THE EFFECT OF MUSCLE LENGTH ON TRANSCRANIAL MAGNETIC STIMULATION-INDUCED RELAXATION RATE IN THE PLANTAR FLEXORS

by

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The Effect of Muscle Length on Transcranial Magnetic Stimulation-Induced Relaxation Rate in the Plantar Flexors

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Abstract

Muscle relaxation rate is an important intrinsic contractile property that influences the neural drive necessary to achieve a desired force. Most relaxation data are obtained at rest through evoked contractions; however, the application of transcranial magnetic stimulation (TMS) during a maximal voluntary contraction (MVC) allows for a more functionally-relevant measurement of muscle relaxation. **PURPOSE:** The purpose of this study was to determine if TMS is a sufficiently sensitive technique to detect changes in relaxation rate with changes in muscle length. A secondary purpose was to qualitatively compare the relaxation rate of whole muscle torque to relaxation rates of single fascicles and the muscle-tendon junction (MTJ) acquired using ultrasound imaging. **METHODS:** Eleven males (24.8 ± 7.0 years; mean ± SD) performed 21 brief (~3s) isometric plantar flexor MVCs in a prone position at full knee extension, pseudo-randomized between 20° dorsiflexion (DF), a neutral ankle position, and 30° plantar flexion (PF). During each MVC, high-intensity TMS (80% stimulator output) was delivered to the motor cortex; ultrasound video recordings captured medial gastrocnemius (MG) fascicle and MTJ length changes. Peak relaxation rate was calculated to be the steepest slope of the TMS-induced drop in torque or rate of length change for MG fascicles and MTJ. **RESULTS:** MVC torque was greater in DF (182.0 ± 40.7 Nm) compared to neutral (147.7 ± 18.9 Nm) and in the neutral compared to PF position (62.2 ± 9.6 Nm). Plantar flexor relaxation rate was slower at PF (-804.02 ± 161.91 Nm/s) compared to neutral and DF (-1895.90 ± 298.31 and -2007.62 ± 692.11 Nm/s, respectively). Similarly, MG fascicle relaxation rate was slower at PF (-2.80 ± 1.10 cm/s) compared to neutral and DF (-5.35 ± 1.10 and -4.81 ± 1.87 cm/s, respectively). Relaxation rate of the MTJ did not differ with ankle angle ($p = 0.06$). **CONCLUSIONS:** Absolute relaxation rate was

markedly slower when the plantar flexor muscles were shortened, indicating the TMS-induced relaxation technique is sufficiently sensitive to detect changes in muscle length. Comparable results were obtained from single fascicles, indicating that ultrasound imaging is suitable for the measurement of evoked contractile properties during voluntary contraction.

Preface

The University of British Columbia's Clinical Research Ethics Board granted ethics approval for this research on July 03, 2015. The ethics approval certificate number for the current study is H15-00716. To date, the research included in this thesis has not been published in full.

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List of Abbreviations

Ag-AgCl	silver-silver chloride
ANOVA	analysis of variance
AT	Achilles tendon
Ca^{2+}	calcium ion
cm	centimetres
CNS	central nervous system
EMG	electromyography
ICC	intraclass correlation coefficient
kg	kilograms
LG	lateral gastrocnemius
MEP	motor evoked potential
MG	medial gastrocnemius
MHz	megahertz
ms	milliseconds
MTJ	Muscle-tendon junction
MVC	maximal voluntary contraction
Nm	Newton metres
ROM	range of motion
rmANOVA	repeated measures ANOVA
s	seconds
SD	standard deviation
SEM	standard error of the mean

SIT	superimposed twitch
TMS	transcranial magnetic stimulation
TS	triceps surae
µm	micrometre
yrs	years

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The past two years have stretched and molded me, both as a student and an individual. I want to extend my gratitude to those who have provided guidance and support throughout my MSc thesis journey, facilitating my academic growth and development.

> Whatever you do in word or deed, do all in the name of the Lord Jesus, giving thanks through Him to God the Father. –Colossians 3:17, NASB

First and foremost, I offer praise and thanksgiving to my Lord Jesus Christ; apart from Him, I can do nothing.

My sincerest thanks goes to my supervisor, Dr. Chris McNeil. You have given me opportunities in academia that few are privileged to have; I am honoured to be your first graduate student. Thank you for your patience with me and your dedication; you have provided everything I need to begin my foray into scientific research – your guidance and support are unequaled. I count myself blessed to study under your supervision.

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To the most important people in my life: Mom and Dad, you are the best parents any kid could ask for. Thank you for your never-ending support, prayers, and love; you have made my dreams possible. Shane, thanks for putting up with me and making me smile – you're a fantastic brother and I know you'll do great things. Keep up the hard work! I love you three more than anything in the world.

By the grace of Christ – to Mom, Dad, and Shane

Chapter 1 Introduction

1.1 Transcranial Magnetic Stimulation

Transcranial magnetic stimulation (TMS) is a non-invasive and painless neurostimulation technique commonly used to assess the motor pathway. It relies on the principles of electromagnetic induction, as established by English physicist Michael Faraday in 1831. Faraday built on the work of scientists before him, to develop the basis for the functional generation of electricity, which is defined by the Law of Induction. Today, electric motors, passive graphics tablets, induction cooktops, and magnetic stimulators are among a few of the many devices to which the principles of electromagnetic induction are applied.

Despite the revolutionizing discovery of electromagnetic induction, the application of magnetic stimulation to the human brain was not introduced for another 150 years. The field of electrophysiology, however, made key advances that more quickly gave rise to a platform for techniques (i.e. electrical stimulation of peripheral nerves) still employed by experimental physiologists today. In 1771, Italian physician Luigi Galvani discovered bioelectricity (animal electricity). His nephew, Giovanni Aldini, later applied these findings in conjunction with an early model of the battery to produce a wide variety of muscular contractions (Parent, 2004). Aldini went on to apply this stimulation technique to the brain of living humans; this research became the foundation for the development of electroconvulsive therapy in 1937 (Horvath et al. 2011).

Using Anthony Barker's initial TMS prototype, P.A. Merton and H.B. Morton were the first to demonstrate the utility of electromagnetic induction for stimulation of the human brain (Barker et al. 1985). This form of stimulation, compared to electrical stimulation, was

painless and provided a more convenient method for analyzing central motor pathways via the activation of the motor cortex (Kobayashi & Pascual-Leone, 2003).

Today, TMS is successfully employed for both clinical and mechanistic research in the fields of neurophysiology, neurology, and psychiatry (Kobayashi & Pascual-Leone, 2003). The therapeutic application of TMS for patients with conditions such as depression and stroke has become one of the hot topics in neurophysiology and psychiatry today (Ridding & Rothwell, 2007). However, for researchers to better understand the effects of TMS within clinical conditions, it is important to obtain information regarding how the healthy neuromuscular system responds to this controlled external stimulus.

TMS elicits an excitatory response (motor evoked potential; MEP) followed by an inhibitory response (silent period), when applied at the level of the motor cortex during a voluntary contraction (Figure 1).

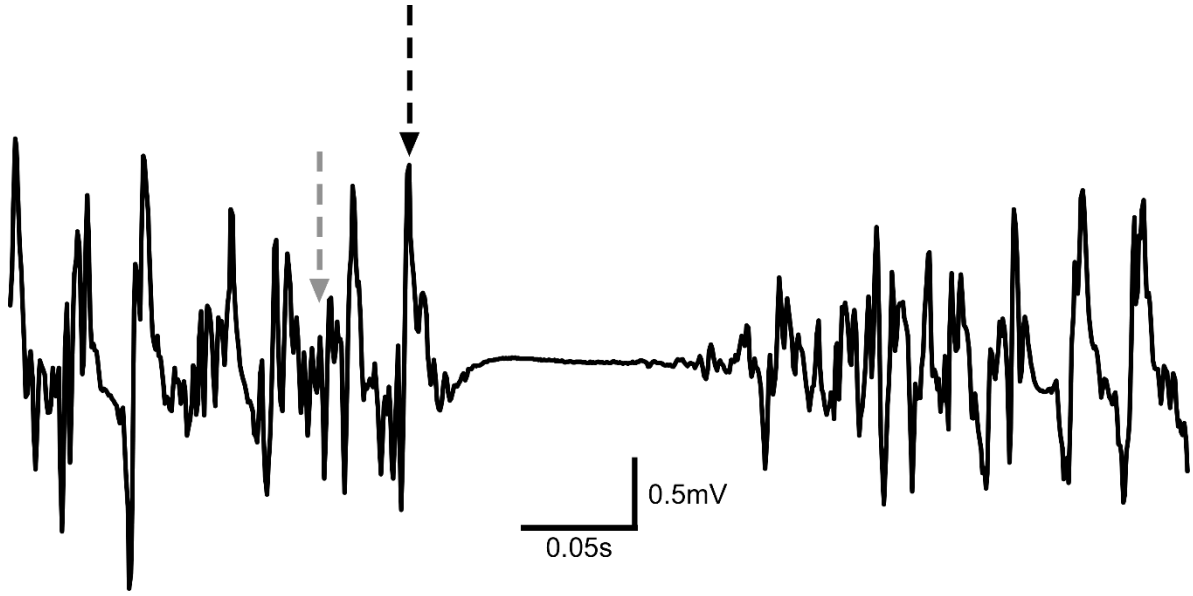

Figure 1: Raw trace of the lateral gastrocnemius EMG response to TMS (80% magnetic output of two magnets connected by a BiStim^2 module) of the primary motor cortex during a maximal voluntary plantar flexor contraction. The gray arrow denotes the stimulus artifact from the delivery of TMS and the black arrow denotes the MEP, followed by a clearly visible silent period (177ms duration).

The TMS-induced silent period is characterized by complete (or near-complete) silence of motoneuron activity and muscle fibre excitation, which is seen as electromyographic (EMG) silence recorded at the level of the muscle. During this period, descending drive from the cortex is interrupted by intracortical (Fuhr et al. 1991; Inghilleri et al. 1993; Roick et al. 1993; Triggs et al. 1993; Ziemann et al. 1993; Schnitzler & Benecke, 1994; von Giesen et al. 1994; Siebner et al. 1998; Werhahn et al. 1999) and spinal mechanisms (Fuhr et al. 1991; Cantello et al. 1992; Inghilleri et al. 1993; Ziemann et al. 1993; Yacyshyn et al. submitted) of inhibition or disfacilitation, and the target muscle involuntarily undergoes relaxation until descending drive has been re-established.

Given the unique ability of TMS to activate motoneurons and their associated muscle fibres (as quantified by the MEP; Figure 1) and transiently interrupt descending drive (as quantified by the silent period; Figure 1), the technique has been successfully adapted for the investigation of intrinsic contractile properties of active whole muscle (Todd et al. 2007), namely peak muscle relaxation rate.

1.2 Intrinsic Muscle Contractile Properties

1.2.1 Muscle Wisdom Hypothesis

The muscle wisdom hypothesis was derived from a set of observations on force and stimulation rate that demonstrated how decreases in muscle relaxation rate paralleled decreases in motoneuron firing rates (Jones et al. 1979; Marsden et al. 1983). Muscle wisdom was first described by Marsden et al. in 1971 and the concept has since been expanded on by a number of researchers. It has been postulated that the fatigue-induced decreases in motoneuron firing rates are not the responsible mechanism for force loss during a sustained maximal contraction. Rather than contributing to force loss, decreased motoneuron firing

rates that match slowing of muscle relaxation may function to optimize muscle performance by avoiding unnecessary energy expenditure through the resulting subtle adjustments in force output (Bigland-Ritchie et al. 1983 a, b; Marsden et al. 1983). Furthermore, it has also been suggested that lower motoneuron discharge rates prevent action potential conduction failure at or distal to the neuromuscular junction (Jones et al. 1979; Marsden et al. 1983).

While the muscle wisdom hypothesis remains well supported, there is some evidence suggesting it does not play a significant role during fatiguing contractions (Barry & Enoka, 2007). First, it has been noted that previous studies (Jones et al. 1979; Marsden et al. 1983) supporting the muscle wisdom hypothesis employed high-frequency stimulation techniques that do not mimic natural motoneuron output as seen during a maximal voluntary contraction (MVC) (Fuglevand & Keen, 2003). It was proposed that high-frequency stimulation may mask force-loss resulting from decreased motoneuron firing rates. Second, motor unit discharge rates during submaximal fatiguing contractions have been shown, often dependent on time of recruitment, to increase or remain unchanged (Nordstrom & Miles, 1991; Garland et al. 1994; Carpentier et al. 2001; Kuchinad et al. 2004).

This evidence does not disqualify the muscle wisdom hypothesis, despite bringing attention to several shortcomings; rather, it emphasizes the variability of the human neuromuscular system. Firing rate adjustments made by motor units are likely task dependent and vary across a given participant population (Barry & Enoka, 2007).

1.2.2 Muscle Relaxation Rate

Both fields of thought regarding muscle wisdom have their strengths (and supporting experimental evidence), and it stands to reason that the potential role of this hypothesis for performance optimization makes relaxation rate an important intrinsic property of muscle fibres (Kleine & Stegeman, 2007). Despite the association between whole muscle relaxation rate and motoneuron firing rate, most relaxation rate data to date have been collected from twitch responses to electrical stimulation of a peripheral nerve or intramuscular nerve fibres (muscle belly) during relaxation. While resting twitch data are valuable, the technique has limited application in defining the functionally-relevant capacity of muscle relaxation when compared to data obtained from a contracting muscle actively being driven by the central nervous system (CNS).

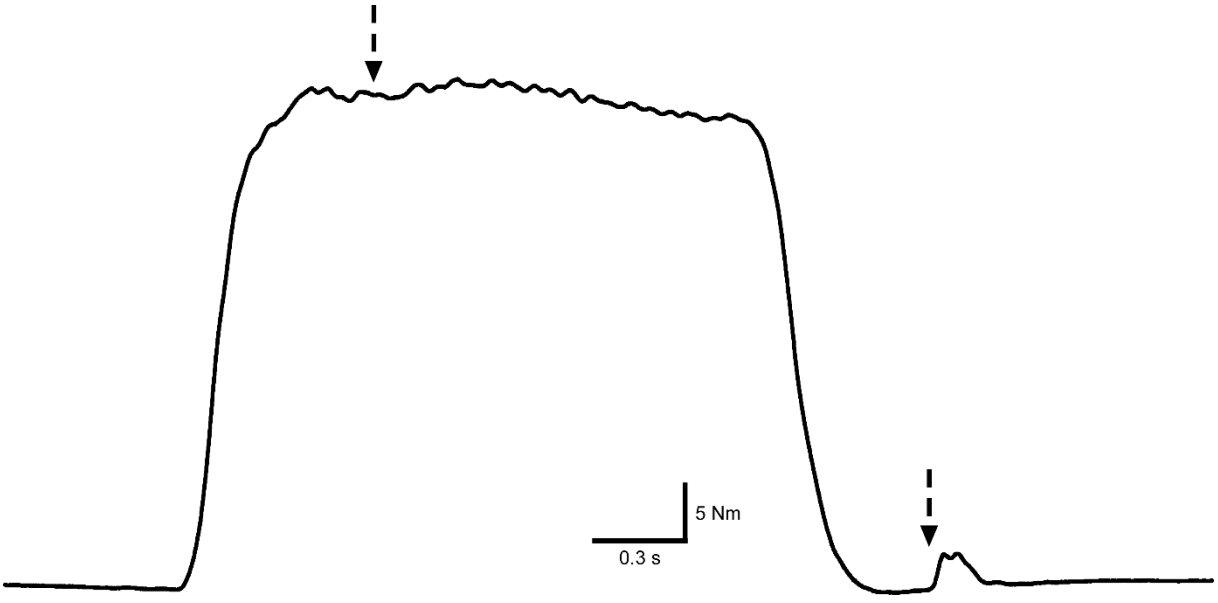

Figure 2: Raw torque data from an elbow flexion MVC. Black arrows indicate delivery of an electrical stimulus (doublet) to the muscle belly of biceps brachii.

As illustrated by Figure 2, an electrical stimulus delivered to intramuscular nerve fibres during a MVC typically does not produce any significant muscle relaxation. However, it should be noted that with peripheral nerve stimulation during a MVC (e.g. to the tibial nerve for activation of the plantar flexors) the subject may exhibit some degree of relaxation (Taylor, 2009). This is caused partially by collisions of antidromic and orthodromic potentials in the motor axons (Inghilleri et al. 1993). Additionally, antidromic potentials briefly impact motoneuron discharge rates by the activation of recurrent inhibition, leading to the generation of inhibitory postsynaptic potentials at the motoneuron level, producing a short EMG silent period (Merton, 1951; Brock et al. 1952; Inghilleri et al. 1993; Herbert & Gandevia, 1999). However, this evoked silent period is not long enough to allow for complete muscle fibre relaxation; therefore, a twitch elicited at rest is required to obtain contractile information when employing electrical stimulation techniques.

Considering this limitation, a growing number of studies have successfully employed TMS to investigate muscle relaxation during a MVC (see Figure 3) by using the silent period to their advantage (e.g. Todd et al. 2007; Butler et al. 2012; McNeil et al. 2013; Molenaar et al. 2013). Results have demonstrated unique physiological differences that were previously masked by less functionally-relevant tests.

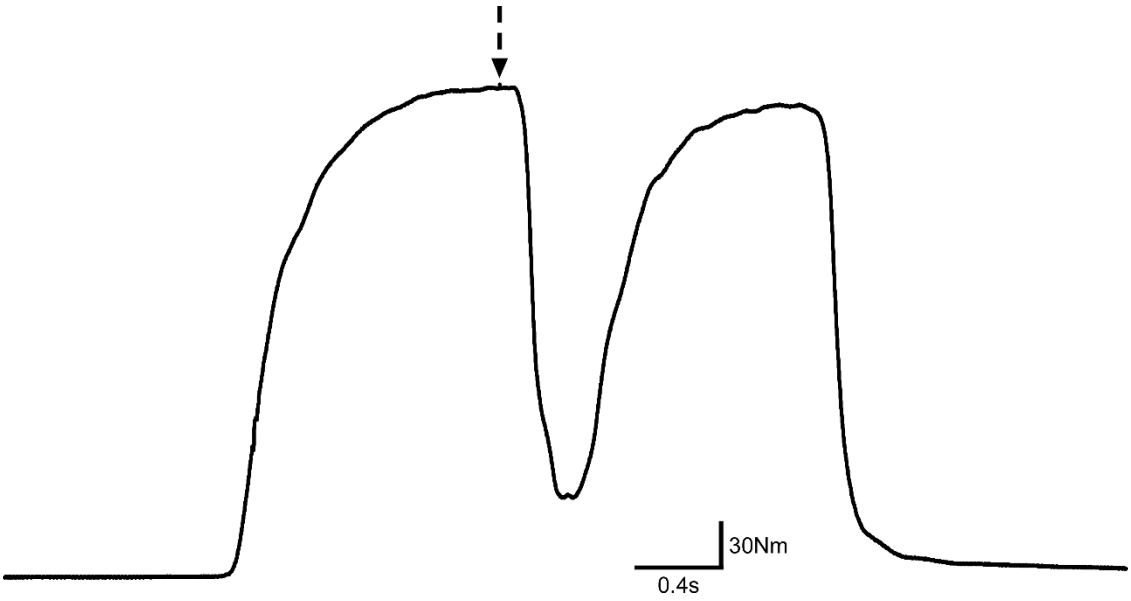

Figure 3: Raw trace of plantar flexor torque during a MVC at 20° dorsiflexion, clearly showcasing TMS-induced relaxation (arrow denotes the timing of TMS delivery). Stimulation intensity set to 80% on a BiStim^2 unit; peak torque measured as 199.4Nm.

For example, a significant age-related slowing of elbow flexor relaxation rate was found with TMS (Hunter et al. 2008; Molenaar et al. 2013) that was not present during the electrically-induced twitch response in relaxed muscle (Doherty et al. 1993; Allman & Rice, 2001; Dalton et al. 2010). This clearly indicates the variable function of muscle contractile properties during different states of descending drive.

The ability to measure TMS-induced maximal relaxation rates has also revolutionized fatigue studies; these data can now be collected during a continuous contraction (Hunter et al. 2006, 2008; Todd et al. 2005, 2007). Previous studies using electrical stimulation were unable to test contractile properties during a fatiguing protocol without requiring an interruption of the ongoing contraction. This makes the usage of TMS attractive to researchers interested in the assessment of fatigue without contamination of the data by the partial recovery which occurs when the task is stopped.

1.2.3 The Role of Calcium in Excitation-Contraction Coupling

While there are many contributing factors to muscle relaxation, such as large-scale whole muscle series elastic component interactions (e.g. variability in the compliance of muscle and tendon) and architectural properties (e.g. pennate versus fusiform muscle fascicles), there are also small-scale ion movements that must be briefly considered.

A biochemical cascade of events follows the propagation of an action potential into the transverse-tubules (a system of sarcolemmal invaginations into muscle fibres). This cascade leads to sarcoplasmic reticulum calcium ion (Ca^{2+}) discharge. Ca^{2+} subsequently drives the contractile apparatus, ultimately resulting in the production of muscle force (Hasselbach, 1964). This conversion of an electrical action potential to mechanical force is referred to as excitation-contraction coupling (first described by Alexander Sandow in 1952).

In addition to having a critical role in the induction of muscle contraction, Ca^{2+} also plays a key part in the process of post-contraction relaxation (which is the pivotal intrinsic contractile property investigated in this thesis) (Brody, 1976). Large-scale muscle relaxation can be broken down into two general intracellular steps (Westerblad & Allen, 2003; Allen et al. 2008): (1) efficient reuptake of Ca^{2+}, which is dependent on internal Ca^{2+} concentrations (i.e. within the myofibril); and (2) Ca^{2+} dissociation from troponin (a tri-protein complex associated with actin filaments) resulting in cross-bridge detachment. Muscle relaxation has been shown to be strongly correlated to the rate of Ca^{2+} reuptake and, subsequently, with the concentration of Ca^{2+}-transport ATPase (Endo, 1977; Dawson et al. 1980; Gollinick et al. 1991).

Based on excitation-contraction coupling research, it is apparent that the intracellular environment of skeletal muscle undergoes rapid changes over the course of a voluntary

contraction. Therefore, it is certain that the delivery of electrical stimulation to a relaxed muscle will test the intrinsic contractile properties of muscle fibres in an intracellular state which is markedly different to that observed during contraction. This further emphasizes the benefit of using TMS to investigate contractile properties of an actively contracting muscle.

1.3 Length-Tension Relationship

In addition to recognizing the important role of intrinsic contractile properties in force production, it is also crucial to understand and appreciate the length-tension relationship of the muscle of interest. This relationship plays a critical role that ultimately influences the neuromuscular system at a number of levels (e.g. at the level of the sarcomere, muscle fascicle, and series elastic component), given that the selection of an optimal muscle length will result in the production of maximal muscle force (Gordon et al. 1966; Kaufman et al. 1989). Sarcomere length and the interaction of the intrinsic contractile proteins, actin and myosin, are key determining factors in force production. Their corresponding roles within an actively contracting muscle have been modeled in two general theories: the sliding filament theory and the cross-bridge theory.

Hugh Huxley founded the sliding filament theory in 1953, following examination of striated frog muscle under an electron microscope. Comparing the data to X-ray diffraction studies, Huxley stated that,

> "…if it is postulated that stretching of the muscle takes place, not by an extension of the filaments, but by a process in which the two sets of filaments slide past each other; extensibility will then be inhibited if the myosin and actin are linked together." (Huxley, 1953)

Huxley brought about the novel proposition that filaments within the muscle are not single units that passively extend, but are rather composed of separate units that slide past each other when stretched. In this manner, force production is then possible by active resistance to stretch, resulting from the ATP-driven interaction of actin and myosin with one another.

Huxley's research in this area later led to the development of the cross-bridge theory of muscle contraction, where he amended his theory to propose that the sliding action of actin and myosin was caused by the breaking and re-forming of independent cross-bridges (rather than direct association of the molecules with one another) (Huxley, 1969). Both theories provided important information regarding muscle length-tension relationships and helped to explain the inverted parabolic appearance of an active sarcomere force-length curve (Figure 4). This curve is shaped by the interaction between actin and myosin during cross-bridge formation, where the overlap between the two proteinaceous filaments resulting in the maximal number of cross-bridges typically produces the greatest maximal force.

1.3.1 Early Force-Length Studies

Early force-length studies pointed to a model where active muscle force decreased as muscle length shortened, with different muscles displaying a variation of the active force-length curve, according to their unique passive extensibility, muscle architecture, and fibre and connective tissue properties (Gans, 1982). These experiments were initially tested in a frog model (Blix, 1893, 1894) and later confirmed, for the first time in humans, with upper-limb amputees (Ralston et al. 1947).

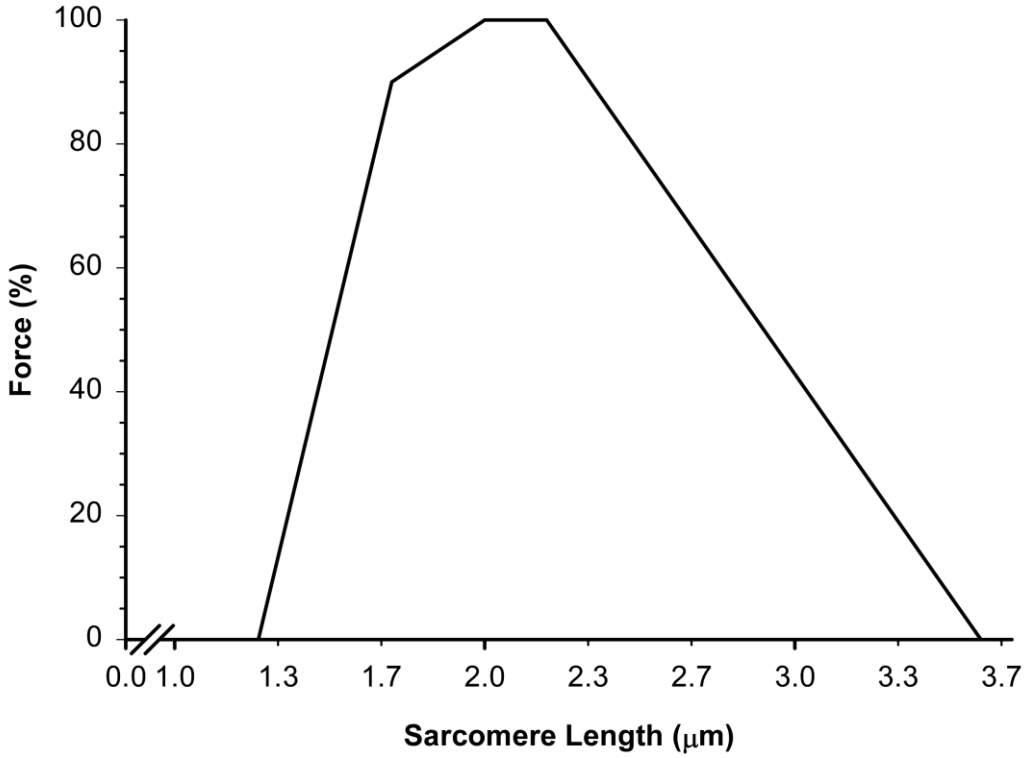

Figure 4: Active length-tension relationship of frog skeletal muscle sarcomere (adapted from Gordon et al. 1996)

There is a steady increase in tension as sarcomeres approach their optimal length, as given by optimal overlap of one myosin strand and one actin strand. From this point, as sarcomere length increases, there is a transition to a period of tension loss as actin strands are forced out of their ideal alignment with myosin (Gordon et al. 1966).

While the general trend of Gordon et al.'s 1966 sarcomere length model (Figure 4) held for application to human skeletal muscle, there were a number of important functional parameters that influenced optimal muscle length-tension relationships. Unlike isolated fibre experiments, *in vivo* length-tension measurements of muscle fascicles (and therefore their associated fibres also) are restricted by anatomical location, muscle architecture (e.g. fascicle

pennation), and in-series components (e.g. associated tendons, aponeuroses, ligaments) (Gans, 1982; Narici et al. 1996; Kawakami et al. 1998, Maganaris et al. 1998; Martin et al. 2001).

1.3.2 Triceps Surae: Human Medial Gastrocnemius

Taking into consideration the function of the length-tension relationship in the optimal generation of force, as well as the key role relaxation holds in the overall functional efficiency of skeletal muscle, the triceps surae (TS) muscle group was selected to be the investigational target in this thesis. Furthermore, the medial gastrocnemius (MG) was selected for ultrasound imaging (see Section 1.4).

The TS is composed of the soleus (originating from the posterior aspect of the fibula and posterior surface of the tibia; inserting onto the Achilles tendon (AT), which continues to insert onto the posterosuperior aspect of the calcaneus) and biarticulate gastrocnemius (originating from the lateral and medial aspects of the femoral condyles; inserting onto the AT). Plantar flexors contribute significantly to bipedal tasks (i.e. walking, running, climbing stairs) (Neptune et al. 2001) and a loss of plantar flexor strength has been associated with increased risk of falling in the elderly (Wolfson et al. 1995). Given the critical role of the soleus and gastrocnemii muscles in human movement, expanding our current knowledge of their intrinsic mechanisms of function will have future implications, particularly in terms of understanding neuromuscular pathologies (see Section 6.1).

1.4 Ultrasound Imaging Technology – Muscle Fascicles

The concept of using sound waves (i.e. ultrasound) as a navigational aid was first hypothesized by the Italian priest and physiologist Lazzaro Spallanzani in 1794, after observing bats in various stages of sensory deprivation (Dijkgraaf, 1960). It wasn't until

1915 that Spallanzani's theory was put to practical use by physicist Paul Langevin to detect icebergs and submarines during World War I. The technique was first used as a medical diagnostic tool in 1942 by neurologist Karl Dussik (Dussik, 1942, 1952), and later adapted for the visualization of biological tissue by John Wild and colleagues in the 1950s (Wild & Neal, 1951).

The first reported use of ultrasound for imaging musculoskeletal structures was published by Karl Dussik; his paper provided the earliest description of muscle fibre anisotropy (i.e. echogenicity dependence on transducer angle), providing the framework for sonomyographic navigation (Dussik, 1958). Since then, ultrasound imaging technology has grown in leaps and bounds, providing clinicians and researchers alike with remarkable and unparalleled access to human skeletal muscle (Kaproth-Joslin et al. 2015).

It wasn't until the mid-1990s that ultrasound technology became prevalent for measuring architectural parameters of skeletal muscle – until that point most data on fascicle length, pennation angle, and muscle volume were obtained from cadavers (Narici, 1999). *In vivo* measurements of architectural parameters made it possible to define the functional boundaries of movement, giving insight into a variety of mechanisms, such as those behind force production and length-tension relationships.

Advancements in the sampling rate and resolution of image acquisition have made it possible to capture *in vivo* architectural changes that occur during electrical stimulation (Nordez et al. 2009) or voluntary isometric contractions (Fukunaga et al. 1997; Ito et al. 1998; Arampatzis et al. 2007; Herbert et al. 2011).

1.5 Combining TMS & Ultrasound Technology

The combination of whole muscle response data (e.g. torque traces) with individual muscle fascicle architectural changes (e.g. changes in fascicle length and pennation angle), as given by ultrasound imaging, allows one to ask the question, "How do whole muscle reactions compare to changes at the fascicle level?" While a small number of studies have paired electrical stimulation and ultrasound technology to investigate contractile properties (Nordez et al. 2009; Maganaris & Paul, 2000), none have paired TMS with ultrasound; therefore the combination of these elements in this thesis is novel.

The lack of *in vivo* muscle fascicle imaging (during stimulation) in the literature, as mentioned above (Section 1.4), is likely due to inadequate sampling rates, low frequencies, and limited temporal resolution of traditional imaging devices (Nordez et al. 2009). However, with technological improvements, it has become feasible to combine stimulation and ultrasound imaging.

Chapter 2 Purposes & Hypotheses

2.1 Purposes

The primary objective of this study was to test the sensitivity of the TMS technique to a change in muscle length by comparing the relaxation rate of the plantar flexor muscles among short (30° plantar flexion), medium (neutral ankle position, 90° between foot and shin) and long lengths (20° dorsiflexion).

This study incorporated ultrasound technology to record length changes in muscle fascicle architecture and tendon excursion. The secondary objective was to determine if the absolute relaxation rates observed at the level of all contributing plantar flexors were reflected at a muscle fascicle and associated muscle-tendon junction (MTJ) level, for the medial gastrocnemius (MG).

2.2 Hypotheses

It was hypothesized that the TMS-induced relaxation rate technique would be sufficiently sensitive to detect changes in whole muscle length (i.e., rates would be slower as muscle length was shortened) and that the relaxation rates of the MG fascicles and associated MTJ would vary with joint angle, thereby following the same general pattern seen with whole muscle torque data.

Chapter 3 Materials & Methods

3.1 Participants

A total of 13 healthy males volunteered (see Table 1 for participant characteristics). Two participants were fully excluded from the study due to suboptimal voluntary activation (Section 3.2.2). Three subjects had a portion of their data excluded based on failure to meet criteria (Section 3.2.3). All volunteers gave written informed consent (see Appendix A) prior to testing and all experimental designs were approved by the University of British Columbia's Clinical Research Ethics Board.

Table 1: Participant characteristics (n = 11); yrs = years, cm = centimetres, kg = kilograms. Data given as mean ± SD.

Age (yrs)	Height (cm)	Weight (kg)	Tibial Length (cm)
24.8 ± 7.0	179.2 ± 9.4	83.6 ± 14.1	41.6 ± 3.3

3.2 Exclusion Criteria

3.2.1 TMS & Injury Screening

All volunteers were required to complete a TMS screening form prior to participating (please see Appendix B for a copy of this form). Volunteers with recent neuromuscular injuries to the right leg or damage to the right hip/knee/ankle were also excluded from the study.

3.2.2 Voluntary Activation Requirements

Participants were required to achieve a voluntary activation (VA) estimate equal to or greater than 90% during maximal voluntary contractions (MVCs) of the plantar flexor muscle group. VA was estimated using the method reported by Taylor and colleagues (2000), and is given as follows:

$$\text{Voluntary Activation (\%)} = \left(1 - \frac{\text{SIT Torque (Nm)}}{\text{Prestimulus Torque (Nm)}}\right) \text{x } 100\%$$

The superimposed twitch (SIT) was defined as any additional force induced by the delivery of TMS (see Figure 5).

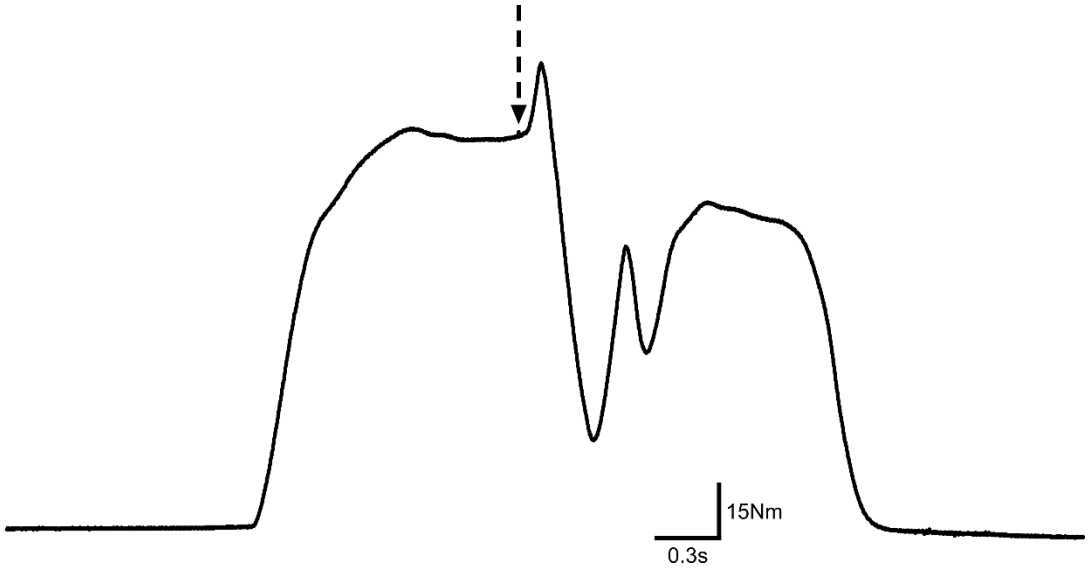

Figure 5: Example of suboptimal VA in an excluded subject. Raw trace of plantar flexor torque during a MVC at 20° dorsiflexion, showing a large superimposed twitch following delivery of TMS (black arrow). Peak pre-stimulus torque was 103.6 Nm, SIT torque was 18.8 Nm, and VA was estimated to be 84.1%.

Two participants from the original thirteen were excluded from the study based on poor VA estimates (<90%) over the course of the protocol. Low VA estimates indicate non-optimal neural drive from the motor cortex to the target muscle, which is due to inadequate recruitment and rate coding of motor units (typically caused by insufficient participant motivation) (Taylor et al. 2000; Todd et al. 2003). As a result, MVC torque output presents as submaximal.

Accurate acquisition of peak whole muscle and *in vivo* muscle fascicle and MTJ relaxation rates using TMS is dependent on muscle fibres contracting maximally (or near-maximally) at the time of the stimulus. To ensure performance and neural drive were near-maximal, VA estimates less than 90% were deemed unacceptable for the present study.

3.2.3 Other Exclusion Cases

There were two additional cases of exclusion regarding specific data sets. In the first case, ultrasound measurement data were excluded (but torque outputs were retained) for one participant, due to the presence of exceptional fascicle curvature. In the second case, MTJ ultrasound measurements were excluded for two participants due excessive bulging of MG during MVC which interfered with probe placement and image acquisition of the MTJ.

3.3 Set-Up

The following variables were determined prior to the experimental protocol: tibial length, passive ultrasound images (Achilles tendon (AT) length, whole muscle length, muscle-tendon junction (MTJ) excursion from neutral, and muscle fascicle length), maximum voluntary contraction (MVC) torque, and TMS coil placement.

Participants lay prone on a HUMAC NORM Extremity System (CSMi, Stoughton, MA) with their right knee extended and their right ankle firmly secured to the dynamometer footplate (ankle axis of rotation was aligned with that of the dynamometer) (Figure 6). Plantar flexor torque was measured and electrical activity (EMG) of the lateral gastrocnemius (LG) (see Figure 7) was recorded via adhesive Ag-AgCl electrodes (10mm diameter) arranged in a bipolar fashion (recording and reference electrodes positioned over the muscle belly, ~2cm from one another; ground electrode was positioned over the patella and secured with a tensor bandage). Visual feedback was provided on a 20 inch monitor.

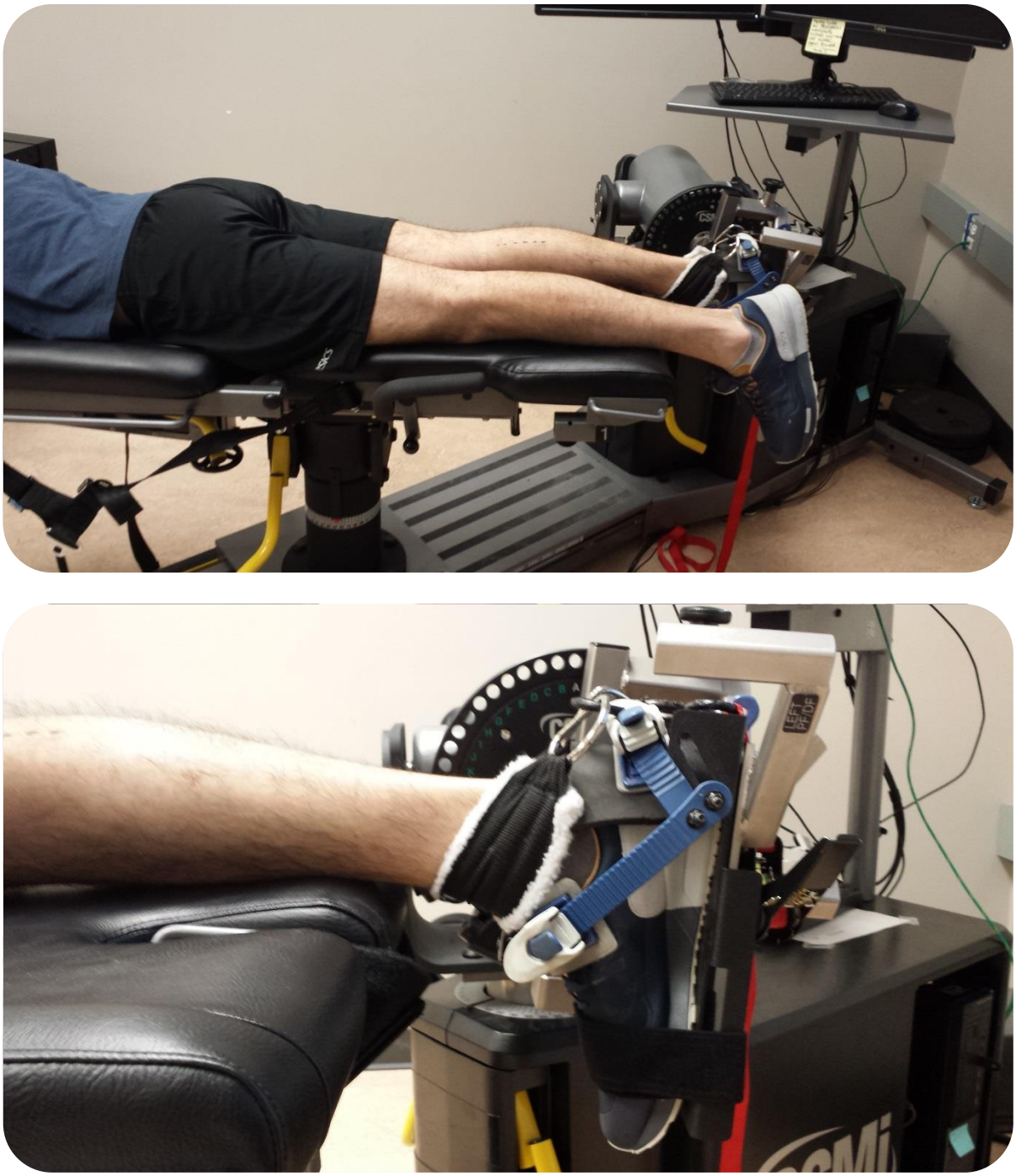

Figure 6: HUMAC NORM participant set-up. (Top) Whole body view. (Bottom) Close-up of ankle set-up; four straps were used to secure the heel to the force plate, preventing excursion or change in ankle angle.

A total of three ankle angles were tested for all components of this experiment: 20° dorsiflexion (DF), a neutral ankle position (0°), and 30° plantar flexion (PF); all angles were pre-programmed into the HUMAC NORM's inherent software as absolute values. The neutral position was pre-programmed to an ankle angle of approximately 90° between the plantar aspect of the foot and the tibia (based on prone participant's natural ankle angle at rest).

3.3.1 Torque and EMG

Torque and EMG signals were sampled online at 1,000 and 2,000 Hz, respectively, using a 16-bit A/D converter (CED Power 1401-3; Cambridge Electronics Design, Cambridge, UK) in combination with Spike2 software (v. 7.10; Cambridge Electronic Design). EMG data were amplified (100×) and bandpass filtered (16–1,000 Hz) using CED 1902 amplifiers (Cambridge Electronic Design).

3.3.2 Ultrasound Imaging: Passive

Images of the medial gastrocnemius (MG) were recorded in real-time B-mode ultrasonography (GE LOGIQ E9, Connecticut, USA) using a ML6-15-D transducer (4.5-15.0 MHz linear array, 13 x 58 mm footprint, 50 mm field of view, and 8 cm depth of field LOGIQview, GE©). Aquasonic clear ultrasound gel was applied to improve acoustic contact between the probe and skin (Parker Laboratories, Inc., New Jersey, USA).

All passive images were repeated 3 times in a pseudo-randomized order at each ankle angle. Standard still images of the MTJ and muscle fascicles were captured, and LOGIQ scanning technology was used to obtain whole AT length and whole MG length. Probe depth was adjusted as necessary to optimize the images.

With the participant relaxed, the location of the MTJ was identified as the point at which the MG and AT merged; probe position was marked on the skin with indelible marker to ensure consistent placement. A long, thin strip of aluminum foil was taped over the skin to act as an anechoic marker. This provided a constant landmark, allowing for measurements to be made across variable ankle angles (see Section 3.5.2). Muscle fascicle images were taken over the center of the MG muscle belly. Probe position was indicated with indelible marker and the probe was adjusted to optimize muscle fascicle clarity.

AT length was scanned from the insertion onto the calcaneus to the MTJ (Figure 9). Whole MG length was scanned from the MTJ to the popliteal crease and normalized to tibial length (Figure 9).

3.3.3 Ultrasound Imaging: Active Contractions

Video recordings of the MG muscle fascicles (for set-up, see Figure 7) and MTJ were taken in real-time B-mode ultrasonography (GE LOGIQ E9, Connecticut, USA) using a ML6-15-D transducer (15.0 MHz linear array, 13 x 58 mm footprint, 50 mm field of view, 77 frames-per-second and 2.8 cm depth of field LOGIQview, GE©). Aquasonic clear ultrasound gel was applied to improve acoustic contact between the probe and skin (Parker Laboratories, Inc., New Jersey, USA). Recordings started immediately prior to each MVC and were ended as soon as the task had been completed. A custom-made foam brace with Velcro straps was used to secure the probe in place during contractions.

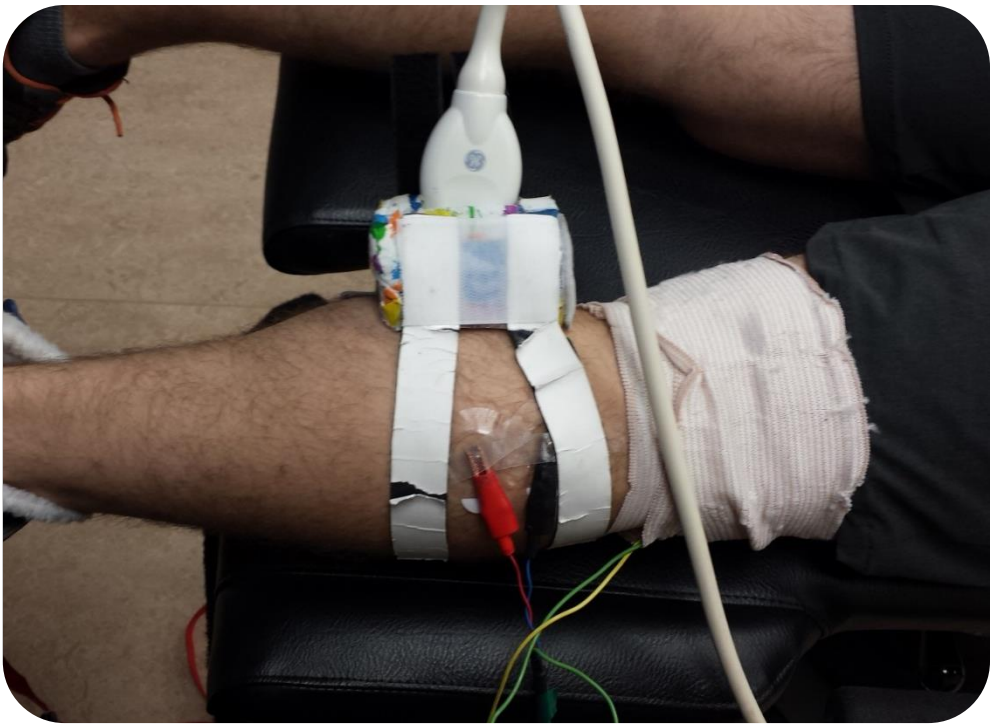

Figure 7: Ultrasound probe set-up for MVC contractions. Electrodes positioned over the lateral gastrocnemius in a bipolar arrangement, with the ground over the patella. A handmade probe-holder was used to secure the ultrasound probe in place over the medial gastrocnemius.

3.3.4 Transcranial Magnetic Stimulation

TMS was delivered using a double cone coil (13cm outside diameter for each cone) attached via a BiStim^2 unit to two Magstim 200^2 stimulators (Magstim, Whitland, UK). The direction of current flow in the coil preferentially activated the motor cortex of the left hemisphere, which innervates the right limb. With the participant performing a 10% submaximal plantar flexion contraction, optimal coil placement was determined by delivering single stimuli (at 40% of stimulator output) every 5-10s as coil position was systematically adjusted.

Beginning at the vertex (middle of the head), the coil was moved first laterally and then in a ventral-dorsal direction as the size of the lateral gastrocnemius motor evoked potentials

(MEPs) was monitored. The location yielding the largest MEP was noted with a dry erase marker for use throughout the remainder of the experiment.

Participants were gradually introduced to high-intensity TMS with the following isometric contraction-TMS intensity pairings: 10% MVC at 40% TMS, 10% MVC at 80% TMS, 50 % MVC at 80% TMS, and 100% MVC at 80% TMS. Stimulus intensity was then fixed at 80% of stimulator output to ensure a maximal relaxation rate (McNeil et al. 2013).

3.4 Experimental Procedures

Data collection began with the determination of tibial length, passive ultrasound images/measurements, and optimal coil position (see Section 3.3.4). Participants then executed 2-3 MVCs with TMS at 80% of the stimulator output to ensure optimal task performance. Strong verbal encouragement and visual feedback were given during all MVCs; participants were instructed to plantar flex "hard and fast" following the click of the TMS. At least 90s of rest separated each contraction.

The experimental protocol (Figure 8) involved 21 brief (~3s) isometric plantar flexor MVCs in a prone position at full knee extension, pseudo-randomized among 20° dorsiflexion (DF), a neutral ankle position, and 30° plantar flexion (PF); i.e., each angle was performed once for every 3 MVCs. During each MVC, high-intensity TMS (80% stimulator output) was delivered to the motor cortex with a double-cone coil attached to two magnets via a BiStim unit. In addition, ultrasound video recordings (see Section 3.3.3 for details) were captured over the MG muscle belly (for 12 consecutive MVCs) and MTJ (for 9 consecutive MVCs), with one recording per MVC. Participants were given a break of ~5 minutes between MG muscle belly and MTJ imaging (or vice versa, as dictated by pre-protocol randomization) to allow for probe placement changes to be made.

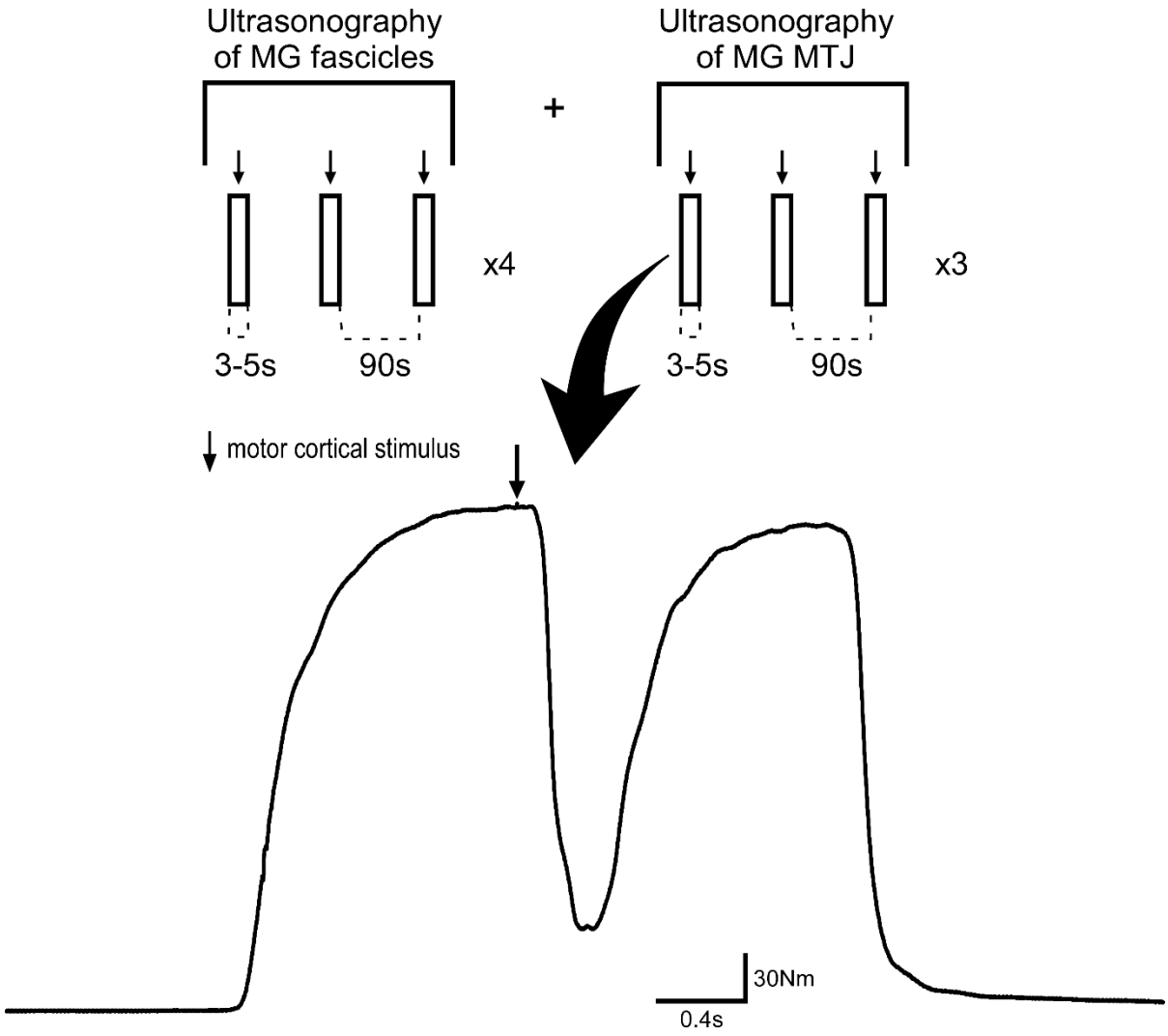

Figure 8: Protocol schematic. (Top) Each rectangle indicates a single 3-5s MVC with TMS (21 in total). MVCs were separated by ≥90s rest. Ankle position was pseudo-randomized (each angle was performed once for every 3 MVCs). One ultrasound video recording of was taken per MVC, with 12 consecutive recordings taken of MG fascicles and 9 consecutive recordings of MG MTJ. (Bottom) Raw trace of plantar flexor torque during a MVC at 20° dorsiflexion, clearly showcasing TMS-induced relaxation (arrow denotes the timing of TMS delivery). Stimulation intensity set to 80% on a BiStim^2 unit; peak torque measured as 199.4Nm.

3.5 Data Analysis

3.5.1 Torque & EMG

Signal software (v. 5.08; Cambridge Electronic Design) was used offline to analyse torque and EMG. Mean torque and voluntary EMG were calculated over 100 ms (in the interval 250 to 150 ms prior to the stimulus). MVC torque was calculated as the peak value of the brief contractions. All torque traces for whole muscle peak relaxation rate calculations were additionally modified with a 3-point smooth feature, inherent to Signal. Whole muscle peak relaxation rate was measured as the steepest negative torque slope over a 10ms interval (5ms on each side of the steepest instantaneous slope) following the delivery of TMS (Molenaar et al. 2013; McNeil et al. 2013). Normalized whole muscle relaxation rate was calculated by dividing the peak whole muscle relaxation rate by the pre-stimulus torque (as measured 5ms before TMS delivery).

3.5.2 Ultrasound Images

Ultrasound images were analysed offline using the GE LOGIQ E9 software inherent to the ultrasound. AT length was measured using an open spline trace from the MTJ to the insertion point on the calcaneus. Whole MG length was measured from the MTJ to the proximal edge of the scan (deliberately stopped at the popliteal crease to allow for consistency among subjects) using a simple straight-line function. See Figure 9 for examples of whole MG and AT scans.

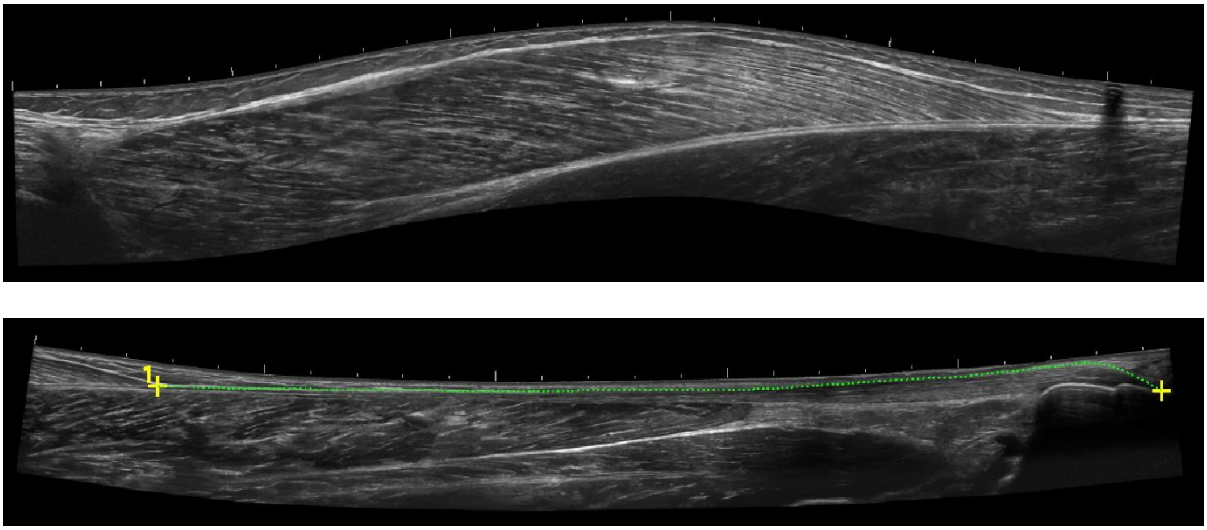

Figure 9: Images taken using an ML6-15-D probe. (Top) LOGIQview scan of the medial gastrocnemius at rest from the AT (right) to the popliteal crease (left); ankle angle set to 20° dorsiflexion and whole muscle length measured 22.0 cm. (Bottom) LOGIQview scan of the Achilles tendon at rest from the MTJ (left marker) to the insertion point at the calcaneus (right marker). The traced line represents an open spline trace of tendon length; ankle angle set to 20° dorsiflexion and tendon length measured 21.9 cm.

MTJ displacement from a neutral ankle angle (for passive changes in ankle angle) and from during-contraction to TMS-induced relaxation (Figure 10) was taken using a straight-line function from the MTJ to the shadow of a static, anechoic marker. Given that shadow edge clarity varied during contractions, a 90° angle marker was drawn parallel from the start of the shadow at the surface of the image to a point below the AT. This ensured less variability of measurements taken within a contraction. The change in MTJ length was averaged for each ankle angle.

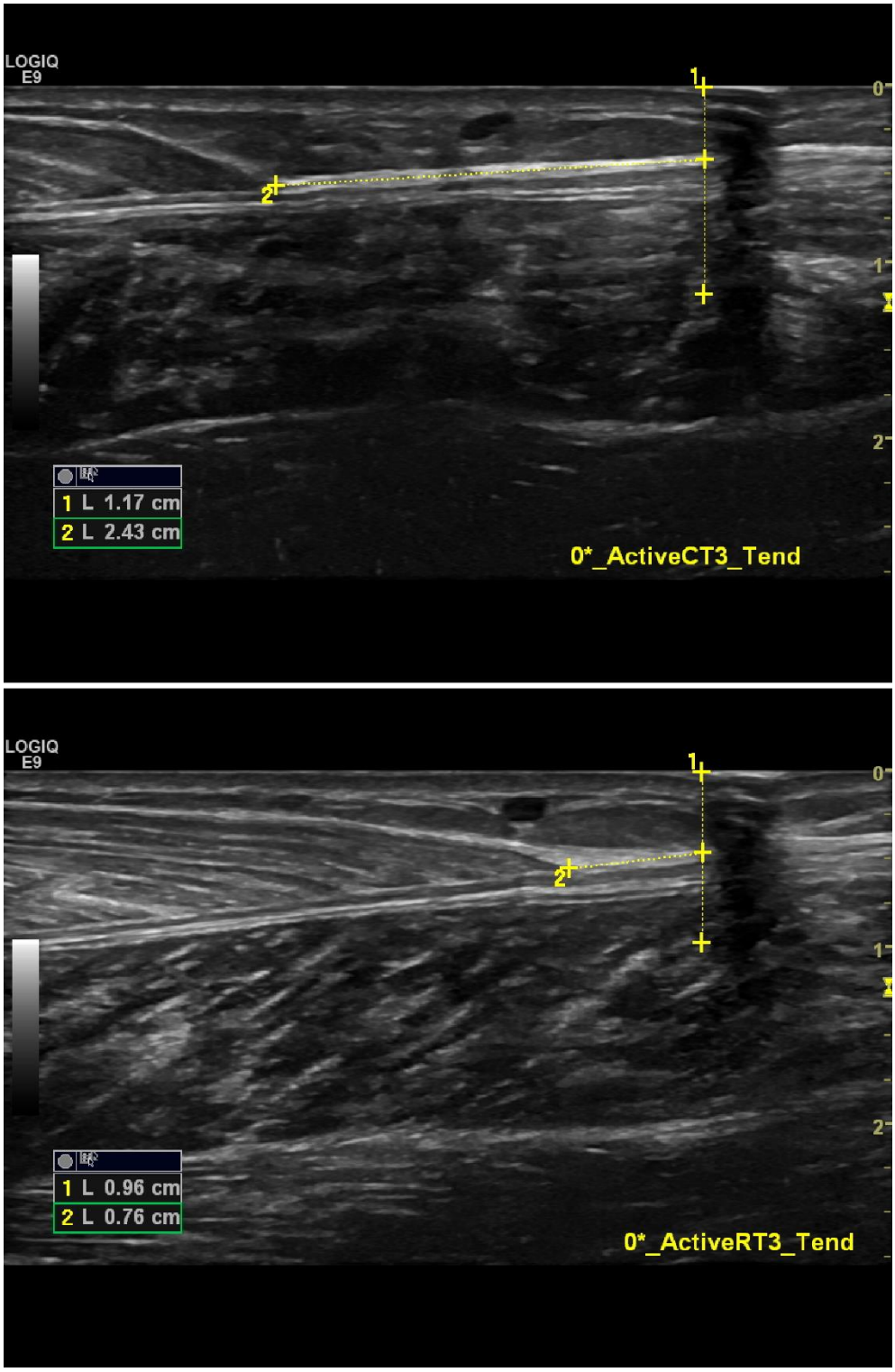

Figure 10: Still images represent captures from a video recording acquired with an ML6-15-D probe. MTJ length change (1.67cm in 0.27s) for a single participant during TMS-induced relaxation at a neutral (0°) ankle angle, from MVC (top) to point of maximal TMS-induced relaxation (bottom).

Muscle fascicle length was measured from the lower to the upper aponeurosis with an open spline trace, enabling adjustments for non-linearity. The Law of Cosines was used to extrapolate muscle fascicle length if the top portion of the fascicle (joining the upper aponeurosis) shifted out of the probe's field-of-view. An inherent angle function was used to measure pennation angle at the point where the muscle fascicle joined the lower aponeurosis.

Relaxation rate was calculated for a given contraction by taking the MG fascicle length change and dividing by the time elapsed during TMS-induced relaxation. Length change was calculated by measuring the length of one or two fascicles immediately prior to TMS and another one or two fascicles (not necessarily the same ones; see next paragraph) at the maximal length after TMS (Figure 11). Measures of the TMS-induced state were taken from the frame prior to the muscle shortening which occurred when the silent period ended and tension was actively produced. At each angle, the difference of the average pre-TMS and TMS-induced relaxation lengths gave an overall average fascicular length change. For each contraction, ultrasound image frame number was noted at both points of length measurement; these values were subtracted from one another and divided by 77 (which was the ultrasound image-acquisition frame rate). Times were then averaged for each ankle angle to obtain a mean value of time elapsed for TMS-induced fascicle relaxation to occur.

Ultrasound image data were averaged as described above because as a single specific fascicle could not be visually tracked over TMS-induced relaxation (80% TMS during a MVC gave a physically intense period of muscle relaxation; this resulted in a small degree of ultrasound probe movement, despite measures taken to secure placement). I compensated for being unable to track specific fascicles by having participants perform multiple (3-4) contractions at each ankle angle and averaging values within a participant.

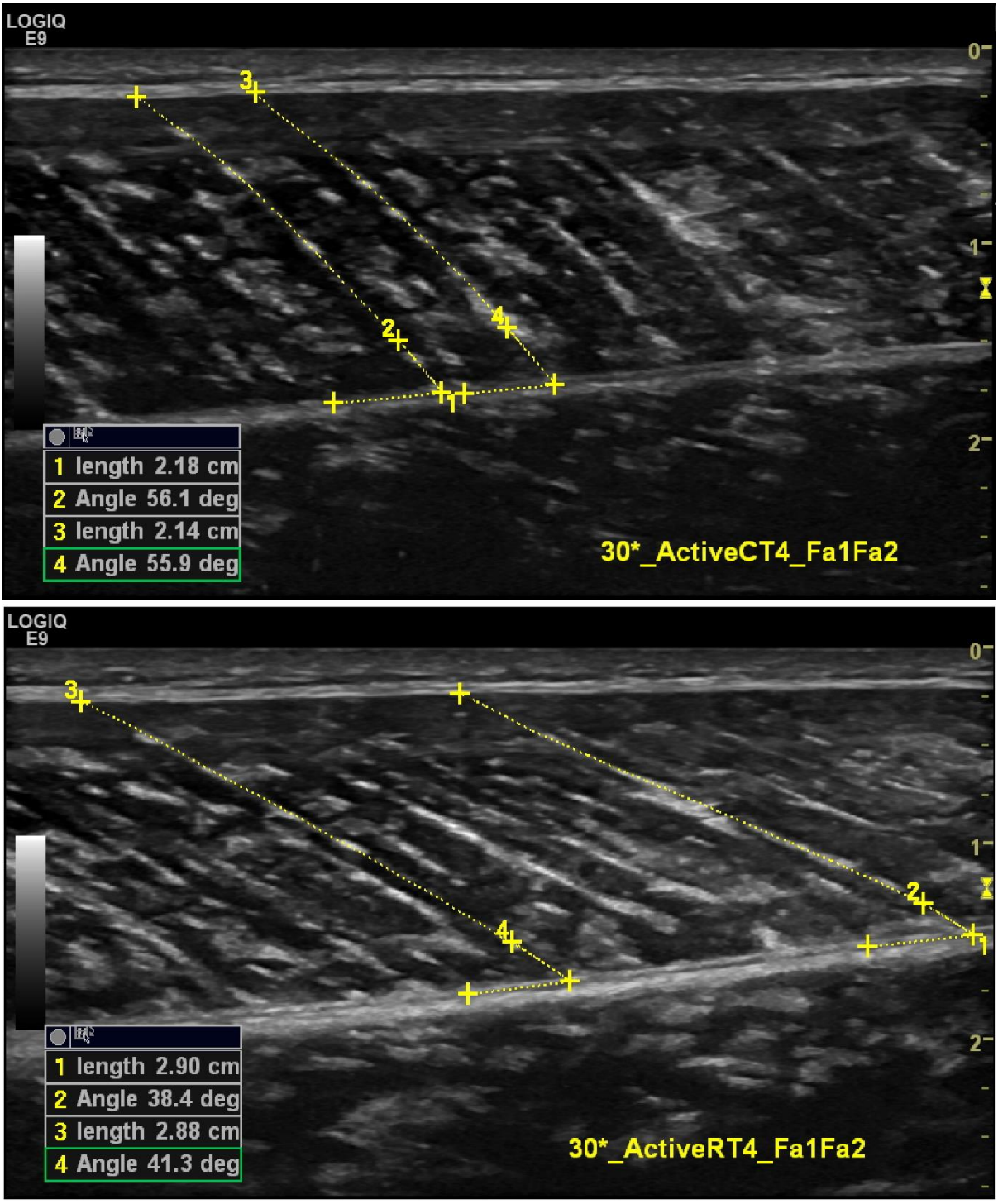

Figure 11: Still images represent captures from a video recording acquired with an ML6-15-D probe. MG muscle fascicle length change and pennation angle change for a single participant during TMS-induced relaxation (duration of 0.30s) at a plantar flexed (30°) ankle angle, from MVC (top) to point of maximal TMS-induced relaxation (bottom).

3.6 Statistical Analysis

Using SPSS (version 22.0, SPSS Inc., Chicago, IL), separate one-way repeated measures (rm) ANOVAs were run to test the effect of ankle angle (DF, neutral, and PF) for MVC torque, absolute plantar flexor relaxation rate, and normalized plantar flexor relaxation rate. A paired-samples t-test of mean MVC torque for the first 6 contractions compared to the last 6 contractions was run to determine whether fatigue developed over the course of the protocol.

To determine the intra-rater reliability for ultrasound measurements, data from two participants were analyzed on two occasions and tested with an intraclass correlation coefficient (ICC). Separate one-way rmANOVAs were run to test the effect of ankle angle for MG and AT length at rest. The impacts of ankle angle and muscle state (rest, during MVC, and TMS-induced relaxation) were assessed with separate two-way rmANOVAs for MG fascicle length and pennation angle. Due to the presence of a significant ankle angle × muscle state interaction for both fascicle length and pennation angle, one-way rmANOVAs were run separately for ankle angle and muscle state. Finally, the effect of ankle angle of MG fascicle and MTJ relaxation rates was tested with separate one-way rmANOVAs.

All one-way rmANOVAs with a main effect of ankle angle or muscle state, were followed by post hoc testing, for which a Bonferroni correction factor was applied to multiple comparisons. Significance for all data was defined as $p < 0.05$. Data are presented as the mean value ± standard deviation (SD) for tables and mean ± standard error of the mean (SEM) for figures.

Chapter 4 Results

TMS-induced absolute relaxation rates for whole muscle and MG muscle fascicles are significantly slower for shortened muscle lengths (PF) compared to longer muscle lengths (DF and neutral) ($p < 0.05$; see text for more details). The TMS-induced relaxation rate for MTJ displacement follows the same trend, despite not achieving statistical significance ($p = 0.06$).

4.1 Whole Muscle Measurements

4.1.1 Maximal Voluntary Torque

A one-way rmANOVA gave a significant main effect of ankle angle for MVC torque; post-hoc testing showed MVC torque was significantly greater for DF (182.07 ± 40.71 Nm) compared to neutral (147.70 ± 18.88 Nm; $p = 0.001$) and PF (62.16 ± 9.55 Nm; $p < 0.001$) and for neutral compared to PF ($p < 0.001$). A paired-samples t-test of mean MVC torque showed no significant difference ($p = 0.101$) between the first 6 contractions (127.97 ± 26.83 Nm) compared to the last 6 contractions (121.31 ± 23.96 Nm), indicating no development of fatigue during the course of the protocol.

4.1.2 Absolute Relaxation Rate

A one-way rmANOVA gave a significant main effect of ankle angle for absolute whole muscle relaxation rate; post-hoc testing showed whole muscle absolute relaxation rates were not significantly different between DF and neutral positions ($p = 1.000$; -2007.62 ± 692.11 Nm/s and -1895.90 ± 298.31 Nm/s, respectively); however, both DF and neutral positions had significantly faster peak relaxation rates compared to PF (-804.02 ± 161.91 Nm/s; $p < 0.001$) (see Figure 12).

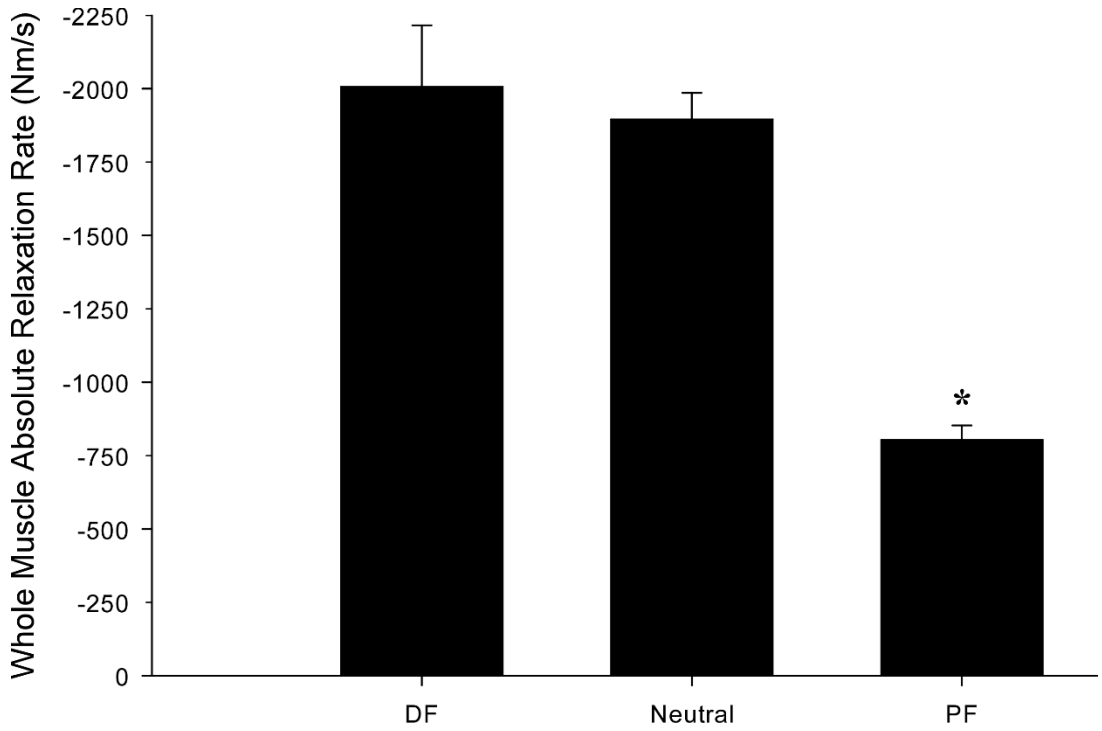

Figure 12: Effect of ankle angle (DF = 20° dorsiflexion, neutral, and PF = 30° plantar flexion) on whole muscle absolute relaxation rate (Newton metres per second, Nm/s). Values are mean ± SEM; n = 11. PF position had a statistically significant (indicated by asterisk; *) slower relaxation rate compared to DF and neutral positions ($p<0.001$).

4.1.3 Normalized Relaxation Rate

A one-way rmANOVA failed to yield a main effect of ankle angle for normalized whole muscle relaxation rates ($p = 0.312$; -11.07 ± 2.95 Nm/s DF, -13.12 ± 2.28 Nm/s neutral, and -13.43 ± 2.33 Nm/s PF).

4.2 Ultrasound Measurements

4.2.1 Intra-Rater Reliability

A two-way mixed, absolute agreement ICC was run on the initial and re-analyzed mean ultrasound fascicle measurements for two participants. The average measures ICC was given as 0.998, indicating excellent intra-rater reliability.

4.2.2 MG & AT Length at Rest

A one-way rmANOVA gave a main effect ($p < 0.001$) for ankle angle on normalized and absolute MG length, as measured at rest from MTJ to popliteal crease (normalized as a percentage of tibial length). Post-hoc testing showed a significant increase in length from the PF position to the DF position ($p < 0.001$; see Table 2).

Table 2: Normalized MG length (% tibial length), absolute MG length (cm), and absolute AT length (cm) for three ankle angles (DF = 20° dorsiflexion, neutral, and PF = 30° plantar flexion). Values are mean ± SD; n = 10; $p < 0.05$. (*significantly different from neutral; †significantly different from DF and neutral; ‡significantly different from PF)

Measurement	DF	Neutral	PF
Normalized MG Length (%)	54.05 ± 4.12*	51.66 ± 3.90	48.26 ± 4.16†
MG Length (cm)	22.38 ± 2.51*	21.39 ± 2.33	19.98 ± 2.28†
AT Length (cm)	22.30 ± 2.43*‡	22.02 ± 2.34	21.91 ± 2.41

A one-way rmANOVA gave a main effect ($p = 0.002$) for ankle angle on absolute AT length, as measured from the calcaneus to the MTJ. Post-hoc testing showed a significant decrease in AT length (see Table 2) from DF to neutral ($p = 0.004$) and from DF to PF ($p = 0.016$), but no significant difference between neutral and PF ($p = 1.000$).

4.2.3 MG Fascicles

A two-way rmANOVA had main effects of ankle angle and muscle state as well as an interaction for MG fascicle length (all $p < 0.001$). Subsequent one-way rmANOVAs for ankle angle and muscle state were significant (both $p < 0.001$) and post-hoc analyses gave $p < 0.001$ for all MG fascicle lengths over 3 levels of ankle angle (DF, neutral, and PF) and 3 levels of muscle state (at rest, during MVC, and TMS-induced relaxation). Table 3 documents MG fascicle lengths, clearly showing that fascicles are longest at rest and shortest

during a MVC, with fascicle lengths increasing during TMS-induced relaxation; however, they remain significantly shorter compared to a muscle at true rest.

Table 3: MG fascicle length (cm) for three ankle angles (DF = 20° dorsiflexion, neutral, and PF = 30° plantar flexion) over three muscle states. Values are mean ± SD; n = 10; $p < 0.01$. (*significantly different from neutral; †significantly different from DF and neutral; § significantly different from resting and TMS-induced relaxation muscle state; # significantly different from resting and MVC muscle state)

Muscle State	DF Fascicle Length (cm)	Neutral Fascicle Length (cm)	PF Fascicle Length (cm)
At Rest	7.28 ± 1.52*	6.35 ± 1.28	4.66 ± 0.95†
During MVC	4.10 ± 0.93*§	3.06 ± 0.56§	2.51 ± 0.42†§
TMS-Induced Relaxation	5.50 ± 1.07*#	4.52 ± 0.75#	3.31 ± 0.59†#

The two-way rmANOVA had main effects of ankle angle and muscle state as well as an interaction for MG pennation angles (all $p < 0.001$). Subsequent one-way rmANOVAs for ankle angle and muscle state were significant (both $p < 0.001$) and post-hoc analyses gave $p < 0.01$ for all MG pennation angles over 3 levels of ankle angle (DF, neutral, and PF) and 3 levels of muscle state (at rest, during MVC, and TMS-induced relaxation), with one exception. During the resting muscle state, pennation angles were not significantly different between DF and neutral ankle angles ($p = 0.058$).

Table 4 documents MG pennation angle variability, clearly showing that these angles are smallest at rest and largest during a MVC, with a trend to decrease from MVC to TMS-induced relaxation; however, they remain significantly larger during TMS-induced relaxation compared to a state of true rest.

Table 4: MG pennation angles (°) for three ankle angles (DF = 20° dorsiflexion, neutral, and PF = 30° plantar flexion) over three muscle states. Values are mean ± SD; n = 10; $p < 0.05$. (*significantly different from neutral; †significantly different from DF and neutral; ‡significantly different from PF; § significantly different from resting and TMS-induced relaxation muscle state; # significantly different from resting and MVC muscle state)

Muscle State	DF Pennation (°)	Neutral Pennation (°)	PF Pennation (°)
At Rest	22.49 ± 2.52	23.91 ± 3.28‡	28.45 ± 4.21†
During MVC	35.68 ± 6.84*§	43.80 ± 7.42§	53.69 ± 7.90†§
TMS-Induced Relaxation	28.73 ± 4.18*#	31.91 ± 4.67#	41.31 ± 5.95†#

4.2.4 Absolute Relaxation Rate

A one-way rmANOVA failed to yield a main effect ($p = 0.108$) of ankle angle for average muscle fascicle relaxation time (0.29 ± 0.05s DF, 0.27 ± 0.06s neutral, and 0.28 ± 0.05s PF). A one-way rmANOVA gave a main effect for average absolute muscle fascicle relaxation rates; these relaxation rates were not significantly different between DF (-4.81 ± 1.87 cm/s) and neutral positions (-5.35 ± 1.10 cm/s; $p = 0.945$); however, both DF and neutral angles had significantly faster peak relaxation rates compared to PF (-2.80 ± 1.10 cm/s; $p = 0.031$ and $p < 0.001$, respectively). See Figure 13.

A one-way rmANOVA gave no main effect ($p = 0.060$) for average MTJ relaxation rates across all ankle angles (6.36 ± 2.97 cm/s DF, 6.87 ± 1.55 cm/s neutral, and 3.89 ± 1.93 cm/s PF).

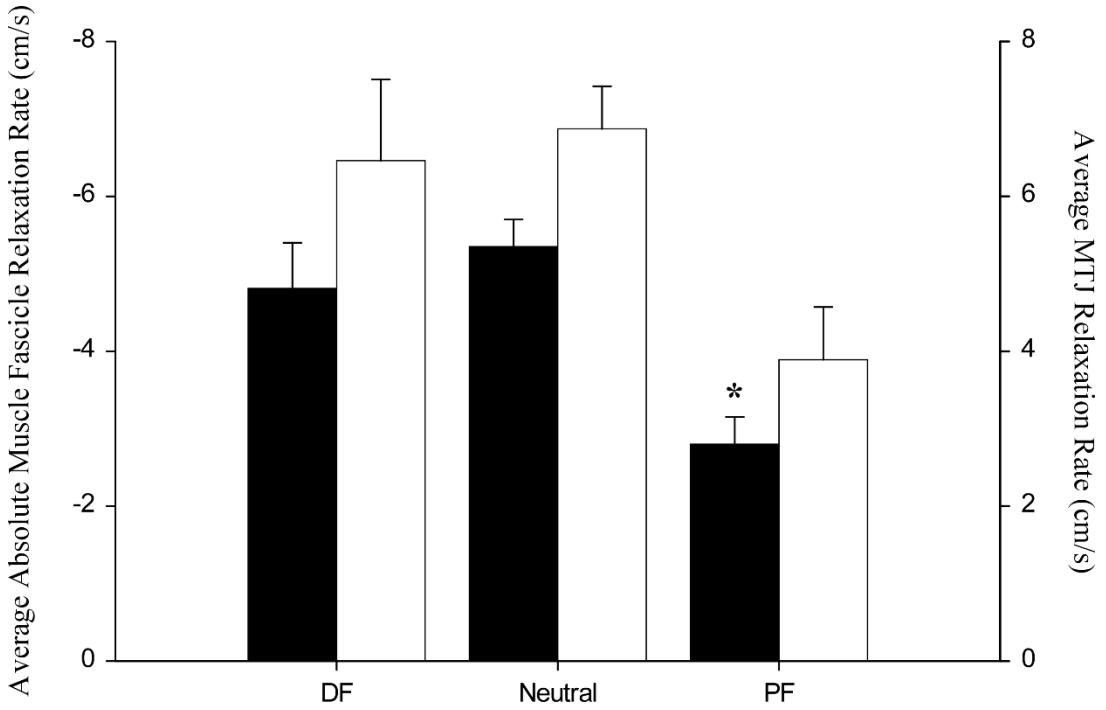

Figure 13: Effect of ankle angle (DF = 20° dorsiflexion, neutral, and PF = 30° plantar flexion) on average absolute MG muscle fascicle relaxation rate (centimetres per second, cm/s; black bars; n = 10) and average MTJ relaxation rate (cm/s; white bars; n = 8). Values are mean ± SEM. PF position had a statistically significant (indicated by asterisk; *) slower muscle fascicle relaxation rate compared to DF and neutral positions ($p<0.05$).

Chapter 5 Discussion

Absolute whole muscle ($p < 0.001$) and muscle fascicle ($p < 0.05$) relaxation rates were markedly slower when the plantar flexors were in a shortened position (30° plantar flexion; PF) compared to a neutral (foot at 90° to tibia) or dorsiflexed (20°) position. This indicates that the TMS-induced relaxation technique is sufficiently sensitive to detect length-tension changes in the triceps surae (TS) muscle group (comprised of the biarticulate gastrocnemius and the soleus) as well as single muscle fascicles of the medial gastrocnemius (MG). It was also shown that TMS paired with ultrasound imaging is a feasible technique to obtain *in vivo* measurements of relaxation in fascicles and of the MTJ (Figure 13). These results support published data which have previously demonstrated the ability of TMS to detect changes in muscle relaxation rate due to effects of sex (Hunter et al. 2006), aging (Hunter et al. 2008), temperature (Todd et al. 2007), fatigue (Todd et al. 2005, 2007), stimulus intensity, and synergist contribution (McNeil et al. 2013).

5.1 TMS-Induced Relaxation Technique

TMS is a more functionally-relevant technique to obtain relaxation rate from skeletal muscle, compared to electrical stimulation delivered at rest. Considering the limitation of electrical stimulation (see Section 1.2.2), a number of studies have successfully employed TMS to investigate muscle relaxation during a maximal voluntary contraction (MVC) by using the silent period (e.g. Todd et al. 2007, Butler et al. 2012, Molenaar et al. 2013, McNeil et al. 2013). Their results have demonstrated unique physiological differences that were previously masked by less functionally-relevant tests. For example, a significant age-related slowing of elbow flexor relaxation rate was found with TMS (Hunter et al. 2008; Molenaar et al. 2013) that was not present during the electrically-induced twitch response in relaxed

muscle (Doherty et al. 1993; Allman & Rice, 2001; Dalton et al. 2010). These studies clearly indicate the state-dependency of muscle contractile properties; e.g., in the absence of descending voluntary drive versus maximal descending voluntary drive during a MVC, as utilized for the TMS-induced relaxation technique.

Prior to this thesis, only one study has investigated the effect of ankle angle (30° plantar flexion, 5° plantar flexion, and 20° dorsiflexion) on contractile properties of the TS (Sale et al. 1982). Using electrical stimulation of the tibial nerve at rest, Sale et al. found half-relaxation time to be slower ($p < 0.001$) at 20º DF (139.0 ± 26.5 ms) compared to 30º PF (89.5 ± 21.4 ms). Interestingly, the results of this present study are opposite, where the 30° PF position was found to have a significantly slower absolute whole muscle rate of relaxation (-804.02 ± 161.91 Nm/s) compared to the 20° DF position (-2007.62 ± 692.11 Nm/s) (see Sections 4.1.2). Furthermore, it was observed that average muscle fascicle relaxation time was not significantly different across ankle angles ($p = 0.108$; 0.29 ± 0.05s DF, 0.27 ± 0.06s neutral, and 0.28 ± 0.05s PF) (see Section 4.2.4).

The data of Sale et al. (1982) are influenced by the size of the electrically evoked twitches; this and the use of absolute time rather than a rate are likely to contribute to the aforementioned disagreement with this present study. In a 30° PF position, resting twitches averaged 0.54 ± 0.19 Nm compared to 13.89 ± 0.75 Nm at 20° DF. The very small twitches elicited in the plantar flexed position likely did not provide meaningful insight into muscle relaxation at this ankle angle (Sale et al. 1982).

5.1.1 Sensitivity to Changes in Length-Tension Relationship

The TMS-induced relaxation technique has previously been shown to be sufficiently sensitive to synergist length changes between a constant-length soleus in conjunction with

optimal (knee extended) and non-optimal (knee flexed) gastrocnemius lengths (McNeil et al. 2013). That is, absolute plantar flexor relaxation rate was significantly slower with the knee flexed compared to extended. While McNeil et al. (2013) accounted for synergist activity, they were unable to make direct interpretations regarding the effect of TMS on the length-tension relationship of the plantar flexors given they changed only gastrocnemius length. In this study, it was demonstrated that TMS is also sensitive to length-tension changes of the TS when all synergists undergo length changes across the same joint (as given by adjustment of ankle angle during full knee extension).

The solitary change of knee angle results in a profound decrease of plantar flexion torque during knee flexion compared to extension due to the dramatic shortening of the gastrocnemius (which is a biarticulate muscle originating from the femoral condyles) (Fugl-Meyer et al. 1979; Cresswell et al. 1995; McNeil et al. 2013). In the present study, ankle (rather than knee) angle was varied, thereby introducing a proportionally equivalent length change across all contributing plantar flexor muscles. These choices in knee and ankle joint angles for my study provide a superior test (compared to McNeil et al. 2013) of TMS sensitivity to changes in the plantar flexor length-tension relationship.

Optimal muscle length selection, as defined by length-tension relationship parameters, results in the production of maximal muscle force (Gordon et al. 1966; Kaufman et al. 1989). The human MG has been shown to operate within an estimated sarcomere length range of 1.4-2.2 μm; therefore, force generation for this muscle is typically confined to the ascending limb of the force-length curve (Figure 4) (Cutts, 1988; Maganaris, 2003). This biomechanical limitation is imposed by the *in vivo* physiologically-attainable range of knee and ankle joint

angles, which enforces bounds upon voluntarily achievable gastrocnemius muscle fibre lengths (Kawakami et al. 1998; Winter & Challis, 2008a).

In one study on the human gastrocnemius (n = 28; 14 males), maximal isometric plantar flexions were performed over 5 ankle angles ranging from 15°DF to 40°PF. The authors found that 3 participants operated over the descending limb of the gastrocnemius force-length curve, 1 operated over the plateau region, and the rest (n = 24) operated on the ascending limb (Winter & Challis, 2008b). Given the majority of subjects operated over the ascending limb of the force-length curve, it follows that as the plantar flexors were stretched to longer fibre lengths, force production would typically continue to increase. This indicates full knee extension and ankle dorsiflexion to be the ideal position for maximal force generation.

In this study (Chapter 4), MVC torque (Section 4.1.1) was significantly greater for DF (182.07 ± 40.71 Nm) compared to neutral (147.70 ± 18.88 Nm; $p = 0.001$) and PF (62.16 ± 9.55 Nm; $p < 0.001$) and for neutral compared to PF ($p < 0.001$). Furthermore, DF and neutral ankle angles both gave significantly faster absolute relaxation rates than PF ($p < 0.001$; Section 4.1.2) for whole muscle torque data. In addition, at the level of the MG muscle fascicles, both DF and neutral angles were significantly faster ($p = 0.031$ and $p < 0.001$, respectively; Section 4.2.3) compared to PF. Likewise, the MTJ followed the same trend, despite not achieving statistical significance ($p = 0.06$; Figure 13). These data support the proposition that the TMS-induced relaxation technique is sensitive enough to investigate intrinsic contractile properties secondary to changes of external factors such as joint position (McNeil et al. 2013).

Previously, it has been hypothesized that an effect of knee angle on plantar flexor torque was primarily due to the mechanism of gastrocnemius unloading over a non-optimal flexed-

knee position (Kennedy & Cresswell, 2001). This implied that a slowing of relaxation rate in the flexed-knee compared to extended-knee position is primarily due to histochemical differences between the soleus and gastrocnemius (McNeil et al. 2013). This present study has extended the findings of McNeil et al. (2013) to show that length changes applied to the TS muscle by adjustments in ankle angle also gives significantly slower relaxation rates at shorter muscle lengths. TS length change, as dictated by variable ankle angles, causes both the gastrocnemius and soleus muscles to function at non-optimal segments of their respective length-tension relationships at 30º PF (see Section 1.5.1 & Section 4.1.1).

The slower relaxation rates observed during knee flexion by McNeil et al. (2013) were ascribed to a greater contribution of the slow-twitch soleus to whole muscle (group) relaxation rate. However, given their limb set-up, it follows that synergist contribution would be predisposed in favour of slow-twitch fibres during knee flexion. In this manner, the gastrocnemius would no longer be engaged to function optimally while the length-tension relationship of the soleus remains relatively unchanged. The avoidance of synergist contribution disparity can be achieved by ensuring proportional contribution of all plantar flexors. In this study, full knee extension paired with changes of the ankle joint angle ensured relatively similar contribution of all plantar flexor muscles and therefore an overall proportional fibre type contribution at each tested angle.

It has been demonstrated that a reduction in muscle length influences onset of motor-unit activation in the MG muscle during flexed knee isometric plantar flexion protocols (Kennedy & Cresswell, 2001). These authors showed that to achieve the same torque output while the knee was flexed compared to extended, the recorded soleus rmsEMG was significantly greater. In addition, during ramped submaximal contractions, MG motor units showed

delayed recruitment thresholds in a flexed knee position compared to extension. Kennedy & Cresswell (2001) proposed that such alteration in recruitment strategy at shortened MG lengths may either reflect a decrease in cortical drive to the MG motoneuron pool or an increase in inhibition or disfacilitation of the MG motoneurons modulated by peripheral afferent feedback.

Previously, there was shown to be no significant difference ($p < 0.05$) for VA of the plantar flexors with the knee flexed (87 ± 8% VA) compared to extended (89 ± 5% VA) (Cresswell et al. 1995). This suggests there was no change in cortical drive, thereby implicating afferent feedback as the likely mechanism of delayed MG motor unit onset in the aforementioned study. VA estimates for my protocol (isometric plantar flexion with the knee extended) were >90% at all ankle angles (Section 3.2.2), therefore inadequate descending drive is not likely to influence the slower relaxation rates at shorter muscle lengths.

5.2 Muscle Fascicles & MTJ

As hypothesized, whole muscle TMS-induced relaxation rates, compared to the MG muscle fascicle and MTJ relaxation rates (see Figures 12 and 13), followed the same trend across ankle angles, with the PF position having the slowest absolute relaxation rate. Analysis of the ultrasound images showed visible changes in fascicle length (increase; Table 3), pennation angle (decrease; Table 4), and MTJ displacement when considered across ankle angles from a MVC to TMS-induced relaxation.

Perhaps the most novel component of this study was the inclusion of ultrasound imaging in conjunction with TMS. Ultrasound technology is particularly useful for capturing the architecture of muscle fascicles in the human gastrocnemius, given their decidedly pennate arrangement and pronounced hyperechoic echogenicity that creates a striking contrast with

the surrounding tissue. Furthermore, the ability to capture ultrasound video makes this technology ideal for the observation of architectural changes to single fascicles. Including the present data, it is now possible to record the changes at the level of muscle fascicles induced by a voluntary (e.g. isometric contraction) (Fukunaga et al. 1997; Ito et al. 1998; Arampatzis et al. 2007; Herbert et al. 2011) or involuntary (e.g. response to TMS or electrical stimulation) movement (Nordez et al. 2009).

The lack of *in vivo* muscle fascicle imaging (during stimulation) in the literature is likely due to inadequate sampling rates, low frequencies, and limited temporal resolution of traditional imaging devices (Nordez et al. 2009). However, with technological improvements, it has become feasible to combine stimulation and ultrasound imaging to capture fast, intrinsic muscle changes. This has been previously demonstrated through the pairing of electrical stimulation with ultrasound technology (Maganaris & Paul, 2000; Nordez et al. 2009). In the present study, ultrasound imaging technology was found to be capable of tracking TMS-induced relaxation in the MG and detecting the influence of muscle length on relaxation rate of single fascicles.

As expected, measurements of MG fascicles (lengths and pennation angles) at rest during passive ankle joint angle manipulation and the subsequent architectural changes from rest to MVC agree with the current literature (e.g., Narici et al. 1996; Fukunaga et al. 1997; Ito et al. 1998; Kawakami et al. 1998; Chow et al. 2000; Maganaris et al. 2002; Hodges et al. 2003; Mademli et al. 2005; Antonios et al. 2008; Herbert et al; 2011). The data of this present study show significant decreases in MG fascicle length and increases in pennation angle across all ankle angles during the transition from rest to MVC. Furthermore, whole MG and AT length

significantly increased ($p < 0.001$; Table 2) during passive stretching as caused by shifting from a plantar flexed to dorsiflexed position.

A significant effect ($p < 0.001$) of both ankle angle and muscle state (i.e. at rest, during MVC, and TMS-induced relaxation) was observed for MG muscle fascicle length (Section 4.2.3). Fascicles did not recover to their initial resting state length during TMS-induced relaxation (see Figure 3). In addition, the absolute time for relaxation of single fascicles (see Section 4.2.4), was not significantly different across ankle angles ($p = 0.108$). This provides evidence that descending drive from the primary motor cortex, which is transiently interrupted by TMS (as quantified by the silent period; Figure 1), recovers within the same time frame, independent of ankle angle.

Therefore, any significant difference between the relaxation rates of the three ankle angles cannot be due to variability in the recovery of descending drive (see also Cresswell et al. 1995 and Section 5.1.1). This indicates that the significantly slower relaxation rates observed in the plantar flexed position are determined primarily by the length-tension relationship of the muscle. Logically it follows that *in vivo*, intracellular changes at the level of the sarcomere must be the determining factors of the overall performance of the MG, in terms of TMS-induced relaxation rate.

Chapter 6 Conclusion

This study incorporated ultrasound technology to record length changes in muscle fascicle architecture and tendon excursion during TMS-induced plantar flexor muscle relaxation. The relationship between ankle joint angle and muscle relaxation rate is influenced by a wide variety of factors, such as the length-tension relationship of the plantar flexors and architectural characteristics of muscle fascicles. This study shows that *in vivo* medial gastrocnemius (MG) muscle fascicle and muscle-tendon junction (MTJ) TMS-induced relaxation rate patterns reflect those seen at a whole muscle level for all plantar flexors, as given by torque traces.

The primary objective was to test the sensitivity of the TMS-induced relaxation technique to a change in muscle length by comparing the relaxation rate of the plantar flexor muscles with the muscles at short (30° plantar flexion), medium (neutral ankle position, 0°) and long lengths (20° dorsiflexion). It was hypothesized that the relaxation rate induced by TMS would be sufficiently sensitive to detect changes in whole muscle length (i.e., rates would slow as the muscle was shortened).

The results (Chapter 4) support the hypothesis and provide further evidence (McNeil et al. 2013) that the TMS-induced relaxation technique is sensitive enough to detect changes in intrinsic contractile properties caused by alterations to joint position. The proposition of McNeil et al. (2013) has been further extended to state that this technique is also sufficiently sensitive to detect changes in the length-tension relationship of the plantar flexor muscle group.

The second objective of this study was to determine whether absolute relaxation rates observed at the level of the whole muscle would be reflected at a muscle fascicle level, for

the MG fascicles and MTJ TMS-induced relaxation rates. It was hypothesized that the relaxation rate of muscle fascicles would vary with joint angle, as seen with whole muscle torque relaxation rate. Additionally, it was hypothesized that MTJ length changes (partially representative of overall muscle-tendon interaction) resulting from TMS-induced relaxation would be comparable to absolute muscle fascicle length changes and that MTJ relaxation rate would also vary with joint angle.

The results (Chapter 4) support the secondary hypotheses, providing evidence in conjunction with previous findings that show plantar flexors perform optimally in an extended knee, dorsiflexed position. *In vivo* changes of muscle architecture were successfully captured using ultrasound technology to record TMS-induced relaxation. Furthermore, it was shown that ankle angle changes have a significant effect on all plantar flexors and MG muscle fascicles TMS-induced relaxation rates. The MTJ also responds similarly, showing a strong trend toward slower relaxation rates at non-optimal points of the MG length-tension relationship.

6.1 Limitations

Ultrasound imaging has advanced significantly since its initial development (see Section 1.4); the machine used for the duration of this study was well-equipped to acquire high-quality images and video of soft tissues, such as skeletal muscle. However, the fastest frame rate choice (77 frames-per-second) for video acquisition was limited by several variables (e.g. image depth, gain, focus), which needed to be consistent across all participants.

In addition, it would have been ideal to time-lock the ultrasound video recordings to the torque acquisition in Spike; however, while such technology does exist, it was unobtainable for this study. It has been previously noted, for electrical stimulation, that there is a time lag between electrical muscle stimulation and onset of fascicle motion of 6.05 ± 0.64 ms (Nordez et al. 2009). Similar lag would also occur for TMS-induced architectural changes associated with relaxation. While it wasn't possible to measure this lag for the present study, one can anticipate that it would be only several milliseconds longer than the latency observed between the delivery of TMS and the appearance of the MEP (see Figure 1; also note that EMG was recorded from the lateral gastrocnemius (LG) for this study, and some small measure of difference can be expected with comparison to the MG).

Ultrasound imaging was used only for the MG muscle. Preferably all components of the triceps surae would have been imaged; however, the protocol and analysis of ultrasound data proved to be a time-intensive process. Given the main goal was to test TMS-induced relaxation technique sensitivity to changes in the length-tension relationship, only one muscle was selected for imaging.

The MG was chosen for ultrasound imaging analysis, rather than the LG or soleus, for a number of reasons. First, the MG is significantly more pennate than the LG (Huijing et al. 1985; Kawakami et al. 1998); this allows more of the whole fascicle length to be captured within the field-of-view of the ultrasound probe, requiring less extrapolation (and therefore introducing less error) of fascicle length measurements. Second, the MG is capable of packing more fascicles within its volume (Kawakami et al. 1998). This gives it a greater potential for force generation and also increases the yield of visible fascicles within the field-of-view of the ultrasound probe. It should be noted that the behaviour of muscle fibres may

not mirror that of muscle fascicles, given that fibres are sub-components of fascicles and they can terminate intrafascicularly, with adjacent fibres not necessarily belonging to the same motor unit (Trotter et al. 1993; Kawakami et al. 1998).

Finally, relaxation rate data for muscle fascicles can only be obtained from adequately pennate muscles at this present time. Fusiform muscles, such as seen in the biceps brachii, have fascicles that run almost parallel across the ultrasound field-of-view. During pilot testing, recording of fascicle relaxation of the biceps brachii was attempted; reliable TMS-induced length changes, however, were unobtainable. As a result, imaging was limited to pennate muscles, of which the MG was selected.

6.2 Implications of TMS for Neuromuscular Pathologies

The measurement of passive or active muscle properties, as given by torque traces or ultrasound imaging, holds a position of clinical interest. The expansion of knowledge in this area is key for improving treatment plans and evaluation of the effectiveness of therapy in patient populations (Segal, 2007). It follows that the techniques used to develop this field should be non-invasive with little to no pain or discomfort involved. TMS has been successfully employed in a number of clinical-based studies that aimed to understand disparities between healthy and patient populations, within the central motor pathway (Oliveri et al. 1997; Ertekin et al. 2001; Floyd et al. 2009). The development of techniques that lead to an improved understanding of the mechanisms of TMS is key to the advancement of knowledge regarding neuromuscular pathologies.

Myotonic disorders are a subset neuromuscular diseases characterized by delayed muscle relaxation following a muscle contraction (e.g. tetanus, stiff-man syndrome, neuromyotonia, myotonia congenita). Patients with these disorders often experience interference with daily

living, as abnormal muscle relaxation rates influence their ability to perform simple activities such as walking or climbing stairs (Trip et al. 2006). However, to date, relevant measures of muscle relaxation have been lacking from the literature. TMS can provide clinicians and researchers with the unique advantage of obtaining functionally-relevant measurements (i.e. absolute muscle relaxation rate) while the muscle is being driven voluntarily by the CNS. The application of this non-invasive, painless technique, in the case of neuromuscular pathologies, has great potential for advancing our knowledge of individual disease mechanisms (Kleine & Stegeman 2007).

6.3 Future Directions

The future of TMS is promising for the field of neuromuscular physiology, bearing in mind the recent advances that have been made with the TMS-induced relaxation technique (summarized in Section 1.2.2 and also implicated in the findings of this present study). Variable-effects masked in resting twitch data and revealed using TMS (e.g. Hunter et al. 2008; Molenaar et al. 2013) advocate for reevaluation of electrical stimulation studies. Hence, the most immediate follow-up step is for researchers to reassess the functional validity of electrical stimulation studies by comparing these data to those acquired using TMS. In addition, the aforementioned implications of TMS for researching neuromuscular pathologies also denotes a potential role in clinical settings.

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Appendices

Appendix A: Participant Consent Form

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PARTICIPANT INFORMATION AND CONSENT FORM

The effect of muscle length on relaxation rate

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1. Invitation to Participate

You are invited to participate in a study to examine the effect of muscle length on the speed your muscle relaxes. You are invited to participate in this study because you are a healthy volunteer between 18-40 years of age.

2. Your participation is voluntary

Your participation is voluntary. You have the right to refuse to participate in this study. If you decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are entitled or are presently receiving. If you are a student at UBC or UBCO, there will be no penalty to your academic status if you choose to withdraw from this study.

Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. Please take time to read this form thoroughly. You are welcome to ask the experimenter and/or Principal Investigator any questions you may have.

If you wish to participate, you will be asked to sign this form. If you do sign the form, you are still free to withdraw at any time without giving a reason for your decision. If you do not wish to participate, you do not have to provide a reason for your decision.

3. Who is conducting this study?

This study is being conducted by Dr. Chris McNeil of the School of Health and Exercise Sciences at UBCO. Internal funds from UBCO and Dr. McNeil's external research grants from Natural Sciences and Engineering Research Council of Canada (NSERC) and Canada Foundation for Innovation (CFI) will fund this study.

4. Background

The speed a muscle relaxes is an important property that helps to optimize muscle performance. Muscle relaxation rate is typically measured by applying electrical stimulation to a nerve when the muscle is at rest. This technique provides valuable information but may not accurately reflect the capacity of the muscle to relax when the muscle fibres are actively contracting (when relaxation rate is most important). To address this concern, an alternative technique was developed. The newer technique measures the relaxation rate of active muscle fibres by applying transcranial magnetic stimulation (TMS) to the brain during a maximal contraction. Electrical stimulation has shown that muscle relaxation rate is affected by factors such as age, temperature, fatigue and muscle length. Recent findings have shown that the newer technique can detect the effects of age, temperature and fatigue but no study has tested if it is sensitive enough to detect changes in muscle length. This study will address this question in order to explore the usefulness of the newer technique and the findings will improve our understanding of muscle properties.

5. Purpose

To examine the effect of muscle length on the rate of muscle relaxation induced by TMS.

6. Who can participate in this study?

You may be eligible to participate in this study if you:

- Are a healthy adult between 18-40 years of age
- Read, understand and speak English

7. Who should not participate in this study?

You will not be eligible to participate in this study if you:

- Are or may be pregnant
- Have a cardiac pacemaker
- Have had brain surgery
- Have or think you may have epilepsy
- Have metal or metallic fragments in the skull (other than dental appliances or fillings)

- Have had any major recent injury or surgery to the limb being tested (e.g., fracture, joint replacement)
- Use any medications with side effects of dizziness, lack of motor control, or slowed reaction time

8. What does the study involve?

If you decide to participate, you will be asked to come to the Integrative Neuromuscular Physiology Laboratory (ART 120) for 1 visit of ~1-2 hours. Please wear shorts to allow for surface electrodes to be placed as described below. During the experimental session, the following procedures (which are described separately) will be performed.

- ☐ To measure the electrical activity of the muscles that point your foot (plantar flexors), surface electrodes will be placed on the skin overlying the calf muscles (triceps surae).

- ☐ To measure the torque (force) produced by the plantar flexor muscles, you will be asked to push your foot against a rigid device equipped with a strain gauge. These contractions (called "maximal voluntary contractions" or MVCs) will require you to try as hard as you can. Your dominant foot will be comfortably and securely strapped into the device. You will be asked to perform repeated (~20-25) brief (~2-5s) MVCs separated by at least 90s of rest. You will not be pushed beyond your limit and you can stop the study at any time.

- ☐ Transcranial magnetic stimulation (TMS) will be delivered to your motor cortex (brain) to briefly interrupt voluntary drive and cause the plantar flexor muscles to relax. A coil which discharges a magnetic pulse will be held in place on top of your head during each MVC. When the brief magnetic pulse is applied, you will hear a click from the stimulator and feel a relaxation of your plantar flexor muscles. You may also feel a brief contraction and relaxation of the muscles of your scalp. Earplugs are available if you find the click to be uncomfortably loud.

- ☐ To observe contraction and relaxation at the level of single muscle fibres, ultrasound images will be taken of the plantar flexors. Ultrasound transmission gel will be applied to the skin overlying the calf muscles and an ultrasound probe will be held in place throughout each contraction.

9. What are my responsibilities?

Your only responsibility as a participant is to decline the invitation to participate in the study if: 1) you do not meet the inclusion criteria listed in section 6; or 2) one of the exclusion criteria listed in section 7 applies to you. If you do participate, you may stop the study at any time for discomfort or any other reason.

10. What are the possible harms and discomforts?

Some participants may develop mild muscle soreness 1-2 days after the exercise.

There is an extremely small risk (~1 in 50,000) of producing an epileptic fit with magnetic stimulation. There have been fewer than 20 events reported since the technique was developed in 1985 and most of these occurred in patients taking pro-epileptogenic medications and some are believed to represent fainting rather than a seizure. All stimulation will comply with published safety guidelines developed in collaboration with the Safety of TMS Consensus Group which includes clinicians and researchers from around the world (S Rossi et al. *Clinical Neurophysiology* 2009; 120:2008-2039). These guidelines set safe standards for the frequency, intensity and duration of stimuli. After large numbers of stimuli some people (fewer than 1 in 20) complain of a mild-headache lasting up to several hours due to scalp muscle contraction. This is unlikely to occur in this experiment because the number of stimuli is small. However, if you do develop a headache, it can be treated with standard non-prescription medications (e.g. acetaminophen).

11. What are the potential benefits of participating?

There are no known benefits to you associated with your participation in this research, although most participants find that the knowledge they acquire during participation in research studies makes for a positive experience.

12. What if new information becomes available that may affect my decision to participate?

If new information arises during the research study that may affect your willingness to remain in the study, you would be advised of this information via your preferred method of communication.

13. What happens if I decide to withdraw my consent to participate?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn; for example, where the data are no longer identifiable (meaning they cannot be linked in any way back to your identity) or where the data have been merged with other data. If you would like to request the withdrawal of your data, please let the Principal Investigator know.

14. Can I be asked to leave the study?

If you do not comply with the requirements of the study, the Principal Investigator may remove you from the study.

15. How will my taking part in this study be kept confidential?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate and UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

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

16. What happens if something goes wrong?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

In the unlikely occurrence of a serious adverse event, the laboratory telephone will be used to call 911 via campus security. If needed, cardiopulmonary resuscitation will be performed by a qualified resuscitator. An automated external defibrillator device is accessible through campus security and in an emergency can be at the laboratory within ~2 min. There is not physician oversight on campus so participants needing emergency care will be taken via ambulance to the KGH which is 14km (~20 minutes) away.

17. What will the study cost me?

You will not incur any costs as a participant in this study. Should you need to pay for on-campus parking, you will be reimbursed at the time of your visit without the need to produce a receipt. You will not be compensated for your participation in this research study.

18. Who do I contact if I have questions about the study during my participation?

If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Dr. Chris McNeil via telephone (250-807-9664) or email (chris.mcneil@ubc.ca).

19. Who do I contact if I have questions or concerns about my rights as a participant?

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by email at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).

20. After this study is finished

Research findings may be disseminated at an academic conference or in a journal article. In all cases, presentation of research findings will primarily involve data based on group means. Any figures which show data from a single participant will simply refer to the individual as "a representative participant." After dissemination of the findings, the raw data obtained in this study will only be available to Dr. McNeil. In accordance with university policy, data will be kept for a minimum of 5 years after it has been published or presented. Data will be stored in a locked room and all electronic files will be password-protected.

Future contact

☐ Using your preferred method of communication, Dr. McNeil may wish to contact you at a later date to participate in other studies. Tick this box if you are willing to be contacted in this manner.

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The effect of muscle length on relaxation rate

PARTICIPANT CONSENT

My signature on this consent form means:

- I have read and understood the information in this consent form
- I have been able to ask questions and have had satisfactory responses to my questions
- I understand that my participation in this study is voluntary
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care that I receive
- I understand that I am not waiving any of my legal rights as a result of signing this consent form

I will receive a signed copy of this consent form for my own records.

I consent to participate in this study.

_____	_____	_____
Participant's Signature	Printed Name	Date

_____	_____	_____	_____
Signature of Person Obtaining Consent	Printed Name	Study Role	Date

Appendix B: Integrative Neuromuscular Physiology Laboratory Transcranial Magnetic Stimulation (TMS) Screening Form

Below is a questionnaire used to exclude participants considered not suitable for transcranial magnetic stimulation (TMS). This information, as well as your identity, will be kept confidential.

Participant Code: _____ Age: _____

PLEASE CIRCLE "YES" or "NO" FOR EACH QUESTION:

Are or may be pregnant	YES	NO
Cardiac pacemaker or other implanted electronic medical devices	YES	NO
Brain surgery	YES	NO
Brain injury	YES	NO
Metal or metallic fragments in the skull (other than dental appliances of fillings)	YES	NO
Personal history of seizure or epilepsy	YES	NO
Family history of seizure of epilepsy	YES	NO
Headaches or hearing problems	YES	NO
Other medical conditions	YES	NO

If you answered "YES" to any of the above questions, please provide details below: