
Electronic Thesis and Dissertation Repository

8-12-2024 1:00 PM

Sex-related Differences in H-reflex Excitability and Inhibition

Jonah D. Dekker, *Western University*

Supervisor: Anita, Christie D., *The University of Western Ontario*

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

© Jonah D. Dekker 2024

Follow this and additional works at: <https://ir.lib.uwo.ca/etd>



Part of the [Motor Control Commons](#)

Recommended Citation

Dekker, Jonah D., "Sex-related Differences in H-reflex Excitability and Inhibition" (2024). *Electronic Thesis and Dissertation Repository*. 10378.

<https://ir.lib.uwo.ca/etd/10378>

This Dissertation/Thesis is brought to you for free and open access by Scholarship@Western. It has been accepted for inclusion in Electronic Thesis and Dissertation Repository by an authorized administrator of Scholarship@Western. For more information, please contact wlsadmin@uwo.ca.

Abstract

The Hoffmann reflex (H-reflex), when elicited in the soleus in healthy males and females, demonstrates sex-specific differences in the neuromodulation of spinal synaptic transmission. This study investigated the sex-related differences in H-reflex excitability and inhibition. Thirty healthy young individuals, 15M (24±2yrs) and 15F (23±3yrs) participated in the study. Surface electrodes were used to elicit and record the H-reflex. Excitability was assessed by stimulating the tibial nerve, beginning at a low-intensity voltage and increasing incrementally until the maximal amplitude of the muscle response was reached. Reciprocal inhibition (RI) was assessed with a conditioning - test stimuli protocol. No significant sex-related differences in excitability were identified; however, males exhibited significantly greater RI than females. This study will add to the growing body of knowledge surrounding differences in the modulation of spinal motor control between sexes and will contribute to the significant gap in neurophysiology research involving females.

Keywords

Sex differences; H-reflex, spinal excitability, reciprocal inhibition, EMG, electrical stimulation, neuromuscular function.

Summary for Lay Audience

The physiological system has been well-defined in scientific literature involving male participants; however, research on females is sparse. This gap in the literature is vitally in need of research as findings from male participants should not be used to explain female neurophysiology. The nervous system's signaling pathway that makes up the spinal reflex loop can be manipulated with the electrical stimulation of a peripheral nerve. The response provides information about how efficiently the signal travels through the spinal cord and to the muscle. Studies on male and female differences in this pathway's activation and resistance to activation, known as excitability and inhibition, respectively, are limited and poorly understood in scientific literature. Therefore, this study aimed to investigate the sex-related differences in spinal reflex excitability and inhibition. We recruited thirty (15F) healthy young adults. Participants visited the lab for a single day of testing. Electrical stimulation of a nerve behind the knee evoked a reaction at the spinal level, and the response was recorded with surface electrodes over the calf muscle. The results showed that males and females do not differ in spinal excitability; however, males had greater spinal inhibition. The findings will help fill the gap in understanding sex-related physiological differences while directing future research on movement control tasks.

Co-Authorship Statement

Anita D. Christie provided feedback and guidance on the entire manuscript, along with overall study design and data analysis. Data were analyzed and interpreted by Jonah D. Dekker

Acknowledgments

The past two years have passed so quickly, yet it's been some of the best years of my life. I want to thank so many people for making this journey as incredible as it was. First and foremost, Anita Christie has been a pillar of support and the best supervisor I could have ever asked for. Her door was always open when I decided to stumble down the hallway from the lab to ask her yet another question. Her way of explaining content while testing my knowledge in the process is like no other. Thank you so much, I could not have done this without you.

To my previous lab mates, Michael and Kherto - thank you for teaching me all about the lab equipment. Our time together was short, but I highly appreciated the time you put in to get me started. Ali, Alicia, and KJ - you guys have been incredibly supportive at every step of the way, from the courses we took together to data collection and write-up and all the fun things we do outside of our school life. I can't thank you enough. Dan and Kathryn - my gym friends, above all else, have made my mornings, lunch breaks, and evenings enjoyable regardless of what activities we get up to. To everyone else on the 4th floor: Kasey, Robin, Nasimi and the Rice boys, you guys have made my time in the office and lab such an enjoyable place to work (and live).

To my mom, thank you for your endless support with the path I've chosen so far. From the day I moved away to London until now, your continuous assistance and advice have helped me persevere through anything. Thank you for listening to me rant and gleam about what has happened to me so far. I can't express enough how much the support you have provided means to me. Thank you for always believing in me.

Table of Contents

Abstract.....	ii
Summary for Lay Audience.....	iii
Co-Authorship Statement.....	iv
Acknowledgments.....	v
List of Tables	viii
List of Figures	ix
List of Appendices	x
List of Abbreviations	xi
Chapter 1.....	1
1 Introduction.....	1
1.1 Neuromuscular function.....	1
1.1.1 Motor unit recruitment and rate coding	1
1.2 Soleus muscle.....	4
1.3 Hoffmann reflex.....	5
1.3.1 Spinal inhibition.....	8
1.3.2 Sex-related differences in H-reflex.....	10
1.4 Purpose.....	11
1.5 References.....	12
Chapter 2.....	18
2 Sex-related differences in H-reflex excitability and inhibition.....	18
2.1 Introduction.....	18
2.2 Methods.....	20
2.2.1 Participants.....	20
2.2.2 Experimental protocol.....	20

2.2.3	Force	21
2.2.4	Surface EMG	21
2.2.5	Electrical nerve stimulation	22
2.2.6	Statistical analysis.....	24
2.3	Results.....	24
2.3.1	Participant Characteristics	24
2.3.2	M-wave	24
2.3.3	H-reflex excitability	26
2.3.4	Reciprocal inhibition.....	26
Chapter 3	28
3	Discussion and Summary.....	28
3.1	Discussion.....	28
3.1.1	M-wave (M-max, M-slope)	28
3.1.2	H-reflex excitability (H-max, H-slope, H-max/M-max, H-slope/M-slope)	29
3.1.3	Reciprocal inhibition.....	30
3.1.4	Methodological considerations	31
3.2	Limitations	33
3.3	Conclusion	33
3.4	Future directions	34
References	35
Appendices	40
Curriculum Vitae	46

List of Tables

Table 1. Participant Characteristics	25
--	----

List of Figures

Figure 1. The Hoffmann reflex pathway. The H-reflex and M-wave are elicited by electrical stimulation of a peripheral nerve and the evoked potential is recorded with EMG. Figure reprinted from (Aagaard et al., 2002).	6
Figure 2. The reciprocal inhibition pathway. Reciprocal inhibition from the tibialis anterior to the soleus, measured by utilizing an H-reflex paired stimulus technique. Dotted lines represent Ia afferent fibres propagating sensory feedback to the soleus motor neuron pool. The solid line with an empty circle represents the transfer of efferent signals from the motor neuron pool to the soleus muscle. The solid line with the filled circle represents an inhibitory interneuron acting upon the soleus motor neuron pool following activation of the tibialis anterior (antagonist muscle). Figure reprinted from (Pierrot-Deseilligny & Burke, 2005)....	10
Figure 3. Timeline of experimental protocol. Outline of the order of experimental procedures on the single day of testing. Horizontal arrows represent time windows. Vertical bars represent maximum voluntary contraction trials. Electrical signals represent elicitation of the H-reflex and M-wave.....	20
Figure 4. Example of H-reflex and M-wave recruitment curve.....	23
Figure 5. M-max amplitude and M-slope. Bars represent the mean values for males and females. Individual data points are shown by circles. Both M-max (left) and M-slope (right) were greater in males than females (* $p \leq 0.004$).	25
Figure 6. H-max amplitude, H-slope, H-max/M-max and H-slope/M-slope ratio. Bars represent the mean values for males and females. Individual data points are shown by circles. H-slope (top right) was greater in males than females (* $p = 0.009$). H-max (top left), H-max/M-max ratio (bottom left), and H-slope/M-slope ratio (bottom right) were not significantly different between sexes.....	26
Figure 7. Reciprocal inhibition. Bars represent the mean values for males and females. Individual data points are shown by circles. Reciprocal inhibition was significantly greater in males than females (* $p \leq 0.01$).	27

List of Appendices

Appendix 1. Ethics Approval.....	40
----------------------------------	----

List of Abbreviations

CSA – Cross-sectional Area

EMG – Electromyography

H-reflex – Hoffmann Reflex

MU – Motor Unit

MVC – Maximal Voluntary Contraction

RI – Reciprocal inhibition

RMS – Root Mean Squared

SOL – Soleus

TA – Tibialis Anterior

Chapter 1

1 Introduction

1.1 Neuromuscular function

The neuromuscular pathway begins in the brain's primary motor cortex, where electrical signals are generated with the end-goal of producing movement (Sanes & Donoghue, 2000). These electrical signals, known as action potentials, propagate through the corticospinal tract to the alpha motor neurons in the spinal cord. From the spinal motor neuron pool, action potentials propagate to the neuromuscular junction and, finally, to the muscle, triggering a contraction. Neuromuscular activation can occur through both voluntary, and involuntary means (Betti et al., 2022; Ugawa, 2020). Voluntary movement depends on descending drive and input from the periphery. However, involuntary movement from reflexes primarily depends on the summation of excitatory and inhibitory afferent input from external sources. Spinal reflexes can be induced mechanically (e.g. tendon tap) or electrically with peripheral nerve stimulation (e.g. Hoffmann reflex (H-reflex)) (Burke, 2016). Specifically, the efficacy of signal transmission from the Ia afferents to alpha motor neurons can be assessed by utilizing the H-reflex (Capaday, 1997). The assessment of signal transmission through the synapse is of the utmost importance as the activation of the alpha motor neuron pool directs all motor output. Understanding how efficiently a signal propagates from afferent to efferent neurons can provide knowledge of the properties of the alpha motor neurons.

1.1.1 Motor unit recruitment and rate coding

Alpha motor neurons contain projections that connect their axons to the extrafusal fibres of muscles (Bessou et al., 1965), allowing for the transfer of excitatory signals that modulate the amount of force produced during a contraction (Henneman & Mendell, 1981). The motor neuron and all the muscle fibres it innervates is called a motor unit (MU). The motor neuron pool receives synaptic input from various peripheral sources, which causes alterations in the resting membrane potential of the pool (Heckman & Enoka, 2012). The summation of the excitatory and inhibitory input to the motor neuron

pool determines whether the depolarization threshold is met and if an action potential is generated and transmitted to the muscle. The magnitude of the contraction in the muscle is dependent on two mechanisms related to the motor neuron: the frequency of action potential transmission and the number of motor neurons activated within the pool (Duchateau & Enoka, 2011). These mechanisms are known as motor unit rate coding and recruitment, respectively, and are the basis for the nervous system's control of movement.

Motor units are recruited in an orderly process based on the diameter of cell body of their motor neuron, demonstrating a concept known as Henneman's size principle (Henneman, 1957). Following this rule, when input is provided to the motor neuron pool, smaller motor neurons are recruited first as they require less overall synaptic input and thus have a lower threshold for activation. However, larger motor neurons require a greater level of excitatory input to meet the threshold for depolarization and to discharge an action potential. During the initiation of a muscular contraction therefore, the descending neural drive and/or afferent input will first activate the lowest threshold motor neurons, which have smaller axon diameters, and innervate fewer muscle fibres that are typically fatigue resistant (Henneman & Mendell, 1981). As the intensity of the neural drive, and therefore the synaptic input, increases larger motor neurons will be recruited to meet the demands of the intended muscle contraction. These larger motor neurons have larger axon diameters, and innervate many more muscle fibres, which are typically less resistant to fatigue (Buchthal & Schmalbruch, 1980). The force produced by a single MU is dependent, in part, on the number and type of muscle fibres it innervates. The contribution of the recruitment of MUs to overall muscle force production is typically greater during the initial phase of a muscle contraction; however, the force level at which rate coding becomes the primary contributor to increased force production is dependent on the muscle (Enoka & Duchateau, 2017).

The amount of synaptic input a motor neuron receives is proportional to the discharge rate of action potentials (Enoka & Duchateau, 2017). The relative contribution of a recruited motor unit to overall force production depends not only on the number of muscle fibers, but also on the firing frequency of action potentials during a given action (Heckman & Enoka, 2012). A muscle twitch is produced when a motor neuron generates

a single action potential and transmits it to the innervated muscle fibres (Mines, 1913). As the rate of action potential discharge increases, the time between each twitch force becomes smaller, and the twitches begin to overlap, resulting in summated force. When a MU is fully activated, it produces a fused tetanic contraction as the frequency of twitches does not allow the muscle enough time to relax between each action potential (Raikova et al., 2007). Rate coding and recruitment work together to allow for a gradual increase in force during a muscle contraction.

Our understanding of sex-related differences in MU behaviour remains limited due to the lack of research investigating neurophysiology in females (Lulic-Kuryllo & Inglis, 2022). Although there have been many advancements in recent years, research utilizing predominantly male participants continues to set the standard for our understanding of neuromuscular function in both sexes. Males and females demonstrate considerable differences in musculoskeletal (Maughan et al., 1983) and biomechanical (Pappas et al., 2007) domains. However, research into neuromuscular differences remains understudied despite sexual dimorphism in other aspects of physiology (Lulic-Kuryllo & Inglis, 2022). Sex-specific differences in motor neuron size and number remains understudied as few studies have investigated the physical properties of motor neurons (Taylor et al., 2022). A study by Yuan et al. 2000 found larger diameter spinal alpha motor neurons in male cadavers compared to females, but no differences in motor neuron number within the pool. Although there is limited evidence, differences in motor neuron size suggest there may be sex-specific modulations in the control of motor output.

Males also have larger cross-sectional area (CSA) of type II muscle fibres, which are linked to higher threshold motor neurons and are associated with greater action potential amplitudes and higher contractile forces (Carter et al., 2001). On the contrary, females have smaller CSA of type II muscle fibres, resulting in lower contractile forces and action potential amplitudes (Landen et al., 2023). Therefore, females may rely on relatively higher firing rates than males to modulate force during low-level contractions (Trevino et al., 2019). Mechanisms that have been associated with differences in MU behaviour (recruitment and rate coding) between sexes include hormonal fluctuations, musculoskeletal differences, and the intrinsic properties of motor neurons (Lulic-Kuryllo

& Inglis, 2022). A better understanding of the factors influencing MU behaviour is essential to filling the gap in understanding of sex-related differences in neurophysiology.

1.2 Soleus muscle

The soleus muscle lies in the superficial posterior compartment of the leg with the gastrocnemius and plantaris muscles. Together, they create the triceps surae muscle complex of the lower leg, commonly known as the calf muscles (Dixon, 2009). The muscle fibres of the soleus extend broadly on the lateral and medial sides of the leg and distally, where the fibres combine to insert into the Achilles (calcaneal) tendon (Zielinska et al., 2023). The soleus contains two heads; one originates more anteriorly on the soleal line and medial border of the tibia, and the other originates more posteriorly from the head and upper third of the body of the fibula (Olewnik, 2020). The tibial nerve innervates the soleus and splits into two distinct branches: posterior and anterior (Loh, 2003). The subdivision of the soleus into anterior and posterior compartments explains its function in postural control. Activation of different regions of the soleus would allow for a change in the direction of force generation depending on the situation (Loh, 2003). It is generally accepted that in humans the soleus is composed of predominantly slow twitch (type I) muscle fibres, making up nearly 90% of its fibre structure (Trappe, 2001). The extensive distribution of type I fibres allows the soleus to function preferentially as a low-activity postural control and walking (Olewnik, 2020).

The soleus has a high muscle spindle density with ~23 spindles/g, relative to the gastrocnemius at ~9 spindles/g, allowing for monosynaptic excitatory spindle feedback from primary and secondary afferent endings (Botterman et al., 1978). The excitability of the spinal motor neuron pool innervating the soleus muscle is highly dependent on muscle length and thus joint angle when initiating a muscular contraction. It has been shown that when the ankle is slightly dorsiflexed relative to neutral, the greatest reflex mechanical gain is produced for the soleus, showing a steep decline with increasing dorsiflexion (Lin, 1997). However, a somewhat plantar flexed position at the ankle produced the greatest reflex electromyography (EMG) gain response, with a decline in amplitude with further dorsiflexion (Lin, 1997).

The primary action of the soleus muscle is plantar flexion of the ankle. The soleus is needed for walking, running, jumping, and standing while playing a vital role in upright postural control, as it maintains activation during standing posture to sustain balance (Olewnik, 2020). Contrary to the gastrocnemius, the soleus is most effective at producing force when the knee joint is flexed (Dixon, 2009). This biomechanical difference is due to the origin of the soleus on the tibia, while the gastrocnemius originates on the femur. When the knee is flexed, large amounts of tendon-joint laxity are produced, significantly reducing the gastrocnemius' force-generating capabilities while isolating the force production of the soleus (Dixon, 2009).

Males often have larger muscles and greater absolute strength when compared to females, even when relative muscle mass and size are considered (Chow et al., 2000; Maughan et al., 1983). Although fibre types and contractile properties have been well-defined for many muscles, little is known about the sex-specific differences within skeletal muscle architecture, and particularly for the soleus muscle. In what is possibly the only study directly examining sex-related differences in soleus muscle properties, Chow et al. (2000) found that males and females demonstrate differences in muscle architecture. Specifically, using ultrasonography, females were found to have longer average fibre bundle lengths, but males had larger pennation angles and thicker muscles. These sex-specific differences in soleus muscle architecture have essential implications concerning force production and performance outcomes.

1.3 Hoffmann reflex

The Hoffmann reflex (H-reflex) is an electrically induced analog to the mechanically induced stretch reflex and is commonly used as an indicator of the excitability and inhibition of the spinal motor neuron pool (Palmieri et al., 2004; Misiaszek, 2003). The difference between the H-reflex and the stretch reflex lies in the H-reflex bypassing muscle spindle discharge and gamma motor neurons, allowing it to be an effective measure of the efficacy of monosynaptic transmission through the alpha motor neuron pool (Knikou, 2008). However, H-reflex responses can be influenced by pre-synaptic inhibition and the intrinsic properties of alpha motor neurons (Zehr, 2002). Therefore, it

can only be an accurate estimate of motor neuron pool excitability when these variables are constant (Palmieri et al., 2004).

The H-reflex can theoretically be elicited in any muscle in the body if it has a peripheral nerve accessible for stimulation (Gajewski & Mazur-Różycka, 2016). The soleus is commonly used for the H-reflex due to ease of access to the tibial nerve, the high density of muscle spindles (Botterman et al., 1978), and the large distribution of slow twitch type I muscle fibres, shown to be the sole contributors to the reflex response (Buchthal & Schmalbruch, 1970).

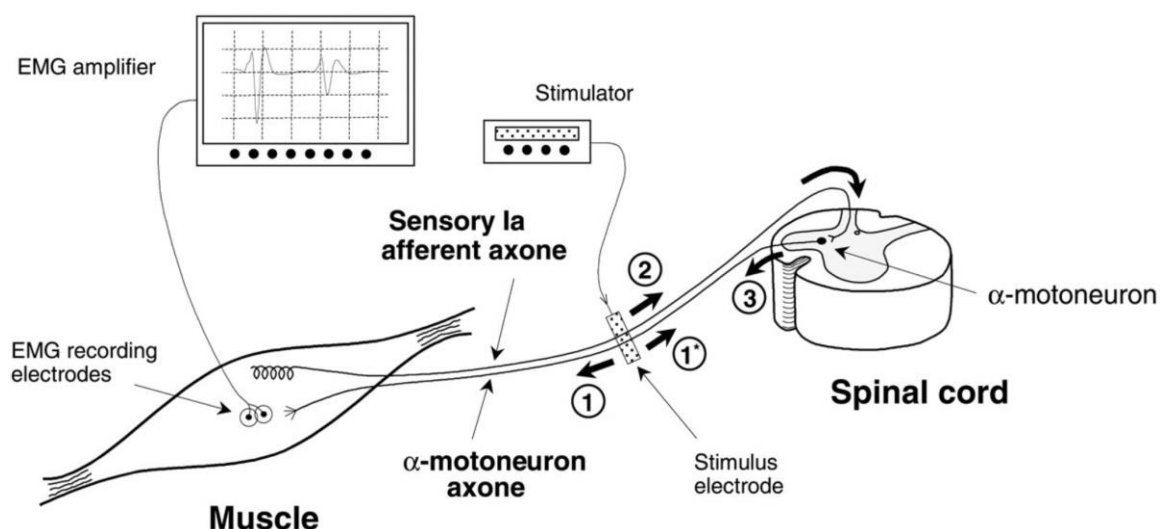


Figure 1. The Hoffmann reflex pathway. The H-reflex and M-wave are elicited by electrical stimulation of a peripheral nerve and the evoked potential is recorded with EMG. Figure reprinted from (Aagaard et al., 2002).

The H-reflex is elicited by electrically stimulating a mixed nerve with a short-duration, low-intensity current to activate the large-diameter Ia afferent fibres selectively (Figure 1). The action potentials evoked within the sensory fibres travel to the spinal cord, where they interact with the motor neuron pool. This gives rise to excitatory post-synaptic potentials, producing action potentials in the alpha motor neurons which propagate to the muscle. The response is recorded at the muscle with electromyography electrodes as a late-latency muscle response of ~35ms (Schieppati, 1986). The large-diameter Ia afferents synapse with the smaller-diameter alpha motor neurons, resulting in orderly

recruitment of MUs through the H-reflex pathway (Zehr, 2002). As the stimulus intensity increases, more Ia afferents are activated and the amplitude of the H-reflex response at the muscle increases, with a greater amplitude response reflecting greater excitability of the alpha motor neuron pool (Aagaard et al., 2002).

Further increases in stimulus intensity will result in direct activation of the large-diameter alpha motor neurons at the point of stimulation, along with the sensory fibres. The action potentials evoked in the motor fibres travel to the muscle and result in a direct response at the muscle (M-wave), recorded as a short-latency muscle response of ~5ms (Schieppati, 1986). The reverse recruitment order of the M-wave, from largest diameter motor neurons to smallest, is in contrast to the orderly recruitment produced through the H-reflex pathway (Knikou, 2008). This difference in recruitment order results in different motor neurons being activated through the H-reflex and the M-wave, at initial appearance of the M-wave.

As stimulus intensity is further increased, the same alpha motor neurons will be activated through the H-reflex pathway and the direct stimulation (M-wave). The antidromic activity of the directly-activated motor neurons will collide with the action potentials from the Ia activation of the reflex pathway, thus attenuating the reflex response. The M-wave will continue to increase in intensity as stimulus intensity increases; however, the reflex response will continue to decrease, until it is no longer present, as the antidromic collision from the motor neurons becomes stronger. At supramaximal stimulus intensity, all motor neurons will be directly activated by the stimulation, evoking action potentials in all available motor neurons and abolishing any reflex volley due to antidromic activity. The state where all motor neurons are directly activated by the stimulation is described as the maximal M-wave (M-max) (Aagaard et al., 2002).

One of the most common indices of reflex excitability is the ratio of the response of the maximum amplitude of the H-reflex (H-max) to the maximum amplitude of the compound muscle action potential (M-max) (Stutzig & Siebert, 2016). H-max represents the maximal activation of the alpha motor neurons through the Ia reflex pathway (Palmieri et al., 2004). On the other hand, M-max represents the complete activation of

the motor neuron pool, and as a result, full muscle activation, as all motor neurons and subsequent muscle fibres are assumed to be activated (Zehr, 2002). However, the methodology for measuring these indices requires careful consideration due to the many factors that influence the parameters of the H-reflex. These include the postural position, stimulation duration and frequency, and anthropometric measures (Burke, 2016). Variations in these parameters make comparisons between H-reflex studies challenging and may explain differences in results between studies (Gajewski & Mazur-Różycka, 2016).

1.3.1 Spinal inhibition

Modulation of synaptic transmission at the lower motor neuron pool is governed by both excitatory and inhibitory inputs from descending cortical drive and peripheral afferents (Betti et al., 2022). Both excitatory and inhibitory inputs can influence the H-reflex response. Known inhibitory modulators of synaptic transmission include pre- and post-synaptic inhibition (Bardoni et al, 2013). Pre-synaptic inhibition acts upon the Ia afferent central terminals on the pre-synaptic membrane, altering neurotransmitter release onto the post-synaptic receptors of the alpha motor neurons (Alford & Schwartz, 2009). This attenuates the H-reflex response at the muscle with no change in motor neuron membrane potential (Bardoni et al., 2013). Presynaptic inhibition is also modulated selectively during voluntary contractions (Iles, 1996). The inhibitory inputs act upon the Ia afferents projecting to alpha motor neurons are reduced during a voluntary contraction or co-contraction, thereby minimizing the influence of pre-synaptic inhibition on synaptic transmission through supraspinal drive (Iles & Roberts, 1987).

In 1941, Renshaw elucidated a form of post-synaptic inhibition, noting that antidromic impulses within alpha motor neuron axons reduced the excitability response of the homonymous motor neuron through recurrent collaterals, later termed Renshaw cells (Renshaw, 1941). These findings demonstrated that ventral horn interneurons mediated inhibitory signals to the motor neuron collaterals of the synergistic alpha motor neuron, acting as a form of post-synaptic inhibition (Renshaw, 1946). There is a descending facilitation of Renshaw cells during low-level contractions and a suppression of these

cells during high-level contractions, which can impact H-reflex responses (Pierrot-Deseilligny & Burke, 2005).

Both Ia pre-synaptic inhibition and recurrent inhibition at the post-synaptic membrane are present during the elicitation of an H-reflex protocol. However, their influence can be reduced by normalizing contraction intensity, in terms of torque, across trials, thereby controlling the effect of pre-synaptic inhibition and recurrent inhibition (Pierrot-Deseilligny & Burke, 2005).

Reciprocal inhibition describes the process whereby the contraction of an agonist muscle is accompanied by the inhibition of its antagonist (Day et al., 1984). This reciprocal behaviour arises from inhibitory interneurons modulating the co-activation of flexor and extensor muscles during a contraction (Iles, 1986). These interneurons are modulated by two primary sources: neural drive from the supraspinal centers and inputs from muscle spindles (Day et al., 1984). The proposed purpose for reciprocal inhibition of Ia interneurons is for the coordinated voluntary contraction and relaxation of agonist and antagonist muscle groups during movement (Lundberg, 1970).

Figure 2 displays the pathway of disynaptic reciprocal inhibition from the tibialis anterior to the soleus muscle motor neurons. The H-reflex pathway can be used to test the amount of reciprocal inhibition. For example, if testing reciprocal inhibition in the soleus muscle, a conditioning stimulus is applied to the deep fibular nerve innervating the tibialis anterior, stimulating the Ia afferents that have monosynaptic excitatory projections to homonymous motor neurons (Figure 2). The conditioned stimulus simultaneously activates inhibitory interneurons synapsing with the motor neurons of the soleus, reducing the excitatory potential of the post-synaptic membrane. A test stimulus is then applied to the tibial nerve innervating the soleus at an interstimulus interval ~100ms, allowing for the inhibitory mechanism of the conditioned stimulus to take place before the test stimulus is applied to the soleus. The amount of reciprocal inhibition acting upon the soleus motor neuron pool can be estimated by the depression in the test H-reflex amplitude when the conditioned stimulus is applied.

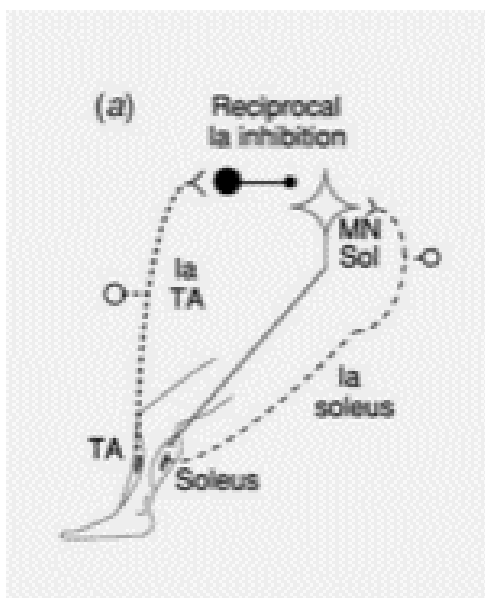


Figure 2. The reciprocal inhibition pathway. Reciprocal inhibition from the tibialis anterior to the soleus, measured by utilizing an H-reflex paired stimulus technique. Dotted lines represent Ia afferent fibres propagating sensory feedback to the soleus motor neuron pool. The solid line with an empty circle represents the transfer of efferent signals from the motor neuron pool to the soleus muscle. The solid line with the filled circle represents an inhibitory interneuron acting upon the soleus motor neuron pool following activation of the tibialis anterior (antagonist muscle). Figure reprinted from (Pierrot-Deseilligny & Burke, 2005).

1.3.2 Sex-related differences in H-reflex

The few studies investigating the sex-related differences in H-reflex excitability and inhibition have come to different conclusions (Hoffman et al. 2018a; Hoffman et al. 2018b; Johnson et al. 2012; Mendonca et al. 2020). A study by Mendonca et al. (2020) found that females have lower H-reflex excitability, but this was dependent on the amount of antagonistic co-activation and resulted in no difference in H-reflex between sexes when co-activation was normalized. Johnson et al. (2012) also found no differences in H-reflex amplitude between the sexes. Hoffman et al. (2018a) found no significant differences in excitability between sexes. However, another study by Hoffman. (2018b) found that females had a greater H-max/M-max ratio than males, indicating greater levels of excitability. These conflicting results may be due to methodological differences in

eliciting H-reflex or the examination of different characteristics of the motor neuron synapse, such as the influence of pre- and post-synaptic inhibition (Johnson et al., 2012).

When the H-reflex is tested alone, it does not account for other influences of synaptic modulation, such as inhibition (Knikou, 2008). For example, although Johnson et al. (2012) found no differences in H-reflex excitability between sexes, males exhibited significantly greater post-synaptic inhibition than females. Similarly, Hoffmann et al. (2018a) also found no differences in excitability, but showed that males had significantly greater pre-synaptic inhibition than females. The discourse surrounding the sexual dimorphism seen at the motor neuron pool makes it difficult to come to definite conclusions as many variables such as sex hormone level, antagonistic co-activation, synaptic inhibition, body positioning, and other methodological conditions were not consistent across studies (Hoffman et al. 2018a; Mendonca et al. 2020; Zehr, 2002). Therefore, to elucidate the mechanisms surrounding the neural control of the motor neuron pool, careful considerations of these variables need to be made when designing a study investigating spinal excitability with the H-reflex. For example, to accurately capture changes in excitability or inhibition following an intervention, it is important to understand potential sex-related differences in physiological baselines.

1.4 Purpose

The purpose of this study was to determine whether healthy young males and females differ in soleus H-reflex excitability and inhibition. We hypothesized that H-reflex excitability would be lower in females than in males and that reciprocal inhibition would be higher in females than males.

1.5 References

- Aagaard, P., Simonsen, E. B., Andersen, J. L., Magnusson, P., & Dyhre-Poulsen, P. (2002). Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. *Journal of applied physiology (Bethesda, Md. : 1985)*, 92(6), 2309–2318. <https://doi.org/10.1152/jappphysiol.01185.2001>
- Alford, S., & Schwartz, E. (2009). Presynaptic inhibition. *Encyclopedia of Neuroscience*, 1001–1006. <https://doi.org/10.1016/b978-008045046-9.00814-7>
- Bardoni, R., Takazawa, T., Tong, C. K., Choudhury, P., Scherrer, G., & Macdermott, A. B. (2013). Pre- and postsynaptic inhibitory control in the spinal cord dorsal horn. *Annals of the New York Academy of Sciences*, 1279, 90–96. <https://doi.org/10.1111/nyas.12056>
- Bessou, P., Emonet-Dénand, F., & Laporte, Y. (1965). Motor fibres innervating extrafusal and intrafusal muscle fibres in the cat. *The Journal of physiology*, 180(3), 649–672. <https://doi.org/10.1113/jphysiol.1965.sp007722>
- Betti, S., Fedele, M., Castiello, U., Sartori, L., & Budisavljević, S. (2022). Corticospinal excitability and conductivity are related to the anatomy of the corticospinal tract. *Brain structure & function*, 227(3), 1155–1164. <https://doi.org/10.1007/s00429-021-02410-9>
- Botterman, B. R., Binder, M. D., & Stuart, D. G. (1978). Functional anatomy of the association between motor units and muscle receptors. *American Zoologist*, 18(1), 135–152. <https://doi.org/10.1093/icb/18.1.135>
- Bryan Dixon J. (2009). Gastrocnemius vs. soleus strain: how to differentiate and deal with calf muscle injuries. *Current reviews in musculoskeletal medicine*, 2(2), 74–77. <https://doi.org/10.1007/s12178-009-9045-8>
- Buchthal, F., & Schmalbruch, H. (1970). Contraction times of twitches evoked by H-reflexes. *Acta physiologica Scandinavica*, 80(3), 378–382. <https://doi.org/10.1111/j.1748-1716.1970.tb04801.x>
- Buchthal, F., & Schmalbruch, H. (1980). Motor unit of mammalian muscle. *Physiological reviews*, 60(1), 90–142.
- Burke D. (2016). Clinical uses of H reflexes of upper and lower limb muscles. *Clinical neurophysiology practice*, 1, 9–17. <https://doi.org/10.1016/j.cnp.2016.02.003>

- Capaday, C. (1997). Neurophysiological methods for studies of the motor system in freely moving human subjects. *Journal of Neuroscience Methods*, 74(2), 201–218. [https://doi.org/10.1016/s0165-0270\(97\)02250-4](https://doi.org/10.1016/s0165-0270(97)02250-4)
- Carter, S. L., Rennie, C. D., Hamilton, S. J., & Tarnopolsky, M. A. (2001). Changes in skeletal muscle in males and females following endurance training. *Canadian Journal of Physiology and Pharmacology*, 79(5), 386–392. <https://doi.org/10.1139/y01-008>
- Chow, R. S., Medri, M. K., Martin, D. C., Leekam, R. N., Agur, A. M., & McKee, N. H. (2000). Sonographic studies of human soleus and gastrocnemius muscle architecture: gender variability. *European journal of applied physiology*, 82(3), 236–244. <https://doi.org/10.1007/s004210050677>
- Bryan Dixon, J. (2009). Gastrocnemius vs. soleus strain: How to differentiate and deal with calf muscle injuries. *Current Reviews in Musculoskeletal Medicine*, 2(2), 74–77. <https://doi.org/10.1007/s12178-009-9045-8>
- Duchateau, J., & Enoka, R. M. (2011). Human motor unit recordings: origins and insight into the integrated motor system. *Brain research*, 1409, 42–61. <https://doi.org/10.1016/j.brainres.2011.06.011>
- Enoka, R. M., & Duchateau, J. (2017). Rate Coding and the Control of Muscle Force. *Cold Spring Harbor perspectives in medicine*, 7(10), a029702. <https://doi.org/10.1101/cshperspect.a029702>
- Gajewski, J., & Mazur-Różycka, J. (2018). The H-reflex as an important indicator in kinesiology. *Human Movement*, 17(2), 64–71. <https://doi.org/10.1515/humo-2016-0018>
- Heckman, C. J., & Enoka, R. M. (2012). Motor unit. *Comprehensive Physiology*, 2(4), 2629–2682. <https://doi.org/10.1002/cphy.c100087>
- Henneman, E. (1957). Relation between size of neurons and their susceptibility to discharge. *Science*, 126(3287), 1345–1347. <https://doi.org/10.1126/science.126.3287.1345>
- Henneman, E., & Mendell, L. M. (1981). Functional Organization of Motoneuron Pool and its inputs. *Comprehensive Physiology*, 423–507. <https://doi.org/10.1002/cphy.cp010211>

- Henneman, E., Somjen, G., & Carpenter, D. O. (1965). Excitability and inhibitability of motoneurons of different sizes. *Journal of neurophysiology*, 28(3), 599–620.
<https://doi.org/10.1152/jn.1965.28.3.599>
- Hoffman, M. A., Doeringer, J. R., Norcross, M. F., Johnson, S. T., & Chappell, P. E. (2018a). Presynaptic inhibition decreases when estrogen level rises. *Scandinavian Journal of Medicine & Science in Sports*, 28(9), 2009–2015.
<https://doi.org/10.1111/sms.13210>
- Hoffman, M., Norcross, M., & Johnson, S. (2018b). The Hoffmann reflex is different in men and women. *Neuroreport*, 29(4), 314–316.
<https://doi.org/10.1097/WNR.0000000000000961>
- Iles J. F. (1986). Reciprocal inhibition during agonist and antagonist contraction. *Experimental brain research*, 62(1), 212–214.
<https://doi.org/10.1007/BF00237419>
- Iles J. F. (1996). Evidence for cutaneous and corticospinal modulation of presynaptic inhibition of Ia afferents from the human lower limb. *The Journal of physiology*, 491 (Pt 1)(Pt 1), 197–207.
<https://doi.org/10.1113/jphysiol.1996.sp021207>
- Iles, J. F., & Roberts, R. C. (1987). Inhibition of monosynaptic reflexes in the human lower limb. *The Journal of physiology*, 385, 69–87.
<https://doi.org/10.1113/jphysiol.1987.sp016484>
- Johnson, S. T., Kipp, K., & Hoffman, M. A. (2012). Spinal motor control differences between the sexes. *European journal of applied physiology*, 112(11), 3859–3864.
<https://doi.org/10.1007/s00421-012-2363-3>
- Knikou M. (2008). The H-reflex as a probe: pathways and pitfalls. *Journal of neuroscience methods*, 171(1), 1–12.
<https://doi.org/10.1016/j.jneumeth.2008.02.012>
- Landen, S., Hiam, D., Voisin, S., Jacques, M., Lamon, S., & Eynon, N. (2023). Physiological and molecular sex differences in human skeletal muscle in response to exercise training. *The Journal of physiology*, 601(3), 419–434.
<https://doi.org/10.1113/JP279499>
- Lin, J. P., Brown, J. K., & Walsh, E. G. (1997). Soleus muscle length, stretch reflex

- excitability, and the contractile properties of muscle in children and adults: a study of the functional joint angle. *Developmental medicine and child neurology*, 39(7), 469–480. <https://doi.org/10.1111/j.1469-8749.1997.tb07467.x>
- Loh, E. Y., Agur, A. M., & McKee, N. H. (2003). Intramuscular innervation of the human soleus muscle: a 3D model. *Clinical anatomy (New York, N.Y.)*, 16(5), 378–382. <https://doi.org/10.1002/ca.10170>
- Lulic-Kuryllo, T., & Inglis, J. G. (2022). Sex differences in motor unit behaviour: A review. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 66, 102689. <https://doi.org/10.1016/j.jelekin.2022.102689>
- Lundberg A (1970) The excitatory control of the Ia inhibitory pathway. In: Andersen P, Jansen JKS (eds) Excitatory synaptic mechanisms. Universitetsforlaget, Oslo, pp 333-340
- Maughan, R. J., Watson, J. S., & Weir, J. (1983). Strength and cross-sectional area of human skeletal muscle. *The Journal of physiology*, 338, 37–49. <https://doi.org/10.1113/jphysiol.1983.sp014658>
- Mendonca, G. V., Pizarat-Correia, P., Gonçalves, A. D., Gomes, M., Correia, J. M., & Vila-Chã, C. (2020). Sex differences in soleus muscle H-reflex and V-wave excitability. *Experimental physiology*, 105(11), 1928–1938. <https://doi.org/10.1113/EP088820>
- Mines G. R. (1913). On the summation of contractions. *The Journal of physiology*, 46(1), 1–27. <https://doi.org/10.1113/jphysiol.1913.sp001577>
- Misiaszek J. E. (2003). The H-reflex as a tool in neurophysiology: its limitations and uses in understanding nervous system function. *Muscle & nerve*, 28(2), 144–160. <https://doi.org/10.1002/mus.10372>
- Olewnik, Ł., Zielinska, N., Paulsen, F., Podgórski, M., Haładaj, R., Karauda, P., & Polguy, M. (2020). A proposal for a new classification of soleus muscle morphology. *Annals of anatomy = Anatomischer Anzeiger : official organ of the Anatomische Gesellschaft*, 232, 151584. <https://doi.org/10.1016/j.aanat.2020.151584>
- Palmieri, R. M., Ingersoll, C. D., & Hoffman, M. A. (2004). The hoffmann reflex:

- methodologic considerations and applications for use in sports medicine and athletic training research. *Journal of athletic training*, 39(3), 268–277.
- Pappas, E., Hagins, M., Sheikhzadeh, A., Nordin, M., & Rose, D. (2007). Biomechanical differences between unilateral and bilateral landings from a jump: gender differences. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, 17(4), 263–268.
<https://doi.org/10.1097/JSM.0b013e31811f415b>
- Pierrot-Deseilligny, E., & Burke, D. (2005). *The Circuitry of the Human Spinal Cord*.
<https://doi.org/10.1017/cbo9780511545047>
- Renshaw, B. (1941). Influence of discharge of motoneurons upon excitation of neighboring motoneurons. *Journal of Neurophysiology*, 4(2), 167–183.
<https://doi.org/10.1152/jn.1941.4.2.167>
- Renshaw, B. (1946). Central effects of centripetal impulses in axons of spinal ventral roots. *Journal of Neurophysiology*, 9(3), 191–204.
<https://doi.org/10.1152/jn.1946.9.3.191>
- Raikova, R., Celichowski, J., Pogrzebna, M., Aladjov, H., & Krutki, P. (2007). Modeling of summation of individual twitches into unfused tetanus for various types of rat motor units. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 17(2), 121–130.
<https://doi.org/10.1016/j.jelekin.2006.01.005>
- Sanes, J. N., & Donoghue, J. P. (2000). Plasticity and primary motor cortex. *Annual Review of Neuroscience*, 23(1), 393–415.
<https://doi.org/10.1146/annurev.neuro.23.1.393>
- Stutzig, N., & Siebert, T. (2017). Assessment of the H-reflex at two contraction levels before and after fatigue. *Scandinavian journal of medicine & science in sports*, 27(4), 399–407. <https://doi.org/10.1111/sms.12663>
- Taylor, C. A., Kopicko, B. H., Negro, F., & Thompson, C. K. (2022). Sex differences in the detection of motor unit action potentials identified using high-density surface electromyography. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 65, 102675.
<https://doi.org/10.1016/j.jelekin.2022.102675>

- Trappe, S. W., Trappe, T. A., Lee, G. A., & Costill, D. L. (2001). Calf muscle strength in humans. *International journal of sports medicine*, 22(3), 186–191.
<https://doi.org/10.1055/s-2001-16385>
- Trevino, M. A., Sterczala, A. J., Miller, J. D., Wray, M. E., Dimmick, H. L., Ciccone, A. B., Weir, J. P., Gallagher, P. M., Fry, A. C., & Herda, T. J. (2019). Sex-related differences in muscle size explained by amplitudes of higher-threshold motor unit action potentials and muscle fibre typing. *Acta physiologica (Oxford, England)*, 225(4), e13151. <https://doi.org/10.1111/apha.13151>
- Ugawa Y. (2020). Voluntary and involuntary movements: A proposal from a clinician. *Neuroscience research*, 156, 80–87.
<https://doi.org/10.1016/j.neures.2019.10.001>
- Yuan, H., Goto, N., Akita, H., Goto, J., & Jin, S. R. (2000). Sexual dimorphism of the motoneurons in the human spinal cord. *Okajimas folia anatomica Japonica*, 77(5), 143–148. https://doi.org/10.2535/ofaj1936.77.5_143
- Zehr E. P. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *European journal of applied physiology*, 86(6), 455–468.
<https://doi.org/10.1007/s00421-002-0577-5>
- Zielinska, N., LaPrade, R. F., & Olewnik, Ł. (2023). Morphological variations of the calcaneal tendon: clinical significance. *Journal of orthopaedic surgery and research*, 18(1), 275. <https://doi.org/10.1186/s13018-023-03748-y>

Chapter 2

2 Sex-related differences in H-reflex excitability and inhibition

2.1 Introduction

The limited work investigating sex-related differences in motor unit (MU) behaviour has created a significant challenge in reaching conclusions about differences in male and female physiology. (Lulic-Kuryllo & Inglis, 2022). Despite advancements in our understanding of neuromuscular function over several decades of research, one significant gap that remains within the literature is that few studies have investigated female neurophysiology. Consequently, previous research using predominantly male participants continues to set the standard of our understanding for both sexes (McNulty et al., 2020). It has been demonstrated that males and females display numerous musculoskeletal and biomechanical differences during motor tasks (Maughan et al., 1983; Pappas et al., 2007). Sex-related difference in neuromuscular physiology may provide a mechanistic explanation for the differences in motor task performance, highlighting the importance of investigating neuromuscular differences between males and females.

Neuromuscular activation can occur voluntarily, relying primarily on descending neural drive, and involuntarily, predominantly via spinal reflexes (Betti et al., 2022; Ugawa, 2020). Spinal reflexes can be elicited through mechanical manipulation, e.g. with a tendon tap, or electrically with peripheral nerve stimulation (Burke, 2016). When electrically elicited, as is the case with the Hoffmann reflex (H-reflex), the input and output of synaptic transmission of the lower motor neuron pool can be estimated (Capaday, 1997). The efficacy of transmission through the lower motor neuron pool is a crucial variable, as all motor output results from the activation of motor neurons (Wolpaw, 2001).

Although the H-reflex has been thoroughly investigated for over a century, few studies have examined potential differences in spinal excitability and inhibition between sexes (Hoffman et al., 2018a; Hoffman et al., 2018b; Johnson et al., 2012; Mendonca et al.,

2020). The studies that have investigated sex-related differences in the H-reflex have found conflicting results. Mendonca et al. (2022) found greater excitability in males than females, indicated by a greater H-max/M-max ratio. However, differences between sexes dissipated when antagonistic co-activation was normalized between males and females. Conflicting with this finding is a study by Hoffman et al. (2018b), who found that females had greater H-max/M-max ratio than males, but only at a time point where males and females had similar natural hormone levels of progesterone and estradiol in their system. Hoffman et al. (2018a) found that despite similar levels of excitability between sexes, females had significantly less pre-synaptic inhibition, particularly when they had greater levels of estrogen in their system. Finally, Johnson et al. (2012) found no differences in excitability but greater recurrent inhibition in males than females.

These conflicting results can likely be attributed to differences in methodologies in eliciting the H-reflex (Zehr, 2002), or examination of different excitatory and inhibitory characteristics of the motor neuron synapse (Johnson et al., 2012). The discourse in results makes creating definite conclusions surrounding sexual dimorphism of spinal excitability and inhibition challenging and highlights a need for further work in this area. A better understanding of the factors influencing MU behaviour is essential in filling the gap in our understanding of sex-related differences in neuromuscular physiology, which can inform future studies. For example, to accurately capture changes in excitability or inhibition following an intervention, it is important to understand potential sex-related differences in physiological baselines.

The purpose of this study was to determine whether healthy young males and females differ in soleus H-reflex excitability and inhibition. We hypothesized that H-reflex excitability would be lower, and inhibition would be higher in females compared with males.

2.2 Methods

2.2.1 Participants

Thirty participants (15 females, 15 males, aged 23.2 ± 2.5 years) were recruited from the university and the local community. Inclusion criteria included: reading and speaking English, normal or corrected-to-normal vision, no history of musculoskeletal or neuromuscular impairments that may affect performance of the protocol, were not taking prescription medication, except for oral contraceptives, and refraining from engaging in exercise or use of alcohol or central nervous system stimulant or depressant pharmacological agents within 12 hours of study participation. The study was reviewed and approved by the Western University Institutional Review Board and all participants provided written informed consent prior to participation.

2.2.2 Experimental protocol

During the single testing session, measures of maximum dorsiflexion and plantar flexion strength were obtained. Neuromuscular excitability and inhibition were then assessed by elicitation of the H-reflex and M-wave from the soleus (Figure 3).

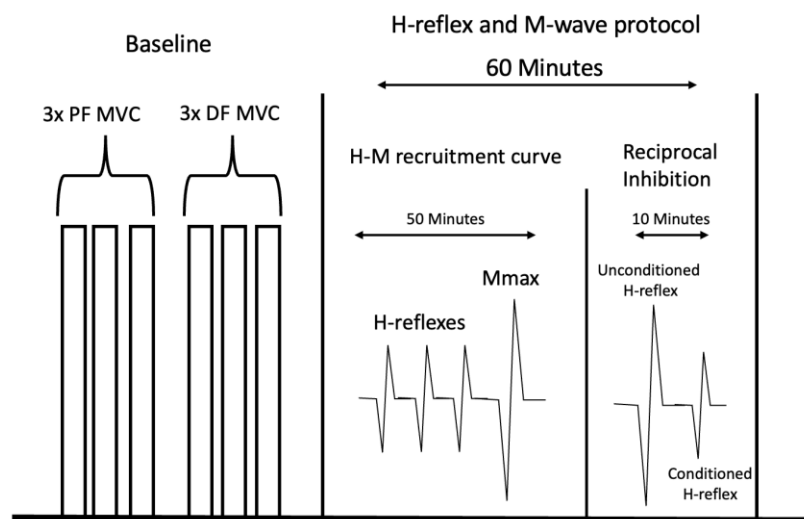


Figure 3. Timeline of experimental protocol. Outline of the order of experimental procedures on the single day of testing. Horizontal arrows represent time windows. Vertical bars represent maximum voluntary contraction trials. Electrical signals represent elicitation of the H-reflex and M-wave.

2.2.3 Force

Participants were asked to identify their dominant lower limb, then subsequently seated in a chair with their dominant leg comfortably placed in a custom-made isokinetic dynamometer equipped with a load cell (SSM-AJ-250; Interface, Scottsdale, AZ, USA). Participants were seated with their hips and knees at 90 degrees of flexion and their foot in neutral position (Knikou & Rymer, 2002; Lin, 1997). The knee angle was chosen to isolate the soleus and reduce the contribution of the gastrocnemius during plantar flexion (Dixon, 2009). Participants were asked to limit unnecessary movements and attempt to maintain an upright seated posture throughout the session. The force signal was amplified (PM-1000; DataQ Instruments, Akron, OH, USA) and sampled at 2000 Hz using a 16-bit analog-to-digital converter (NI USB-6251; National Instruments, Austin, TX, USA), and stored for offline analysis.

Baseline testing involved three maximal effort plantar flexion and three maximal effort dorsiflexion contractions. Additional trials were performed if there was a >10% difference between any two trials. The highest value from each of plantar flexion and dorsiflexion were set as the 100% MVC. Participants produced contractions at 10% MVC throughout H-reflex testing and were provided with a visual target on a computer screen.

A custom-written Matlab program (version 2023a; Mathworks Inc, Natick, MA, USA) analyzed a 500ms window and calculated mean force during TA and SOL MVCs.

2.2.4 Surface EMG

Prior to the application of electrodes, the skin was gently cleaned with an abrasive gel (NuPrep®) and wiped with alcohol to reduce signal impedance. A bipolar electrode configuration with an inter-electrode distance of 1cm, was placed over the lateral aspect of the SOL and over the proximal aspect of the tibialis anterior (TA) muscle bellies. Ground electrodes were secured over the patella and proximal aspect of the tibia. These EMG signals were amplified and sampled at 48kHz using a clinical EMG cart (Nicolet EDX EMG, Natus, WI, USA) for acquisition of H-reflex and M-wave responses. For continuous EMG sampling during contractions, a wireless 4-pin surface EMG electrode (Galileo wireless EMG, Delsys Inc., Natick, MA, USA) was attached to the medial aspect of the SOL with a reference electrode attached to the medial aspect of the knee. A

wireless bipolar surface EMG electrode (Avanti wireless EMG, Delsys Inc., Natick, MA, USA) was attached to the distal aspect of the TA. Surface EMG signals were sampled at 2000Hz and stored on a personal computer for offline analysis of EMG during background contractions, using DasyLab software (Data Acquisition System Laboratory, DasyTec, USA, Inc., Amherst, NH, USA).

A custom-written Matlab program was used to analyze the EMG activity. The RMS amplitude of EMG was calculated over a 500ms window during TA and SOL MVCs. Co-activation of the TA and SOL were measured during the reciprocal inhibition protocol. It was calculated as the relative RMS EMG from the TA expressed as a percentage of the relative SOL RMS EMG, during a 500ms window prior to stimulation.

2.2.5 Electrical nerve stimulation

To elicit H-reflex and M-wave responses in the SOL, a bipolar stimulating arrangement was employed, where the cathode electrode was placed over the tibial nerve in the popliteal fossa and the anode on the distal thigh, superior to the patella. Through these electrodes, 1ms square wave pulses were delivered to the nerve. The cathode position that elicited the greatest response with the lowest stimulus intensity was chosen. The stimulus intensity was lowered until no muscle response was present. Starting at this subthreshold stimulus intensity, an H-reflex and M-wave recruitment curve was generated by gradually increasing the stimulus intensity by 1.2 mA until there was no further increase in the peak-to-peak amplitude of the M-wave. Three stimuli were delivered at each intensity, with 8-10 s between stimuli, to reduce the effects of post-activation depression (Zehr, 2002). Participants were instructed to produce a plantar flexion contraction to 10% MVC prior to each stimulation and relaxed immediately after the stimulus.

Reciprocal inhibition of the soleus was elicited by performing a paired stimulus protocol, with a conditioning stimulus applied to the common fibular nerve. A stimulating electrode was placed over the common fibular nerve distal to the fibular head. This electrode placement was used to ensure stimulation of the deep fibular branch of the common fibular nerve in order to limit excitation of the peroneal muscle group. The maximal electrical response (M-max) of the TA was identified by applying electrical

stimuli at increasing stimulus intensities until there was no further increase in peak-to-peak amplitude of the M-wave.

A custom-written Matlab code was used to calculate the peak-to-peak amplitude of H-reflex and M-wave, which were averaged across the three trials at each intensity and used to generate a recruitment curve (Figure 4). Peak values of each curve were taken as H-max and M-max. A line was fit to the ascending limb of each curve from 10% to 90% of maximum and the slope of the line was calculated (H-slope and M-slope). The ratio of H-max/M-max and H-slope/M-slope were then calculated.

To elicit reciprocal inhibition of the SOL H-reflex, a conditioning stimulation of the common fibular nerve was provided 100 ms prior to stimulation of the soleus (test). The conditioning stimulus intensity was set to 50% of the TA's M-max, while the test stimulus intensity was set to 10% of the soleus' M-max (Johnson et al., 2012). A total of 10 trials of paired stimulus inhibition were completed. Five trials of the test stimulus alone were completed both before and after 10 trials of conditioned+test stimuli. The 10 test trials and ten conditioned+test trials were each averaged for each participant. Reciprocal inhibition was then calculated as: $(1 - \text{Test reflex} / \text{Conditioned reflex}) \times 100\%$ (Johnson et al., 2012).

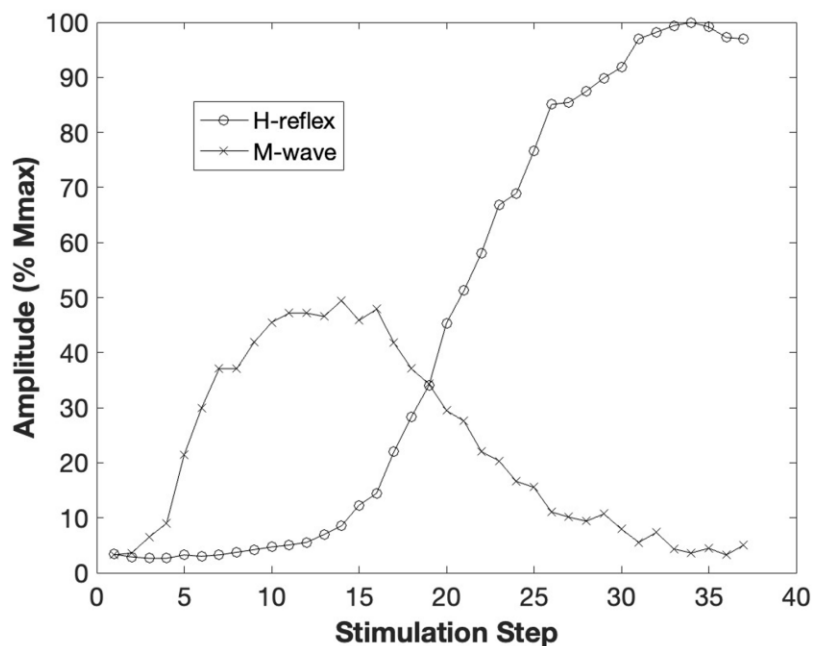


Figure 4. Example of H-reflex and M-wave recruitment curve.

2.2.6 Statistical analysis

Independent samples t-tests were used to compare each primary outcome variable between sexes (H-max, M-max, H-max/M-max, H-slope, M-slope, H-slope/M-slope, % inhibition). Effect sizes were calculated to display the magnitude of difference and presented as Cohen's d for most measures (Cohen, 1988). Cohen's d effect sizes were interpreted as small ($d > 0.2$), medium ($d > 0.5$), and large ($d > 0.8$) effects. For SOL and TA RMS, and TA/SOL co-activation, Hedge's g was calculated to account for bias in unequal sample size (Lakens, 2013). Hedge's g used the same interpretation as Cohen's d effect sizes. Statistical analyses were performed using SPSS (Version 24; IBM SPSS Statistics, Armonk, NY, USA). Significance was set at $p \leq 0.05$ and data are displayed as mean \pm SD for all measures.

2.3 Results

2.3.1 Participant Characteristics

Participant characteristics are presented in Table 1. Males and females differed in height ($p < 0.001$, $d = 2.80$) and mass ($p < 0.001$, $d = 1.71$), as males were significantly taller and heavier than females. There was no significant difference in age between groups ($p = 0.28$, $d = 0.40$). Males had higher PF ($p = 0.014$, $d = 0.95$) and DF ($p < 0.001$, $d = 2.46$) MVC values than females. Males also had greater SOL RMS EMG ($p = 0.008$, $g = 1.38$), but not significantly different in TA RMS EMG ($p = 0.13$, $g = 0.72$). No significant differences were identified in TA/SOL co-activation between sexes ($p = 0.82$, $g = 0.12$).

2.3.2 M-wave

Mean M-max amplitude and M-slope are displayed in Figure 5. M-max was significantly greater in males than females ($p = 0.006$, $d = 1.08$). M-slope was also significantly greater in males than females ($p = 0.004$, $d = 1.15$).

Table 1. Participant Characteristics

	Males (n=15)	Females (n=15)
Age (years)	23.7 ± 2.3	22.7 ± 2.7
Height (cm)*	181.0 ± 6.9	163.3 ± 5.7
Mass (kg)*	76.9 ± 8.9	61.5 ± 9.0
MVC PF (N)*	875.5 ± 227.3	663.0 ± 218.8
MVC DF (N)*	163.3 ± 40.3	80.9 ± 25.0
SOL RMS (μV)*	73.8 ± 21.4	43.9 ± 20.3
TA RMS (μV)	221.4 ± 45.75	178.5 ± 61.8
TA/SOL co-activation (%)	29.6 ± 13.4	31.9 ± 20.6

Soleus (SOL) and tibialis anterior (TA) root mean squared (RMS) amplitude include 7M and 12F participants. DF=dorsiflexion; PF=plantar flexion

TA/SOL co-activation include 5M and 10F participants

Values are presented as mean ± SD.

**Males significantly greater than females ($p < 0.05$).*

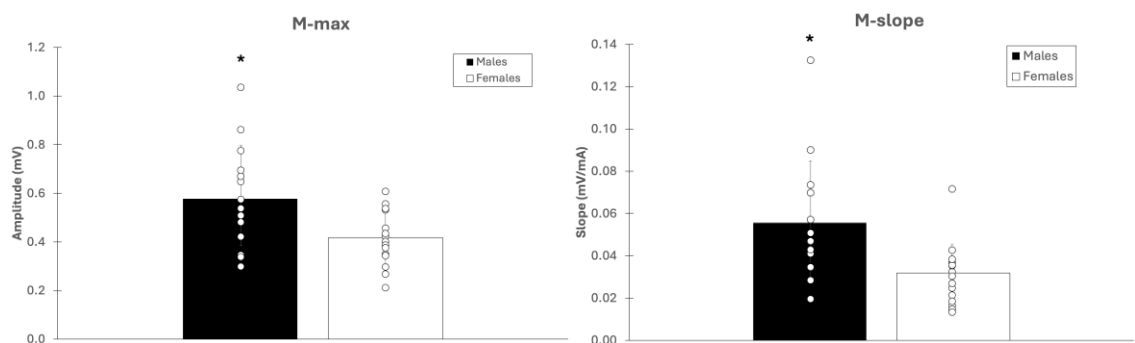


Figure 5. M-max amplitude and M-slope. Bars represent the mean values for males and females. Individual data points are shown by circles. Both M-max (left) and M-slope (right) were greater in males than females ($*p \leq 0.004$).

2.3.3 H-reflex excitability

Mean H-max amplitude, H-slope, H-max/M-max and H-slope/M-slope ratio are displayed in Figure 6. The H-max amplitude ($p=0.24$, $d=0.43$), H-max/M-max ratio ($p=0.13$, $d=0.57$), and H-slope/M-slope ratio ($p=0.91$, $d=0.04$) were not significantly different between sexes. However, H-slope was significantly greater in males than females ($p=0.009$, $d=1.08$).

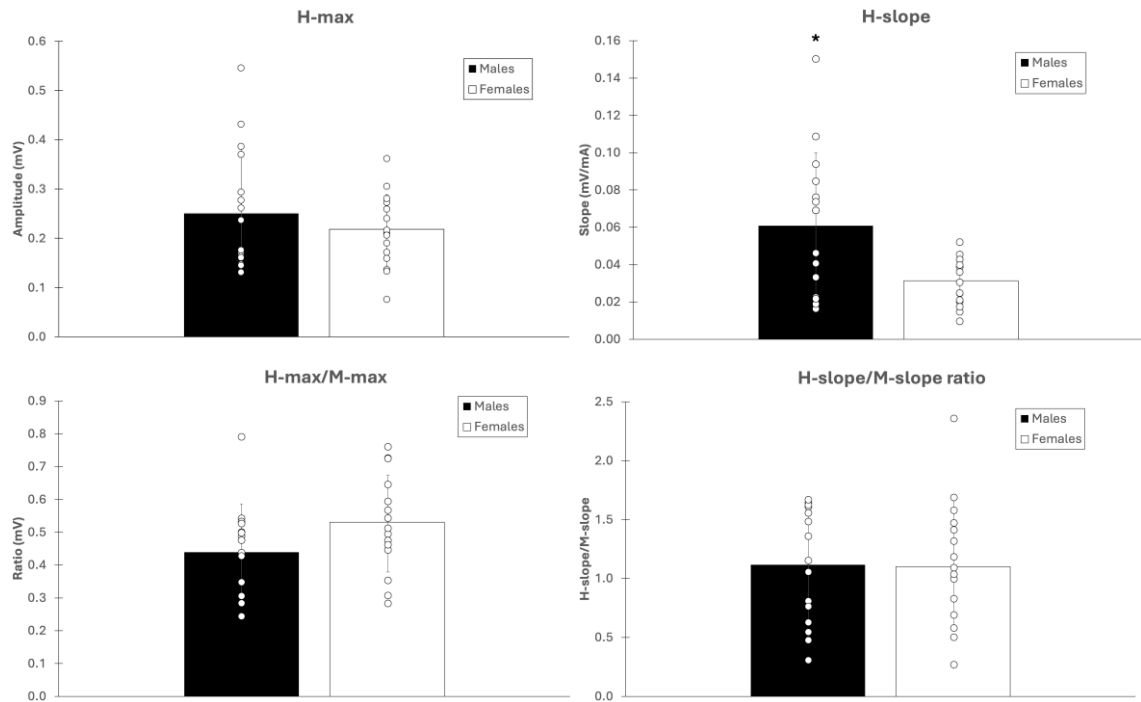


Figure 6. H-max amplitude, H-slope, H-max/M-max and H-slope/M-slope ratio. Bars represent the mean values for males and females. Individual data points are shown by circles. H-slope (top right) was greater in males than females (* $p=0.009$). H-max (top left), H-max/M-max ratio (bottom left), and H-slope/M-slope ratio (bottom right) were not significantly different between sexes.

2.3.4 Reciprocal inhibition

Reciprocal inhibition results are displayed in Figure 7. One male participant was deemed to be an extreme outlier and was therefore removed from analysis for this measure. Reciprocal inhibition was significantly greater in males than females ($p=0.018$, $d=0.93$).

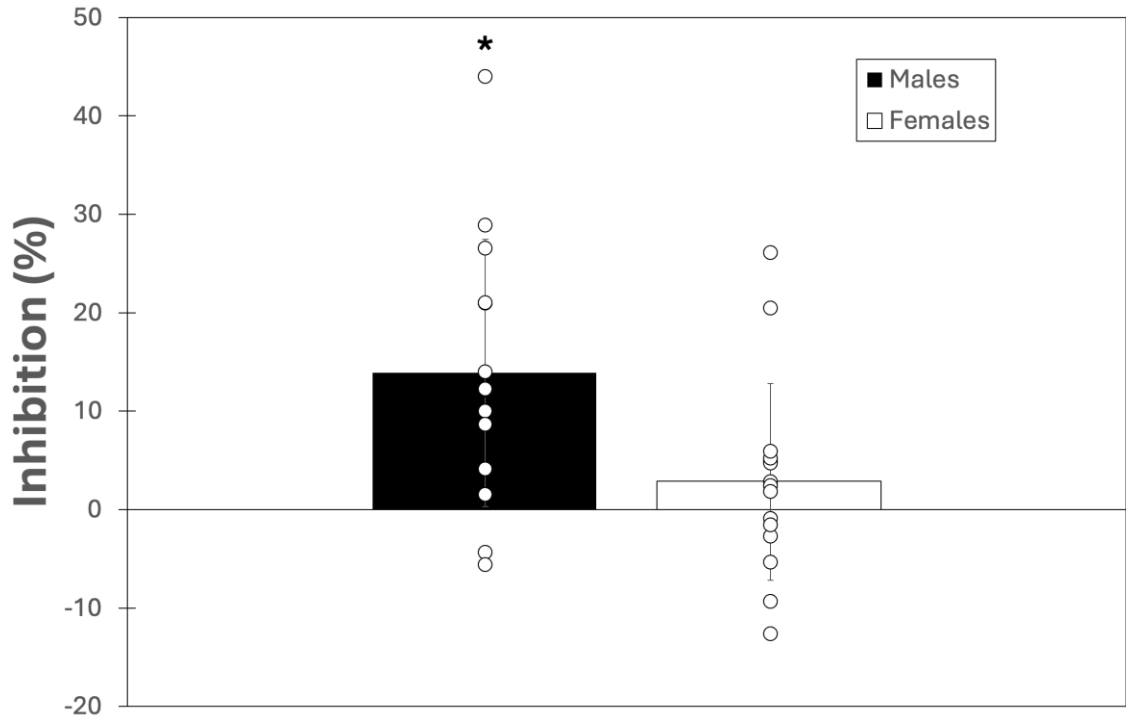


Figure 7. Reciprocal inhibition. Bars represent the mean values for males and females. Individual data points are shown by circles. Reciprocal inhibition was significantly greater in males than females ($*p \leq 0.01$). An extreme outlier (greater than three standard deviations from the mean) from the male participant group was removed from the data, which did not change the outcome.

Chapter 3

3 Discussion and Summary

3.1 Discussion

This study aimed to investigate the sex-related differences in H-reflex excitability and inhibition in young, healthy individuals. No significant differences in H-reflex excitability (i.e. H-max/M-max and H-slope/M-slope) were identified between sexes. Males were found to have significantly greater M-max, M-slope, and H-slope than females. Contrary to our hypothesis, reciprocal inhibition was found to be significantly greater in males than females.

3.1.1 M-wave (M-max, M-slope)

M-max is a commonly reported in H-reflex studies. It represents the complete activation of the motor neuron pool, resulting in full muscle activation, as all motor neurons and subsequent muscle fibres are assumed to be activated (Zehr, 2002). Findings from the present study showed that males exhibited a significantly greater M-max amplitude than females. This finding is consistent with previous work investigating sex-related differences in H-reflex (Mendonca et al., 2020). The sexual dimorphism present in these studies might be explained by males typically having larger muscles size and larger diameter of individual muscle fibres than females (Miller et al., 1993). Therefore, the larger diameter muscle fibres in males would produce larger action potentials when activated, resulting in a greater EMG amplitude of evoked responses. Although we did not measure muscle size in the current study, anthropometric data showed that males were significantly heavier, taller, and had greater maximal force, suggesting evidence for greater muscle mass in males.

Measuring the slope of the M-wave recruitment curve showed that males had a significantly greater M-slope than females. M-slope provides information about the recruitment properties of the soleus muscle. It is an alternative measure to the M-max, used to define the evoked muscle response as the slope of the regression line or rate of change in motor neuron excitability as a function of increased stimulus intensity (Christie et al., 2004; Funase et al., 1994). The finding of a greater slope in males than females is within reason, as M-max was also found to be significantly greater in males. Males would

then be activating muscle fibres of larger diameter than females per increment in stimulus intensity than females to reach their M-max. Thus, it would have a steeper slope indicated by a greater rate of change in excitability at the level of the muscle fibres.

3.1.2 H-reflex excitability (H-max, H-slope, H-max/M-max, H-slope/M-slope)

No significant differences in H-max were found between sexes in the current study. The H-max is defined as the measurement of maximal reflex activation and reflects the number of motor units that can be activated at any one point in time through the reflex arc (Palmieri et al., 2004). We also found no difference between sexes in the H-max/M-max ratio in the current study, indicating there were no differences in H-reflex excitability between sexes, contrary to our hypothesis.

The ratio of the evoked response is a standardized method of reporting the H-reflex as it is expressed relative to an individual's M-wave, allowing for comparisons across participants (Palmieri et al., 2004). The finding of no significant differences in H-reflex excitability agrees with studies by Johnson et al. (2012) and Hoffman et al. (2018a), who also found no significant differences in H-reflex excitability between sexes. However, it disagrees with Mendonca et al. (2020) and Hoffman et al. (2018b), who found that males and females differ significantly in H-max/M-max. Differences across studies may be due to differences in participant positioning and/or the presence or absence of background muscle contractions (Hoffman et al., 2018a; Johnson et al., 2012; Mendonca et al., 2020; Zehr, 2002), as described in more detail below. Interestingly, Mendonca et al. (2020) found that males exhibited greater excitability than females, while Hoffman et al. (2018b) found the opposite. Mendonca et al. (2020) suggested that the between-sex differences were strongly related to greater antagonistic co-activation of the tibialis anterior in females, as the difference dissipated when co-activation was accounted for. In the present study no significant differences in co-activation between sexes were observed in a subset of participants. Our finding is therefore in line with the conclusion of Mendonca et al. (2020), which suggested that when males and females displayed similar levels of co-activation, differences in H-reflex excitability became insignificant.

We also found that males exhibited a significantly greater slope of the H-reflex (H-slope) than females. As defined in the previous section, the slope is the rate of change of motor neuron excitability measured from the ascending limb of the H-reflex recruitment curve. Our finding aligns with previous work (Mendonca et al., 2020). This is an interesting finding as H-slope was significantly different between sexes, but H-max was not. The advantage of the H-slope over H-max is that it is minimally affected by the collision of H-reflex Ia afferent discharge and the antidromic activity of M-wave alpha motor neurons (Funase et al., 1994). Therefore, it can provide a reasonable estimate of the recruitment properties of the motor neuron pool. However, when expressed relative to M-slope there was no sex-related difference, suggesting the difference is at the level of the muscle and not at the motor neuron pool. Similar to H-max/M-max, there were no significant differences identified between sexes for the ratio of slopes (H-slope/M-slope), providing additional support that there were no significant differences in H-reflex excitability between sexes.

3.1.3 Reciprocal inhibition

Reciprocal inhibition of the soleus muscle was accomplished utilizing a paired stimulus technique. A conditioning stimulus excited the nerve innervating the antagonistic muscle prior to the test (C-T) stimulus exciting the nerve innervating the soleus. Reciprocal inhibition is known to occur from the activation of inhibitory interneurons connecting to the post-synaptic cleft of the motor neurons innervating the agonist muscle (Iles, 1986). Based on the results acquired within the present experiment, the paradigm used to exhibit RI was successful for most participants, resulting in an attenuated H-reflex.

Previous research has identified greater pre-synaptic and recurrent inhibition in males than in females (Hoffman et al., 2018a; Johnson et al., 2012). The current study results show that males exhibited significantly greater reciprocal inhibition, a form of post-synaptic inhibition at the neuron pool during soleus H-reflex responses. Although our study replicated the reciprocal inhibition protocol performed in the study by Johnson et al. (2012), our results differed, as they demonstrated no significant differences between sexes. A possible explanation for the discrepancies in the responses, according to Hoffman et al. (2018), is the influence of neuroactive sex hormones on the central

nervous system. The predictable nature of female (~28 days) and male (24 hours) hormonal cycles allows for investigation into how neuroactive sex hormones, such as estrogen, may influence synaptic transmission and, thus, motor control. Hoffman et al. (2018a) suggested that as estrogen levels rise during the first phase of the menstrual cycle, inhibitory neurotransmitter γ -Aminobutyric acid (GABA) levels within the central nervous system would be reduced. These assumptions are founded on brain studies (Corvino et al., 2015; Ottem et al., 2004) where estrogen either upregulated or downregulated the function of GABA. Therefore, by reducing estrogen interaction, which reduces the function of GABA, the inhibitory influence on the alpha motor neuron pool would be reduced, resulting in less inhibition in females. In contrast it has been suggested that testosterone metabolites can act as GABA receptor agonists, resulting in greater inhibition (Bitran et al., 1993). These findings, although completed in animal models, can provide some evidence supporting our finding of greater inhibition in males compared to females.

The results of the reciprocal inhibition protocol showed that six female participants and two males displayed facilitation of the H-reflex rather than the intended and expected attenuation. Capaday et al. (1990) described concerns about the C-T method used to activate the inhibitory pathway modulating the post-synaptic membrane of the lower motor neuron pool. Previous research has suggested that the conditioning stimulus may not be sufficient to elicit a response in some individuals. The H-reflex produced by the test stimulus is attenuated because of disynaptic reciprocal inhibition. The large excitatory post-synaptic potential causes a synchronous discharge of alpha motor neurons, producing an H-reflex. However, the small to moderate inhibitory post-synaptic potentials produced by the inhibitory Ia afferents may not be strong enough to hyperpolarize the post-synaptic membrane and prevent depolarization (Capaday, 1997). Therefore, the intended result to inhibit the test stimulus may be undetectable when attempting to reduce a large excitatory synchronous volley in some individuals.

3.1.4 Methodological considerations

The methodology for measuring these indices requires careful consideration due to the many factors that influence the parameters of the H-reflex. These include the postural

position, stimulation duration and frequency, muscle activation, and anthropometric measures (Burke, 2016). Variations in these parameters make comparisons between H-reflex research challenging and may explain differences in results between studies (Gajewski & Mazur-Różycka, 2018). Like Mendonca et al. (2020), the present study recorded all H-reflexes during a background level of muscle activation to normalize descending drive to the motor neuron pool and ensure males and females maintained similar levels of motor neuron excitability (Zehr, 2002). Although mentioned within their limitations, Johnson et al. (2012) and Hoffman et al. (2018a) evoked all H-reflexes at rest. When the H-reflex is recorded at rest, it does not account for supraspinal input or inhibitory inputs post-synaptic effects while also reducing the applicability of the results to functional movement (Knikou, 2008). A counterpoint provided by Palmieri et al. (2004) to evoking H-reflexes during a light contraction is that it can alter muscle geometry, therefore altering the H-reflex without affecting the neural drive. The leg of the participant was secured within a dynamometer used to measure isometric plantar flexion, and the contraction participants were instructed to contract at 10% of their MVC. Therefore, changes in muscle geometry would have been minimal during elicitation of the H-reflex.

The stimulation duration was set as a 1ms duration pulse, and frequency was set to evoke an H-reflex every ~10 seconds (Palmieri et al., 2004), similar to Hoffman et al. (2018a) and Johnson et al. (2012) in order to reduce the influence of post-activation depression. The joint angles used in the present study were 90 degrees of hip flexion and knee flexion (180 degrees is full extension) and anatomically neutral position for the ankle (Hoffman et al., 2018a; Johnson et al., 2012). Participant postural positioning at the hip, knee, and ankle varied significantly between the few studies investigating sex-related differences in H-reflex. Mendonca et al. (2020) had hips and knee at 120 and ankle at 110 of plantar flexion, Johnson et al. (2012) reported a semi-recumbent position at the hip, 60 degrees of knee flexion and neutral ankle, and Hoffman et al. (2018a) placed participants in 5 degrees of hip flexion, fully extended knee and neutral ankle angle. The aforementioned studies show a wide variable in the joint angles chosen, which may help explain differences in results between studies. Especially since varying postural conditions (seated to standing) have displayed progressive inhibition of the H-reflex (Angulo-

Kinzler et al., 1998). In order to isolate the soleus as the primary contributor to force and reduce the activation of the gastrocnemius during voluntary contraction, the knee in our study was placed in a flexed position (Dixon, 2009). Lastly, the postural conditions in order to minimize the outside influence on the H-reflex were followed as outlined in the available literature (Pierrot-Deseilligny & Burke, 2005).

3.2 Limitations

As we did not measure hormone levels, either by salivary or blood samples, we cannot definitively conclude that there is a relationship between neuroactive sex hormones and H-reflex excitability and inhibition measures within this study. Further, this study used surface electromyography to record muscle activity. It is known that females tend to have greater subcutaneous adipose distribution (Hattori et al., 1991), which may impede the signal quality and filtering, resulting in a reduced peak-to-peak amplitude of evoked potentials (Doheny et al., 2010). However, since we normalized EMG responses, our findings should not have been affected by this sex-related discrepancy. Lastly, although we followed standard procedures by eliciting the H-reflex during muscle activity to normalize excitability and other inhibitory inputs to the pool, we did not directly measure pre-synaptic inhibition (Hultborn et al., 1987) nor recurrent inhibition (Zehr, 2002). These variables have been shown to have a more profound influence as muscle activation increases. Therefore, we cannot confirm their presence had no significant influence on our findings.

3.3 Conclusion

In this study it was hypothesized that males would display greater H-reflex excitability and lower reciprocal inhibition than females. The findings demonstrate no significant sex-related difference in H-reflex excitability between males and females. This finding was unexpected, but our results can potentially be explained by the lack of difference in co-activation, similar to the findings by Mendonca et al. (2020). Contrary to the hypothesis, males were found to have significantly greater reciprocal inhibition than females. The findings of the present study suggest that there may be an influence of neuro-modulatory sex hormones on the spinal motor neuron pool. This finding could also result from male and female participants responding unexpectedly to the conditioning

stimulus of the reciprocal inhibition protocol. Further research investigating sex-related differences in H-reflex excitability and inhibition is needed to better understand the discrepancies between male and female neurophysiology at the level of the spinal motor neuron pool.

3.4 Future directions

Reciprocal inhibition was significantly greater in males than females. The underlying mechanism for this discrepancy is speculated to be in part influenced by neuroactive sex hormones. There is conflicting evidence surrounding the hormonal influence on spinal inhibition (Casey et al., 2016; Hoffman et al., 2018a). However, Hoffman et al. (2018a) have provided preliminary evidence supporting this theory, stating that the hormone estrogen interacts with the inhibitory neurotransmitter GABA, attenuating its effect on inhibitory interneurons and thus reducing the overall impact of inhibition on the spinal motor neuron synapse. There is also evidence supporting the theory that greater levels of testosterone play an agonistic role on GABA receptors, resulting in greater inhibition (Bitran et al., 1993). The current study did not directly measure neuroactive sex hormones; thus, we cannot conclude their influence on our H-reflex excitability and inhibition measures. Therefore, future research should investigate the influence of sex hormones on spinal inhibition in both males and females.

Understanding how hormonal fluctuations influence these responses will provide researchers with greater insight into how to better control for these intrinsic factors to elicit the H-reflex under controlled conditions appropriately. Additionally, future work should include other measurements of inhibition, as demonstrated by Johnson et al. (2012), who included pre- and post-synaptic inhibitory measurements within their H-reflex protocol. Investigating additional variables of the motor neuron synapse can provide researchers with a broader view of the reflex transmission from afferent to efferent, hopefully narrowing down the sex-related difference for future work.

References

- Angulo-Kinzler, R. M., Mynark, R. G., & Koceja, D. M. (1998). Soleus H-reflex gain in elderly and young adults: Modulation due to body position. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences*, 53A(2).
<https://doi.org/10.1093/gerona/53a.2.m120>
- Burke D. (2016). Clinical uses of H reflexes of upper and lower limb muscles. *Clinical neurophysiology practice*, 1, 9–17. <https://doi.org/10.1016/j.cnp.2016.02.003>
- Capaday, C., Cody, F. W., & Stein, R. B. (1990). Reciprocal inhibition of soleus motor output in humans during walking and voluntary tonic activity. *Journal of Neurophysiology*, 64(2), 607–616. <https://doi.org/10.1152/jn.1990.64.2.607>
- Capaday, C. (1997). Neurophysiological methods for studies of the motor system in freely moving human subjects. *Journal of Neuroscience Methods*, 74(2), 201–218. [https://doi.org/10.1016/s0165-0270\(97\)02250-4](https://doi.org/10.1016/s0165-0270(97)02250-4)
- Casey, E., Reese, M., Okafor, E., Chun, D., Gagnon, C., Nigl, F., & Dhaher, Y. Y. (2016). Influence of menstrual cycle and oral contraceptive phase on spinal excitability. *PM&R*, 8(9), 860–868.
<https://doi.org/10.1016/j.pmrj.2016.01.013>
- Christie, A., Lester, S., LaPierre, D., & Gabriel, D. A. (2004). Reliability of a new measure of H-reflex excitability. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*, 115(1), 116–123.
[https://doi.org/10.1016/s1388-2457\(03\)00306-7](https://doi.org/10.1016/s1388-2457(03)00306-7)
- Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences* (2nd ed.). Routledge. <https://doi.org/10.4324/9780203771587>
- Corvino, V., Di Maria, V., Marchese, E., Lattanzi, W., Biamonte, F., Michetti, F., & Geloso, M. C. (2015). Estrogen administration modulates hippocampal GABAergic subpopulations in the hippocampus of trimethyltin-treated rats. *Frontiers in cellular neuroscience*, 9, 433.
<https://doi.org/10.3389/fncel.2015.00433>
- Betti, S., Fedele, M., Castiello, U., Sartori, L., & Budisavljević, S. (2022). Corticospinal

excitability and conductivity are related to the anatomy of the corticospinal tract. *Brain structure & function*, 227(3), 1155–1164.

<https://doi.org/10.1007/s00429-021-02410-9>

- Bitran, D., Kellogg, C. K., & Hilvers, R. J. (1993). Treatment with an anabolic-androgenic steroid affects anxiety-related behavior and alters the sensitivity of cortical GABAA receptors in the rat. *Hormones and behavior*, 27(4), 568–583. <https://doi.org/10.1006/hbeh.1993.1041>
- Bryan Dixon, J. (2009). Gastrocnemius vs. soleus strain: How to differentiate and deal with calf muscle injuries. *Current Reviews in Musculoskeletal Medicine*, 2(2), 74–77. <https://doi.org/10.1007/s12178-009-9045-8>
- Doheny, E. P., Caulfield, B. M., Minogue, C. M., & Lowery, M. M. (2010). Effect of subcutaneous fat thickness and surface electrode configuration during neuromuscular electrical stimulation. *Medical Engineering & Physics*, 32(5), 468–474. <https://doi.org/10.1016/j.medengphy.2010.03.004>
- Frey-Law, L. A., & Avin, K. G. (2013). Muscle coactivation: A generalized or localized motor control strategy? *Muscle & Nerve*, 48(4), 578–585. <https://doi.org/10.1002/mus.23801>
- Funase, K., Imanaka, K., & Nishihira, Y. (1994). Excitability of the soleus motoneuron pool revealed by the developmental slope of the H-reflex as reflex gain. *Electromyography and clinical neurophysiology*, 34(8), 477–489.
- Gajewski, J., & Mazur-Różycka, J. (2018). The H-reflex as an important indicator in kinesiology. *Human Movement*, 17(2), 64–71. <https://doi.org/10.1515/humo-2016-0018>
- Hattori, K., Numata, N., Ikoma, M., Matsuzaka, A., & Danielson, R. R. (1991). Sex Differences in the Distribution of Subcutaneous and Internal Fat. *Human Biology*, 63(1), 53–63. <http://www.jstor.org/stable/41464142>
- Hoffman, M. A., Doeringer, J. R., Norcross, M. F., Johnson, S. T., & Chappell, P. E. (2018a). Presynaptic inhibition decreases when estrogen level rises. *Scandinavian Journal of Medicine & Science in Sports*, 28(9), 2009–2015. <https://doi.org/10.1111/sms.13210>
- Hoffman, M., Norcross, M., & Johnson, S. (2018b). The Hoffmann reflex is different in

- men and women. *Neuroreport*, 29(4), 314–316.
<https://doi.org/10.1097/WNR.0000000000000961>
- Hultborn, H., Meunier, S., Pierrot-Deseilligny, E., & Shindo, M. (1987). Changes in presynaptic inhibition of Ia fibres at the onset of voluntary contraction in man. *The Journal of physiology*, 389, 757–772.
<https://doi.org/10.1113/jphysiol.1987.sp016681>
- Iles, J. F., & Roberts, R. C. (1987). Inhibition of monosynaptic reflexes in the human lower limb. *The Journal of physiology*, 385, 69–87.
<https://doi.org/10.1113/jphysiol.1987.sp016484>
- Johnson, S. T., Kipp, K., & Hoffman, M. A. (2012). Spinal motor control differences between the sexes. *European journal of applied physiology*, 112(11), 3859–3864.
<https://doi.org/10.1007/s00421-012-2363-3>
- Knikou, M., & Rymer, W. Z. (2002). Effects of changes in hip joint angle on H-reflex excitability in humans. *Experimental Brain Research*, 143(2), 149–159.
<https://doi.org/10.1007/s00221-001-0978-4>
- Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Frontiers in psychology*, 4, 863.
<https://doi.org/10.3389/fpsyg.2013.00863>
- Lin, J. P., Brown, J. K., & Walsh, E. G. (1997). Soleus muscle length, stretch reflex excitability, and the contractile properties of muscle in children and adults: a study of the functional joint angle. *Developmental medicine and child neurology*, 39(7), 469–480. <https://doi.org/10.1111/j.1469-8749.1997.tb07467.x>
- Lulic-Kuryllo, T., & Inglis, J. G. (2022). Sex differences in motor unit behaviour: A review. *Journal of electromyography and kinesiology : official journal of the International Society of Electrophysiological Kinesiology*, 66, 102689.
<https://doi.org/10.1016/j.jelekin.2022.102689>
- Maughan, R. J., Watson, J. S., & Weir, J. (1983). Strength and cross-sectional area of human skeletal muscle. *The Journal of physiology*, 338, 37–49.
<https://doi.org/10.1113/jphysiol.1983.sp014658>
- McNulty, K. L., Elliott-Sale, K. J., Dolan, E., Swinton, P. A., Ansdell, P., Goodall, S.,

- Thomas, K., & Hicks, K. M. (2020). The effects of menstrual cycle phase on exercise performance in EUMENORRHEIC Women: A systematic review and meta-analysis. *Sports Medicine*, *50*(10), 1813–1827.
<https://doi.org/10.1007/s40279-020-01319-3>
- Mendonca, G. V., Pezarat-Correia, P., Gonçalves, A. D., Gomes, M., Correia, J. M., & Vila-Chã, C. (2020). Sex differences in soleus muscle H-reflex and V-wave excitability. *Experimental physiology*, *105*(11), 1928–1938.
<https://doi.org/10.1113/EP088820>
- Miller, A. E., MacDougall, J. D., Tarnopolsky, M. A., & Sale, D. G. (1993). Gender differences in strength and muscle fiber characteristics. *European Journal of Applied Physiology and Occupational Physiology*, *66*(3), 254–262.
<https://doi.org/10.1007/bf00235103>
- Ottem, E. N., Godwin, J. G., Krishnan, S., & Petersen, S. L. (2004). Dual-phenotype GABA/glutamate neurons in adult preoptic area: Sexual dimorphism and function. *The Journal of Neuroscience*, *24*(37), 8097–8105.
<https://doi.org/10.1523/jneurosci.2267-04.2004>
- Palmieri, R. M., Ingersoll, C. D., & Hoffman, M. A. (2004). The hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. *Journal of athletic training*, *39*(3), 268–277.
- Pappas, E., Hagins, M., Sheikzadeh, A., Nordin, M., & Rose, D. (2007). Biomechanical differences between unilateral and bilateral landings from a jump: gender differences. *Clinical journal of sport medicine : official journal of the Canadian Academy of Sport Medicine*, *17*(4), 263–268.
<https://doi.org/10.1097/JSM.0b013e31811f415b>
- Pierrot-Deseilligny, E., & Burke, D. (2005). *The Circuitry of the Human Spinal Cord*.
<https://doi.org/10.1017/cbo9780511545047>
- Ugawa Y. (2020). Voluntary and involuntary movements: A proposal from a clinician. *Neuroscience research*, *156*, 80–87.
<https://doi.org/10.1016/j.neures.2019.10.001>
- Wolpaw, J. R. (2001). Motor neurons and spinal control of movement. *Encyclopedia of Life Sciences*. <https://doi.org/10.1038/npg.els.0000156>

Zehr E. P. (2002). Considerations for use of the Hoffmann reflex in exercise studies. *European journal of applied physiology*, 86(6), 455–468.
<https://doi.org/10.1007/s00421-002-0577-5>

Appendices

Appendix 1. Ethics Approval



Date: 4 July 2023

To: Professor Anita Christie

Project ID: 122743

Review Reference: 2023-122743-81183

Study Title: Sex-related Differences in the Nervous System's Control of Muscle

Application Type: HSREB Initial Application

Review Type: Full Board

Meeting Date: 16/May/2023 13:00

Date Approval Issued: 04/Jul/2023 15:02

REB Approval Expiry Date: 04/Jul/2024

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
IPAQ_English_self-admin_long	Online Survey	19/Apr/2023	1
Data Collection Sheet	Other Data Collection Instruments	05/May/2023	1
In Class Recruitment Script	Recruitment Materials	23/May/2023	1
Protocol and Research Plan	Protocol	12/Jun/2023	2
Recruitment Email Script	Email Script	12/Jun/2023	2
Scheduling and Exclusion Email	Email Script	12/Jun/2023	2
Consent Form	Written Consent/Assent	14/Jun/2023	3
Flyer	Recruitment Materials	26/Jun/2023	3
Screening Form	Online Survey	26/Jun/2023	3
Follow-up Email Script with Consent Form	Email Script	26/Jun/2023	3

Documents Acknowledged:

Document Name	Document Type	Document Date	Document Version
Budget	Study budget	05/May/2023	1

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:

Ms. Nicola Geoghegan-Morphet, Ethics Officer on behalf of Dr. Emma Duerden, HSREB Vice-Chair, 04/Jul/2023 15:02

Reason: I am approving this document

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations, See [Electronic System Compliance Review](#))

Appendix 2. The American Physiological Society Copywrite Approval

8/23/24, 11:54 AM

RightsLink Printable License

THE AMERICAN PHYSIOLOGICAL SOCIETY LICENSE TERMS AND CONDITIONS





Aug 23, 2024

This Agreement between Jonah Dekker ("You") and The American Physiological Society ("The American Physiological Society") consists of your license details and the terms and conditions provided by The American Physiological Society and Copyright Clearance Center.

License Number	5854800626305
License date	Aug 23, 2024
Licensed Content Publisher	The American Physiological Society
Licensed Content Publication	Journal of Applied Physiology
Licensed Content Title	Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses
Licensed Content Author	Per Aagaard, Erik B. Simonsen, Jesper L. Andersen, et al
Licensed Content Date	Jun 1, 2002
Licensed Content Volume	92
Licensed Content Issue	6
Type of Use	Thesis/Dissertation
Requestor type	author
Format	electronic

8/23/24, 11:54 AM

RightsLink Printable License

Portion	figures/tables/images
Number of figures/tables/images	1
Will you be translating?	no
World Rights	no
Title of new work	Sex related differences in H reflex excitability and inhibition
Institution name	University of Western Ontario
Expected presentation date	Aug 2024
Portions	Table 1, Page 22
The Requesting Person / Organization to Appear on the License	Jonah Dekker
	 er
Requestor Location	 Canada Attn: Mr. Jonah Dekker
Billing Type	Invoice
	 er
Billing Address	 Canada Attn: Mr. Jonah Dekker
Total	0.00 CAD

Appendix 3. Cambridge University Press Copywrite Approval



PARTIES:

1. **Cambridge University Press** (Licensor); and
2. **Jonah Dekker** (Licensee).

Thank you for your recent permission request. Some permission requests for use of material published by the Licensor, such as this one, are now being facilitated by PLSclear.

Set out in this licence cover sheet (the **Licence Cover Sheet**) are the principal terms under which Licensor has agreed to license certain Licensed Material (as defined below) to Licensee. The terms in this Licence Cover Sheet are subject to the attached General Terms and Conditions, which together with this Licence Cover Sheet constitute the licence agreement (the **Licence**) between Licensor and Licensee as regards the Licensed Material. The terms set out in this Licence Cover Sheet take precedence over any conflicting provision in the General Terms and Conditions.

Free Of Charge Licence Terms

Licence Date: 12/07/2024
 PLSclear Ref No: 95744

The Licensor

Company name: Cambridge University Press
 Address: University Printing House
 Shaftesbury Road
 Cambridge
 CB2 8BS
 GB

The Licensee

Licensee Contact Name: Jonah Dekker
 Licensee Address: [REDACTED]

Licensed Material

title: The Circuitry of the Human Spinal Cord Its Role in Motor Control and Movement Disorders
 ISBN: 9780521825818
 publisher: Cambridge University Press

Are you requesting permission to reuse the cover of the publication?	No
Figure number & title	Figure 1.11 (a)
Page numbers	25
Name of illustrator	Emmanuel Pierrot-Deseilligny and David Burke
Are you the author of the content that you are requesting to reuse?	No
Will you be changing or editing the image?	No

For Use In Licensee's Publication(s)

usage type	Book, Journal, Magazine or Academic Paper-Thesis or Dissertation
Will your dissertation be placed in an online repository?	Yes
Author	Jonah Dekker
Language	English
Title of dissertation/thesis	Sex-related differences in H-reflex excitability and inhibition
University or institution	University of Western Ontario
Unlimited circulation?	Yes

Rights Granted

Exclusivity:	Non-Exclusive
Format:	Thesis or Dissertation
Language:	English
Territory:	UK & Commonwealth
Duration:	Lifetime of Licensee's Edition
Maximum Circulation:	Unlimited

GENERAL TERMS AND CONDITIONS

1. Definitions and Interpretation

1.1 Capitalised words and expressions in these General Terms and Conditions have the meanings given to them in the Licence Cover Sheet.

1.2 In this Licence any references (express or implied) to statutes or provisions are references to those statutes or provisions as amended or re-enacted from time to time. The term including will be construed as illustrative, without limiting the sense or scope of the words preceding it. A reference to in writing or written includes faxes and email. The singular includes the plural and vice versa.

2. Grant of Rights

2.1 The Licensor grants to Licensee the non-exclusive right to use the Licensed Material as specified in the Licence Cover Sheet.

2.2 The rights licensed to Licensee under this Licence do not include the right to use any third party copyright material incorporated in the Licensed Material. Licensee should check the Licensed Material carefully and seek permission for the use of any such third party copyright material from the relevant copyright owner(s).

2.3 Unless otherwise stated in the Licence Cover Sheet, the Licensed Material may be:

2.3.1 subjected to minor editing, including for the purposes of creating alternative formats to provide access for a beneficiary person (provided that any such editing does not amount to derogatory treatment); and/or

2.3.2 used for incidental promotional use (such as online retail providers' search facilities).

2.4 Save as expressly permitted in this Licence or as otherwise permitted by law, no use or modification of the Licensed Material may be made by Licensee without Licensor's prior written permission.

3. Copyright Notice and Acknowledgement

3.1 Licensee must ensure that the following notices and acknowledgements are reproduced prominently alongside each reproduction by Licensee of the Licensed Material:

3.1.1 the title and author of the Licensed Material;

3.1.2 the copyright notice included in the Licensed Material; and

3.1.3 the statement "Reproduced with permission of The Licensor through PLSclear."

4. Reversion of Rights

4.1 The rights licensed to Licensee under this Licence will terminate immediately and automatically upon the earliest of the following events to occur:

4.1.1 the Licensed Material not being used by Licensee within 18 months of the Licence Date;

4.1.2 expiry of the Licence Duration; or

4.1.3 the Maximum Circulation being reached.

5. Miscellaneous

5.1 By using the Licensed Material, Licensee will be deemed to have accepted all the terms and conditions contained in this Licence.

5.2 This Licence contains the entire understanding and agreement of the parties relating to its subject matter and supersedes in all respects any previous or other existing arrangements, agreements or understandings between the parties whether oral or written in relation to its subject matter.

5.3 Licensee may not assign this Licence or any of its rights or obligations hereunder to any third party without Licensor's prior written consent.

5.4 This Licence is governed by and shall be construed in accordance with the laws of England and Wales and the parties hereby irrevocably submit to the non-exclusive jurisdiction of the Courts of England and Wales as regards any claim, dispute or matter arising under or in relation to this Licence.

Curriculum Vitae

- Name:** Jonah D. Dekker
- Post-secondary Education and Degrees:** McMaster University
Hamilton, Ontario, Canada
Bachelor of Science Kinesiology – Honors (2018-2022)
- The University of Western Ontario
London, Ontario, Canada
Master of Integrative Biosciences in Kinesiology (2022-present)
Supervisor: Dr. Anita D. Christie
- Honours and Awards:** Province of Ontario Graduate Scholarship
2022-2023, 2024-2025
- Western Graduate Research Scholarship
The University of Western Ontario
2022-2023, 2023-2024
- Dean’s Honour List
McMaster University (2022)
- Related Work Experience** Graduate Teaching Assistant (2022-Present)
The University of Western Ontario
- KINESIOL 2230: Introduction to Exercise Physiology
 - KINESIOL 3330: Laboratory in Exercise Physiology
 - KINESIOL 3495: Practical Aspects of Strength and Conditioning
 - KINESIOL 3347: Growth and Development
- Publications:**
Zhou, J., Wituik, C., Al-Salem, M., Aljarbou, A., **Dekker, J.**, Karimi, S., & Mertz, D. (2023). Determining coronavirus disease 2019 (COVID-19) community incidence threshold for preoperative testing. *Infection Control & Hospital Epidemiology*, 44(7), 1196-1198. doi:10.1017/ice.2022.206
- Presentations:**
Dekker J. D., Christie A.D., “Sex-related Differences in H-reflex Excitability and Inhibition”. Poster Presentation at the American College of Sports Medicine. In-person 2024.