FATIGABILITY AND ADAPTATION TO MAXIMAL ECCENTRIC EXERCISE IN YOUNG FEMALES AND MALES

by

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Abstract

In addition to fatigue, performance of unaccustomed eccentric (ECC) exercise causes temporary muscle damage, initiating a protective response to minimize damage from a subsequent bout (i.e., the repeated-bout effect; RBE). Indirect evidence suggests females experience greater muscle damage following ECC maximal voluntary contractions (MVCs). If true, this could induce a larger RBE for females than males; however, the RBE has not been compared between the sexes. **PURPOSE:** To compare fatigue and damage induced by ECC MVCs as well as the magnitude of the RBE in females and males. **METHODS:** Twelve males (21.6 ± 2.2yrs; 1.8 ± 0.1m; 82.4 ± 12.4kg) and twelve females (23.5 ± 3.0yrs; 1.6 ± 0.1m; 60.1 ± 4.1kg) performed two bouts (separated by four weeks) of 200 ECC MVCs of the dorsiflexors (60°/s from an ankle angle of 90° to 30° of plantar flexion; 4 sets of 50 reps; 1min rest between sets). Isometric (ISO) MVC torque and the ratio of ISO torque responses to low vs. high frequencies of electrical stimulation (10:100Hz) were compared before and after (2, 3, 5, and 10min, as well as 2, 4, and 7d) the fatiguing protocol. **RESULTS:** Measures of fatigue (ECC MVC torque) and damage (ISO MVC torque and 10:100Hz ratio) did not differ for females and males for bout one (all $P > 0.05$). Pooled data revealed decreases of 31.6 ± 13.4% for ECC MVC torque, 25.2 ± 13.9% for ISO MVC torque (2min post-exercise) and 53.6 ± 9.0% for 10:100Hz ratio (10min post-exercise). A two-way repeated measures ANOVA determined no main effect of sex or bout for any measure except the 10:100Hz ratio. Recovery of the ratio improved between bouts for both females and males ($P < 0.05$). **CONCLUSION:** Our results suggest that an initial bout of ECC MVCs causes equivalent fatigue and muscle damage of the dorsiflexors for females and males. After bout two, acute decreases in the 10:100Hz ratio are attenuated for both groups, suggesting a similar RBE for both females and males.

Lay Summary

Despite overwhelming similarities, differences exist in the neuromuscular systems of males and females. Females tend to experience less muscle fatigue during static tasks. However, static tasks are seldom performed during daily activities making dynamic contractions more functionally relevant. Few studies exist in this area, but the data suggest no sex differences exist for fatigability from lengthening (eccentric) contractions. Eccentric contractions are of particular interest because they are effective for developing strength and preventing injury. Exposure to new eccentric exercise will cause muscle damage, indicated by impaired torque production in the hours and days after the exercise. Luckily, muscle damage is significantly reduced after a subsequent bout of the same exercise weeks-to-months later (i.e., the "repeated-bout effect"; RBE). This study will advance our understanding of sex-related differences in fatigability to eccentric exercise and examine if muscle impairments following eccentric contractions translate to a change in the magnitude of the RBE.

Preface

The University of British Columbia's Clinical Research Ethics Board granted approval for research on August 28th, 2017. The ethics approval certificate number for the current study is H17-01631. Portions of the research data presented in this thesis are included in a methods-focussed article recently accepted by the Journal of Applied Physiology (JAPPL-00840-2018R2). The specific questions addressed in this thesis have not yet been published in full.

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List of Abbreviations

ANOVA	analysis of variance
BL	baseline
Ca^{2+}	calcium ion
d	days
DHPR	dihydropyridine receptor
E-C	excitation-contraction
ECC	eccentric
Hz	Hertz
ISO	isometric
kg	kilograms
m	metres
mA	milliamps
MVC	maximal voluntary contraction
Nm	Newton metres
PLFFD	prolonged low-frequency force depression
PT	peak torque
RBE	repeated-bout effect
RyR	ryanodine receptor
s	seconds
SD	standard deviation
SEM	standard error of the mean
SIT	superimposed twitch
µs	microseconds
yrs	years
VA	voluntary activation

Acknowledgements

I am incredibly lucky to have had the education I've received thus far. Not only because of the quality, but because of the mentality of the individuals who have delivered the lessons. I have been blessed with the opportunity to learn from people who love their jobs. To this, I owe my enthusiasm for science research. I would like to acknowledge Dr. Trevor Day for introducing me to the study of human physiology and providing me with guidance in both academia and life for the past six years. Your dedication to student's learning and the pursuit of science communication is inspiring. I would like to thank my committee members, Dr. Jennifer Jakobi and Dr. Brian Dalton for dedicating their time and advice to my learning throughout the last two years. In addition, I would like to thank Dr. Tanya Forneris and Dr. Gregory duManoir for their overwhelming efforts to expand my teaching capabilities. To all four of you, your love for teaching is infectious and I cherish the lessons you've passed on to me. I am very grateful to my lab mates, Alexandra Yacyshyn and Luca Ruggiero for their constant patience and guidance; I owe a great deal of my success to you both. Last, but not least, I would like to extend my sincerest gratitude to my supervisor, Dr. Chris McNeil. Thank you for taking a chance on me two years ago and allowing me to learn from you; both from your expertise in neuromuscular research and from your extensive knowledge in teaching. I truly value your patience and the insights you've given me into pursing a career in academia. You gave me the freedom to explore various scientific topics and continue to pursue my career in music, all while studying for my master's degree and for that, I am forever grateful. I would also like to acknowledge the support of the UBC Okanagan Aboriginal Graduate Fellowship for funding this thesis and to all the individuals who participated in my study.

Dedication

To my mother, father, little sister, and my BFFL.

Chapter 1: Introduction

1.1 Motor Control of Human Skeletal Muscle

One of the many functions of skeletal muscle is to generate force and produce movement in response to descending voluntary command from the central nervous system. From the motor system of the brain all the way to the muscle fibre, force output relies on the faithful transmission of an electrical signal from the primary motor cortex, along efferent nerves (motor neurons) and into the muscle fibre to complete excitation-contraction (E-C) coupling (see next section for further details). In addition to descending drive from the central nervous system, information from afferent nerves residing within the periphery also influence activation of skeletal muscle, especially from a fatiguing muscle (Amann & Dempsey, 2008). Intrinsic properties of the motor cortex, corticospinal tract, motoneurons, peripheral nerves, or the muscle fibres themselves could all influence force production. Because human skeletal muscle is responsible for many important physiological actions and processes, such as (but not limited to) locomotion, balance and postural control, thermogenesis, and breathing, decrements of any kind in one's ability to exert force or power will undoubtedly impinge upon daily activities.

1.2 Excitation-Contraction Coupling

The process by which skeletal muscle converts an electrical impulse (action potential) into mechanical force is known as E-C coupling (first described by Sandow, 1952). There are multiple steps that must take place in order for this translation to occur; as such, there are multiple opportunities for impairments to arise (Bellinger et al., 2008). E-C coupling begins when an action potential reaches the synaptic terminal of a motor axon and triggers the release of acetylcholine, which binds to the associated muscle fibre and initiates electrical transmission of the action potential along the membrane (sarcolemma) of said muscle fibre. Once the action potential propagates along the sarcolemma and into the transverse tubules (t-tubules; invaginations of the sarcolemma into the muscle fibre), voltage-gated ion channels (dihydropyridine receptors; DHPRs), which are closely associated with Ca^{2+} release channels (ryanodine receptors; RyR) embedded into the walls of the sarcoplasmic reticulum, undergo a conformational change to release Ca^{2+} into the intracellular matrix. The increase in myoplasmic Ca^{2+} assists in cross-bridge cycling which ultimately results in force development. Improper Ca^{2+} release and reuptake from the sarcoplasmic reticulum leads to reductions in force (Allen, Lamb, & Westerblad, 2008). Failure

in the interaction between the DHPRs and RyR (which leads to impaired Ca^{2+} release from the sarcoplasmic reticulum) has been established as an area susceptible to damage following strenuous eccentric contractions (Balnave, Davey, & Allen, 1997; Bellinger et al., 2008; Warren, Hayes, Lowe, Prior, & Armstrong, 1993).

1.3 Neuromuscular Fatigue

Neuromuscular fatigue can be defined as any exercise-induced decrease in force or power generated by a muscle in response to volitional effort, regardless of task performance or completion (Bigland-Ritchie, 1981, p. 130). Depending on the nature of the fatiguing task, decrements of force can occur due to changes anywhere along the motor pathway or within the muscle itself and can be classified as central or peripheral in nature (Gandevia, 2001).

The extent to which descending drive from the cortex activates motor neurons in response to volitional effort is known as voluntary activation (VA; Gandevia, 2001). VA can be calculated via the interpolated twitch technique (Merton, Hospital, & Square, 1954) where electrical stimulation is delivered to the peripheral nerve or muscle belly during a maximal voluntary contraction (MVC). In response to fatiguing exercise, impaired VA of a muscle has been termed central fatigue (CF; Gandevia, 2001) and occurs from impairments superior to the neuromuscular junction. Impairments in VA (CF) can be observed as a post-exercise increase in the superimposed twitch (SIT) force, which indicates less descending drive. Peripheral fatigue (PF) refers to force reductions occurring from processes at or distal to the neuromuscular junction (Allen et al., 2008; Gandevia, 2001) and can be detected using peripheral nerve or motor point stimulation. The magnitude of muscle fatigue, as well as the contribution of central and peripheral mechanisms, depends on the parameters of the task. Commonly manipulated parameters include: contraction intensity (percentage of MVC force); contraction type (i.e., isometric vs dynamic); duration and timing (sustained vs intermittent and work-rest ratio); the muscle group being tested; and differences in the population being testing (e.g., age and sex differences). In general, exercise-induced neuromuscular fatigue is transient in nature and recovers following the cessation of exercise within minutes to hours (depending on the nature of the task) in comparison to muscle damage (Allen, 2001). Exercise-induced muscle injury (damage) causes prolonged weakness that can last for days following exercise due to structural impairments within the muscle fibres (Friden et al. 1984), leading to impaired E-C coupling (i.e., E-C uncoupling) for reasons unrelated to increases in metabolic byproducts (Kamandulis et al., 2017; Nishikawa, 2016).

1.4 Exercise Induced Muscle Damage

One of the earliest studies to report exercise-induced muscle damage was published in 1898 by an American physician, Theodore Hough, who first suggested micro-tears in the connective tissue were responsible for pain experienced by the participants following maximal dynamic contractions (Hough, 1898). Since then, it has been well established that exercises involving high-force, lengthening (eccentric) contractions are highly effective for inducing reversible injury in muscle unaccustomed to such exercise (Allen, 2001; Nosaka & Clarkson, 1995).

The decline in performance associated with exercise-induced muscle damage has been found to coincide with structural impairments of the cellular membrane, sarcomeres, and impaired Ca^{2+} homeostasis and E-C uncoupling (Balnave, Davey, & Allen, 1997; Clarkson & Hubal, 2002; Proske & Morgan, 2001; Warren et al., 1993). Common markers of muscle damage include a loss in MVC force production, morphological changes detected via light and electron microscopy (i.e., z-line streaming), elevated enzymatic and protein levels within the blood such as creatine kinase (CK) and myoglobin (Mb), and delayed onset of muscle soreness (DOMS; Warren, Lowe & Armstrong, 1999). In addition, unaccustomed lengthening contractions have been found to elicit a greater impairment of electrically-evoked torque at a low vs. high frequency stimulation due to impaired Ca^{2+} homeostasis (Cheng, Place, & Westerblad, 2017; Clarkson & Hubal, 2002; Skurvydas et al., 2016). This phenomena indicates greater impairments in force production at low-intensity tasks compared to near-maximal tasks and was originally termed Low Frequency Fatigue (LFF; Edwards, Hill, Jones, & Merton, 1977). Edwards and colleagues found that impaired MVC force of the knee extensors, as well as adenosine triphosphate (ATP) and phosphocreatine (PC) muscle content, had recovered by 60 min post eccentric exercise but a measure of calcium handling was still impaired (i.e., LFF; Edwards et al., 1977). Because exercise-induced muscle damage is the result of mechanical strain on various components of the muscle fibre, eccentric contractions (which can involve forceful lengthening of the muscle during contraction) have been found to provoke greater force impairments and histological damage than isometric or concentric contractions in humans (Skurvydas et al., 2016). However, the term has since been rephrased as Prolonged Low-Frequency Force Depression (PLFFD) in order to establish this fatigue occurs during (not because of) low frequency stimulation (Allen et al., 2008).

Direct assessment of exercise-induced muscle damage *in vivo* requires biopsies or magnetic resonance imaging (MRI) and these techniques are not without limitations. When determining the

mechanisms responsible for force loss *in vivo* (without the use of microscopy, MRI, or blood samples), making the distinction between transient fatigue and damage is difficult; however, if damage exists, the distinction becomes more apparent in the minutes, hours and days after exercise. This is likely due to the secondary loss in force production due to an inflammatory response that follows the initial structural changes which occur from exercise-induced muscle damage (Clarkson & Hubal, 2002; Hubal, Chen, Thompson, & Clarkson, 2008).

An interesting phenomenon occurs when an individual performs repeated bouts of eccentric exercise that induces fatigue and damage, impairments are significantly reduced if the same exercise is performed weeks-to-months later (i.e., the "repeated bout effect"; RBE; Nosaka, 1995). It is still unclear whether or not the RBE occurs because of protective effects from the initial mechanical insult, from the inflammatory response, from an improvement in the recovery phase of a subsequent bout of exercise or from a combination of all three (Goodall et al., 2017; Hubal et al., 2008; Hyldahl, Chen, & Nosaka, 2017; McHugh, 2003). Neural, mechanical and cellular adaptations have all been suggested as the mechanisms responsible for the RBE (Goodall et al., 2017; McHugh, 2003). A number of studies have investigated sex- (Hubal, Rubinstein, & Clarkson, 2008; Kerksick, Taylor, Harvey, & Willoughby, 2008; Lee et al., 2017; MacIntyre, Reid, Lyster, & McKenzie, 2000; Power, Dalton, Rice, & Vandervoort, 2010; Sewright, Hubal, Kearns, Holbrook, & Clarkson, 2008; Stupka et al., 2000; Wiecek, Maciejczyk, Szymura, & Szygula, 2017) and age- (Chen, Chen, Liu, & Nosaka, 2014; Lin et al., 2018) related differences following damaging eccentric contractions which collectively have equivocal conclusions regarding the magnitude of fatigue and muscle damage in females and males following subsequent bouts of eccentric exercise.

1.5 Sex Differences in Muscle Physiology, Fatigue, and Damage

Females tend to have a greater proportional area of slow-twitch muscle fibres compared to males (Simoneau et al., 1985), which could be responsible for a greater reliance on oxidative metabolism for energy production in females (Russ, Lanza, Rothman, & Kent-Braun, 2005). One of the functional outcomes of this difference is a greater resistance to muscle fatigue during static (isometric) tasks (Enoka & Duchateau, 2008; Hunter, 2014). However, isometric tasks are seldom performed during daily activities so it is more functionally-relevant to investigate fatigue during dynamic contractions. Relatively few studies exist in this area, but the data suggest no sex-related differences exist for fatigability during protocols of shortening (concentric) contractions (Senefeld,

Yoon, Bement, & Hunter, 2013) or lengthening (eccentric) contraction (Hubal et al., 2008; Rinard, Clarkson, Smith, & Grossman, 2000). In both these studies, impairment was physiologically greater in females at the end of the protocol so more contractions (25% more) may have revealed a statistical difference because the groups were diverging. Furthermore, two other studies (Power, Dalton, Rice, & Vandervoort, 2013; Sewright et al., 2008), found females generated less isometric torque during a maximal voluntary contraction (MVC) in the recovery period after damaging eccentric exercise. Hence, it is important to further examine the influence of sex on fatigue and muscle damage induced by damaging eccentric exercise. Lower isometric MVC torque after eccentric exercise implies that females have greater acute damage compared to males (Warren et al., 1993; Warren et al., 1999). Depending on the mechanism(s) responsible, this could influence the efficacy of the RBE.

To date, no study has tested if the protective adaptations of the RBE differ in adult males and females following damaging eccentric contractions. Chen and colleagues, in two separate studies, have explored the RBE in pre-pubescent, pubescent and post-pubescent males (Chen et al., 2014) and females (Lin et al., 2018). Both studies found the extent of exercise-induced damage increased with age, and that the RBE was similar across all age groups. However, one study used MVCs while the more recent study used submaximal dynamic contractions to induce muscle damage and fatigue, therefore, few conclusions can be drawn to speculate if sex-related differences do exist.

Chapter 2: Purposes and Hypotheses

2.1 Purposes

The purposes of my thesis are to: 1) examine sex-related differences to a high-volume bout of fatiguing eccentric exercise; and 2) compare the magnitude of the RBE between females and males when a second bout of eccentric exercise is performed four weeks after the damaging initial bout. Specifically, the thesis will aim to determine if a greater decrease in isometric MVC torque for females than males after bout one translates to enhanced adaptation and greater protection against damage from the second bout.

2.2 Hypotheses

For the initial bout of eccentric exercise, we hypothesized that, in comparison to males, females will show evidence of greater muscle fatigue (as measured by the loss of eccentric MVC torque during the protocol) and greater muscle damage (as measured by isometric MVC torque and PLFFD in the days after the protocol). Following the initial bout of exercise, females will exhibit a greater relative adaptation to eccentric exercise such that no sex differences will exist for fatigue and indirect markers of muscle damage during and after the second bout

Chapter 3: Methods & Materials

3.1 Participants

Twenty-four young recreationally active individuals participated in this study. Twelve of whom were females (mean ± SD; age: 23.5 ± 3.0 years; height: 1.6 ± 0.1 m; body mass: 60.1 ± 4.1 kg) and 12 males (age: 22 ± 2.3 years; height: 1.8 ± 0.1 m; body mass: 82.4 ± 12.4 kg). Phase of menstrual cycle and oral contraceptive use was not controlled for based on previous literature who found varying hormone levels during the menstrual cycle did not affect contractile properties of the muscle (de Jonge, Boot, Thom, Ruell, & Thompson, 2001) or indirect measures of muscle damage following eccentric exercise (Oosthuyse & Bosch, 2017). All participants gave written informed consent prior to becoming familiarized with the protocol.

3.2 Exclusion Criteria

Individuals were excluded from participation in this study if they experienced any recent major injury or surgery to their dominant leg, had a neuromuscular disease (e.g., amyotrophic lateral sclerosis, muscular dystrophy, multiple sclerosis, myasthenia gravis), experienced significant exercise-induced muscle soreness of the dorsiflexors within the last nine months, or took any medications that affected motor control and/or reaction time. Additionally, individuals were also excluded if they were regularly involved in extreme lower body physical activities (i.e., distance trail running or cross-country skiing) which may alter the magnitude of their repeated-bout effect.

3.3 Study Design

All testing was completed in the Integrative Neuromuscular Physiology Laboratory at the Okanagan campus of The University of British Columbia. Participants were required to complete isometric neuromuscular function testing before and at multiple time intervals (2, 3, 5 and 10 minutes as well as 2, 4, and 7 days) following a bout of fatiguing eccentric contractions. Acute recovery measures only included isometric contractions (both voluntary and electrically evoked) while recovery measures on day 2, 4 and 7 included both isometric and dynamic (eccentric) contractions. The same sequence of testing was performed four weeks after the first bout of exercise in order to complete the second bout. Participants had eight visits total which were all

performed at a similar time of day (± 2.5 hour). Participants were asked to refrain from partaking in any unaccustomed lower limb activities, which were considered strenuous to the participant, between the two bouts of exercise.

3.4 Experimental Set-Up

All isometric contractions were performed at 30° of plantar flexion on a custom-built, isometric dynamometer. The foot of the dominant leg was secured to the footplate by a Velcro strap across the toes and another over the dorsum of the foot. The knee joint was adjusted to 90° and a c-clamp was tightened over the distal portion of the thigh to restrict movement during dorsiflexor contractions. The eccentric fatigue protocol was performed on a HUMAC NORM multi-joint dynamometer (CSMi, Stoughton, MA, USA), with the axis of rotation of the torque motor aligned with the malleoli of the ankle of the dominant leg. Participants sat in a reclined position, with an angle of 120° at the hip and 90° at the knee. As with the isometric dynamometer, the foot of the dominant leg was secured to the footplate by Velcro straps over the toes and instep. Non-elastic adjustable shoulder, waist, and thigh straps were used to minimize extraneous movement during the fatiguing protocol.

3.4.1 Common Fibular Nerve Stimulation

Data collection began with the participant seated comfortably at the isometric dynamometer in order to determine the maximal peak torque (PT) of a twitch in response to a single electrical stimulus delivered to the common fibular nerve while the dorsiflexors were relaxed. Stimulation current was increased incrementally with successive stimuli until the PT of the resting twitch reached a plateau. Stimulus intensity was then adjusted to 115% of that required to evoke the maximal twitch, in order to ensure activation of all motor axons throughout the protocol. Square-wave electrical stimuli (pulse width of 500 μs; 100-400V; 10-35 mA) were delivered to the common fibular nerve using a computer-triggered stimulator (DS7AH, Digitimer) to induce electrically-evoked contractions while the participant was seated. Stimuli were delivered using a bar electrode (279-930-24TP, Chalgren Enterprises, Gilroy, CA, USA) that was held against the skin, distal to the fibular head. The optimal site of stimulation was marked with permanent ink to ensure similar placement for all visits. Optimal pressure and placement of the bar electrode was checked at the beginning of each visit (once stimulation intensity was

determined) by delivering a single train of 100Hz to ensure the torque response reached a plateau. The ink landmark was retraced each time the participant visited the lab to avoid fading.

3.4.2 Baseline Testing

Once the stimulator output was determined, baseline isometric data were collected from a sequence that involved a brief (~2-3 s) isometric (ISO) MVC followed by electrically-evoked contractions. A single stimulus was manually delivered during the MVC to test for the presence of a SIT and, ~1-2 s after the participant relaxed from the MVC, another single stimulus was delivered followed, at 1 s intervals, by 1 s trains at 10 and 100 Hz. For familiarization purposes, participants performed one to two practice sequences with electrical stimulation set to half the required current (see section 3.4.1). To collect the baseline data, the sequence of contractions was performed three times, with 90 seconds of rest between sequences. Visual feedback and strong verbal encouragement were given during each MVC.

3.4.3 Fatiguing and Recovery Protocol

All eccentric contractions consisted of an active eccentric motion (from an ankle angle of 90 ° until 30 ° of plantarflexion at 60 °/s), followed by a passive concentric motion (30 °/s) to return to a neutral ankle position (90 °). The eccentric and concentric movements were separated by a 1 second delay. In order to start the eccentric phase with maximal effort, participants were asked to initiate an MVC during the 1 second delay in a neutral ankle position following the upward movement of the concentric phase. Before initiating the fatigue protocol, maximal eccentric torque of the dorsiflexors was determined by 3-5 practice MVCs. To induce fatigue, participants performed 200 eccentric MVCs (4 sets of 50 repetitions; 3 s rest between repetitions and 1 min rest between sets) of the dorsiflexors. Throughout the protocol, the maximal eccentric torque was continuously displayed on an oscilloscope to provide feedback to the participant. Baseline testing, the fatigue protocol and all recovery measures are represented in figure 1 (protocol schematic).

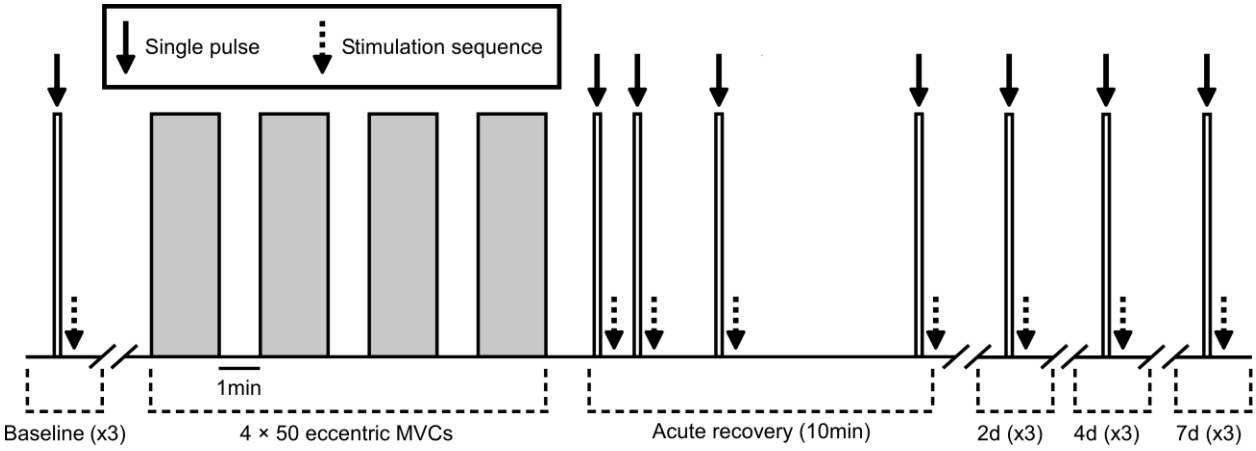

Figure 1. Protocol Schematic. Beginning from the left, the thin vertically orientated rectangles represent ISO MVCs performed at 30 ° of plantar flexion. Individuals received a single pulse (solid arrow) of electrical stimulation during the MVC followed by the stimulation sequence (single pulse, 1s train of 10 Hz and 100 Hz) while at rest (dashed arrow). The fatiguing protocol is shown by the four, large, grey rectangles with 1 min rest between sets. Each grey rectangle represents 1 set of 50 ECC MVCs. This protocol schematic was completed twice, with four weeks between bouts.

3.5 Data Reduction and Analysis

All torque data were sampled online using Spike 2 software (version 8; Cambridge Electronic Design) and analyzed off-line using Signal software (version 5.08; Cambridge Electronic Design). The torque produced during voluntary and electrically-evoked isometric contractions was measured by a linear strain gauge connected to the footplate (MLP-300; Transducer techniques, Temecula, CA, USA). The signal was then amplified (×1000) (CED 1902, Cambridge Electronic Design, Cambridge, UK), sampled at 1000 Hz using a 16-bit A/D converter (CED 1401-3; Cambridge Electronic Design), and recorded using Spike 2 software (version 8; Cambridge Electronic Design). Peak voluntary torque and superimposed twitch torque were calculated from each isometric MVC to obtain ISO MVC torque and assist with the calculation of VA (see below), respectively. For each eccentric MVC, area under the curve was used to calculate ECC MVC torque. During the fatiguing protocol of bout one and two, ECC MVC torque was averaged from the first and last five contractions of each set. The average from the first five contractions of the first set was considered the baseline value and all other values were normalized to this value. For the prolonged recovery days (i.e., 2, 4 and 7 days) an average of five ECC MVCs were obtained and expressed as a percentage of baseline. All isometric variables measured before

the fatiguing protocol as well as at 2, 4 and 7 days post-fatigue were averaged from three contractions. Isometric variables measured during the acute recovery time points (2, 3, 5 and 10 min) represent only one contraction. All post-fatigue measurements (acute and prolonged) were normalized to baseline values except VA, which is already expressed as a percentage. In order to calculate voluntary activation of the dorsiflexors during each isometric MVC, peak torques of the superimposed twitch and resting twitch were used in the following equation: VA = [1 − (superimposed twitch ÷ resting twitch)] × 100%

3.5.2 Statistical Analysis

Two main comparisons were made in this study: (1) the comparison between males and females for each dependent variable during and after the first bout of exercise; and (2) the comparison between the first and second bouts to determine the magnitude of the repeated-bout effect. SPSS software (version 24) was used to conduct all statistical analyses. Normality of data was examined using skewness, kurtosis, and the Shapiro-Wilks test. If independent data were found not to be normally distributed, comparisons were made using a Mann-Whitney *U* test. Unpaired-samples *t*-tests were used to compare males and females for baseline values of ISO and ECC MVC torque, VA, torque responses from 10 and 100 Hz stimulation, and the PLFFD ratio (10:100 Hz torque) for bout one and two, separately. Data collected from bout one have been expressed as a percentage of baseline and then compared using a two-way repeated measures ANOVA, with sex as a between-subjects factor and time as a within-subjects factor. Separate ANOVAs were performed for acute (2, 3, 5 and 10 minutes) vs. prolonged (2, 4 and 7 days) recovery time points. If only a main effect of time was found, data were pooled for females and males and paired-samples *t*-tests and a Dunnett's table were used to determine which time points were different from baseline. If a significant main effect of group or group × time interaction was found, separate one-way ANOVA tests were run for each group to determine the effect of time as described in the previous sentence and a Tukey's *post hoc* test was performed to determine at which point were values different. To compare normalized data between bout one and two, three-way repeated measures ANOVAs were used to identify if a significant interaction existed between time, bout and sex. If a main effect of bout or bout × time interaction was found, separate one-way ANOVA tests were conducted to determine the effect of time during each bout and if significant, a paired-samples *t*-test and a Dunnett's table were used to determine which recovery time points were different from baseline. All data are reported as mean ± SD in the text, and as mean ± SEM in the figures. The significance level was set at $P \leq 0.05$ and effect size of the ANOVA results are reported as eta-squared (η^2).

Chapter 4: Results

4.1 Baseline Measurements for Bout One

Baseline values of ISO and ECC MVC torque, electrically-evoked torque responses (10 Hz, 100 Hz, and 10:100 Hz PT) and VA are presented for each sex in Table 1. The absolute ISO and ECC MVC peak torque values of males were 60.7% and 63.9% greater compared to those of females ($P < 0.05$). VA was not different between the sexes prior to fatigue ($P = 0.51$). The torque responses to the 10 and 100 Hz trains were larger for males compared to that of females (62% and 61.8%, respectively; $P < 0.05$). Consequently, the 10:100 Hz ratio was not different between females and males ($P = 0.79$).

Table 1. **Baseline ankle dorsiflexor characteristics separated by group.**

Sex	ECC MVC (N·m)	ISO MVC (N·m)	VA (%)	10 Hz (N·m)	100 Hz (N·m)	10:100 HZ
Male	57.6 ± 8.7	47.8 ± 9.4	98.9 ± 0.72	17.9 ± 3.4	36.4 ± 9.0	0.51 ± 0.09
Female	36.8 ± 6.2*	29 ± 5.3*	99.1 ± 0.90	11.1 ± 3.4*	22.5 ± 5.3*	0.51 ± 0.09

Mean resting absolute values (± SD) are presented above. ISO MVC, isometric maximal voluntary contraction torque; ECC MVC, eccentric maximal voluntary contraction peak torque; VA, voluntary activation; 10 Hz, peak torque of 1 s tetanus at 10 Hz; 100 Hz, peak torque of a 1 s tetanus at 100 Hz; 10:100 Hz, peak torque of both 1 s tetani presented as a ratio. * denotes a value significantly lower in females than males ($^*P < 0.05$).

4.2 Maximal Voluntary Contraction Torque

The fatiguing protocol of 200 maximal eccentric contractions from bout 1 led to a significant reduction in ISO and ECC maximal voluntary contraction torque. A total of 24 individuals completed the first bout of eccentric contractions but one file with ECC MVC responses was lost due to human error (11 females, 12 males; Figure 2A). Isometric MVC data were collected from all 24 participants at all recovery time points (Figure 3A).

4.2.1 Fatiguing Eccentric Contractions

A two-way repeated measures ANOVA indicated no sex × time interaction ($F_{3.2, 66.8} = 0.73$, $\eta^2 = 0.03$, $P = 0.55$), no main effect of sex ($F_{1, 21} = 0.18$, $\eta^2 = 0.008$, $P = 0.68$), but a main effect of time ($F_{3.2, 66.8} = 60.1$, $\eta^2 = 0.74$, $P < 0.05$; Figure 2A) for ECC MVC torque during the fatiguing

protocol. With data from the two groups pooled (i.e., males and females), ECC MVC torque was impaired by 31.6% by the end of the fatiguing ptocol (S4). During the long-term recovery period (2, 4 and 7 days later), there was no sex × time interaction ($F_{2.6, 55.4} = 0.84$, $\eta^2 = 0.04$, $P = 0.47$), no main effect of sex ($F_{1, 21} = 0.16$, $\eta^2 = 0.008$, $P = 0.69$), and no main effect of time ($F_{2.6, 55.4} = 0.64$, $\eta^2 = 0.03$, $P = 0.57$; Figure 2A). *Post hoc* analyses indicated ECC MVC torque recovered to baseline by day 2 ($P < 0.05$). ECC MVC torque responses from bout two are also presented below (Figure 2B); however, results are reported in the Repeated-Bout Effect section of the results (4.5.1).

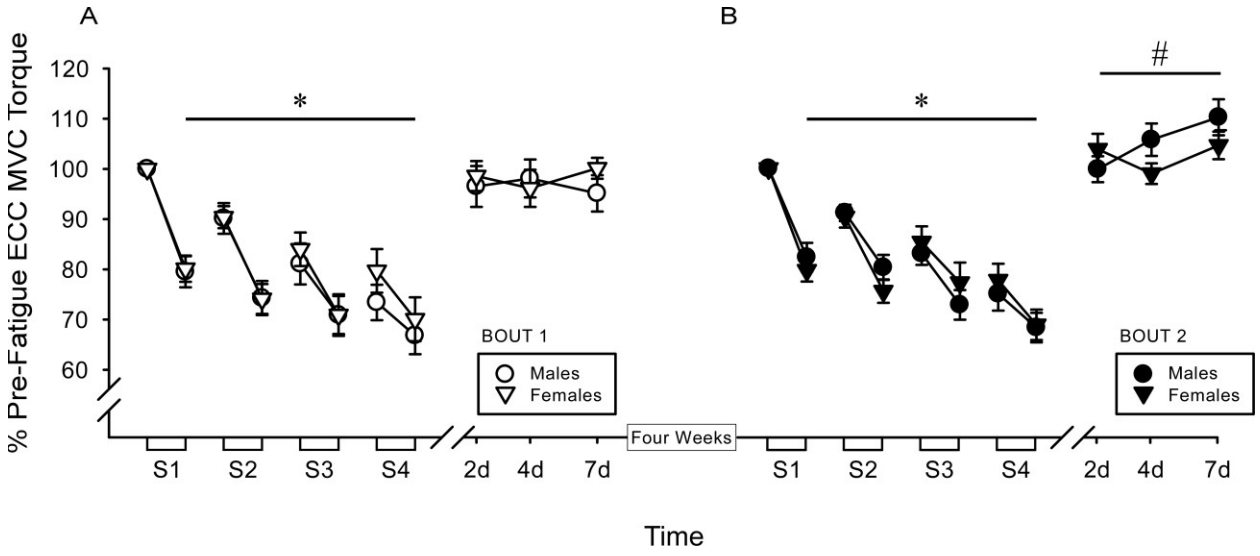

Figure 2. Normalized eccentric MVC torque during and after the fatigue protocol for males (circles) and females (triangles). Data from bout one (A; open symbols; 12 males and 11 females) and bout two (B; closed symbols; 10 males and 9 females) are presented with each symbol representing the mean (± SEM) of five contractions from the beginning and end of each of the four sets (i.e., S1, S2, etc.) and from the recovery visits on day two, four and seven. All data points are normalized to the first five contractions of the fatiguing protocol for each bout (first symbols of S1). ECC MVC torque was impaired during the fatiguing protocol of both bouts (denoted by *) and recovered by day 2 days for both groups following bout one and two (*$P < 0.05$). ECC MVC torque was significantly greater during the long-term recovery time points after second bout compared to the first bout (# $P < 0.05$).

4.2.2 Isometric Maximal Voluntary Contraction Torque

During the acute recovery time period, there was no sex × time interaction ($F_{1.8, 39.5} = 1.5$, $\eta^2 = 0.06$, $P = 0.23$), no main effect of sex ($F_{1, 22} = 1.9$, $\eta^2 = 0.008$, $P = 0.18$), and a main effect of

time ($F_{1.8, 39.5} = 51.4$, $\eta^2 = 0.70$, $P < 0.05$) for ISO MVC torque (Figure 3A). The fatiguing protocol significantly impaired ISO MVC torque by 25.2 ± 14% for both groups at two minutes which modestly recovered to 21.3% by 10 minutes post-fatigue. During the days following the fatiguing exercise, there was no sex × time interaction ($F_{1.9, 42.1} = 1.5$, $\eta^2 = 0.06$, $P = 0.25$), no main effect of sex ($F_{1, 22} = 1.1$, $\eta^2 = 0.05$, $P = 0.30$), and a main effect of time ($F_{1.9, 42} = 9.2$, $\eta^2 = 0.30$, $P < 0.05$; Figure 3A). *Post hoc* analyses determined all acute and long-term time points were significantly different from baseline values ($P < 0.05$; Figure 3A). Below, ISO MVC torque responses are presented for bout one (Figure 3A) and bout two (Figure 3B), with bout two responses reported in the Repeated-Bout Effect section (4.5.2).

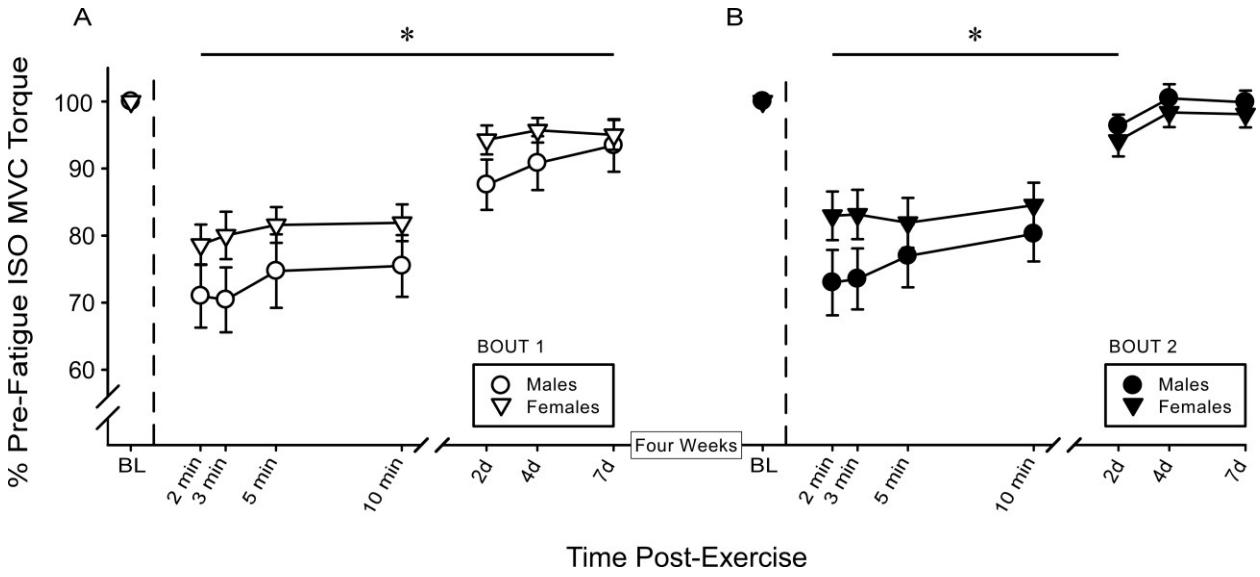

Figure 3. Fatigue-induced changes in normalized isometric (ISO) peak MVC torque for males (circles) and females (triangles). The vertical dashed line in both panels represents time of the fatiguing protocol in relation to baseline (BL) and recovery measures. ISO MVC responses from bout one (A; open symbols; 12 males and 12 females) and bout two (B; closed symbols; 12 males and 10 females) are presented with all values representing means ± SEM. For data pooled across the sexes, ISO MVC torque had not recovered by 7 days after bout one (A) but was not different from BL by 4 days after bout two (B). (*$P <$ 0.05).

4.3 Prolonged Low-Frequency Force Depression

Following bout one, the 10 Hz tetanus peak torque response was markedly impaired two minutes following exercise (38.1% from baseline) and continued to decrease until ten minutes post-exercise (57.2%; Figure 4A). For PT of the 10 Hz train during the minutes following

damaging eccentric contractions, there was no sex × time interaction ($F_{1.5, 32.4} = 0.37$, $\eta^2 = 0.02$, $P = 0.63$) or main effect of sex ($F_{1, 22} = 0.15$, $\eta^2 = 0.007$, $P = 0.71$) with a main effect of time ($F_{1.5, 32.4} = 228.3$, $\eta^2 = 0.91$, $P < 0.05$; Figure 4A). *Post hoc* analyses determined all recovery time points were lower than baseline ($P < 0.05$), meaning the 10 Hz train did not recover to baseline within one week post-exercise. For PT of the 100 Hz train, statistical analysis indicated no sex × time interaction ($F_{1.9, 42.6} = 1.11$, $\eta^2 = 0.05$, $P = 0.34$) or main effect of sex ($F_{1, 22} = 0.69$, $\eta^2 = 0.03$, $P = 0.41$) with a main effect of time ($F_{1.9, 42.6} = 16.1$, $\eta^2 = 0.42$, $P < 0.05$; Figure 4C) following the fatiguing protocol. *Post hoc* analyses determined the 100 Hz PT responses recovered by day 2 ($P < 0.05$). With respect to the 10:100 Hz PLFFD ratio during the minutes following exercise, there was no sex × time interaction ($F_{1.7, 38.3} = 0.9$, $\eta^2 = 0.04$, $P = 0.42$) or main effect of sex ($F_{1, 22} = 1.2$, $\eta^2 = 0.05$, $P = 0.28$) but there was a main effect of time ($F_{1.7, 38.3} = 201.9$, $\eta^2 = 0.90$, $P < 0.05$; Figure 5A). There was also no sex × time interaction ($F_{3, 59.8} = 1.6$, $\eta^2 = 0.07$, $P = 0.19$) or main effect of sex ($F_{1, 22} = 0.73$, $\eta^2 = 0.03$, $P = 0.40$) but there was a main effect of time ($F_{3, 59.8} = 13.9$, $\eta^2 = 0.39$, $P < 0.05$; figure 5A) during the days following exercise. *Post hoc* analyses determined all recovery time points following bout one were significantly lower than baseline ($P < 0.05$), meaning the 10:100 Hz ratio did not recover to baseline within one week post-exercise. Two minutes following the fatiguing contractions, the 10:100 Hz ratio fell to 70.6 ± 16.2% of baseline values for both groups and continued to decrease until 10 minutes post-exercise (46.9 ± 9.1% of baseline values). Impairment and recovery of the PLFFD ratio as well as the individual 10 and 100 Hz train PT responses following bout one and two are presented below. The results from bout two are reported in the Repeated-Bout Effect section of the results (4.5.3).

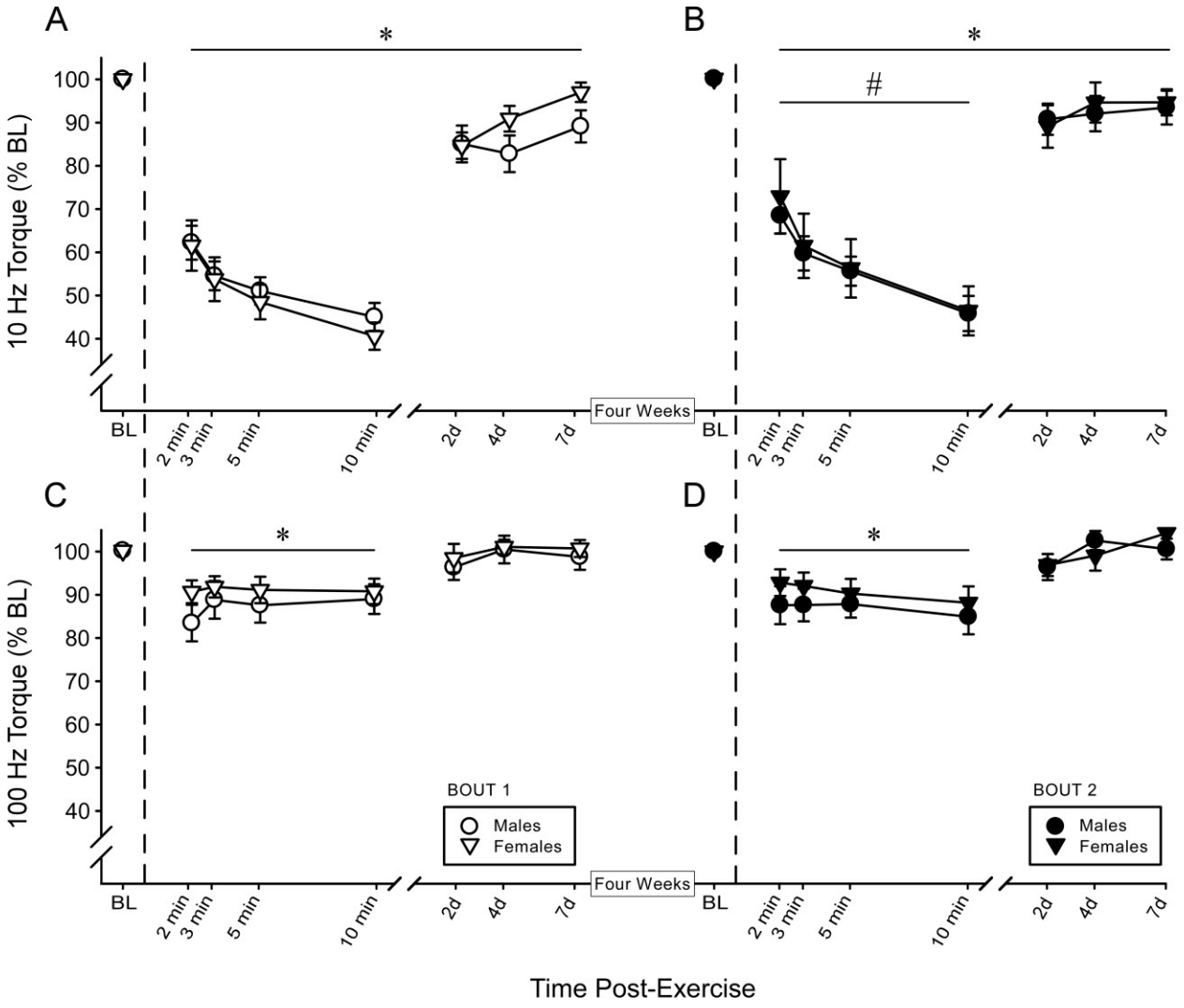

Figure 4. Fatigue-induced changes in normalized 10 (A and B) and 100 (C and D) Hz peak torque (PT) for males (circles) and females (triangles). For bout one (A and C; open symbols; 12 males and 12 females) and bout two (B and D; closed symbols; 12 males and 10 females), values are means ± SEM and the vertical dashed line represents the time of the fatiguing protocol with respect to baseline and recovery measures. Following the first and second fatiguing protocol, PT of the 10 Hz train was significantly impaired and did not return to baseline before 7 days (A and B). PT from the 100 Hz train was also significantly impaired following the first and second fatiguing protocol but returned to baseline values before 2 days (C and D; $*P < 0.05$). During the acute recovery, peak torque of the 10 Hz train was significantly higher following bout two (B) compared to bout one ($\#P < 0.05$).

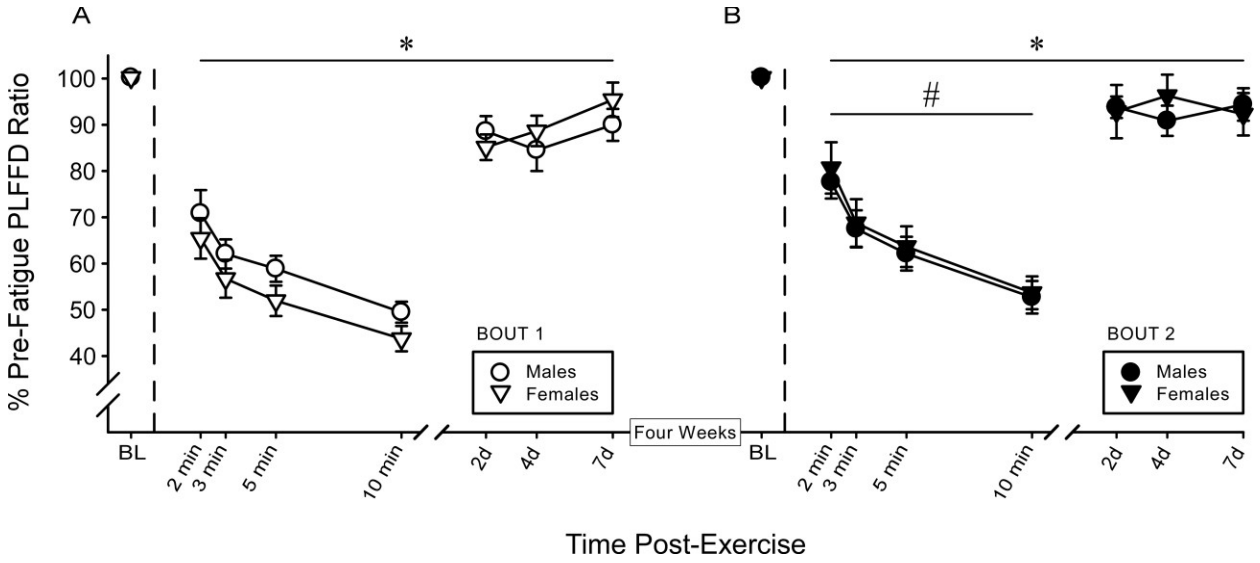

Figure 5. Fatigue-induced changes in normalized prolonged low-frequency force depression (PLFFD) ratio for males (circles) and females (triangles) during bout 1 (A; open symbols; 12 males and 12 females) and bout 2 (B; closed symbols; 12 males and 10 females). Values are means ± SEM and the vertical dashed lines represent time of the fatiguing protocol in relation to baseline (BL) and recovery measures. The 10:100 Hz ratio did not recovery by 7 days following the first (A) or second bout (B) for both groups (*$P < 0.05$). The acute recovery time points after bout two are significantly higher than acute recovery values after bout one (#$P < 0.05$).

4.4 Voluntary Activation

Following the first fatiguing protocol of ECC contractions, a two-way ANOVA indicated there was no sex × time interaction ($F_{2.6, 56.6} = 1.21$, $\eta^2 = 0.05$, $P = 0.31$), no main effect of sex ($F_{1, 22} = 3.45$, $\eta^2 = 0.14$, $P = 0.08$), but a main effect of time ($F_{2.6, 56.6} = 5.53$, $\eta^2 = 0.20$, $P < 0.05$; Figure 6A) for VA during the acute recovery time points. With data pooled for both groups, VA was significantly reduced from baseline at two minutes post-fatigue (declined from 99.0 ± 0.8% to 94.5 ± 6.1%) and still lower than baseline at 10 min post-fatigue (95.5 ± 4.6%; Figure 6A). *Post hoc* analysis determined VA was impaired during the acute recovery time points and recovered to baseline measures by day 2 ($P < 0.05$). During the days following the damaging eccentric contractions, there was a sex × time interaction ($F_{3, 66} = 3.6$, $\eta^2 = 0.14$, $P < 0.05$) with a main effect of sex ($F_{1, 22} = 5.4$, $\eta^2 = 0.20$, $P < 0.05$), but no main effect of time ($F_{3, 66} = 1.3$, $\eta^2 = 0.06$, $P = 0.29$; Figure 6A) for VA. VA was higher for females in the days following fatigue but *post hoc* analysis revealed no specific time points were different from one another. Below, bout one (A) and bout

two (B) are presented with the results reported in the Repeated-Bout Effect section of the results (4.5.4).

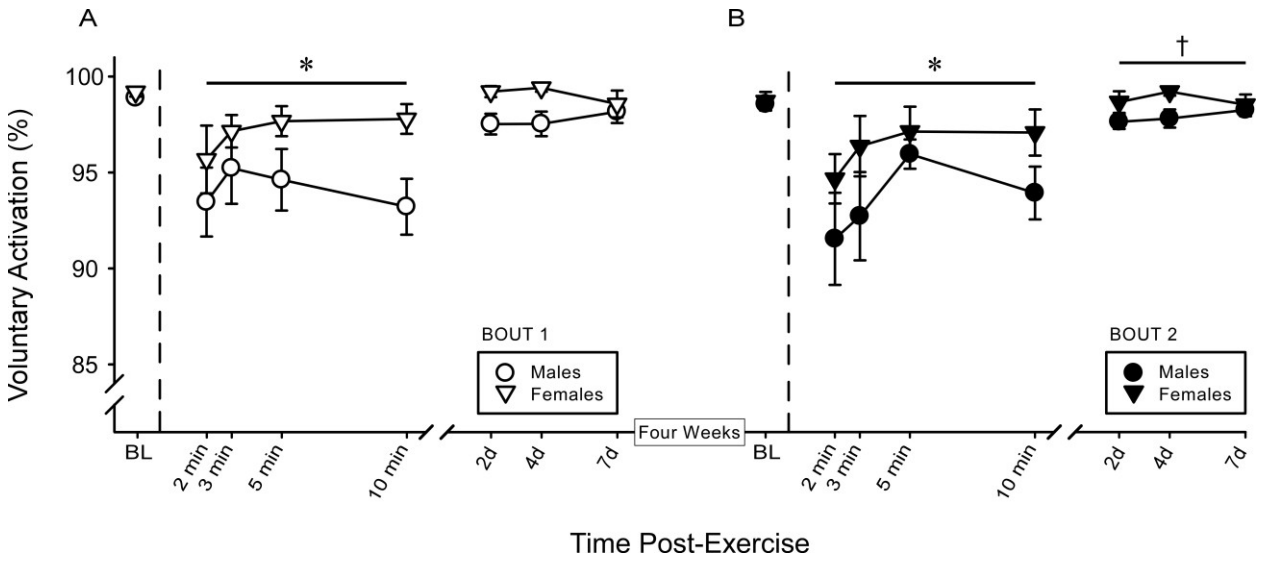

Figure 6. Fatigue induced changes in voluntary activation for males (circles) and females (triangles) during bout 1 (A; open symbols; 12 males and 12 females) and bout 2 (B; closed symbols; 12 males and 10 females). Values are means ± SEM and the vertical dashed lines represent time of the fatiguing protocol with respect to baseline and recovery measures. VA was significantly impaired by two minutes post-fatigue and recovered by 2 days following the first (A) and second (B) bout for both groups (*P < 0.05). In the days following the fatiguing protocol, there was a group × time interaction and a main effect of sex (†P < 0.05).

4.5 Repeated-Bout Effect

Of the 24 participants who performed the first bout of eccentric exercise, all 12 males and 10 females completed the second bout; however, due to human error and technical difficulties, the second bout of ECC MVC data from two males and one female participant were unobtainable. Thus, for the RBE ECC MVC data, 10 males and 9 females were included in the analysis. With regards to RBE ISO MVC, PLFFD, and VA measures, data were recorded from all 12 males and 10 females. Baseline values from the second bout of eccentric exercise for ISO and ECC MVC torque, electrically-evoked torque responses (10 Hz, 100 Hz, and 10:100 ratio) and VA were not different from baseline values prior to the first bout. The absolute ISO and ECC MVC torque values of males were 64.6% and 67.1% greater compared to those of females before performing bout two (P < 0.05). VA was not different between the sexes prior to fatigue. The PT responses of

the 10 and 100 Hz train were greater for males compared to those of females (62.3% and 62%, respectively; $P < 0.05$). Consequently, like the first bout, the 10:100 Hz ratio prior to fatigue was not different between females and males. In order to investigate if a RBE occurred, a three-way factorial ANOVA (group × bout × time) was performed to identify if a significant interaction effect existed for bout or sex for ECC and ISO MVC torque, PT responses from the 10 and 100 Hz trains, the 10:100 Hz (PLFFD) ratio, and VA.

4.5.1 Maximal Eccentric Voluntary Contraction

Statistical analysis revealed there was no group × bout × time interaction ($F_{3.9, 66.9} = 0.84$, $\eta^2 = 0.05$, $P = 0.50$), no main effect of bout ($F_{1, 17} = 0.95$, $\eta^2 = 0.05$, $P = 0.34$) or sex ($F_{1, 17} = 0.00$, $\eta^2 = 0.00$, $P = 0.99$) for ECC MVC torque during bout two of eccentric exercise (Figure 2B). Similar to bout one, there was a main effect of time ($F_{3.2, 55.1} = 86.4$, $\eta^2 = 0.84$, $P < 0.05$) in bout two. When comparing bout one and two, the ~2.2% difference in ECC MVC torque impairment by the end of the fatiguing protocol (S4) was not significant. During the days following the second bout of damaging eccentric contractions, there was a time × bout interaction ($F_{3, 51} = 4.3$, $\eta^2 = 0.20$, $P < 0.05$), and a main effect of bout ($F_{1, 17} = 5.92$, $\eta^2 = 0.26$, $P < 0.05$) with no main effect of time ($F_{3, 51} = 0.79$, $\eta^2 = 0.05$, $P = 0.50$). From pooled data across the days following the second bout of eccentric exercise, ECC MVC torque recovered by two days post-fatigue and was a 3.8% improvement compared to bout one (Figure 2B).

4.5.2 Maximal Isometric Voluntary Contraction

For ISO MVC torque during the second acute recovery period, there was no group × bout × time interaction ($F_{2.2, 44.1} = 0.80$, $\eta^2 = 0.04$, $P = 0.47$), no main effect of bout ($F_{1, 20} = 2.07$, $\eta^2 = 0.09$, $P = 0.17$) or sex ($F_{1, 20} = 1.7$, $\eta^2 = 0.08$, $P = 0.21$). Similar to bout one, there was a main effect of time for bout two ($F_{1.5, 29.1} = 53.2$, $\eta^2 = 0.73$, $P < 0.05$; Figure 3B). The ISO MVC torque was not different from bout 1 (~3.7% improvement between bouts; Figure 3A and B) two minutes after eccentric exercise. During the days following the fatiguing protocol, there was no group × bout × time interaction ($F_{3, 60} = 1.4$, $\eta^2 = 0.06$, $P = 0.26$), no main effect of bout ($F_{1, 20} = 3.1$, $\eta^2 = 0.14$, $P = 0.09$) or sex ($F_{1, 20} = 0.41$, $\eta^2 = 0.02$, $P = 0.53$). Similar to bout one, there was a main effect of time for ISO MVC following bout two ($F_{3, 60} = 10.8$, $\eta^2 = 0.35$, $P < 0.05$; figure 3B). *Post hoc* analysis determined ISO MVC following the second bout of eccentric contractions did not recover until 4 days post-exercise ($P < 0.05$).

4.5.3 Prolonged Low-Frequency Force Depression

Statistical analysis of the acute recovery time points revealed the individual 10 Hz PT responses between bout one and two had no group × bout × time interaction ($F_{2.1, 42.3} = 0.69$, $\eta^2 = 0.03$, $P = 0.51$), a bout × time interaction ($F_{2.1, 42.3} = 4.3$, $\eta^2 = 0.02$, $P < 0.05$), and no bout × group interaction ($F_{1, 20} = 1.1$, $\eta^2 = 0.05$, $P = 0.31$). There was a main effect of bout ($F_{1, 20} = 5.2$, $\eta^2 = 0.21$, $P < 0.05$), no main effect of sex ($F_{1, 20} = 0.07$, $\eta^2 = 0.004$, $P = 0.79$), and a main effect of time ($F_{1.4, 27.4} = 216.7$, $\eta^2 = 0.92$, $P < 0.05$) in the minutes following exercise. There was ~10% improvement in the 10 Hz train PT response in the first two minutes, a 7.84% improvement at three minutes, and a 7.0% improvement at five minutes following the second bout of eccentric exercise compared to the first (Figure 4B). Days following the second bout of eccentric exercise, statistical analysis determined there was no group × bout × time interaction ($F_{3, 60} = 0.70$, $\eta^2 = 0.03$, $P = 0.56$), no bout × time interaction ($F_{3, 60} = 2.1$, $\eta^2 = 0.09$, $P = 0.12$), and no bout × group interaction ($P = 1$) for the 10 Hz PT response. There was no main effect of bout ($F_{1, 20} = 2.2$, $\eta^2 = 0.10$, $P = 0.15$), no main effect of sex ($F_{1, 20} = 0.97$, $\eta^2 = 0.05$, $P = 0.34$), and a main effect of time ($F_{3, 60} = 10.8$, $\eta^2 = 0.35$, $P < 0.05$). *Post hoc* analysis determined that following the second bout of eccentric exercise, the 10 Hz PT response did not recover before 7d post-fatigue ($P < 0.05$).

For the 100 Hz PT responses following bout two, statistical analysis of the acute recovery time points revealed no group × bout × time interaction ($F_{2.3, 46.2} = 0.2$, $\eta^2 = 0.01$, $P = 0.94$), no bout × time interaction ($F_{2.3, 46.2} = 1.6$, $\eta^2 = 0.07$, $P = 0.21$), and no bout × group interaction ($F_{1, 20} = 0.22$, $\eta^2 = 0.01$, $P = 0.65$). There was no main effect of bout ($F_{1, 20} = 0.19$, $\eta^2 = 0.009$, $P = 0.67$), no main effect of sex ($F_{1, 20} = 0.46$, $\eta^2 = 0.02$, $P = 0.51$), and a main effect of time ($F_{1.8, 35.1} = 20.0$, $\eta^2 = 0.50$, $P < 0.001$). *Post hoc* analysis indicated the PT response from the 100 Hz train was lower than baseline during the acute recovery period ($P < 0.05$). In the days following eccentric exercise, statistical analysis of the 100 Hz PT response revealed no group × bout × time interaction ($F_{3, 60} = 0.28$, $\eta^2 = 0.01$, $P = 0.84$), no bout × time interaction ($F_{3, 60} = 0.53$, $\eta^2 = 0.03$, $P = 0.66$), and no bout × group interaction ($F_{1, 20} = 0.01$, $\eta^2 = 0.001$, $P = 0.91$). There was no main effect of bout ($F_{1, 20} = 0.44$, $\eta^2 = 0.02$, $P = 0.52$), no main effect of sex ($F_{1, 20} = 0.02$, $\eta^2 = 0.001$, $P = 0.89$), and a main effect of time ($F_{3, 60} = 3.0$, $\eta^2 = 0.13$, $P < 0.05$). By day 2, PT of the 100 Hz train recovered to within 4% of baseline values.

Statistical analysis of the acute recovery time points revealed the 10:100 Hz ratio between bout one and two had no group × bout × time interaction ($F_{2.2, 43.9} = 2.1$, $\eta^2 = 0.09$, $P = 0.14$), a

bout × time interaction ($F_{2.2, 43.9} = 6.7$, $\eta^2 = 0.25$, $P < 0.05$), and no bout × group interaction ($F_{1, 20} = 3.0$, $\eta^2 = 0.13$, $P = 0.10$). There was a main effect of bout ($F_{1, 20} = 11.4$, $\eta^2 = 0.36$, $P < 0.05$), no main effect of sex ($F_{1, 20} = 0.30$, $\eta^2 = 0.02$, $P = 0.60$), and a main effect of time ($F_{1.7, 34.4} = 201$, $\eta^2 = 0.91$, $P < 0.05$). For data pooled between groups, there was ~9.1% improvement in the PLFFD ratio in the first two minutes, 9.0% by three minutes, and 7.5% by ten minutes following the second bout of eccentric exercise compared to the first (Figure 5B). Days following the second bout of eccentric exercise, statistical analysis of the PLFFD ratio revealed no group × bout × time interaction ($F_{3, 60} = 0.7$, $\eta^2 = 0.03$, $P = 0.56$), no bout × time interaction ($F_{3, 60} = 2.1$, $\eta^2 = 0.09$, $P = 0.11$), and no bout × group interaction ($P = 1$). There was no main effect of bout ($F_{1, 20} = 2.2$, $\eta^2 = 0.10$, $P = 0.15$), no main effect of sex ($F_{1, 20} = 0.97$, $\eta^2 = 0.05$, $P = 0.34$), and a main effect of time ($F_{3, 60} = 10.8$, $\eta^2 = 0.35$, $P < 0.05$). *Post hoc* analysis determined that the PLFFD ratio did not recover before 7 days following the second bout of eccentric contractions ($P < 0.05$).

4.5.4 Voluntary Activation

Following statistical analysis of voluntary activation, no group × bout × time interaction ($F_{4, 80} = 0.88$, $\eta^2 = 0.04$, $P = 0.48$), no bout × time interaction ($F_{4, 80} = 1.0$, $\eta^2 = 0.05$, $P = 0.41$), and no bout × group interaction ($F_{1, 20} = 0.03$, $\eta^2 = 0.001$, $P = 0.87$). No main effect of bout ($F_{1, 20} = 0.48$, $\eta^2 = 0.02$, $P = 0.50$) or sex ($F_{1, 20} = 2.50$, $\eta^2 = 0.11$, $P = 0.13$) was found; however, there was a main effect of time ($F_{2.2, 44.9} = 8.3$, $\eta^2 = 0.29$, $P < 0.05$). Similar to bout one (Figure 6A), VA was impaired at 2, 3, 5 and 10 minutes following damaging eccentric contractions for both groups (Figure 6B). Days following the fatiguing eccentric contractions, there was no group × bout × time interaction ($F_{2.1, 42.6} = 0.52$, $\eta^2 = 0.03$, $P = 0.67$), no time × bout interaction ($F_{2.1, 42.6} = 0.30$, $\eta^2 = 0.02$, $P = 0.76$) but there was a time × group interaction ($F_{2.1, 42.7} = 4.42$, $\eta^2 = 0.18$, $P < 0.05$). There was no main effect of bout ($P = 0.44$), group ($P = 0.08$) or time ($P = 0.12$) and *post hoc* analysis revealed no specific time points were different between the groups. During the second bout, VA recovered by 2 days post-fatigue (figure 6B).

Chapter 5: Discussion

5.1 Impairments in Neuromuscular Function

Contrary to the hypotheses, females did not experience significantly greater impairments in eccentric MVC torque during the fatiguing protocol nor did they experience a greater impairment to isometric MVC torque compared to males after the protocol. Results from the present study also indicate females and males experienced a similar magnitude of the repeated bout effect following unaccustomed eccentric exercise in the dorsiflexors. The eccentric exercise protocol elicited impairments in all measures during and after bout one. Evidence of a RBE is indicated by an accelerated recovery time of the isometric MVC torque, greater recovery of eccentric MVC torque in the days after the exercise protocol, as well as an attenuated impairment of 10 Hz peak torque and the 10:100 Hz ratio (PLFFD) in the minutes following the second bout of eccentric exercise.

5.1.1 Fatiguing Eccentric Contractions

In the present study, both females and males experienced a similar impairment in their ability to generate maximal ECC torque during a bout of unaccustomed dorsiflexor exercise. By the end of the fatiguing protocol, eccentric contraction torque was impaired by ~31% but recovered within two days. Findings from Hubal et al. (2008), insinuated that with a longer protocol (i.e., more contractions), females would present with a greater impairment to ECC MVC torque loss compared to males in the elbow flexors as they were trending in such a way. Although in a different muscle group, we hypothesized to see a greater impairment to ECC MVC torque in females in response to 200 dorsiflexions which is 50 contractions more than what previous studies used to induce damage in the dorsiflexors (McNeil, Vandervoort, & Rice, 2007; Power et al., 2010; Power, Dalton, Rice, Vandervoort, 2012; Power et al., 2013).

5.1.2 Maximal Isometric Voluntary Contractions

It has been known for decades that unaccustomed lengthening contractions lead to structural damage of the muscle fibre and impairments in force production for both animal (Balnave et al., 1997; Lännergren, Westerblad, & Bruton, 1996) and human (Clarkson & Hubal, 2002; Hubal, Rubinstein, & Clarkson, 2007; Martin, Millet, Lattier, & Perrod, 2004) models. In humans, it appears that high-intensity eccentric contractions elicit membrane and myofibril

damage, which ultimately impairs voluntary, as well as electrically-evoked, force production (Clarkson & Hubal, 2002). A prolonged decrease of maximal isometric force production is one of the most commonly used and most reliable indirect measures of muscle damage in humans (Warren et al., 1999). We saw a modest decrease in isometric MVC torque (~25%) which is comparable to previous findings from Power and colleagues who saw a 28% reduction in MVC strength following 150 eccentric contractions at 80% of MVC torque (Power et al., 2010).

Various studies have assessed neuromuscular function following a bout of damaging eccentric exercise (Goodall et al., 2017; McNeil, Allman, Symons, Vandervoort, & Rice, 2004; Power et al., 2013; Prasartwuth, Taylor, & Gandevia, 2005), with only a few specifically investigating sex-related differences (Hubal, Rubinstein, & Clarkson, 2008; Lee et al., 2017; Sewright et al., 2008; Stupka et al., 2000). These studies have equivocal results with regards to isometric voluntary strength, with one reporting no sex-related differences within the knee extensors (Lee et al., 2017) while the other reported a greater impairment within the elbow flexors of females (Sewright et al., 2008). Lee and colleagues found a ~39% reduction in maximal voluntary isometric torque which did not recover before 4 days post fatigue (Lee et al., 2017). Although no statistical sex differences were found, males from that particular study experienced more of an impairment to isometric MVC compared to that of females, which is comparable to the results of my study (female and male participants experienced a 21.4% and 29.0% reduction two minutes into recovery, respectively). Interestingly, Sewright and colleagues saw an immediate reduction of 57.8% and 50.4% in isometric MVC torque for females and males, respectively, which was significantly lower in females. For almost ten days following eccentric exercise, impairments to isometric MVC were physiologically lower in females compared to males although this was not statistically different (Sewright et al., 2008). Hubal and colleagues found no differences between males and females and did not report measures in the days following fatigue (2008). However, towards the end of the 50 fatiguing eccentric contractions, it appeared that the eccentric MVC torque of the females was becoming more impaired than the males and with additional contractions, may have become significantly different. Discrepancies between these two studies may be due to the structure of the fatiguing protocols; both included 50 eccentric MVCs of the elbow flexors but the study which found no significant differences allowed for greater rest time (~3 minutes more; Hubal et al., 2008) compared to the other study (Sewright et al., 2008).

Results from the two aforementioned studies can lead one to predict a fatiguing protocol with a sufficient amount of damaging contractions may tease out any possible sex differences.

The dorsiflexors were chosen for this particular study for ease of accessibility to stimulate the peroneal nerve (for the 10:100 Hz ratio), as well as for the high voluntary activation levels reported in this particular muscle group (Klass, Baudry, & Duchateau, 2007). However, it appears that the dorsiflexors are less susceptible to exercise induced damage compared to other muscle groups, possibly due to frequent use for locomotion. The loss of isometric MVC torque in the present study is comparable to previous studies that examined the dorsiflexors (loss of 27 - 32%; McNeil et al., 2004) and found no statistical differences between the sexes (loss of 28%; Power et al., 2010).

5.1.3 Prolonged Low-Frequency Force Depression

Isometric MVC torque loss is considered to be the best indirect measure of muscle damage (Warren et al., 1999). However, because unaccustomed, high-intensity eccentric contractions cause mechanical disruption (E-C uncoupling) that leads to a reduction in the amount of Ca^{2+} released per action potential, a brief MVC is likely to underestimate the magnitude of muscle damage as an MVC leads to saturation of intracellular Ca^{2+}. Furthermore, MVCs are uncommon and unnecessary for everyday tasks and it is therefore more functionally relevant to assess force output (and force loss) at motor unit discharge rates (MUDRs) which are similar to everyday tasks when Ca^{2+} handling is most impaired (Allen et al., 2008). High frequency stimulation represents the plateaued region of the force- Ca^{2+} relationship where small changes in intracellular calcium lead to minimal changes in force production (much like during an MVC) whereas changes in intracellular calcium lead to a greater change in force production during low frequency stimulations (Allen et al., 2008). Because of this, the 10:100 Hz ratio (PLFFD) may be a better indirect measure of muscle damage as it incorporates both ends of the force-frequency relationship during impaired Ca^{2+} handling.

No sex-related differences were found with the magnitude of PLFFD following eccentric contractions of the dorsiflexors. On average, both groups experienced a 32% impairment of the 10:100 Hz ratio from baseline to two minutes post-fatigue, which continued to decrease, reaching a nadir of 54% at 10 minutes post-fatigue. A decrease in the low to high frequency stimulation ratio following eccentric damage has been attributed to E-C uncoupling from a disruption in the interaction between the RYR and the DHPR (Allen et al., 2008; Balnave & Allen, 1995;

Lännergren et al., 1996), ultimately leading to impairments in Ca^{2+} handling and reduced cross-bridge formation at low frequencies. The reduction in the 10 Hz peak torque response was 57% from baseline whereas the 100 Hz peak torque was only reduced by 10% from baseline at 10 minutes of recovery. This supports the notion that the contractile components of the muscle fibre are still relatively functional at high MUDRs, with saturation of myoplasmic [Ca^{2+}] leading to negligible isometric torque loss. The reduction in the amount of Ca^{2+} released per action potential is more apparent at low frequencies (i.e., low MUDRs) leading to greater reductions in force. With the 10 Hz peak torque response being more impaired and for longer than the 100 Hz during recovery, our results suggest greater impairments in force production at low compared to high frequency stimulation.

Previous studies have assessed prolonged low-frequency force depression in humans following unaccustomed lengthening contractions in the elbow flexors (Janecki et al., 2014) and the dorsiflexors (McNeil et al., 2004; Ruggiero et al., 2019) but none have compared females to males across multiple bouts of eccentric exercise. Although not statistically different, it is interesting to note the degree of PLFFD experienced by females and how this changed following the second bout of exercise in comparison to males. Females presented with numerically greater PLFFD following the first bout (35.4% compared to 29.4%, respectively) and had a larger repeated bout effect compared to that of males. Two minutes following the fatiguing protocols, the decrement in the PLFFD ratio between the two bouts improved by 6.8% for males and 17.4% for females. Previous findings suggest female connective tissue is more susceptible to damaging eccentric contractions within the elbow flexors due to females experiencing a greater loss in range of motion than males (Rinard et al., 2000). Further investigations including direct measures of muscle damage (i.e., muscle biopsies) are needed to assess the role of connective tissue damage and calcium handling impairments.

5.1.4 Voluntary Activation

Our results have shown that repeated maximal eccentric contractions significantly impair voluntary activation to the dorsiflexors in both males and females for the first 10 minutes following exercise. With no differences found between the sexes, average impairment in voluntary activation was 5.5 ± 6.1% at two minutes following the first bout and was not different following the second bout.

The presence of central fatigue was somewhat of an unexpected finding considering previous studies which performed high-intensity ECC MVCs of the dorsiflexors found no impairments in voluntary activation (McNeil et al., 2004; Power et al., 2010; Power et al., 2012). It is important to note however, that the impairments of VA between my results and those reported by Power and colleagues (~ 5% decrease; 2010) are similar. The discrepancy in results may be due to differences in fatiguing protocols. Power and colleagues set the intensity of the eccentric contractions to be 80% of isometric maximal voluntary strength for 150 contractions whereas participants from my study completed 200 maximal eccentric voluntary contractions. More contractions could have placed a greater metabolic demand on the fatiguing muscle which in turn could increase the firing rate of fatigue-sensitive group III/IV afferent nerves which has been found to impair VA (e.g., Kennedy et al., 2014).

It is interesting to note, although not statistically different from one another, the males experienced a numerically greater impairment in their ability to voluntarily contract the dorsiflexors maximally compared to that of females. Not only was this the case following both bouts of exercise, VA for males became increasingly more impaired during the acute recovery phase, but not for females. Previous studies have indicated that males have more of an inflammatory response (i.e., more inflammatory markers present in the skeletal muscle tissue) following eccentric contractions compared to females (Kerksick et al., 2008; Stupka et al., 2000). The influx of various leukocytes from arterial blood into the damaged muscle tissue could increase excitability of III/IV afferents which could inhibit voluntary drive (Kamandulis, Skurvydas, Brazaitis, Škikas, & Duchateau, 2010; Kennedy, McNeil, Gandevia, & Taylor, 2014), although this is only speculative. Some studies have found impairments to VA following eccentric exercise in muscles other than the dorsiflexors (Goodall et al., 2017; Kamandulis et al., 2010; Lee et al., 2017; Prasartwuth et al., 2005). Albeit, the magnitude of VA impairment found in my study is considerably less compared to the aforementioned studies, all of which saw reductions of no less than ~10%. The varying levels of VA may be due to differences in muscle groups since VA of the dorsiflexors has previously been found to be high before and after fatigue (Power et al., 2010). Similar to our results, Lee and colleagues recently found no sex differences with impairments in VA following eccentric contractions of the knee extensors (2017). Interestingly, females who participated in the aforementioned study had a numerically greater impairment in VA compared to males following eccentric contractions of the knee extensors which is opposite to the

observation from my study where males had a numerically lower VA level following exercise. With recent studies suggesting changes occur within the central nervous system following damaging eccentric contractions (Goodall et al., 2017; Lee et al., 2017), the conflicting results of VA levels following eccentric exercise warrants further investigation.

5.2 The Repeated-Bout Effect

Results from the current study indicate females and males experience a similar level of muscle damage and fatigue in response to unaccustomed eccentric contractions. Consequently, there were no sex differences in the magnitude of the repeated bout effect of the dorsiflexors.

As a group, impairments to the 10 Hz tetani (but not the 100 Hz tetani) were significantly less following the second bout of eccentric exercise compared to the first during the first ten minutes of recovery. Subsequently, the decrease in the 10:100 Hz ratio was also significantly less following the second bout of exercise. It has been suggested that the mechanism responsible for greater impairments in torque during low compared to high frequency stimulation is impaired Ca^{2+} release from the sarcoplasmic reticulum (Kamandulis et al., 2017). A proposed mechanism of eccentric induced muscle damage (E-C uncoupling) includes the disruption of sarcomeres, leading to membrane damage (Proske & Morgan, 2001). There is evidence to suggest that the addition of sarcomeres in series reduces the mechanical strain endured by the muscle fibres following the second bout, and in doing so, shifts the optimal angle required of the muscle to produce peak force (Proske & Morgan, 2001). However, the protective effects of the RBE appear to outlast the shift in optimal angle (Chen, Nosaka & Sacco, 2006) suggesting other mechanisms, such as tendon compliance, that may be contributing to the RBE (Hyldahl et al., 2017).

With regards to centrally-mediated mechanisms assessed via voluntary activation, results in the current literature are equivocal where some studies have found no neural adaptations (Kamandulis et al., 2010) while other studies have found changes to VA following a repeated bout of eccentric contractions (Goodall et al., 2017). Results from the current study revealed there were no differences of VA between bouts, for either group. This insinuates the mechanisms responsible for the RBE of the dorsiflexors are likely mechanical in nature rather than neural, which has recently been implied (Goodall et al., 2017) from attenuated responses in VA of the elbow flexors.

Chapter 6: Conclusion

6.1 Limitations and Considerations

Neither menstrual cycle or oral contraceptives were controlled for in the current study which may have an effect on performance (Bambaeichi, Reilly, Cable, & Giacomoni, 2004) and recovery time following eccentric contractions (Savage & Clarkson, 2002), respectively. Four weeks was chosen as the time between bouts in order to test females during the same time within their menstrual cycle for both bouts. Additionally, de Jonge and colleagues found that muscle contractile characteristic were not affected by fluctuations in female reproductive hormones throughout the menstrual cycle (2001). It is also important to note that estrogen has previously been suggested to have protective effects with regards to muscle fatigue during metabolically demanding exercise (Carter, Dobridge, & Hackney, 2001). If estrogen is beneficial when metabolic wastes accumulate following exercise (such as reactive oxygen species; ROS), one possible reason for an absence of sex differences in our study is due to the energy-conserving nature of eccentric contractions as they are less metabolically demanding compared to concentric or isometric contractions (Nishikawa et al., 2018), however this is only speculative.

6.2 Future Directions

Considering the RBE magnitude from the current study, it would be interesting to see if the same results are obtained from a muscle group that is more prone to eccentric-induced damage; e.g., the elbow flexors (Hubal et al., 2008). Although not statistically different, it is interesting to note that females had a numerically greater impairment to the 10 Hz tetanus and to the 10:100 Hz ratio after the first bout, and had a greater improvement in the 10:100 Hz ratio (almost double) following the second bout compared to males. On the other hand, males experienced the numerically greater impairment to ISO MVC but this is merely an observation and not a statistically significant finding. Isometric MVC torque has been considered the most reliable, accurate indirect measure to assess muscle injury following damaging eccentric contractions (Warren et al., 1999). With more recent evidence to suggest that there can be a central component to fatigue as a result of damaging eccentric contractions (Goodall et al., 2017), isometric MVC torque loss would be inadequately assessing peripheral and central impairments. Finally, results from this study highlight the importance of using more than just a maximal isometric contraction or resting twitch to obtain information regarding peripheral impairments. Studies have previously

utilized the resting twitch as the main indicator for peripheral fatigue (e.g., Goodall et al., 2017), however, only using a twitch will overestimate the magnitude of force impairment (Ruggiero et al., 2019). From our results, we suggest the 10:100 Hz ratio could be a more accurate representation of muscle damage. It would be worthwhile to directly compare the 10:100 Hz ratio and ISO MVC torque to more direct measures of muscle damage obtained from muscle biopsy samples.

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Appendix 1: Participant Information and Consent Form

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a place of mind

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The impact of sex on the repeated bout effect

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1. Invitation to Participate

You are invited to participate in a study investigating the influence of sex on fatigue from dynamic exercise. You have been invited to participate in this study because you are a healthy young individual 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, at any time, throughout the study.
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. Dr. McNeil's external research grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) will fund this study.

4. Background/Justification?
It is well established that females fatigue less than males during tasks which involve contractions where the muscle stays the same length (isometric contractions). However, it is unclear if females also experience less fatigue during dynamic exercise where the muscle shortens (concentric contractions) or lengthens (eccentric contractions). Eccentric exercise is relevant to study because it is effective for developing strength and preventing injury. This study will determine if sex differences in fatigue result in different adaptations to dynamic exercise. Findings from this experiment will improve our understanding of how the nervous system and muscles function in males and females.

5. Purpose
The purpose of this study is to determine if females and males fatigue and recover differently to repeated maximal voluntary eccentric contractions.

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 had any major recent injury or surgery to your dominant leg
- Have a neuromuscular disease (e.g., amyotrophic lateral sclerosis, muscular dystrophy, multiple sclerosis, myasthenia gravis)
- Have recently (in the last 9 months) experienced significant exercise-induced soreness of the muscles that lift your toes
- Are regularly involved in extreme lower body physical activities
- Use any medications with side effects of 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 8 visits over a 5-week period. Session 1 will last 60-90 minutes and involve tests of muscle function before, during, and after a fatigue protocol. Sessions 2-4 (2, 4 and 7 days after session 1) will take ~30 minutes each as they involve only the

baseline tests. Sessions 5-8 will repeat sessions 1-4 and take place 4 weeks after session 1. You will be asked to wear shorts to allow for surface electrodes to be placed as described below. During the experimental sessions, the following procedures (which are described separately) will be performed.

- ☐ To measure the electrical activity of the muscles that raise the foot (dorsiflexors) and lower the foot (plantar flexors), surface electrodes will be placed on the skin overlying the muscle belly and tendon of a shin muscle (tibialis anterior) and a calf muscle (soleus).

- ☐ To measure the voluntary torque (force) produced by the dorsiflexor muscles of your dominant leg, you will be asked to pull (try to lift your toes) against a rigid device equipped with a strain gauge. You will be seated in a semi-reclined position with your foot comfortably and securely strapped to a foot plate. These contractions (called "maximal voluntary contractions" or MVCs) will require you to try as hard as you can. Each session, you will be asked to perform 3-5 brief (2-3 second; s) isometric MVCs separated by at least 60s of rest. You will also be asked to perform 3-5 brief (1s) eccentric MVCs (30° range of motion and velocity of 60°/s) separated by at least 60s of rest. For the fatigue sessions, you will be asked to perform an additional 200 eccentric MVCs (4 sets of 50 contractions, with 1s rest between contractions and 1 minute of rest between sets). Between each set as well as 1, 3, 5, and 10 minutes after the final set, you will be asked to perform a brief isometric MVC. At each recovery time point, you will also be asked to perform a brief eccentric MVC. You will not be pushed beyond your limit and you can stop the study at any time.

- ☐ Electric stimulation of the nerve leading to the dorsiflexors (fibular nerve) will be used to assess responsiveness of the muscle fibres. Using a bar electrode held over the nerve (at the outside of the leg, just below the knee), stimuli will be delivered as single pulses or as 1s trains of 10 or 50Hz (pulses per second). During all brief isometric MVCs, a single stimulus will be delivered to measure your nervous system's ability to drive the dorsiflexors to their full potential (voluntary activation). Approximately 1s after each MVC, a sequence of stimuli (single pulse, 10Hz, and 50Hz; 1s rest between each), will be delivered to assess contractile function. The stimulation will cause a brief contraction of the dorsiflexor muscles of the dominant lower limb. You may feel moderate local discomfort under the electrodes, but this is very brief (less than 1s) and no long-term problems have been reported with this stimulation.

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?

Electric stimulation is achieved with isolated and grounded electric stimulators designed specifically for humans. Some participants perceive these stimuli to be uncomfortable (e.g., a pain rating of 2-4 out of 10) but they are very brief and cause no injury. The initial bout of fatiguing eccentric exercise will lead to

moderate muscle soreness 1-4 days after the session. The feeling will be similar to that experienced after unaccustomed exercise. Soreness will be much less following the second fatigue bout.

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 on the UBCO campus, 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 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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a place of mind

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The impact of sex on the repeated bout effect

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.

Printed Name	Participant's Signature	Date

Printed Name	Signature of Person Obtaining Consent	Study Role	Date