MUSCLE FIBER COMPOSITION AND MOTOR UNIT RECRUITMENT PATTERNS IN ADULT MALES AND FEMALES

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B.S., University of Kansas, 2016

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ABSTRACT

SEX-RELATED DIFFERENCES IN MUSCLE FIBER COMPOSITION AND MOTOR UNIT RECRUITMENT PATTERNS

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It is unknown whether firing rate patterns differ between sexes in extended isometric contractions. **Purpose:** Therefore, the purposes of this study are to examine potential differences between untrained males and females for (1) motor unit (MU) activity during a single extended contraction (2) MU activity during a subsequent extended contraction, specifically the relationships between motor unit action potential amplitudes ($MUAP_{AMPS}$) vs. recruitment threshold (RT), firing rates vs. RT, and firing rates versus $MUAP_{AMPS}$, in addition to the root mean square of the electromyographic readings (EMG_{RMS}). (3) To determine if % myosin heavy chain (MHC) isoform or muscle cross sectional area (mCSA) explain differences between two repetitions of extended isometric contractions. **Methods:** Ultrasonography was used to obtain subjects' muscle cross sectional area (mCSA), subcutaneous fat (sFAT) and echo intensity (mEI) from 50% upper leg length of the vastus lateralis (VL). MU activity was analyzed from two extended isometric contractions at 40% maximum voluntary contraction (MVC) by the decomposition of the EMG signal from the surface of the skin. Muscle biopsies were also performed from the muscle belly of the VL, to be used for MHC analysis. **Results:** There were no significant differences between groups in age (female = 19.8 ± 1.3 yrs, male = 19.5 ± 1.2 yrs; P = 0.577) or body mass (female = 66.3 ± 14.14 kg, male = 79.35 ± 16.63 kg; P = 0.070), but there were significant differences in height (female = 165.21 ± 6.16 cm, male = 180.69 ± 8.31

cm; $P < 0.001$) and MVC strength (female = 125.9 ± 29.3 N, male = 209.1 ± 75.8 N; $P = 0.003$). No significant differences existed for mCSA between sexes (female = 19.02 ± 3.88 cm^2, male = 26.52 ± 9.67 cm^2; $P = 0.069$) There were no significant interactions for sex × % MHC isoform ($P = 0.107$), but there was a significant main effect for % MHC isoform ($P = 0.028$). Percent MHC I was greater (45.45 ± 9.53%) than % MHC IIA (37.16 ± 10.34%) collapsed across sexes. For EMG_{RMS}, there was no significant interaction for sex × repetition ($P = 0.987$). There was a significant main effect for sex ($P = 0.001$), but there was no significant main effect for repetition ($P = 0.447$), with males having greater EMG_{RMS} at steady force than the females collapsed across repetitions. For the MFR vs. RT, $MUAP_{AMP}$ vs RT, and MFR vs. $MUAP_{AMP}$ relationships, there were no significant two-way interactions (sex × repetition) for the slopes, y-intercepts, *A* terms, or *B* terms. However, for the slopes of the MFR vs. RT relationship, there were significant main effects for sex ($P = 0.005$) and repetition ($P = 0.037$), where the slopes were more negative for females (-0.276 ± 0.020 pps/%MVC) than males (-0.184 ± 0.024 pps/%MVC). The slopes were more negative for the first repetition (-0.264 ± 0.022 pps/%MVC) than the second repetition (-0.197 ± 0.22 pps/%MVC). There was also a main effect for sex for the y-intercepts ($P = 0.020$); females had greater y-intercepts (22.57 ± 0.44 pps) than the males (20.86 ± 0.543). For the $MUAP_{AMP}$ vs. RT relationship, there was a significant main effect for sex ($P = 0.027$) for the y-intercepts; the males had greater y-intercepts (0.023 ± 0.014 mV) than the females (0.015 ± 0.009 mV). For the $MUAP_{AMP}$ vs. MFR relationship, there was a significant main effect for sex ($P = 0.040$) for the *B* terms; males (-4.48 ± 0.52 pps/mV) had less negative *B* terms than females (-5.92 ± 0.43 pps/mV). **Discussion:** Although there were minimal sex-related differences observed for the MU relationships, this result was not entirely unexpected due to the lack of significant differences between sexes for %MHC isoform or mCSA. The more negative slopes

and *B* terms exhibited by the females in the MFR vs. RT and MFR vs. $MUAP_{AMP}$ relationships indicate that females potentially must recruit a larger percentage of their MU pool to maintain the necessary force level. The lack of differences between groups for the changes in firing rates or $MUAP_{AMPS}$ from the 1st to 2nd repetition is most likely due to the similarities in % MHC isoform or mCSA between groups. However, there were changes in firing rates collapsed across sex from the 1st to 2nd repetition, which was likely a function of larger MUs participating earlier in the contraction.

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CHAPTER I

INTRODUCTION

Background

Since the publication of the "Henneman size principle" (Henneman, 1957), it has been well accepted that motor units (MUs) are recruited in an orderly fashion by size from smallest to largest during isometric contractions. Therefore, MUs with the smallest diameter are the first to be activated during a contraction, and progressively larger-diameter MUs are recruited to maintain force. As the contraction proceeds, each MU also adjusts its firing rate; studies examining firing rates in relation to recruitment threshold (RT) have reported variable patterns depending on the method of stimulation and statistical analysis. When observing electrically stimulated decerebrate cats, it is reported that lower-threshold (earlier-recruited, smaller) MUs demonstrate lower firing rates than higher-threshold (later-recruited, larger) MUs (Eccles, Eccles, & Lundberg, 1958). This firing rate pattern (referred to as the after-hyperpolarization control scheme) has also been observed in humans when relationships were constructed across contractions and subjects (Barry, Pascoe, Jesunathadas, & Enoka, 2007; Gydikov & Kosarov, 1974; Moritz, Barry, Pascoe, & Enoka, 2005). However, when MU data is analyzed using separate contractions and subjects, the relationship is inverted; low-threshold MUs demonstrate higher firing rates than high-threshold MUs at all force levels and time points in a contraction (C. J. De Luca & Contessa, 2012; C. J. De Luca & Hostage, 2010; C. J. De Luca, LeFever, McCue, & Xenakis, 1982; Hu, Rymer, & Suresh, 2014; Kanosue, Yoshida, Akazawa, & Fujii, 1979; Masakado, 1994; Masakado, Akaboshi, Nagata, Kimura, & Chino, 1995; Masakado, Kamen, & De Luca, 1991; McGill, Lateva, & Marateb, 2005; Monster & Chan, 1977; Person & Kudina, 1972; Rose & McGill, 2001; Stashuk & de Bruin, 1988; Tanji & Kato, 1973). This pattern has

been referred to as the onion-skin control scheme and demonstrates significant biomechanical advantages over the after-hyperpolarization scheme (C. J. De Luca, Adam, Wotiz, Gilmore, & Nawab, 2006; Carlo J De Luca & Contessa, 2015; C. J. De Luca & Erim, 1994). The onion-skin scheme allows lower-threshold MUs, which are less fatigable, to be used to a greater extent in maintaining a target force. This allows higher-threshold MUs, which are more fatigable, to be used more sparingly.

Additionally, the RT of MUs are linearly related to their action potential amplitudes (AP_{AMPS}) (Pope, Hester, Benik, & DeFreitas, 2016). The AP_{AMPS} have been correlated with the diameter of muscle fibers that comprise a MU and, thus, provides an indirect measure of MU size (Hakansson, 1956). This allows relationships to be made between $MUAP_{AMP}$ and firing rate; this relationship is important because while MUs can be recruited at differing RTs (especially during longer contractions when fatigue is a potential factor), $MUAP_{AMP}$ remains relatively consistent and can give more insight about firing rates of individual MUs across contractions (Hu, Rymer, & Suresh, 2013). It has been reported that during exhausting contractions, the firing rate vs. RT relationship becomes significantly more negative as the contraction proceeds, indicating that higher-threshold MUs are recruited during fatigue in order to maintain a targeted force level. Adam and De Luca (2005) demonstrated this pattern when subjects performed 20% MVC contractions to exhaustion.

In previous literature, it has been observed that young, untrained females have higher % MHC I and lower % MHC II than young, untrained males (Staron et al., 2000), as well as smaller muscle cross sectional area (mCSA) (Kubo et al., 2003; Trevino et al., 2018). These architectural factors have potential implications for fatigue resistance and MU firing rate patterns during repetitive contractions. Presumably, the larger mCSA of men is due to larger MUs across the

spectrum, but especially the highest-threshold MUs. While the lowest-threshold (smallest) MUs are typically of comparable size between sexes, the highest-threshold (largest) MUs are often much larger in males (Trevino et al., 2018). This is presumably due to greater percentage of % MHC II fibers (which have been shown to have greater capacity for hypertrophy (Nilwik et al., 2013)) and leads to larger mCSA and greater ability to produce force. These characteristics make sex-related differences in firing rate characteristics difficult to predict. Larger % MHC I for females could allow lower-threshold MUs to be used longer without necessary recruitment of higher-threshold MUs, and therefore maintain higher firing rates than males throughout the contraction, due to the lowered fatigability of MHC I (Hunter, 2009). However, if the females possess a smaller MU pool, they could be required to use a higher operating point to maintain the same force as the males (Contessa & De Luca, 2013), resulting in relatively lower firing rates at steady torque for females than males.

Purpose

The purposes of this study are to examine potential differences between untrained males and females for (1) MU behavior during a single extended contraction (2) MU behavior during a subsequent extended contraction, specifically the relationships between $MUAP_{AMPS}$ vs. RT, firing rates vs. RT, and firing rates versus $MUAP_{AMPS}$, in addition to the root mean square of the elctromyographic readings (EMG_{RMS}). MUs will be observed in the vastus lateralis (VL) muscle of the quadriceps, at a 40% MVC intensity sustained for 45 seconds of steady force, performed twice with a short (<10 seconds) rest period. (3) To determine if % myosin heavy chain (MHC) isoform or muscle cross sectional area (mCSA) explain differences between two repetitions of extended isometric contractions. We hypothesize that (1) the females will demonstrate smaller

slope values for both the $MUAP_{AMP}$ vs. RT and firing rates vs. RT relationships due to greater % MHC I isoforms and (2) females will demonstrate higher firing rates across subsequent contractions due to delayed recruitment of high-threshold MUs.

Hypothesis and Specific Aims

Hypothesis

Females will demonstrate higher firing rates at steady torques and smaller $MUAP_{AMPS}$ for the same RT than males in initial and subsequent fatiguing contractions. The relationship between firing rate and RT will be more negative for men than for women during both initial and subsequent fatiguing contractions.

Specific Aim #1

To evaluate differences between men and women in MU activity during a single submaximal fatiguing contraction.

Specific Aim #2

To evaluate differences between MU behavior during a subsequent submaximal fatiguing contraction, specifically the relationships between $MUAP_{AMP}$ and RT, firing rates at steady torque and recruitment threshold, and firing rates at steady torque and $MUAP_{AMP}$.

Operational Definition of Terms

Isometric MVC force (MVC force) – peak force achieved during a MVC. Expressed in Newtons (N).

Surface Electromyography (sEMG) – a recording of MUAPs moving across the sarcolemma as detected by a surface electrode. The amplitude and frequency of the signal contains information

that has physiological implications about the recruitment, firing rates, AP_{AMPS}, and AP durations (AP_{DURS}) of individual MUs during muscle actions.

Delimitations

Eighteen untrained individuals (8 men, 10 women) volunteered for this study. All participants completed a written informed consent prior to testing. Subjects reported no current or ongoing injuries or neuromuscular disease related to the lower limb.

Assumptions

Theoretical assumptions

1. Subjects gave full effort on all isometric MVC tests.
2. Subjects accurately achieved and maintained force during isometric submaximal muscle actions.
3. There were no significant training status differences in the VL between the men and women due to increased body weight or lifestyle.
4. All equipment was calibrated and functioning properly throughout all tests and testing sessions.
5. Myosin heavy chain analysis gathered from biopsies are representative of the entire VL.

Statistical assumptions

1. The population from which the samples were drawn is normally distributed.
2. The data meet the assumption of sphericity, which requires that repeated measures data have both homogenous variance and covariance.
3. The data was based on a parametric scale, either interval or ratio.

Limitations

Subjects were recruited by word of mouth and from departmental classes, and therefore cannot necessarily be considered random. Additionally, sEMG does not detect all active MUs during any contraction, and therefore the total MU pool used in analysis includes only those detected, and not necessarily all MUs contributing to muscle force. Finally, myosin heavy chain was only analyzed from a small sample of the VL.

CHAPTER II

LITERATURE REVIEW

Motor Unit Behavior and the Onion-Skin Control Scheme

De Luca & Erim (1994)

The purpose of the article was to construct a model that synthesized the currently available information in order to explain the effect of the nervous system's regulation of motor unit (MU) behavior within a muscle. Using a decomposition technique, mean firing rate (MFR) was calculated as a continuous time signal to provide an estimate of the average firing intensity of the MU at a given time during the contraction. It was observed that firing rates were modulated symbiotically with other concurrently activated MUs. In an analysis of over 300 contractions, the researchers observed nearly no time delay between peaks of firing rates, leading them to develop the concept of the "common drive." The common drive concept states that all the motoneurons belonging to the motoneuron pool receive the same net drive at any point. This is achieved by central nervous system regulation of the excitatory and inhibitory inputs to the pool. Individual MU properties determine the recruitment threshold and firing rates, in agreement with Henneman's size principle. Therefore as excitation increases, progressively larger MUs (higher-threshold MUs) are recruited as firing rates of all recruited MUs increase. As excitation decreases, the higher-threshold MUs are derecruited first, followed by progressively smaller MUs (in the reverse order as observed at recruitment). An additional property, known as the "onion-skin" phenomenon, dictates the relationship of firing rates during isometric contractions. This observation states that the firing rates of earlier-recruited MUs are greater than those of later-recruited MUs at every point in the contraction. Even as excitation increases and firing rates increase for all activated MUs, the earlier-recruited MUs will always maintain greater

firing rates than those of the later-recruited MUs. While seemingly paradoxical to the traditional understanding of MU behavior (i.e. that MU control schemes should be designed to maximize force output and therefore larger/later-recruited MUs should fire at a higher rate), the onion-skin scheme implies that the neuromuscular system might reserve capacity for force generation in addition to maximizing sustainability of force by relying more heavily on less fatigable MUs (lower-recruited). This model, the combination of the common drive concept and the onion-skin scheme, outlines the basic rules governing force production, and is a helpful tool in speculating the general behavior of MUs under different conditions.

De Luca & Contessa (2015)

The purpose of this study was to apply a model of muscle force generation to compare force characteristics produced by the onion-skin and after-hyperpolarization (AHP) schemes of motor unit (MU) behavior during constant-force contractions. The AHP scheme, which states that larger (higher-threshold) MUs exhibit higher firing rates than smaller, lower-threshold MUs, would theoretically optimize force generation capacity. The AHP scheme has been observed during electrical stimulation of anesthetized cat muscle, as well as in humans when MU data is grouped across subjects and contractions, rather than examining each relationship individually. The more recently developed onion-skin scheme analyzes firing rate vs. recruitment threshold relationships in individual subjects and contractions. It states that the firing rates of earlier-recruited MUs are greater than those of later-recruited MUs at every point in the contraction. Even as excitation increases and firing rates increase for all activated MUs, the earlier-recruited MUs will always maintain greater firing rates than those of the later-recruited MUs. This scheme theoretically maximizes sustainability of force by relying more heavily on less fatigable MUs

(lower-recruited). A mathematical model was applied using data provided for both MU control schemes. This model was applied to a theoretical human first dorsal interosseous (FDI) and the vastus lateralis (VL) in order to compare muscles involved in low-force level activities (FDI) and high-force level activities (VL). When comparing results between the two schemes, it was shown that the onion-skin scheme produced greater absolute force than the AHP scheme between 0-60% maximum voluntary contraction (MVC). While approaching and at maximal input excitation, the AHP scheme is capable of generating greater absolute forces (~20% in the FDI and ~30% in the VL) because all MUs are entirely fused. In addition, the onion-skin scheme produced greater force steadiness due to its lowered force variability at low input excitations. In a final observation of MU endurance, a simulation was performed where both muscles sustained 50% MVC until fatigue, and time to fatigue was observed. The AHP scheme produced quicker time to fatigue than the onion-skin scheme due to its faster firing rates (especially of larger, more fatigable MUs). Based on the results of this simulation, the researchers determined that the onion-skin scheme produced a greater biomechanical advantage. This advantage is based on the increased force steadiness, quicker force generation, and greater force sustainability associated with the onion-skin scheme. Because this scheme does not easily generate maximum force capacity, it allows for a reserve of energy that could be advantageous in a fight-or-flight response.

Trevino et al. (2016)

The purpose of this study was to examine the possible relationships between myosin heavy chain (MHC) isoform content of the vastus lateralis (VL) and motor unit (MU) firing rates during an isometric leg extensor trapezoidal muscle action at 40% maximum voluntary

contraction (MVC). 12 participants, 6 men (age = 21.67 ± 2.80 yr) and 6 women (age = 19.67 ± 1.37 yr) volunteered for this study. During the experimental visit, participants performed a submaximal isometric contraction at 40% MVC. For the trapezoidal muscle action, force was increased at 10% MVC/s for 4 seconds (to reach 40% MVC), held at a steady force for 12 seconds, then decreased to baseline at 10% MVC/s for 4 seconds. Thus, the duration of each contraction was 20 seconds. Muscle actions were completed using a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY), force was measured by a load cell (LC; Omegadyne, Sunbury, OH, USA), and surface electromyography (EMG) of the VL was recorded using a 5-pin electrode (Delsys, Boston, MA, USA). The EMG signals were decomposed using Precision Decomposition III algorithm into constituent MU action potential trains. MHC isoform content was determined using biopsies from the VL and analyze with sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Using decomposed firing rate data, several regressions were created: (1) firing rate at recruitment vs. recruitment threshold, (2) mean firing rate vs. recruitment threshold, (3) firing rate at derecruitment vs. derecruitment threshold. Percent MHC I was also correlated with these relationships. The % MHC I isoform content was significantly correlated with relationship (1) (slopes: $r = 0.741$, $P = 0.006$; y-intercepts: $r = -0.577$, $P = 0.050$), the slopes of relationship (2) ($r = 0.039$, $P = 0.905$), and relationship (3) (slopes: $r = -0.701$, $P = 0.011$; y-intercepts: $r = -0.597$, $P = 0.040$). Although there were significant correlations between % MHC I and the slopes and y-intercepts of relationships (1) and (3), only a small number (16%) of these individual subject relationships were significant. When the mean firing rate vs. recruitment threshold was analyzed for each subject, it was found that those with greater % MHC I had less negative slopes, implying that lower-threshold MUs were more heavily relied upon

during the contraction than those with lower % MHC I. These results indicate that the physical properties of the motoneuron play a prominent role in MU firing rate characteristics.

Hu et al. (2013)

The purpose of this study was to characterize recruitment and firing rate organization of concurrently active motor units (MUs) of differing action potential sizes in order to provide a more complete data set regarding firing rate modulation. The study was performed on the first dorsal interosseous (FDI) muscle of the hand and used the spike-triggered averaging method of motor unit action potential (MUAP) extraction from the surface electromyogram (sEMG). Eight subjects (4 men, 4 women) volunteered to participate in the study. The subject was asked to perform four submaximal isometric trapezoidal contractions at 20%, 30%, 40%, and 50% of their maximum voluntary contraction (MVC). Each force was performed five times with 30 seconds rest in between each; each force block was performed separately. sEMG signals were recorded from each contraction from each subject while seated in a Biodex chair (Biodex Medical systems, Shirley, NY, USA) with a custom-made setup to record isometric abduction of the index finger using a 6 degrees-of-freedom load cell (ATI; no. 3226) to record force signals. For the MUAP vs. recruitment threshold relationship, it was observed that higher MUAP amplitudes were observed at higher thresholds during higher levels of muscle contraction. The r^2 values for these relationships for each contraction ranged from 0.46±0.03 - 0.55±0.05, and were all significant ($P > 0.05$), which is consistent with the size principle. An inverse relationship was observed between the mean firing rate and MUAP amplitude. The r^2 values for these relationships for each contraction ranged from 0.41±0.03 - 0.52±0.04, and this relationship is in line with the onion-skin scheme of MU control.

Miller et al. (2017)

The purpose of this study was to determine the direction and magnitude of time-related changes in motor unit (MU) firing rates and their relationship to recruitment threshold in the first dorsal interosseous (FDI), especially between elderly and young populations. Twenty-two young subjects (YG, 12 men, 10 women, age = 22.6±2.7 years) and fourteen elderly subjects (AG, 7 men, 7 women, age = 62.1±4.7 years) volunteered for participation in this study. During the experimental trial, subjects were seated with their right hand in a custom-made restraint in order to isolate the muscle action of the FDI. Abduction force of the FDI was measured by a force transducer (MB-100; Interface, Inc., Scottsdale, AZ, USA) and electrical stimulation was delivered to the ulnar nerve using a high-voltage constant-current stimulator (Digitimer DS7AH, Welwyn Garden City, UK). Maximal evoked twitch force (TF) and maximal voluntary contraction (MVC) force were determined. A 50% MVC trapezoidal isometric contraction was performed, with twitches evoked at rest immediately before and immediately after the contraction in order to determine difference in TF. Surface electromyographic (sEMG) readings were recorded (Bagnoli 16-channel EMG system, Delysis Inc.) via 5-pin surface array electrodes, and signals were decomposed into their constituent MU action potential trains using the precision decomposition III algorithm. TF was significantly greater ($P = 0.003$) for YG (0.0608±0.253 N) than for AG (0.314±0.167 N). Additionally, for the slopes of the mean firing rate (MFR) vs. recruitment threshold (RT), there was a significant two-way interaction (group x time, $P = 0.005$); the YG exhibited significantly lower values at time point 1 than time point 2 ($P < 0.001$), but no significant differences from time point 2 to time point 3 ($P = 0.467$). There were no significant differences across time points for the the AG ($P = 0.142$). There was also a significant two-way interaction (group x time, $P = 0.009$) for the y-intercepts of the MFR vs. RT

relationship; the YG demonstrated significantly higher values at time point 1 than time point 2 ($P < 0.001$), with no difference from time point 2 to time point 3 ($P = 0.999$). The AG demonstrated no differences across time points ($P = 0.530$). There were also no significant differences between groups at any time point ($P = 0.605$-0.835). These results indicate that individuals with greater TF potentiation were more likely to exhibit changes in firing rate behavior during an isometric contraction. The absence of changes in the AG indicates that firing rate adjustments seen in the YG over the course of the contraction could be explained by the presence of minimal twitch potentiation.

Stock et al. (2012)

The purpose of this study was to investigate the influence of fatigue on the mean firing rate (MFR) vs. recruitment threshold (RT) relationships for motor units (MUs) of the vastus lateralis (VL) and vastus medialis (VM). Twelve men (age = 22.1±1.4 years; weight = 78.9±10.4 kg) and 7 women (age = 21.6±1.2 years; weight = 65.4±13.1 kg) volunteered to participate in the study. Subjects participated in one experimental trial. Subjects were seated and strapped into a custom-made chair made for lower body isometric strength testing. Force was measured by a load cell (Model SSM-AJ-500; Interface, Scottsdale, Arizona) attached to the ankle cuff and was used to determine maximum voluntary contraction (MVC) strength during a 3-second contraction. A 50% MVC trapezoidal isometric contraction was performed with force increasing for 4 seconds (12.5 %MVC/second), constant force for 12 seconds, and force decreasing for 4 seconds (-12.5 %/second). The fatiguing protocol was performed immediately afterwards, and involved ten 10-second isometric MVCs of the leg extensors with 10-second rest periods between each MVC. Immediately after the fatigue protocol, a new 3-second MVC was

measured, and then another 50% trapezoidal isometric contraction was performed using the initial MVC. Surface electromyographic (sEMG) signals were detected from the VL and the VM with 5-pin surface array EMG sensors (Delsys, Inc., Boston, Massachusetts). The EMG signals were decomposed into their constituent MU action potential trains using the Precision Decomposition III algorithm and were used to calculate MFR curves for each motor unit. For the MFR vs. RT relationship, there was a significant increase in the linear slope coefficients after the fatiguing protocol for the VL (fresh: -0.420±0.165 pps/%MVC; fatigued: -0.301±0.102 pps/%MVC), but not for the VM (fresh: -0.446±0.189 pps/%MVC; fatigued: -0.337±0.164 pps/%MVC). Additionally, the fatiguing protocol resulted in a significant decrease in y-intercepts for this relationship in the VL (fresh: 29.4±6.0 pps; fatigued: 26.3±4.3 pps) but not the VM (fresh: 31.7±9.7 pps; fatigued: 28.5±7.7 pps). The fatiguing protocol resulted in an MVC force reduction of 18.6±10.8%. The authors contend that the increased slope coefficient and decrease y-intercept value for the MFR vs. RT relationship of the VL is due to recruitment of higher-threshold MUs and/or indication of increased drive to motor neuron pool to compensate for mechanical changes at the cellular level. Additionally, the authors noted that even after fatigue the MFR values for higher-threshold MUs were always lower than those of earlier-recruited MUs.

Muscle Architecture: Cross-Sectional Area and Echo Intensity

Doherty (2001)

The purpose of this review was to discuss the influence of aging on skeletal muscle mass and strength, especially differences due to sex-related changes. Age-related loss in strength is both a result of and highly correlated with loss in muscle mass. Although men have a larger total

skeletal muscle mass than women, men also exhibit larger degrees of sarcopenia (age-related decreases in skeletal muscle mass) than women. In one study, the prevalence of sarcopenia was 13-24% in individuals 65-70 years, and >50% in individuals older than 80 years. This study also demonstrated the higher prevalence for men over age 75 (58%) than for women (45%). Biopsy studies have noted that type II myosin heavy chain (MHC) fiber area can be reduced by 20-50%, while type I MHC fiber area reduction is more moderate, 1-25%. One study reported that fiber area declined to a greater extent in women (MHC I: 25%; MHC II: 45%) than for men (MHC I: 15%; MHC II: 19%). However, these reductions in fiber size are moderate in comparison to decreases in muscle mass. A few studies detailed the differences between sexes in muscle quality (specific tension or strength per unit muscle mass) as an assessment of functionality rather than strength. Most studies report an age-related decline in muscle quality. In one study, type I and type IIA MHC fibers were adjusted for size and compared between older men and women, suggesting that muscle quality is different between sexes during aging.

Kubo et al. (2003)

The purpose of this study was to compare muscle architecture characteristics (muscle thickness, pennation angle, and fascicle length) between groups of different age and sex. Although it is consistently documented that muscle mass and strength decrease with age, decreases in functionality appear to be more related to changes in muscle architecture. 311 subjects volunteered to participate and were divided into 4 separate groups (young = 20-39 years; old = 60-85 years): young men (N = 67), young women (N = 46), old men (N = 54), and old women (N = 144). Architectural characteristics were measured using B-mode ultrasonography (SSD-500, Aloka, Japan). Muscle thickness (MT) and pennation angle (PA) were measured at

the approximate muscle belly of the vastus lateralis (VL), medial gastrocnemius (MG), and long head of triceps brachii (TB) muscles. Fascicle length (FL) was calculated as $FL = MT * \sin^{\alpha-1}$, where α is the PA of the muscle. Limb length was significantly positively correlated with MT of all muscles ($P < 0.01$), PA of all muscles ($P < 0.01$), and FL of the MG. Significant positive correlation was observed between PA and MT of all muscles ($P < 0.01$), as well as relative MT (to limb length) of all muscles ($P < 0.01$). MT was significantly less in young women than young men for all muscles ($P < 0.001$), old men than young men for the VL and MG ($P < 0.001$), old women than old men for all muscles ($P < 0.05$), and old women than young women for all muscles ($P < 0.001$). PA was significantly less in young women than young men for all muscles ($P < 0.001$), old men than young men for the VL ($P < 0.001$), old women than old men for the VL and TB ($P < 0.05$), and old women than young women for the VL ($P < 0.001$). FL was significantly less in young women than young men for all muscles ($P < 0.05$), old men than young men for the VL and MG ($P < 0.01$), old women than old men for the MG and TB ($P < 0.01$), and old women than young women for the MG ($P < 0.01$). The authors concluded that in the VL, decrease in MT with age was significant, and that there are significant sex-related differences in the FL of the VL.

Ahtianen et al. (2010)

The purpose of this study was to examine the reliability and validity of the panoramic B-mode ultrasonography (US) method for detection of training-induced changes in muscle cross-sectional area (mCSA). US is an easily accessible, minimally invasive, and relatively inexpensive option for muscle imaging than other methods (i.e. magnetic resonance imaging (MRI) or computed tomography (CT)). No previous studies had investigated the reliability and

validity of US compared to a "gold standard" measurement. 27 untrained adult male participants volunteered for this study; 20 subjects were placed in an experimental resistance training group and seven were placed in a non-training control group. US and MRI images of the vastus lateralis (VL) muscle were taken on the same day for each subject after 3 days rest from physical activity, both at time points before and after the training period. Body positioning was the same for both imaging techniques. US was performed by a portable B-mode device (GE Logiq e, USA) using a 10-MHz linear-array probe. The transducer was moved manually at a continuous rate from lateral to medial borders, and three separate scans were taken (the value was recorded as the mean of the two closest values). The experimental resistance training protocol was performed for 21 weeks, two times per week, using total-body heavy resistance exercises designed for muscle hypertrophy. When measured by MRI, mCSA of the VL changed by 13±6% ($P < 0.001$) for the experimental group and -3±4% ($P > 0.05$) for the control group. When using US, mCSA of the VL changed by 14±7% ($P < 0.001$) for the experimental group and -1±6% ($P > 0.05$) for the control group. The differences between measurements (US and MRI) were not statistically significant. Additionally, repeatability was calculated from the repeated scans performed on the subjects. For US intra-day repeatability, the ICC was 0.997, SEM 0.38 cm^2 and SDD 1.1 cm^2. Although there were minor differences between MRI and US, the differences were not statistically significant and probably represent differences in how the algorithm processes panoramic mCSA measurements. This data also demonstrates that US measurements are highly reliable and a valid method for detecting training-induced changes in mCSA for the VL.

Noorkoiv et al. (2010)

The purpose of this study was to test the validity and reliability of extended-field-of-view ultrasonography (EFOV US) for measuring the cross-sectional area (mCSA) of the quadriceps. The EFOV method is theoretically less prone to error than the image-matching technique of US devices that do not have panoramic capabilities. Six male subjects volunteered to participate for this study; both thighs for each subject were measured, making the sample size 12. B-mode and axial-plane US (Aloka SSD-α10, software number 6.1.0, Aloka Co., Ltd., Tokyo, Japan) images were taken with a 10 MHz linear-array probe (60-mm width) using EFOV mode. Measurements were taken at five points along the line from the central point of the patella to the medial aspect of the anterior superior iliac spine; 10, 20, 30, 40, and 50% of the total distance were used. Images were taken with the US at each point, and then 2 hours later using axial plane computed tomography (CT) (Siemens SOMATOM Definition 1009134374 Dual Source 64 Slice CT, Siemens AG, Berlin, Germany). The ICCs between the EFOV US method and the CT images were calculated for the 10, 20, 30, 40, and 50% points, respectively: 0.987, 0.951, 0.994, 0.984, 0.998. For the same measurement locations, the ICC values for inter-day reliability were 0.982, 0.998, 0.998, 0.990, and 0.991. Additionally, low intra- and inter-experimenter reliability for the EFOV method were reported. This data shows that EFOV US measurements are a reliable and valid method of measuring mCSA in the quadriceps. This is in agreement with many other concurrent studies on this method. The authors suggest that because distal regions of the thigh are more tightly curved, it makes the EFOV method less reliable at these sites. Therefore, they recommend that the EFOV technique be used between 30-50% of total thigh length to ensure that the surface of the thigh allows accurate and repeatable muscle scanning.

Fiber Type Composition and Fatigue Mechanisms

Staron et al. (2000)

The purpose of this study was to analyze data collected from muscle biopsies of the vastus lateralis (VL) muscle to define normative parameters on muscle fiber types and sizes of untrained young men and women. 150 subjects participated in this study (men = 95, women = 55); all were college-aged, healthy, and untrained. Muscle biopsies were extracted from the superficial portion of the vastus lateralis muscle by the percutaneous needle biopsy technique (Bergstrom 1962). Biopsies were analyzed using myofibrillar adenosine triphosphatase (mATPase) histochemistry. Six fiber types were distinguished based on staining intensities: I, IC, IIC, IIA, IIAB, and IIB; they were then collapsed into three major types: I, IIA, and IIB. Additionally, myosin heavy chain (MHC) analysis was performed on most of the biopsy samples (men = 95, women = 26) using sodium dodecyl sulfate (SDS)-polyacrylamide electrophoretic techniques. Only one statistically significant difference was observed between sexes for fiber type percentages; women had a significantly greater type IC percentage (1.1±2.2%) than men (0.4±0.6%). However, when collapsed into major fiber types, significant differences existed in fiber type percentage between men and women in type I (men = 36.2±11.6%; women = 44.0±11.6%) and type IIA (men = 41.2±9.4%) fibers. Cross-sectional area (μm^2) values were significantly greater for all fiber types in men than women. MHC content percentages were significantly different between men and women for MHC I (men = 33.9±11.4%; women = 41.0±12.9%) and MHC IIa (men = 46.3±3%; women = 36.0±9.9%). Additionally, the relative MHC content for men was MHC IIA>MHC I>MHC IIB, while the relative MHC content for women was MHC I=MHC IIA>MHC IIB. The authors concluded that all fiber types are larger (based on cross-sectional area) in men than women, and that differences do exist in fiber type

distribution; slow fibers occupy a greater area in women than men, and fast fibers occupy a greater area in men than women.

Douris et al. (2006)

The purpose of this study was to determine the relationship between an individual's type II fiber percentage in the quadriceps and their ability to perform repetitions to exhaustion at a particular load. 22 untrained women volunteered to participate in this study. Type II fiber % was estimated by a noninvasive method involving measurements of fat-free mass (by skinfold calipers) and isokinetic dynamometry. A 1 repetition maximum (1RM) was determined for each subject for the leg extension. After 1RM determination and a 15-minute rest period, subjects performed repetitions until failure at 70% 1RM on cue with a metronome. The number of total repetitions performed was used for subsequent data analysis. Type II fiber % was correlated to repetitions performed, 1RM, body weight, and fat free mass. Only the type II fiber % vs. repetitions performed relationship was significant ($r = -0.48$, $P = 0.02$). The authors suggest that fiber type % is an important factor in fatigue resistance and time to failure in knee extensions. Individuals with higher % type II fibers will fatigue more quickly and can perform fewer repetitions than individuals with lower % type II fibers.

Carter, et al. (2001)

The purpose of this study was to investigate differences in muscle enzyme adaptations following endurance training between sexes. Several previous studies have indicated that males and females have different preferential usage of substrates. For this study, fourteen subjects volunteered for the study and were used for analysis (men = 8; women = 6). The training

protocol involved 7 weeks of cycle training, using 60-minute sessions at 60% VO_2max. Two muscle biopsies were taken, one before and one after training, from the vastus lateralis muscle using a modified Bergstrom needle (5mm diameter) with suction modification. Training resulted in an increase in VO_2max, 3-β-hydroxyacyl CoA dehydrogenase, citrate synthase, succinate cytochrome C oxidoreductase, and cytochrome c oxidase for both men and women ($P < 0.01$), but there was not a significant difference between sexes in the amount of increase. Additionally, body fat percentage significantly decreased for both sexes after training ($P < 0.001$). Training had no significant effect on mean fiber size, fiber area %, or fiber type % in men or women. However, significant differences between sexes existed for mean fiber size of type II muscles (men > women, $P < 0.05$), type I % fiber area (women > men, $P < 0.05$), and type II % fiber area (men > women, $P < 0.05$). The authors concluded that while men and women do not adapt to endurance training differently, women's pre-existing higher % type I fiber area may preferentially utilize a greater proportion of lipid energy during submaximal exercise.

Bigland-Ritchie & Woods (1984)

The purpose of this review was to establish the evidence that mechanisms of muscular fatigue are more present at the cellular contractile level than the central nervous system (CNS). The authors defined fatigue as “any reduction in the force generating capacity of the total neuromuscular system.” It was generally determined in studies on isolated muscle that decline of force occurs when ATP synthesis can no longer keep pace with the rate of ATP utilization. Additionally, the excitability of the muscle membrane/conduction system may be impaired due to improper maintenance of the electrolyte gradient. In analysis of single motor unit (MU) firing rates, mean firing rate decreased simultaneously with force; however, this was not found to be

the only explanation of decline in force. Rather, relaxation time was also prolonged by 50% in a study on the adductor pollicis muscle. These results suggest that the CNS regulates the firing rate to match changes in muscle contractile speed due to fatigue, rather than the CNS input being the root cause of fatigue. Additionally, different muscles with different predominant fiber types respond differently to fatigue; firing rates in each muscle respond proportionally to decrease in contractile speed, further supporting the notion that the CNS matches events at the cellular level rather than causing them.

Sex-Related Differences in Fatigue

Hunter (2009)

The purpose of this study was to determine the magnitude of sex-related differences in fatigue between different tasks. Most studies demonstrate a greater resistance to fatigue in women than men. Some suggested potential mechanisms of this phenomenon include lowered muscle mass, lowered sympathetic activation, lowered glycogen utilization, and higher type I fiber percentage in women. Across all studies, the average magnitude of sex-related difference in sustained isometric contractions is ~23%, with women being less fatigable than men in all cases where a difference was observed. However, the observed difference between sexes was observed to a greater extent at low contraction intensities (i.e. 20% maximum voluntary contraction (MVC) of the elbow flexors) than higher intensities (i.e. 80% MVC). The magnitude of the difference at even low-force contractions decreased when men and women were matched for strength, indicating that total muscle mass/strength plays a significant role in time to failure. This could be due to greater perfusion in female muscle during contractions. The magnitude of sex-

related differences is greater in intermittent contractions (~33%) than sustained isometric contractions. During intermittent contractions, sufficient blood flow is not a factor as it is in sustained contractions. Additionally, greater type I fiber percentage is directly proportional to time to fatigue during intermittent contractions, and studies have shown that women generally possess a greater percentage of type I fibers. This was demonstrated during several electrical stimulation studies in which women were found to have lower peak relaxation rates for the same muscle (both elbow flexors and knee extensors), indicating larger percentage of slow muscle fibers. In a study comparing isometric and dynamic contractions between males and females, it was found that no sex-related differences existed for submaximal (50% MVC) dynamic contractions, even when a difference was demonstrated with isometric contractions at the same intensity ($r^2 = 0.0005$). It is hypothesized that this is due to females' larger proportion of type I fibers, which slows shortening velocity and could impact fatigue during dynamic contractions. When analyzing studies that compared sex-related differences amongst various muscle groups, it was generally found that the magnitude of the sex-related difference is not consistent; this can be attributed to a variety of factors. When analyzing fatiguing contractions in the elbow flexors, voluntary activation showed a similar decline between men and women; however, when analyzing fatiguing contractions in the knee extensors, men demonstrated a greater decline in voluntary activation (22%) than women (9%). The general conclusion of these analyses was that the component of the CNS that causes these sex-related differences is different between muscle groups. Finally, the author analyzed studies comparing sex-related differences in fatigue between age groups. It was found that the difference in time to failure disappeared with elderly adults, with the composite results more closely matching those of young women; this indicates that there are changes in fatigability with age, and these changes affect men to a greater extent.

Yoon et al. (2007)

The purpose of this study was to analyze the difference in voluntary activation during fatigue between men and women at both high and low contraction intensities in the elbow flexors. Eighteen young adults (women: n = 9, men: n = 9; age 21-33 years) volunteered to participate in this study. Subjects performed three visits; the first was a familiarization, succeeded by two experimental sessions that involved a fatiguing contraction of the elbow flexor muscle in the nondominant arm at either 20% or 80% maximal voluntary contraction (MVC) force (experimental protocols were at least 7-10 days apart). Subjects were seated with the elbow joint flexed to a 90º with the forearm horizontal to the ground; the hand and forearm were placed in a modified wrist-hand-thumb orthosis (Orthomerica, Newport Beach, California). The force developed by the wrist was measured in a vertical direction by a transducer (JR-3 Force-Moment Sensor; JR-3 Inc., Woodland, California). The force exerted was displayed on a screen for the subject to see in order to trace the target force level for as long as possible. EMG signals were recorded with bipolar surface electrodes (Ag-AgCl, 8-mm diameter; 16 mm between electrodes) on the biceps brachii, brachioradialis, and triceps brachii muscles. Heart rate and blood pressure were also monitored as a measurement of peripheral and central adjustments. Electrical stimulation was used to evoke force in the biceps brachii and analyze voluntary activation (VA). Activation was achieved by a constant-current stimulator (Digitimer DS7AH, Welwyn Garden City, UK) that delivered a rectangular pulse of 100-μs duration and a maximal amplitude of 400 V. The stimulation intensity was set to 10% above the level required to produce a resting twitch of maximal amplitude so that the level of stimulation was supramaximal. During each experimental visit, subjects were tested for (1) supramaximal levels of electrical stimulation, (2) assessment of MVC torque and voluntary activation for the elbow flexor muscles, (3)

performance of an MVC of the elbow extensor muscles, (4) brief submaximal isometric contractions of the elbow flexor muscles to determine the EMG-force and voluntary activation-torque regulations, (5) performance of a fatiguing contraction at either 20% or 80% MVC force, which was terminated when the subject could no longer sustain within 10% of the targeted force for 3-5 seconds, (6) a twitch contraction, a recovery MVC with the elbow flexor muscles, and another twitch contraction. Men had a shorter time to task failure than women for the 20% MVC contraction (10.6±2.0 min vs. 17.0±8.7 min, respectively), but a similar time for the 80% MVC contraction (25.0±6.5 s vs. 24.3±6.6 s, contraction intensity × sex, $P < 0.05$). There were no significant differences between men and women in voluntary activation decline ($P > 0.05$). There were no differences in the EMG activity across experimental days, nor any interactions of sex × contraction intensity. RMS EMG of the biceps brachii was greater for women than men at the end of the 20% MVC contraction, but not for the 80% MVC (contraction intensity × time × sex, $P < 0.05$). The rate of increase in RPE was more gradual for women than men (0.95±0.2/min vs. 0.68±0.2/min, $P < 0.05$) for the 20% MVC task. The authors hypothesized that muscle perfusion may explain the sex difference in time to failure for the sustained contractions at low forces. Because there was a similar reduction between men and women in twitch amplitude after both fatiguing contractions, the authors suggest that the average rate of peripheral fatigue was much greater during 80% MVC than the 20% MVC contraction.

Wüst et al. (2008)

The purpose of this study was twofold; the first objective was to use electrical stimulation (thus avoiding motivation bias) to determine differences in resistance to peripheral fatigue between men and women. Additionally, the authors wanted to determine whether differences in

fatigue between sexes could be attributed to blood supply within the muscle. There have been many reasons attributed to the difference observed in fatigue resistance between men and women, including differences in motivation/ability to sustain central drive, differences in blood supply to the working muscle, and intrinsic differences in the composition and fatigue characteristics of the fibers making up the muscle. Sixty-four adult (ages 19-45 yrs) subjects volunteered for the study (men: N = 29; women: N = 35). Anatomical cross-sectional area (ACSA) of the quadriceps was measured via MRI (E-scan, ESAOTE Biomedica, Genova, Italy). Torque measurements were measured using a cybex norm dynamometer (Ronkonkoma, new York, NY, USA) and obtained at multiple joint angles (60, 70, 80, 90 degrees) in randomized order to determine optimal joint angle, which was used for the duration of the study. Percutaneous electrical stimulation (square wave, pulse width 50 μs; DSV Digitimer Stimulator, Digitimer Ltd, Welwyn Garden City, UK) was applied to the proximal region of the quadricep (anode) and distal third of the upper thigh (cathode). Resistance to fatigue was measured by a series of electrically evoked isometric contractions (standard protocol: 60 contractions, 30 Hz, 1 s on, 1 s off); stimulation intensity had been previously determined so that a 1 s 100Hz tetanus produced approximately 28.6% of the MVC value. Additionally, a subset of the subjects (men: N = 12; women = 18) performed the standard protocol under ischemic conditions using a pneumatic thigh cuff (Accoson, Harlow, UK). MVC strength and ACSA were lower in women than men, but voluntary activation were not significantly different. Contractile speed (assessed by rate of relaxation) at 30 Hz was significantly slower ($P = 0.002$) in women ($-14.1 \pm 1.8\ s^{-1}$) than men ($-15.6 \pm 2.4\ s^{-1}$). For the standard protocol, torque declined significantly more ($P < 0.05$) in men (37.7±10.7%) than in women (29.9±10.0%). For the protocol performed under ischemic conditions, the results were similar, with torque declining significantly more ($P < 0.05$) in men

(76.9±10.8%) than women (59.5±16.9%). Additionally, the pooled data reflected that there was a significant correlation ($r = -0.37$, $P < 0.005$) between MVC and fatigue resistance. The authors concluded that the data collected supports the previous findings that women are more resistant to fatigue than men, even when excluding motivational bias or CNS drive. They also concluded that the difference in fatigability between sexes cannot be attributed to differences in blood flow. They suggest that a potential cause of the observed difference is due to differences in fiber type percentages. This fiber difference has been previously observed, and the present study determined that female subjects had slower rates of relaxation (a characteristic of greater percentage type I fibers), and that this rate of relaxation was significantly related to fatigue resistance.

Fulco, et. al (1999)

This study aimed to examine the differences in fatigue between men and women when the groups were matched for maximum voluntary contraction (MVC) strength. Although previous studies have analyzed men and women at equal percentages of MVC strength, the authors believe that ignoring differences in absolute strength ignores potentially significant differences in O_2 demand to the exercising muscle that could play an important role in fatigability. 28 subjects (women: N = 17; men: N = 11) volunteered for the study. Maximal voluntary contraction force (MVC) of the adductor pollicis muscle was significantly lower ($P < 0.05$) in women than men, but from these groups, an equal number of male and female subjects (N = 9) were matched for strength and used for further analysis. Each subject visited the testing center twice, once for a familiarization session and once for the definitive experimental protocol. For the experimental protocol, subjects had their right hand and arm secured into supination with

fingers flexed and thumb abducted. A force transducer (model SSM-250, Interface, Scottsdale, AZ; sensitivity 1.5 mV kg^{-1}) was attached and interfaced with an amplifier (model 13-421202, Gould, Cleveland OH; 90% response time in 2 ms), chart recorder (model 2200, Gould), and oscilloscope. Subjects could visually observe the oscilloscope at all times in order to maintain the force trajectory. Each subjects performed 3 MVC trials to determine their highest possible MVC force. The subjects performed a series of 5-s static contractions at 50% MVC, followed by 5-s rest. After every minute (6 contractions), a 5-s MVC was performed instead of the 50% MVC. When subjects could no longer maintain the target force for 5 seconds, they were considered exhaustion. After exhaustion, a final MVC was recorded and was recorded again every 3 minutes during recovery. Adductor pollicis muscle strength was not different ($P > 0.05$) between women and men (132±5 N; 136±4 N, respectively). After the first minute of contractions, MVC force had fallen significantly more ($P < 0.01$) for men (-27±4 N) than women (-9±2 N). Exhaustion occurred at nearly identical force levels ($P > 0.05$) for men and women (75±3 N; 73.3±3 N), but women's time to exhaustion (14.7±1.6 min) was approximately twice as long ($P < 0.05$) as that of the men's (7.9±0.7 min). The authors concluded that fatigue differences must originate from some sex-related difference rather than strength differences. Additionally, the more rapid recovery of MVC force for the women implied that at least part of women's increased endurance ability is due to greater capacity for recovery between contractions.

CHAPTER III

METHODS

Subjects

20 healthy participants (8 male, mean ± SD age = 19.5 ± 1.2 years, height = 180.31 ± 10.32 cm, body mass = 77.39 ± 12.29 kg; 12 female, mean ± SD age = 19.9 ± 1.2 years, height = 165.15 ± 6.56 cm, body mass = 68.95 ± 12.69 kg) participated in this investigation. None of the participants had participated in any form of structured exercise program for the previous 6 months. None of the participants had any history of current or ongoing neuromuscular diseases or musculoskeletal injuries specific to the ankle, knee, or hip joints. This study was approved by the University's institutional review board for human subjects research. Each subject read a signed an informed consent form and completed a health status questionnaire prior to the study beginning.

Testing Timeline

Participants attended three laboratory sessions. Visit one included the completion of a health history questionnaire and informed consent form and subjects became familiar with the isometric strength testing measurements, such as submaximal isometric trapezoidal muscle actions, and isometric maximal muscle actions. Visit 2 included ultrasound imaging to determine subcutaneous fat and cross-sectional area. Visit 3 included experimental isometric strength testing of the leg extensors and a muscle biopsy.

Ultrasound

Ultrasound was used to measure anatomical muscle cross-sectional area (mCSA) and subcutaneous fat (sFAT) of the VL. Distance from the anterior superior iliac spine to the superior border of the patella on the right leg was measured and a mark was placed at 50% of total leg length. The participants then laid in a supine position for 10 minutes in order to allow fluid shifts to occur. A portable brightness mode (B-mode) ultrasound imaging device with a multi-frequency linear-array probe (12 L-RS; 5-13 MHz; 38.4-mm field of view) in conjunction with GE logiq e Logic View software was used to generate real-time images of the VL. Equipment settings included gain (68 dB), frequency (10 MHz), and depth (6.0 cm) to optimize image quality and were held constant across all subjects. Great care was taken to ensure consistent minimal pressure was applied with the probe to the skin to avoid any muscle compression. A generous amount of water-soluble transmission gel was applied to the skin to reduce possible near-field artifacts and enhance acoustic coupling. A custom foam support was positioned perpendicular to the longitudinal axis of the thigh to ensure ultrasound probe movement in the transverse plane. The Logiq-view function was used to obtain a single mCSA image of the VL. All ultrasound imaging analyses was performed using ImageJ software (Version 1.46r, National Institutes of Health, Bethesda, MD, USA). Each image was scaled from pixels to cm using centimeter marks inlaid in the ultrasound image. For mCSA (cm^2), the muscle was outlined using the polygon function (Figure 1), with care taken to exclude the surrounding fascia. sFAT was quantified as the distance between the skin and the superficial aponeurosis of the muscle (Figure 2).

Muscle Biopsy

After experimental testing, muscle biopsies were taken from the VL at the midpoint of the thigh (similar position of EMG electrode), midway between the inguinal ligament and the patella on the right leg, with the percutaneous needle biopsy methods of Bergstrom (1962). After careful cleaning of the sample site, a local anesthetic (2% lidocaine) was injected cutaneously, and a small incision was made through the skin and deep fascia with a no. 11 scalpel. The sample was taken with a traditional Bergstrom needle (Pelomi Medicals, Albertslund, Denmark), utilizing the double chop and suction method (Evans, Phinney, & Young, 1982; Staron et al., 1990). The subjects was required to return to the laboratory 24–48 h after the biopsy procedures to ensure that the incision was healing properly. Muscle samples were frozen with isopentane cooled in liquid nitrogen for later analysis of % MHC isoform. Percent MHC isoform was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Carraro & Catani, 1983; Perrie & Bumford, 1986). Muscle samples were weighed using an AX105 DeltaRange balance (Mettler Toledo, Columbus, OH), and then added to lysing buffer [10% (wt/vol) glycerol, 5% (vol/vol) β-mercaptoethanol, 2.3% (wt/vol) SDS in 62.5 mmol/l Tris·HCl, pH 6.8] using a 10 mL/g sample dilution. The sample-lysing buffer mixture was then mechanically homogenized with a mortar and pestle (Kimble Chase LLC, Vineland, NJ) until the sample was completely dissolved. Prepared samples were then placed on ice and vortexed (~5 s) every 10 minutes for 30 minutes. After 30 minutes, prepared samples were centrifuged (~10 s) and frozen. After at least 2 hours of freezing (-80 C), homogenate solutions were further diluted (1:20) with lysing buffer. To determine % MHC isoform, small amounts of extracts (10 μl) were loaded on 4–8% gradient gels with 4% stacking gels, run overnight (18–24 h) at 120 V, and stained with Coomassie blue. The MHC isoforms (types I, IIA, and IIX) were identified according to their molecular masses (Staron &

Hikida, 1992; Staron & Johnson, 1993) (Figure 3). The % MHC areas and total % MHC II area (% MHC IIA + % MHC IIX) were used for sex comparisons and the correlations with the data derived from the EMG signals. Strong relationships ($r > 0.82$) were reported between the histochemically determined percentage fiber type area and the electrophoretically determined MHC content for the VL in males and females (Andersen & Aagaard, 2000; Fry, Allemeier, & Staron, 1994; Staron et al., 2000). It has been reported that the fiber type distribution of the whole VL muscle in humans is not different along the axis of the muscle (Lexell, Downham, & Sjostrom, 1983) and that the differences in variation with single-fiber measurements from muscle biopsies account for < 2% of a change in fiber type proportion (Williamson, Gallagher, Carroll, Raue, & Trappe, 2001).

Isometric Strength Testing

Each participant was seated with restraining straps over the pelvis, trunk, and contralateral thigh, and the lateral condyle of the femur was aligned with the input axis of a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) in accordance with the Biodex User's Guide (Biodex Pro Manual, Applications/Operations, 1998). All isometric leg extensor strength assessments were performed on the right leg at a flexion of 90°. Isometric strength for the right leg extensor muscles was measured using the torque signal from the Biodex System 3 isokinetic dynamometer.

During the experimental trials, participants performed three isometric maximal voluntary contractions (MVCs) with strong verbal encouragement for motivation followed by two, consecutive submaximal isometric trapezoid muscle actions at 40% relative to the maximum recorded MVC strength. The highest torque output for visit 3 determined the maximal torque

output for each participant and the force level for the 40% MVC submaximal isometric trapezoid muscle actions for the isometric strength testing visit. For all isometric trapezoid muscle actions, the force increased at 10% MVC/s to the desired torque level, where it was held during a 45 s plateau and then decreased to baseline at a rate of -10% MVC/s (Figure 4). Therefore, the duration of each contraction was 53 s and participants were given an ~6 s rest interval between repetitions for the 3 s queiescent period required prior to and following the contraction. Participants were instructed to maintain their force output as close as possible to the target force presented digitally in real time on a computer monitor.

Electromyographic Recording

During the isometric muscle actions, surface EMG signals were recorded from the VL using a 5-pin surface array sensor (Delsys, Boston, MA). The pins have a diameter of 0.5 mm and were positioned at the corners of a 5 x 5 mm square, with the fifth pin in the center. Prior to sensor placement, the surface of the skin was prepared by shaving, removing superficial dead skin with adhesive tape (3M, St Paul, MN), and sterilizing with an alcohol swab. The sensor was placed over the VL muscle at 50% of the distance between the greater trochanter and the lateral condyle of the femur with adhesive tape. The reference electrode was placed over the left patella. The signals from four pairs of the sensor electrodes were differentially amplified and filtered with a bandwidth of 20 Hz to 9.5 kHz. The signals were sampled at 20 kHz and stored on a computer for off-line analysis.

EMG Decomposition

For detailed information regarding the signal processing of the EMG signals, refer to De Luca et al. (2006) and Nawab (2006). Action potentials were extracted into firing events of single MUs from the four separate EMG signals via the PD III algorithm as described by De Luca et al. (2006). This algorithm is designed for decomposing EMG signals into their constituent MU action potential trains. The accuracy of the decomposed firing instances was tested with the reconstruct-and-test procedure (Nawab, Chang, & De Luca, 2010). Only MUs with >90% accuracies were used for further analysis. In addition, the firing rate curve of each MU was computed by low-pass filtering the impulse train with a unit area Hanning window of 2-s duration (C. J. De Luca & Contessa, 2012; C. J. De Luca & Hostage, 2010). For each MU, 4 parameters were extracted from the firing rate data: 1) recruitment threshold (RT, expressed as %MVC), 2) mean firing rate (MFR, pulses per second [pps]) 3) MU action potential amplitude ($MUAP_{AMP}$, mV), and 4) MUAP duration ($MUAP_{DUR}$, ms). The MFR was calculated as the average value of the mean firing rate trajectory during a 10 second interval between 2 and 12 s during the constant torque segment of each contraction. An average 0.10 ms epoch of force that began at the first discharge of the MU was selected as the RT for the MU. The averages of the peak-to-peak amplitude and the duration from each of the four action potential waveforms were used to calculate $MUAP_{AMP}$ and $MUAP_{DUR}$, respectively (Figure 5).

EMG Amplitude

Channel 1 of the 4 bipolar EMG channels from the 5-pin surface array sensor was selected for the time-domain (amplitude) analyses. The time period that corresponded with the interval analyzed for the MU data was isolated and averaged for subsequent amplitude and force

calculations. The EMG signals were bandpass filtered (fourth-order Butterworth) at 10–500 HZ. The time domain of the EMG signal was calculated with the root mean square (RMS) function for each steady force segment of the plateau for the isometric muscle actions. Offline processing was performed with custom-written LabVIEW version 16 software (National Instruments).

Statistical Analysis

For the subjects' demographic data, independent t-tests were used to determine potential differences between sex, weight, height, age, and MVC. To determine significant interactions between % MHC isoforms and sex, a two-way ANOVA was used. Only MHC isoforms I and IIA were used for analysis, as the value for MHC IIX is dependent on the values of I and IIA. For EMG_{RMS}, significant interactions between sex and repetition were analyzed with a two-way ANOVA. For the MU data, linear regressions were performed on the MFR vs. RT, $MUAP_{AMP}$ vs. RT, and $MUAP_{DUR}$ vs. RT relationships, whereas, exponential regressions were performed on the MFR vs. $MUAP_{AMP}$ relationships (Contessa, De Luca, & Kline, 2016). Only MUs recruited during the linearly increasing force segment were included in analysis; MUs recruited during steady force were excluded. Slope and y-intercept values were calculated for each linear relationship, while *A* and *B* terms were calculated for the exponential relationship. Significant interactions between sex and repetition for each MU parameter were analyzed with two-way ANOVAs. When appropriate, follow-up analyses for the ANOVA models were performed using dependent samples t-tests with Bonferroni corrections. For all individual relationships, slopes and y-intercepts and the *A* and *B* terms were calculated using Microsoft Excel version 2013 (Microsoft, Redmond, WA). The level of significance was set at $P \leq 0.05$ for the statistical tests. Statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, New York).

CHAPTER IV
RESULTS

Subject data

There were no significant differences between groups in age (female = 19.8 ± 1.3 yrs, male = 19.5 ± 1.2 yrs; P = 0.577) or body mass (female = 66.3 ± 14.14 kg, male = 79.35 ± 16.63 kg; P = 0.070). However, significant differences did exist between sexes for height (female = 165.21 ± 6.16 cm, male = 180.69 ± 8.31 cm; P < 0.001) and MVC strength (female = 125.9 ± 29.3 N, male = 209.1 ± 75.8 N; P = 0.003) (Table 1).

Ultrasound

Significant differences existed between sexes for sFAT (female = 1.55 ± 0.53 cm, male = 0.88 ± 0.73 cm; P = 0.027) and mEI (female = 63.26 ± 10.98 au, male = 51.06 ± 10.39 au; P = 0.029). However, no significant differences existed for mCSA between sexes (female = 19.02 ± 3.88 cm^2, male = 26.52 ± 9.67 cm^2; P = 0.069) (Table 1).

Myosin heavy chain analysis

There were no significant interactions for sex × % MHC isoform (P = 0.107). There was no significant main effect for sex (P = 0.497), but there was a significant main effect for % MHC isoform (P = 0.028). Percent MHC I fiber area was greater (45.45 ± 9.53%) than % MHC IIA (37.16 ± 10.34%) collapsed across sexes (Figure 3) (Table 2).

EMG_{RMS}

There was no significant interaction for sex × repetition (P = 0.987). There was a significant main effect for sex (P = 0.001), but there was no significant main effect for repetition (P = 0.447) (Figure 6). Males had greater EMG_{RMS} at steady force than the females collapsed across repetitions.

MU data

For the first repetition, 36.4 ± 8.0 and 39.4 ± 6.5 MUs were included with RT ranges from 5.01 ± 7.11 to 29.2 ± 7.09% and 4.81 ± 3.46 to 32.34 ± 3.82% MVC for the females and males. Due to recruitment during steady force, 26 (of 469 total) MUs were excluded for the females, and 11 (of 326) were excluded for the males. For the second contraction, 36.3 ± 10.7 and 33.8 ± 14.1 MUs were recorded with RT ranges from 5.36 ± 5.86 to 29.46 ± 6.93% and 5.75 ± 2.63 to 34.26 ± 4.75% MVC for females and males. Due to recruitment during steady force, 21 (of 459) MUs were excluded for the females, and 66 (of 338) were excluded for the males. All relationships possessed RT ranges >15% MVC (Table 3).

MU relationships

All subjects' relationships were significant for the MFR vs. RT (REP 1, r = -0.946 ± 0.021; REP 2, r = -0.931 ± 0.040), the $MUAP_{AMP}$ vs. RT (REP 1, r = 0.791 ± 0.082; REP 2, r = 0.766 ± 0.072) and the $MUAP_{AMP}$ vs. MFR (REP 1, r = 0.799 ± 0.106; REP 2, r = 0.793 ± 0.066) relationships.

For the MFR vs. RT relationship, there were no significant two-way interactions (sex × repetition) for the slopes (P = 0.112) or y-intercepts (P = 0.633). However, there were significant

main effects for sex (P = 0.005) and repetition (P = 0.037) for the slopes. The slopes were more negative for females (-0.276 ± 0.020 pps/%MVC) than males (-0.184 ± 0.024 pps/%MVC) (Figure 4). The slopes were more negative for the first repetition (-0.264 ± 0.022 pps/%MVC) than the second repetition (-0.197 ± 0.22 pps/%MVC). For the y-intercepts, there was no main effect for repetition (P = 0.280), however, there was a main effect for sex (P = 0.020). Females had greater y-intercepts (22.57 ± 0.44 pps) than the males (20.86 ± 0.543) (Figure 7A).

For the $MUAP_{AMP}$ vs. RT relationship, there were no significant two-way interactions (sex × repetition) for the slopes (P = 0.076) or y-intercepts (P = 0.386). There were also no main effects for sex (P = 0.243) and repetition (P = 0.173) for the slopes or repetition (P = 0.095) for the y-intercepts. There was, however, a significant main effect for sex (P = 0.027) for the y-intercepts. The males had greater y-intercepts (0.023 ± 0.014 mV) than the females (0.015 ± 0.009 mV) (Figure 7B).

For the $MUAP_{AMP}$ vs. MFR relationship, there were no significant interactions for sex × repetition for the *A* terms (P = 0.704) or *B* terms (P = 0.983). There were also no main effects for sex (P = 0.066) and repetition (P = 0.344) for the *A* terms or repetition (P = 0.569) for the *B* terms. There was, however, a significant main effect for sex (P = 0.040) for the *B* terms. Males (-4.48 ± 0.52 pps/mV) had less negative *B* terms than females (-5.92 ± 0.43 pps/mV) (Figure 7C).

CHAPTER V

DISCUSSION

There were no sex-related differences for mCSA and % MHC isoforms, however, there were differences in MFRs at steady torque. Females possessed greater firing rates at the steady torque than the males. There were no significant changes in $MUAP_{AMPS}$ from the 1st to 2nd repetition, however, there was a change in MFRs when expressed as a function of RT but not $MUAP_{AMP}$.

Contrary to previous literature, the male and female subjects for this study did not have significant differences in mCSA (Doherty, 2001; Frontera et al., 2000; Kubo et al., 2003; Miller, MacDougall, Tarnopolsky, & Sale, 1993) (Table 1) or % MHC isoforms (Miller et al., 1993; Staron et al., 2000) (Figure 3). However, there can be great variability within sex for these measurements, especially with small sample sizes (Maughan, Watson, & Weir, 1983). Although not significant, the differences in the means do reflect previous literature with the males having larger mCSAs and greater % MHC II isoforms.

Previously, the slopes from the $MUAP_{AMPS}$ vs. RT relationships have accounted for a portion of the sex-related differences in mCSA and % MHC isoform area (Trevino et al., 2018). Presently, however, there were no differences in mCSA or % MHC isoform between sexes and there were no significant sex × repetition differences observed for the $MUAP_{AMP}$ vs. RT relationship. However, the y-intercepts for the males were larger than the females, while the slope values were not significantly different between sexes (Figure 8B). This could imply that MUs for males were larger at every recruitment threshold, although the differences in MU size between males and females was not large enough to constitute a significant difference in mCSA. In a closer examination of the relationships, the second repetition for the males appears to be

primarily contributing to the significant main effect for sex between y-intercepts (Figure 9B). The mean (SD) y-intercept values for the 1st repetition for the males (0.019 ± 0.011 μV) and females (0.014 ± 0.012 μV) were similar, but greater differences did exist for the 2nd repetition (males = 0.028 ± 0.016 μV; females = 0.017 ± 0.006 μV). Similar to mCSA, the non-significant differences in the slopes between sexes (males = 0.003 ± 0.002 μV/%MVC; females = 0.002 ± 0.001 μV/%MVC) from the $MUAP_{AMP}$ vs. RT relationships did reflect previously reported differences (Trevino et al. 2018).

In the present study, the MFR vs. RT relationship did not exhibit significant sex × repetition differences, but did demonstrate main effects for sex (slope and y-intercept) (Figure 8A). The females exhibited greater firing rates of the lower-threshold MUs, however, the higher-threshold MUs did not differ as a function of sex. To date, few other studies have investigated the possible sex-related differences between firing rates and RT. Peng & Tenan (2018) and Tenan et al. (2013) have previously investigated potential sex-related differences initial firing rates (IFR), rather than MFR vs. RT relationships. However, evidence would suggest (Wray et al., *in review*) that the IFR analysis can be extrapolated to the MFR vs. RT relationship, especially at moderate-to-high contraction intensities. The results of the MFR vs. RT relationships in the present study were consistent with what has been previously reported (Wray et al. *in review*) for sex-related differences in y-intercepts. Additionally, studies investigating IFR found that females exhibited slightly higher IFRs than males for a 75% MVC in the vastus medialis (Peng et al., 2018), indicating that females would most likely have significantly higher y-intercepts than males in the MFR vs. RT relationship. However, these sex-related differences were only observed at higher contraction intensities. At 10% MVC (Wray et al. *in review*) and 30% MVC (Tenan et al., 2013), IFRs were not significantly different between sexes. It appears

that the 40% MVC included in the present study was sufficiently high enough of intensity to observe these sex-related differences in firing rates.

Furthermore, when MFR was analyzed as a function of $MUAP_{AMP}$, there were no significant sex × repetition differences, but there was a main effect for sex in the *B* terms. The males (-4.48 ± 0.52 pps/mV) had less negative *B* terms than females (-5.92 ± 0.43 pps/mV). However, the similarities in the *A* terms indicate that for similarly-sized MUs, those recruited for the males could be at a lower overall recruitment threshold than females. Therefore, while the slope for the MFR vs. RT relationship changed slightly, this can be explained by a shift in operating point (Contessa & De Luca, 2013), rather than individual MUs firing at a higher rate. While the firing rates for the smallest MUs were similar, as the sizes of the amplitudes (and therefore the sizes of the MUs) increased, the males maintained a higher MFR than the females for each increment in MU size. This indicates that the females were recruiting a higher percentage of MUs to maintain the necessary force level.

The higher firing rates of the lower-threshold MUs observed in the MFR vs. RT relationship may be an indication that the females were at a greater operating point of motor control (Contessa & De Luca, 2013). This could be the result of greater co-contraction of the leg flexors and/or non-uniform differences in the twitch forces of the MUs, such as, the lower-threshold MUs were of similar size and strength with males possessing larger MUs that were stronger in comparison to the females. Such a phenomenon would require greater excitation to the motoneuron pool to maintain the same relative strength for the females. This increased excitation would cause an increase in the firing rates of the lower-threshold MUs and recruitment of larger, higher-threshold MUs with lower firing rates, effectively shifting the operating point to the right for the females.

There was also a main effect for repetition for the MFR vs. RT relationship, with slopes decreasing from repetition 1 (males = -0.243 ± 0.052 pps; females = -0.284 ± 0.070 pps) to repetition 2 (males = -0.126 ± 0.091 pps; females = -0.268 ± 0.043 pps). Of note, it would appear that changes in the slopes for the males was the primary contributor to the main effects, whereas, the slopes for the females did not change to the same extent as the males (Figure 9A). Nonetheless, the EMG_{RMS} did not significantly increase during the second repetition. Therefore, it appears that there was not a significant increase in the recruitment of larger MUs during the linearly increased force segment of the second repetition (Martinez Valdes, Negro, Falla, De Nunzio, & Farina, 2018). The less negative slopes with no change y-intercepts suggests that the larger higher-threshold MUs displayed an increase in firing rates at steady force. However, the strict inclusion criteria for MUs resulted in the exclusion of MUs that were recruited during steady force. There were considerably more excluded MUs in the second repetition. It can be assumed that these later-recruited MUs were larger and, therefore, maintained a lower firing rate, which if included could have ultimately created a more negative slope value from the MFR vs. RT relationship that would match second repetition.

For the MFR vs. $MUAP_{AMP}$ relationship, no significant main effects were observed between repetitions in either *A* or *B* terms. This, taken with the significant difference in slope in the MFR vs. RT relationship, implies that the recruitment position was being altered during the second repetition; i.e. the same MUs were being recruited earlier, as often happens during long contractions at any intensity (Adam & De Luca, 2005). Therefore, firing rates of a given MU were not achieving greater firing rates, but rather the recruitment position was changing, which supports the findings of Contessa et al. (2013).

Although there were minimal sex-related differences observed for the MU relationships, this result was not entirely unexpected due to the lack of significant differences between sexes in % MHC isoforms or mCSA. Previously, these differences in % MHC isoforms and mCSA have explained the differences in the properties of MUs (sizes and firing rates) (Hunter, 2009; Trevino et al., 2016; Trevino et al., 2018). However, while not statistically significant, differences in % MHC isoform and mCSA trended in the expected directions (males % MHC I area < females % MHC I area; males mCSA > females mCSA). These non-significant differences were also observed in the MU relationships. It was hypothesized that significantly greater % MHC II for the males would manifest into a greater change in $MUAP_{AMP}$ and MFR vs. RT relationships in comparison to the females as a result of lower resistance to fatigue. However, there were no differences in % MHC II and, therefore, it would be expected that there would be no sex-related differences in the responses of MUs during the elongated repetitive contractions as reported in the present study.

In summary, differences in firing rates did exist between groups, similar to what has been previously reported (Peng et al., 2018). In contrast, the males and females did not exhibit significant differences in % MHC isoforms or mCSA unlike previous research. Therefore, the lack of differences between groups for the changes in firing rates or MU action potential amplitudes from the 1st to 2nd repetition is not surprising (Figure 9). The firing rates did change (collapsed across sex) from the 1st to 2nd repetition and was likely a function of larger MUs participating earlier in the contraction.

Table 1. Mean±SD values for subject demographics (height, weight, and age) and from B-mode ultrasound images of the vastus lateralis muscle [muscle cross-sectional area (mCSA), and subcutaneous fat (sFAT)] for the female and male groups.

	n	Height (cm)	Weight (kg)	Age (years)	mCSA (cm^2)	sFAT (cm)
Females	10	165.21±6.16	66.03±14.14	19.83±1.34	19.02±3.88	1.55±0.532
Males	8	180.69±8.31 *	79.35±16.63	19.5±1.20	26.52±9.67	0.875±0.73 *

* males significantly different from females ($P < 0.05$)

Table 2. Mean±SD values for myosin heavy chain (MHC) isoform area percentages for females and males.

	MHC I (%)	MHC IIA (%)	MHC IIX (%)
Females	46.68±10.77	34.20±11.40	19.12±10.6
Males	43.62±7.61	41.60±6.99	14.78±7.76

Table 3. Motor unit (MU) recruitment ranges, MU counts, and electromyographic amplitude (EMG_{RMS}) for repetitions (Rep) 1 and 2 for the females and males.

	Repetition 1			Repetition 2		
	Recruitment Range (%MVC)	MU Count	EMG_{RMS} (μV)	Recruitment Range (%MVC)	MU Count	EMG_{RMS} (μV)
Females	5.01±7.11 - 29.24±7.09	36.42±8.01	20.05±5.65	5.36±5.86 - 29.46±6.93	36.25±10.66	21.82±6.35
Males	4.81±3.46 - 32.34±3.82	39.38±6.50	28.23±9.25	5.75±2.63 - 34.26±4.75	33.75±14.10	30.07±8.44

Figure 1. Cross-sectional scan of the vastus lateralis muscle via ultrasonography demonstrating measurement parameters using ImageJ software.

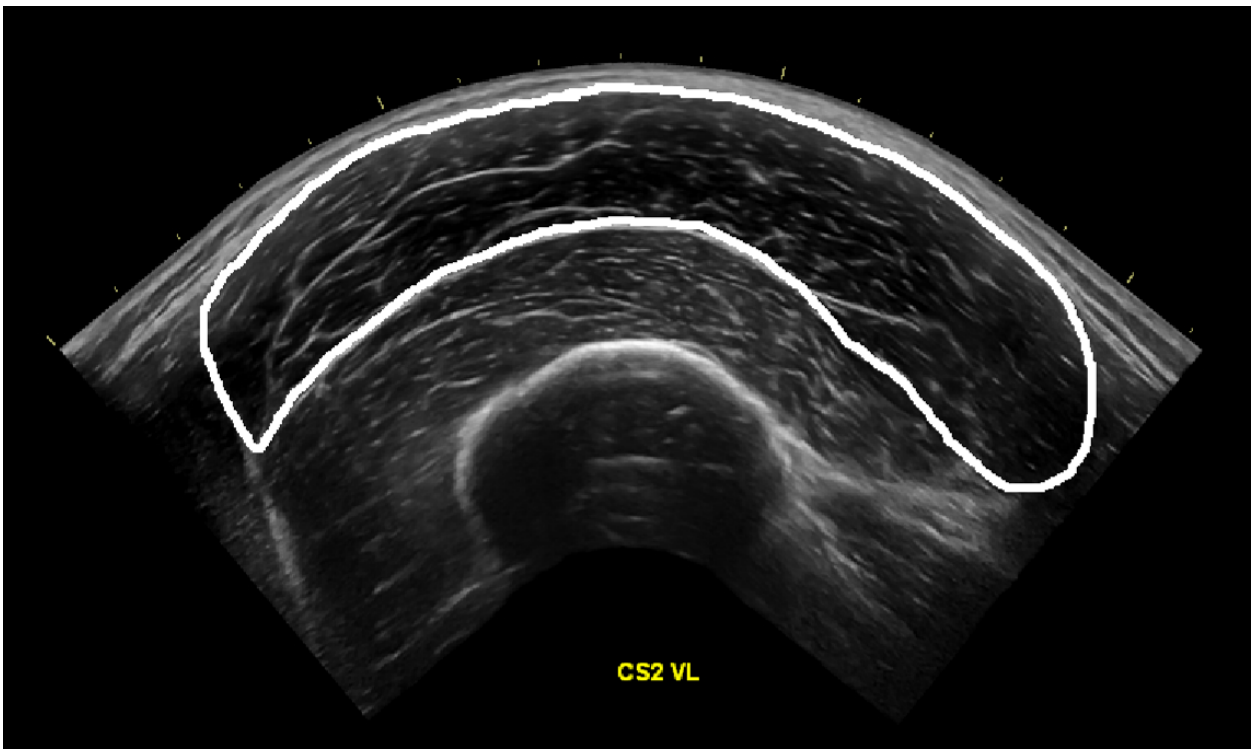


Figure 2. B-mode scan of the vastus lateralis muscle via ultrasonography demonstrating measurement parameters using ImageJ software.

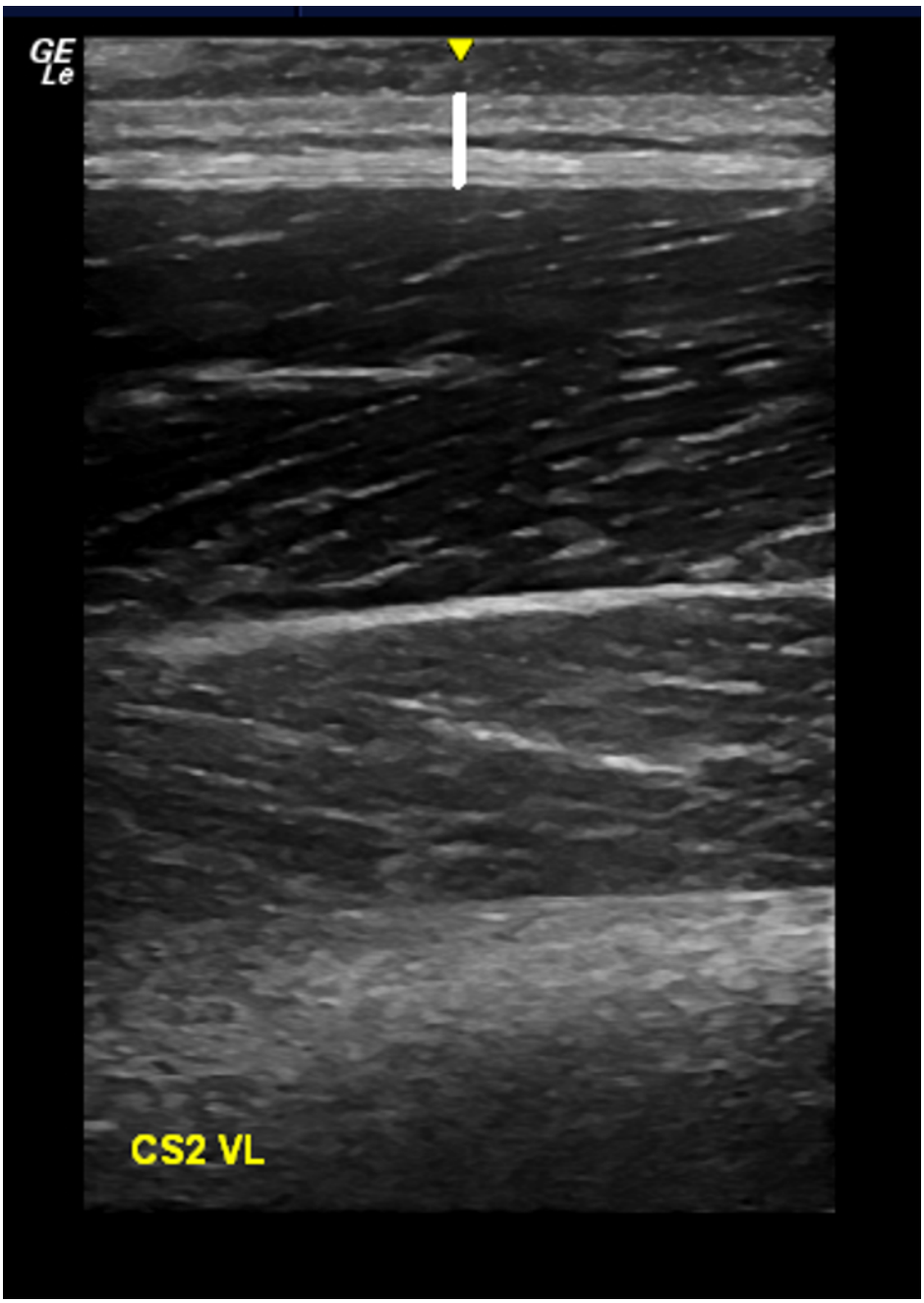


Figure 3. Means and standard deviations for % myosin heavy chain (MHC) isoforms (I, IIA, IIX) for the females and males.

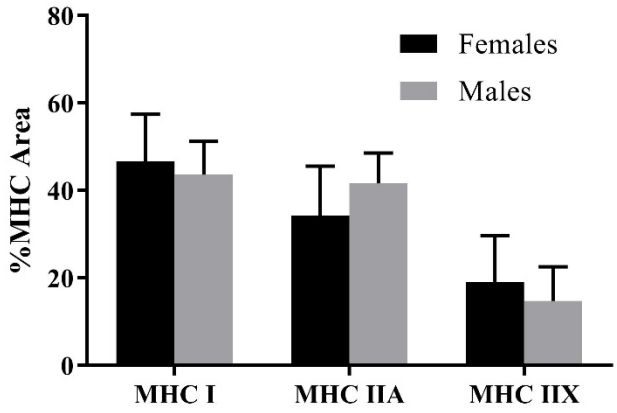


Figure 4. Top graphs: An example of the average firing rate plots of detected motor units (MUs) recorded from the five-pin surface array sensor for the vastus lateralis during isometric trapezoidal contraction at 40% maximal voluntary contraction (MVC) for one participant for repetitions 1 and 2. The black line shows the force signal as it appeared to the participant during the trial. The gray curves represent the average firing rates in pulses per second (pps) across time for each MU. The dotted lines demonstrate the epoch at which the mean firing rate vs. recruitment threshold relationship was calculated. Bottom graphs: The individual subject's plotted mean firing rate vs. recruitment threshold relationship for repetition 1 and 2.

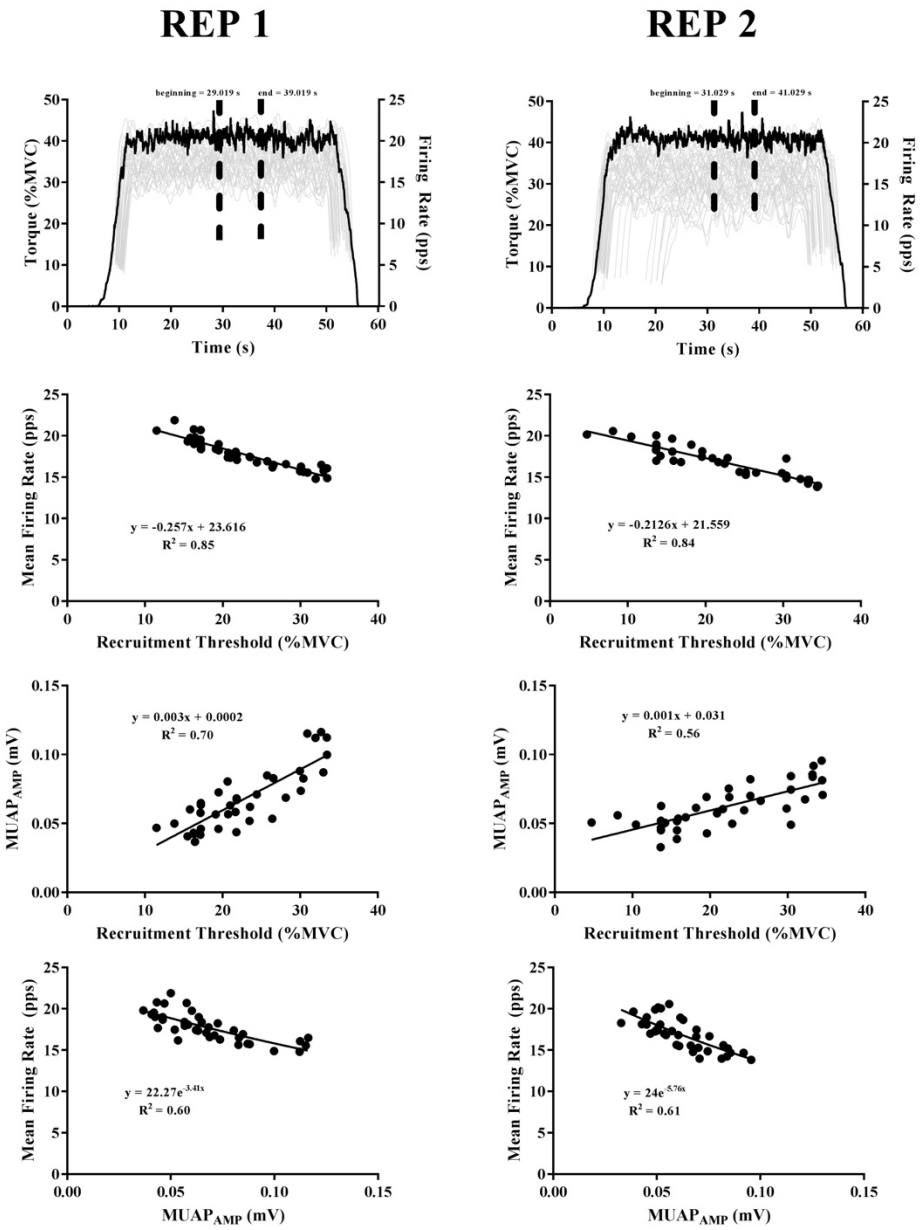


Figure 5. The motor unit action potential (MUAP) waveforms from channels (CH) 1–4 for one participant during repetitions 1 and 2. Measurement methods for MUAP amplitude ($MUAP_{AMP}$) and duration ($MUAP_{DUR}$) are illustrated by gray bars and provided for solid black line.

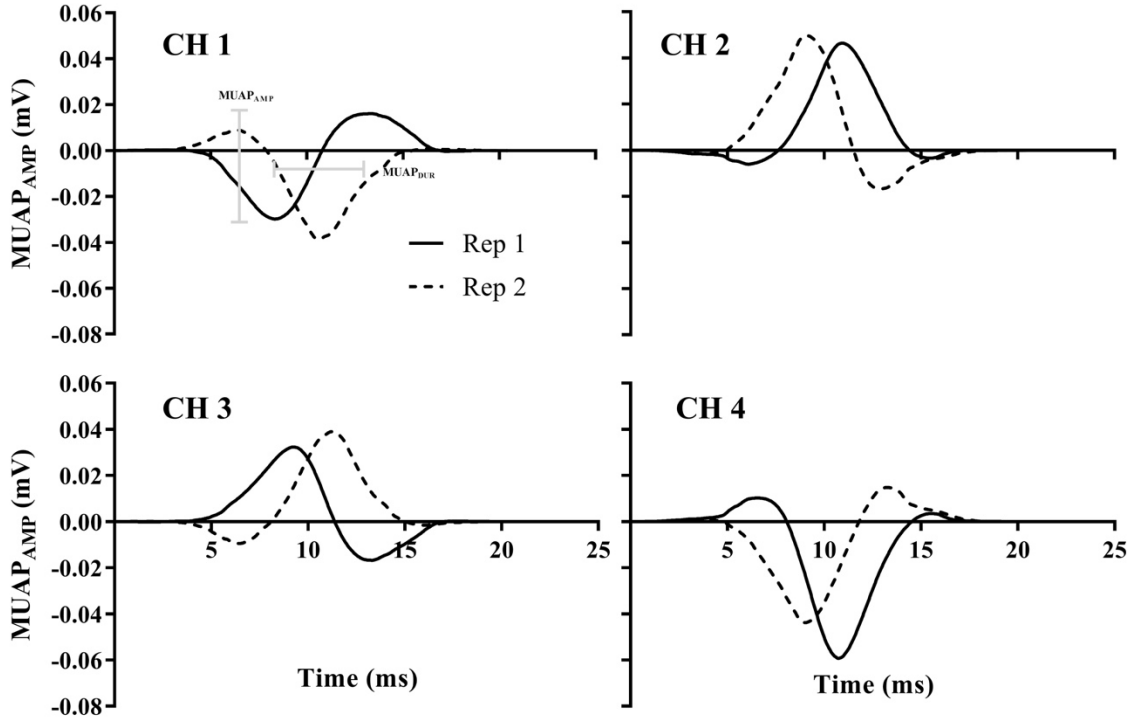


Figure 6. Mean and standard deviations for electromyographic amplitude (EMG_{RMS}) recorded for repetitions 1 and 2 for the females and males. * Indicates significant differences between sexes ($P < 0.05$).

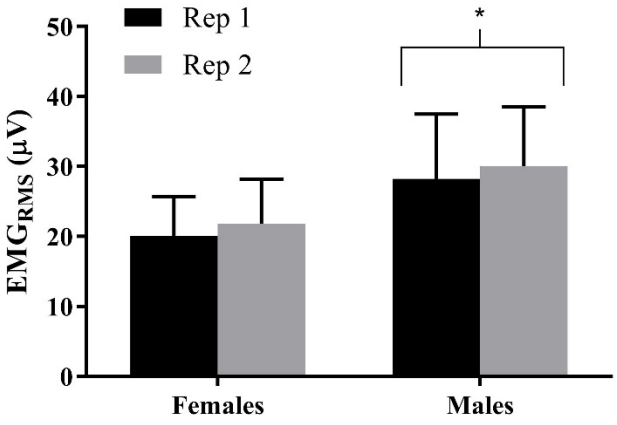


Figure 7. Means and standard deviations for slopes and *y*-intercepts of the (A) mean firing rate [MFR, pulses per second (pps)] vs. recruitment threshold (RT, % maximum voluntary contraction), (B) motor unit potential sizes [$MUAP_{AMP}$ (mV)] vs. RT, and the (C) MFR vs. $MUAP_{AMP}$ relationships. † Indicates significant differences between sexes ($P < 0.05$)

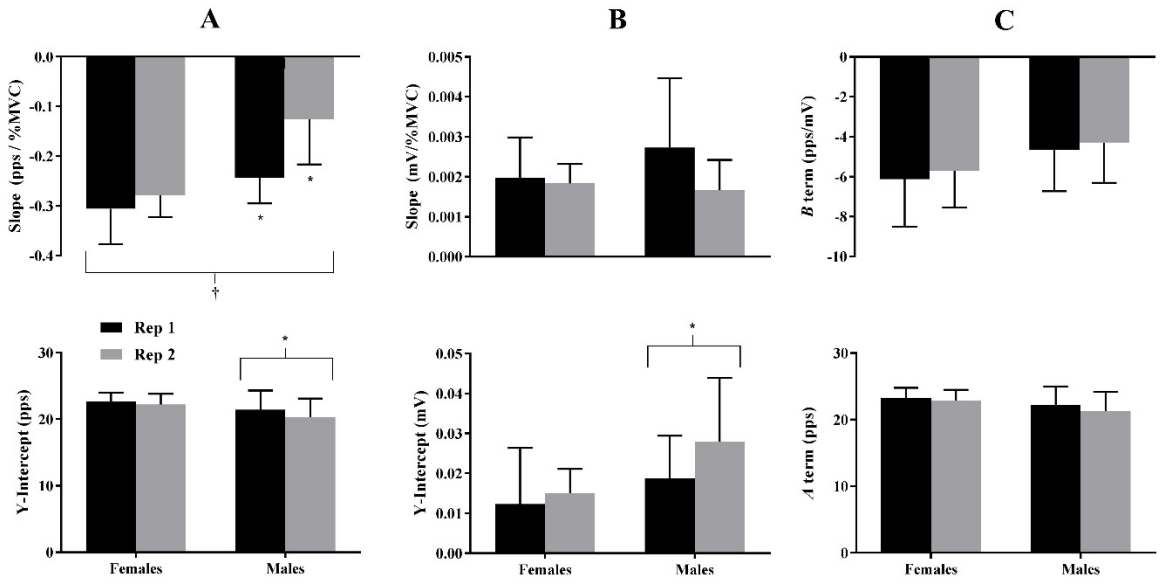


Figure 8. The mean and standard deviation of the predicted (A) mean firing rate [MFR, pulses per second (pps)] vs. recruitment threshold [RT, expressed as percentage of maximum voluntary contraction (%MVC)] relationships calculated from the slopes and *y*-intercepts for each individual, (B) mean and standard motor unit action potential size ($MUAP_{AMP}$, mV) vs. RT relationships calculated from the slopes and *y*-intercepts for each individual, and the (C) mean and standard deviations MFR vs. $MUAP_{AMP}$ relationships calculated from the *A* and *B* terms from each individual for the recorded RT ranges for each group for the first repetition. The composite patterns for the females were calculated (gray dashed line) to the average highest RT reported for the males for illustrative purposes.

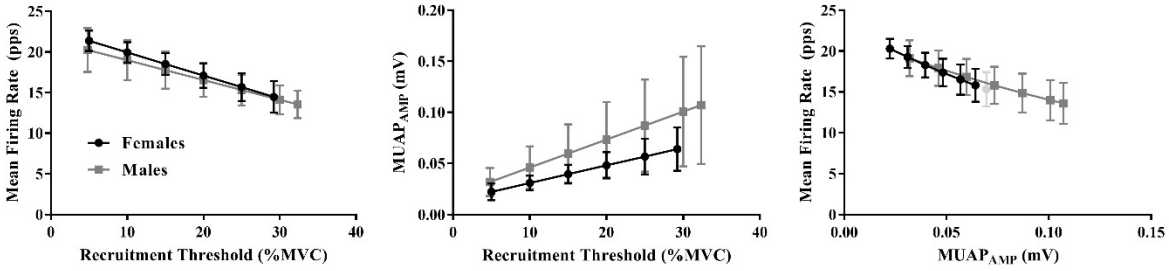
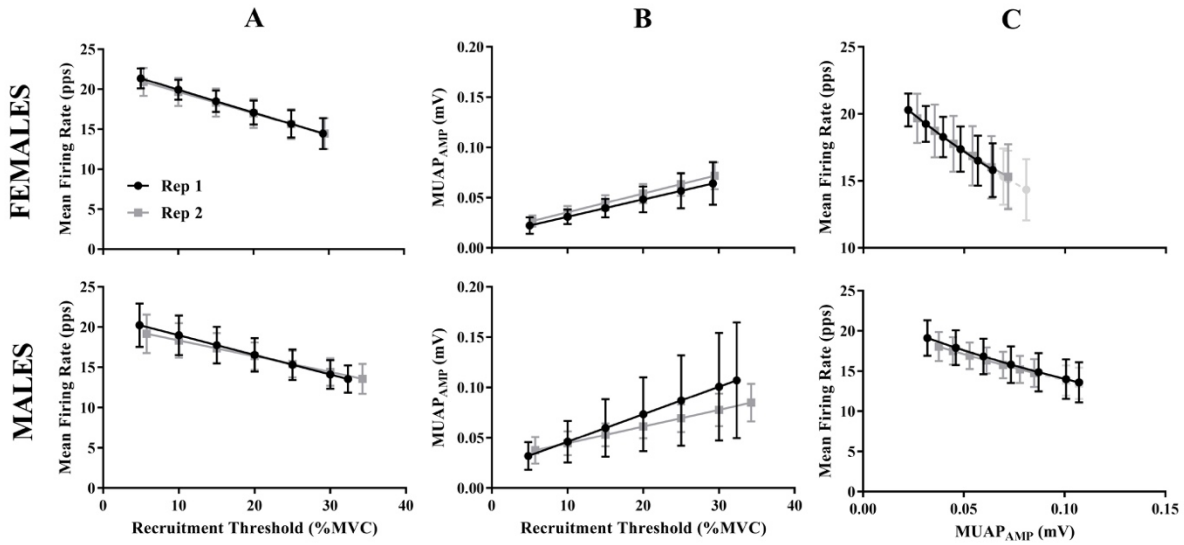


Figure 9. The mean and standard deviation of the predicted (A) mean firing rate [MFR, pulses per second (pps)] vs. recruitment threshold [RT, expressed as percentage of maximum voluntary contraction (%MVC)] relationships calculated from the slopes and *y*-intercepts for each individual, (B) mean and standard motor unit action potential size ($MUAP_{AMP}$, mV) vs. RT relationships calculated from the slopes and *y*-intercepts for each individual, and the (C) mean and standard deviations MFR vs. $MUAP_{AMP}$ relationships calculated from the *A* and *B* terms from each individual for the recorded RT ranges for each group for both repetitions. The composite patterns for the females were calculated (gray dashed line) to the average highest RT reported for the males for illustrative purposes.



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APPENDIX

Informed Consent

Effect of aerobic training on motor unit behavior of the vastus lateralis

INTRODUCTION
The Department of Health Sport and Exercise Science at the University of Kansas supports the practice of protection for human subjects participating in research. The following information is provided for you to decide whether you wish to participate in the present study. You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or the University of Kansas.

PURPOSE OF THE STUDY
You are being asked to participate in this study because you're a healthy adult male or female between the ages of 18 and 40 years. The purpose of this study is to examine the effects of 10 weeks of aerobic cycling training on muscle function and muscle fiber type. The secondary purposes of this study are to examine the effects of the aerobic training on body composition, muscle quality and cardiovascular fitness. Electromyography (EMG) and mechanomyography (MMG) sensors will be placed on your thigh muscles to monitor muscle function during the maximal and submaximal strength tests. Dr. Gallagher or Dr. Fry will perform muscle biopsy procedures on your right quadriceps muscles (thigh muscles). Muscle fiber type information will be quantified from the muscle biopsy. Images of your thigh muscles will be taken with an ultrasound machine to determine muscle size. Bioimpedance spectroscopy will be used to determine body composition (i.e. lean mass and fat-mass). A VO_{2MAX} will be completed to determine the cardiovascular fitness levels. The aerobic cycling training program will be for 10 weeks, with four sessions a week for 30-40 minutes each. All measurements will be taken prior to, at the midpoint, and following the 10 week aerobic cycling training. You may choose to participate in the aerobic cycling training (up to 45 participants) or not participate in the aerobic cycling training (up to 10 participants). If you choose not to participate in the aerobic cycling training, testing visits will be taken at the same time point as the individuals that are participating in the aerobic cycling training (i.e pre-, mid-, and poststudy).

PROCEDURES
A time-line of the testing procedures and an overview of the testing sequence for the 9 testing days are presented below. All procedures will be conducted in the Neuromechanics Laboratory and the Applied Physiology Laboratory at the University of Kansas and will be supervised by trained personnel. Visit 1 will last approximately 60 minutes and we will collect your baseline information (height, weight, etc.), determine if you are eligible for the study, and familiarize you with the neuromuscular testing for the right thigh muscles (leg extensors). Visits 4 and 7 will last approximately 15 minutes and we will refamiliarize you with the neuromuscular testing for the right thigh muscles (leg extensors). Visits 2, 5 and 8 will last approximately 60 minutes, and will include Bioimpedance spectroscopy measurements to determine body composition, ultrasound imaging of the thigh muscles and a VO_{2MAX} test on a cycling ergometer to determine cardiovascular fitness levels. Visit 3, 6 and 9 will last approximately 1.5 hours, and will consist of neuromuscular testing and a muscle biopsy. Visits 1, 2 and 3 will be completed during the first week of the study, and prior to the 10 week aerobic cycling training if you choose to participate in the aerobic cycling training. Visits 4, 5 and 6 will be completed after the first 5 weeks of training for the participants who chose to do the aerobic cycling training, whereas these same visits will occur 5 weeks after visits 1, 2, and 3 for the participants who chose not to participate in the aerobic cycle training. Visits 7, 8 and 9 will be completed following the conclusion of the 10 week cycle ergometer training for the participants who choose to do the aerobic cycling training, whereas these same visits will

occur 5 weeks after visits 4, 5, and 6 for the participants who chose not to do the aerobic cycling training. The 9 testing days and 40 cycle ergometer training sessions (10 weeks, 4x's/wk) will require a total of 49 visits by participants who choose to do the aerobic cycling training. The participants who choose not to participate in the aerobic cycling training will require a total of 9 visits (9 testing days).

Testing visits will be taken at the same time point regardless if you choose to participate in the aerobic cycling training program or not (i.e pre-, mid-, and post-study).

Visit 1: Consent form, pre-exercise testing health and questionnaire determination of eligibility, demographics (height, weight, etc.), and familiarization with testing measurements.
Visit 2: Bioimpedance spectroscopy measurements
Ultrasound imaging of the thigh muscles
VO_{2MAX} test in the Applied Physiology Laboratory
Visit 3: Perform isometric maximal and submaximal strength testing
Muscle biopsy in the Applied Physiology Laboratory

Cycle Ergometer Training: Participants who choose to participate in the aerobic cycling training will perform weeks 1 – 5 of aerobic training on a cycle ergometer at a frequency of 4 sessions per week. The duration will be 30-40 minutes at an intensity (60-80%) of the maximal heart rate elicited during the previous $V0_{2MAX}$ test. The training sessions will take place on the second floor of the Robinson Center, which is the same building as the Neuromechanics and Applied Physiology Laboratories. If you choose not to participate in the aerobic cycling training, you will not have any training requirements during these weeks.

Visit 4: Familiarization with testing measurements.
Visit 5: Bioimpedance spectroscopy measurements
Ultrasound imaging of the thigh muscles
VO_{2MAX} test in the Applied Physiology Laboratory
Visit 6: Skinfold thicknesses will be taken at the site of the EMG and MMG electrode placement
Perform isometric maximal and submaximal strength testing
Muscle biopsy in the Applied Physiology Laboratory

Cycle Ergometer Training: Participants who choose to participate in the aerobic cycling training program will perform weeks 6 – 10 of aerobic training on a cycle ergometer at a frequency of 4 sessions per week. The duration will be 30-40 minutes at an intensity (60-80%) of the maximal heart rate elicited during the previous $V0_{2MAX}$ test. The training sessions will take on the second floor of the Robinson Center, which is the same building as the Neuromechanics and Applied Physiology Laboratories. If you choose not to participate in the aerobic cycling training, you not have any training requirements during these weeks.

Visit 7: Familiarization with testing measurements.
Visit 8: Bioimpedance spectroscopy measurements
Ultrasound imaging of the thigh muscles
VO_{2MAX} test in the Applied Physiology Laboratory
Visit 9: Perform isometric maximal and submaximal strength testing
Muscle biopsy in the Applied Physiology Laboratory

Strength testing - Participants may experience some physical stress from the muscle actions performed in this study. Participants will be positioned in a strength testing machine for the leg extensor muscles. The strength testing machine used in this study is similar to the equipment used in physical therapy

clinics. During testing, participants will be seated a position similar to sitting in a chair. The non-tested leg will be comfortably strapped down to the strength testing machine while the tested leg will have a cushioned pad comfortably strapped to the shin of their tested leg. Participants will push the shin of their right leg against the cushioned pad (similar to a kicking motion) to measure right leg strength. After you are positioned and prior to the strength testing, EMG and MMG electrodes will be placed on the skin of your right thigh to monitor muscle function of the thigh muscles. Following 2-4 warm-ups, you will perform 2-3 maximal strength tests which will require you to push your right shin as hard as you can with your thigh muscles. Three minutes of rest will be given between all maximal strength tests in the study.
Then in a random order, you will perform submaximal tests at a low intensity (10-40%) and high intensity (50-80%) of your maximal strength. One to two minutes of rest will be given between the submaximal strength tests. Your leg will not move during these initial strength tests. In addition, you will complete 2-3 maximal strength tests were you kick out as hard and fast as possible and your leg will move. These tests will be performed at a fast and slow speed. All procedures will be tested on the right leg.

Body Composition – Bioimpedance spectroscopy (BIS) measurements will be taken for the assessment lean mass and fat-mass. BIS is used to assess total body water, and is a valid, reliable, and non-invasive assessment to predict body composition. Prior to the BIS assessment, your hydration status will be determined using a handheld device. If you are not properly hydrated, you will be asked to collect your urine and water will be provided if you are not properly hydrated. For the BIS measurements, you will rest on your back with electrodes positioned on the right hand and foot. Unnoticeable currents will be sent between the pair of electrodes and lean mass and fat mass will be calculated with the measured impedance of the current. The BIS uses technology similar to handheld devices in gyms and bathroom scales used to measure body fat, but provides a more valid and reliable measurement. The BIS measurements will take approximately 15 minutes.

Ultrasound imaging – Ultrasound (US) will be used to assess muscle size and quality of the thigh muscles. Participants will rest on their back and a pad will be placed around their right leg. US gel will be placed over the area of the thigh to be imaged, and a probe will be lightly swept across the skin to obtain panoramic scans. The US machine is similar is to equipment used in doctor offices and by other research institutions. The US scans will take approximately 15 minutes.

VO_{2MAX} Testing – Testing will be completed on cycle ergometer. The bike seat will be set at a comfortable position and foot straps will be secured to prevent your feet from slipping during the test. Following a 2 minute warmup at a low-intensity, the resistance will be slightly increased every minute. You will be encouraged to maintain 70 revolutions per minute (rpm), but the test will end when you can no longer maintain 60 rpm. A mouthpiece, attached to headgear to hold it in place, will be used to measure the volume of gas concentrations of inhaled and exhaled air. A nose clip will also be used to ensure that breathing is occurring entirely through the mouth. This test is designed to achieve a maximal effort and is generally considered the best indicator of cardiovascular fitness.

Muscle Biopsy –You have been informed that one of the purposes of this study is to measure the muscle fiber type from the muscle samples that we collect. By obtaining a small sample of your muscle tissue (size of a pencil eraser or small finger nail), the different types of proteins in your muscle may be determined which will be helpful in the evaluation of health and exercise performance. All muscle tissue samples (biopsies) will be taken from the outer portion of the front of the thigh using a needle biopsy technique by either Philip Gallagher or Andy Fry. Philip Gallagher, PhD., Associate Professor of HSES, and Andrew Fry, PhD., Professor of HSES, have performed over 400 muscle biopsies over the past seven years and have assisted on over 1000 biopsies over the past ten years on various populations (athletes,

sedentary people, elderly etc.) with no significant complications and nothing more than minimal adverse reactions. The procedure is being overseen by Jeff Burns, M.D., who is a medical doctor in Neurology at the KU Medical Center in Kansas City, Kansas. Dr. Burns supervises the procedure, but will not be physically present for the biopsies. The total size of the muscle biopsy will be approximately the size of a pea. You will be placed on an examination table lying down on your back (supine) so that the muscles of the leg are relaxed. The skin will be thoroughly cleaned with antiseptic solution (Betadine) using sterile cotton swabs after which a surgical cover will be placed around the sampling site. If you are allergic to Betadine, an alternative antiseptic solution will be used to clean the skin. A small amount (2ml or 2cc) of a local aesthetic (2% Lidocaine) will be injected into the tissue under the skin around the site to be sampled. During this injection you may feel a slight burning sensation. If you are allergic to the local anesthetic, or have had allergic reactions to other anesthetics (i.e.: Novocain) then you will be disqualified from the study. Following the injection of the numbing agent into your thigh, a minimum of five (5) minutes will be allowed to pass to ensure adequate time for the agent to take effect in the area where the incision will be made. A small incision (1 cm) will then be made in the skin overlying the muscle and the biopsy needle inserted into the middle of the muscle (muscle belly) at a depth of 3 cm (about 1 inch). During the time that the sample is being taken (about 5 seconds) you may feel some deep pressure and cramping that will be moderately painful. You have been informed that if you have been previously diagnosed as having a bleeding disorder, a blood clotting problem, take blood thinning medication or have sensitivity to scarring, you will tell the researcher and not participate in the biopsy procedure.
Following the biopsy procedure, firm and constant pressure will be placed on the wound to stop any bleeding. The incision site will be closed with a Steri-Strip and covered with a large Band-Aid and the site compressed using a 10 cm strip of elastic tape for a period of 24-hrs. You have been informed that the procedure will take about 20 min.
Sterile disposable instruments and sterile gloves will be used for the preparation of the site and the reusable biopsy needle will be thoroughly sterilized (via steam and heat autoclave) after each biopsy. Approximately 100-120 mg (size of the exposed lead on a pencil) of skeletal muscle tissue will be removed. With the invasive skeletal muscle biopsy technique there is the possibility of a blood related infection (HIV, Hepatitis B), but the reusable needles will be cleaned and sterilized using an autoclave, a generally accepted method of cleaning surgical instruments.

Cycle Ergometer Training – Participants have the choice to opt-in or opt-out of the aerobic cycling training. Participants who opt-in for the training will perform 10 weeks of aerobic cycling training on a cycle ergometer at a frequency of 4 sessions per week for a total of 40 training sessions. Participants who opt-out of the aerobic cycling training will not have any training requirements for the duration of the study. Each session will last 30-40 minutes at an intensity of 60-80% of the maximal heart rate elicited during the most recent VO_{2MAX} test. Heart rate will be monitored via a heart rate monitor and all training sessions will take on the second floor of the Robinson Center, which is the same building as the Neuromechanics and Applied Physiology Laboratories. Participants may experience some physical stress from the cycle ergometer training.

Please check a box below to indicate if you would like to opt-in or opt-out of the aerobic cycling training.

- ☐ I choose to participate in the 10 week aerobic cycling training program.
- ☐ I choose to not participate in the 10 week aerobic cycling training program.

RISKS
As a participant there is the potential to experience some physical stress during and muscle soreness following the maximal voluntary contractions, submaximal contractions, VO_{2MAX} test, and the cycle ergometer training. In addition, you may have skin abrasions due to shaving and cleansing the skin with

alcohol prior to electrode placement.

Muscle Biopsy - The use of local anesthetics will result in a slight burning sensation, lasting approximately 5 seconds. There is a risk of allergic reactions to the local injection (1 in 1 million). There is a small chance of bleeding from the biopsy site. The principle concern would be prolonged bleeding which would produce a bruise in the area. This would extend the muscle soreness, but is adequately treated with rest, ice, a compression bandage, and keeping the leg elevated as much as possible. Nausea, dizziness, and fainting can occur (1 in 100) during the biopsy process. As a result, the subjects will be in a supine position during the biopsy procedure. There is a risk of infection (1 in 1000) and irritation associated with the biopsy procedure. The use of aseptic techniques, careful cleaning of the skin and keeping the area dry will minimize the risk of infection. There is a risk of bruising (1 in 100) from the biopsy procedure. This will be minimized by placing ice over the site following the procedure and by applying a compression bandage on the site for the 24 hours following the biopsy. In some instances (1 in 200), some motor nerves may be damaged which may cause local muscle atrophy (decrease in the size of muscle fibers, with a small dimple on the skin). There is likely to be a small scar where the biopsy is performed. This scar usually dissipates over a period of 6-12 months at which time the scarring is very modest.

FOLLOW UP CARE

Following the procedure you will be provided with a biopsy care sheet, extra bandages and contact information for Philip Gallagher, Ph.D., Andrew Fry, Ph.D., and Trent Herda, Ph.D. After 24-hrs you must report back to the testing coordinator to check the wound. At this time the bandage will be removed and properly disposed of and a new sterile dressing placed over the wound. You will again be asked to report to the test coordinator after 3-days and be contacted via phone one week after the biopsy or whenever necessary to ensure normal recovery. The biopsy procedure often results in a small fine scar at the site of the incision, however, all care post-treatment will aid in reducing the potential for scarring. All care will be taken to aid in the healing of the wound. The entire biopsy procedure will be performed under sterile conditions. All testing staff and associated personnel will be trained in first aid and will be familiar with emergency procedures.

There have been no other major complications reported in the scientific literature as a result of taking small tissue samples from the skeletal muscle using the percutaneous needle biopsy technique described above. This procedure has been performed on numerous subjects by qualified personnel in many institutions worldwide with only slight discomfort being reported. During the muscle biopsy it is common to feel a strong cramping sensation in the muscle while the biopsy is being performed. However, muscle function is not impaired. In fact, subjects have been reported to continue participation in sporting events immediately following a muscle biopsy. It is common for subjects to experience mild soreness, moderate pain and bruising near the biopsy site, similar to a “Charlie-horse” the day after the procedure. In order to allow the incisions to heal properly and minimize the risk of infection, you should not get the biopsy site wet for 24-hours and avoid prolonged exposure to water for 4-days. Daily showers are acceptable (after the first 24-hours), but baths, swimming, sauna’s etc. should be avoided for 4-days following the biopsy procedure.

BENEFITS

You will not directly benefit from participating in this study. However, you will gain an increased understanding of your skeletal muscle function. Specifically, you will learn about your level of muscular strength, body composition, cardiovascular fitness, and how aerobic training influences neuromuscular function. A copy of all personal data from the tests will be provided to you and your data will be completely explained to you by a member of the investigation team.

PAYMENT TO PARTICIPANTS

There are 3 biopsies for this study and you will be compensated $60 for every biopsy. You will not receive financial compensation for the other activities in the study. Investigators may ask for your social security number in order to comply with federal and state tax and accounting regulations.

PARTICIPANT CONFIDENTIALITY
Your name will not be associated in any publication or presentation with the information collected about you or with the research findings from this study. Instead, the researcher(s) will use a study number or a pseudonym rather than your name. Your identifiable information will not be shared unless (a) it is required by law or university policy, or (b) you give written permission. Tissue samples will be destroyed 10 years after completion of the study. Permission granted on this date to use and disclose your information remains in effect indefinitely. By signing this form you give permission for the use and disclosure of your information for purposes of this study at any time in the future.

INSTITUTIONAL DISCLAIMER STATEMENT
In the event of injury, the Kansas Tort Claims Act provides for compensation if it can be demonstrated that the injury was caused by the negligent or wrongful act or omission of a state employee acting within the scope of his/her employment.

REFUSAL TO SIGN CONSENT AND AUTHORIZATION
You are not required to sign this Consent and Authorization form and you may refuse to do so without affecting your right to any services you are receiving or may receive from the University of Kansas or to participate in any programs or events of the University of Kansas. However, if you refuse to sign, you cannot participate in this study.

CANCELLING THIS CONSENT AND AUTHORIZATION
You may withdraw your consent to participate in this study at any time. You also have the right to cancel your permission to use and disclose further information collected about you, in writing, at any time, by sending your written request to: Trent J. Herda, 1301 Sunnyside Avenue 101BE, Robinson Center, Lawrence, KS 66045.
If you cancel permission to use your information, the researchers will stop collecting additional information about you. However, the research team may use and disclose information that was gathered before they received your cancellation, as described above.

QUESTIONS ABOUT PARTICIPATION
Questions about procedures should be directed to the researcher(s) listed at the end of this consent form.

PARTICIPANT CERTIFICATION:
I have read this Consent and Authorization form. I have had the opportunity to ask, and I have received answers to, any questions I had regarding the study. I understand that if I have any additional questions about my rights as a research participant, I may call (785) 864-7429 or (785) 864-7385, write the Human Subjects Committee Lawrence Campus (HSCL), University of Kansas, 2385 Irving Hill Road, Lawrence, Kansas 66045-7568, or email irb@ku.edu.

I agree to take part in this study as a research participant. By my signature I affirm that I am at least 18 years old and that I have received a copy of this Consent and Authorization form.

_____ _____
Type/Print Participant's Name Date

_____
Participant's Signature

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Health History Questionnaire

PRE-EXERCISE TESTING HEALTH & EXERCISE STATUS QUESTIONNAIRE

Name _____ Date_____

Home Address_____

Phone Number _____ Email _____

Birthday (mm/dd/yy)____/_____/_____

Person to contact in case of emergency_____

Emergency Contact Phone _____

Personal Physician _____ Physician's Phone_____

Gender_____ Age _____(yrs) Height _____(ft)_____(in) Weight_____(lbs)

Does the above weight indicate: a gain____ a loss____ no change____ in the past year?
If a change, how many pounds?_____(lbs)

A. JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

Joint Areas
( ) Wrists
( ) Elbows
( ) Shoulders
( ) Upper Spine & Neck
( ) Lower Spine
( ) Hips
( ) Knees
( ) Ankles
( ) Feet
( ) Other_____

Muscle Areas
( ) Arms
( ) Shoulders
( ) Chest
( ) Upper Back & Neck
( ) Abdominal Regions
( ) Lower Back
( ) Buttocks
( ) Thighs
( ) Lower Leg
( ) Feet
( ) Other_____

B. HEALTH STATUS (✓Check if you currently have any of the following conditions)

( ) High Blood Pressure
( ) Heart Disease or Dysfunction
( ) Peripheral Circulatory Disorder
( ) Lung Disease or Dysfunction
( ) Acute Infection
( ) Diabetes or Blood Sugar Level Abnormality
( ) Anemia
( ) Hernias

( ) Arthritis or Gout
( ) Edema
( ) Epilepsy
( ) Multiply Sclerosis
( ) High Blood Cholesterol or Triglyceride Levels
( ) Allergic reactions to rubbing alcohol
( ) Thyroid Dysfunction
( ) Pancreas Dysfunction
( ) Liver Dysfunction
( ) Kidney Dysfunction
( ) Phenylketonuria (PKU)
( ) Loss of Consciousness

* *NOTE: If any of these conditions are checked, then a physician's health clearance will required.*

C. PHYSICAL EXAMINATION HISTORY
Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of physical exertion? _____YES _____NO
If YES, what limitations were recommended?_____
_____

D. CURRENT MEDICATION USAGE (List the drug name, the condition being managed, and the length of time used)

MEDICATION	CONDITION	LENGTH OF USAGE
_____	_____	_____
_____	_____	_____

E. PHYSICAL PERCEPTIONS (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA	SED		PA	SED	
( )	( )	Chest Pain	( )	( )	Nausea
( )	( )	Heart Palpitations	( )	( )	Light Headedness
( )	( )	Unusually Rapid Breathing	( )	( )	Loss of Consciousness
( )	( )	Overheating	( )	( )	Loss of Balance
( )	( )	Muscle Cramping	( )	( )	Loss of Coordination
( )	( )	Muscle Pain	( )	( )	Extreme Weakness
( )	( )	Joint Pain	( )	( )	Numbness
( )	( )	Other_____	( )	( )	Mental Confusion

F. FAMILY HISTORY (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)
( ) Heart Disease

( ) Heart Attacks or Strokes (prior to age 50)
( ) Elevated Blood Cholesterol or Triglyceride Levels
( ) High Blood Pressure
( ) Diabetes
( ) Sudden Death (other than accidental)

G. EXERCISE STATUS

Do you regularly engage in running? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

How many miles per week do you typically run? _____

What is your fastest 5 km time? _____

What is your fastest 10 km time? _____

What is your fastest mile time? _____

What is your fastest times at other distances not listed? _____

Do you regularly lift weights? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours

What is your back squat 1 repetition maximum (RM)? _____

What is your deadlift 1 RM? _____

What is your power clean 1 RM? _____

What are your other 1 RMs that are not listed? _____

Do you regularly play recreational sports (i.e., basketball, racquetball, volleyball, etc.)? YES NO

How long have you engaged in this form of exercise? _____ years _____ months

How many hours per week do you spend for this type of exercise? _____ hours