participate in the study, stop participating or cancel your previously given consent, without stating any reason for this and at any time during the study. This will have no negative consequences to you.

If you stop participating in the study or if you cancel your consent, the personal data, samples and other information collected on you up to that point will be used as part of the research material as far as it is necessary in order to ensure relevant research outcomes.

3. **Progress of the study**

Each participant will attend the laboratory at least in 4 sessions: 1.) familiarization session. 2.) SSC fatigue protocol, pre- and immediate post- fatigue NMFA, and post-2h NMFA. 3.) post-2 days NMFA. 4.) post 7-days NMFA. If possible, the follow-up assessment of neuromuscular function will be carried out every day up to seven days after the SSC fatigue protocol. As abovementioned in the introduction section, there is a certain degree of inter-subject variability regarding the time course of the second drop in neuromuscular function according to the bimodal recovery pattern. Thus, by extending the follow-up measure every day up to 7 days after the completion of the SSC fatigue protocol, we will be able to limit as much as possible the time course recovery variability between subjects in this regard.

Approximately one week prior to the fatigue protocol, subjects will visit the lab for a familiarization session. For maximal DJs and submaximal continuous rebounding subjects will be instructed to keep the arms folded across their chest to limit upward propulsion of the upper body, allow their knees to passively flex freely during the rebound airborne phase to reduce fatigue of the hip and knee extensors, and to limit their knee angle to a maximum of 90° during ground contact. Subjects will be introduced to the sledge apparatus and the optimal dropping height for drop jumps (DJs) will be determined as follows. After being instructed to rebound jump as high and as fast as they could, subjects will be dropped from 10 cm intervals and the rebound jump height will be recorded. Dropping heights will be increased until the rebound height no longer improved, and the dropping height with the corresponding highest rebound height will be determined to be the optimal dropping height and marked on the side of the sledge apparatus. In addition, subjects will perform a series (e.g., 20 x 2) of submaximal continuous jump in order to get familiar with the submaximal protocol. Subjects will be also shown how to perform and practice MVCs of the plantarflexors on

the custom-made ankle dynamometer. Subjects will be introduced to percutaneous nerve stimulation and TMS. Furthermore, hotspot identification for TMS will be carried out.

Neuromuscular Function Assessment. Measures of neuromuscular function for the assessment of central and peripheral fatigue will be evaluated before and after the SSC fatigue protocol and every day up to seven days using TMS of the motor cortex and electrical stimulation of the femoral nerve, with evoked responses recorded with surface EMG. In order to effectively investigate the SSC exercise-induced fatigue, the post-SSC fatigue neuromuscular function assessment will be completed within 2.5 min of exercise cessation. The rapid nature of this procedure is necessary to capture the magnitude of fatigue induced by the exercise before it dissipates. Prior to each neuromuscular function assessment, except for the one at the completion of the SSC fatigue protocol, rMT (TMS) and motor threshold (PNS) will be determined in order to identify the final stimulation intensity for the respective session.

Each neuromuscular function assessment will consist of:

1) 10 single pulse TMS + 3 electrical stimuli in a randomized order upon 5% MVC, with a 5s interstimulus interval.

2) 2 ankle plantarflexors IMVC with 1-min of rest in between. Whenever the difference in force production between the two IMVCs is above 5%, an additional IMVC will be performed. At the completion of the SSC fatigue protocol only 1 ankle plantarflexors IMVC will be performed.

3) 1 supramaximal PNS upon ankle plantarflexors IMVC as explained in the respective section.

4) TMS at 100%, 75%, and 50% MVC with 5-sec or rest in between contractions. The contraction and stimulation at 100% MVC will be performed first, followed by stimulation upon 50 and 75% MVC in a randomized order.

5) 2 trains of impulses with a frequency of 20 Hz (1-s duration) and 100 Hz (0.8-s duration) to the medial gastrocnemius and soleus.

SSC Fatigue Protocol. Subjects will perform a standardized, moderate-intensity warm-up on a cycle ergometer for 10 min. In addition, they will perform approximately 20 submaximal drop jumps from a height of 20 cm. Subjects then will perform 100 successive maximal bilateral DJs (one DJ every 5 s) from the optimal dropping height, split into 5 sets of 20 with 2 min rest given between sets (See Fig. 1). A researcher will catch the sledge at the end of the jump and reposition it to the optimal dropping height between each maximal DJ. Immediately after the completion of the maximal DJs, the subjects will begin an incremental continuous submaximal rebounding starting at a height corresponding to 50% of the initial maximal rebound height for a duration of 5 minutes. At the completion of these 5 minutes of continuous submaximal rebounding, the target rebound height will be increased to 60% of the initial maximal rebound height for an additional 5 minutes. Eventually at the completion of this step, the target rebound height will be increased to 70% of the initial maximal rebound height for an additional 3 minutes. From this point onwards, the progression will consist of stepwise 10% increments (e.g., 70, 80, 90%) of the target rebound height up to 90% of the initial maximum rebound height, with each step lasting 3 minutes (See Fig. 1). Before every step increment a maximal DJ, test will be performed. Rebound jumping continued until either 50% of maximal rebound height could not be attained or volitional fatigue. Verbal encouragement and feedback will be given to each participant by the researchers.

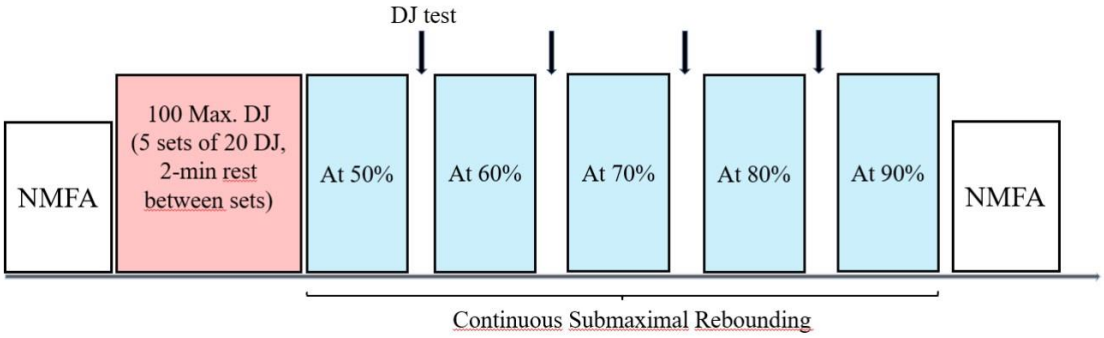


Figure 1. Schematic illustration of the SSC fatigue protocol.

4. **Possible benefits from the study**

The benefits of this research are more significant to science and to an individual than its possible risks. Subjects are able to increase their knowledge about their own force production capabilities and muscle fatigue characteristics. This research will bring new knowledge about the neural mechanisms of SSC type of muscle fatigue and especially about their recovery.

5. **Possible risks, harm, and inconvenience caused by the study as well as preparing for these**

Surface EMG provides a non-invasive, painless, and global measurement of muscle activity. Following the application of surface EMG electrodes, some participants may develop allergic reactions as well as a rash on the site of placement after the removal of the electrodes, but eventually, it should completely heal within a few days post-measurement. Maximal and submaximal SSC fatigue protocol will result in quite heavy and taxing on participants since they will get close to or at exhaustion, therefore the risk of muscle strain and injury is potentially in place. However, the sledge will help the participant maintain a more or less appropriate execution form. Moreover, each protocol will take place under the supervision of the research group providing constant feedback to the participant, and eventually interrupting the protocol when potentially about to create harm to the participant. Transcranial magnetic stimulation (TMS) is a non-invasive technique increasingly utilized to, but not limited to, investigate cortical excitability. As mentioned in section 3.3, participants also have to go through a TMS checklist in order to be cleared to participate in the current experiment. Whenever TMS could create harm to a participant, this one will not be admitted in the current experiment. Nevertheless, TMS stands as a safe stimulation technique, the occurrence of side effects post-cortical stimulation via TMS cannot be completely excluded. Aside discomfort during the stimulation, the most common side effects reported in the literature are headache and neck pain. The intensity of pain experienced varies from subject to subject, depending on individual susceptibility, coil design, stimulation location, intensity, and frequency. Reported head/neck pains are largely believed to occur due to muscle tension, generated either by the stimulation itself or the posture assumed during longer protocols. During discharge, the TMS coil produces a deceptively loud clicking noise (~120–140 dB). Although seemingly innocuous, repeated exposure to this intense sound can lead to acoustic trauma. However, participants and operators will be provided with earplugs in order to prevent these potential adverse effects. Other reported side effects, are episodes of syncope and fainting, occurring for several reasons in addition to stimulation such as anxiety, physical discomfort, and/or psychological discomfort. Participants will be closely monitored for any signs of syncope or related

symptoms (i.e., dizziness, light-headedness, faint feelings) (Najib et al., 2014). In the event of syncope, stimulation will be ceased immediately, and qualified assistance promptly offered. Some other side effects have been reported, although exceedingly rare, such as seizure, mood, and dental pain. In the current experiment a limited number of stimulations will be administered, therefore exponentially limiting the risk of developing the above-mentioned adverse effects. Peripheral nerve stimulation (PNS) is a non-invasive technique utilized to, but not limited to, investigate nerve conduction or pain management. As well as TMS, PNS stands out as a relatively safe stimulation technique, although, by stimulating the muscle in the periphery the risk of developing headache or fainting episodes is mainly bypassed. Primarily, PNS could induce discomfort and local feeling of numbness during or following stimulation. The same precautions and safety measures for TMS will be followed even during PNS.

6. **Study-related costs and compensations to the subject as well as research funding**

No rewards will be paid for participation in the study.

7. **Informing about research results and research outcomes**

The outcomes and results of the current experiment will be presented in the master's thesis and potentially used to publish a paper. After the work, anyone will be able to review the manuscript. Each participant's privacy will be ensured at any point in the final manuscript. Background information (i.e., age, weight, height, and body composition) and results will be presented as mean and standard deviation, therefore guaranteeing the anonymity of the participants. Participants' names will not appear at any point in the data collection sheets/files and/or final manuscript. Participants will be randomly assigned numbers and/or initials. For this reason, it will not be possible to deduce the identity of the participants for the results presented in the manuscript. All the ethical guidelines will be followed to ensure fairness, accountability, anonymity, and validity. Data will be exclusively stored on the university's hard disk, and not made public for any reason whatsoever.

8. **Insurance coverage for research subjects**

The University of Jyväskylä has insurances for its activities and research subjects.

The set of insurance includes a malpractice insurance, an operational liability insurance, and an optional insurance against accidents. During the research activities, the subjects are covered by the insurance for accidents, damages and injuries inflicted by an external cause. The accident insurance is valid during measurements and on trips integrally connected to them.

9. **Contact person for further information**

Tutkimuksen vastuullinen johtaja
Janne Avela
Professori
Jyväskylän yliopisto, Liikuntatieteellinen tiedekunta
Puh. +358 50 505 4654

Sähköposti: janne.avela@jyu.fi

Yhteyshenkilö
Jacopo Bascherini
Puh. (+39) 345 1563058
Sähköposti: jacopo.j.bascherini@student.jyu.fi

CONSENT TO PARTICIPATE IN SCIENTIFIC RESEARCH

Corticomotor and corticospinal excitability contributions to neuromuscular fatigue following exhaustive stretch-shortening cycle actions and associated recovery pattern

I understand that participation in the study is voluntary and that I can pause or stop taking part in the study at any time without giving a reason, and there will be no negative consequences for me. The research materials collected about me up to the point of pausing or stopping may still be used in the study.

By giving my consent I accept that data and materials will be collected from me as described in the information sheet.

By giving my consent to participate in this study:

I consent to photographs/videos being taken of me for research purposes, but they have been processed in such a way that I cannot be identified. Photographs will only be used in scientific presentations. Videos will be taken for motion capture and, thus for kinematic analyses.

Yes ☐ No ☐

I confirm that I will not participate in face-to-face data collection if I have flu symptoms, fever, am recovering from illness, or am feeling otherwise unwell.
Yes ☐

I confirm that I have received the information sheet about the the content of the study, how it will be conducted and what it means in my part, as well as the privacy notice. I have also had the opportunity to ask the researchers further questions. I have therefore been adequately informed about the content of the survey, how it will be conducted and what it means for me, as well as about the processing of my personal data which are processed on the basis of public interest as set out in the law.

I have had sufficient time to consider my participation in the study. I have not been pressured or tempted to take part in the research.

I have considered the above points and have decided that I want to participate in the study / those sections where I have ticked "yes".

Yes ☐ No ☐

Signature of the participant, name in block capitals, and date

Y-tunnus:
02458947
Sähköposti:
etunimi.sukunimi@jyu.fi

Puhelin:
(014) 260 1211
Faksi:
(014) 260 1021

Jyväskylän yliopisto
PL 35
40014 Jyväskylän yliopisto
www.jyu.fi

Contact details:

Janne Avela
Professori
Jyväskylän yliopisto, Liikuntatieteellinen tiedekunta
Puh. +358 50 505 4654
Sähköposti: janne.avela@jyu.fi

Yhteyshenkilö
Jacopo Bascherini
Puh. (+39) 345 1563058
Sähköposti: jacopo.j.bascherini@student.jyu.fi

Paper or recorded consent is stored in accordance with data security guidelines, as is other personal data.