9-10-2020 9:00 AM

The effects of medial gastrocnemius muscle fatigue on regional modulation of the ankle plantarflexors during standing external perturbations

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology

Abstract

The ankle plantarflexor muscles (soleus, medial, and lateral gastrocnemius) exhibit direction-specific regional modulation of muscle activity during external perturbations. This study sought to investigate the effect of medial gastrocnemius muscle fatigue on plantarflexor muscle activation patterns. Unipedal balance was tested during external perturbations that challenged standing balance in different directions before and after low-frequency fatigue was induced to the medial gastrocnemius via electrical stimulation. High-density surface electromyography was used to determine the amplitude and barycenter of muscle activation. It was hypothesized that the central nervous system would compensate for a loss of force-generating capacity in the medial gastrocnemius by modulating the activity of the soleus and lateral gastrocnemius to maintain balance. The soleus experienced an increase in muscle-activation amplitude and a proximal barycenter shift when the medial gastrocnemius was fatigued. However, the direction-specific regional modulation of plantarflexor muscle activity was not significantly affected by medial gastrocnemius fatigue.

Keywords

Medial Gastrocnemius, Lateral Gastrocnemius, Soleus, Regional Modulation, High-Density Surface Electromyography, Muscle Fatigue, Balance

Summary for Lay Audience

The triceps surae is a group of three ankle plantarflexor muscles (soleus, medial gastrocnemius, and lateral gastrocnemius) located in the calf. Together, these three muscles play a fundamental role in the maintenance of standing balance. Although it was previously believed that the three muscles function as a collective group, it has been discovered that the central nervous system (CNS) can modulate the activity in each muscle during balance tasks, a phenomenon known as regional modulation. It is understood that the CNS can modulate the activity of the triceps surae so that muscles located in mechanically advantageous regions are preferably activated during tasks that challenge standing balance. However, it remains unknown how muscle fatigue affects regional modulation of muscle activity in the triceps surae. To answer this question, participants performed a single-leg balance test before and after fatigue was induced in the medial gastrocnemius via electrical stimulation. During the balance test, participants were pulled in different directions and instructed to maintain their balance on one leg. The results showed that muscle activity was modulated depending on the direction of pull so that muscles located in mechanically advantageous regions were preferably activated. This result was observed before and after the medial gastrocnemius was fatigued, suggesting that muscle fatigue in one of the ankle plantarflexor muscles did not change the regional modulation of muscle activation. However, there was also a marked increase in the activation level of the soleus muscle during the balance testing when the medial gastrocnemius was in a state of fatigue. This suggests that the soleus muscle may compensate to help maintain balance when the medial gastrocnemius is compromised. In summary, not only has this study demonstrated a persisting ability to modulate muscle activity in the triceps surae despite fatigue, but it has also revealed the compensatory role of the soleus muscle in maintaining standing balance when the medial gastrocnemius is not functioning at full capacity.

Table of Contents

List of Tables

List of Figures

List of Appendices

Chapter 1

1 Introduction

1.1 Background and Rationale

Upright standing posture is maintained by controlling the center of mass (COM) within the base of support (BOS) (Pollock et al., 2000; Winter, 1995). The central nervous system (CNS) accomplishes this by activating motor units in different muscle groups. The triceps surae is a group of three ankle plantarflexor muscles, the soleus, medial and lateral gastrocnemius (SOL, MG, and LG, respectively), located in the posterior compartment of the shank, that is fundamental for the maintenance of upright standing posture and the production of locomotion (Winter, 1995).

The inverted pendulum model is a traditional biomechanical framework that is often used to illustrate postural control during quiet stance (Winter, 1995). In Winter's model, the human body is likened to an inverted pendulum that continuously sways back and forth during quiet standing, pivoting about the ankle joint (Winter, 1995). The premise of Winter's classic model is that muscles act as springs to move the center of pressure (COP) in phase with the COM to maintain balance as the body sways (Winter et al., 1998). This model emphasizes the role of ankle strategy, a fixed-support postural control strategy, in which the distally-located ankle plantarflexors act as the primary muscle group in controlling postural sway (Gage et al., 2004; Winter, 1995; Winter et al., 1998). One problem that arises from the inverted pendulum model is that it assumes the ankle plantarflexors are activated in unison as a collective synergist group and overlooks the unique properties of each individual muscle. For example, SOL is primarily composed of slow-twitch motor units, which are resistant to fatigue and ideal for tasks requiring low-level tonic activity such as its principle function of controlling antero-posterior sway during quiet standing (Duysens et al., 1991; Edgerton et al., 1975; Gollnick et al., 1974; Mochizuki et al., 2005). In contrast, the gastrocnemii have a higher distribution of fast-twitch motor units that are capable of producing larger propulsive forces at higher firing rates (Butler & Dominy, 2016; Edgerton et al., 1975). During quiet standing, the motor units in the gastrocnemius are recruited intermittently and are most frequently triggered

when the body sways fast and forwards (Vieira et al., 2012). Thus, the inverted pendulum model may be an oversimplified approach to upright postural control insofar as it fails to recognize the ability for the CNS to modulate the activity of the SOL, MG, and LG independently according to the task.

Further evidence opposing the perception that the triceps surae muscles are activated as a collective plantarflexor group comes from the finding that the CNS can preferentially recruit motor units in different regions within a single muscle. This phenomenon is known as regional modulation and has been observed in the triceps surae in response to external perturbations. Cohen et al. (2020) demonstrated that the activity of the ankle plantarflexors is modulated in response to external perturbations in a direction-specific manner. That is, when the perturbation direction moved from left to right, not only did the gastrocnemius muscle opposite to the direction of pull of the perturbation show the highest relative amplitude of activation, the barycenter of muscle activation within MG, LG, and SOL also shifted to oppose the direction of the perturbation. The results of that study suggested that the CNS preferentially activates motor units located in mechanically advantageous regions within the ankle plantarflexors in a task-specific manner (Cohen et al., 2020).

1.2 Objective and Hypothesis

It remains unknown how regional modulation is affected by muscle fatigue, as a loss of force-generating capacity in one muscle may alter how the CNS modulates muscle activation in non-fatigued muscles. Investigating the effect of fatigue on muscle activation patterns will contribute to our understanding of the ability of the CNS to modulate motor output to compensate for changing conditions in muscles (i.e., muscle fatigue or injury). Therefore, the purpose of this study is to investigate the effect of muscle fatigue on regional modulation of the activity of SOL, MG, and LG during external perturbations. This study will answer the question: Can the CNS adapt to a loss of force-generating capacity in one muscle by altering how it activates the other two plantarflexors? It is hypothesized that the CNS will compensate for a loss of force-generating capacity in the fatigued muscle (MG) by modulating the activation patterns in the two non-fatigued muscles (LG and SOL) to maintain balance following perturbations.

Thus, if the MG is at a mechanical advantage to resist perturbations to the right, it is anticipated that the barycenter will shift medially in the LG and SOL and for the amplitude of activation to increase in the LG and SOL, particularly for rightward perturbations, when the MG is in a state of fatigue.

Chapter 2

2 Literature Review

2.1 Balance, Stability, and Postural Control

Balance is often thought to be synonymous with stability and postural control. However, it is important to differentiate between these terms for the purpose of understanding the neurological basis of human movement and motor control. Balance is a term used to describe a state of equilibrium in which the resultant forces acting upon an object are equal to zero (Newton's First Law) (Pollock et al., 2000; Winter, 1995). In the case of humans, balance is a multidimensional concept that describes the dynamics of one's body posture (body orientation relative to the gravitational vector) to prevent falling (Pollock et al., 2000; Winter, 1995). The CNS controls human balance through sensory and motor systems that regulate the position of a person's COM relative to the BOS. Balance is maintained as long as the COM falls within the BOS. In contrast, a person is unbalanced and prone to falling as the COM approaches the limits of stability or is entirely displaced outside of the bounds of the BOS (Pollock et al., 2000).

Stability is defined as the ability of an object/person to maintain, achieve, or restore a specific state of balance (Pollock et al., 2000). Thus, an object/person is said to be stable if the COM falls within the BOS. Humans can increase their stability by ensuring their COM is in the center of their BOS, lowering their COM, widening their BOS, or adding another supporting point of contact (e.g., holding a railing or using a cane) (Pollock et al., 2000). Given that stability is enhanced with more points of contact and a larger BOS, it is clear why stability is decreased when humans stand in a single-leg stance as opposed to a natural double-leg stance.

Postural control (sometimes referred to as balance control) is the maintenance of balance during any posture or activity (Pollock et al., 2000). In the case of upright stance, humans have a relatively high COM (near the midsection) and narrow BOS (between their two feet during double-leg stance) (Winter, 1995). The maintenance of stability is further complicated during single-leg upright stance, in which there is only one point of contact with the support surface and an even smaller BOS (only within the bounds of the single

foot). When the CNS detects a displacement of the COM (as detected by one of the three major sensory systems involved in balance and posture, namely the visual, vestibular, and somatosensory systems), certain postural control strategies are implemented to restore stability (Winter, 1995).

2.2 Postural Control Strategies

Postural control strategies function to restore stability by returning the displaced COM to the center of the BOS. This is accomplished via changes in muscle activity and adjustments in the COP—the point location of the vertical ground reaction force vector (Winter, 1995). The COP represents the weighted average of all the pressures over the surface of the area in contact with the ground and is completely independent of the COM (Winter, 1995). From a biomechanics perspective, the COP is used as a kinematic measurement to quantify the postural sway and displacements of the COM.

There are two main categories of postural control strategies: reactive (compensatory) and predictive (anticipatory) (Maki & McIlroy, 1997; Pollock et al., 2000). A predictive postural control strategy involves increased muscle activity or voluntary movements in anticipation of an expected perturbation (Maki & McIlroy, 1997; Pollock et al., 2000). These anticipatory postural adjustments most frequently occur following voluntary movements (i.e., locomotion), which comprise the majority of disturbances to balance and posture (Massion, 1994). In contrast, reactive postural control strategies involve compensatory movements/muscle activations in response to a perturbation that may or may not have been expected (Maki & McIlroy, 1997; Pollock et al., 2000).

Reactive postural control strategies can be further divided into two distinct classes of strategy: fixed-support strategies and change-in-support strategies (Maki & McIlroy, 1997). Change-in-support strategies involve moving the upper or lower limbs to make new contact with support surfaces, thus altering the BOS (Maki & McIlroy, 1997). Examples of change-in-support strategies include taking a step to change or widen the BOS or grasping with a hand to increase the BOS by adding another point of contact. Fixed-support strategies differ from change-in-support strategies in that they act to control the displaced COM without altering the BOS (Maki & McIlroy, 1997). The two

primary fixed-support strategies are the ankle and hip strategies (Horak & Nashner, 1986).

In the ankle strategy, compensatory torques are created about the ankle joint, shifting the COM forward or backward to restore equilibrium (Horak & Nashner, 1986). The hip strategy involves shear horizontal forces against the support surface about the hip joint (Horak & Nashner, 1986). When standing on a normal support surface, fixed-support strategies begin in a distal to proximal sequence, with the ankle strategy being activated first (Horak & Nashner, 1986). When the support surface is small in relation to foot size, a proximal to distal sequence can be observed, beginning with the hip strategy (Horak & Nashner, 1986). Other studies investigating fixed-support strategies suggest that the ankle strategy is primarily used to control antero-posterior (AP) sway in the sagittal plane via the ankle plantarflexors, whereas the hip strategy controls medio-lateral (ML) sway in the frontal plane via the hip abductors/adductors (Winter et al., 1998). This concept was derived from Winter's classic inverted pendulum model discussed below.

2.3 Inverted Pendulum Model

Winter (1995) was the first to illustrate the relationship between the COP and COM in the inverted pendulum model, which explains how the CNS controls balance during quiet standing. The inverted pendulum model proposes that humans are constantly swaying back and forth in the sagittal plane during quiet standing (Winter, 1995). As the body sways, the COM is constantly being displaced in the anterior and posterior directions. When the COM approaches the limits of the BOS, the COP must corral the COM back within the boundaries of the BOS. Winter's model emphasizes the role of the ankle strategy by proposing that the COP is adjusted by increasing or decreasing plantarflexor muscle activity to move the COM in the appropriate direction (Winter et al., 1998). For example, when an individual sways forwards, the COM is displaced anteriorly, causing the posterior plantarflexor muscles to be activated in a reactive spring-like fashion to increase the COP (Winter, 1995; Winter et al., 1998). The muscle activity generated in the plantarflexors causes the COP to move anteriorly in front of the displaced COM in order to corral the COM back into a neutral position in the middle of the BOS, thus

restoring stability and maintaining standing balance. This theory is known as the stiffness control of balance (Winter et al., 1998).

One problem with the inverted pendulum model and this theory of postural control is that it assumes that the three plantarflexor muscles act in unison as a single plantarflexor muscle group, either by increasing or decreasing muscle activity together. This model fails to recognize the unique properties of the three primary plantarflexor muscles (MG, LG, and SOL) and their ability to be independently modulated and differentially activated according to the external task demands.

2.4 Ankle Plantarflexor Muscles

Comprising the bulk of the calf muscle, the triceps surae is a group of three ankle plantarflexor muscles (MG, LG, and SOL) located in the posterior compartment of the shank. These three muscles play a fundamental role in maintaining upright posture and human locomotion.

The gastrocnemius is a superficial muscle that supplies the primary propulsive forces for human locomotion, such as walking, running, and jumping (Butler & Dominy, 2016). It consists of two heads, the medial and lateral gastrocnemius, respectively. The MG and LG are separated by a broad layer of connective tissue known as an aponeurosis. The gastrocnemius is a dual joint muscle, as it crosses both the knee and ankle joints to assist in knee flexion and ankle plantarflexion. The MG originates on the posterior surface of the medial femoral condyle and the LG originates on the posterolateral surface of the lateral femoral condyle. Both the MG and LG insert onto the calcaneus via the achilles tendon. The MG is generally a larger muscle than the LG in terms of length, volume, and physiological cross-sectional area (Fukunaga et al., 1992). Both heads of the gastrocnemius muscle are innervated by the tibial nerve (S1 and S2) and receive their blood supply from the posterior tibial artery.

The soleus is the deepest and largest muscle of the triceps surae. It is a postural muscle that plays a critical role in opposing the force of gravity to maintain upright postural control and assisting in locomotion (Agur et al., 2003). The SOL is a single-joint muscle

that acts at the ankle joint to produce plantarflexion. It originates on the posterior surface of the fibular head and along the medial border of proximal tibial shaft and inserts onto the calcaneus via the achilles tendon. The SOL shares the same source of innervation as the gastrocnemii (tibial nerve S1 and S2) and receives its blood supply from the sural and posterior tibial arteries.

2.5 Motor Unit and Muscle Fiber Characteristics of the Plantarflexors

Although the muscles of the triceps surae are located very close in proximity and all perform the common action of plantarflexion, the distribution of muscle fibers and motor unit types amongst the triceps surae is quite varied (Tucker et al., 2005). A motor unit consists of a single motor neuron and all of the muscle fibers that it innervates. All of the muscle fibers are of the same type within a single motor unit. Motor units are classified into three different types: type S (slow, small, fatigue-resistant), type FR (fast, intermediate in size, fatigue-resistant), and type FF (fast, large, fatigable) (Burke, 1981). According to Henneman's size principle, motor units are recruited in order of increasing size and fatigability, meaning that the small slow motor units are recruited at lower thresholds than large fast motor units (Henneman et al., 1965). Slow motor units are composed of type 1 (slow oxidative; SO) muscle fibers which are specialized for sustained aerobic contractions with lower force outputs. In contrast, the fast motor units contain type 2 muscle fibers that are specialized for rapid contractions with greater force outputs. Type 2 muscle fibers can be further subdivided into one of two groups: type 2a (fast oxidative glycolytic; FOG), namely muscle fibers that can produce rapid contractions for longer periods before experiencing fatigue; and type 2b (fast glycolytic; FG), namely muscle fibers that can only produce strong rapid contractions for a very limited period of time before becoming fatigued (Monti et al., 2001; Peter et al., 1972).

The soleus is mainly composed of slow twitch motor units (approximately 70%) and is often referred to as the slow twitch synergist to its neighboring gastrocnemius muscle (Edgerton et al., 1975). Although these slow twitch motor units have a lower recruitment threshold and produce lower force outputs, they are highly resistant to fatigue (Burke, 1981; Edgerton et al., 1975; Garnett et al., 1979; Walmsley et al., 1978). In comparison,

the gastrocnemius has a more equal distribution of slow (type I) motor units and fast twitch (type II) motor units (approximately 50%) (Edgerton et al., 1975). These fast twitch motor units are larger in size, have a high discharge frequency and recruitment threshold, and produce greater force outputs, but are susceptible to fatigue (Burke, 1981; Edgerton et al., 1975; Garnett et al., 1979; Walmsley et al., 1978).

The varied distributions of motor units amongst the triceps surae allow each of the three muscles to be specialized for different functional purposes during quiet stance and locomotion. The slower twitch properties of the motor units in the soleus lend it to be more tonically active during quiet stance, thus providing static background plantarflexion torque to counteract the gravitational body load (Héroux et al., 2014; Mochizuki et al., 2006). In contrast, the gastrocnemius muscle produces strong propulsive forces and is activated more intermittently, generating dynamic plantarflexion torques to stabilize the body during movement (Héroux et al., 2014; Woollacott et al., 1984).

In terms of muscle architecture, all three muscles of the triceps surae have a pennate muscle fiber arrangement with slight variations in pennation angle between muscles and within specific regions of individual muscles. The muscle fibers in the MG and LG are heavily pennated throughout the muscle bellies but become more parallel in the distal regions near the insertion point onto the achilles tendon (Narici et al., 1996). Additionally, previous studies have revealed that the muscle fibers in the MG and SOL have larger pennation angles than those in the LG (Kawakami et al., 1998). Although the ability for the CNS to preferentially activate certain muscles can be observed in many muscle groups throughout the human body, the triceps surae serves as an excellent example for how anatomical differences in muscle architecture and motor unit properties between the MG, LG, and SOL may serve as a mechanical advantage for specific tasks (Héroux et al., 2014).

2.6 Independent Modulation of the Plantarflexors

Historically, it was believed that the triceps surae functioned as a single plantarflexor group. However, recent evidence has revealed that this may not be the case. Using surface and intramuscular electromyography (EMG), studies have found that surface potentials

have high spatial selectivity, suggesting that the activity of the human LG and MG is independently modulated during the control of upright stance via selective activation of motor units (Vieira et al., 2010b). During quiet standing, the MG and SOL have a much lower recruitment threshold and are preferentially activated over the LG (Héroux et al., 2014). The absence of LG activity during quiet stance suggests that the motoneurons of the triceps surae are modulated independently to optimize anatomical variations between each muscle. During dynamic tasks involving plantarflexion (i.e., calf-raise exercises), the MG is preferentially activated to a greater extent than the LG or SOL (Kinugasa et al., 2005). These differences in the activation patterns of individual muscles indicate that each plantarflexor muscle is independently modulated according to the task as their activations are not always synchronized or uniform (Segal & Song, 2005; Vieira et al., 2010b).

The above examples demonstrate the differential activation that exists between the three major plantarflexor muscles, in which some muscles have greater levels of activation than others during various tasks. This observation of differential activation refers to the notion that groups of muscles that would normally be predicted to act in unison due to their common location and function, may actually be differentially recruited and independently modulated depending on the task (Segal & Song, 2005). Furthermore, not only are there differences between muscles, but there are also regional differences in activation *within* a single muscle, in which certain regions of a specific muscle are activated more or less than others. This phenomenon is known as regional modulation and is of central importance to this research project.

2.7 Regional Modulation of the Plantarflexors

Regional modulation of muscle activity refers to the notion that the CNS can preferentially recruit motor units located in mechanically advantageous regions *within* a muscle in a task-dependent manner. This concept diverges from the classical size principle, in which excitatory inputs are distributed evenly, thus recruiting all motor units within a motor neuron pool in a predictable order according to size (small to large) (Henneman et al., 1965). Rather, research by McMillan and Hannam (1992) on regional modulation in the human masseter muscle suggested that the activation pattern of a motor

neuron pool can be modulated via uneven sensory inputs and alterations in synaptic efficacy, wherein individual motor units can be selectively recruited for specific functional tasks.

Research on the human triceps surae has revealed heterogeneity of plantarflexor muscle activation associated with force production in different directions with changing foot positions, suggesting that sub-volumes of the plantarflexor muscles are differentially activated for specific biomechanical purposes (Staudenmann et al., 2009). This notion is supported by previous work by Henry et al. (1998), showing that the activation of the MG and SOL occurs in a direction-specific manner during support surface translations, with maximal activation occurring in response to diagonal translations as opposed to antero-posterior or lateral translations. Regional modulation of plantarflexor muscle activity has also been observed in the MG and LG during quiet stance, in which discrete regions of each muscle were found to undergo sequential activation (Vieira et al., 2010b).

Other studies using magnetic resonance imaging (MRI) concur that there is a nonuniform pattern of muscle activation within the triceps surae, suggesting that sub-volumes of the MG, LG, and SOL undergo differential activation during exercise tasks (Kinugasa et al., 2005; Segal & Song, 2005). More recent research conducted by Cohen et al. (2020) provides additional evidence regarding regional modulation of the ankle plantarflexors during external perturbations. Results from that study suggested that motor units in the MG, LG, and SOL are recruited in a direction-specific manner, in which the highest relative amplitude of activation is located in the region of muscle furthest away from the direction of the perturbation (Cohen et al., 2020).

2.8 High-Density Surface Electromyography (HDS-EMG)

High-density surface electromyography (HDS-EMG) is an innovative approach to studying muscle activity over a large surface area. This technique involves the application of a grid of several densely-spaced monopolar electrodes which map the myoelectrical activation over a broad superficial area on the skin, thus allowing for the detection of regional differences in muscle activation patterns (Merletti et al., 2008; Oliveira et al., 2009). This technique is often preferred over traditional EMG methods (i.e., bipolar

surface EMG) because the large array of electrodes provides a more representative and accurate depiction of muscle activity as a whole by recording muscle activity from a wide range of motor units (Merletti et al., 2008).

In a 2009 study, researchers established that HDS-EMG can be used as a reliable tool to study the heterogeneity of muscle activation patterns during voluntary contractions of the triceps surae (Staudenmann et al., 2009). Additionally, Vieira et al. (2015) demonstrated the advantages of HDS-EMG for studying regional differences in plantarflexor muscle activity by using a grid of 128 electrodes to quantify changes in the amplitude of electrically evoked responses within different regions of the human MG. The results of that study indicated that distal regions of the MG were activated to a greater extent than others during increasing levels of electrical stimulation (Vieira et al., 2015). Intramuscular EMG or traditional bipolar surface EMG recordings would not have been able to detect the regional differences in muscle activity, as both of these methods can only record activity from a small area.

2.9 Muscle Fatigue

Muscle fatigue is defined as any exercise-induced reduction in the ability of a muscle to generate force or power (Gandevia, 2001). Fatigue has both central and peripheral components, which occur at the neural and muscle level, respectively. Central fatigue involves a decline in voluntary activation of a muscle during an exercise task. It is manifested as a decrease in maximal voluntary contraction force without a reduction in maximal evocable force (i.e., muscle force produced that is not augmented by additional electrical stimulation) (Vøllestad, 1997). Central fatigue is often measured using a technique called twitch interpolation, which involves the addition of electrical stimulation during the peak force produced by a maximal voluntary contraction. An increase in force produced by the additional electrical stimulation implies that the muscle is capable of generating more force than the person is able to produce voluntarily.

To contrast, peripheral fatigue occurs as a result of changes in processes at or distal to the neuromuscular junction. Factors contributing to the development of peripheral fatigue include reductions in excitation-contraction coupling, cross-bridge force, ATP hydrolysis,

blood flow, alterations in the availability and consumption of oxygen and energy sources, and metabolite accumulation (Carroll et al., 2017; Gandevia, 2001; Vøllestad, 1997).

Recovery time following exercise-induced declines in force production largely depend on the nature of the exercise task (Carroll et al., 2017). In the case of sustained maximal voluntary contractions, force can rapidly fall to below 50% within the first few minutes of exercise. It is presumed that this rapid decrease in force is, in part, due to lack of blood flow during sustained maximal contractions, as there is a prompt partial recovery of strength that occurs immediately upon the cessation of the contraction that does not occur when muscles remain under ischemic conditions. This observation leads to the assumption that muscular reperfusion is the primary mechanism of initial recovery in muscles following a fatiguing exercise task. Further recovery (unaffected by reperfusion) occurs at a much slower rate and can take upwards of five minutes before recovering to 80% (Carroll et al., 2017; Gandevia, 2001). The majority of the decline in sustained maximal voluntary force production can be attributed to peripheral causes of fatigue occurring distal to the neuromuscular junction, while central factors of fatigue (i.e., failure of voluntary activation) are estimated to account for the remaining 25% of total force reduction (Gandevia et al., 1996; Merton, 1954).

In comparison, during sustained submaximal voluntary contractions, target force can be maintained for longer periods of time before signs of fatigue begin to develop. A common indicator of central fatigue is an increase in perceived level of effort during sustained submaximal contractions with no further increase in force output. This coincides with the notion that central mechanisms of fatigue contribute substantially more to the loss of force during sustained submaximal versus maximal voluntary contractions (Smith et al., 2007). This is likely due to the nature of motor unit recruitment during sustained submaximal contractions, in which additional motor units are available for recruitment to maintain force as lower threshold motor units become fatigued (Adam & De Luca, 2005). This recruitment strategy would not be possible during maximal contractions, wherein all available motor units would be recruited at high rates from the onset of a sustained maximal voluntary contraction until fatigue sets in and firing rates progressively slow down or stop firing entirely (Peters & Fuglevand, 1999).

2.10 Measures of Muscle Fatigue

Muscle fatigue can be assessed using a variety of methods, each of which reveals different information about the underlying processes contributing to the generation of muscle force.

2.10.1 Maximal Voluntary Contractions

Maximal voluntary contractions (MVCs) are commonly used to measure fatigue in humans. All of the following central and peripheral processes contribute to the production of force during MVCs: supraspinal processes occurring in the CNS, excitation of motor neurons and muscle fibers, calcium release and binding, cross-bridge cycling, and energy usage and regeneration (Vøllestad, 1997). The main downfall to using MVCs to assess fatigue is that voluntary force output is highly influenced by central factors such as lack of motivation, even with constant encouragement and feedback (Gandevia et al., 1995). Twitch interpolation allows for the assessment of maximal evocable force via electrical stimulation during MVCs and is often considered the standard for assessing the maximum voluntary force-generating capacity of a muscle or group of muscles. This method also has its limitations in that it is difficult to assess force produced by individual muscles during voluntary contractions, as synergists and antagonist muscles can contribute to force output in an unpredictable manner (Vøllestad, 1997).

2.10.2 Tetanic Force

Electrical stimulation offers a reliable measure of force-generating capacity without the interference of supraspinal factors. Brief trains without overly high frequencies (i.e., less than 50 Hz) are recommended to assess tetanic force to avoid any neuromuscular transmission block that can arise with excessive stimulation (Jones, 1996; Vøllestad, 1997). Tetanic force is often considered to be a more reliable measure of force production in cases where a single twitch produces a force that is too small to accurately measure (Vøllestad, 1997).

2.10.3 Twitch Force

There are cases in which twitch force is a preferred estimate of fatigue over tetanic force. In cases where the twitch force is large enough or the equipment is sensitive enough to accurately measure small twitches, twitch force may be a superior estimate of fatigue. This is believed to be largely due to the observation that there is a faster decline in twitch force than tetanic force, the former having a disproportionately longer recovery time that can take hours or, in extreme cases, days to recover fully described as low-frequency fatigue (Edwards et al., 1977; Jones, 1996).

2.10.4 Electromyographic Recordings

EMG is a technique in which electrical activity is recorded from muscles using electrodes either attached to the skin above a superficial muscle (i.e., surface EMG) or inserted into a muscle (i.e., intramuscular EMG). The electrical activity recorded from muscles can be used to determine the amplitude and timing of muscle activation (voluntary and/or evoked).

Muscle compound action potentials (M-waves) are commonly evoked by applying an electrical stimulus to a nerve trunk and can be recorded as an EMG response from surface electrodes placed over the muscle of interest (Rodriguez-Falces & Place, 2018). The resulting M-wave represents the synchronous sum of action potential produced by the motor units (within the electrode's recording range) whose motor axons were depolarized by the electrical stimulus (Rodriguez-Falces & Place, 2018). M-waves can be used to detect peripheral fatigue as they do not directly involve the CNS. This can be done by investigating changes in neuromuscular propagation that occur during fatiguing contractions, in which fluctuations in M-wave amplitude can be attributed to changes in neuromuscular propagation of the action potential from the stimulated nerve to the muscle fibers (Enoka & Stuart, 1992; Rodriguez-Falces & Place, 2018). Thus, a decrease in M-wave amplitude is suggestive of peripheral failure.

2.11 Plantarflexor Muscle Fatigue and Postural Stability

It is widely accepted that muscle fatigue reduces postural stability. This observation holds true for the ankle plantarflexor muscles following fatigue protocols, wherein postural stability is diminished in both AP and ML directions, with the greatest increases in postural sway occurring during single-leg stance (Bisson et al., 2010; Grey et al., 2013). It has been hypothesized that the musculature surrounding the knee and hip joints may play a compensatory role in correcting posture when the plantarflexors are functioning at a reduced capacity (Grey et al., 2013). This notion is supported by evidence that participants may adopt a more flexed knee and hip posture in addition to using the ankle strategy as the primary postural control strategy during single-leg stance following ankle plantarflexor muscle fatigue (Boyas et al., 2013). Most studies investigating the effects of ankle plantarflexor fatigue on postural control utilize voluntary exercise as a means of inducing fatigue to the three plantarflexor muscles as a group. Unfortunately, this method does not allow for the study of the effects of localized muscle fatigue to individual muscles on postural control.

2.12 Electrically Induced Muscle Fatigue

Transcutaneous neuromuscular electrical stimulation is one method that can be used to induce targeted peripheral muscle fatigue, allowing researchers to selectively target and fatigue individual muscles without affecting nearby muscle groups. Conventional studies aimed at investigating the effects of muscle fatigue often instruct participants to perform voluntary contractions until there is a decline in force. The downfall to central and voluntarily induced muscle fatigue is that multiple synergist muscles will experience a loss in force-generating capacity simultaneously. However, electrical stimulation permits the study of peripheral fatigue isolated to one single muscle. Further benefits of using electrical stimulation to induce muscle fatigue include a greater impairment of voluntary force following electrical stimulation compared to conventional exercise protocols and longer recovery times (Galea, 2001; Paillard et al., 2010).

Although there are several modifiable parameters involved in electrical stimulation (e.g., pulse duration, current/voltage amplitude, duty cycle, etc.), stimulation frequency has

been shown to be the primary determinant of muscle fatigue (Bickel et al., 2012; Gorgey et al., 2009). Thus, electrically induced muscle fatigue protocols typically fall into one of two categories: high-frequency fatigue (HFF) and low-frequency fatigue (LFF). HFF is characterized by significant reductions in force when tested with high frequency stimulation and rapid recovery of force when the test stimulation frequency is reduced. The force loss associated with HFF is believed to be a result of a build-up of extracellular potassium (Jones, 1996). LFF is defined as a disproportionate loss of force that occurs when tested with low frequency stimulation (e.g., 20 Hz), which is much less pronounced or absent when tested at high frequencies (e.g., 80 Hz), with a very slow recovery (Edwards et al., 1977). While it is possible for LFF to develop following voluntary exercise or electrical stimulation with high or low frequencies, the way in which researchers discern HFF from LFF is by testing the force output produced by a high versus low frequency electrical stimulus (Edwards et al., 1977; Jones, 1996). The effects of LFF have been found to be most prominent when stimulated at frequencies ranging from 10–30 Hz, which are similar to naturally occurring firing rates of skeletal muscle (Edwards et al., 1977; Jones, 1996). LFF can produce dramatic force deficits often exceeding 50% with extremely slow recovery times that can take hours or, in extreme cases, days for a full recovery to occur (Edwards et al., 1977; Jones, 1996; Russ et al., 2002). This long-lasting depression of force is understood to be a consequence of a reduction in calcium release from the sarcoplasmic reticulum following low-frequency stimulation (Jones, 1996). In addition to using low-frequency trains (i.e., 20 Hz), it has also been suggested that intermittent electrical stimulation with constant currents and contractions of short durations causes muscle fatigue to develop at faster rates with the greatest force decrements (Bergstrom & Hultman, 1988; Binder-Macleod & Russ, 1999; Matsunaga et al., 1999; Russ et al., 2002).

Chapter 3

3 Methods

This study was conducted at Western University in the Wolf Orthopedics Biomechanics Laboratory (WOBL). All materials and methods used in this study were approved by the Western University Health Sciences Research Ethics Board. Each participant provided informed written consent prior to participation.

3.1 Study Design

This is a repeated measures study in which the same participants performed a unipedal balance test before and after a fatigue protocol. Participation in this study required one visit to the lab for a single testing session of approximately 120 minutes. All participants were compensated with $20.00 CAD for their time.

3.2 Participants

This study included nine healthy adult volunteers recruited from Western University between the ages of 20–28 years of age (three females and six males). To be included in this study, participants were required to meet the following criteria: 1) be able to stand independently on one leg without physical support; 2) have no acute injury to their dominant leg that may impair balance; and 3) have no health conditions that may negatively impact the ability to balance independently on one leg (e.g., dizziness, peripheral neuropathies or concussions sustained within the past six months, etc.).

3.3 Experimental Procedure

3.3.1 Initial Setup

The experiment began by performing an ultrasound scan of the participant's right leg to locate the borders of the MG, LG, and SOL (NextGen LOGIQ^{TM} e R7 Series Ultrasound System, GE Medical Systems, China). After the ultrasound scan, the participant was positioned supine in a Biodex dynamometer (Biodex Manual Systems Inc., Shirley, New York, USA) and the MVC of isometric plantarflexion was recorded (details described in section 3.3.3). The MVC was recorded prior to the balance-testing and fatigue protocol to

minimize the possibility of potentiation. The MG motor point was also identified at this time using a stimulating probe to aid with the placement of the recording electrodes (details described in section 3.3.3).

Prior to applying any electrodes, the leg was thoroughly cleaned with an abrasive paste, and shaved if necessary, to ensure optimal recordings with minimal noise. Hydrogel self-adhesive reusable electrodes were used for electrical stimulation. For MG stimulation, a small (1x2 cm) electrode (cathode) was placed over the MG motor point and a larger (1.5x3 cm) electrode (anode) was affixed on the superior aspect of the MG. For tibial nerve stimulations, two square (2.5x2.5 cm) electrodes were used with the cathode placed in the popliteal fossa and the anode positioned superior to the patella on the anterior thigh.

Three HDS-EMG grids were placed over the muscle bellies of the MG, LG, and SOL. Each grid was prepared using a conductive cream spread onto a sheet of disposable foam (KIT08MM1305 and KIT10MM0808, OTBioelettronica, Torino, Italy) that adhered the grid to the participant's leg. Two 64 channel semi-disposable adhesive matrices (8 columns x 8 rows) with an interelectrode distance of 10 mm (OTBioelettronica Model GR10MM0808) were placed over the MG and LG. The MG grid was applied first and was positioned approximately 1 cm proximal to the motor point. The LG grid was placed directly beside the MG grid at the same height. Both grids were positioned in line over the bulk of each muscle belly where the muscle fibers are consistently pennate, thus avoiding the distal region where muscle fibers are aligned on a more parallel plane, which may affect EMG recordings (Gallina et al., 2013). The aponeurosis separating the MG and LG was used as a landmark to guide the grid placement so that nine columns were over the MG and seven columns were over the LG to accommodate the larger surface of the MG. Therefore, the MG and LG had a total of 72 and 56 channels, respectively.

A third semi-disposable 64 channel adhesive matrix (13 columns x 5 rows) with one electrode missing (the most medial and distal electrode) and an interelectrode distance of 8 mm (OTBioelettronica Model GR08MM1305) was placed over the SOL. This grid was placed in line with the achilles tendon, approximately 1 cm inferior to the gastrocnemius insertion point.

Each grid was attached to its own connector box, which were all connected to the HDS-EMG amplifier (256-channel EMG-USB2 with OTBioLab software v.2.0.5) via ribbon cables and to their respective reference electrodes. Two disposable self-adhesive hydrogel electrodes (1x1.5 cm) (Covidien Kendall H59P) were used as reference: one, for the MG/LG, was adhered to the patella, and the other, on the right medial malleolus, for the SOL. A common ground strip electrode was secured around the participant's right ankle. All EMG signals were recorded in monopolar modality. The force signal used for load-drop detection was also recorded with the HDS-EMG amplifier.

Four surface bipolar EMG electrodes were placed on the MG, LG, SOL, and tibialis anterior (TA) (Trigno Avanti wireless sensors, Delsys Inc., Natick, Massachusetts, USA). The sensor on the TA was positioned over the muscle belly, whereas the sensors for the MG, LG, and SOL were placed distal to the grids. The bipolar surface electrodes were used to record M-waves from the MG, LG, and SOL during the fatigue protocol and muscle activation from the TA during the balance testing. The digital signals from wireless EMG sensors were recorded with Spike2 v.9.03 software via Delsys Talker (Cambridge Electronic Design Ltd., Milton, Cambridge, UK) at 2000 Hz.

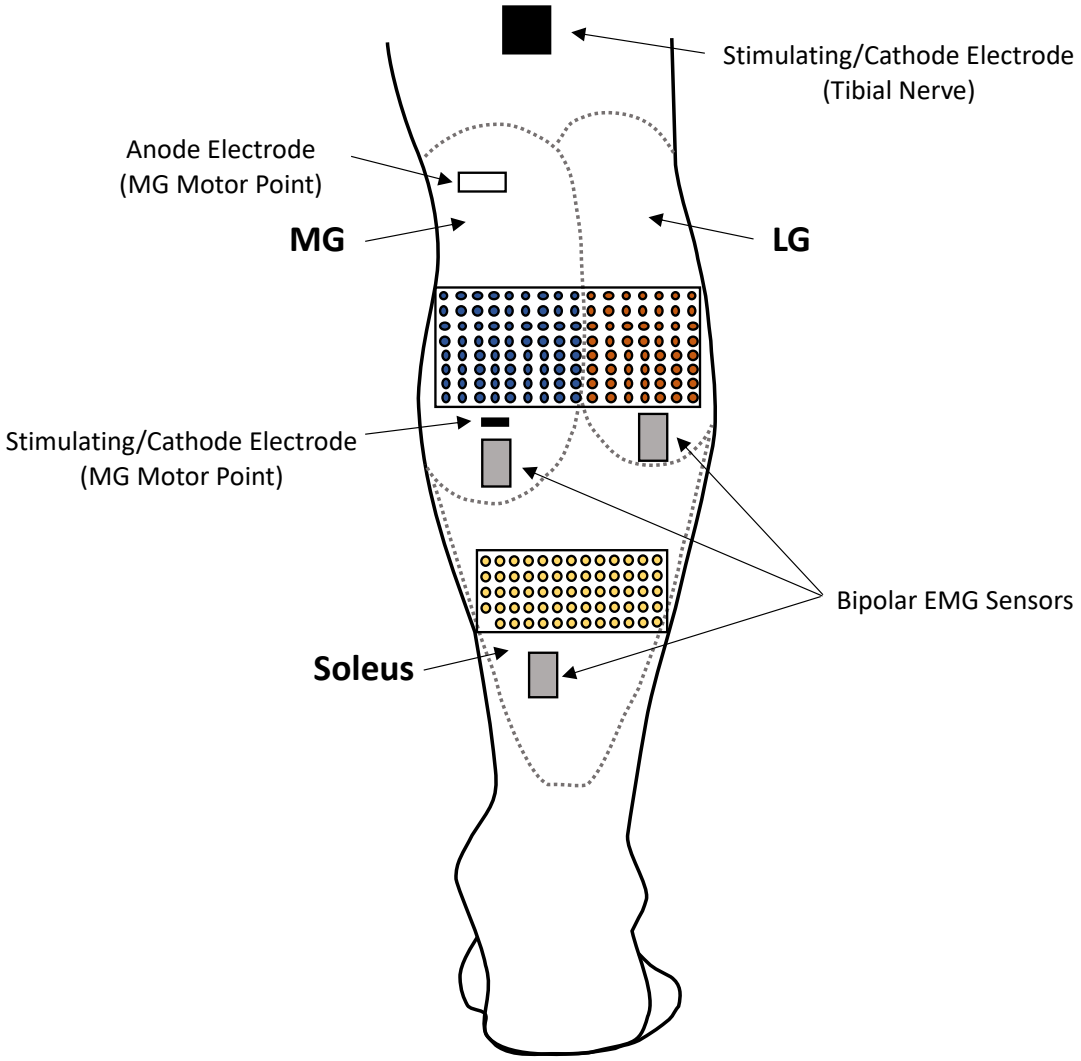

Figure 1. Schematic of HDS-EMG grids and electrode placement. HDS-EMG grids depicted as large rectangles with each circle representing a single recording channel (MG (blue circles): 72 channels, LG (red circles): 56 channels, SOL (yellow circles): 64 channels). Bipolar EMG sensors (grey) positioned distal to each grid. MG motor point cathode/stimulating electrode (black rectangle) positioned over point of peak torque amplitude (located outside of grid), anode electrode (white rectangle) on proximal aspect of MG. Tibial nerve cathode/stimulating electrode (black square) positioned in popliteal fossa (with anode electrode on anterior aspect of distal quadriceps, not pictured).

3.3.2 Balance-Testing Protocol

Participants stood barefoot in a unipedal stance on their right leg on a force platform (AccuGait, AMTI, Watertown, MA, USA) from which COP data were collected with a sampling rate of 1000 Hz. The right leg was used for all participants as it was the preferred leg, and research suggests that there is no difference in unilateral postural stability between the dominant and non-dominant leg in healthy young adults (Hoffman et al., 1998). Participants experienced an external perturbation in one of three directions (60° to the left, 60° to the right, and 0° to the front). Diagonal perturbation directions of 60° to the left and right were selected based on research suggesting that the plantarflexor muscles are maximally active in response to diagonal surface translations (Henry et al., 1998).

Perturbations were initiated when an external load weighing 1% of the participant's body mass was dropped from 40 cm into a basket below via a cable-pulley mechanism that attached to a belt secured around the participant's pelvis. The height of the cable-pulley mechanism was adjusted to match the height of the participant's hips. A force transducer (MLP-150, Transducer Techniques, Temecula, CA, USA) in line with the cable-pulley mechanism detected when the load was dropped into the basket. The force signal for load drop detection and the eight analog signals from AccuGait force platform were collected through Power1401 with Spike2 software.

Each participant completed five load-drop trials in each of the three perturbation directions, which were randomized across participants. Participants were instructed to maintain their balance on one leg (the right leg) for ten seconds after the load was dropped into the basket. If the participant lost their balance during the ten seconds (i.e., took a step, put their left foot down, or grabbed onto something), the trial was excluded and repeated. If the participant was able to successfully maintain their balance for ten seconds following the load drop, the trial ended, and the participant was allowed to briefly put their left foot on the ground before reassuming their unipedal stance for the next trial.

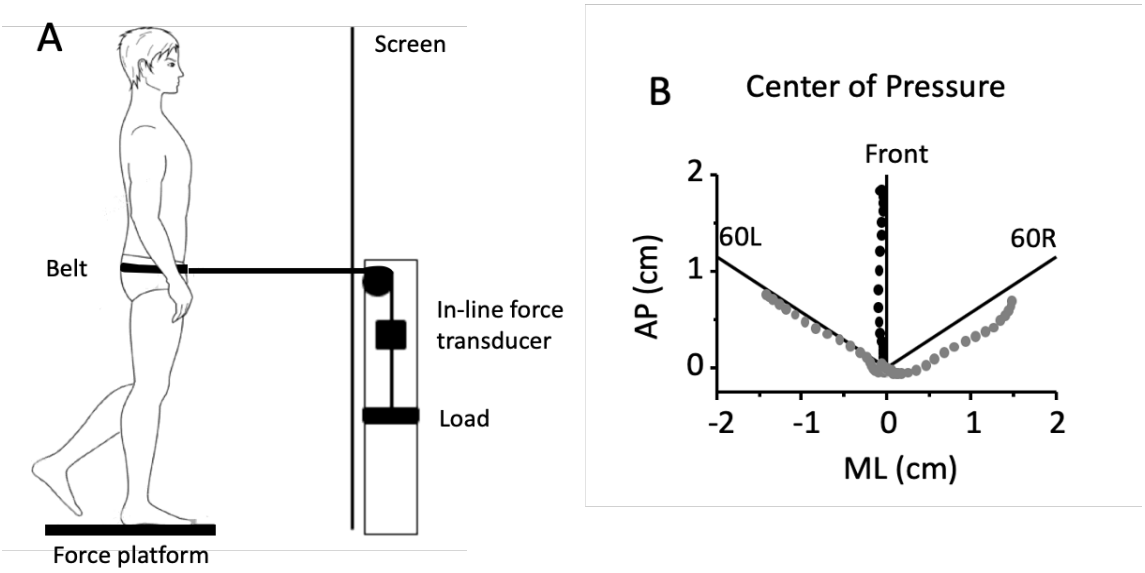

Figure 2. A) Experimental setup for balance-testing protocol with the participant in position for anterior pull (front direction). B) Three perturbation directions in degrees relative to anterior (0°). 60L (participants are facing right and pulled from 60° to their left), Front (participants are facing directly forwards and pulled from 0° in anterior direction), and 60R (participants are facing left and pulled from 60° to their right).

3.3.3 Fatigue Protocol Setup

The fatigue protocol was conducted with participants positioned supine with their right knee fully extended and right ankle fixed at 10° dorsiflexion in a Biodex System 3 dynamometer using an ankle attachment. The Biodex torque signal was collected through Power1401 with Spike2 software. The ankle was slightly dorsiflexed in an effort to lengthen the MG as fatigue has been shown to be greater at longer muscle lengths following a low-frequency electrical stimulation fatigue protocol (Gohary et al., 2016; Lee et al., 2007). Noncompliant straps were used to secure the participant's torso to minimize extraneous upper body movements. The right foot was securely fixed in the ankle attachment with a ratchet ankle binding and Velcro straps.

To record the MVC torque, participants remained supine in the Biodex and were instructed to cross their arms over their chest while plantarflexing their ankle against the footplate to maximal torque production and to hold this contraction for three to five seconds. Participants were provided with visual feedback of their torque production and were verbally encouraged during each MVC. Each participant performed a minimum of two MVCs separated by one minute of rest. If the peak torque differed by more than 10%, another trial was recorded. The MVC was measured from baseline to peak from the trial producing the largest plantarflexion torque. The MVC was only used to set the stimulation intensity for the fatiguing trains (≥20% of MVC torque) and was not repeated post-fatigue. Visual feedback was removed for the remainder of the fatigue protocol.

Three constant current single channel stimulators were used for different stimulations throughout the fatigue protocol described in detail below. The three stimulators allowed for quick transitions between fatiguing trains and test pulses. All stimulators were triggered through Power 1401 with Spike2. The first stimulator (DS7AH; Digitimer, Welwyn Garden City, UK) was used to deliver a single pulse with a supramaximal current intensity to the tibial nerve. Stimulation of the tibial nerve elicited M-waves and a twitch torque from all three plantarflexor muscles. M-waves were recorded to monitor the progression of fatigue and to ensure that the fatigue was isolated to the MG. Another stimulator (Digitimer Model DS7AH) was used to deliver a high-frequency 80 Hz doublet pulse to the MG motor point. The doublet stimulation of the MG motor point elicited

brief high-frequency fused torques from the MG. The MG motor point was located at the start of the experiment using a stimulating probe that delivered the same 80 Hz doublet pulse with a constant current intensity. To identify the motor point, the stimulating probe was moved within the boundaries of the MG until the point of largest torque amplitude, as measured by the dynamometer, was found. The third stimulator (Digitimer Model DS7A) was used to deliver low-frequency fatiguing trains and a test train (20 Hz) to the MG motor point. The current intensity of this stimulator was adjusted to produce a minimum of 20% of the participant's maximal voluntary contraction (MVC); some participants tolerated 30%. The torque produced from the 80 Hz doublet was compared to the torque produced from the 20 Hz test train and the difference in torque amplitude was used to establish LFF, defined as a disproportionate loss of force found to occur when stimulated at low frequencies (20 Hz), that is absent or much less pronounced when stimulated at high frequencies (80 Hz) with a slow recovery of hours or even days (Edwards et al., 1977).

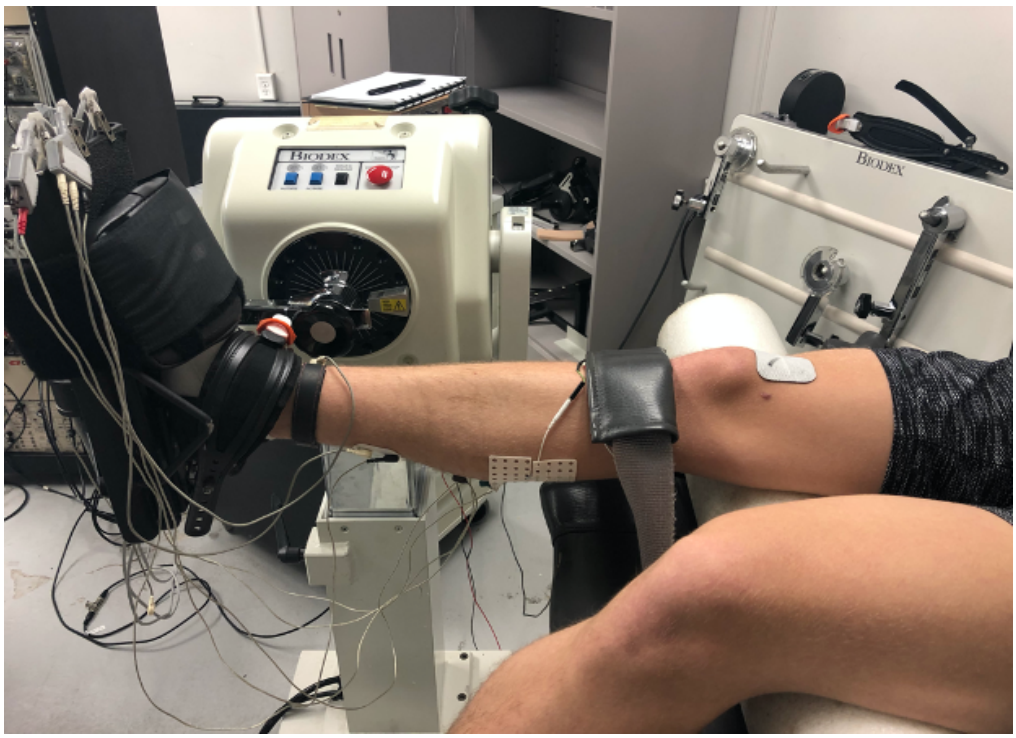

Figure 3. Experimental setup for fatigue protocol. Participant's right foot secured against footplate with right ankle slightly dorsiflexed, knee and hip fully extended.

3.3.4 Fatigue Protocol

Three different testing stimuli were used to monitor the progression of fatigue. These testing stimuli were recorded pre-fatigue and after each six-minute long train. The first testing stimulus was a test train delivered to the MG motor point (10 pulses at 20 Hz, 200 μs duration, current intensity ≥20% of MVC torque). The torque produced by the 20 Hz test train served as the fatigue indicator and the fatiguing protocol ended when the torque fell to below 50% of the pre-fatigue value. The second testing stimulus was a supramaximal single pulse delivered to the tibial nerve (100 μs duration, current intensity set to 20% above that required to evoke the MG maximal M-wave) which produced maximal M-waves and a collective twitch torque from the MG, LG, and SOL. The maximal intensity was obtained by incrementally increasing the current until there was no further increase in the peak-to-peak amplitude of the MG M-wave. The third testing stimulus was a doublet pulse delivered to the MG motor point (2 pulses at 80 Hz, 100 μs duration, current intensity set to 20% above that required to evoke the maximal MG torque) which produced a brief fused tetanic torque from the MG. The maximal doublet intensity was found by incrementally increasing the current until there was no further increase in torque amplitude.

Once the three pre-fatigue testing stimuli were recorded, the fatiguing trains began. The fatiguing stimulation was given to the MG motor point and consisted of ten-pulse trains (20 Hz, 200 μs, current intensity set to produce ≥20% of MVC torque) delivered once every second for six minutes to produce LFF (Binder-Macleod & Russ, 1999). At the end of each six-minute stimulation cycle, the three testing stimuli were repeated to monitor the progression of fatigue. When the torque from the 20 Hz test train dropped to below 50% of the pre-fatigue value, an LFF testing period began to determine whether LFF was induced in the MG. The required range of the six-minute cycles was between three to six, with an average of five cycles required.

Maintenance of LFF was determined by delivering the three testing stimuli every three minutes for at least for six minutes while the participant rested. Every three minutes, the torque was measured from the 20 Hz test train and compared to the torque from the 80 Hz

doublet as an indicator of LFF. M-waves were also monitored for any signs of recovery. If there were signs of recovery after the first three minutes of rest (e.g., recovery of torque or M-wave amplitude), additional six-minute fatigue cycles were required before conducting a new LFF testing period. If, at the end of six minutes of rest, the torque from the 20 Hz test train remained disproportionately depressed relative to the torque from the 80 Hz doublet (torque from 20 Hz test train was below 70% of the pre-fatigue value), and there was no significant recovery of the M-waves, one or two final fatigue cycles were conducted prior to beginning the post-fatigue balance testing. A pictorial representation of the fatigue protocol is outlined in Figure 4.

The LFF testing period was used to ensure that the MG would remain in a state of fatigue for the duration of the six to ten-minute balance-testing protocol. During pilot testing, longer rest periods of up to twelve minutes revealed that the recovery of the torque from the 20 Hz test train was no different between six and twelve minutes of rest. Thus, a shorter six-minute LFF testing period was preferred. The loss of torque produced by LFF has been documented to last hours or days, in extreme cases. Accordingly, if the torque remained depressed after six-minutes of rest, it is reasonable to assume that the torque would continue to be depressed for the duration of the balance testing when the participant was in a unipedal stance, requiring activation of the fatigued MG.

This protocol was developed to allow for a more direct and reliable comparison of pre-fatigue and post-fatigue balance testing under the same conditions by incorporating the entire experiment into a single testing session. Had this experiment been conducted on two separate occasions, the signals from the grids would be less comparable due to slight variations in recording position between the two testing sessions. Further, the LFF testing period was implemented in part to replace the need for a post-fatigue MVC as an index of force recovery. The MVC was not recorded at the end of the fatigue protocol to avoid potentiation of muscle activity during the balance testing and to reduce the amount of time from the end of the fatigue protocol to the start of the balance testing. Conducting an MVC upon completion of the post-fatigue balance testing was also deemed unreliable as it would be too difficult to return the participant to the Biodex and ensure the exact same body position, ankle joint angle, strap tightness, etc., as for the pre-fatigue measures.

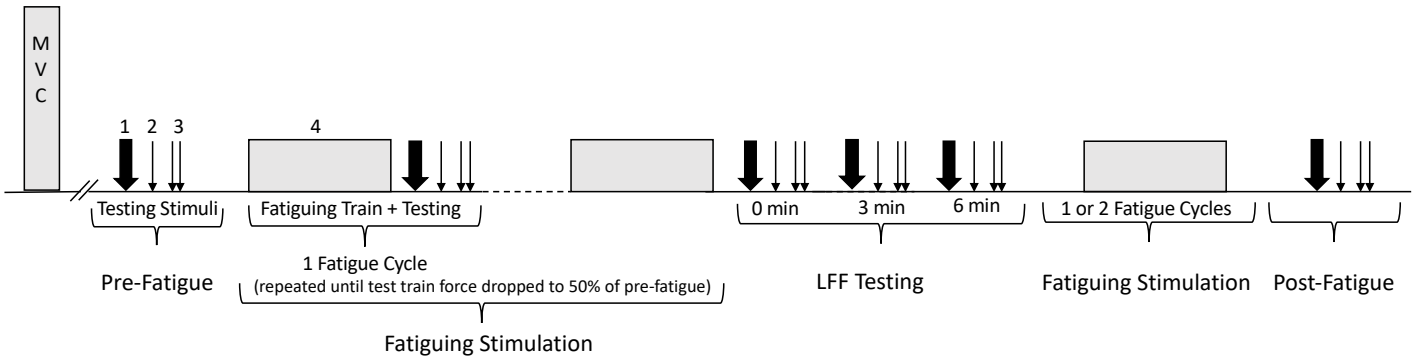

Figure 4. Illustration of fatigue protocol. The maximal voluntary contraction (MVC) of isometric plantarflexion was used to set the stimulation intensity of the fatiguing trains to ≥20% of the MVC torque. The three testing stimuli were conducted after each 6-minute low-frequency fatiguing train (4) and consisted of: 1) 20 Hz test train delivered to MG motor point, 2) a supramaximal single pulse delivered to the tibial nerve, and 3) a supramaximal 80 Hz doublet delivered to MG motor point. A low-frequency fatigue (LFF) testing period was conducted to ensure that the MG was in a state of LFF that would persist throughout the post-fatigue balance testing.

3.4 Data Analysis

3.4.1 HDS-EMG Analysis

MATLAB R2016b (The MathWorks, Inc., Natick, MA, USA) was used to analyze all HDS-EMG signals. All HDS-EMG signals were bandpass filtered (Butterworth filter, 4th order, 20–400 Hz cut-off) and analyzed in single differential configuration, resulting in 63, 49, and 51 EMG signals for MG, LG, and SOL, respectively. For some participants, due to anatomical differences in the muscles, the most lateral (LG and SOL) or most medial (MG) column was located outside of the muscle borders (identified via ultrasound). These channels were excluded from the analysis. All recordings were carefully inspected and any noisy signals (due to poor contact with the skin) were either interpolated from adjacent channels or excluded from the analysis (a maximum of 10% of channels per grid).

Baseline EMG activity was calculated from 500 ms prior to the onset of the perturbation. EMG activity in response to perturbations was calculated from 70–280 ms after each perturbation, when peak EMG activity occurred (Gallina et al., 2016). The timing of the onset of each perturbation (i.e., when the load was dropped) was determined from the filtered force transducer signal of the load hitting the basket (low-pass, Butterworth second order; 8 Hz cut-off) when it crossed a threshold calculated as mean +2 SD (Cohen et al., 2020). To improve the signal-to-noise ratio for detecting the response to perturbations, the full-wave rectified EMG signals collected from each channel were averaged across the five perturbations for each perturbation direction (Gallina et al., 2016).

The amplitude of EMG signals in response to perturbations was calculated for the MG, LG, and SOL for each perturbation direction as the average rectified value (ARV) of each channel during the 70–280 ms epoch following the load drop. The overall activation of the MG, LG, and SOL was calculated from the mean ARV of all channels within each grid during the five perturbations per direction for each muscle before and after the fatigue protocol.

The barycenter of muscle activation was calculated from each grid to provide an estimate for the identification of regional activation within each muscle. The barycenter is the centroid of EMG activity or point of peak muscle activation calculated from channels with amplitudes above 70% of the ARV from the channel with the highest activity within that muscle (Vieira et al., 2010a). The location of the barycenter has an X (medio-lateral axis) and Y (proximo-distal axis) coordinate representing the respective column and row within the grid where the EMG activity is highest, as calculated from the cluster of channels exceeding 70% of the peak activity.

3.4.2 Bipolar Surface EMG Analysis

The bipolar surface EMG recordings were analyzed using Spike2 software. M-wave amplitude (mV) was measured from peak-peak for each muscle. M-wave area (mV·ms) was measured using the modulus function in Spike2, to determine the area from the start to the end of the M-wave.

The TA EMG signals were recorded during balance testing to investigate any effects of MG fatigue on antagonist muscle activity. The signals were bandpass filtered (Butterworth filter, 4th order, 20–400 Hz cut-off), rectified and averaged for the five perturbations in each direction. The root mean square (RMS) of the TA EMG amplitude was calculated from 70–280 ms after the load drop (the same time frame used to analyze the HDS-EMG signals) (Figure 5, row 4). The EMG amplitude was not measured from the bipolar sensors of the plantarflexors as the HDS-EMG signals provided a more accurate measure of muscle activity due to the preferable positioning of the grids over the muscle belly.

3.4.3 Torque Analysis

All torque signal analyses were performed via Spike2. The torque amplitudes from the 80 Hz doublet, 20 Hz test train, and single pulse were expressed as a percentage of the torque during MVC. The half-relaxation times of all torque signals were measured from the midpoint between the baseline and the peak torque.

3.4.4 COP Analysis

COP data were analyzed using Spike2. The eight signals recorded from the AccuGait force platform were used to calculate the COP excursions in the X (antero-posterior) and Y (medio-lateral) directions. In the AP direction, positive values represent anterior COP excursions and negative values represent posterior excursions. In the ML direction, a positive value indicates a COP excursion to the participant's right, whereas negative values represent a deviation to the participant's left. The peak-to-peak amplitude of COP excursions was measured from the first deflection occurring immediately after the load drop to the next deflection occurring no later than 400 ms after the load drop.

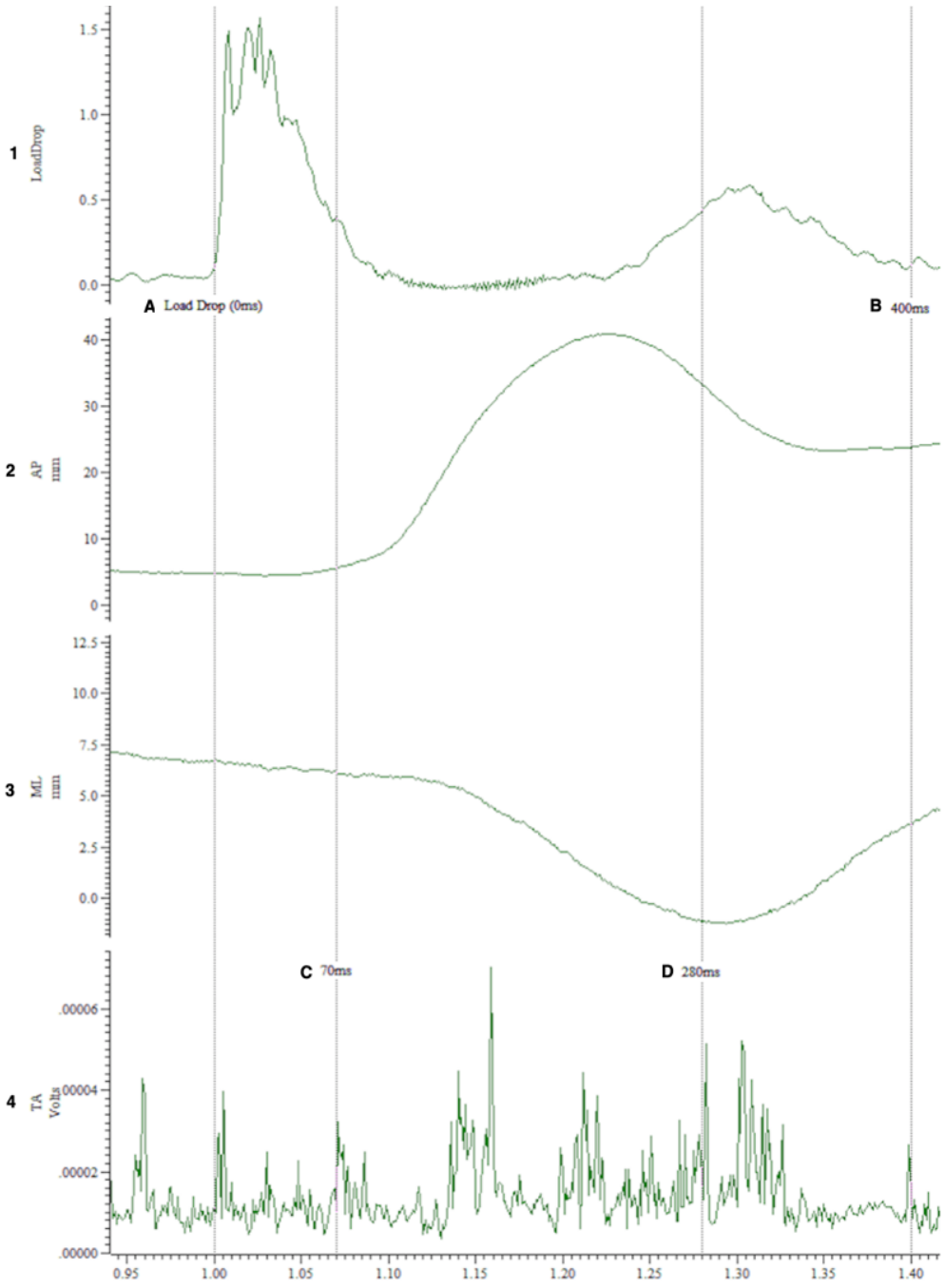

Figure 5. Screenshot showing the force signal from the load drop (row 1), the AP COP signal (row 2), the ML COP signal (row 3) and the EMG signal recorded from the TA (row 4). The amplitude of the first COP deflection was measured from baseline to the maximum peak (AP) or minimum peak (ML) occurring from 0 ms (cursor A) to 400 ms (cursor B) after the load drop. The RMS amplitude of the TA EMG signal was measured from 70 ms (cursor C) to 280 ms (cursor D) after the load drop.

3.5 Statistical Analysis

All statistical analyses were performed using SPSS v.24 (IBM Corp, Armonk, NY) and Microsoft Excel v.16.36. To examine the difference in M-wave amplitude and area, as well as torque amplitude and half-relaxation time from pre-fatigue to post-fatigue, separate paired-samples T-tests were conducted. COP data were analyzed with separate two-way repeated measures analyses of variance (ANOVA) comparing the effect of perturbation direction (60L, front, 60R) and fatigue (pre and post) for the AP and ML directions.

To determine the regional differences in muscle activation of each MG, LG, and SOL muscle from the HDS-EMG grids, a separate two-way repeated measures ANOVA was conducted for EMG amplitude, X and Y coordinates of the barycenter to evaluate the effect of fatigue (pre- vs. post-fatigue), and direction of perturbation (60L, front, 60R). A two-way repeated measures ANOVA was also conducted to investigate the effect of fatigue and perturbation direction on the EMG amplitude of the TA.

The alpha level for the statistical tests was set to $p = .05$. If the assumption of sphericity was not met (Mauchly's test of sphericity $p < .05$), Greenhouse-Geisser corrections were used. If a statistically significant result was found, post-hoc pairwise comparisons were conducted with a Bonferroni correction. All data are reported as mean ± SD, unless otherwise noted.

Chapter 4

4 Results

4.1 Fatigue

The following results support that the fatigue protocol was successful in inducing selective LFF in the MG without inadvertently fatiguing the other heads of the triceps surae in the process.

4.1.1 M-Wave

There was a significant decrease in the MG M-wave amplitude ($t(8) = 4.97$, $p = .001$), from pre-fatigue to post-fatigue. The MG M-wave area also decreased significantly ($t(8) = 3.30$, $p = .01$) from pre-fatigue to post-fatigue. The M-wave amplitude did not change significantly in the LG ($t(8) = 0.84$, $p = .424$), nor in the SOL ($t(8) = -0.51$, $p = 0.623$), from pre-fatigue to post-fatigue. There was also no significant change in M-wave area in the LG ($t(8) = -0.04$, $p = 0.965$), nor in the SOL $t(8) = -0.93$, $p = 0.378$.

Table 1. Means and standard deviations of M-wave amplitude and area of MG, LG, and SOL.

	M-Wave Amplitude (mV)		M-Wave Area (mV·ms)	
	Pre-Fatigue	Post-Fatigue	Pre-Fatigue	Post-Fatigue
MG	2.73 ± 1.32	2.30 ± 1.30*	12.05 ± 6.62	10.72 ± 6.73*
LG	2.71 ± 0.87	2.60 ± 0.96	14.87 ± 4.57	14.91 ± 4.79
SOL	1.27 ± 0.66	1.32 ± 0.65	4.55 ± 1.70	4.95 ± 1.52

(* denotes significant difference from pre-fatigue to post-fatigue)

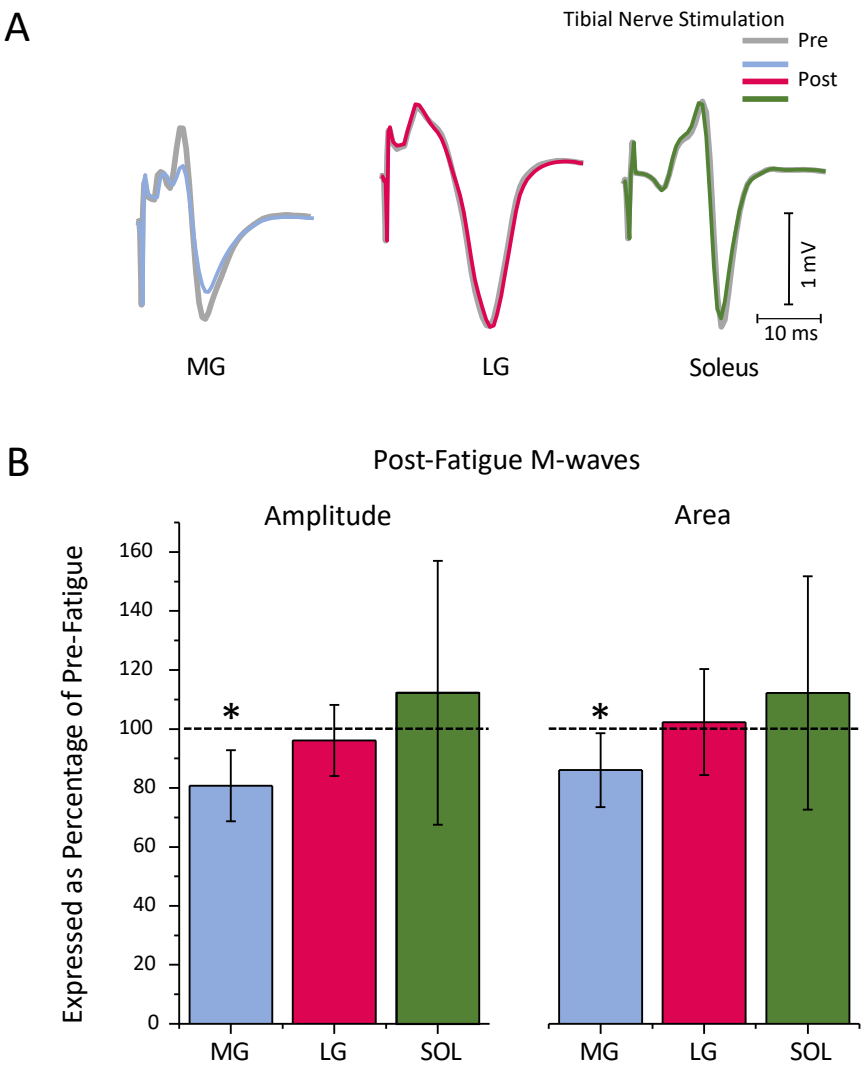

Figure 6. A) Traces of MG (blue), LG (red), and SOL (green) M-waves compared to pre-fatigue (grey) from one participant. B) Mean amplitude and area of post-fatigue M-waves of MG, LG, and SOL expressed as a percentage of pre-fatigue values.

4.1.2 Torque

The MVC torques of the nine participants ranged from 68.31 Nm to 141.05 Nm with an average of 105.57 ± 27.65 Nm. The torque amplitude from the 20 Hz test train delivered to the MG motor point decreased significantly ($t(8) = 7.28$, $p < .001$) from pre-fatigue to post-fatigue. The half-relaxation-time of the torque from the test train increased significantly ($t(8) = -3.08$, $p = .015$) from pre-fatigue to post-fatigue. The torque produced by the 80 Hz doublet delivered to the MG motor point decreased significantly in amplitude ($t(8) = 6.6$, $p < .001$) from pre-fatigue to post-fatigue but did not change significantly in half-relaxation time ($t(8) = -0.13$, $p = .894$). The same outcome was observed for the twitch torque produced by the single pulse delivered to the tibial nerve, which also decreased significantly in amplitude ($t(8) = 7.49$, $p < .001$) from pre-fatigue to post-fatigue but did not change significantly in half-relaxation time ($t(8) = 0.87$, $p = 0.410$). At the end of the six-minute LFF testing period, the torque amplitude produced by the 20 Hz test train remained depressed at 55% (± 10) of pre-fatigue while the mean torque produced by the 80 Hz doublet was only depressed to 88% (± 7) of its pre-fatigue value.

Table 2. Means and standard deviations of torque amplitude and half-relaxation time from ten pulses at 20 Hz, 2 pulses at 80 Hz (both delivered to MG motor point), and from single pulse delivered to tibial nerve.

	Torque Amplitude (% MVC)		Torque Half-Relaxation Time (ms)	
	Pre-Fatigue	Post-Fatigue	Pre-Fatigue	Post-Fatigue
Ten pulses at 20 Hz (MG motor point)	30.35 ± 5.81	11.18 ± 3.72*	145.8 ± 25.8	175.94 ± 52.31*
2 pulses at 80 Hz (MG motor point)	25.20 ± 5.74	21.18 ± 4.82*	122.09 ± 30.83	123.14 ± 17.57
Single pulse (tibial nerve)	14.35 ± 2.40	12.08 ± 1.76*	123.24 ± 18.62	119.01 ± 15.66

(* denotes significant difference from pre-fatigue to post-fatigue)

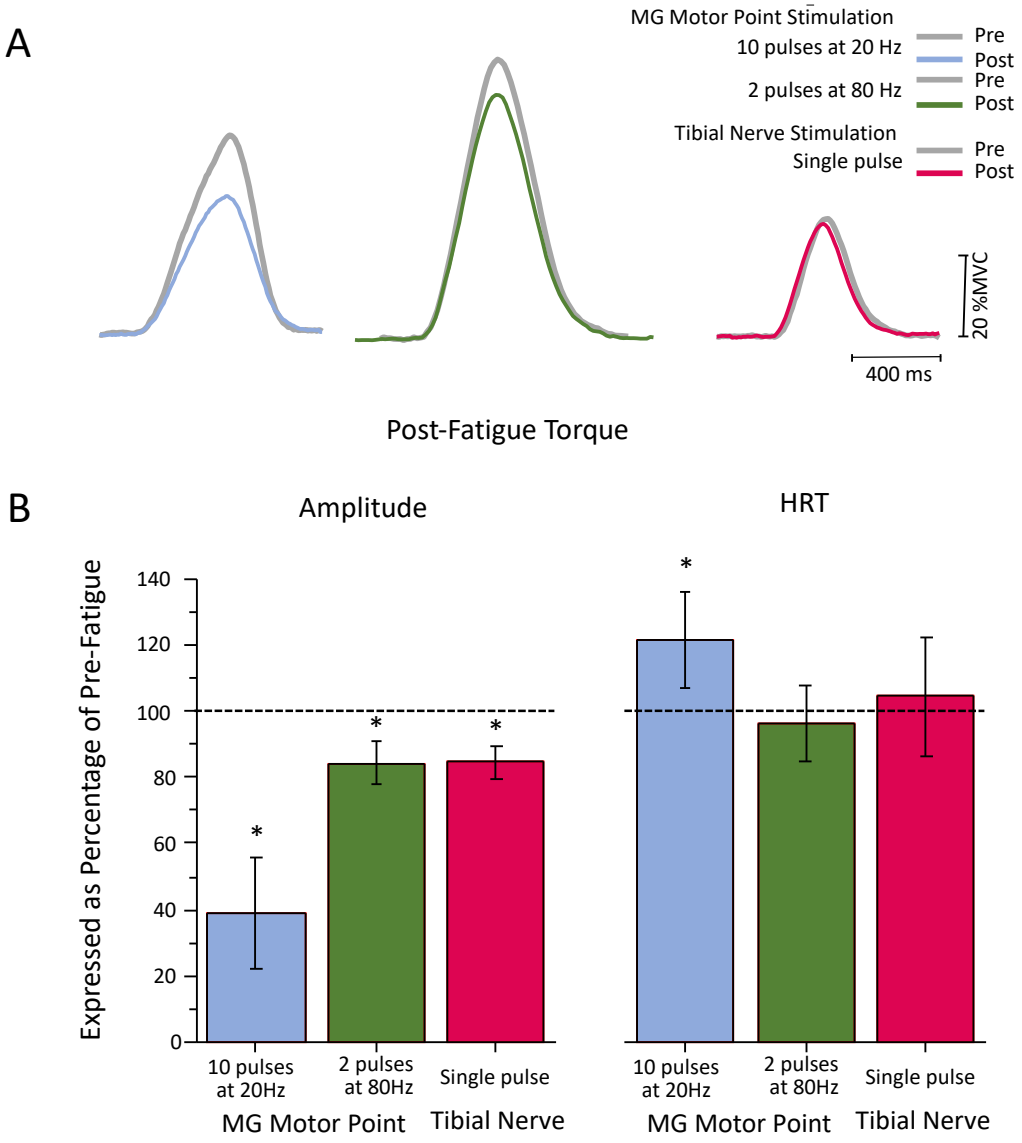

Figure 7. A) Torque traces from one participant from 10 pulses at 20 Hz delivered to MG motor point (test train; blue), two pulses at 80 Hz delivered to MG motor point (doublet; green), and single pulse delivered to tibial nerve (red) compared to pre-fatigue torque (grey). B) Graph depicts mean post-fatigue torque amplitude and half-relaxation time of 10 pulses at 20 Hz, two pulses at 80 Hz, and single pulse expressed as a percentage of pre-fatigue values.

4.2 Balance Testing

4.2.1 HDS-EMG Amplitude

A two-way repeated measures ANOVA comparing the effects of fatigue and perturbation direction on the EMG amplitude of the MG revealed that there was a significant main effect of direction on the amplitude of activation in the MG (p = .001; Figure 8). Post-hoc tests using a Bonferroni correction revealed that the MG amplitude was significantly lower for 60L compared to 60R (p = .021) and front (p = .002). The EMG amplitude was not significantly different between perturbations of 60R and front (p = 1.000). Therefore, the perturbation directions of 60R and front elicited statistically significantly greater amplitudes of activation in the MG compared to 60L. The EMG amplitude of the MG was not statistically different from pre-fatigue to post-fatigue (p = .335). There was also not a statistically significant interaction between fatigue and direction of perturbation for the EMG amplitude of the MG (p = .094), indicating that the regional modulation of MG by direction of perturbation was unchanged with MG fatigue

For the LG, there was a statistically significant main effect of direction (p = .017; Figure 8). Post-hoc comparisons revealed that the amplitude for 60R was significantly lower than in the 60L condition (p = .006) but the EMG amplitude was not significantly different for 60L and front (p = 1.000). Therefore, the LG EMG amplitude was significantly greater when perturbed 60L versus 60R. However, the EMG amplitude was not significantly different (p = .072) from pre-fatigue to post-fatigue, albeit there was a trend for increased amplitude post-fatigue. Further, there was not a statistically significant interaction between fatigue and direction (p = .332), thus the regional modulation of LG by direction of perturbation was unchanged with MG fatigue.

There was a statistically significant main effect of fatigue on the EMG amplitude of the SOL (p = .041; Figure 8), in which the amplitude of activation of SOL was greater post-fatigue versus pre-fatigue. There was also a statistically significant main effect of direction on the EMG amplitude of the SOL (p = .045). Post-hoc testing showed that the amplitude of the SOL was significantly greater for the 60R condition compared to the front (p = .048). There was no significant difference between 60L and front (p = .08) or

60L an 60R (p = 1.00). The interaction between fatigue and direction did not have a statistically significant effect on the EMG amplitude of the SOL (p = .172); thus, the regional modulation of SOL by direction of perturbation was unchanged with MG fatigue.

The signals recorded from the TA of eight participants revealed that there was a significant main effect for direction of perturbation (p = .003). Post-hoc analyses showed that the TA EMG amplitude was highest for 60R, which was significantly higher than the amplitude for both 60L (p = .025) and front (p = .005), but the TA amplitude was not significantly different between 60L and front (p = .398). The amplitude of the TA activity was not statistically different (p = .093) pre- versus post-fatigue. There was also no significant interaction between fatigue and direction (p = .591).

Table 3. EMG Amplitude: Degrees of freedom (*df*), F-statistic (*F*), and p-value (*p*) for the main effects of fatigue and directions, and the interaction between fatigue and direction from a two-way repeated measures ANOVA.

	Fatigue			Direction			Fatigue x Direction		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
MG	1, 8	1.05	.335	2, 16	11.5	.001*	1.21, 9.68	3.34	.094
LG	1, 8	4.3	.072	2, 16	5.35	.017*	2, 16	1.18	.332
SOL	1, 8	5.94	.041*	2, 16	3.8	.045*	2, 16	1.97	.172
TA	1, 7	3.77	.093	1.13, 7.92	17.24	.003*	2, 14	0.55	.591

(* denotes significance)

Table 4. Means and standard deviations of EMG amplitude (μV) of MG, LG, SOL, and TA between condition of fatigue and direction of perturbation.

	Fatigue		Direction		
	Pre-Fatigue	Post-Fatigue	60L	Front	60R
MG (μV)	79.28 ± 29.38	86.49 ± 22.13	52.83 ± 19	94.73 ± 26.69	101.1 ± 41.2
LG (μV)	52.61 ± 19.65	61.43 ± 19.02	61.63 ± 19.67	61.47 ± 20.4	47.97 ± 20.08
SOL (μV)	40.1 ± 11.54	48.29 ± 18.66	51.33 ± 25.04	35.14 ± 9.37	46.12 ± 15.94
TA (μV)	22.57 ± 8.41	19.37 ± 7.5	13.99 ± 5.19	10.27 ± 4.88	38.64 ± 18.43

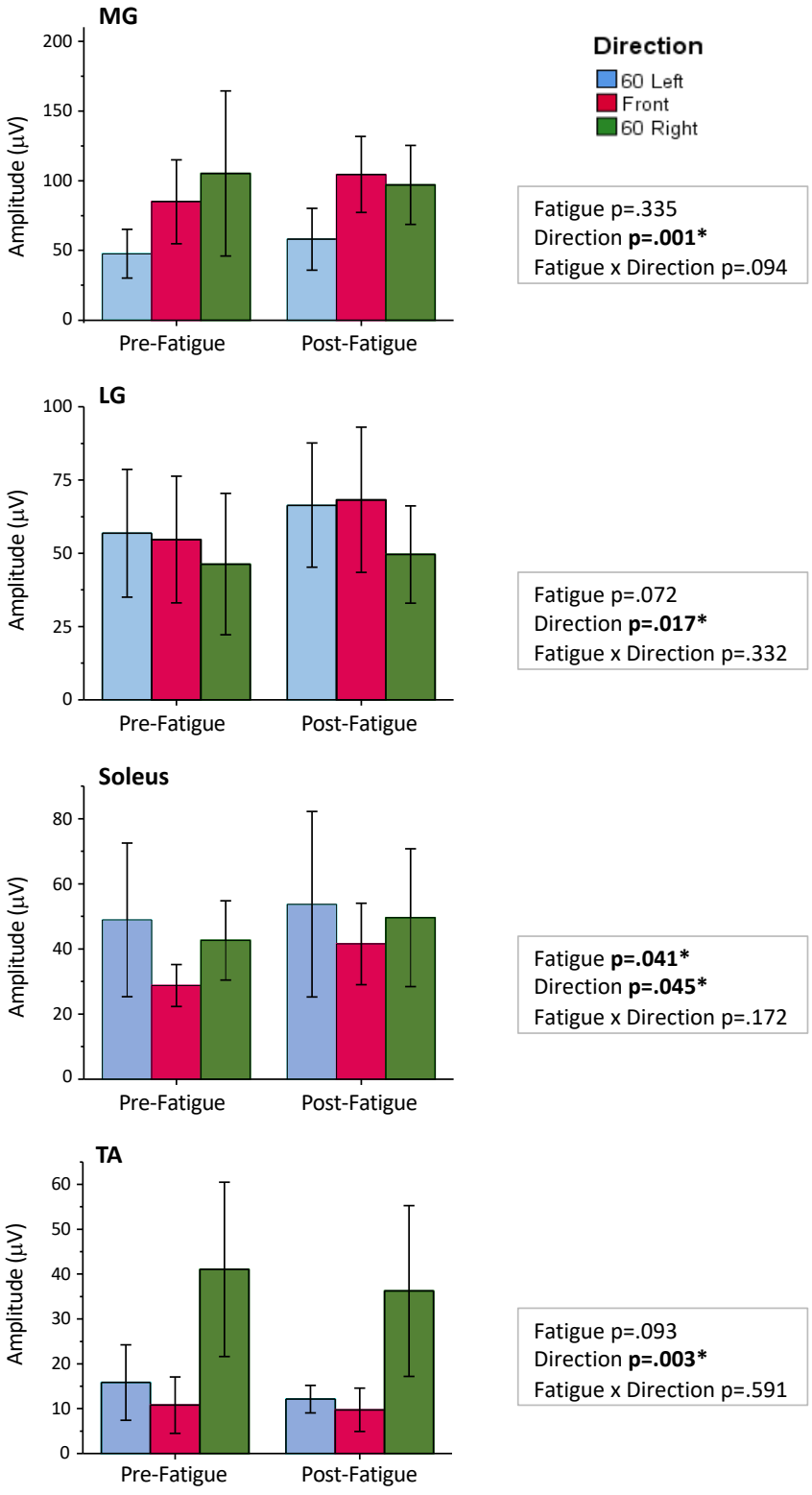

Figure 8. EMG amplitude of MG, LG, SOL, and TA pre-fatigue and post-fatigue across the three perturbation directions.

4.2.2 Medio-lateral Barycenter Coordinate (X-Axis)

The medio-lateral coordinate of the barycenter refers to the location of the centroid of EMG activity along the x-axis within each grid on the MG, LG, and SOL. It is denoted by column number (ranging from 1–9 in the MG, 10–16 in the LG, and 1–13 in the SOL), where lower column numbers are located more medially, and higher column numbers are located more laterally.

A two-way repeated measures ANOVA revealed that there was no statistically significant main effect of fatigue ($p = .140$) on the ML coordinate of the barycenter in the MG. Thus, the location of the barycenter in the ML axis did not differ significantly from pre-fatigue to post-fatigue. There was also not a statistically significant main effect of direction ($p = .337$). Therefore, the mean location of the MG barycenter did not change significantly between the three perturbation directions: 60L, front, and 60R. The interaction between fatigue and direction was not found to be statistically significant ($p = .952$).

There was a significant main effect of perturbation direction on the location of the ML coordinate of the barycenter in the LG ($p < .001$). Post-hoc tests with Bonferroni corrections showed that the LG barycenter was positioned significantly more medially for 60R perturbations compared to 60L ($p = .002$) and front perturbations ($p = 0.16$). The difference in location of the barycenter was not statistically significant between perturbations of 60L and front ($p = .076$). When comparing the pre- and post-fatigue, there was no significant main effect of fatigue on the ML coordinate of the barycenter in the LG ($p = .388$), nor was there a statistically significant interaction between fatigue and direction ($p = .349$).

As for the SOL, there was a statistically significant main effect of direction of perturbation on the ML coordinate of the SOL barycenter ($p < .001$). Post-hoc comparisons revealed that the barycenter was positioned most laterally for 60L perturbations, which was significantly different from the front ($p = .002$) and from 60R perturbations ($p < .001$). The barycenter was located most medially for the 60R condition, which was statistically significantly different from the front condition ($p = .004$). Therefore, it can be concluded that the ML coordinate of the SOL barycenter was

positioned more laterally when perturbed 60L and more medially when perturbed 60R. The medio-lateral coordinate of the barycenter was not statistically significantly different (p = .330) from pre-fatigue to post-fatigue. There was no statistically significant interaction effect between fatigue and direction on the ML coordinate of the SOL barycenter (p = .177).

Table 5. Medio-lateral coordinate of barycenter (within x-axis): Degrees of freedom (*df*), F-statistic (*F*), and p-value (*p*) for the main effects of fatigue and directions, and the interaction between fatigue and direction from a two-way repeated measures ANOVA.

	Fatigue			Direction			Fatigue x Direction		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
MG	1, 8	2.69	.140	2, 16	1.17	. 337	2, 16	0.05	.952
LG	1, 8	0.83	.388	2, 16	16.75	.0001*	2, 16	1.13	.349
SOL	1, 8	1.07	.330	2, 16	56.84	.0001*	2, 16	1.94	.177

(* denotes significance)

Table 6. Means and standard deviations of the medio-lateral coordinate of barycenter in MG, LG, and SOL between condition of fatigue and direction of perturbation. Each value represents the column number of the barycenter position in the mediolateral direction (x-axis). Lower column numbers are positioned more medially and higher column numbers more laterally within each grid.

	Fatigue		Direction		
	Pre-Fatigue	Post-Fatigue	60L	Front	60R
MG (column #1–9)	4.59 ± 0.86	4.26 ± 1.18	4.3 ± 1.13	4.58 ± 1.1	4.38 ± 0.86
LG (column #10–16)	13.19 ± 1.16	13.38 ± 0.87	14.73 ± 0.94	13.21 ± 1.58	11.91 ± 1.28
SOL (column #1–13)	6.78 ± 1.36	6.4 ± 1.87	9.46 ± 1.24	6.24 ± 2.23	4.07 ± 1.7

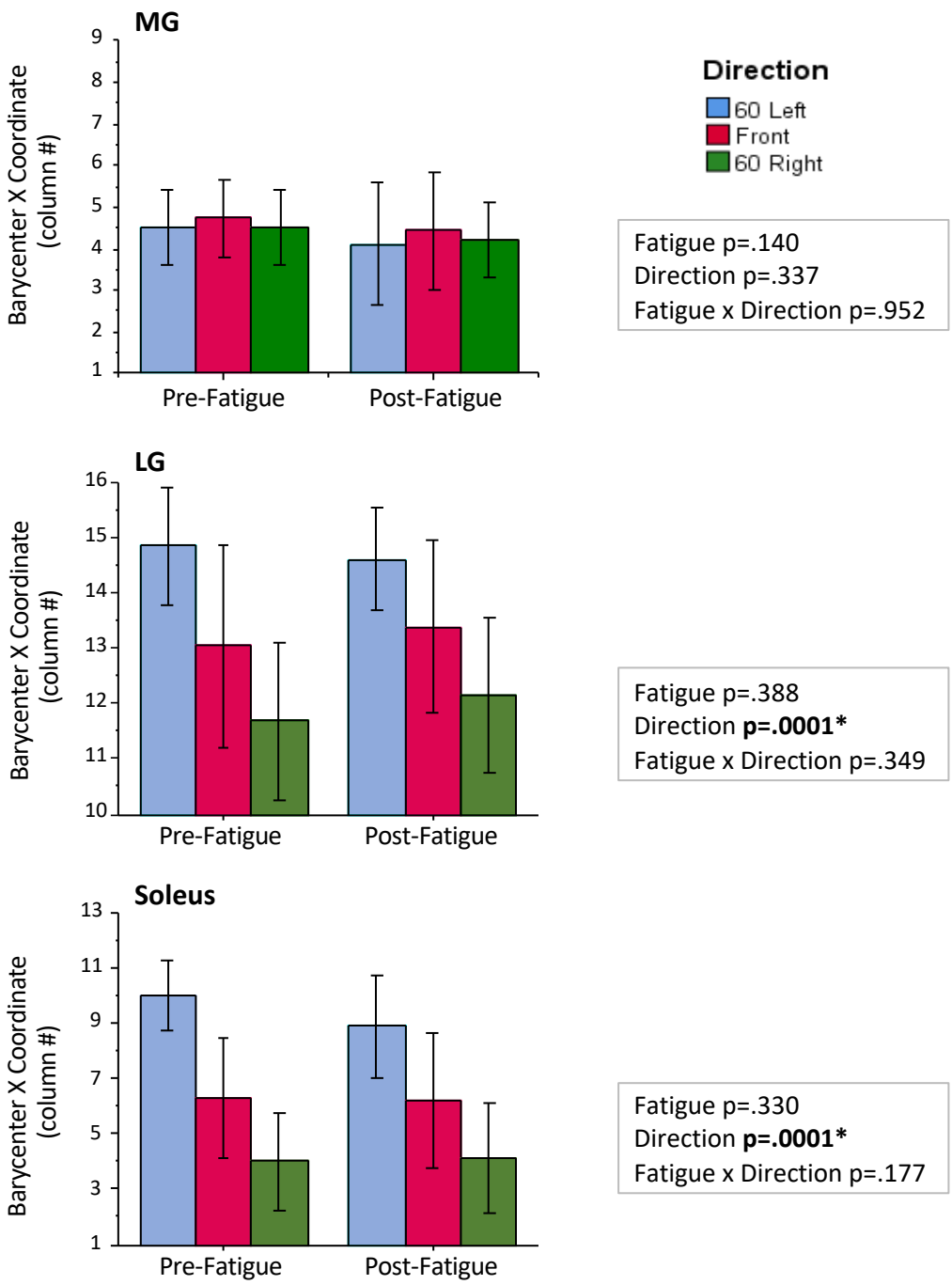

Figure 9. Medio-lateral coordinate of barycenter in x-axis of MG, LG, and SOL pre-fatigue and post-fatigue across the three perturbation directions

4.2.3 Proximo-distal Barycenter Coordinate (Y-Axis)

The proximo-distal coordinate of the barycenter refers to the location of peak EMG activity in the y-axis (ranging from row 1 to 7 in the MG and LG and row 1 to 4 in the SOL), where lower row numbers are positioned distally and higher row numbers are positioned proximally within each grid.

The results of a two-way repeated measures ANOVA comparing the effect of fatigue and direction on the location of the barycenter in the y-axis MG revealed that there was not a statistically significant difference (p = .285) from pre- to post-fatigue. Although perturbation direction did not have a statistically significant main effect, there was a trend observed (p = .063). Post-hoc analyses between the three perturbation directions were statistically nonsignificant and could not confirm the implication of the perturbation direction on the proximo-distal location of the MG barycenter. The interaction between fatigue and direction was also found to be statistically nonsignificant (p = .201).

The same results were found for the LG, in which there was no significant effect of fatigue (p = .341), no significant effect of direction (p = .161), and no significant interaction (p = .888). Therefore, the location of the LG barycenter in the y-axis did not change significantly from pre-fatigue to post-fatigue, nor did it differ significantly between the three perturbation directions.

As for the location of the barycenter in the y-axis of the SOL, there was a significant main effect of fatigue (p = .05). Therefore, the barycenter was located significantly more proximally in the SOL post-fatigue. However, there was no significant effect of direction (p = .089) and no significant interaction between fatigue and direction (p = .189). Thus, the barycenter location did not change significantly in the y-axis of the SOL across the three perturbation directions.

Table 7. Proximo-distal coordinate of barycenter (within y-axis): Degrees of freedom (*df*), F-statistic (*F*), and p-value (*p*) for the main effects of fatigue and perturbation directions, and the interaction between fatigue and direction from a two-way repeated measures ANOVA.

	Fatigue			Direction			Fatigue x Direction		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
MG	1, 8	1.31	.285	1.22, 9.79	4.17	.063	2, 16	1.78	.201
LG	1, 8	1.02	.341	2, 16	2.05	.161	2, 16	0.12	.888
SOL	1, 8	5.29	.05*	2, 16	2.83	.089	2, 16	1.85	.189

(* denotes significance)

Table 8. Means and standard deviations of proximo-distal coordinate of barycenter in MG, LG, and SOL between condition of fatigue and perturbation direction. Each value represents the row number of the barycenter position in the proximodistal direction (y-axis). Lower row numbers are positioned more distally and higher row numbers more proximally within each grid.

	Fatigue		Direction		
	Pre-Fatigue	Post-Fatigue	60L	Front	60R
MG (row #1–7)	5.08 ± 0.83	4.85 ± 1.01	4.68 ± 0.87	5.09 ± 0.97	5.13 ± 0.94
LG (row #1–7)	3.91 ± 1.1	4.12 ± 1.22	3.98 ± 1.39	4.36 ± 1.24	3.7 ± 1.14
SOL (row #1–4)	2.93 ± 0.53	3.25 ± 0.65	3.29 ± 0.52	2.99 ± 0.62	3 ± 0.67

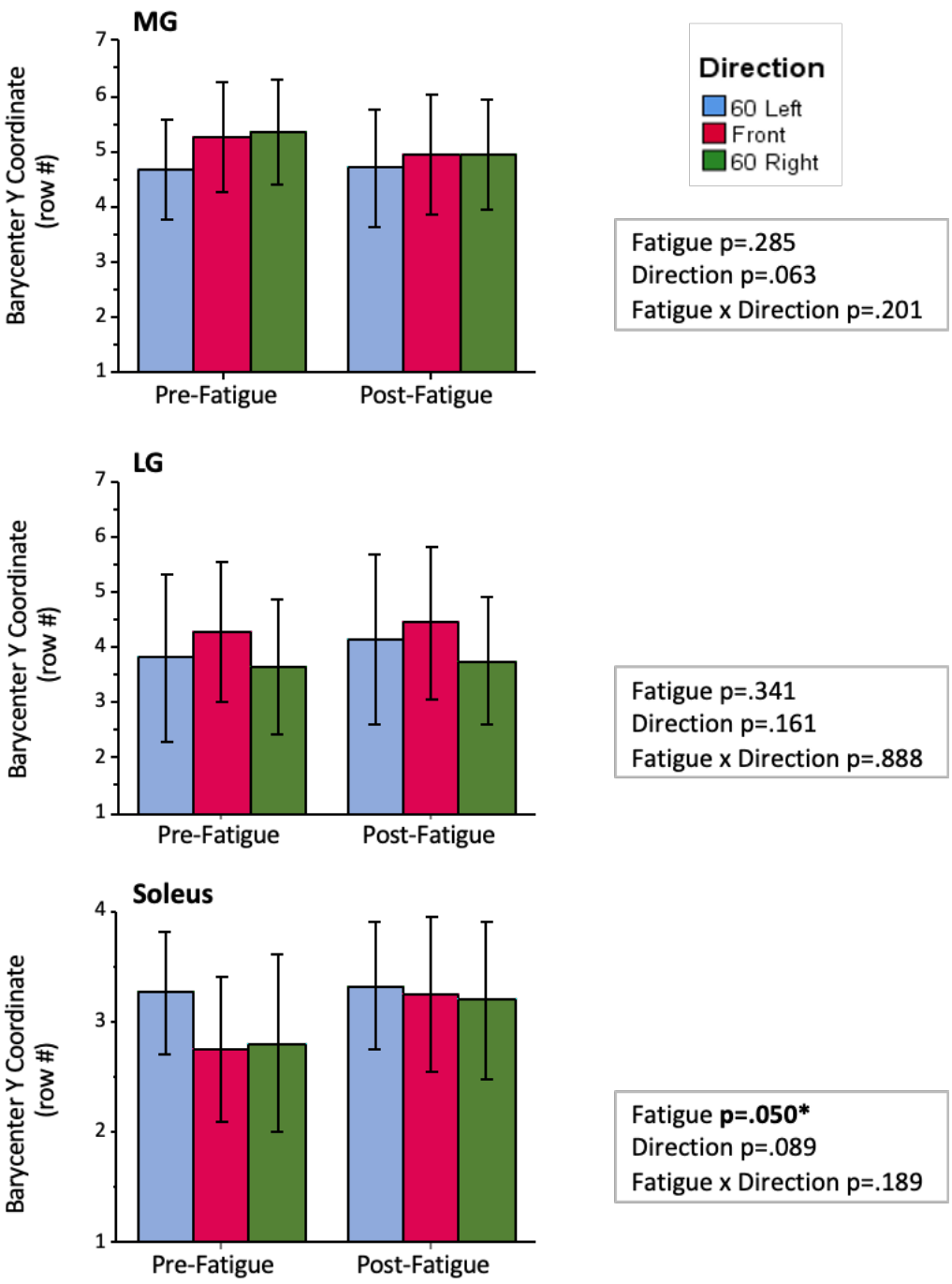

Figure 10. Proximo-distal coordinate of the barycenter in the y-axis of MG, LG, and SOL pre-fatigue and post-fatigue across the three perturbation directions.

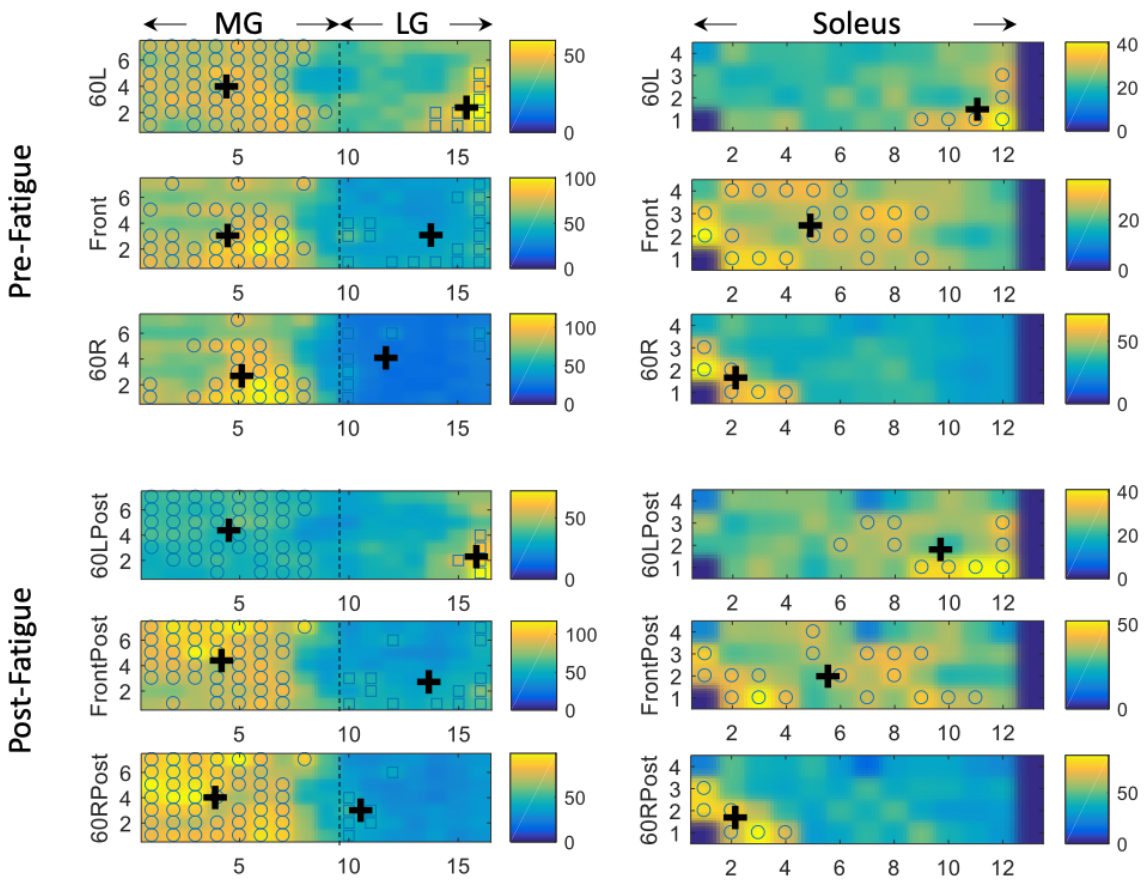

Figure 11. HDS-EMG activity maps from one participant. The legend on the right side of each grid shows the scale of the amplitude of each muscle, wherein brighter yellow/orange colours represent higher levels of activity, while the darker blue colour denotes lower levels of activity. The circle outlines on the MG and SOL and the square outlines on the LG indicate the channels with amplitudes of activation exceeding 70% of the peak activity within each muscle, from which the barycenter (denoted by the bold black cross) is calculated.

4.2.4 COP Amplitude

Two separate two-way repeated measures ANOVAs comparing the effect of fatigue and perturbation direction on the mean amplitude of COP excursions were conducted for the AP and ML directions. The following COP results are taken from seven participants as the data from two participants were compromised. For the AP direction, there was a significant main effect of fatigue (p = .017), a significant main effect of direction (p = .032), but no significant interaction (p = .967). Therefore, the COP excursion amplitude in the AP direction was significantly greater pre- versus post-fatigue, meaning that the COP deviated more anteriorly when the MG was in a non-fatigued state. In addition, the COP excursion amplitude was significantly greater for front compared to 60R (p = .034).

For the ML direction, there was a significant main effect of direction (p < .001) but no significant main effect of fatigue (p = .548) and no significant interaction (p = .463). Accordingly, the COP excursion amplitude was not significantly different from pre-fatigue to post-fatigue, both of which showed a slight deviation to the left. The COP excursion amplitude was significantly different for 60R and front (p = .022). The COP excursion for 60L was also significantly different from the front condition (p = .012) and significantly different from the 60R condition (p = .002).

Table 9. Amplitude of COP excursions in antero-posterior (AP) and medio-lateral (ML) axis: Degrees of freedom (df), F-statistic (F), and p-value (p) for the main effects of fatigue and directions, and the interaction between fatigue and direction from a two-way repeated measures ANOVA.

	Fatigue			Direction			Fatigue x Direction		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*p*
AP	1, 6	10.7	.017*	1.07, 6.43	7.25	.032*	2, 12	0.03	.967
ML	1, 6	0.4	.548	2, 12	29.74	<.001*	2, 12	0.82	.463

(* denotes significance)

Table 10. Means and standard deviations of COP excursion amplitude (mm) in the AP and ML directions. In the AP direction, positive values represent anterior sways, while negative values represent posterior sways. For the ML direction, positive values represent sways to the participant's right and negative values indicate sways to the participant's left.

	Fatigue		Direction		
	Pre-Fatigue	Post-Fatigue	60L	Front	60R
AP (mm)	32.93 ± 1.76	30.05 ± 1.62	29.80 ± 0.93	36.48 ± 3.24	28.19 ± 1.39
ML (mm)	-2.65 ± 1.42	-2.04 ± 1.78	9.41 ± 2.48	-5.19 ± 2.40	-11.25 ± 1.67

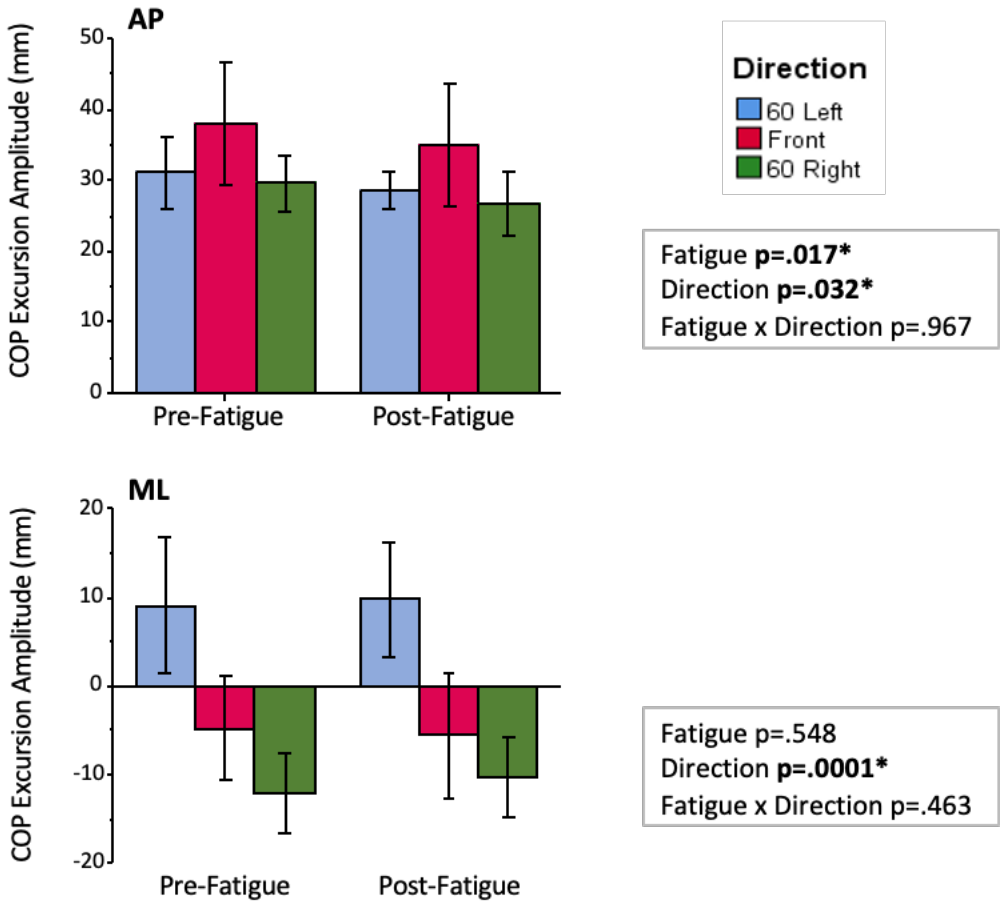

Figure 12. Mean amplitude (mm) of COP excursions in AP and ML directions pre-fatigue and post-fatigue across the three perturbation directions.

Chapter 5

5 Discussion

5.1 Fatigue

The results from the fatigue protocol demonstrated that the MG was effectively rendered into a state of LFF. The amplitude and area of the MG M-wave decreased significantly by 16% and 11% (respectively) from pre-fatigue to post-fatigue. The M-wave is a commonly used index of neuromuscular propagation and muscle excitability (Enoka & Stuart, 1992). The observed decrease in M-wave amplitude and area is consistent with previous findings that attribute an overall reduction in M-wave size to impaired neuromuscular propagation and reduced muscle membrane excitability (Cupido et al., 1992; Fuglevand et al., 1993; Galea, 2001; Milner-Brown & Miller, 1986). The M-wave amplitude and area did not change significantly in the LG or SOL. The significant reduction of M-wave amplitude and area in the MG but not the LG or SOL indicates that peripheral fatigue was isolated to the MG and did not affect the transmission of action potentials in the LG or SOL. Other studies aiming to induce targeted muscle fatigue to a single muscle head (LG) have reported the successful use of M-waves to identify the progression of fatigue and verify that synergist muscles were not unintentionally fatigued (Stutzig & Siebert, 2015). The present findings support this technique and indicate that repetitive low-frequency electrical stimulation produced peripheral muscle fatigue localized to the MG.

The torque produced by the 20 Hz ten-pulse test train delivered to the MG motor point decreased significantly in amplitude by 63% and increased significantly in half-relaxation time by 21% from pre-fatigue to post-fatigue. Reductions in tetanic force output are a measure of peripheral fatigue as tetanic force generation occurs in the absence of central drive (Vøllestad, 1997). In addition, increased relaxation times are an important feature of muscle fatigue following repeated activity that is reflective of reduced rates of cross-bridge detachment (Westerblad & Allen, 1993). Stimulation frequencies of 20 Hz are ideal for inducing and testing for fatigue as normal voluntary activity typically occurs at relatively low firing rates within this range (Bigland-Ritchie et al., 1986). Although there was a decrease in torque amplitude produced by the high frequency (80 Hz)

supramaximal doublet, the reduction in mean torque amplitude was modest (16% decline) in comparison to the torque produced by the low-frequency train, and there was no increase in the associated HRT. The disproportionate loss of force observed when stimulated at a low-frequency (20 Hz) with less pronounced force loss when stimulated at a higher frequency (80 Hz) is a clear sign of LFF (Edwards et al., 1977; Jones, 1996). Thus, the results support that LFF was successfully induced to the MG.

LFF is attributed to a reduced release of calcium from the sarcoplasmic reticulum (Bigland-Ritchie et al., 1986; Edwards et al., 1977; Vøllestad, 1997). A key feature of LFF is the slow recovery time, from which it can take several hours to recover fully. It is reasonable to assume that the long-lasting effects of LFF preserved the fatigue in the MG throughout the duration of the post-fatigue balance testing, which took no more than ten minutes to complete. The LFF testing period confirmed that the mean torque amplitude of the MG produced by the 20 Hz test train remained depressed at 55% of the pre-fatigue value after six-minutes of rest. Therefore, if the MG remained fatigued after six-minutes of rest, it is reasonable to assume that the MG would remain fatigued to the same, if not a greater, extent during the post-fatigue balance testing when the participant was standing on one leg, which would require the involvement of the fatigued MG.

One concern that arises with tetanic stimulation, even at force outputs as low as 10% of the maximal force, is the risk of cutting off blood supply, as the intramuscular pressure is increased during contractions (Wesche, 1986). This can create a problem insofar as fatigue may be overestimated when muscles are placed under ischemic conditions during constant-force contractions, but force may recover quickly when the contractions cease and reperfusion occurs. During intermittent stimulation there is less occlusion of the blood supply (Vøllestad, 1997; Vøllestad et al., 1990). Therefore, an intermittent fatigue cycle allows for adequate aerobic energy release, thus providing a more reliable measure of true low-frequency muscle fatigue as opposed to temporary force loss caused by ischemia (Vøllestad, 1997; Vøllestad et al., 1990).

The reduction in mean twitch torque amplitude produced by the supramaximal single pulse at the tibial nerve was relatively small but still statistically significant (16% decline). Since this pulse was delivered to the tibial nerve, the resultant twitch torque

would be the sum torque from all three plantarflexor muscles combined. Thus, the post-fatigue twitch torque would not be dramatically reduced as only one out of three muscles was functioning at a reduced capacity (MG). Accordingly, the mean HRT of the plantarflexor twitch torque did not change significantly.

5.2 Balance Testing

5.2.1 EMG Amplitude

The results indicated that the EMG amplitude of the MG was significantly greater when perturbed 60R compared to 60L (63% difference). The opposite was found for the LG, as the EMG amplitude was significantly greater when perturbed 60L compared to 60R (25% difference). Therefore, the perturbation direction of 60R caused higher activity in the MG, whereas the perturbation direction of 60L caused higher activity in the LG. This is consistent with research that the gastrocnemius head opposite to the direction of perturbation shows the highest relative amplitude of activation (Cohen et al., 2020). The modulation of EMG activity according to perturbation directions implies that the gastrocnemius head furthest away from the direction of pull is located in a more mechanically advantageous position for creating plantarflexion torques to oppose the direction of pull and maintain balance. This direction-specific modulation of the activation of the gastrocnemius was maintained even when the MG was in a state of fatigue. Therefore, the results suggest that direction-specific modulation of muscle activity persists in the triceps surae even when one muscle is in a state of fatigue.

The EMG amplitude of the MG did not change significantly from pre-fatigue to post-fatigue. The literature proposes that EMG amplitude is likely to increase following prolonged submaximal contractions as motor unit firing rate increases and additional larger motor units are recruited to compensate for the loss of force-generating capacity in the exercising muscle (Adam & De Luca, 2005; Conwit et al., 2000; Dorfman et al., 1990). That the results did not show an increase in post-fatigue EMG amplitude of the exercising MG suggests that there may be another compensatory mechanism at play.

Despite the evidence supporting the relatively low contribution of the LG to standing balance, it was hypothesized that both the LG and SOL would show increased activation

during the post-fatigue balance testing in order to compensate for the fatigued MG, which is the primary gastrocnemius head involved in standing under normal circumstances (Héroux et al., 2014). While the results show a statistically significant increase in SOL activation post-fatigue, the LG did not change.

One possible explanation as to why an increase in LG activation was not observed might be a potential change in postural strategy post-fatigue, which could put the gastrocnemius in a mechanically disadvantaged position. Previous studies have reported that when the plantarflexor muscles become fatigued, participants change their postural strategy to incorporate more hip and knee flexion in order to maintain postural stability during single-leg stance (Boyas et al., 2013). If the postural strategy changed post-fatigue to adopt a more flexed position of the knee, the gastrocnemius EMG would be depressed as surface EMG signals are up to 5x smaller when the knee is flexed versus extended (Avancini et al., 2015). Because the gastrocnemius crosses both the ankle and knee joint, it mechanically produces the greatest plantarflexion torque when the knee is fully extended and the muscle fibers are at their optimal length for maximal force production (45% of total plantarflexion torque) (Cresswell et al., 1995; Kawakami et al., 1998). Consequently, the force production from the gastrocnemius decreases by approximately 15% when the knee is in a flexed position (Fukunaga et al., 1992). If there was more knee flexion post-fatigue, the gastrocnemius would be in a disadvantaged position, so an increase in EMG amplitude may not be observed. Nevertheless, this theory cannot be validated without kinematic data, which were not recorded in the study.

It is important to note that while the 17% increase in mean EMG amplitude of the LG from pre- to post-fatigue was not statistically significantly, the *p*-value shows a trend that is approaching significance ($p = .072$). Therefore, an increase in the sample size may confirm that the LG EMG amplitude is higher post-fatigue. If this were the case, it could be inferred that an increase in LG activation post-fatigue compensates for the fatigued MG during balance tasks.

The SOL showed a significant 20% increase in EMG amplitude post-fatigue, when the MG was functioning at a reduced capacity. Regardless of fatigue, a main effect of direction was observed in the SOL, in which a higher amplitude of activation occurred in

response to diagonal perturbations (60L and 60R) compared to front. The post-fatigue increase in activation of the SOL was observed across all perturbation directions, including the front condition. Given that the MG was most active for perturbation directions of 60R and front, the post-fatigue increase in SOL activation for these directions may aid in maintaining postural stability when the MG is functioning at a reduced capacity. Therefore, increases in post-fatigue SOL activation would be adept at regulating AP sway following perturbations given that the primary role of the SOL during standing balance is to regulate AP sway (Duysens et al., 1991).

The activity of the antagonist TA muscle was modulated by direction of perturbation. The EMG amplitude of the TA was significantly higher for perturbation directions of 60R compared to 60L and front, regardless of MG fatigue. The literature suggests that TA ankle inversion torques contribute to stability in the frontal plane, especially when a narrow BOS is assumed (e.g., unipedal stance) (Lemos et al., 2015). Given that perturbation directions of 60R produced the greatest ML sway, the heightened TA EMG amplitude during 60R perturbations is likely a mechanism to regulate ML sway and maintain balance in the frontal plane. The TA findings provide no evidence of increased co-contraction about the ankle joint as a compensatory post-fatigue strategy.

5.2.2 Barycenter Location

The position of the barycenter of the LG and SOL was modulated across perturbation directions in the ML direction (X-coordinate). In both the LG and SOL, perturbation directions of 60L were associated with a more lateral position of the barycenter, while perturbation directions of 60R had a more medial position of the barycenter. This is consistent with research showing that the barycenter within the LG and SOL shifts in the horizontal axis to oppose the direction of perturbation (Cohen et al., 2020). Additionally, the present study demonstrates that this pattern of the barycenter shifting away from the direction of perturbation is maintained in the LG and SOL when the MG is in a state of fatigue. This observation suggests that muscle fatigue of one head of the gastrocnemius has limited influence on the medio-lateral position of the barycenter in the non-fatigued muscles. Accordingly, the notion that the CNS can preferentially modulate the activity of

certain muscle regions located in mechanically advantageous positions persists even in the presence of synergist muscle fatigue.

The SOL was the only muscle showing modulation of the barycenter location in the proximo-distal direction (y-axis), in which a proximal shift was observed for all post-fatigue perturbation directions. This significant change of the barycenter in the proximo-distal direction was only observed in the SOL (not the MG or LG). This observation is consistent with previous findings showing that of the three plantarflexor muscles, a proximo-distal barycenter shift only occurred in the SOL (Cohen et al., 2020). The proximal shift in SOL barycenter may help compensate to maintain balance when the MG is in a state of fatigue by modulating the point of peak activity closer to the MG, thereby shifting the point of peak force production closer to a mechanically advantageous position.

Although there was no significant change in the barycenter location of the MG before and after fatigue, nor between the perturbation directions, previous studies that have shown a difference in the medio-lateral position of the MG barycenter for perturbation directions of 60R versus 60L used a larger sample size (n = 12) and only found a small shift of approximately 6 mm (less than the distance between 2 adjacent channels) (Cohen et al., 2020). Therefore, it is not surprising that the results from fewer participants (n = 9) did not show a significant shift in the MG barycenter location.

5.2.3 Postural Sway

Contrary to previous studies reporting impaired postural stability following plantarflexor muscle fatigue (Boyas et al., 2013; Grey et al., 2013; Paillard, 2012; Rojhani-Shirazi et al., 2019; Springer & Pincivero, 2009), the results of this experiment show smaller COP excursions during the post-fatigue balance testing. Other studies have suggested that this may be accounted for by a change in postural control or kinematics. According to Alderton and Moritz (1996), calf muscle fatigue may trigger compensatory mechanisms such as increased muscle spindle reflex activity or increased stiffness to maintain balance. The EMG data collected from the TA does not show any evidence of increased co-contraction post-fatigue, suggesting that increased stiffness of the ankle joint was not a

compensatory mechanism during the post-fatigue balance testing. Boyas et al. (2013) suggested that while participants attempt to maintain the ankle strategy as the principal postural control strategy, they may also employ more ankle and hip flexion to compensate for plantarflexor fatigue during unipedal stance. This corroborates the prevailing view that the ankle strategy is the dominant strategy governing postural control in the sagittal plane (Horak & Nashner, 1986). Thus, research supports that the ankle strategy would be effective in controlling postural sway following perturbations in the pre-fatigue balance testing when the plantarflexors are functioning at full capacity. However, the observed reduction in AP sway post-fatigue suggests that compensation via a change in postural strategy may be operating in addition to the ankle strategy when the MG is fatigued.

5.3 Limitations and Future Research Directions

Although COP data were recorded during balance testing, there were no kinematic data. Therefore, any hypotheses regarding alterations in postural strategy (i.e., increased knee flexion post-fatigue) are purely speculative. Future studies should record kinematic data to confirm if a change in postural strategy is a compensatory mechanism post-fatigue. Further, it has been reported that larger EMGs can be detected in the proximal region of the gastrocnemius with the knee flexed (Avancini et al., 2015); in this study, however, the placement of the grids may have been a limiting factor in recording proximal muscle activity as the grids did not cover the entire proximo-distal span of the muscles. Previous studies investigating regional modulation within the ankle plantarflexors in a larger sample (n = 12) have found a significant shift in the medio-lateral position of the barycenter to oppose the direction of perturbation in all three muscles. In the present study, with a smaller sample size (n = 9), a medio-lateral barycenter shift opposite to perturbation direction was only observed in the LG and SOL, but not in the MG. Additionally, increasing the sample size may lead certain observations (i.e., increased LG amplitude post-fatigue) to become statistically significant with a higher power. Thus, a small sample size was a limitation to this study. Finally, while the medio-lateral position of the barycenter was modulated in the LG and SOL despite the presence of MG fatigue, it remains unknown if regional modulation of the barycenter would persist if the LG or

SOL were in state of fatigue. Therefore, future studies should investigate the effect of muscle fatigue induced to the LG or SOL on regional modulation of muscle activation.

5.4 Conclusion

It was hypothesized that when the MG was in a state of fatigue, the LG and SOL would experience compensatory increases in amplitude of activation and a medial barycenter shift, particularly for 60R perturbation directions, for which the MG is at a mechanical advantage. The findings of the present experiment suggest that the CNS compensates for a loss of force-generating capacity in the fatigued MG by increasing the amplitude of activation in the SOL and shifting the barycenter of peak activation towards the proximal region of the SOL to maintain unipedal balance following external perturbations. Additionally, regional modulation of EMG activity during directional external perturbation persists in the presence of MG fatigue, wherein the gastrocnemius muscle opposite to the direction of perturbation has a higher relative amplitude of activation, and the barycenter within the LG and SOL shifts to oppose the direction of perturbation. Therefore, although the compensation did not occur in the way it was originally hypothesized, the results of this study suggest that the CNS can adapt to a loss of force-generating capacity in one of the ankle plantarflexor muscles by modulating the activation patterns of the non-fatigued muscles to maintain unipedal balance during external perturbations.

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Appendices

Appendix 1. Initial ethics approval notice.

Western Research

Research Ethics

Western University Health Science Research Ethics Board
HSREB Delegated Initial Approval Notice

Principal Investigator: Dr. Jayne Garland
Department & Institution: Health Sciences\Health & Rehabilitation Sciences,Western University

Review Type: Delegated
HSREB File Number: 108977
Study Title: Control of human standing posture

HSREB Initial Approval Date: April 03, 2017
HSREB Expiry Date: April 03, 2018

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Revised Western University Protocol	Version 2	2017/03/24
Revised Letter of Information & Consent	Version 3	2017/03/24
Other	Research Proposal - NSERC	2012/10/31
Data Collection Form/Case Report Form		
Other	UBC NSERC original approval - Received for information	2013/09/04
Other	UBC current ethics certificate - Received for information	2016/07/05

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

[redacted]

[redacted]

Appendix 2. Ethics approval form with amendments.

Western Research

Date: 22 October 2019

To: Jayne Garland

Project ID: 108977

Study Title: Control of human standing posture

Application Type: HSREB Amendment Form

Review Type: Delegated

Full Board Reporting Date: November 5, 2019

Date Approval Issued: 22/Oct/2019

REB Approval Expiry Date: 03/Apr/2020

Dear Jayne Garland ,

The Western University Health Sciences Research Ethics Board (HSREB) has reviewed and approved the WREM application form for the amendment, as of the date noted above.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Consent for Controls	Consent Form	01/Oct/2019	1
Consent NSERC 2013 UWO V6	Consent Form	01/Oct/2019	6
ethics 108977 V6 Amendment including research plan	Protocol	01/Oct/2019	6

REB members involved in the research project do not participate in the review, discussion or decision.

The Western University HSREB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Karen Gopaul, Ethics Officer on behalf of Dr. Philip Jones, HSREB Vice-Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix 3. Letter of information and subject consent form.

Western HealthSciences

LETTER OF INFORMATION
SUBJECT CONSENT FORM

Project Title: Control of Human Standing Posture

Principal Investigator: Jayne Garland PhD, Physiotherapist (PT), Faculty of Health Sciences, Western University, [redacted]

Co-investigators: Tanya Ivanova PhD, Faculty of Health Sciences, Western University, [redacted]

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BACKGROUND AND PURPOSE

You are being invited to participate in this study investigating the control of standing balance as a healthy adult, or an older adult who is at risk of falling, or a person who has recently sustained your first concussion. We know that poor balance is a significant risk factor for experiencing a fall in the elderly and for success return to play post-concussion. Your calf muscles are very important in the maintenance of standing posture. We are conducting a series of experiments to investigate how your ankle muscles are controlled by your brain during standing, with the hopes that this understanding will improve balance in those at risk of falling.

DETAILS OF THE STUDY

This study involves four experiments which will take place in the Wolf Biomechanics Orthopaedic Laboratory (WOBL) which is situated in Room 1210 at 3M Centre of Western University. The study comprises four different experiments. The experiment in which you are being invited to participate is identified in the square below; you do not need to participate in all four experiments. Each experiment lasts 90 minutes.

☐ *Experiment 1: Responses to disturbances in balance on a force platform*

You will be asked to stand as still as possible on a force platform (a square, flat device level with the ground that measures your amount of body sway) for approximately 2 minutes. Next, you will have a belt strapped to your waist and weights of different heaviness (from 1% to 5% of your body weight) will be applied to the belt in different directions, pulling you forwards, sideways, backwards. You will not know the exact size of the weight or the direction of pull in advance. The pull is meant to provide a very small change in your standing balance but will be small enough so you will not need to step to catch your balance. To ensure your safety, you will be secured into an overhead safety harness. Rest breaks can be taken during testing.

[redacted]

During these movements, we will be measuring the activity of your calf muscles with three grids of surface recording electrodes, which are called High Density Surface Electromyography (HDS- EMG) and will be placed on the skin covering your calf muscles. Gel will be used between your skin and the grids. We may need to shave a small patch of skin to ensure adequate contact between the electrodes and your skin. In addition to the electrodes, we will also be taping reflective markers on your skin over your ankle, knee, hip, and trunk to measure how your body moves in response to the balance disturbances. The cameras will capture only the movement of these markers, and you will not be able to be identified by these images. Please note that you will be required to wear loose fitting shorts that can be rolled up to the mid-thigh.

If you have had a concussion and have consented to be included in the Fowler Kennedy Concussion Registry, your concussion assessment and history will be obtained from the registry. If you are not participating in the registry, at the beginning of your experiment we will ask you questions about your concussion history and perform a concussion assessment, called the SCAT5. The SCAT 5 includes tests of your concentration, balance, walking ability.

You will be asked to complete the Community Balance and Mobility (CB&M) Assessment. The CB&M requires you to perform various balancing activities (e.g. one legged stance, crouch walk, run). We will call you 6 weeks and 3 months after your testing to ask if you have sustained any injuries or symptoms since the testing.

Experiment 2: Influence of fatigue on responses to disturbances in balance

This experiment involves one visit to the laboratory. You will complete a balance test with a belt strapped to your waist and weights of 2% of your body weight will be applied to the belt in different directions, pulling you to the front, and sideways to the left and right. The pull is meant to provide a very small change in your standing balance and will be small enough so you should not need to step to catch your balance. For just in case, an investigator will stand beside you to support you if you lose your balance. Next, one of your ankle muscles will be fatigued electrically stimulating the muscle while you are otherwise resting in a seated position. The balance test with weights of 2% of your body weight will be repeated immediately after the fatigue task. The electrical stimulation may be repeated between balance testing directions if the fatigue starts to wear off. During the experiment, we will be measuring the activity of your calf muscles with grid electrodes on your skin and observing your postural sway by having you stand on a force platform.

Experiment 3: Role of the spinal cord in controlling standing balance

This experiment involves two visits to the laboratory. You will be asked to stand on a force platform for approximately 15 minutes. During this time, 300 brief electrical pulses will be given every 1-3 seconds with two electrodes placed on the skin on the outside of your ankle. These pulses can be felt but are not painful. The response of your ankle muscles to these pulses tell us how balance is controlled in the spinal cord. Next, these pulses will be repeated with you sitting with your leg straight in front of you. You will be asked to contract your calf muscle by pushing your foot down with a small force of 10% of your maximum. On the second day, you will perform the same tasks except the pulses

will be given at the back of your knee. Again, you will feel these pulses but they are not painful.

A sterilized electrode will be placed under your skin to monitor the motor unit activity from your calf muscle. The motor unit consists of the nerve that starts in your spinal cord and the muscle fibers that contract to produce force. The recording electrode is very small and is placed in the muscle, temporarily, with a needle. The pain that may be felt with this procedure is typically less than that experienced during standard blood tests. Your skin will be cleaned with alcohol swabs prior to inserting the electrode. The electrodes do not send electricity to you. Surface recording electrodes will also be placed on your skin to monitor the calf muscle activity. Gel will be used between your skin and the surface electrodes.

Experiment 4: Role of the brain in controlling standing balance

You will be asked to stand as still as possible on a force platform. You will complete a balance test with a belt strapped to your waist and weights of 3% of your body weight will be applied to the belt in different directions, pulling you forwards, sideways to the left, sideways to the right. To ensure your safety, you will be secured into an overhead safety harness. The pull will be controlled by you by pushing a button or by the investigator so you will not know exactly when or in which direction the pull will occur. There will be a total of 100 of these pulls.

A cap, similar to a bathing cap, with small disks (electrodes) will be placed on your head to record brain waves from your scalp. This equipment is called an “EEG”. EEG electrodes will also be positioned behind each ear, beside each eye, and beneath one eye on the cheek. Small red marks may be apparent at one or more of the electrode locations once the cap is removed. These marks go away quickly. We will also be measuring the activity of your calf muscles with grid electrodes on your skin and observing the movement of your body with reflective markers.

PARTICIPATION CRITERIA:

You are invited to participate if you are 1) between 16 and 50 years of age and able to stand independently for two minutes without physical support, 2) between the ages of 14 and 50 years of age and have sustained your concussion between 3 days and 6 weeks ago, have been cleared by your physician to return to non-contact training drills, or 3) 70 years of age or older. People who have any health conditions which significantly impact their ability to stand (e.g. dizziness, acute leg injuries or peripheral neuropathies) or sustained additional concussions within the past 6 months will not be able to participate in this study.

RISKS/SIDE-EFFECTS:

Possible discomforts within the study are possible skin irritation during the placement of the electrodes or the reflective tape, or during the skin preparation prior to placement (e.g. shaving the skin). You may experience some muscle soreness in your calf from the prolonged standing or after the fatigue experiment but this shouldn’t last more than a day or two. In Experiment 2, the electrical stimulation causes a prickly sensation and an abrupt muscle contraction that can be uncomfortable but not painful. Please inform us if

you find the electrical stimulation painful as we can adjust the settings to make it more comfortable. In Experiment 3, the insertion of the fine-wire electrode feels like a sharp pin-prick; there is a remote chance of bruising or infection at the needle site. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

In the unlikely event of a medical emergency during any of the assessments, the research personnel will call 911.

BENEFITS:

Although there is no expected direct benefit to you from participating in the study, the findings from this study may contribute to our understanding of how to prevent falls in older adults or how to detect balance problems post-concussion.

REIMBURSEMENT:

We appreciate your involvement in this study and do not want you to incur any cost associated with travel to the laboratory. You will be reimbursed for any out-of-pocket expenses such as parking, taxi fare etc. that you may incur as the result of your participation in this study. Please retain your receipts. If you are participating in Experiment 2, you will be provided a small $20.00 honorarium.

CONFIDENTIALITY:
Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada, *the Western Research Ethics Board,* and Lawson's Quality Assurance Education Program for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study (i.e. data collected, questionnaires), so that your identity [i.e. your name or any other information that could identify you] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to Dr. Garland.

VOLUNTARY PARTICIPATION:

Participation in the study is completely voluntary. You may refuse to participate or withdraw from the study at any time with no effect on you, including clinical care. You are not obligated to provide any reason for your withdrawal, should you choose to do so. Participation in this study does not prevent you from participating in other research studies in the future. If you are willing to be contacted in the future for other research studies, please indicate this on the consent form. You can always withdraw this consent to be contacted in the future, should you change your mind. In this case, your name and contact information will be removed from our records. Participation in any future research is completely voluntary and will have no bearing on the results of the current research project. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else, and you do not release the study doctors or participating institutions from their legal and professional responsibilities.

FURTHER QUESTIONS?
Please contact one of us, at the address below or by phone, to ask any questions you may have about the study.

Principal Investigator: S. Jayne Garland, PT PhD Professor and Dean Faculty of Health Sciences Western University

Arthur and Sonia Labatt Health Sciences [redacted]

Co-investigator:
Tanya Ivanova, PhD
Lab Manager
Neural Control of Force Production and Movement Lab Faculty of Health Sciences, Western University

Wolf Lab/ Fowler Kennedy Sports Clinic [redacted]

If you have any questions about your rights as a research participant or the conduct of this study, you may contact The Office of Research Ethics [redacted], email: [redacted]

Please keep this information letter for future reference.

CONSENT FORM

Control of Human Standing Posture

• I have read and understood the subject information and consent form.

• I have had the opportunity to ask questions and have had satisfactory responses to my questions.

• I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.

• I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.

• I understand that I am not waiving any of my legal rights as a result of signing this consent form.

• I understand that there is no guarantee that this study will provide any benefits to me
My signature indicates that I consent to participate in this study.

_____ _____ _____

Print Name
Signature of Person Obtaining Consent:

Signature Date

_____ _____ _____

Print Name Signature Date

Possibility of future research

There may be future opportunities for you to participate in ongoing research. If you are interested in being contacted, please check the appropriate box below. If contacted, you will be asked to read a new letter of information and sign a new consent form.

□ Please do not keep my name and contact information. I do not wish to be contacted in the future.
□ Please keep my name and contact information so that I may be contacted to learn about future research opportunities or have access to my data in the future.

Copy of Study Results
I would like a copy of the study results. Yes □ No □ If yes, please write your mailing address below.

_____

_____

_____

_____

RECEIPT

I,_____ acknowledge that I received $20 honorarium for my participation in the study "*Influence of fatigue on responses to disturbances in balance"*

Signature:

Date:

Curriculum Vitae

Name:	Nicole Grzywnowicz
Post-secondary Education and Degrees:	Wilfrid Laurier University Waterloo, Ontario, Canada 2014–2018 B.A. Honours Kinesiology and Physical Education The University of Western Ontario London, Ontario, Canada 2018–2020 M.Sc. Candidate, Kinesiology, Integrative Biosciences
Honours and Awards:	Dean's Honour Roll – High Distinction Graduate Wilfrid Laurier University 2014–2018 Western Graduate Research Scholarship Western University 2018–2020
Related Work Experience	Graduate Teaching Assistant Western University Faculty of Health Sciences, School of Kinesiology 2018–2020
Presentations:	The effects of muscle fatigue on regional modulation of the ankle plantarflexors *Oral Presentation* Exercise Neuroscience Group McMaster University, Hamilton, ON 2019