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Sex Comparisons of External, Anatomical and Methodological Factors on Motor Unit Firing Behavior

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A thesis submitted in partial fulfillment of the requirements for the Doctor of Philosophy degree in Kinesiology

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Abstract

Motor unit (MU) recruitment and firing rate (MUFR) modulation are the primary factors governing force control. Differences in the anatomical distributions of α -motoneurons, muscle properties, and perception of fatigue between males and females may cause sexually dimorphic MU firing behaviors. The purpose of this dissertation was to further examine external, anatomical, and methodological factors that may influence observations of sex-related differences in neuromuscular function. In chapter 2, sex differences in how mental fatigue may influence MU firing behaviors were examined. Mental fatigue did not significantly alter MU firing behavior, maximal force, or force tracing ability in either males or females. This study utilized various isometric contractions and found that females, but not males improved in their performance of tracing a novel, variable force tracing task. In chapter 3 the relationship between muscle pennation angle and MUFR was examined in the tibialis anterior of males and females. There were no significant sex-related differences in MUFR or pennation angle. Higher firing rates were associated with higher pennation angles only in males. Chapter 4 was an investigation of how the number of motor units in a recording impacts the calculation of mean MUFR in males and females. Mean MUFR of the entire active MU pool was estimated at submaximal and maximal contraction intensities through bootstrapping. The results from this study revealed that to achieve at least 50% probability of accurately reflecting the mean MUFR of the active pool, a minimum number of 4-7 MUs should be included in the calculation, depending on the contraction intensity in males and females. Overall, these data contribute to our understanding of sex-related differences in neuromuscular physiology and suggest that MU firing behavior is not significantly affected by mental fatigue or number of motor units recorded in males and females. However, pennation angle may influence MUFR differently in males and females.

Keywords

firing rate, mental fatigue, pennation angle, force, sex differences, neuromuscular function, motor unit, force

Summary for lay audience

Voluntary muscular contraction is controlled by signals generated within the neuromuscular system. The signals to muscle come from regions of the spinal cord and are directly influenced by signals generated in the brain. The spinal cord control of muscle has unique patterns determined, in part, by the qualities of the muscle to which it is connected. Researchers can measure the final stage of these signals at the muscle using electromyography. Differences in how males and females respond to mentally fatiguing tasks may result in an alteration of signals sent to the spinal cord from the brain, and the characteristics of their muscles may influence the signals sent to the muscle from the spinal cord. An individual's sex, muscle characteristics and perception of fatigue can all contribute to alterations to the signals within the neuromuscular system. Furthermore, electromyographic recordings are limited in their ability to record the activity of an entire muscle. This dissertation had three objectives. The first was to determine if there were sex-based differences in muscle control after a mentally fatiguing task. The second was to determine if there was relationship between neuromuscular control patterns and the muscle fiber arrangement in males or females. The third objective was to use statistical methods to estimate how a common method of describing spinal control of muscle compares to an estimated mean used to represent the activity of the entire muscle. Neuromuscular control patterns were unaffected by the perception of fatigue, and significantly related to muscle properties only in males. There were also no sex-related differences when estimating the activity of the entire muscle.

Co-authorship statement

This thesis contains material from three manuscripts where Michael Marsala is the primary author. A version of chapter 2 has been accepted for publication in *Experimental Physiology*. Chapters 3 and 4 are in preparation for journal submission. Michael Marsala was the primary author, and contributor for study design, participant recruitment, data acquisition and analysis, and drafting manuscripts. Chapter 4 was written using data collected by Greig Inglis, and David Gabriel was a co-contributor to study design. Dr. Anita D. Christie is also co-author on all chapters, contributing to study design, data interpretation, and the reviewing and revision of manuscripts.

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List of abbreviations

CSA – Cross-Sectional Area

CV – Coefficient of Variation

CVISI – Coefficient of Variation of the Inter-spike Interval(s)

EMG – Electromyography

FDI – First Dorsal Interosseus

FS – False Starts

IPAQ – International Physical Activity Questionnaire

METS – Metabolic Equivalent of Task

MFI – Multidimensional Fatigue Inventory

MT – Muscle Thickness

MUFR – Motor Unit Firing Rate

MVC – Maximal Voluntary Contraction

PSQI – Pittsburgh Sleep Quality Index

PVT – Psychomotor Vigilance Task

RMSE – Root Mean Squared Error

RT – Reaction Time

TA – Tibialis Anterior

TTL – Transistor-transistor Logic

Chapter 1

1 Introduction

1.1 General overview of neuromuscular function

Voluntary movement is generated through a train of electrical impulses delivered from the primary motor cortex through the spinal cord and ultimately triggering muscular contraction (Brouwer & Ashby, 1990; Merton & Morton, 1980; Watson et al., 2009). These electrical impulses that originate within the primary motor cortex are propagated down corticospinal neurons, and synapse with α -motoneurons in the spinal cord where the train of electrical impulses is further propagated to skeletal muscle (Brouwer & Ashby, 1990; Colbert & Pan, 2002; Evarts, 1968; Fuglevand et al., 1993). The neuromuscular system consists of the anatomical components necessary for both voluntary and involuntary control of muscle, including the primary motor cortex, upper and lower motoneurons, muscle fibers, and sensory neurons, among other structures. Voluntary control of muscle involves an integration of both descending drive and sensory feedback from internal and external stimuli. Alterations in any of these components can influence the output of the system, which characterizes neuromuscular function, or the ability to produce controlled force through neural activation of muscles. Outcome measures used to define neuromuscular function in dissertation include the mean and variability of force and motor unit firing rate (MUFR).

The neuromuscular system can be generalized into two levels: central and peripheral. The central level of the neuromuscular system consists of the brain and the spinal cord, and the peripheral level are the nerves branching from the spinal cord and the muscle they innervate. Central influences on neuromuscular function can include factors such as neurotransmitter levels in the brain which can be altered by prolonged cognitive effort (Boksem & Tops, 2008; Martin et al., 2018). Peripherally, the influence of skeletal muscle properties on neuromuscular function are more widely studied and include factors such as the architecture of the muscle itself (Martinez-Valdes et al., 2022; Zierath & Hawley, 2004).

1.1.1 Motor unit recruitment and rate coding

The corticospinal tract transmits the action potentials necessary for voluntary movement through synaptic connections directly to neurons in the spinal cord called α -motoneurons (Lemon, 2008; Watson et al., 2009). The α -motoneurons integrate this descending information with afferent dendritic sensory input of both excitatory and inhibitory nature to modulate the output that directly controls muscular contraction (Binder et al., 2002; MacIntosh et al., 2006). Summation of all excitatory and inhibitory input changes the resting membrane potential of α -motoneurons (Rekling et al., 2000). With sufficient excitatory input a threshold potential is reached, resulting in generation of efferent action potentials that are propagated through axonal connections to groups of muscle fibers by each individual motoneuron (Heckman & Enoka, 2012). Each spinal motoneuron and the associated groups of muscle fibers are called motor units (Heckman & Enoka, 2012) and are described as the final common pathway, ultimately responsible for the control of force during muscular contraction (Liddell & Sherrington, 1925).

Skeletal muscles utilized in voluntary contractions are innervated through groups of motor neurons specific to each muscle. The muscle units, which are the groups of innervated muscle fibers unique to each motor unit, receive the action potentials delivered by the motoneurons through the neuromuscular junction (Heckman & Enoka, 2012). Regardless of the purpose of a muscular contraction, the same principles of motor unit activation are followed. As the required force for a contraction increases, motor units are gradually recruited to contribute to muscular contraction. This orderly recruitment of motoneurons demonstrates the concept referred to as the size principle, directly pertaining to the diameter of the cell bodies of the α -motoneurons (Henneman, 1957). The size of the α -motoneuron is related to the net excitatory input needed to trigger an action potential. An inverse relationship exists between the resistance of a cell membrane to excitatory input (i.e., current) and the size of the soma of the α -motoneuron. The smallest α -motoneurons have the greatest resistance to current, resulting in a greater change in voltage for a given amount of current, according to Ohm's Law, resulting in less current required to reach the threshold for an action potential to be generated (Henneman et al., 1965a; Hursh, 1939; Solomonow, 1984). In an example of a muscular

contraction where force increases linearly from rest to maximal effort, the smallest α -motoneurons attain their excitatory threshold first and are recruited first to contribute to the output of force (Henneman et al., 1965b, 1965a). As descending drive and afferent input summate at the α -motoneuron pool, larger numbers of α -motoneurons are recruited along a gradient of size, with the largest α -motoneurons recruited last (Henneman, 1957). Additionally, fewer and smaller muscle fibers are generally innervated by smaller diameter α -motoneurons, providing the ability to produce controlled force (Conwit et al., 1999; Henneman et al., 1965b). Motor unit recruitment is one method by which motor units contribute to the control of force.

The second primary method through which motor units control the output of force is through the manipulation of firing rates. A single motor unit action potential triggers the muscle electrically and chemically to produce a single twitch force. Singular twitches do not last long enough to produce the tension required for movement such as gait or for prolonged voluntary force output. Motor units instead produce successive firings of action potentials at a rate faster than the muscle can relax, creating an overlap of twitch forces called a fused tetanus (Celichowski & Grottel, 1993). The rate of motor unit firing is measured in pulses per second. Firing rate increases with the intensity of a contraction (Milner-Brown et al., 1973a) and works in parallel with the orderly recruitment of motor units to control force output of a muscle. In linearly increasing force, the spatial, or physical, aspect of motor unit recruitment will saturate prior to maximal force output and as such, the pool of motor units further activates the muscle temporally through rate coding, increasing the firing rate of active motor units to achieve maximal force output (Hatze, 1977). Motor unit firing behavior is the term used to describe the various aspects of motor unit firings, such as the mean or peak firing rate and the variability of the firing rate.

1.1.2 Electromyography (EMG)

The assessment of neural activation of muscle has benefitted greatly from recent improvements in technology. Electromyographic recordings can be obtained from electrodes placed either on the surface of the skin or placed intramuscularly (Farina et al., 2004; Merletti et al., 2008; Merletti & Farina, 2008). The source of these signals comes

from the repeated firings of motor unit action potentials generated across muscle fiber membranes. Recordings of action potentials are multiphasic, meaning that the shape of the action potentials can involve multiple phases of positive or negative values. (Hodgkin & Huxley, 1939; Richfield et al., 1981). The shape of an action potential in an EMG recording is the result of a moving change in membrane potential caused by the traversal of Na^+ and K^+ ions across the sarcolemma, and the position of the EMG electrode relative to the active muscle fibers (Farina et al., 2004; Nielsen & Clausen, 2000). Surface EMG provides the summation of action potentials from all concurrently active motor units within the recording field of a sensor at any moment in time (Sanders et al., 1996). This resultant signal is called the EMG interference pattern, as the action potentials of separate motor units detected by an electrode fire closely in time, and their summation leads to a stochastic signal with a mean of nearly zero (Richfield et al., 1981; Sanders et al., 1996).

Motor unit action potentials vary in size and shape based on the electrophysiological traversal of the action potential across the muscle membrane. The duration of an action potential is dependent on the velocity with which it travels across the muscle, called muscle fiber conduction velocity (Hursh, 1939). The amplitude of an action potential is also strongly associated with the muscle fiber conduction velocity, with larger amplitude action potentials in muscle fibers with greater conduction velocities. (Arendt-Nielsen et al., 1989; Henneman, 1985; Milner-Brown et al., 1973b; Tanji & Kato, 1973).

Electromyographic signals can be recorded from the surface of the skin, or intramuscularly, using needle or fine wire techniques. Regardless of the technique, EMG electrodes record action potentials from a distance, and the electrical potentials must be conducted through a volume of tissue between muscle fibers and the electrode. This tissue has passive resistance to the conductance of electrical signals, functionally acting as a "low-pass filter" (Gygi & Moschytz, 1997). This low-pass filter effect alters the amplitude and frequency content of EMG signals, flattening and lengthening action potential shapes as the distance between the electrode and the active muscle fibers increases (Barkhaus & Nandedkar, 1994; Farina et al., 2004). Although the filtering effect of tissue is greatly reduced when the electrode is closer to the muscle fibers via intramuscular electrodes, the detection volume of these electrodes is smaller than that of

surface electrodes, limiting the volume of muscle fibers from which action potentials are recorded (Merletti & Farina, 2008). The area of muscle from which an electrode can detect action potentials, called the “pickup area,” can be altered by electrode configurations such as electrode size and distance between contact points, when used in bi-polar configuration (Fuglevand et al., 1992). The limited pickup area of both surface and intramuscular electrodes make it difficult to obtain measurements of the individual activity of every active motor unit within a muscle (Gygi & Moschytz, 1997; Lefever et al., 1982; Lefever & De Luca, 1982; Merletti & Farina, 2008).

Identifying singular motor unit action potentials from an EMG signal is a complicated process. Historically, the activity of individual motor units could not be identified from the interference pattern generated by surface EMG recordings. However, improvements in technology have allowed researchers to decompose the EMG signal into its constituent motor unit action potential trains first from intramuscular recordings (Lefever et al., 1982; Lefever & De Luca, 1982) and more recently, from surface EMG recordings (Chae et al., 2001; Merletti et al., 2008; Nawab et al., 2010). Surface EMG decomposition in particular, has yielded relatively high numbers of decomposed motor units from each participant (Nawab et al., 2010). Regardless of the method used, the recent improvements in decomposition of motor unit firings from the EMG signal has increased the accessibility of the field to new researchers. However, despite improvements in EMG decomposition, there remains a large variance in the number of motor units sampled across recording techniques, muscles, and participants within a study. There is currently no examination of how well various numbers of recorded motor units represent the activity of the entire active motor unit pool during a contraction.

1.2 Neuromuscular control of force

Isometric contractions offer a wide variety of force trajectory methodologies that provide information about motor unit firing behaviors. As the influence of descending drive and other synaptic inputs onto α -motoneurons in participants cannot be directly measured in live humans (Araki & Terzuolo, 1962), assigned force output trajectories for voluntary force tracing can be used to indirectly represent a relative amount of descending drive (Farina & Negro, 2015; Fuglevand et al., 1993; Kirk et al., 2021; Knight & Kamen,

2007). Contraction intensity and force prescription are typically represented as percentages of force from an individual's maximal voluntary contraction (MVC). Linearly increasing force templates can provide structured observations of motor unit recruitment thresholds (Park et al., 2016). Static assignments of force, where force is held at a percentage of MVC, allows for comparisons of MUFR in a steady state of motor unit activity (de Luca et al., 1996; Inglis & Gabriel, 2020; Orssatto et al., 2021; Parra et al., 2020). As MUFR and recruitment are both maximally saturated to achieve a maximal voluntary force output, relative percentages of this maximal force can be used to compare motor unit responses across levels of effort (Parra et al., 2020).

Prescription of a pre-determined force profile is important in experimental design for neuromuscular research. Neuromuscular function can change considerably between isometric and dynamic contractions (Kossev et al., 1992; Merlo et al., 2005; Pasquet et al., 2005; Trontelj, 1993). For example, changes to the length of a muscle during dynamic contractions can influence MUFR and conduction velocity (Maton, 1980; Merlo et al., 2005; Oliveira & Negro, 2021; Pasquet et al., 2005). Further, movement of the muscle below a surface EMG electrode during dynamic contractions can also cause displacement of fibers relative to the electrode which can alter the EMG signal. In contrast, isometric conditions provide a relatively stable structure of muscle, where factors that influence motor unit firing behavior and their recordings, such as muscle length, changing geometry of the muscle and relative sensor location can be controlled (Farina et al., 2002; Jensen et al., 1993; Kossev et al., 1992; Manal et al., 2006; Pasquet et al., 2005).

Variable force contractions, while still isometric, represent a complicated control process of MUFR modulation, recruitment and de-recruitment, and agonist-antagonist reciprocal activation (De Luca & Mambrito, 1987; Iyer et al., 1994; Park et al., 2016). These factors may be more relevant to activities of daily living than static contractions, as many tasks involve continuous adjustments to force levels. As such, changes to neuromuscular function observed during force-varying contractions offer an expanded examination of the factors influencing force control.

As the primary source of control for muscle, the firing behavior of motor units can be used to describe neuromuscular control of force. The relationship between MUFR and force output provides an expected pattern, where increased MUFR results in increased force (Conwit et al., 1999; Fuglevand et al., 1999). Expected patterns provide the basis for experimental conditions to identify unexpected alterations to these patterns in response to experimental conditions, for example during fatiguing conditions where MUFR can be decreased (Carpentier et al., 2001). The average MUFR, described as the activity of a group of motor units, is a common metric used to compare neuromuscular function between different muscles, populations and conditions (Bellemare et al., 1983; Fling et al., 2009; Kowalski et al., 2022; Kowalski & Christie, 2020; Macefield et al., 1996; Parra et al., 2020). Variability, or fluctuations in the firing rate of motor units are largely responsible for the fluctuations in a target force output (Farina & Negro, 2015; Moritz et al., 2005). These aspects of motor unit behavior and their relationship to force output provide multiple avenues of design for experimental study of the neuromuscular system.

1.3 Muscular control of force

Innervation and control of muscle are provided by the motoneurons in the spinal cord; however, the muscle fibers and their associated qualities also play an important part in force generation. Muscle fibers exist along a spectrum of contractile speed, fatiguability and twitch torque and are categorized based on their associated myosin heavy chain content and contractile speed (Bárány, 1967; Fry et al., 1994; Scott et al., 2001). Muscle fibers are grouped into two categories based on their speed of contraction; Type I and Type II fibers representing slow and fast twitch fibers, respectively, with 2 subcategories of type II: IIA, or IIB (Engel, 1998; Pette et al., 1999; Scott et al., 2001). Further investigation and development of staining methods revealed a continuum of fibers that can be further classified into up to seven categories (for review see Pette et al., 1999). Despite the numerous categories based on the myosin ATPase staining intensities of muscle fibers, the role of muscle in the neuromuscular system can be described conceptually through “fast” and “slow” type II and type I fibers with the acknowledgement that this is a generalization. The “slow” and “fast” in the description of

fiber types is a general reference to the speed and duration of a muscle twitch in response to a single depolarization of the innervating nerve (Sica & McComas, 1971). Slow twitch muscle fibers have a prolonged time to both the peak twitch force and return to a relaxed state after a twitch relative to faster twitch fibers. Muscle fibers with faster contractile properties also tend to have higher twitch torques. A fused tetanus in muscle is dependent on an overlap of force output between action potentials; the prolonged contraction times of type I fibers in response to a single stimulus require a lower firing rate to achieve tetanic contraction (Fuglevand et al., 1999; Raikova et al., 2007). The inverse applies for fast twitch muscle fibers which reach their peak force and relax quickly relative to slower muscle fibers.

The properties of skeletal muscle fibers are closely related to the size of the soma of α -motoneurons in the spinal cord by which they are innervated (Clark, 1931; Henneman & Olson, 1965). Recalling that recruitment threshold is dependent on the size of the α -motoneuron, there exists a relationship between muscular properties of contractile speed and twitch torque with neuronal properties of recruitment threshold and conduction velocity (Calancie & Bawa, 1985; Hursh, 1939; Milner-Brown et al., 1973b; Riek & Bawa, 1992). Faster and stronger twitch forces are strongly associated with the motor units recruited towards the end of a contraction (Andreassen & Arendt-Nielsen, 1987; Goldberg & Derfler, 1977). The firing rates of motor units are also suggested to be related to their recruitment thresholds (Bigland & Lippold, 1954; Gydikov & Kosarov, 1974). At lower contraction intensities, lower threshold motor units fire earlier and thus increase firing rates with increases in force, such that they are higher than the later recruited motor units at the moment of their recruitment (Moritz et al., 2005). The distributions of motor unit sizes and muscle fiber types differ depending on the muscle examined. For example, the tibialis anterior (TA) is comprised of approximately 73% type I fibers, while the first dorsal interosseus is approximately 50% type I fibers (Hwang et al., 2013; Porter et al., 2002). The TA is also estimated to have greater innervation ratios than the first dorsal interosseus (Duchateau & Enoka, 2022), suggesting larger motor units (i.e., greater number of muscle fibers per motoneuron) in the TA (Gordon et al., 2004; McPhedran et al., 1965; Wuerker et al., 1965). As such, maximal firing rates

and twitch properties can vary depending on the muscle examined (Bellemare et al., 1983; Kirk et al., 2021).

1.3.1 Muscle architecture

On a larger scale, the architecture of different muscles can influence force output. While the nervous system provides the stimulus required to generate sarcolemmal action potentials, the muscle-tendon complex is necessary to effectively generate force across the muscle (Ross et al., 2021). The muscle-tendon complex is the complete system of the muscle fibers within each muscle and the connective tissue through which muscle contractile force is applied to the skeleton for motion. There are various qualities of the muscle-tendon complex that can influence force output, such as the overall size of the muscle and internal organization of the fibers.

The size of the muscle, quantified by measures of its cross-sectional area (CSA) or muscle thickness (MT), has strong correlations to muscular strength (Abe et al., 2015; Alway et al., 1990; Franchi et al., 2018; Maughan et al., 1983; Stock et al., 2017). Increases in CSA and MT are primarily driven by hypertrophy, or enlargement, of existing muscle fibers (MacDougall et al., 1979). Within each muscle, fibers are bundled together into groups called muscle fascicles, which are independent in organization relative to muscle unit groups (Borg & Caulfield, 1980; Sjöström et al., 1986). The fascicles of some muscles present at an angle to the axis (aponeurosis) through which force is applied to the tendon (Rodríguez-Rosell et al., 2018). Muscles with angled fascicles are called pennate, and the angle at which the fascicles attach to the central aponeurosis is the pennation angle (Kwah et al., 2013). The angular attachment of pennate fibers within a muscle allows a larger number of muscle fibers to be attached to the central aponeurosis (Jones & Rutherford, 1987). The density of a muscle, fiber length, and the angle of pennation within a muscle are used to calculate the physiological CSA. Accordingly, the pennation angle can contribute to a greater number of muscle fibers in the same CSA, resulting in greater force (Wickiewicz et al., 1983). Indeed, pennation angle has been shown to increase following resistance training and is suggested to contribute to observed increases in strength (Aagaard et al., 2001). Researchers observed that the CSA of single muscle fibers increased by 16%, and the pennation angle by $\sim 2^\circ$

compared to total muscle CSA increases of only 10% (Aagaard et al., 2001). Pennation angle can also be easily assessed in human participants using ultrasonography (Hodges et al., 2003).

1.4 Fatigue

Fatigue is a universally experienced symptom in everyday life and can impact the function of the neuromuscular system. The sensation of “weariness”, as it can be described subjectively, is a multifactorial physical or mental state (Aaronson et al., 1999). Performance fatigue and perceived fatigue interact to impair physical and cognitive function (Enoka & Duchateau, 2016). In the framework of athletic performance and neuromuscular function, performance fatigue is defined as the reduction in the muscular capacity to produce force (Enoka & Duchateau, 2016; Kent-Braun et al., 2012). A relatively simple method of identifying performance fatigue is through a reduction in a produced vs. prescribed force, such as an inability to repeat an MVC, or an inability to maintain a target force level after a prolonged period of time (Kent-Braun, 2009; Kent-Braun et al., 2012). However, the source of performance fatigue within the neuromuscular system can vary. Central fatigue can be seen in alterations in the activation of the primary motor cortex and motor unit activity, and peripheral fatigue can be the cause of alterations in one or many of potential muscular components (Bigland-Ritchie, 1981; Kent-Braun et al., 2012). However, perceived fatigue is specific to the mental state and trait of an individual and can influence athletic and cognitive performance (Boksem & Tops, 2008; Habay et al., 2021; Marcora et al., 2009). Mental fatigue is a psychophysiological state resulting from prolonged periods of sustained attention and cognitive activity which is a common symptom of modern workplaces (Boksem et al., 2005; Caldwell et al., 2019). Understanding the mechanisms behind the decrements observed during mentally fatiguing conditions can aid in the development of appropriate methods of mitigating its detrimental effects on athletic and cognitive performance.

1.4.1 Mental fatigue and neuromuscular function

Mental fatigue results in the impairment of the ability to regulate attention (Boksem & Tops, 2008; Grillon et al., 2015). The influence of mental fatigue on performance seems to be driven by the perception of effort and the subsequent changes in the brain associated with prolonged cognitive activity (Boksem & Tops, 2008; Hopstaken et al., 2016; Martin et al., 2018). Multiple tasks requiring prolonged attention and cognitive activity have been used to induce mental fatigue. Two of the most common tools include the Psychomotor Vigilance Task, and the prolonged Stroop task (Smith et al., 2019). An advantage of the Psychomotor Vigilance Task is that mental fatigue is evident after 20-30 minutes, while other tasks require 60-90 minutes (Smith et al., 2019). Regardless of the tool used, reductions in task performance and subjective ratings of fatigue are commonly used to quantify mental fatigue in participants (Boksem & Tops, 2008; Lim et al., 2010; Lim & Dinges, 2008).

Effects of mental fatigue on neuromuscular function have been observed through changes in MUFR, EMG amplitude, and skill-based performance (Bray et al., 2008, 2012; Habay et al., 2021; Kowalski & Christie, 2020). Currently, the proposed mechanism for performance decrements with mental fatigue is that alterations in motivation to continue a task are driven by changes in dopamine and adenosine in the brain with prolonged cognitive effort (for review see Martin et al., 2018). Changes in motivation and neurotransmitter concentrations after a prolonged task could potentially change neuromuscular activation strategies of mentally fatigued participants as seen in changes to EMG activity after a mentally fatiguing task (Bray et al., 2008; Pageaux et al., 2015). Mental fatigue does not seem to have a strong influence on peripheral factors such as contractile properties (Kowalski et al., 2022; Kowalski & Christie, 2020). Previous examinations of MUFR in response to mental fatigue have only been performed in the TA (Kowalski et al., 2022; Kowalski & Christie, 2020).

1.5 Sex-related differences in neuromuscular function

The size principle of motor unit recruitment is made possible by the distribution of motoneurons of variable diameter (Henneman et al., 1965b). Measuring the distributions

of size and quantity of these motoneurons in humans is challenging as these procedures require dissection of the spinal cord. As such, relatively few measurements of motoneuron composition have been performed in humans (Irving et al., 1974; Kawamura, Okazaki, et al., 1977; Kawamura, O'Brien, et al., 1977; Yuan et al., 2000). From the few existing studies, it has been demonstrated through autopsy that males generally have larger average α -motoneuron diameter than females (Yuan et al., 2000). Despite this difference in relative size of the α -motoneurons, there does not appear to be sex-related differences in total number of motor units (Gawel & Kostera-Pruszczyk, 2014; Yerdelen et al., 2006). However, it is possible that the difference in distribution of size of α -motoneurons may result in difference in motor unit firing behaviors used to control force.

These differences are further compounded by the interaction of motor unit sizes and behaviors on EMG signals and decomposition. Computerized estimations of motor unit activity have recently suggested that surface decomposition has a tendency to oversample motor unit action potentials of larger amplitudes (Caillet et al., 2022). However, the exact effect of this on actual female motor unit decomposition is unknown. A recent review by Lulic-Kuryllo & Inglis in 2022 identified only 11 publications on sex differences in MUFR and recruitments. Additionally, from 68 studies retrieved, only 18 had the sex-separated data available, and only 10 were from female-only samples.

Difference in motor unit firing behavior between males and females varies considerably. For example, contraction intensity dependent patterns have been seen when comparing firing rates in males and females. In the vastus lateralis and vastus medialis, females had higher firing rates than males at 10% and 25% MVC in the former, and higher firing rates up to 75% MVC in the latter (Guo et al., 2022; Peng et al., 2018). In the TA, MUFR were higher in females relative to males at submaximal contraction intensities, but this result was reversed at maximal intensities (Inglis & Gabriel, 2020). Understanding how neuromuscular control may be different between males and females will aid in our understanding of neuromuscular physiology, which can potentially be applied to disease states and rehabilitative and preventative strategies unique to males and females (Drost et al., 2006; Hunter, 2014; Phan et al., 2019; Shultz & Perrin, 1999).

1.6 Sex-related differences in control of force

Despite the difficulties in quantifying motoneuron size and quantity in humans, assessments of motor unit activity, EMG, and force provide insight into sex-related differences in neuromuscular function. Sex-based comparisons of motor unit firing behavior have shown that females can sometimes demonstrate faster firing rates than males at similar levels of force output when matched for strength (Inglis & Gabriel, 2020). The initial firing rate at recruitment and the variability of firing rates in females are also higher than in males (Inglis & Gabriel, 2021; Peng et al., 2018). Further, motor unit firing behavior in the form of rapid, unsustainable levels of motor unit firing, referred to as doublets (Simpson, 1969), occur more frequently in females than in males (Inglis & Gabriel, 2021), and are thought to have an influence on the rate of force development (Aagaard, 2003; Christie & Kamen, 2006; Van Cutsem et al., 1998). Females also exhibit less fatigability in certain tasks and muscles, such as single-limb isometric contractions, and differences in contractile function (Hunter et al., 2006; Hunter, 2014). The differences in contractile function and fatiguability in females are often attributed to larger proportions of type I fibers in females than males (for review see Hunter, 2014).

The variability of force output and MUFR can also provide insight into sex-related differences in motor unit firing behavior and force control. Force output during isometric contractions fluctuates around a target value, and the quantification of this fluctuation is the variability of force, also referred to as force steadiness, with less variable force representing greater steadiness. Absolute values of force variability increase with increasing contraction intensity (Brown et al., 2010). However, when adjusted relative to a participant's maximal force output, the relationship of relative force variability and relative contraction intensity are inverted; as contraction intensity is increased, force variability decreases up to 50-75% of a participant's MVC (Brown et al., 2010; Inglis & Gabriel, 2021; Tracy & Enoka, 2002). Force variability is thought to be a function of both the variability of MUFR and the number of active motor units during a contraction (de C. Hamilton et al., 2004; Farina & Negro, 2015; Jesunathadas et al., 2012). Previous observations show that females have higher mean MUFR, but also higher relative force

variability. (Inglis & Gabriel, 2020, 2021; Jakobi et al., 2018). Of note, although female force output is more variable, males and females exhibit the same pattern of force variability across contraction intensities, with an increase in force variability from low to moderate forces (5-80% MVC) and a reduction with higher relative forces (Inglis & Gabriel, 2021; Jakobi et al., 2018; Yoon et al., 2014).

1.6.1 Sex-related differences in muscle architecture

Both type I and type II muscle fiber CSA is greater in males than females, with a larger difference observed in type II fibers (Simoneau & Bouchard, 1989). As type II fibers are typically greater in size and tension generating capability, this has a significant influence on the discrepancy in muscular size and strength between males and females (Brunner et al., 2007; Miller et al., 1993). Sex-related differences also exist in pennation angle, with smaller angles and smaller absolute change in pennation angle from rest to maximal contraction in females compared with males (Manal et al., 2006). Differences in pennation angle have recently been suggested as a potential factor contributing to the sex-related differences in MUFR (Inglis & Gabriel, 2020), however, currently, no studies have examined both MUFR and pennation angle in males and females.

1.6.2 Sex-related differences to mental fatigue

Sex differences may exist in how mental fatigue affects neuromuscular function. The recorded changes to endurance performance after mental fatigue are present without any changes to cardiovascular mechanisms or blood lactate (Marcora et al., 2009; Pageaux et al., 2015). The lack of these changes suggests that other factors contribute to performance degradations with mental fatigue. Females report higher ratings of subjective fatigue (Engberg et al., 2017; Manierre et al., 2020; Schwarz et al., 2003) and as such a sex-specific influence on neuromuscular function could be present (Pageaux & Lepers, 2018). A previous study has demonstrated greater increases in subjective fatigue in females after a mentally fatiguing task when compared to males (Kowalski et al., 2022). Force output in females is more variable with generally a higher resistance to fatigue in sustained isometric contractions (Hunter et al., 2009; Jakobi et al., 2018). Further evidence of sex-related differences in motor unit behavior in females, such as increased variability and

average of MUFR, suggest that central mechanisms altered by mental fatigue may cause different responses in males and females (Inglis & Gabriel, 2020, 2021; Jakobi et al., 2018). Previous examinations of sex-related differences in motor unit firing behavior during steady state isometric contractions in the TA suggest that neither males nor females experience substantial changes to neuromuscular function in the presence of mental fatigue (Kowalski et al., 2022; Kowalski & Christie, 2020). However, this interaction of sex and mental fatigue has only been examined in the TA. As upper and lower limb muscles have differences in motor representation within the primary motor cortex (Roux et al., 2020), different innervation ratios (Duchateau & Enoka, 2022) and different fiber type distributions (Hwang et al., 2013; Porter et al., 2002) the influence of mental fatigue may yield different results when examined in a different muscle.

1.7 Purpose

The overall objective of this dissertation was to further examine sex-related differences in factors that can impact assessments of neuromuscular function. Specifically, these factors included: (i) a cortical influence through the examination of the impact of mental fatigue on neuromuscular function, ii) the influence of muscle architecture through the examination of the relationship between muscle pennation angle and neuromuscular function, and iii) the influence of the methodological limitations on measures of neuromuscular function through the impact of the number of recorded motor units on MUFR calculations.

Chapter 2 was an investigation of the sex-specific influence of mental fatigue on neuromuscular function in the first dorsal interosseus, in static and time-varying contractions. We examined sex-related differences in MUFR and their variability before and after a mentally fatiguing condition. It was hypothesized that i) participants would increase in error during a complex force tracing task after a mentally fatiguing task; and ii) females would have a larger increase in this task-error along with increased force and MUFR variability compared to males.

Chapter 3 was an examination of sex-specific differences in muscle architecture and MUFR. We examined pennation angles and MUFR of male and female participants across a range of contraction intensities from sub-maximal to maximal intensities. The hypotheses were: i) pennation angle and MUFR would both increase with the contraction intensity with a positive correlation and ii) females would have less change in pennation angle with larger increases in MUFR across contraction intensities, compared to males.

Chapter 4 examined how MUFR calculated with varying numbers of motor units influences the mean firing rate calculation and how this may be different by sex. This analysis was performed on a previously collected data set that included decomposed MUFR from multiple contractions at various intensities in equally sized groups of males and females. The hypotheses were: i) higher numbers of motor units would increase the probability of obtaining a firing rate representative of the entire active motor unit pool would increase; ii) the number of motor units required to obtain a firing rate representative of the entire active motor unit pool would increase with increasing contraction intensity; and iii) the number of motor units for each level of probability would be the same between males and females.

1.8 References

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Chapter 2

2 Neuromuscular behavior following mental fatigue in the first dorsal interosseus

2.1 Introduction

Mental fatigue is a psychophysiological state that occurs after prolonged periods of cognitive activity or sustained attention (Boksem et al., 2005). Mental fatigue can lead to an inability to efficiently pay attention to a task (Boksem et al., 2005; Boksem & Tops, 2008) and is characterized by a combination of subjective ratings of increased fatigue and perceived exertion, or behaviorally through changes to attention to a task (Boksem et al., 2005, 2005; Boksem & Tops, 2008; Lim et al., 2010; J. Van Cutsem et al., 2017) (Boksem et al., 2005; Boksem & Tops, 2008; Lim et al., 2010; J. Van Cutsem et al., 2017). Boksem and colleagues (2008) described the process of mental fatigue as a subconscious feeling when the energy cost of a task outweighs the perceived benefits. These changes in perception of effort reduce motivation and alertness (Boksem et al., 2005; Lorist et al., 2002; van der Linden & Eling, 2006) and result in a reduction in attention that can lead to increased reaction time (Marcora et al., 2009) and greater task error (Lorist et al., 2000).

Further assessments of mental fatigue have demonstrated reductions in endurance performance (Pageaux & Lepers, 2018; J. Van Cutsem et al., 2017), sport-related decision making, and psychomotor performance (Duncan et al., 2015; Habay et al., 2021). For example, a reduction in time to exhaustion was observed in participants who had an increased perception of effort in a cycling task after 30-90 minutes of a mentally fatiguing task, despite no changes in cardiorespiratory mechanisms or blood lactate measurements (Marcora et al., 2009; Pageaux et al., 2015). In another study on self-paced endurance, running speed was reduced and participants reported higher levels of perceived exertion during the exercise task after 30 minutes of an incongruent Stroop task with no change in heart rate or blood lactate (Pageaux et al., 2013). The lack of observed changes in cardiorespiratory factors amidst performance reductions suggest other factors contribute to the changes in performance with mental fatigue.

Previous research on neuromuscular function has shown that mental fatigue may influence performance of submaximal, but not maximal isometric contractions, nor contractile properties (Kowalski et al., 2022; Kowalski & Christie, 2020; Pageaux et al., 2015). In submaximal isometric handgrip tasks, increased surface electromyographic (EMG) activity and a reduced time to exhaustion were observed, with no change in EMG amplitude observed during a maximal effort (Bray et al., 2008, 2012). In contrast, a reduction in surface EMG amplitude was observed in the TA at 10% MVC, with no change in motor unit firing rate (MUFR) after a mentally fatiguing task (Kowalski et al., 2022). Collectively, these results indicate that the consequences of mental fatigue on neuromuscular function remain unclear and are potentially different between muscles.

Outcomes of neuromuscular function can vary across muscles, and physiological differences in both the spinal cord and at the muscular level can influence MUFR. The difference in muscles from motoneuron size distributions, and muscle fiber type change the proportion of recruitment and firing rate contributions to specific force output for each muscle (Del Vecchio et al., 2018; Moritz et al., 2005). The first dorsal interosseous (FDI) and the tibialis anterior (TA), specifically, have different ranges of MVC at which recruitment of new motor units saturates. In the FDI, recruitment has been reported to occur up to 70% MVC (De Luca & Hostage, 2010) and up to 90% in the TA (Van Cutsem et al., 1997). The differences in recruitment ranges can change the contribution of firing rate to force output (De Luca et al., 1982; Feiereisen et al., 1997; Milner-Brown et al., 1973). Relative to the FDI, the TA has a higher innervation ratio, greater cross-sectional area, and a different primary function (fine motor control vs. gait) (Allen et al., 2014; Duchateau & Enoka, 2022; Johnson et al., 1973). Given the anatomical, physiological, and functional differences between the FDI and TA, the neuromuscular function of the FDI could have a different response to mental fatigue than the TA.

To date, motor unit firing behavior in response to mental fatigue has only been assessed in the TA. Other studies including surface EMG measures during mental fatigue in the upper limb have been performed on the forearm during handgrip tasks (Bray et al., 2008, 2012). These studies have employed static isometric contractions at an assigned force level. These static contractions provide clear measurements for neuromuscular function

but lack applicability to the variable motion of daily activities or exercise. Motor unit firing behavior is different during various phases of a contraction depending on the trajectory of the force output (Knight & Kamen, 2004; Park et al., 2016). Variable force contractions and the associated modulation of firing rates could therefore have different responses than static contractions to mentally fatiguing conditions,

Motor unit firing rates are also different between males and females, with the latter having higher MUFs at similar force levels (Lulic-Kuryllo & Inglis, 2022). Compared to males, females have higher levels of subjective feelings of physical and mental fatigue (Engberg et al., 2017; Manierre et al., 2020; Schwarz et al., 2003). Females also have greater increases in reports of subjective fatigue following a cognitive task compared to males (Kowalski et al., 2022). Higher ratings of subjective fatigue and perceived exertion as well as differences in modulations of firing rate (Inglis & Gabriel, 2021) and force steadiness (Jakobi et al., 2018) compared to males, suggests there could also be sex-specific responses to mental fatigue. In a previous study, a sex-specific response was observed, in which MUFs declined following mental fatigue in males but not females, during contractions at 50% MVC (Kowalski & Christie, 2020). However, a follow up study found no sex differences (Kowalski et al., 2022).

The purpose of this study was to further explore sex-related differences in the impact of mental fatigue on neuromuscular function in the first dorsal interosseous (FDI) during both static and variable force contractions. We sought to expand on previous assessments using a variable force contraction in the FDI. As task error increases with mental fatigue (Lorist et al., 2000) we hypothesized that the performance of a complex force tracing task would show an increase in error after a mentally fatiguing task. As well, we hypothesized that females would have a larger increase in task-error in conjunction with their higher force variability, and greater increases in motor unit firing variability in the presence of mental fatigue, compared to males.

2.2 Methods

2.2.1 Participants

Twenty-five participants, aged 23 ± 2.9 years, were recruited from the local university community (13 female and 12 male). All participants reported normal or corrected-to-normal vision, were free of any musculoskeletal or neurological impairments, and were not taking any medication which may alter cognitive or neuromuscular function. Prior to enrollment, each participant provided written informed consent according to procedures approved by the Health Sciences Research Ethics Board at the University of Western Ontario.

2.2.2 Experimental protocol

Prior to starting the protocol, all participants completed three surveys, the Pittsburgh Sleep Quality Index (PSQI), the Multidimensional Fatigue Index (MFI) and the International Physical Activity Questionnaire (IPAQ). Participants then visited the lab on two separate days, one day where the protocol was performed with a mentally fatiguing task, and another day for a control condition. The order of conditions was randomized across days.

On the first visit to the lab participants were acquainted with the setup and after obtaining an initial maximal voluntary contraction (MVC) a practice session was performed. The practice session consisted of 12 force tracing tasks. The first and last trial was a series of contractions to plateaus at 10, 20 and 50% MVC for 1 second each, and the middle 10 trials were a sine wave force template, centered around 20% MVC. This practice session was performed on the first visit only. The experimental protocol consisted of participants tracing a series of steady force and variable force (sine wave) templates before and after a mentally fatiguing task or control condition. The order of the conditions and force tracings were randomized for each participant. An MVC was then performed at the end to assess fatigue.

Self-reported fatigue on 10-point Likert scale was recorded (1 = not tired at all, 10 = very tired; Kowalski and Christie, 2020) before and after the mentally fatiguing and control conditions.

2.2.3 Questionnaires

The PSQI is a self-report questionnaire used to measure sleep and sleep quality in seven domains: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, sleep medication use, and daytime dysfunction. For each question, participants provide a rating from 0-3 with lower scores indicating healthier sleep and a total score across all questions of 5 or above indicates poor sleep quality (Buysse et al., 1989). The MFI is used to assess general fatigue in a self-report questionnaire of 20 items with higher scores indicating greater fatigue (Smets et al., 1995). The IPAQ is used to gather data on health-related physical activity in 5 domains: job-related physical activity, transportation physical activity, housework/house maintenance and family physical activity, recreational/sport/and leisure-time physical activity, and time spent sitting.

2.2.4 Sustained attention task

Participants performed the Psychomotor Vigilance Task (PVT) to induce mental fatigue. Participants were seated 2 meters away from a computer monitor used to display the PVT. Every 2-10 seconds, a red number counter on a black screen appeared and began to count up in milliseconds (Dinges & Powell, 1985). Participants were instructed to click the left mouse button in their dominant hand as fast as possible and performed the PVT for 30 minutes. When the PVT is performed for 20 minutes or more, a slowing of reaction time (RT) and reduction in task accuracy occurs indicating mental fatigue (Lim et al., 2010). False starts (FS) where participants click with no stimulus and lapses (RT > 500ms) were also recorded (Lee et al., 2010; Lim et al., 2010).

2.2.5 Control task

For the control condition, participants watched a 30 minute clip of the *Earth* documentary which follows the migration paths of four animal families (Fothergill & Linfield, 2007). This control task has been used previously as an emotionally neutral condition for

participants (Kowalski et al., 2022; Pageaux et al., 2013). Sound was transmitted through noise-cancelling headphones and the documentary was displayed on the same screen utilized for the PVT.

2.2.6 Force

Participants were seated adjacent to a custom-built apparatus designed to measure abduction force of the first finger and their force was displayed in real-time on a monitor 2 meters in front of them. All participants used their non-dominant FDI with their hand pronated in the device. Participants traced 4 different force curves: 3 trapezoidal force curves at 10%, 20% and 50% of their MVC, and a sinusoidal condition at $20 \pm 5\%$ MVC at a rate of 0.15 Hz. All force templates traced had a 3 second flat period before the ramp, with a ramp up and down of 10% MVC/second and participants performed two of each force tracings before (pre) and after (post) the PVT or documentary. These force tracings were randomized for each participant on each day, and the same randomized order was used for the pre and post conditions on each day. The timeline of the protocol is presented in Figure 2.1. A custom-written Matlab (2021a) program was used to calculate the root-mean-squared-error (RMSE), mean force and the coefficient of the variation (CV) of the force. All force outcomes were measured within the middle 3 seconds of the trapezoidal conditions, and the middle period (~6.6 seconds) of the sinusoidal condition. The average of the two repetitions was used for each pre and post measurement.

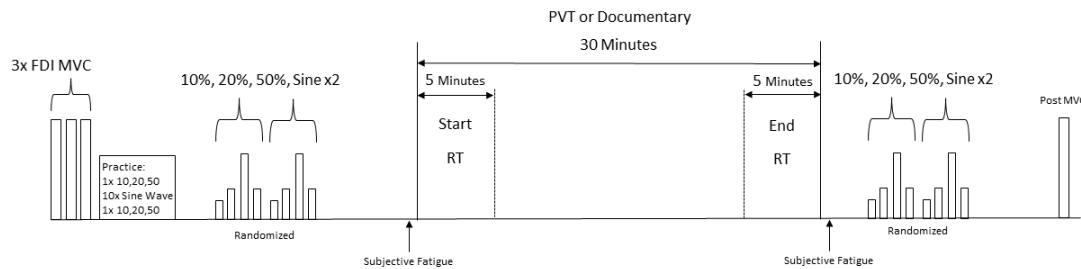


Figure 2.1 Timeline of experimental protocol

Outline of the order of experimental procedures on the first testing day. The second day did not involve the practice block. Vertical arrows represent time points and repetitions of subjective fatigue assessments. Horizontal arrows represent time windows.

2.2.7 EMG signal and motor unit processing

A wireless 4-pin surface EMG electrode (Galileo wireless EMG, Delsys Inc., Natick, MA, USA) was attached to the posterior of the FDI muscle with a reference electrode attached to the back of the wrist. Surface EMG signals were sampled at a rate of 2222 Hz, band-pass filtered between 20-450 Hz (Delsys Trigno Wireless System, Delsys inc., Natick, MA, USA) and collected on EMGWorks acquisition software (version 1.2.2, Delsys Inc., Natick, MA, USA). The skin at both the electrode and reference sites were abraded (Nuprep®) and swabbed with alcohol prior to application of the sensor. The sensors were held in place with double-sided tape, fit for the electrodes. The EMG signals were decomposed into their motor unit firing trains using the Precision Decomposition III algorithm (Neuromap v1.2; Delsys De luca et al., 2006; Nawab et al 2010) and tested for accuracy using the Decompose-Synthesize-Decompose-Compare test (De Luca and Contessa, 2012) using Neuromap software (Delsys inc., Natick, MA, USA). Motor units below 80% accuracy were excluded from analysis. Motor unit firings were further excluded if they had fewer than 10 discharges, an inter-pulse intervals less than 10

milliseconds (doublet discharges), or an inter-pulse interval greater than 200 milliseconds. Motor unit firing rate and CVISI were calculated from the average of all motor units recorded within the windows of interest. A custom-written Matlab program was used to calculate MUFR and the variability of the inter-spike intervals (CVISI). The average of these values from each of the two trials pre and post for each condition were used. An example of the output of motor unit firings during a variable force contraction is presented in Figure 2.2.

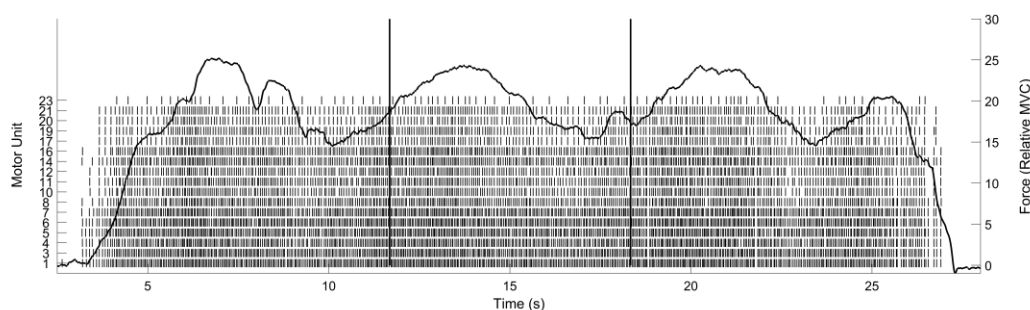


Figure 2.2 Example sine wave force and motor unit decomposition

Sample force and motor unit data. Example of a variable force trial for one participant. The identified motor unit firings from the surface decomposition presented underneath a participant's force tracing recording. Motor unit number assigned from the decomposition program is on the left y-axis. The vertical bars represent the window in which Mean, RMSE, CV of force, MUFR, and CVISI were calculated.

2.2.8 Statistical analysis

A two-way (time, sex) repeated-measures ANOVA was used to determine differences in PVT outcomes (RT, Lapses, and FS). A three-way (time, sex, condition) repeated-measures ANOVA was used to evaluate differences in RMSE, mean force, CV of force, MUFR and CVISI. Independent samples t-tests were used to compare participant characteristics between sexes, and the survey results from the MFI, PSQI and IPAQ. Statistical analyses were performed with SPSS (Version 28; IBM SPSS Statistics, Armonk, NY, USA). Outliers within each sex for each force condition were excluded if they were below the 1st quartile – 1.5* the interquartile range, or above the 3rd quartile + 1.5* the interquartile range. Significance was set at $p \leq 0.05$, and all data are presented as mean \pm SD. Effect sizes are presented as partial eta squared (η^2_p) and interpreted as small

($\eta^2_p = 0.01$), medium ($\eta^2_p = 0.6$) and large ($\eta^2_p = 0.14$) (Cohen, 1988). Due to technical issues, one male and one female were excluded from the analysis of 10% MVC.

2.3 Results

2.3.1 Participant characteristics

Participant characteristics and survey results are presented in Table 1. Males were taller and heavier than females ($p < 0.02$) with no significant difference in BMI ($p = 0.9$). Males had higher MVC ($p < 0.001$) with no differences between sexes in MFI ($p = 0.6$), PSQI ($p = 0.8$) or IPAQ scores ($p = 0.2$).

Table 2.1 Characteristics for all 25 participants

	Females	Males
Age (years)	22.5±2.6	24.1±3.1
Height (m)*	1.65±0.04	1.81±0.06
Weight (kg)*	67.1±15	79.5±8.4
BMI (kg/m ²)	24.3±5	24.1±2.2
MFI Total	61±4.8	59.8±5.6
PSQI	5.3±2.9	4.9±3.4
IPAQ (Continuous, METS/week)	4083.1±3091.1	2248.1±2727
MVC Pre (N)*	19±4.6	31±4.8
MVC Post (N)*	18.1±4.3	28.7±5.1
Motor Units (%MVC)		
10%		
Pre	11.7±7.4	9±3.7
Post	11.6±6.5	8.9±4.21
20%		
Pre	16.4±8.5	14.7±5
Post	17.1±9.0	14.3±5.3
50%		
Pre	31.1±12.0	27.3±7.1

Post	31.1±12.2	24.2±8.9
Sine Wave		
Pre	28.5±8.9	25.4±7.9
Post	30.4±10.2	26±8.1

Note. * Indicates significant difference between sexes ($p < 0.05$). Data are mean \pm SD.

2.3.2 Mental fatigue

The outcomes from the PVT are presented in Table 2. During the last five minutes of the PVT, participants had a slower reaction time compared to the first five minutes ($p = 0.001$, $\eta^2 = 0.47$). Males had faster RT than females ($p = 0.02$, $\eta^2 = 0.20$), with no significant time by sex interaction ($p = 0.5$). There were no significant main effects of time ($p \geq 0.08$), sex ($p = 0.2$), or time by sex interaction ($p \geq 0.5$) for the number of lapses or false starts.

Subjective fatigue reports increased over time on both days ($p \leq 0.001$, $\eta^2 = 0.41$), with a main effect of day ($p = 0.02$, $\eta^2 = 0.11$) in which subjective fatigue reports on the PVT day were higher than the documentary day. There was a main effect of sex on subjective fatigue ($p = 0.05$, $\eta^2 = 0.08$) in which females reported higher feelings of subjective fatigue. There were no significant interactions of time and sex ($p = 0.3$), time and day ($p = 0.06$), day and sex ($p = 0.7$), or time, sex and day ($p = 0.9$) on subjective fatigue.

Table 2.2 Psychomotor vigilance task outcomes

	Start/Pre	End/Post
Subjective Fatigue ^{*‡†}		
PVT		
Females	4.8±1.7	6.2±1.3
Males	3.7±1.3	5.6±1.5
Documentary		
Females	4±1.3	4.6±1.2
Males	3.4±1.2	4.4±2.2
Reaction Time [†]		
Females	269.4±22.1	318.1±48.7
Males	249.8±32.8	286.5±32.8
Lapses		
Females	0.7±0.8	1.1±1.9
Males	1.1±1	2±2.4
False Starts		
Females	0.7±0.5	0.3±0.5
Males	0.8±0.5	0.5±0.5

Note. *indicates significant difference between sexes ($p<0.05$). †indicates significant effect of time. ‡ indicates significant effect of days ($p<0.05$). Data are mean \pm SD.

2.3.3 Force and neuromuscular function

2.3.3.1 Maximal voluntary contraction

For each force level, any participants with outliers for RMSE, CV, or mean force within each sex were excluded. For MVC, one female and one male were excluded as outliers. There was a main effect of time on MVC ($p<0.001$, $\eta^2=0.27$) where MVC was reduced over time, with a significant effect of sex ($p<0.001$, $\eta^2=0.64$) where males were higher than females. There was no significant effect of day ($p=0.9$), nor any significant interactions ($p\geq 0.07$).

2.3.3.2 10% Contraction intensity

Force outcomes for 10% MVC are presented in Figure 2.3. For the 10% MVC contractions, 4 females and 3 males were excluded from the analysis as outliers and one female and one male were excluded due to technical issues with collection, therefore the analysis was performed on 7 females and 7 males. Mean and CV of force had no significant main effect of time ($p \geq 0.3$, $\eta^2 \leq 0.05$), sex ($p \geq 0.06$, $\eta^2 \leq 0.14$) or day ($p \geq 0.8$, $\eta^2 \leq 0.004$). Although the sex effect was not significant for CV of force, there was a large effect size ($\eta^2 = 0.14$), as the mean CV was greater in males. There were no significant interactions for mean ($p \geq 0.2$, $\eta^2 \leq 0.08$) or CV of force ($p \geq 0.3$, $\eta^2 \leq 0.05$). The RMSE of force at 10% had no main effects of time ($p = 0.8$, $\eta^2 = 0.002$), sex ($p = 0.8$, $\eta^2 = 0.003$), or day ($p = 0.9$, $\eta^2 \leq 0.001$) with no significant interactions ($p \geq 0.2$, $\eta^2 \leq 0.06$).

On the documentary day, 133 motor units were identified before the intervention and 143 after. On the PVT day, 143 motor units were identified before the intervention and 139 after. Motor unit firing behavior outcomes for 10% MVC are presented in Figure 2.4. Motor unit firing rate at 10% significantly increased over time ($p = 0.04$, $\eta^2 \geq 0.16$), with no significant effect of sex ($p = 0.06$, $\eta^2 = 0.09$), or day ($p = 0.9$, $\eta^2 = 0.001$). There were no significant interactions for MUFRR ($p \geq 0.1$, $\eta^2 \leq 0.09$). The CVISI had no significant effects of time ($p = 0.09$, $\eta^2 = 0.11$), sex ($p = 0.08$, $\eta^2 \geq 0.12$) or day ($p = 0.2$, $\eta^2 \geq 0.06$). There were also no significant interactions for CVISI ($p \geq 0.09$, $\eta^2 \leq 0.11$).

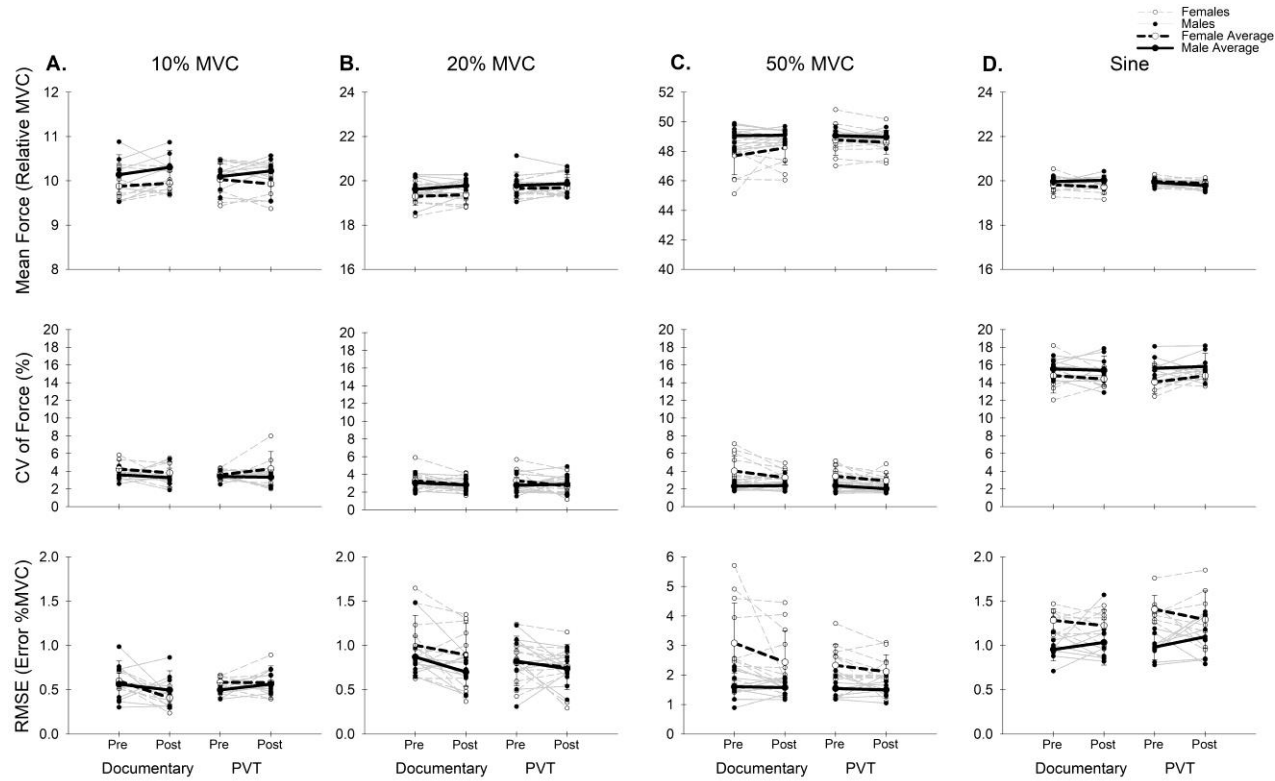


Figure 2.3 Force outcomes

Individual data are presented in gray lines, and averaged data presented in black. **A: 10% MVC.** There were no significant changes over time, nor any effects of sex or day or interactions. **B: 20% MVC.** RMSE was significantly reduced over time ($p=0.02$) in both sexes. **C: 50% MVC.** In both sexes, CV of force was reduced over time ($p=0.03$). Males had higher mean force ($p=0.005$), lower CV of force ($p<0.001$) and less error ($p<0.001$) than females. **D: Sine Wave.** Males had less error than females ($p<0.001$), but there was an improvement (reduction) in error in females ($p<0.05$). Males also had higher CV of force ($p=0.005$).

2.3.3.3 20% Contraction intensity

Force outcomes for 20% MVC are presented in Figure 2.3. During the 20% MVC contractions, 2 females and 2 males were excluded from the analysis as outliers, therefore the analysis was performed on 11 females and 10 males. There was no significant change over time in mean ($p=0.2$, $\eta^2=0.05$) or CV of force ($p=0.09$, $\eta^2=0.07$). Males had higher mean force compared to females ($p=0.04$, $\eta^2=0.1$), but there was no significant difference in CV of force ($p=0.6$, $\eta^2\geq 0.008$). There were no significant interactions for mean force ($p\geq 0.4$, $\eta^2\leq 0.08$) or CV of force ($p\geq 0.2$, $\eta^2\leq 0.05$). The RMSE of force at 20% was reduced over time ($p=0.02$, $\eta^2=0.13$), with no significant main effects of sex ($p=0.2$, $\eta^2=0.03$) or day ($p=0.2$, $\eta^2=0.08$) and no significant interactions ($p\geq 0.3$, $\eta^2\leq 0.03$).

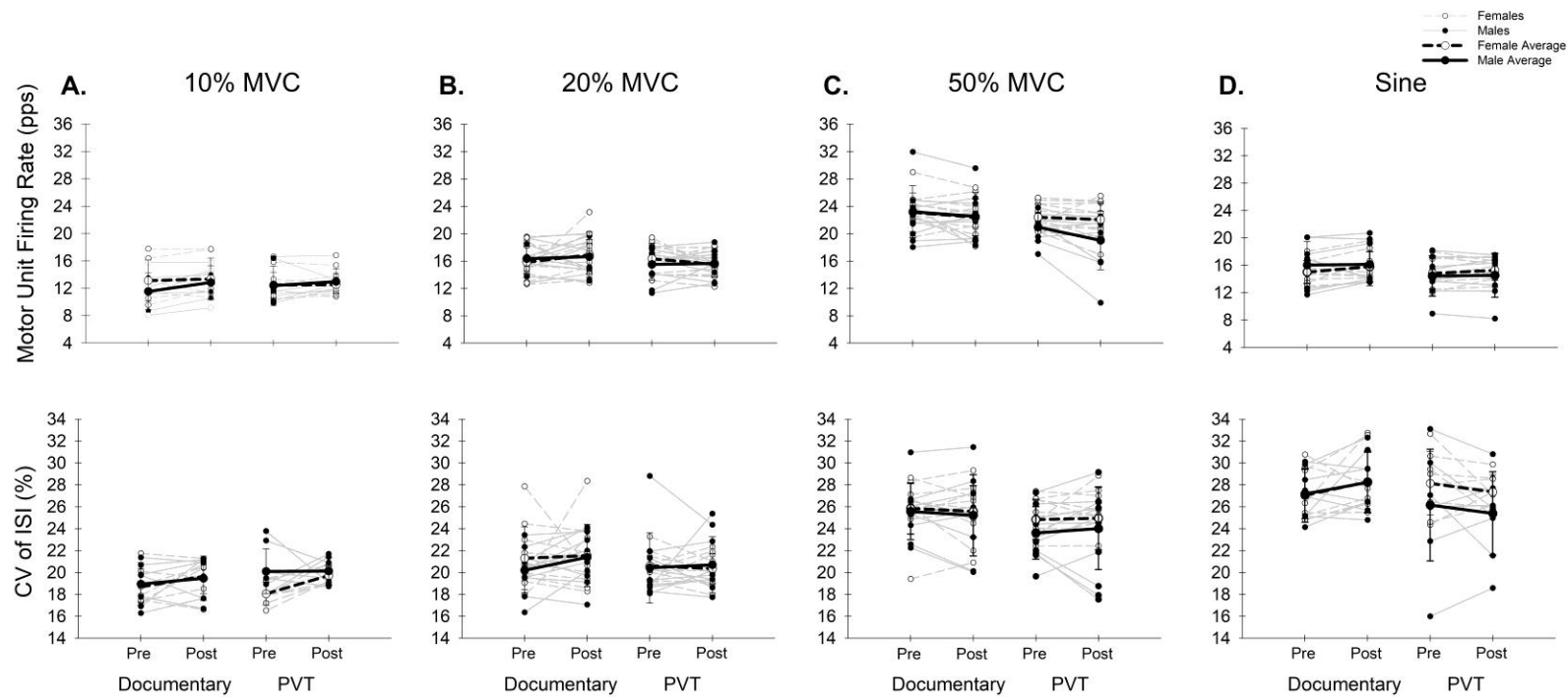


Figure 2.4 Motor unit firing behavior

Individual data are presented in gray lines, and averaged data presented in black. **A:** 10% MVC. Motor unit firing rate was higher at post compared to pre ($p = 0.04$) in both sexes. No significant changes over time, nor any effects sex, or day or interactions. **B:** 20% MVC. No significant changes over time, nor any interactions for MUF. **C:** 50% MVC. Motor unit firing rate at 50% MVC was lower at post compared to pre ($p = 0.02$). There were no significant effects of sex or day or interactions. **D:** Sine wave. There were no significant changes over time, nor effects of sex or day or interactions.

On the documentary day, 346 motor units were identified before the intervention and 370 after. On the PVT day, 342 motor units were identified before the intervention and 337 after. Motor unit firing behavior outcomes for 20% MVC are presented in Figure 2.4. Motor unit firing rate at 20% had no significant main effects of time ($p \geq 0.6$, $\eta^2 = 0.006$), sex ($p = 0.8$, $\eta^2 = 0.001$), or day ($p = 0.3$, $\eta^2 = 0.03$). There were no significant interactions for MUFR ($p \geq 0.2$, $\eta^2 \leq 0.04$). The CVISI at 20% had no significant effects of time ($p = 0.4$, $\eta^2 = 0.01$), sex ($p = 0.7$, $\eta^2 = 0.005$) or day ($p = 0.3$, $\eta^2 = 0.02$). There were also no significant interactions for CVISI ($p \geq 0.4$, $\eta^2 \geq 0.02$).

2.3.3.4 50% Contraction intensity

Force outcomes for 50% MVC are presented in Figure 2.3. During the 50% MVC contractions, 1 female and 3 males were excluded as outliers. Therefore, the analysis was performed on 12 females and 9 males. Over time, mean force did not significantly change ($p = 0.6$, $\eta^2 = 0.009$), but CV of force was significantly reduced ($p = 0.03$, $\eta^2 = 0.11$). There was a significant effect of sex for both mean force ($p = 0.005$, $\eta^2 = 0.19$) and CV of force ($p < 0.001$, $\eta^2 = 0.35$) in which males had a higher mean force and were less variable than females. There was no significant effect of day on mean force ($p = 0.2$, $\eta^2 = 0.05$) or CV of force ($p = 0.2$, $\eta^2 = 0.04$). There were no significant interactions for mean force ($p \geq 0.1$, $\eta^2 \leq 0.07$) or CV of force ($p \geq 0.2$, $\eta^2 \leq 0.05$). The RMSE of force at 50% did not significantly change over time ($p = 0.06$, $\eta^2 = 0.09$). Compared to females, males had significantly lower RMSE values ($p \leq 0.001$, $\eta^2 = 0.34$). There was no significant effect of day on RMSE ($p = 0.1$, $\eta^2 = 0.05$). There were also no significant interactions for RMSE ($p \geq 0.1$, $\eta^2 \leq 0.06$).

On the documentary day, 647 motor units were identified before the intervention and 633 after. On the PVT day, 609 motor units were identified before the intervention and 515 after. Motor unit firing behavior outcomes for 50% MVC are presented in Figure 2.4. Motor unit firing rate at 50% significantly decreased over time ($p = 0.01$, $\eta^2 = 0.15$), but there were no significant effects of sex ($p = 0.2$, $\eta^2 = 0.04$) or day ($p = 0.6$, $\eta^2 = 0.09$). There were no significant interactions for MUFR ($p \geq 0.2$, $\eta^2 \leq 0.05$). There were no significant

effects of time ($p=0.9$, $\eta^2<0.001$), sex ($p=0.4$, $\eta^2=0.02$), or day ($p=0.1$, $\eta^2=0.05$) on CVISI. There were also no significant interactions for CVISI ($p\geq 0.3$, $\eta^2\leq 0.2$) during the 50% contractions.

2.3.3.5 Sinusoidal contraction

Force outcomes for the sinusoidal contractions are presented in Figure 2.3. During the sinusoidal contractions, 5 females and 4 males were excluded as outliers. Therefore, the analysis was performed on 8 females and 8 males. Neither mean force ($p=0.2$, $\eta^2=0.07$), nor CV of force ($p=0.7$, $\eta^2=0.005$) significantly changed over time. Males had more variable force than females ($p=0.005$, $\eta^2=0.24$), but mean force was not significantly different between sexes ($p=0.3$, $\eta^2=0.04$). There was no significant effect of day on mean force ($p=0.9$, $\eta^2=0.001$) or CV of force ($p=0.9$, $\eta^2<0.001$). There were no significant interactions for mean force ($p\geq 0.1$, $\eta^2\leq 0.07$) or CV ($p\geq 0.2$, $\eta^2\leq 0.05$) of force in the sinusoidal contraction. The RMSE did not significantly change over time ($p=0.9$, $\eta^2=0.001$), but there was a significant effect of sex ($p<0.001$, $\eta^2=0.46$) in which males were more variable than females. There was no effect of day on RMSE ($p=0.2$, $\eta^2=0.05$). There was a significant interaction of time and sex on the RMSE during the sinusoidal contraction ($p=0.05$, $\eta^2=0.13$) where males had increased error over time and females reduced their error over time. There were no additional significant interactions for RMSE ($p\geq 0.1$, $\eta^2\leq 0.06$).

On the documentary day, 437 motor units were identified before the intervention and 506 after. On the PVT day, 424 motor units were identified before the intervention and 410 after. Motor unit firing behavior outcomes for the sinusoidal contractions are presented in Figure 2.4. Motor unit firing rate during the sinusoidal contraction had no effect of time ($p=0.2$, $\eta^2=0.07$) sex ($p=0.9$, $\eta^2<0.001$) or day ($p=0.3$, $\eta^2=0.04$). There were no significant interactions for MUFR ($p\geq 0.3$, $\eta^2\leq 0.05$). There was no significant effect of time ($p=0.7$, $\eta^2=0.005$), sex ($p=0.3$, $\eta^2=0.03$), or day ($p=0.3$, $\eta^2=0.03$) on CVISI. There were no significant interactions for CVISI ($p\geq 0.06$, $\eta^2\leq 0.11$) during the sinusoidal contractions.

2.4 Discussion

In this study, we sought to further investigate potential sex-related differences in the impact of mental fatigue on neuromuscular function in the FDI. The PVT successfully induced mental fatigue, as indicated by a slowed reaction time and higher reported SF. In this study, we did not observe any significant changes to force or motor unit firing behavior in the FDI that were specific to mental fatigue. Motor unit firing rates increased over time on both days in the 10% condition and decreased in the 50% conditions with no change to the mean force, and a decrease in the force variability. However, these changes were not specific to the mental fatigue condition. We did observe that females reduced their error in tracing a sinusoid force pattern, whereas males increased their error.

2.4.1 Mental fatigue

Our thirty-minute PVT task seems to have successfully induced mental fatigue, as indicated by a significant slowing of RT. On both days, participants reported an increase in subjective fatigue, with higher reported values observed on the PVT day at both time points. The large change in RT is similar to previous assessments of mental fatigue using the PVT (Kowalski et al., 2022; Kowalski & Christie, 2020; Morris & Christie, 2020). Females reported greater subjective fatigue than males at both time points irrespective of mental fatigue, supporting previous findings that females report greater subjective fatigue (Engberg et al., 2017). Our observed increase in subjective fatigue over time was not specific to mental fatigue, although this did trend towards significance on the PVT day. Previous studies observed increases of 14-19.5% in subjective fatigue reports after sustained attention tasks (Pageaux et al., 2015; Kowalski & Christie, 2020), which is slightly lower than our changes of 12-33% change in subjective fatigue. These larger changes could have been driven by our population's scores on the PSQI where females and males reported an average of 5.3 and 4.9, respectively, where 5 and higher is considered poor sleep. Our average MFI scores of 61 for females and 59.8 for males were similar to previous assessments of neuromuscular function and mental fatigue, ranging from 59.3-60 (Kowalski & Christie, 2020; Morris & Christie, 2020).

2.4.2 Maximal force

The sex difference in MVC in our participants was large, similar to previous assessments of MVC in the FDI (Parra et al., 2020). Maximal voluntary contraction was also reduced over time by an average of 5%, which, while significant, is a much lower change compared to the fatigue observed in isometrically fatiguing studies on the FDI (McManus et al., 2015). There was also no combination of any other changes in mean or variability of force over time to indicate that fatigue was an influence in performance. Other studies performed on dorsiflexion and knee extension found no difference in MVC over time (Kowalski et al., 2022; Kowalski & Christie, 2020; Pageaux et al., 2013, 2015). However, in the hand, significant reductions were found in the present study and during an isometric handgrip task (Bray et al., 2012) in contrast to others showing no change (Bray et al., 2008; Mehta & Parasuraman, 2014). This variability in the outcome of MVC assessments in the hand, especially in contrast to assessments in lower limb muscles, warrants further study.

2.4.3 Submaximal force

During the submaximal contractions, we did not observe any changes to RMSE, CVISI, or mean force that were unique to the mentally fatiguing condition. This is consistent with previous findings on force steadiness in the FDI following mental fatigue (Budini et al., 2022) and handgrip steadiness (Shortz & Mehta, 2017). Males in our study had higher mean force than females at 20% MVC, and less error and RMSE with higher mean force during the 50% MVC trapezoidal contractions. This is in line with previous sex-based comparisons with lower CV of force and RMSE at in males (Jakobi et al., 2018; Inglis & Gabriel 2021). A reduction in CV of force and RMSE over time occurred at 50% and 20%, respectively, although these were not unique to mental fatigue. This reduction was not observed in the sinusoidal contraction. The practice session included a focus on the sinusoidal contraction, and less on the static contractions which could have led to this change over time.

At 10% MVC, there were no significant changes to the force output, but there was a trend for male CV of relative force to be higher than females. Despite any changes to force

outcomes, there was a significant increase in MUFR over time. The average firing rate at the pre time point was about 12.4 pulses per second, and the post time point was about 13 pulses per second. The effect size of this change was large, and the increase in MUFR with a lack of differences in mean, CV, and RMSE of force is in contrast to a previous assessment of MUFR in the TA, where a decline in average force was found with no change in MUFR after mental fatigue (Kowalski et al., 2022). A previous study on the TA also suggested that mental fatigue could have changed recruitment or synergistic muscle activity (Kowalski et al., 2022). However, a lack of significant contributors of synergistic muscle activity in the FDI indicates that this is likely not a factor in this study. These contrasting outcomes indicate support that these outcomes are different across muscles.

At 20% MVC, we observed a reduction in RMSE over time in both sexes, and males traced the templates with a higher relative mean force than females. This reduction in RMSE was not accompanied by other changes in force performance. We did not observe any changes to MUFR on the PVT or Documentary day, in contrast to previous studies in the TA, where a reduction in MUFR was observed in response to the same conditions (Kowalski et al., 2022; Kowalski & Christie, 2020). The different responses in MUFR to mentally fatiguing conditions between studies could potentially be attributed to the muscles examined.

The 50% MVC condition had significant changes over time to both the CV of force, and the MUFR, as well as sex differences in RMSE, mean, and CV of force. Males were observed to have less RMSE force, higher relative mean force than females, and both sexes had reduced CV of force while tracing the force template than females. There was a trend for RMSE at 50% to reduce over time in both sexes, but it failed to reach significance. This decline in MUFR at 50% MVC is in-line with one previous assessment of MUFR in the TA (Kowalski & Christie, 2020), but not when compared to the another assessment which included a documentary condition as well (Kowalski et al., 2022).

A novel observation in the present study was observed in the performance of the sinusoidal force tracing. Similar to the complex sinusoidal wave utilized in the FDI

previously (Knight & Kamen, 2007), a flat rate of 0.15hz was utilized. Males had less error in force in performing the task yet were more variable during their performance. Interestingly, over time, males had an increase in RMSE and females had a decrease. Our females also reported higher levels of subjective fatigue and scored over 5 on the PSQI on average, which could have potentially had an influence on their performance. Another assessment between males and females in the FDI noted sex differences in the size of recruited motor units starting around 18% MVC (Parra et al., 2020). Our variable force contraction varied around this percentage, and potentially this sex difference in size of motor units during the variable force condition could have contributed to the sex-specific improvement. In another study using a force-tracing task with a complex sinusoidal wave, a group of female participants increased their performance through a reduction in RMSE by 52.8% after 15 trials which was accompanied by improvements in modulation frequencies of discharge rate specific to the task (Knight & Kamen, 2004). A lack of sex differences in MUFR and the CVISI with an improvement in RMSE in female performance during the variable force condition, observed in the current study, could indicate sex differences in the modulation of firing rates during variable force-matching tasks at 0.15 Hz.

2.4.4 Limitations

Most of our PVT outcomes were indicative that the PVT successfully induced mental fatigue. However, we observed an increase in subjective fatigue reported on both days, contrary to a similar protocol using the same control stimulus (Kowalski et al., 2022). Our participants also did not experience any significant changes in false starts or lapses, potentially indicating different levels of mental fatigue in response to the PVT in our participants compared to the previous study. The PVT is a simple reaction time task, and as such, the length of the task combined with simpler nature against other tasks like the Stroop was potentially not enough to induce levels of mental fatigue required to observe neuromuscular changes. The length of the protocol following the PVT trial could also have exceeded the duration of the effects of mental fatigue, however, our protocol was completed within 10-12 minutes before the suggested 15-45 minute range of suggested recovery time from mental fatigue (Magnuson et al., 2021).

2.5 Conclusion

Overall, our findings indicate that there is a lack of significant changes to neuromuscular function in response to a mentally fatiguing task, as there was no unique change compared to a control condition. Although there is a lack of significant changes to motor unit firing behavior in response to mental fatigue, there is evidence to suggest that increased feelings of subjective fatigue can cause different adaptations to neuromuscular function according to the muscle. The changes we observed were not unique to the mental fatigue condition. Changes over time were observed during both a mentally fatiguing task, and a mentally neutral documentary. Females in our study improved in their performance of tracing a sinusoid force pattern, while males had a reduction in performance over time. Sex differences were observed in force tracing performance of a variable force task, and these results indicate that more sex-specific comparisons of performance during variable force contractions should be made.

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Chapter 3

3 Sex-related differences in the relationships between motor unit firing rate and pennation angle

3.1 Introduction

Gradation of force is controlled through the recruitment and rate coding of motor units, where both the number of recruited motor units and their rate of firing increase with increasing force (Henneman et al., 1965b; Milner-Brown et al., 1973). The amount of force an individual motor unit contributes to a contraction is dependent on its firing rate at a given moment, and the number of muscle fibers it innervates (Bodine et al., 1987; Kanda & Hashizume, 1992; Rack & Westbury, 1969). However, there seems to be differences in motor unit firing rates (MUFR) between males and females, with some reports of higher firing rates in females than males at a similar force levels and others with no differences (Inglis & Gabriel, 2020; Taylor et al., 2022). One potential explanation for a sex-related difference in MUFR is a difference in muscle architecture, in that females may have altered motor unit firing behavior to account for differences in the muscle, such as pennation angle. There are well documented differences between males and females in muscle architecture, where males typically have larger cross-sectional areas and larger pennation angles (Carter et al., 2001; Manal et al., 2008; Trevino et al., 2023). Although average MUFR and pennation angle increase with contraction intensity (Inglis & Gabriel, 2020; Manal et al., 2006, 2008), it is unknown if the relationship between MUFR and pennation angle is different for males and females.

Some studies have provided evidence to suggest that females have a higher number of smaller motor units contributing to force at submaximal intensities (Cioni et al., 1994; Krishnan & Williams, 2009). In general, females seem to have different rates of change in MUFRs across contraction intensities, with lower firing rates at maximal contraction intensities (Inglis & Gabriel, 2020). However, when males and females are matched for strength, females have higher firing rates at submaximal and maximal intensities (Inglis & Gabriel, 2020, 2021). Other motor unit firing behaviors, such as doublet discharges and variability in firing rate, were also higher in females than males (Inglis & Gabriel,

2021). Despite differences in motor unit firing behaviors, motor unit number estimations have not been shown to be different between males and females (Yerdelen et al., 2006).

Architectural properties and the size of muscle are also different between males and females. Males have lower proportions of type I muscle fibers, and the cross-sectional area of all muscle fibers are smaller in females (Simoneau & Bouchard, 1989). A larger range of pennation angles, with a greater average value has been reported in males compared with females (Manal et al., 2006), but fascicle length is similar to females (Deng et al., 2021). The higher pennation angles in males contribute to larger physiological cross-sectional areas, representing more muscle fibers arranged in parallel for force production (Jones & Rutherford, 1987; Wickiewicz et al., 1983). In comparison to females with smaller pennation angles and muscle thickness, stronger relationships between pennation angle and muscle thickness to rate of torque development was observed in males (Trevino et al., 2023).

Relationships between pennation angle and EMG from submaximal to maximal intensities at various muscle lengths have also been observed (Manal et al., 2006, 2008; Mela et al., 2001). MUFrs have been suggested as a strategy to compensate for the low-pass filtering of the contractile characteristics of muscle (Baldissera et al., 1998). As pennation angle is a mechanism to allow for the arrangement of more muscle fibers within a given cross-sectional area, a lower pennation angle, as typically seen in females, could necessitate higher firing rates to achieve similar force output as males (Inglis & Gabriel, 2020). Indeed, sex-related differences in motor unit behavior have been suggested to be the result of compensation for differences in muscle architecture (Inglis & Gabriel, 2020). Pennation angle is an easily observed aspect of muscle architecture (Cho et al., 2017), and although it is closely related to force output alongside MUFr, there has been no concurrent observation of MUFr and pennation angle in males and females.

The purpose of this study was to assess differences in pennation angle and MUFr in the tibialis anterior (TA) in males and females to determine if there is a relationship between the two. Pennation angle and MUFr were measured at the same time during submaximal

and maximal contraction intensities. We hypothesized that MUFRRs on average would be higher in females at lower contraction intensities and higher in males during maximal contractions. As pennation angle has been shown to have a larger range in males, we expected a more prominent relationship between MUFRR and pennation angle in males compared to females.

3.2 Methods

Thirty-one healthy participants (16 female, 15 male) aged 19-32 years were recruited from the local university. Participants were free of neuromuscular disorders or injury and refrained from recreational drug and alcohol intake and exercise for 24 hours prior to visiting the lab. All participants provided written informed consent prior to participation and the protocols and procedures were approved by the Health Sciences Research Ethics Board at the University of Western Ontario.

3.2.1 Experimental setup

Testing occurred during one visit to the laboratory. The experimental protocol started with an assessment of maximal voluntary contraction (MVC) force of the ankle dorsiflexors. After the MVC assessment, participants performed a series of isometric dorsiflexion contractions to 20, 40, 60, 80, or 100% of MVC in random order. Three trials were performed at each force level and measures of MUFRR and pennation angle were obtained during the contractions. At the end of the protocol, an additional MVC was performed to assess fatigue.

3.2.2 Force

Participants were seated in a testing chair, with their right foot strapped to a custom-built apparatus designed to measure dorsiflexion and plantar flexion force and their hip and knee angles at 90 degrees. The ankle joint was set to 20 degrees of plantar flexion. The apparatus was equipped with a load cell beneath the foot plate (SSM-AJ-250; Interface, Scottsdale, AZ, USA) which was amplified (PM-1000; DataQ Instruments, Akron, OH, USA) and sampled at 2224 Hz using a 16-bit A/D converter (NI USB-6251; National

Instruments, Austin, TX, USA) and DASyLab software (Data Acquisition System Laboratory, DasyTec, USA, Inc., Amherst, NH, USA). The force was also low-pass filtered at 50Hz to provide real-time feedback to the participant using DASyLab software.

Prior to performing the assigned contractions for the experimental protocol, participants performed a minimum of three MVCs, each lasting 4-5 seconds with 3 minutes of rest between contractions. Additional trials were performed if the peak force varied greater than 10% across trials, and the highest peak was identified as each participant's MVC and used to set the display for real-time feedback of force throughout the remainder of the protocol. Force was displayed on a bar graph with targets at 20%, 40%, 60%, 80%, and 100% MVC. For 20% and 40% MVC contractions, participants held the contractions for 60 seconds, and for 60%, 80% and 100% MVC contractions, participants were instructed to hold the contraction as best as they could for 10 seconds. The order of contractions was randomized and then repeated three times to attain three recordings for each contraction intensity. Three minutes of rest was provided between all contractions. A custom-written Matlab program (version 2022a; Mathworks Inc, Natick, MA, USA) was used to average the force in a 1-second window around the time when the pennation angle was measured. Any contractions where mean force was not within 10% of the target were excluded from further analysis.

3.2.3 Pennation angle

Images of the TA were collected using a β -mode ultrasound imaging device (GE Logiq e, USA) and a multifrequency linear-array probe (12L-RS; 5-13 MHz, 38.4 mm field of view). A frequency of 10 MHz and depth of 4 cm were used for all participants. After the placement of the surface EMG electrodes, the ultrasound probe was placed in an apparatus to maintain the probe's elevation distal to the electrode on the participants' TA. The recording site for pennation angle was manually identified in the longitudinal plane, and the apparatus was adjusted to maintain the probe's position immediately distal to the EMG sensor. Care was taken to ensure a thick layer of ultrasound gel between the participants' skin and the ultrasound probe to prevent any compression effect on the

muscle. Between MVC trials, three static images of the participants' TA at rest were obtained. If the probe was moved or the image was unclear, the probe and apparatus were adjusted to regain image clarity.

Throughout the testing protocol, images were obtained during all contractions by recording a 9-second video at 56 frames per second. Participants were instructed to contract at the ultrasound operator's instruction, and manual adjustment was made during each contraction to attain a clear view of pennation angles within each participants' TA. The time of recording was manually recorded using a TTL pulse from a stimulator to align the force and EMG recordings to the ultrasound recording. Ultrasound recordings were analyzed offline. Within the recording from each contraction, 3 pennation angles were identified and measured during the plateau in force. Pennation angles were selected based on the visibility of their attachment to the central aponeurosis of the muscle. Only the pennation angle from fascicles with a clear attachment to or near to the central aponeurosis were used. An example image is presented in Figure 3.1. Using ImageJ software (Version 1.53k, National Institutes of Health, Bethesda, MD, USA), the angle between visible muscle fascicles and the aponeurosis was measured. The frame within the video and the total number of frames for each video were recorded for time alignment with the force and EMG data. The average of all three pennation angles within each image were used for each trial and then averaged across trials at each contraction intensity.

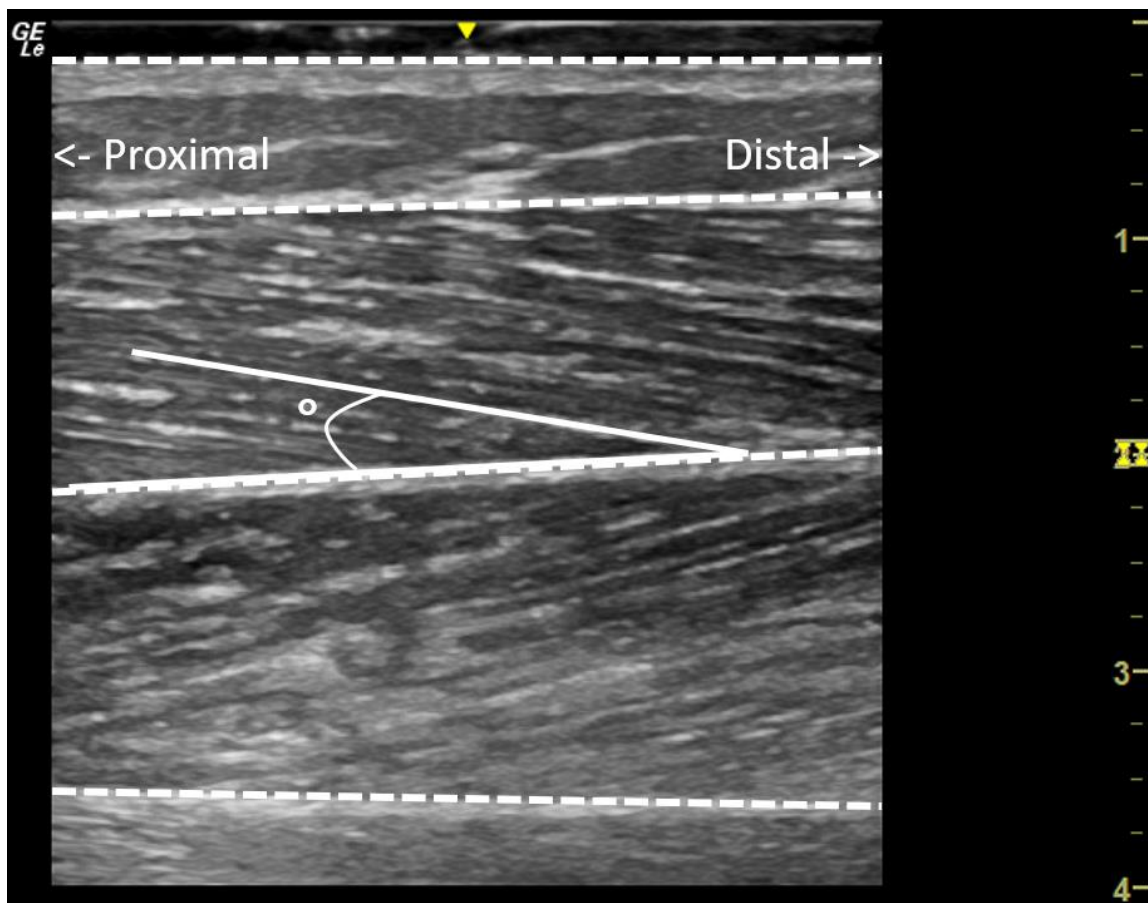


Figure 3.1 Example pennation angle measurement

Example pennation angle from the central region of a female participant's TA during a 40% contraction. An example tracing of pennation angle is presented as the measure of degrees from the central dotted line indicating the muscle's central aponeurosis. The left side of the image is proximal; the right side of the image is distal, and the top of the image is the surface of the muscle. The top-most dotted line traces the surface of the skin, and the one below it traces the fascia separating the muscle from subcutaneous tissue. The bottom most dotted line traces the fascia of the muscle deeper in the body. The scale on the right represents 4 cm of depth for the recording.

3.2.4 EMG signal and motor unit processing

A wireless 4-pin surface EMG electrode (Galileo wireless EMG, Delsys Inc., Natick, MA, USA) was attached to the skin over each participant's TA with a reference electrode attached to either medially or laterally of the patella depending on individual musculature and available space in the leg apparatus. The sensor location was abraded (Nuprep®) and wiped with an alcohol swab prior to sensor placement, and the sensors were held in place

with double-sided tape fit for the electrodes. All surface EMG signals were sampled at 2222hz with a band-pass filter at 20-450 Hz using EMGworks Acquisition software (4.7.7; Delsys Inc, Natick, MA) simultaneously with DASYlab software. The EMG signal was decomposed into motor unit firing trains using the Precision Decomposition III algorithm within Neuromap software (version 1.2.2; Delsys De luca et al., 2006; Nawab et al 2010) and tested for accuracy using the Decompose-Synthesize-Decompose-Compare test (De Luca and Contessa, 2012) using Neuromap software (version 1.2.2, Delsys Inc). Motor units below 80% accuracy were excluded from analysis.

Motor unit firings were excluded from further analysis if they had fewer than 10 discharges or inter-pulse intervals less than 10 milliseconds (doublet discharges) or greater than 200 milliseconds (Christie et al., 2009; Christie & Kamen, 2010). A custom-written Matlab program was used to exclude motor units, calculate mean and peak MUFR, and to align motor unit firing times according to the stimulator TTL pulse. These were calculated within a 1-second window around the selected frame from the ultrasound recording with the measured pennation angle for each contraction. The reciprocal of the inter-pulse intervals for each motor unit firing train was used to calculate firing rate and then averaged across trials for each contraction intensity. Peak MUFR was calculated as the mean of the 5 fastest consecutive inter-pulse intervals identified from a point-by-point moving window in each of the included motor unit firing trains. Peak MUFRs were then averaged across trials for each contraction intensity. An example of the identified motor unit firings and window of interest is presented in Figure 3.2.

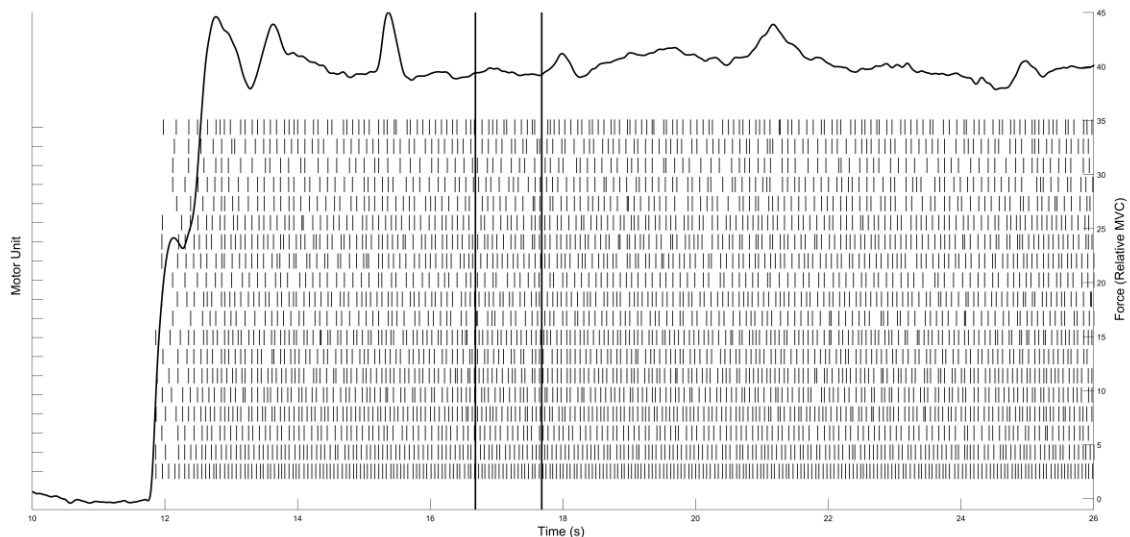


Figure 3.2 Decomposed motor units and force window example

An example 40% condition for a participant. The x-axis represents time, and the right y-axis represents the participant's force output. Vertical lines represent the window used for force and MUFR analysis. Pennation angle was recorded in the center of the 2-second window presented. Motor unit firings are presented as vertical dashes, each representing an individual firing used in the analysis of mean and peak MUFR.

3.2.5 Statistical analysis

Two-tailed independent samples t-tests were used to examine sex-related differences in participant characteristics (age, height, weight, BMI) and baseline pennation angles. Outcome variables of pennation angle, mean MUFR, and peak MUFR were assessed for normality and analyzed with a two-way (sex, contraction intensity) repeated measures ANOVA. A two-way (time, sex) repeated measures ANOVA was performed on the MVC assessments to compare MVC before and after the protocol in males and females. When assumptions of normality or sphericity were violated, Greenhouse-Geisser corrections were used. Post-hoc comparisons with Bonferroni correction were used where necessary. Outliers were identified as data points that were more than 1.5x the interquartile ranges above or below the third and first quartiles and were excluded from analysis.

A two-sample K-S test for normality was performed on the distributions of mean MUFR and peak firing rate and pennation angle. The K-S test on pennation angle and mean

MUFR, and peak MUFR was significant, and therefore Kendall's tau was calculated to assess the relationship between pennation angle and each of mean and peak MUFR (Long & Cliff, 1997). All statistical analyses were performed in R 4.3.0 (R Core Team, 2023). Significance was set at $p \leq 0.05$ and all data are presented as mean \pm SD. Partial eta squared values are presented as effect sizes for all ANOVA outcomes are presented as η^2 and interpreted with $\eta^2=0.01$ as small, $\eta^2=0.06$ as medium, and $\eta^2=0.14$ as a large effect. The strength of correlations from Kendall's tau were interpreted as $\tau \geq 0.1$ as small, $\tau \geq 0.3$ as medium, and $\tau \geq 0.5$ as strong.

3.3 Results

3.3.1 Participant characteristics

Participant characteristics, MVC values, and motor unit decomposition yields are presented in Table 3.1. Motor unit exclusion criteria resulted in 4 female participants being excluded from further analysis. One male was excluded due to an inability to measure pennation angles at 80% and 100% MVC, and one male was excluded due to failure to reach the target force level during the 100% MVC conditions. Two females and three males were further excluded as outliers during analysis. Therefore, the results for all analyses were performed on 10 females and 9 males.

Table 3.1 Participant characteristics

	Females (n=10)	Males (n=9)
Age (years)	22.7±2.3	22.2±1.8
Height (m)*	1.65±0.06	1.84±0.06
Mass (kg)*	61.2±11.1	82.7±10.3
BMI (kg/m ²)	22.3±3.2	24.4±2.5
MVC Pre (N)*	124.6±27.3	258.5±84.1
MVC Post (N)*	131.1±32.9	245.7±44.0
Motor Unit Yield		
20%	17.2±10	30.3±16.8
40%	36±13.7	31.5±17.4
60%	15±8.7	13.4±4.2
80%	19.1±6.6	16.1±6.2
100%	17±10.4	11.7±7.8

Note. * Indicates significant difference between sexes ($p < 0.001$). Data are mean \pm SD.

3.3.2 Maximal force

The outcomes for female and male force are presented in Table 3.1. There was a significant effect of sex on MVC ($p < 0.001$, $\eta^2 = 0.38$) where males were stronger than females. There was no significant effect of time on MVC ($p = 0.68$, $\eta^2 = 0.01$) nor a significant interaction of sex and time ($p = 0.21$, $\eta^2 = 0.09$), suggesting the testing protocol did not induce fatigue.

3.3.3 Motor unit firing rate

Both mean and peak MUFR analyses were computed using Greenhouse-Geisser corrections and are presented in Figure 3.3. There was no significant effect of sex

($p=0.09$, $\eta^2=0.16$) on mean MUFR. There was a significant effect of relative contraction intensity ($p<0.001$, $\eta^2=0.71$) where mean MUFR increased with increasing contraction intensity. There was also a significant interaction of sex and relative contraction intensity ($p=0.04$, $\eta^2=0.16$). Post-hoc analysis revealed that mean firing rate in males and females was greater at 100% compared with 20% MVC ($p\leq 0.002$). However, females had a significant increase from 60% to 80% MVC ($p=0.005$) while males did not ($p=0.99$). Mean firing rates plateaued in both sexes from 80% to 100% MVC.

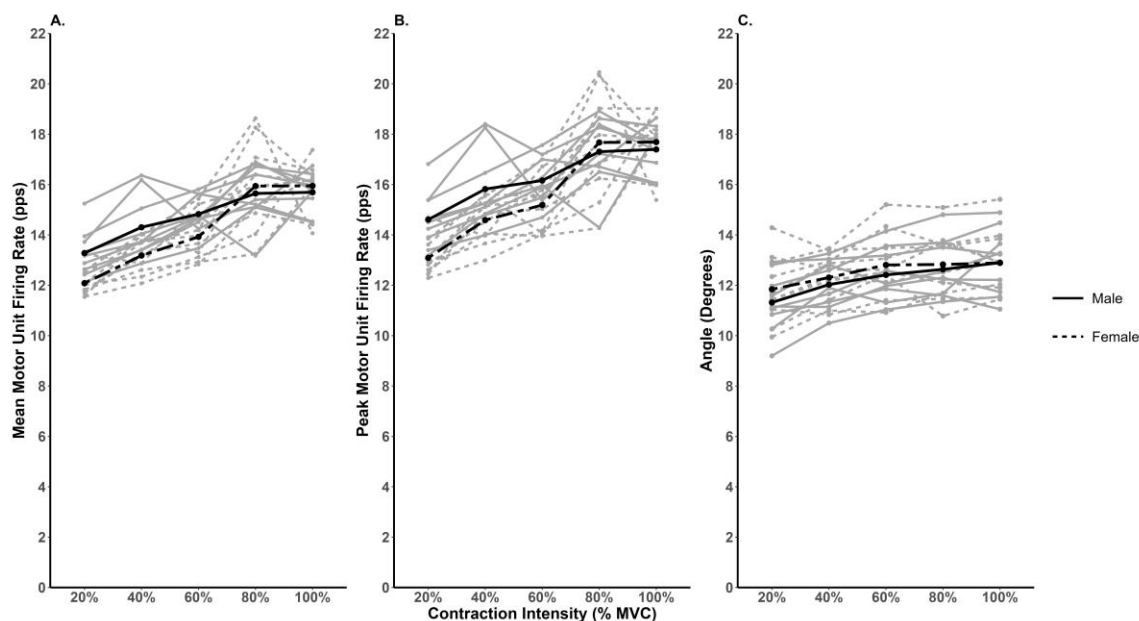


Figure 3.3 Motor unit firing and pennation angle measurements across contraction intensity

Data from individual participants are presented in gray. Black lines represent averages for each sex. **A:** Contraction intensity had a significant effect on mean MUFR, where both males and females had significantly higher mean firing rates at 100% than 20% MVC. Females had significant increase between 60% and 80% MVC, whereas males did not. Mean MUFR plateaued at 80% MVC for both sexes. **B:** Contraction intensity had a significant effect on peak MUFR, where both males and females had significantly higher peak firing rates at 100% than 20% MVC. Females had significant change between 60% and 80% MVC, whereas males did not. Mean MUFR plateaued at 80% MVC. **C:** Pennation angle had significant increase from 20% MVC to 100% MVC. There was no effect of sex nor interaction of sex and contraction intensity.

For peak MUFR, there was no significant effect of sex ($p=0.11$, $\eta^2=0.14$). There was a significant effect of relative contraction intensity ($p<0.001$, $\eta^2=0.70$), where peak MUFR increased with increasing contraction intensity. There was also a significant interaction of sex and relative contraction intensity ($p=0.04$, $\eta^2=0.15$). Post-hoc analysis revealed that peak firing rate at 100% MVC was greater than 20% in both sexes ($p\leq 0.003$). Females had a significant increase from 60% of MVC to 80% MVC ($p=0.002$) while males did not ($p=0.44$). In both sexes, peak firing rate plateaued from 80% to 100% MVC ($p\geq 0.99$).

3.3.4 Pennation angle

The coefficient of variation of the pennation angle across all three measurements included in the calculation of pennation angle was 4.9%. Pennation angle analyses were performed using Greenhouse-Geisser corrections and are presented in Figure 3.3. There was no significant effect of sex on pennation angle ($p=0.59$, $\eta^2=0.02$). There was a significant effect of relative contraction intensity ($p<0.001$, $\eta^2=0.53$) where the pennation angle was significantly higher at 100% compared to 20% MVC. Post-hoc analysis revealed that pennation angle plateaued at 60% MVC ($p\geq 0.09$). There was no significant interaction of sex and relative contraction intensity ($p<0.56$, $\eta^2=0.04$).

3.3.5 Correlation between firing rate and pennation angle

Correlational data are presented in Figure 3.4. In males, pennation angle had significant positive correlations of medium strength with mean firing rate ($\tau=0.33$, $p=0.001$) and peak firing rate ($\tau=0.31$, $p=0.003$). There was no significant correlation between pennation angle and mean MUF_R in females ($\tau=0.10$, $p=0.31$) nor peak MUF_R ($\tau=0.09$, $p=0.34$). Correlations with force revealed that males had weak but significant positive correlations between pennation angle and relative contraction intensity ($\tau=0.22$, $p=0.03$) and absolute force ($\tau=0.27$, $p=0.009$). Females did not have significant correlations between pennation angle and relative contraction intensity ($\tau=0.17$, $p=0.08$) nor absolute force ($\tau=0.15$, $p=0.14$).

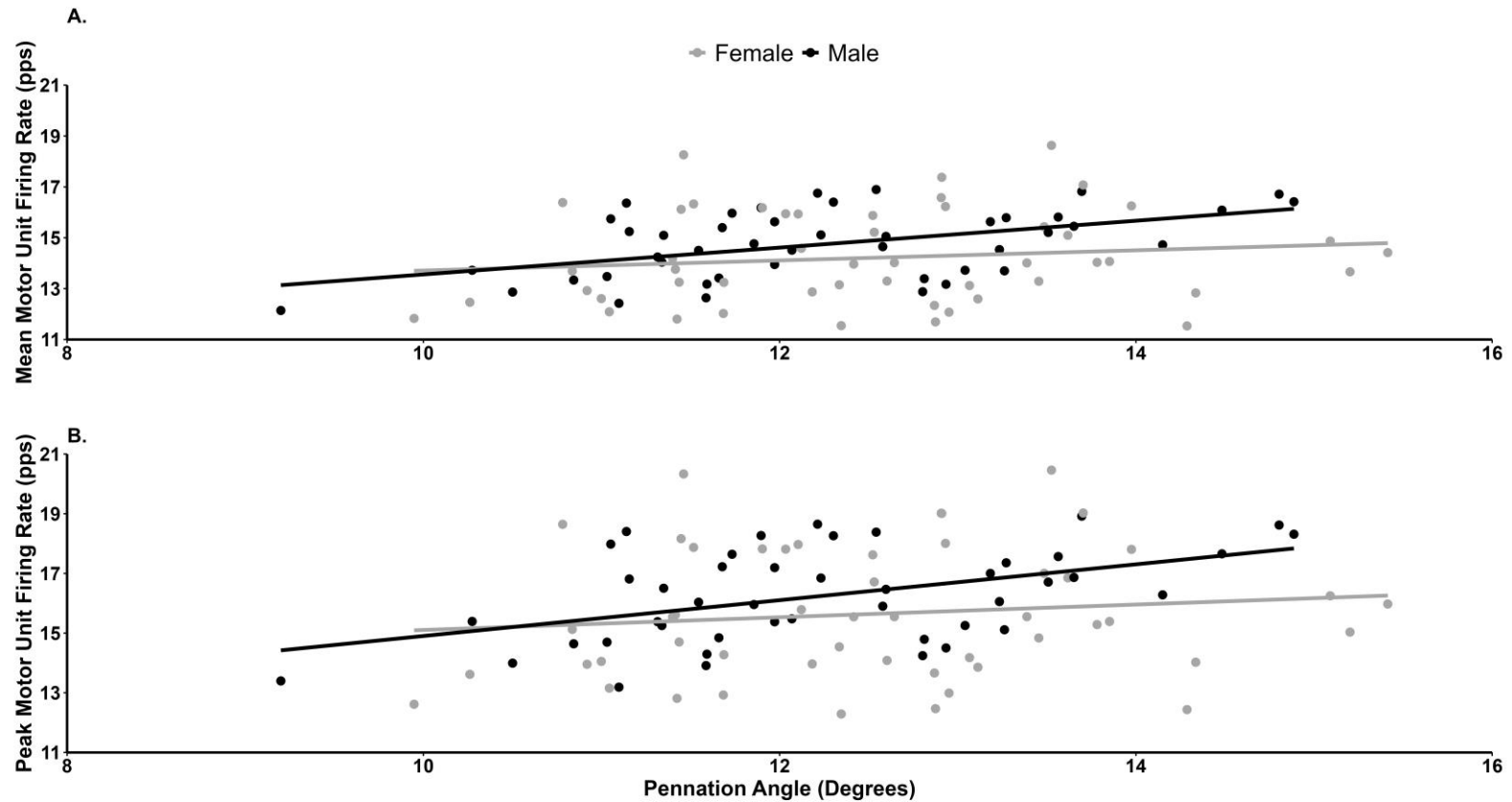


Figure 3.4 Correlation between pennation angle and firing rate

Data are presented for both males and females. **A:** Pennation angle and its relationship to mean firing rate. **B:** Pennation angle and its relationship to peak firing rate. Males had significant positive correlations between pennation angle and both mean and peak MUFR. Females had non-significant positive correlations between pennation angle and mean and peak MUFR.

3.4 Discussion

The purpose of this study was to examine the potential relationship between MUFR and pennation angle in males or females. There were no significant sex-based differences in MUFR or pennation angle at any contraction intensity. Both sexes displayed a plateau in mean and peak MUFR at 80% MVC and in pennation angle at 60% MVC. However, there was a sex-specific change in MUFR, where both mean and peak firing rates for females significantly increased from 60% to 80% prior to plateauing, while males did not display the same level of change. Males also had significant correlations between pennation angle and both motor unit firing outcomes and force, while females did not.

In contrast to some previous studies (Inglis & Gabriel, 2020; Peng et al., 2018), we did not find a difference between sexes in MUFR. Although this result was contrary to our hypothesis, other studies have also reported similar firing rates in males and females (Harwood et al., 2014; Inglis & Gabriel, 2020; Lulic-Kuryllo & Inglis, 2022). The pattern of increase in firing rate across contraction intensities was different between sexes, with greater increases from 60-80% MVC in females, which is in line with previous findings (Inglis & Gabriel, 2020). This sex-related difference in the change in firing rate across contraction intensities could be due to differences in motor unit recruitment, as it has been suggested that females rely on a larger number of smaller motor units in the early phase of recruitment (Cioni et al., 1994; Guo et al., 2022). Such differences in recruitment may influence changes in MUFR as contraction intensity increases.

Overall, the MUFRs obtained in this study (~16 pps) are lower than some previous studies, demonstrating values of up to ~35 pps in the TA (Connelly et al., 1999; Kirk et al., 2021). This discrepancy in values is likely due to differences in recording techniques across studies. Typically, studies reporting higher MUFR (Connelly et al., 1999; Kirk et al., 2021) have employed intramuscular motor unit recording techniques. However, other studies using surface decomposition techniques, report similar MUFR values (~12-16 pps) (Hoshizaki et al., 2020; Taylor et al., 2022) as observed in the current study. Similar trends are present in other muscles, such as the quadriceps, where lower mean firing rates

are observed with surface motor unit recordings (Beausejour et al., 2023; Chalchat et al., 2019) compared with other studies using indwelling techniques (Contessa et al., 2009). Differences in frequency content of the surface compared with the indwelling signal (Gygi & Moschytz, 1997; Caillet et al., 2022; Hu et al., 2013) and/or differences in decomposition algorithms may contribute to these differences in MUFR between techniques (Beausejour et al., 2023; De Luca & Contessa, 2012; Sherman et al., 2023; Sterczala et al., 2018).

The range of pennation angles in our study aligns with previous ranges of pennation angles in the TA for males and females from $\sim 8\text{-}20^\circ$ (Hodges et al., 2003; Maganaris & Baltzopoulos, 1999; Manal et al., 2006). Although a lack of sex-related differences in pennation angle is unusual in many muscles (Manal et al., 2006, 2008; Trevino et al., 2023), it has been demonstrated that the overall range in the TA is low and is similar in males and females (Manal et al., 2006). Although greater pennation angle is often associated with higher force, males and females in our study had similar pennation angles, despite significantly higher force in males. A similar pennation angle with large differences in force could be due to muscle fiber types or other muscle architectural properties such as such as cross-sectional areas, muscle thickness, or tendon compliance, all of which have a significant influence on force output (Brunner et al., 2007; Erdemir et al., 2007; Miller et al., 1993; Rekabizaheh et al., 2016; Scott et al., 2001; Trevino et al., 2023). Males are known to have larger proportions of type II fibers (Brunner et al., 2007; Miller et al., 1993), and as such their force generating abilities would be higher, which would allow higher force outputs with similar pennation angle.

Our results suggest a sex-specific relationship between MUFR and pennation angle, as only males had a significant relationship between these two factors. This relationship aligns with previous evidence suggesting muscle architecture is more strongly correlated with measurements of muscle activation in males than females (Trevino et al., 2019, 2023). Mechanomyography is a tool used to measure the oscillations of muscle during contractions and is suggested to represent the mechanical component of neural activation (Coburn et al., 2005). The slope of mechanomyography RMS and force/torque has been shown to be more strongly related to pennation angle in males compared with females

(Trevino et al., 2023). Although mechanomyography is an indirect measure of neural activation, these results do align with the results of the current study, demonstrating a relationship between MUFR and pennation angle which was significant in males, but not females. This sex-specific relationship could be influenced by the joint angle utilized in the present study. Twenty degrees of plantar flexion was chosen to match previous assessments of MUFR in the TA (Inglis & Gabriel, 2020). However, it is possible this joint angle resulted in different relative muscle lengthening between males and females, which has been observed to influence the relationship between EMG and pennation angle (Manal et al., 2006, 2008). An angle of 30° has been suggested to produce the optimal fiber length for the TA and could influence sex-specific architectural parameters (Manal et al., 2006). As shorter muscle lengths can both show greater changes in architectural parameters and MUFRs (Hodges et al., 2003; Pasquet et al., 2005), different muscle lengths may produce different strengths of the relationships between firing rate and pennation angle. Further study is required to evaluate this relationship in males and females in muscles with larger ranges of pennation angles and at varying muscle lengths.

3.4.1 Limitations

Although part of the instructions during the experimental protocol was to focus on isolating the TA, we were unable to account for the influence of accessory muscles contributing to dorsiflexion force. The influence of toe extension from the extensor hallucis longus and extensor digitorum longus on both TA strength and EMG activity is significant (Jeon & Jang, 2020). As such, if participants relied on accessory dorsiflexor muscles, this could affect the measurements of pennation angle and MUFR.

The number of excluded participants in this study is also a limitation in the interpretation of results. A priori estimates were used to determine the original sample size and the combination of exclusion criteria in force and motor unit analyses, as well as technical difficulties recording pennation angles in some participants brought the sample size down considerably, increasing the risk of type II error.

3.5 Conclusion

Our results demonstrate similar MUFrs and pennation angles between males and females in the TA. The relationship between firing rate and pennation angle was sex-specific, with a significant relationship in males but not females. This relationship provides further evidence that males and females may rely on different motor unit firing strategies to accommodate differences in muscle. Further research into this relationship is necessary in other muscles with wider ranges of pennation angles. Different joint angles should also be employed to examine the potential sex-specific influence of muscle length on the relationship between MUFr and muscle architecture.

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Chapter 4

4 The influence of the number of motor units on firing rate calculations

4.1 Introduction

Electromyography (EMG) is a widely-used method of measuring muscle activation. Electromyographic recordings can be collected from either the surface of the skin or below the skin, intramuscularly. The EMG signal is composed of all active motor unit action potentials within the pickup area of the sensor and can be decomposed into its constituent motor unit firing trains, providing a method of quantifying motor unit firing behavior (De Luca et al., 1982b). The most common outcome variable of motor unit firing decomposition being MUFR. Motor units have typically been recorded through an invasive method using needles or wires under the surface of the skin (De Luca et al., 1982a). However, recent advances in technology now provide validated methods of decomposing motor unit action potentials through recordings from the surface of the skin (De Luca et al., 2006; Merletti et al., 2008; Nawab et al., 2010). This new technology has increased the number of investigations into motor unit firing behavior and reports of MUFR. For example, a search for published studies in humans using the keywords “motor unit discharge/firing rate” and “motor unit behavio(u)r” provided more than double the number of publications in the last 20 years compared to the previous 33 years. The increase in availability of decomposition methods requires a more in-depth understanding of the data presented.

Motor unit firing rate is typically reported as the mean firing rate of all motor units within a period of interest during a voluntary contraction. Calculations of MUFR rely on a relatively small number of motor units compared to a muscle’s total, as recording with either surface or indwelling EMG electrodes cannot capture the entire pool of active motor units during a voluntary contraction (Merletti et al., 2016). In young healthy adult males, motor unit number estimates in the tibialis anterior (TA) range between 90 and 200 with considerably variability across studies (Boe et al., 2009; McNeil et al., 2005; Power et al., 2010). The numbers of decomposed motor units used in the calculation of

firing rates varies across studies but is consistently below the total motor unit number estimates. For example, motor units sampled from participants can vary from an average of 4-8 motor units decomposed from intramuscular electrodes (Christie & Kamen, 2006; Inglis et al., 2011) to 11-20 motor units using high-density surface EMG decomposition (Martinez-Valdes et al., 2020), and up to 20-40 reported from other surface EMG algorithms (Nawab et al., 2010). Some researchers have proposed a minimum number of 10 motor units for inclusion in analysis in their methodology (Madarshahian et al., 2021; Madarshahian & Latash, 2021). The numbers of decomposed motor units used in the calculation of firing rate therefore varies widely across studies. Additionally, little, if any, research to date provides an examination of the effect of the numbers of motor units included on the calculated firing rate.

In young, healthy individuals, there has been no reported difference between males and females in the estimated total number of motor units within a muscle (Gawel & Kostera-Pruszczyk, 2014; Yuan et al., 2000). However, in recordings of motor unit firing behavior, there is a trend in the literature for larger numbers of motor units to be decomposed in males than females (Lulic-Kuryllo & Inglis, 2022). From these recordings, there are reports where females have lower MUFs compared to males (Christie & Kamen, 2010; Inglis & Gabriel, 2020; Lulic-Kuryllo & Inglis, 2022; Piasecki et al., 2021). However, when matched for strength, females had higher MUFs than males during both submaximal and maximal contractions (Inglis & Gabriel, 2020). These differences between male and female MUFs and number of decomposed motor units highlight the importance of understanding the influence of the number of recorded motor units on the calculation and interpretation of MUF.

The purpose of the present study was to provide an analysis of the probability that average firing rates calculated from sub-sets of motor units represent the firing rate of the entire active motor unit pool. We used physiological firing rates obtained from the TA and compared subsets of these MUFs to statistical estimates of the mean firing rate of the active motor unit pool during submaximal and maximal contractions. Given the discrepancies in the number of motor units and mean firing rates reported between sexes in a given contraction, we also compared these probability outcomes between males and

females. We hypothesized that with increasing numbers of motor units included, the calculated firing rates would better match the firing rate of the estimated pool of active motor units. Further, we hypothesized that due to a lack of significant sex differences in motor unit number estimation, the number of motor units required for higher probabilities of reflecting the mean firing rate of the entire pool would be similar between males and females.

4.2 Methods

4.2.1 Participants

This study involved secondary analysis of previously-collected data. The original data were collected from 48 participants, 24 males (22 ± 2.06 years) and 24 females (21.54 ± 1.69) years and are reported in a previous publication (Inglis & Gabriel, 2020). Written informed consent was acquired prior to participation. All participants were free of neuromuscular and orthopedic disorders.

4.2.2 Data collection

Data acquisition methods have been described in detail elsewhere (Inglis & Gabriel, 2020). The decomposition of the motor units was performed using EMGLab (McGill et al., 2005). Briefly, data collection was performed on a second visit, following a familiarization session at least 24 hours prior. All participants performed 3 8-second maximal voluntary contractions (MVC) of the ankle dorsiflexors, and the maximum force value was used as the MVC (Inglis et al., 2011). Participants then performed a series of 8-second submaximal contractions at 20%, 40%, 60%, and 80% of the MVC with each intensity repeated 3 times. Motor units were decomposed from intramuscular EMG recordings in the TA from each contraction and manually checked a second time by a single operator. Motor unit firing rate was calculated from the reciprocal of the inter-pulse intervals of firings collected within a 2-second window of the steadiest force that was within 2.5% of the target. Inter-pulse intervals less than 10 ms and greater than 200 ms long were excluded from the calculation of firing rate. Three minutes of rest was provided between every contraction.

4.2.3 Data bootstrapping and processing

For each contraction intensity, the individual MUFs for all three trials at each contraction intensity were combined for each participant to create the sample in which the bootstrapped distribution for each participant was created. The number of motor units used in the bootstrapping process at each contraction intensity for each participant are presented in Table 1. Each iteration of the bootstrap distribution was created from a sample of 1.0×10^5 firing rate means, generated from the combined motor units per contraction (Table 4.1). A 95% two-tailed, bias-corrected and accelerated confidence interval (CI) was applied to each distribution (Efron, 1987). The normality of individual participant/contraction intensity distribution was then assessed. After the bootstrapped distributions and confidence intervals were created, a random sample of MUFs were compared to the confidence interval.

Table 4.1 Motor unit counts by MVC %

Sex	Participant	20%	40%	60%	80%	100%
Male	1	30	50	53	72	71
	2	34	39	46	70	45
	3	29	44	52	58	56
	4	31	46	45	44	59
	5	33	37	68	77	79
	6	15	26	45	39	47
	7	43	50	72	68	41
	8	43	59	46	67	67
	9	31	41	51	48	68
	10	48	68	82	76	55
	11	44	51	65	59	76
	12	41	27	55	57	71
	13	40	68	80	76	72
	14	53	53	59	67	63
	15	57	62	46	72	65
	16	51	59	57	77	72
	17	34	51	62	57	61
	18	37	54	64	63	64
	19	38	39	50	82	47
	20	32	42	41	37	34
	21	26	42	38	39	31
	22	35	26	22	15	21
	23	33	31	31	13	19
	24	28	36	27	22	21
Female	25	47	59	55	34	30
	26	28	36	27	22	21
	27	44	54	38	37	19
	28	22	22	11	26	20

29	19	28	25	18	14
30	29	53	48	57	19
31	20	30	45	40	25
32	30	37	44	50	39
33	52	70	70	75	72
34	38	63	50	76	79
35	45	70	65	91	55
36	29	29	40	34	33
37	61	52	51	34	33
38	41	39	60	75	70
39	53	53	66	66	70
40	73	59	63	66	72
41	51	61	64	66	90
42	19	36	51	64	82
43	26	65	60	78	74
44	24	37	52	66	80
45	32	53	69	66	75
46	13	29	34	59	83
47	41	34	62	52	40
48	32	48	59	78	82
Total	1755	2223	2466	2685	2582

4.2.4 Probability distributions

With n as the total number of MUFRRs within each condition for each participant, 1.0×10^5 MUFRR means (Davison & Hinkley, 1997; Kirk et al., 2021) were generated by sampling

every number of identified motor units between 1 and n . For example, at 20% MVC in participant 1 in Table 4.1, this process was repeated 30 times, creating 30 distributions of 1.0×10^5 means. The first distribution is created by sampling a single MUFR 1.0×10^5 times, and the final distribution is created by sampling 30 motor units 1.0×10^5 times.

The distributions from each of the $n \times 1.0 \times 10^5$ MUFR means were then compared to the bootstrapped confidence interval of the mean MUFR for each participant at each contraction intensity. The ratio of means that fell within the confidence intervals was then created to provide a probability for each number of sampled MUFRs. The probability of each number of sampled firing rates was then averaged across all participants in all contraction intensities, and then compared between males and females. Outcomes for probability levels of 50, 60, 70, 80, and 90% were generated. All processing was performed in Matlab version 2022a (Mathworks, Natick, Mass., USA).

4.2.5 Statistical analysis

Normality of bootstrapped distributions was assessed using a one sample Kolmogorov-Smirnov test. A three factor (Contraction intensity, Probability Level, Sex) repeated measures ANOVA was performed, using the probability levels of 50%, 60%, 70%, 80%, and 90%. Post-hoc pair-wise comparisons with Bonferroni corrections were performed for significant outcomes. All statistical tests were performed in SPSS v28 with α set at 0.05 for significance.

4.3 Results

Individual plots of the probability that each number of sampled motor units results in a firing rate within the bootstrap confidence intervals are presented in Figure 4.1. Sex separated data for 50%, 60%, 70%, 80%, and 90% probability levels of firing rates within the bootstrapped confidence intervals are presented in Figure 4.2. There was a significant effect of relative contraction intensity on the number of motor units ($p < 0.001$, $\eta^2 = 0.22$), where higher numbers of motor units are required to calculate a firing rate that is within the bootstrapped confidence interval as relative contraction intensity increases.

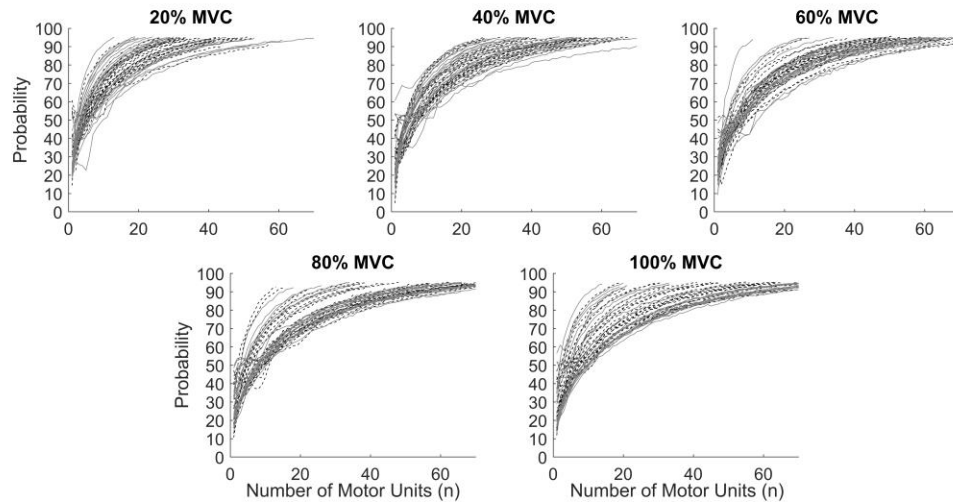


Figure 4.1 Probability distributions for each motor unit sample
Individual lines represent data for each participant.

There was also a significant effect of the level of probability ($p < 0.001$, $\eta^2 = 0.82$; Figure 4.3) and a significant interaction of relative contraction intensity and probability ($p < 0.001$, $\eta^2 = 0.10$; Figure 4.3) with higher numbers of motor units required to calculate firing rates with higher probability of landing within the bootstrapped confidence interval. Post-hoc comparisons across contraction intensities at 50% probability revealed that the number of motor units required at 60% and 80% MVC were significantly higher than the number of motor units required at 20% MVC for males and females ($p < 0.05$; Figure 4.2). For 60%, 70%, 80%, and 90% probability, the number of motor units at 40% MVC and above were significantly greater than the number of motor units required at 20% MVC ($p < 0.05$; Figure 4.2).

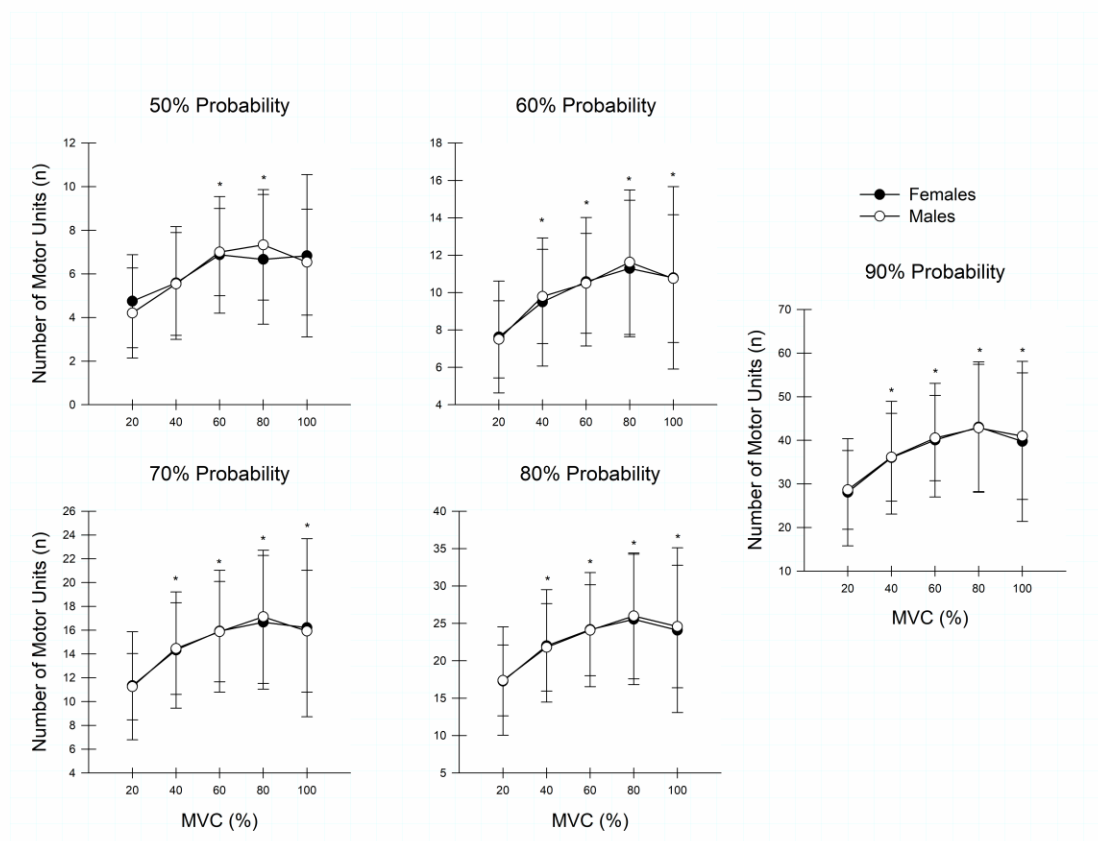


Figure 4.2 Number of motor units required across contraction intensities to fall within each level of probability

Data for males and females are shown separately. * Indicates significantly different from 20% MVC for both sexes ($p < 0.05$). Values are mean \pm SD.

There was no significant effect of sex on the number of motor units required to fall within the bootstrapped confidence interval ($p = 0.97$, $\eta^2 < 0.01$). The interactions of sex and probability level, as well as sex and relative contraction intensity, were not significant ($p = 0.99$, $\eta^2 < 0.01$). There was no significant three-way interaction of relative contraction intensity, sex, and probability level ($p = 0.99$, $\eta^2 < 0.01$).

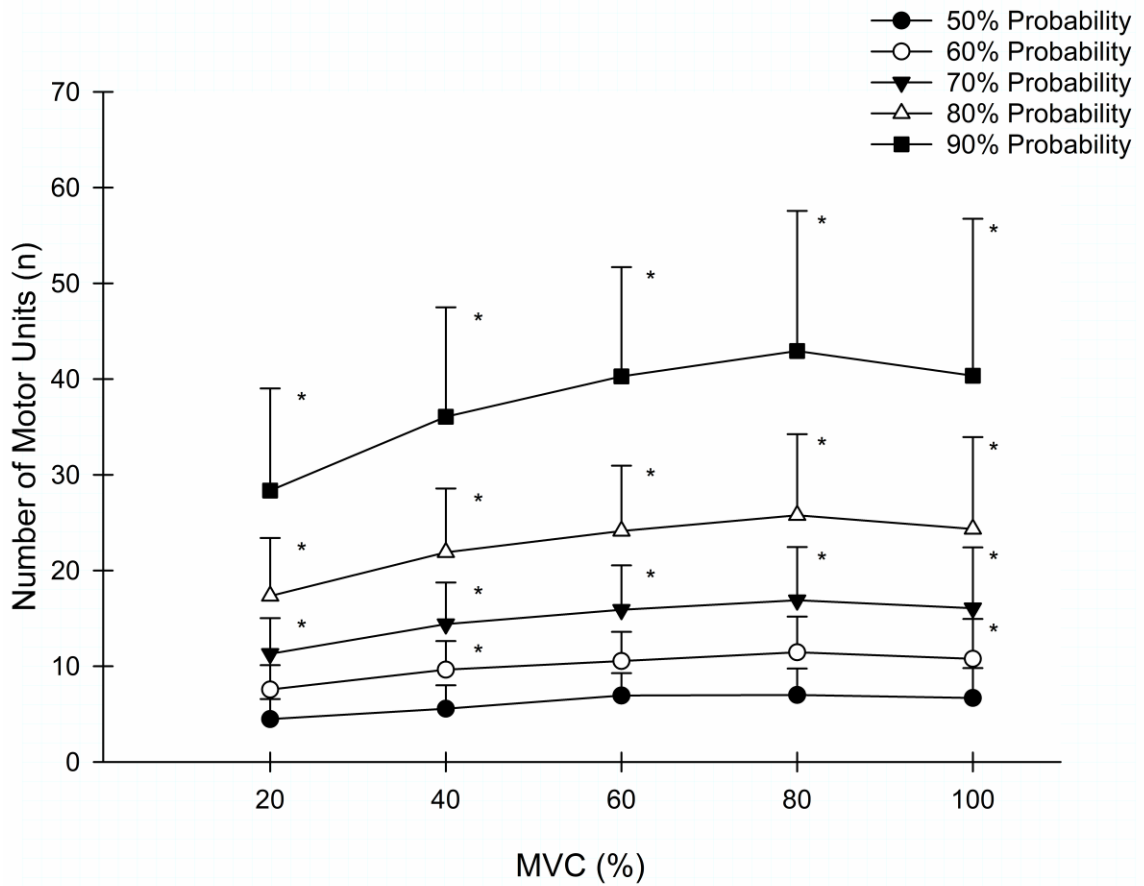


Figure 4.3 Number of motor units required across contraction intensities with male and female data combined

Averaged data for the required number of motor units across contraction intensities are shown for each probability level. * Indicates significantly different from the previous probability level ($p < 0.05$). Values are mean \pm SD.

4.4 Discussion

The purpose of this study was to provide insight into how the calculation of mean MUFR is influenced by the number of motor units included in the calculation. We hypothesized that increasing numbers of motor units would result in higher probabilities of matching the firing rate of the estimated pool of active motor units, and that males and females would require similar numbers of motor units in the calculation of MUFR. The results support these hypotheses where greater numbers of motor units were required for both higher contraction intensities and the probability of matching the estimated means. We also observed no differences between males and females in the required number of motor units to achieve a given level of probability of matching the estimated mean.

Current methods of motor unit acquisition and decomposition provide large ranges of number of motor units identified per contraction. For example, in the original data used for the current study, for 20% MVC contractions, the number of identified motor units ranged from 5-18 across participants (Inglis & Gabriel, 2020). Other studies report 4-14 motor units when using indwelling recordings (Christie & Kamen, 2006, 2009; Inglis & Gabriel, 2020) and 11-22 motor units when using surface EMG decomposition methods (Martinez-Valdes et al., 2020; Parra et al., 2020). Based on the results of the present study, the previous reports of 4-22 motor units would result in 50%-80% probability that the calculated mean firing rates would fall within the confidence intervals of the estimated population mean firing rate, depending on the contraction intensity. Higher numbers of motor units involved in a calculation of MUFR can be achieved through multiple trials at a given contraction intensity for each participant (Inglis & Gabriel, 2020; Kowalski et al., 2022).

As the data from this study are based on only the TA, the application of these results to other muscles may not be appropriate. Although these results do not provide a single value of the “required” number of motor units, the results do provide useful outcomes for future research. First and most importantly, statistical and conceptual support for methodological design are provided from these results. The representativeness of sampled motor unit populations has been previously challenged, with results that provided a substantial increase in the sensitivity of analysis when over 20 motor units are

sampled (Podnar, 2004). The 20 motor units suggested by Podnar (2004) fall within the 80% probability level in the current study. The results of this study further support the benefits of including greater numbers of motor units and provide additional information with the inclusion of multiple contraction intensities. A secondary outcome from these results are the avenues for further research, including examination of lower contraction intensities, different muscles, and different populations.

4.4.1 Sex-related differences

Differences in neuromuscular function between males and females are well documented, including lower MUFR and greater variability of firing rate in females (Inglis & Gabriel, 2020b, 2021; Jakobi et al., 2018; Lulic-Kuryllo & Inglis, 2022). Existing literature suggests that, compared with males, females tend to have a distribution of α -motoneurons that reflects a larger proportion of smaller α -motoneurons (Yuan et al., 2000). Despite different size distributions, the total number of α -motoneurons and the estimated total number of motor units are not significantly different between males and females (Gawel & Kostera-Pruszczyk, 2014; Yerdelen et al., 2006; Yuan et al., 2000). However, age-related loss of α -motoneurons and the sequential neuromuscular remodeling seems to result in a greater loss in females (Gawel & Kostera-Pruszczyk, 2014). A generalized reduction in motor units that is greater in females provides a strong avenue of future research from this study, focused on aging populations.

Despite the lack of documented differences in the number of motor units between males and females, recordings of motor units often result in a lower yield from female participants (Lulic-Kuryllo & Inglis, 2022; Martinez-Valdes et al., 2020, 2021). Based on the results of the current study, this difference in motor unit yield between sexes would result in different levels of probability that the calculated firing rates represent the possible firing rate of the active motor unit pool. For example, in one study (Taylor et al., 2022), the mean number of reported motor units that were included in calculations of firing rates for females and males were 13.4 and 27.7, respectively. In this example, based on the motor unit numbers, the probability that the mean MUFR is within a 95% confidence interval of the estimated mean of the active pool would be 70% for females

and 90% for males. Low levels of probability are not necessarily an indication of poor methodology; however, our results suggest that the often-reported differences in the number of recorded motor units between males and females may be an important consideration in interpretation of sex-related differences in MUFRs.

4.4.2 Limitations

The main limitation of this study lies in the assumptions made by bootstrapping methodology and the decomposition process. The strength of a bootstrapped distribution to reflect the true population from which the sample was made is dependent on the accuracy of the original sample (Efron & Tibshirani, 1993) and the accuracy of our original sample cannot be specifically quantified. The methodology of combining all 3 trials per contraction intensity implemented in this study was meant to address this assumption by increasing the sample from which the confidence interval was generated. Furthermore, the resultant motor unit decomposition was manually inspected after decomposition with strict inclusion criteria of motor unit behavior to provide firing rates most representative of the contraction intensity (Christie & Kamen, 2009, 2010; Stashuk, 2001). Finally, the results in the present study were obtained through analysis on MUFRs decomposed from the TA in young, healthy individuals. The results could change depending on the muscle from which the motor units are recorded and the age of the participants (Kirk et al., 2021). Future studies should focus on different muscles, as well as the influence of age.

4.5 Conclusion

The results of the present study provide justification for methodology aimed at increasing the number of motor units recorded per contraction when calculating MUFR. These results provide evidence for inclusion of greater numbers of motor units in the calculation of firing rate at higher contraction intensities. A lack of sex-related differences in our results also implies that researchers should strive for equal numbers of motor units recorded from males and females, as the number of motor units required for each level of probability was similar between sexes. More work is necessary to understand the sex-

related difference in motor unit yield that is often observed in studies of motor unit behavior. The methodology of the current study should be expanded to other muscles and groups of individuals (e.g., older adults) with documented lower numbers of motor units.

4.6 References

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Chapter 5

5 Discussion

This dissertation examined the influence of mental fatigue and pennation angle on motor unit firing rates (MUFR) in males and females. Motor unit firings in the first dorsal interosseous (FDI) were compared before and after a mentally fatiguing task at submaximal intensities in chapter 2, and motor unit firings in the tibialis anterior (TA) were examined for a relationship to pennation angle at submaximal and maximal intensities in chapter 3. The fourth chapter used previously collected data from the TA to examine how MUFR calculated from different numbers of motor units compared to an estimated distribution of MUFRs used to represent the total activity of motor units in a muscle. No effect of mental fatigue was found on motor unit firing behavior in the FDI, however, there was a significant effect of sex where females improved in their performance of tracing a variable force template in chapter 2. There was a positive correlation between MUFRs and pennation angles in males in chapter 3. No sex-related differences were found in the number of motor units needed to approximate the mean firing rate of the motor unit pool at submaximal and maximal contraction intensities in chapter 4. The results of this dissertation provide further evidence of sex differences in motor unit firing behavior, although this may not be different in response to mental fatigue. Males and females also require similar numbers of motor units to get similar estimates of whole muscle activity.

In chapter 2, we found that there was no significant effect of a mentally fatiguing task compared to a documentary control condition. Males and females in our study also had higher levels of subjective fatigue after both mental fatigue and control conditions, with higher reports overall on the mental fatigue day. Females also reported higher levels of subjective fatigue, which aligns with previous studies where females report higher levels of fatigue (Engberg et al., 2017), however, it was not unique to the mentally fatiguing condition. Both sexes had pre-to-post increases in average firing rate at 10% and decreases at 50% MVC, however, these changes were not unique to the mental fatigue condition. There was a sex-specific difference over time where females improved in their force tracing in the variable force template. The mechanisms leading to a female-specific

improvement in tracing a variable force template in the FDI, however, is unclear in the present study. There is evidence to suggest females utilize higher numbers of smaller motor units to contribute to force in lower limb muscles (Cioni et al., 1994; Guo et al., 2022). Larger numbers of motor units contributing to force is known to reduce coefficient of variation (CV) of force by reducing independent input on the active motor unit pool (Farina & Negro, 2015; Taylor et al., 2003). In this variable force condition, males also had significantly more variable force compared to females alongside the sex-specific change in performance, which is contrary to expected higher force variability in females (Jakobi et al., 2018; Pereira et al., 2015; Yoon et al., 2014). Female-only studies show that significant reductions in MUFR modulation outside of the frequencies of complex sinusoidal force tracing template are associated with improvements in the tracing performance (Knight & Kamen, 2004, 2007). A sex-effect of variable force and associated MUFR modulations is suggested as a topic for further investigation.

The Psychomotor Vigilance Task (PVT) utilized in chapter 2 is a sustained attention task measuring reaction time, lapses, and false starts. Our intervention resulted in a significant increase in participant reaction time, suggesting our intervention was successful as a mentally fatiguing task as measured by an increase in reaction time (Lim et al., 2010). Our participants, however, did not have significant changes to their lapses which has been suggested as another objective measure of mental fatigue (Lee et al., 2010). Despite this, our protocol utilized a 30-minute task of the PVT and a control condition, the *Earth* documentary, for the same duration. Previous examinations of neuromuscular function and mental fatigue utilized 22-30 minutes of the PVT and observed significant changes to reaction time, lapses, and false starts (Kowalski et al., 2022; Kowalski & Christie, 2020). The change in subjective fatigue over time was also not unique to the PVT day, which is also similar to previous studies on mental fatigue utilizing the *Earth* documentary (Kowalski et al., 2022). This provides supporting evidence for previous suggestions that 30 minutes of the *Earth* documentary could have other effects on neuromuscular function potentially caused by prolonged sitting (Baker et al., 2018; Chandrasekaran et al., 2021; Hachard et al., 2020; Kowalski et al., 2022).

These conflicting outcomes between the mental fatigue and control conditions, and a lack of significant changes to lapses could have been related to our participant's high scores on the Multidimensional Fatigue Inventory (MFI). The MFI scoring in chapter 2 is higher than some previous studies utilizing the PVT as a mentally fatiguing condition (Kowalski et al., 2022; Lee et al., 2010) but not others (Kowalski & Christie, 2020). Our participants for this study were recruited 18 months into the Covid-19 pandemic. The effects of lockdown procedures and self-isolation are known to increase levels of reported mental fatigue which could have influenced the similar increase in mental fatigue after both the control and mentally fatiguing condition (Leigh-Hunt et al., 2017; Torrente et al., 2022). Regardless, even though the PVT task utilized in chapter 2 was longer than other studies, the effect of mental fatigue resulting from the PVT in our protocol was not significant on neuromuscular function. The novel force tracing tasks utilized in the non-dominant hand could have been challenging enough to participants that the effects of mental fatigue present were not enough to suppress participant's focus on the task.

The objective of chapter 3 was to establish whether there exists a relationship between MUFR and pennation angle in males and females, and only in males was the relationship significant. Compared to previous studies where males and females had significantly different pennation angles in the TA (Manal et al., 2006), our participants had similar pennation angles and MUFRs, yet only males had a significant relationship between the two. A male-only relationship between MUFR and pennation angle is not unexpected. Relationships between pennation angle and the mechanical activity of the muscle as represented by mechanomyography amplitude had significant, moderate correlations with pennation angle and muscle thickness in the vastus lateralis (Trevino et al., 2023). As mechanomyography activity is suggested to represent the mechanical component of neural activity in the muscle (Coburn et al., 2005), larger and stronger muscles in males would explain a stronger relationship in males. Although muscle thickness is not presented in our study, males generally have larger and thicker muscles than females (Trevino et al., 2019, 2023), and maximal force output in our males was significantly higher than the females. A stronger relationship in males between MUFR in our study would then follow these trends. However, there was a significant difference in MUFR and pennation angle between 20% and 100% maximal voluntary contraction in males and

females. Another study in the TA found sex-driven differences in MUFR when a subset of participants were matched for force, with no mean differences in firing rates at submaximal and maximal contraction intensities (Inglis & Gabriel, 2020). Our results suggest that females may utilize different motor unit firing strategies, such as doublets (Inglis & Gabriel, 2021) to compensate for differences in muscle size, but further investigation is required.

Chapter 4 was an examination of how mean MUFR, computed from various numbers of motor units firing, compared to an estimated mean used to represent the total pool of active, unrecorded motor unit firing trains. The data utilized in this chapter was acquired from another study using intramuscular electrodes to record the EMG signals used in decomposition. A common trend in all three chapters is that the methodology in reporting motor unit firing behaviors used the average calculated from multiple trials at the same contraction intensity. This methodology provides both larger numbers of motor units for analysis and reduces the influence of potential outliers or equipment failures from single trials. Higher numbers of motor units are essential in studies of motor unit firing behavior on an average scale, as limitations imposed by the filtering effects of tissue make it difficult to reliably record the activity of distinct motor units across the entire muscle (Gygi & Moschytz, 1997). This issue is compounded by a trend in the literature for lower numbers of motor units decomposed from female participants, both through sampling methodologies favoring males and through commonly lower motor unit yield in females (Del Vecchio et al., 2020; Guo et al., 2022; Lulic-Kuryllo & Inglis, 2022; Taylor et al., 2022). Although there are a few studies with equal or greater motor unit yield in females (Christie & Kamen, 2006; Knight & Kamen, 2008), the evidence to date has been provided from male only or sex-collapsed data (Lulic-Kuryllo & Inglis, 2022).

Regardless of this difference, the results from chapter 4 suggest that the probability of matching an estimated distribution of means from which the recorded MUFR was sampled requires similar numbers of motor unit firing trains for males and females. Within the probability levels, increasing contraction intensity also required higher numbers of motor units. The results from chapter 4 also provide insight into the probabilities for MUFR reported in Tables 2.1 and 3.1. In chapter 2, motor unit numbers

ranged from 9-31 from 10% to 50% MVC, resulting in a range of 70%-90% probability of reflecting the population mean. In chapter 3, motor unit numbers ranged from 11-36, resulting in a 60-90% probability. The influence of contraction intensity from the results was expected, as increases in motor unit recruitment with contraction intensity would result in a larger sample of potential motor units active during a contraction. The tested samples and means of MUFs were taken from a pooled collection of motor units that were combined from multiple contractions, and the results provide justification for methodological steps designed to increase motor unit yield for motor unit behavior analysis. However, the exact motor unit counts for probability and contraction intensity may vary between muscles. Factors, such as age or different muscles, may change the exact numbers required for the probability results presented in this dissertation. Age is an established influence on motor unit behavior and the number of motor units in participants, and the distribution of motor unit sizes in different muscles may change the relative contribution of rate coding and recruitment between muscles (Bellemare et al., 1983; Knight & Kamen, 2007; Macefield et al., 1996; McNeil & Rice, 2018).

5.1 Future research

The results of chapter 2 suggest a potential sex-related difference in modulation of force during a variable force contraction at low to moderate intensities. However, the results do not offer a mechanistic explanation for this observation. An examination of this motor unit firing behavior revealed suppressed rate modulation in older females compared to young (Knight & Kamen, 2007), and this comparison has not been made between males and females. Furthermore, this dissertation focused on samples from young, healthy adults. Results from this population help describe neuromuscular function in general, however, the effect of age is a defining factor in alterations of neuromuscular function. Changes in the neuromuscular system with age and the interaction with sex have been observed to change all aspects of neuromuscular function investigated in this dissertation, such as motor unit firing behavior, muscle architecture, and the perception of fatigue (Avlund, 2010; Christie & Kamen, 2006; Engberg et al., 2017; Hepple & Rice, 2016; Knight & Kamen, 2007). Further work should therefore expand these studies to older adult populations. The range of pennation angles in chapter 3 were also relatively low

compared to ranges observed in other lower limb muscles (Manal et al., 2006, 2008; Trevino et al., 2023). Despite the small range of pennation angles in the TA and observed in chapter 3, positive relationships were observed with MUFR. Muscles with larger ranges of pennation angle and different relative contributions of recruitment and rate coding may reveal further sex-specific relationships between MUFR and pennation angle in submaximal and maximal contraction intensities. The estimation of probability in how many motor units are required to match the estimated mean may also be different across muscles and with age. The effect of age and sex may provide different results, as the neuromuscular system undergoes significant changes which result in lower numbers of motor units, specifically in females (Gawel & Kostera-Pruszczyk, 2014).

5.2 Limitations

The results in chapter 2 suggest that the effect of the PVT and the *Earth* documentary on neuromuscular function may be the same. This provides evidence that there is no significant effect on neuromuscular function unique to mental fatigue in this comparison. However, previous studies using the *Earth* documentary observed a similar lack of changes and suggested that the effect of sitting may confound this control condition from specifying the influence of mental fatigue (Kowalski et al., 2022). In chapter 3, females had a positive but not significant correlation between MUFR and pennation angle, and further studies may elucidate further evidence about this relationship. Muscle, fascicle length, and subcutaneous fat thickness were not recorded, which may influence these variables as noted when using surface EMG (Del Vecchio et al., 2020; Martinez-Valdes et al., 2022). Chapter 4 also utilized estimates of the distributions of potential MUFR means used to represent the entire muscle. The bootstrapping method utilized to generate the distributions is sensitive to the accuracy of the original recorded data, and it is unknown how well this sample represents the true motor unit population.

5.3 Conclusion

In summary, this dissertation demonstrated no sex-related differences in MUFRs in the FDI or TA. There was no apparent sex-related difference in the effects of mental fatigue nor the number of motor units recorded on MUFR. There are potential sex-related

differences caused by anatomical factors, as evident by the significant relationship between MUFR and pennation angle in males but not females. Female-specific improvements in the tracing of our sine wave condition suggest that other sex-specific differences in neuromuscular function during variable or cyclical isometric contractions. These results provide both avenues for future research and methodological support for neuromuscular studies

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Appendices

Appendix 1 Chapter 2 ethics approval



Date: 20 August 2020

To: Professor Anita Christie

Project ID: 116216

Study Title: Sex Differences in Motor Unit Firing Behavior and Force Control in the FDI Following Mental Fatigue

Application Type: HSREB Initial Application

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 07/Jul/2020

Date Approval Issued: 20/Aug/2020

REB Approval Expiry Date: 20/Aug/2021

Dear Professor Anita Christie

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. All other required institutional approvals must also be obtained prior to the conduct of the study.

Documents Approved:

Document Name	Document Type	Document Date	Document Version
Data Collection Form	Other Data Collection Instruments	19/Jun/2020	1
IPAQ_English_self-admin_long	Online Survey	28/Jul/2020	1
MFI	Online Survey	28/Jul/2020	1
Pittsburgh_Sleep_Quality_Index	Online Survey	28/Jul/2020	1
Mental state and trait	Online Survey	28/Jul/2020	1
Recruitment Email v2	Email Script	13/Aug/2020	2
Email 3 with survey and schedule	Email Script	13/Aug/2020	1
Follow up email with consent	Email Script	13/Aug/2020	1
FDI flier	Recruitment Materials	13/Aug/2020	1
Screening_Form - qualtrics	Online Survey	13/Aug/2020	1
FDI consent form v3	Written Consent/Assent	18/Aug/2020	3
Protocol and Research Plan v3	Protocol	18/Aug/2020	3

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
Rationale References	References	14/Jun/2020	1
FDI-Mental Fatigue budget	Study budget	13/Aug/2020	2

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

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,

Appendix 2 Chapter 3 ethics approval



Date: 13 June 2022

To: Professor Anita Christie

Project ID: 120894

Review Reference: 2022-120894-67555

Study Title: Sex differences in the relationships between pennation angle and firing rate

Application Type: HSREB Initial Application

Review Type: Delegated

Meeting Date / Full Board Reporting Date: 28/June/2022

Date Approval Issued: 13/June/2022 16:04

REB Approval Expiry Date: 13/June/2023

Dear Professor Anita Christie

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above mentioned study as described in the WREM application form, as of the HSREB Initial Approval Date noted above. This research study is to be conducted by the investigator noted above. **All other required institutional approvals and mandated training must also be obtained prior to the conduct of the study.**

Documents Approved:

Document Name	Document Type	Document Date	Document Version
TA_Screening	Online Survey	19/May/2022	1
Protocol and Plan	Protocol	25/May/2022	1
TA follow up email	Email Script	09/June/2022	2
Recruitment Email	Email Script	09/June/2022	2
TA Flier	Recruitment Materials	10/June/2022	2
Ultrasound Consent form	Written Consent/Assent	10/June/2022	3

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
Study Rationale References	References	18/May/2022	1
TA-PenAng Budget	Study budget	19/May/2022	1

No deviations from, or changes to, the protocol or WREM application should be initiated without prior written approval of an appropriate amendment from Western HSREB, except when necessary to eliminate immediate hazard(s) to study participants or when the change(s) involves only administrative or logistical aspects of the trial.

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.

Electronically signed by:

Appendix 3 Chapter 4 ethics approval



Brock University



Bioscience Research Ethics Board

Certificate of Ethics Clearance for Human Participant Research

DATE: 8/3/2012
 PRINCIPAL INVESTIGATOR: GABRIEL, David - Kinesiology
 FILE: 12-027 - GABRIEL
 TYPE: Ph. D. STUDENT: Greig Inglis
 SUPERVISOR: David Gabriel

TITLE: Sex Differences in motor unit discharge rates at various force levels

ETHICS CLEARANCE GRANTED

Type of Clearance: NEW

Expiry Date: 8/30/2013

The Brock University Bioscience Research Ethics Board has reviewed the above named research proposal and considers the procedures, as described by the applicant, to conform to the University's ethical standards and the Tri-Council Policy Statement. Clearance granted from 8/3/2012 to 8/30/2013.

The Tri-Council Policy Statement requires that ongoing research be monitored by, at a minimum, an annual report. Should your project extend beyond the expiry date, you are required to submit a Renewal form before 8/30/2013. Continued clearance is contingent on timely submission of reports.

To comply with the Tri-Council Policy Statement, you must also submit a final report upon completion of your project. All report forms can be found on the Research Ethics web page at <http://www.brocku.ca/research/policies-and-forms/research-forms>.

In addition, throughout your research, you must report promptly to the REB:

- Changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- All adverse and/or unanticipated experiences or events that may have real or potential unfavourable implications for participants;
- New information that may adversely affect the safety of the participants or the conduct of the study;
- Any changes in your source of funding or new funding to a previously unfunded project.

We wish you success with your research.

Approved:



Brian Roy, Chair
 Bioscience Research Ethics Board

Note: Brock University is accountable for the research carried out in its own jurisdiction or under its auspices and may refuse certain research even though the REB has found it ethically acceptable.

If research participants are in the care of a health facility, at a school, or other institution or community organization, it is the responsibility of the Principal Investigator to ensure that the ethical guidelines and clearance of those facilities or institutions are obtained and filed with the REB prior to the initiation of research at that site.

Curriculum Vitae

Name: Michael Marsala

Post-secondary Education and Degrees: University of Central Florida
Orlando, Florida, United States
2016-2017 B.S.

University of Central Florida
Orlando, Florida, United States
2018-2019 M.S.

The University of Western Ontario
London, Ontario, Canada
2019-2023 Ph.D.

Related Work Experience Data and Quality Analyst
St. Joseph's Healthcare London
2023-present

Teaching Assistant
The University of Western Ontario
2019-2023

Research Assistant
The University of Western Ontario
2019-2023

Publications:

Marsala, M. J., Belfry, S., Orange, J. B., & Christie, A. D. (2023). Sex-related differences in functional fitness outcomes in older adults. *Journal of Aging and Physical Activity*, 31(4), 556–567. <https://doi.org/10.1123/japa.2022-0071>

Shirzad, M., Tari, B., Dalton, C., Van Riesen, J., **Marsala, M. J.**, & Heath, M. (2022). Passive exercise increases cerebral blood flow velocity and supports a postexercise executive function benefit. *Psychophysiology*, 59(12), e14132.

Conference Presentations:

Marsala, M. J., Christie, Anita D. Motor unit firing behavior following mental fatigue in static and variable-force contractions. poster presentation at the international Society of Electrophysiology and Kinesiology, 2022.

Marsala, Michael J., Belfry, Shannon., Orange, Joseph B., Christie, Anita D. FACSM. Sex differences in five years of functional fitness in older adults. Poster presentation at The American Society of Sports Medicine, 2021.

Marsala, M. J., Christie, A. D., Stock, M. S., Fukuda, D. H., & Garcia, J. M. (2020). Measurement of muscle thickness in the forearms of rock climbers using ultrasound: Poster presentation at The American Society of Sports Medicine, 2020.