

The effects of muscle quality and continuous cycling
on motor unit behavior of the vastus lateralis

By

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ABSTRACT

Introduction: Recent advancements in surface electromyographic signal decomposition and the inclusion of ultrasonography have presented the opportunity for a deeper understanding of motor unit behavior. However, the influence of sex and muscle composition on motor unit recruitment patterns has yet to be investigated. In addition, little is known regarding the neuromuscular adaptations from endurance training on motor unit behavior during submaximal tasks and long duration activities. **Methods:** Three distinct experiments were conducted to expand the current literature on motor unit behavior. The first project investigated the influence of sex and muscle quality on motor unit action potential sizes for 10 males and 10 females. The second project longitudinally investigated the effects of 10-weeks of continuous cycling on motor unit firing rate behavior and recruitment patterns for 23 sedentary individuals. The third project longitudinally investigated the effects of 5-weeks of continuous cycling on motor unit firing rate behavior and recruitment patterns during consecutive, long duration contractions for 25 sedentary individuals. **Conclusions:** For project one, the greater muscle quality and motor unit action potential sizes exhibited by males suggests greater muscle fiber sizes of the higher-threshold motor units. For project two, participants displayed increases in maximal aerobic capacity and decreases in maximal strength. The decreases in maximal strength were associated with alterations in motor unit firing rate patterns and increases in muscle activation to match pre-training absolute torques. For project three, participants displayed increases in maximal aerobic capacity and decreases in maximal strength. Following 5 weeks of training, there were time and repetition dependent change in motor unit firing rate and likely recruitment patterns to compensate for losses in strength.

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REVIEW OF LITERATURE

Size Principal of Recruitment

Henneman (1957)

The authors of this study performed experiments on cats with spinal cords that had been transected distal to the obex. Electrical stimulation was applied to the dorsal roots of the large nerve trunks to elicit reflexes in the lumbar ventral roots. The authors reported two phases were observed when shocks with sufficient strength were used: a relatively synchronized discharge followed by a second or more of rhythmical firings. A closer examination of the rhythmical firings indicated amplitudes of the impulses varied directly with the diameters of the fibers. The authors concluded that less excitation is necessary for smaller motor neurons to discharge than larger motor neurons and, thus, the susceptibility of the motor neuron to discharge is related to its size.

Monster and Chan (1977)

For this study, the authors used surface and intramuscular electromyographic (EMG) recordings to investigate the firing rate behavior of the left middle finger extensor digitorum communis (EDC) muscle during steady isometric contractions for eight adults ages 19 to 30 years old. Monster and Chan reported a systematic relationship between the motor unit (MU) size, recruitment order and firing rate characteristics. For example, MUs were recruited in order of size with earlier recruited MUs displaying greater firing rates than later recruited MUs. In addition, the firing rates of the smallest, earliest recruited MUs were more likely to become fused than the larger, later recruited MUs and, thus, Monster and Chan suggested the larger MUs are

more effective for quick adjustments in force whereas the smaller units are more suited for maintaining force during a steady contraction.

Garnett, O'Donovan, Stephens, and Taylor (1979)

In this study, the authors investigated MU organization in human medial gastrocnemius for 13 participants, ages 22 – 46 years. The MU were isolated with controlled intramuscular microstimulation and plantarflexion torque was measured. The muscle was stimulated for 2 – 5 hours to deplete the muscle fibers a glycogen and was followed by a muscle biopsy. MUs were divided into three groups: (1) type S (slow, small, and fatigue resistant), (2) type FR (fast, intermediate sized, and fatigue resistant), and (3) FF (fast, large, and fatigable). The type S MUs had longer contraction times (> 99 ms vs. < 85 ms) and smaller twitch forces (11.5 ± 5.0 vs. 23.3 ± 35.1 g wt) and greater fatigue resistance than type F units. The results suggest that during slowly increasing voluntary contractions, MUs are recruited by order size and at low force levels, both type S and FR are contributing to force production. Furthermore, above approximately 30% of maximal strength, type FF would be additionally recruited.

Orderly Recruitment of Motor Unit Action Potentials

Olson, Carpenter, and Henneman (1968)

This study examined the relationship between the amplitude of motor unit action potential (MUAP) sizes and their recruitment thresholds for triceps surae in 19 adult cats. Single, double, and steel wire electrodes were used to record EMG. The muscle potentials were led through amplifiers and photographed on moving film. Action potentials were paired and rank

correlated, which displayed a strong tendency of the EMG amplitude for a higher-threshold MU to be greater than that of a lower-threshold MU. In addition, factors such as fiber diameter, fiber number, and fiber dispersion influenced the size of the EMG potentials. Furthermore, the authors stated the size of a MU and its fiber type determine the density of the fiber, which determines the amplitude of the signal.

Hu, Rymer, and Suresh (2013)

In this study, the authors investigated the recruitment and firing rate organization of multiple MUs with different action potential sizes for the first dorsal interosseous (FDI) using surface EMG signal decomposition techniques. Spike triggered averaging methods were used by the authors to estimate MU action potential sizes with the firing times of MUs as recruitment triggers. Eight right hand dominant individuals with no neurological disorders (4 men, 4 women) completed 3 maximal voluntary contractions (MVCs) followed by four blocks of trials with five repetitions in each block. For each repetition, participants completed isometric trapezoidal muscle actions at 20%, 30%, 40% and 50% MVC. The results indicated that MU size, as indicated by peak-to-peak amplitude of the MUAP, increased linearly with recruitment threshold. Therefore, the size principal was apparent during the voluntary force contractions. In addition, MU mean firing rates during the targeted force were inversely related to MUAP size and recruitment threshold. The authors proposed that surface EMG signal decomposition in conjunction with spike triggered averaging may provide an alternative method to examine MU pool organization properties.

Pope, Hester, Benik, and DeFreitas (2016)

The authors investigated the use of the MUAP size vs. recruitment threshold relationship from surface EMG signal decomposition and muscle cross sectional area (mCSA) with ultrasonography (US) to measure hypertrophy of higher-threshold MUs following a strength training program. Ten participants completed 8 weeks of high-intensity strength training for the leg extensors. US images and MU recording were obtained pre- and post-training intervention. The training program resulted in significant increases in muscle size, strength, and the slopes of the MUAP size vs. recruitment threshold relationship. In addition, 84% of the variance in the change in the slopes of the MUAP size vs. recruitment threshold relationships could be explained by the increases in mCSA recorded by US imaging. Therefore, the findings suggest the MUAP size vs. recruitment threshold relationships can be used to investigate the size principal. Furthermore, the surface EMG signal decomposition can be used to assess longitudinal changes to MUAP sizes across the recruitment threshold range.

Surface Electromyographic Signal Decomposition

De Luca, Adam, Wotiz, Gilmore, and Nawab (2006)

Typically, previous studies decomposing indwelling EMG signals could only investigate low contraction intensities yielding three or less MUs due to the difficulty associated with distinguishing similar action potential shapes when four or more MUs are active. Therefore, the firing rate characteristics of higher-threshold MUs, or lower- and higher-threshold MUs during higher contraction intensities were not well understood. In this study, the authors introduce a new technique for decomposing surface EMG signals into individual MUAP trains with a four differential channel surface array sensor. This technique, termed Precision Decomposition III, is

capable of decomposing several simultaneous MUAP trains from real surface EMG signals. Signals were detected from the orbicularis oculi and platysma and where feedback to the subject provided by the root-mean-squared value of the EMG signal whereas, for the tibialis anterior, subjects maintained a constant force at 20-50% MVC. The Precision Decomposition III algorithm was able to identify at least three MUAP trains as well as recruitment and derecruitment thresholds. The firing rate characteristics of the orbicularis oculi, platysma and tibialis anterior were consistent with earlier indwelling studies with earlier-recruited, lower-threshold MUs displaying greater firing rates than later-recruited, higher-threshold MUs. Recent advancements in the Precision Decomposition algorithm have yielded over 60 MUs during isometric contractions and, thus, provide greater resolution to data investigating MU behavior.

Common Synaptic Input and the Onion Skin Control Scheme

Farmer, Bremner, Halliday, Rosenberg, and Stephens (1993)

The authors examined the firings of paired MUs recorded from either the first or second dorsal interosseous, and the biceps brachii in sixteen healthy controls aged 21 – 56 and eleven patients aged 49 – 74 who reported a clinically unilateral stroke between 1 and 9 months previously. Subjects performed weak isometric contractions so individual MUs recorded from each needle discharged at a steady rate of approximately 10 pulses per second. Cross-correlations were performed in the time domains for the spike trains of the MU pairs with the results compared to the coherence analysis performed on the same spike train data. Farmer et al. reported a common synaptic input to the MU pair as central peaks were exhibited in the cross-intensity functions.

De Luca and Erim (1994)

In this study, the authors discuss the phenomenon of common drive. The concept of common drive suggests the central nervous system (CNS) does not control the firing rates of individual motor units, but supplies the entire motor unit pool with a common synaptic input during voluntary contractions. The MU pool is recruited in order of size and their susceptibility to excitation will result in different firing rates during voluntary contractions. It has been suggested common drive relieves the CNS from deciding which MUs to selectively activate during a task.

In addition, De Luca and Erim (1994), among others, report earlier recruited MUs have greater firing rates than later recruited MUs. Plotting the firing rates of MUs as a function of contraction time results in an orderly nesting of firing rate curves under each other where earlier recruited MUs display greater firing rates than later recruited MUs and, thus, this behavior has been termed “onion skin.” It is well understood that later-recruited, higher-threshold MUs display higher-amplitude, shorter duration force twitches and, thus, would require greater firing rates to tetanize and produce maximal force than lower-threshold MUs. However, higher-threshold MUs are fatigued faster than the lower-threshold MUs and would not be able to maintain a target force. Therefore, it appears the neuromuscular system is designed to optimize force and duration, not solely force production.

De Luca and Hostage (2010)

This study used surface EMG signal decomposition technology to investigate the MU control properties of the vastus lateralis (VL), tibialis anterior, and first dorsal interosseous (FDI) during isometric trapezoidal muscle actions at 20, 50, 80, and 100% MVC. The relationships

between the firing rates at the target force level and the recruitment thresholds were analyzed with linear regression models for all contractions. For these relationships, the y-intercept represents the average firing rates of the lowest-threshold MUs, whereas the slope represents the overall MU firing rate behavior. For all subjects, muscles, and contraction intensities, the slopes of these relationships were negative, indicating that earlier recruited MU had greater average firing rates at the target force level than later recruited MUs. This behavior has been termed “the onion skin” because the MU firing rates, plotted as a function of time, look like the layering pattern of an onion.

Among muscles, there were differences in MU behavior. At maximum force, firing rates for the FDI were greater than the VL and the tibialis anterior. In addition, the average of the slopes for the four contraction intensities were similar for the VL (-0.30 ± 0.11) and for the tibialis anterior (-0.25 ± 0.05), whereas the slopes for the FDI (-0.40 ± 0.08) were more negative. The greater standard deviations of the slopes for the VL suggests greater inter-subject variability in MU behavior compared to the tibialis anterior and the FDI.

This study indicated a consistent MU control scheme among muscles and the differences in the parameters of the control scheme among muscles may be explained by differences in the physical properties and the functional roles of the muscle. For all muscles and contraction intensities, the earlier recruited, lower-threshold MUs displayed greater firing rates than the later recruited, higher-threshold MUs. It has been suggested this MU control scheme is not designed to produce maximal force but to optimize the ability to generate and maintain force.

Previous research has reported a linear relationship between recruitment threshold and firing rates when the nerves of anesthetized cats are electrically stimulated, and when MU data is grouped from different subjects and contractions performed on different days or at different target forces. This had led to the notion that higher-threshold MUS have greater firing rates than lower-threshold MUs. However, when motor unit firing rate characteristics are observed for each contraction and subject during isometric voluntary contractions, an inverse relationship exists between recruitment threshold and MU firing rates. The purpose of this study was to clarify the differences in observations and provide a model for MU firing rate behavior during isometric contractions. Eight participants (24.38 ± 5.21 yrs) completed isometric trapezoidal contractions at 100%, 80%, and 50% MVC. EMG was recorded from the VL and the FDI and the EMG signal was decomposed to extract the individual firing events of single MUs. The higher-threshold MUs, recruited with greater levels of excitation, had lower firing rates than the lower-threshold MUs at recruitment and target force. In addition, higher-threshold MUs had slower firing rate increases than lower-threshold MUs. Therefore, at any time and force, the firing rates of the higher-threshold MUs will be lower than lower-threshold MUs. The authors report favorable aspects of this control scheme for daily activities: (1) a greater ability to maintain force, and (2) a greater economy during activities of daily living, which are typically performed at low force levels.

The Influence of Endurance Training on Muscle Fiber Types

Jansson, Sjödín, and Tesch (1978)

This study follows up on previous animal research that has reported shifts in muscle fiber types due to cross-innervation and electrical stimulation. The authors investigated fiber type changes due to different types of physical training. Four long-distance runners completed a training program that was composed of two periods: (1) aerobic training (13-20 weeks; 70-80% VO_{2MAX}), and (2) anaerobic training (7-13 weeks; 2-3 times a week at 90-100% VO_{2MAX}). Biopsies from the VL were taken prior to the study and following both training periods and myofibrillar actomyosin ATPase (mATPase) was used to identify the fiber types. Two participants completed aerobic training followed by anaerobic training, whereas the order was reversed for the other two participants. Following anaerobic training, subjects displayed lower percentages of type I and higher percentages of type IIC fibers than after the aerobic training. In addition, three subjects had higher percentages of type IIA + IIB fibers with an increased IIB/IIA ratio following the anaerobic training. The authors proposed that type II fibers had converted to type IIC fibers due to aerobic training and a conversion in the reverse direction had occurred due to anaerobic training. Furthermore, the authors stated the possibility of fibers transforming between type I and II fibers is supported by an earlier observation of a well-trained cross-country skier in which the percentage of type I fibers decreased from 81% to 57% during 6 weeks of immobilization post-injury. The findings support the conversion of type I to type II muscle fibers as a result of specific training.

Simoneau, Lortie, Boulay, Marcotte, Thibault, Bouchard (1985)

The effects of 15 weeks of strenuous high-intensity intermittent training was measured on muscle fiber type proportions and fiber areas. Twenty-four sedentary (14 women and 10 men) and 10 control (4 women and 6 men) completed a 15 week training program consisting of

continuous and interval work patterns 15s to 90s on a cycle ergometer. Muscle biopsies for the VL were taken before and after the training program. The tissue was sectioned for mATPase techniques. In addition, 300 fibers were classified for each specimen and mean muscle fiber areas were determined. The high-intensity training program resulted in a significant increase in the proportion of type I fibers and a significant decrease in type IIb fibers. In addition, the areas of the type I and IIb fibers significantly increased following the training. The results of this study suggest that skeletal muscle fiber type proportion and fiber areas for the VL can be altered by high-intensity intermittent training for adult sedentary individuals.

Howald, Hoppeler, Classen, Mathieu, and Straub (1985)

The authors investigated the influence of endurance training on VO_{2MAX} , muscle fiber type, mitochondrial density, and intracellular lipid density. Ten sedentary subjects (5 males and 5 females) performed 6 weeks of cycle ergometer training for 6 weeks, 5 times a week for 30 mins at 70% of baseline VO_{2MAX} levels. Prior and after training, two muscle biopsies were taken from the VL and cross-sectioned for identification of the fiber types (type I, IIA, IIB, and IIC) by histochemical staining of ATPase. Five individual fibers of each type (type I, IIA, IIB) from each sample were processed for electron microscopy to estimate the volumes of different cellular components per unit of volume. Paired samples t-tests indicated the training program significantly increased VO_{2MAX} , type I fibers, mitochondrial volume density for all three fiber types, and volume density of intracellular lipid droplets in type IIA and IIB fibers, whereas type IIB were significantly decreased. Therefore, high intensity endurance training leads to changes in muscle fiber type and an enhancement of the oxidative capacity in all muscle fiber types.

Trappe, Harber, Creer, Gallagher, Slivka, Minchev, and Whitsett (2006)

In this study, the authors investigated the effects of marathon training on single muscle fiber contractile properties for a group of recreational runners. Seven participants (4 males and 3 females; ages 22 ± 1 yrs) completed a 16 week training plan that increased in volume for the first 13 weeks and concluded with a 3 week taper leading into the marathon race. Before, after 13 weeks, and post-taper participants were tested for single muscle fiber experiments, oxidative enzyme activity, and maximal oxygen uptake. A muscle biopsy of the lateral gastrocnemius was taken for all three time points. Following the taper, MHC composition significantly increased for MHC I fibers (48 ± 6 to $56 \pm 6\%$), significantly decreased for MHC I/IIa hybrid fibers (7 ± 1 to $2 \pm 1\%$), and significantly decreased total MHC hybrid fibers (24 ± 7 to $13 \pm 4\%$) compared to pre-training. Therefore, a 13 weeks of aerobic exercise led to changes in the MHC content of the involved muscle.

Konopka, Trappe, Jemiolo, Trappe, and Harber (2011)

The purpose of this study was to investigate MHC plasticity in older individuals with aerobic exercise training. Eight sedentary women (70 ± 2 years) completed 12 weeks of aerobic training on a cycle ergometer. The duration (20-45 minutes), intensity (60-80% heart rate reserve), and frequency (3-4 sessions per week) were progressively increased throughout the duration of the study. Before and after the 12 week aerobic training program, a muscle biopsy was performed on the VL to measure the effect of the training program on MHC isoform content and single muscle fiber MHC distribution. Paired samples t-tests indicated MHC isoform content for the VL significantly increased from pre- ($54\% \pm 4\%$) to post-training ($61\% \pm 2\%$). In addition, percent type IIx MHC isoform content significantly decreased post-training ($4\% \pm 1\%$)

compared to pre-training ($8\% \pm 1\%$). For the MHC analysis of single muscle fibers, aerobic training elicited a significant increase in type I fiber distribution ($42\% \pm 4\%$ to $52\% \pm 3\%$) and a significant decrease in MHC IIa fiber distribution ($48\% \pm 3\%$ to $38\% \pm 4\%$). The findings of this study further support the ability of the muscle to change the MHC isoform content following an aerobic training program.

Influence of the Physical Properties of the Motor Unit

Pool on Contractile Properties

De Luca, LeFever, McCue, and Xenakis (1982)

In this study, the authors investigated MU behavior of the FDI and deltoid muscle during triangular isometric contractions at 40 and 80% MVC for Olympic caliber swimmers and powerlifters, nationally known pianists, and healthy controls. For all triangular isometric contractions, there was a highly ordered recruitment and derecruitment MU control scheme. For example, there were inverse relationships between (1) recruitment and derecruitment order, and (2) threshold force and peak firing rates. In addition, MU firing rates at recruitment were greater than at derecruitment for both muscles with lower-threshold MUs tending to be derecruited at lower force levels while higher-threshold MUs showed a propensity to be derecruited at higher force levels. The greater firing rates at recruitment versus derecruitment combined with higher derecruitment than recruitment forces may be explained by an increase in mechanical efficiency of the MU after repetitive stimulation during the contraction, which has been termed potentiation.

De Luca et al. also reported between-muscle differences, such as greater firing rates at recruitment for the deltoid than the FDI and greater peak firing rates for the FDI than the deltoid. There were also within-muscle differences at recruitment and derecruitment as a function of training status. For the deltoid, the powerlifters had lower firing rates at recruitment and greater firing rates at derecruitment compared to the other groups, whereas the swimmers had greater firing rates at recruitment compared to all other groups for the FDI. Therefore, De Luca et al. proposed the CNS does not control the firing rates of individual MUs. Rather, the motor neuron pool is provided a common synaptic input during voluntary contractions and the mechanical properties of the motor neuron pool, particularly the ratio of fast-twitch and slow-twitch fibers and their influence on force twitches and fatigability, results in adjustments of the common synaptic input to the motor neuron pool to match a target force level.

Orizio and Veicsteinas (1992)

This study investigated the influence of fiber type composition for the VL on mechanomyographic (MMG) amplitude and frequency among seven sprinters, seven long distance runners, and seven sedentary individuals during a MVC of the leg extensors sustained to exhaustion. The sprinters displayed greater MVC strength at the beginning of the task but lower effort time than the long distance runners and sedentary individuals. At the onset of the sustained contraction, MMG amplitude and frequency were greater for the sprinters in comparison to the long distance runners and sedentary individuals. Throughout the contraction, there was a clear reduction in MMG amplitude for the sprinters and sedentary individuals while there was a compression in the power spectra towards the lower frequencies. This behavior was also reported

in the long distance runners but was less pronounced. The authors suggested the results were a result of the differences in the percentages of fast twitch fiber area among the three groups.

Taylor, Bronks, Smith, and Humphries (1997)

The purpose of this study was to locate the sites of muscular fatigue during submaximal dynamic work under severe hypoxia at a work level where metabolite accumulation exceeds steady-state levels. A secondary aim was to investigate the influence of fiber composition on fatigue during hypoxic conditions. Eight males performed cycle ergometry under normal and normobaric hypoxia with integrated EMG measured for the VL. MVCs were performed after each trial to quantify changes in force, conduction velocity, electromechanical delay, median frequency, and maximal integrated EMG. Muscle biopsies were performed on the VL and MHC was quantified with sodium dodecyl-polyacrylamide gel electrophoresis. Under the environmental normobaric hypoxia trial, the subjects with higher type II MHC accumulated greater amounts of blood lactate and displayed greater reductions in median frequency and conduction velocity despite minimal differences in training status among participants. The authors suggested the participants with greater amounts of type II MHC composition displayed greater myoelectric fatigue during the hypoxic trial due to an increased ability to produce lactate and a decreased ability to consume lactate by the type II fibers.

Swallow, Gosker, Ward, Moore, Dayer, Hopkinson, Schols, Moxham, and Polkey (2007)

In this study, the authors proposed repetitive magnetic stimulation of the femoral nerve could be used to induce and quantify quadriceps endurance in eight normal subjects and eight subjects with advanced chronic obstructive pulmonary disorder. The quadriceps were provided

stimuli at 30 Hz, a duty cycle of 0.4 (2 sec on, 3 sec off), for 50 trains with force and surface EMG measured throughout testing and quadriceps twitch forces from the supramaximal magnetic stimulation of femoral nerve was measured before and after the protocol. In order to validate this method, in vivo contractile properties of the VL was compared with MHC isoform protein expression. There was a positive correlation between the time for force to decrease to 70% of the baseline and the proportion of type I fibers. Thus, MHC is associated with the fatigability of the muscle fiber.

Beck, Housh, Fry, Cramer, Weir, Schilling, Falvo, and Moore (2007)

The purpose of this study was to investigate the influence of MHC isoform content on EMG and MMG amplitude and mean power frequency responses. Five resistance- (age \pm SD; 23.2 ± 3.7 yrs) and aerobically-trained (32.6 ± 5.2 yrs) males completed a 30 second submaximal isometric muscle action of the VL at 50% MVC followed by a muscle biopsy of the VL. Type IIa MHC was significantly greater ($p < 0.05$) for the resistance-trained individuals ($59.0 \pm 4.2\%$ vs. $27.4 \pm 7.8\%$) whereas, type I MHC was significantly greater ($p < 0.05$) for the aerobically-trained participants ($72.6 \pm 7.8\%$ vs. $40.9 \pm 4.3\%$). The absolute and normalized EMG amplitude and mean power frequency patterns of responses versus time for the aerobically- and resistance-trained participants were similar with increases and decreases, respectively. However, the resistance-trained exhibited relatively stable absolute and normalized MMG amplitude and MPF across time whereas the aerobically-trained participants displayed increases in MMG amplitude and decreases in MMG MPF. In addition, MMG amplitude and MPF values for the resistance-trained subjects were greater than the aerobically-trained subjects. The authors suggested the relatively stable absolute and normalized MMG amplitude and mean power

frequency values for the resistance-trained participants may reflect the recruitment and fatigue induced drop out of fast-twitch MUs since there were significant increases in EMG amplitude and decreases in EMG MPF. The linear increases in absolute and normalized MMG amplitude and linear decreases in absolute and normalized MMG mean power frequency across the 30 second fatiguing task for the aerobically-trained individuals may be explained by the influence of greater type I MHC for the VL. For example, the increase in MMG amplitude across time may suggest lower fatigue-induced dropout of fast twitch MUs, while the decrease in MMG mean power frequency over time could be explained by greater firing rates and longer twitch durations of the MUs due to greater amounts of type I MHC for the VL. Thus, the physical properties of the MU pool, particularly the ratio of fast-twitch and slow-twitch contractile proteins and their influence on fatigability and force twitches, play a prominent role on firing rate characteristics at a steady force.

Herda, Housh, Fry, Weir, Schilling, Ryan, and Cramer (2010)

This study investigated the log-transformed EMG and MMG amplitude-force relationships for aerobically-trained, resistance-trained, and sedentary individuals during an isometric ramp contraction from 5% to 90% MVC. Muscle biopsies, skinfold thickness measurements, and EMG and MMG were collected for the VL. Linear regressions were performed on the log-transformed EMG- and MMG-amplitude vs. force relationships and the slopes and the antilog of the y-intercept were calculated. Analysis of the muscle biopsies indicated the aerobically-trained individuals had the greatest percentage of type I fiber area, the resistance-trained had the greatest percentage of type IIa fiber area, and the sedentary individuals had the greatest percentage of type IIx fiber area. The aerobically-trained had lower slopes for

the MMG amplitude-force relationships compared to the resistance-trained and sedentary individuals. The authors suggested the slopes for the log-transformed MMG amplitude-force relationships may have reflected the differences in fiber area-related MU activation strategies between individuals with predominantly type I vs. type II fiber area in the VL. For example, the MMG amplitude-force force relationships for the aerobically-trained group plateaued earlier than the resistance-trained and sedentary groups. These findings suggest the aerobically-trained group, which had significantly higher amounts of type I fiber area, may begin rate coding earlier in the force spectrum than the resistance-trained and sedentary groups (predominately type II fiber area).

Herda, Siedlik, Trevino, Cooper, and Weir (2015)

The authors examined the influence of chronic training (> 3 years) on MU firing rates for the VL. Five resistance- and five aerobically-trained individuals performed an isometric trapezoidal contraction at 40% and 70% MVC. Surface EMG was recorded for the VL and the EMG signal was decomposed to extract the firing events of single MUs. The aerobically-trained individuals had greater firing rates during the 40% and 70% contraction than the resistance-trained individuals. In addition, the difference in the mean MU firing rates were becoming larger with increments in the recruitment threshold between the chronic training groups. The findings suggest that differences in MU mean firing rate behavior can occur within a muscle as a result of chronic training status and/or type I% MHC area.

Trevino and Herda (2015)

This study investigated the MMG amplitude-force relationships for 5 aerobically-trained, 5 resistance-trained, and 5 sedentary individuals during an isometric trapezoidal muscle action at 60% MVC of the leg extensors that included a linearly increasing, steady force, and linearly decreasing segment. MMG was recorded from the VL and slopes were calculated from the natural log-transformed MMG amplitude-force relationships for the linearly increasing and decreasing segments for each participant. In addition, MMG amplitude was averaged for the entire steady force segment. MMG amplitude for the steady force segment was not different among groups. However, the slopes for the resistance-trained and sedentary individuals during the linearly increasing and decreasing segments were significantly greater ($P < 0.05$) than the aerobically-trained individuals. Therefore, the resistance-trained and sedentary individuals displayed greater acceleration in the MMG amplitude-force relationships during the linearly increasing and decreasing muscle actions. The authors suggested the aerobically trained relied primarily on MU firing rates and/or had a greater magnitude of active muscle stiffness via fusion of twitches at the higher force levels than the resistance-trained and sedentary individuals. Thus, MU activation and deactivation control strategies were influenced by training status.

Meijer, Jaspers, Rittweger, Seynnes, Kamandulis, Brazaitis, Skurvydas, Pisot, Simuni, Narici, and Degens (2015)

This study investigated the contractile properties of single muscle fibers among body-builders, power athletes, and control subjects via biopsies taken from the VL. Maximal isotonic contractions were performed with single muscle fibers that were typed by sodium dodecyl sulfate poly-acrylamide gel electrophoresis. The muscle fibers of the body builders and the power athletes generated greater maximal isometric tension than the sedentary subjects. Across all

subjects, the force generated for type I fibers was significantly lower than IIA and IIA/IIx fibers. These findings suggest that MHC isoform content would influence twitch forces of the MU.

Trevino, Herda, Fry, Gallagher, Vardiman, Mosier, and Miller (2016)

The authors investigated the influence of MHC isoform content on the mechanical behavior of the VL during isometric trapezoidal muscle actions at 40% and 70% MVC. Muscle biopsies were collected and MMG was recorded from the VL, respectively. For the linearly increasing and decreasing segments, linear regressions were fit to the natural-log transformed MMG amplitude–force relationships and MMG amplitude was selected during the steady force segment at the targeted force. Correlations were performed among type I percent MHC isoform content and the slopes from the MMG amplitude–force relationships during the linearly increasing and decreasing segments and MMG amplitude at the targeted force. For the 40% isometric trapezoidal contraction, correlations were significant among type I percent MHC isoform content and the slopes from the MMG amplitude–force relationships during the linearly increasing and decreasing segments, and MMG amplitude for the steady force segment. This is the first study to report relationships between type I percent MHC isoform content and MMG amplitude of muscle in vivo. The significant relationships occurred primarily during the lower intensity contraction, which was likely the result of force being produced by predominately the lower-threshold MUs. The authors suggested that higher type I percent MHC isoform content reduced acceleration in MMG amplitude during the 40% MVC and the amplitude during the steady force segments.

Trevino, Herda, Fry, Gallagher, Vardiman, Mosier, and Miller (2016)

This study investigated the relationships among the MHC isoform content of the VL and MU firing rates at recruitment, targeted force, and derecruitment. Twelve participants completed a 40% isometric trapezoidal muscle action that included a linearly increase, steady force, and decreasing segment. During testing, surface EMG signals were collected for the VL and later decomposed into the firing events of single MUs. Slopes and y-intercepts were calculated for 1) firing rates at recruitment vs. recruitment threshold, 2) mean firing rates at steady force vs. recruitment threshold, and 3) firing rates at derecruitment vs. derecruitment threshold relationships for each subject. Following testing, participants gave a muscle biopsy of the VL and electrophoretic techniques were utilized to quantify type I MHC isoform content. Correlations were performed among type I MHC isoform content and the slopes and y-intercepts for the three relationships. Type I MHC isoform content was correlated with the slopes of the mean firing rate vs. recruitment threshold relationships at targeted forces, particularly the higher-threshold MUs. This study supports the hypothesis that the physical properties of the motorneuron pool influence MU firing rate characteristics.

Endurance Training and Motor Unit Behavior of the Vastus Lateralis

Vila-Chã, Falla, and Farina (2010)

The authors investigated MU behavior during submaximal contractions after six weeks of endurance or resistance training. Thirty healthy men (age, 26.0 ± 3.8 yr) who did not regularly participate in endurance or resistance training were randomly assigned to an endurance-, resistance-training, or control group. The intensity of the training programs progressively increased and participants had three experimental sessions (pre-, mid-, and post-program

completion). For each experimental session, participants performed maximal and randomly ordered submaximal (10%, 30% MVC, and 30% to task failure) isometric knee extensions. Surface and intramuscular electrodes were used to acquire EMG signals for the VL, vastus medialis obliquus (VMO), and biceps femoris muscles during the maximal and submaximal isometric contractions. The intramuscular EMG recordings were decomposed and mean discharge rates were computed. In addition, MU conduction velocity was investigated from the surface EMG signals. Six weeks of endurance training significantly increased time to task failure by $29.7 \pm 13.4\%$, but did not change MVC or maximal rate of force development RFD. Conversely, 6 weeks of strength training significantly increased MVC force by $17.5 \pm 7.5\%$ and maximal RFD by $33.3 \pm 15.9\%$, but did not influence time to task failure. In addition, MU conduction velocity increased during the 30% contraction for both groups following their 6 week training programs with no differences between the groups. For the 10% MVC, average MU discharge rate of the VL was not influenced by training. However, for the 30% MVC, the average MU discharge rate significantly decreased for the endurance-trained, whereas the opposite was true for the strength trained. For the same MUs that could be identified during the 10% and 30% MVC, the difference in the discharge rates between the lower and higher force increased following strength training but decreased following endurance training. The findings in this study suggest 6 weeks of endurance- and strength-training elicit different alterations in discharge rates, conduction velocity, and fatigability of the MU.

Vila-Cha, Falla, Correia, and Farina (2012)

In this study, the effect of separate strength and endurance training programs on MU firing rates and fiber membrane properties during fatiguing contractions was investigated. Thirty

healthy men (26.0 ± 3.8 yr) without regular strength or endurance training experience were randomly assigned to one of three groups: endurance-, resistance-trained, and control group. The training programs were 6 weeks long for a total of 18 sessions with progressive increases in load intensity. Participants performed an experimental session before and after completing the training program. For each experimental visit, participants completed two MVCs followed by a constant isometric contraction at 10% MVC for 70 sec followed by a 30% MVC to task failure after a 15 minute rest. Surface and intramuscular EMG was recorded for the VL, VMO, and the biceps femoris. MU conduction velocity and average rectified values were recorded for the first ten seconds of each contraction and the last ten seconds of the 10% MVC. Intramuscular signals were decomposed and the MU discharge rates and interspike interval variability were computed for the MU spike trains. Six weeks of endurance training significantly increase time to task failure by 29% without any change in MVC strength. For the strength trained participants, MVC force increased by 16% but time to task failure was unchanged. These same variables did not change for the control group. Regardless of intervention group, MU firing rates decreased over time throughout for the 30% MVC. After 6 weeks of training, both groups exhibited greater MU conduction velocities for the 30% MVC. In addition, the endurance group showed a lower decrease in MU conduction velocity than the strength trained group.

Martinez-Valdes, Falla, Negro, Mayer, Farina (2017)

This study utilized a novel technique of high-density surface EMG decomposition for MU tracking to investigate the influence of an endurance or high-intensity interval (HIIT) training program on MU behavior for the VL and VMO. Eighteen recreationally active healthy men were randomly assigned to an endurance or HIIT group. Pre- and post-training, participants

performed MVCs, followed by randomly ordered contractions at 10, 30, 50, and 70% MVC maintained for 20, 20, 15, and 10 sec respectively. In addition, a 30% MVC to task failure (10% < targeted force) was performed. EMG signals were recorded from the VL, VMO, and biceps femoris with an electrode grid and decomposed offline with an extensively validated method. The mean discharge rates were calculated during the steady torque segment and recruitment thresholds were defined as the time when the MU began discharging action potentials. A tracking method was used identify and match MUs that were present in the pre- and post-intervention trials. An incremental exercise test to exhaustion was performed 24 hours after the strength and EMG measurements to assess maximal aerobic capacity. The training protocols included 6 training sessions over 14 days. For the endurance protocol, training consisted of 90-120 mins of continuous cycling at 65% VO_{2MAX} , whereas the HIIT group participants completed eight to twelve, 60 sec bouts of high-intensity cycling at 100% peak power output with 75 sec of cycling at 30 watts for recovery. Only the HIIT group increased peak torque while both groups significantly increased VO_{2MAX} following their training program. The endurance group increased their time to task failure following training whereas the strength group showed no difference after training. EMG amplitude during the 10% and 30% MVC was not different post-training for either group, however, only the HIIT showed increases for the 50%, 70%, and MVCs. For the 10% and 30% MVC, discharge rates were not influenced by either training program. Conversely, the firing rates for the VL and VM increased for the 50% and 70% only for the HIIT group. These findings suggest that HIIT and endurance training elicit different neuromuscular adaptations. The authors hypothesized the findings were a result of differences in the exercise intensities and training volumes between the programs.

Motor Unit Behavior during Prolonged and/or Repeated Contractions

Dorfman, Howard, and McGill (1990)

This study examined MU behavior during submaximal fatiguing contractions of the left brachial biceps. Ten healthy adults (5 men and 5 women; ages 24-52 years) performed a MVC followed by a steady isometric contraction at 20% MVC for a total of 45 mins with a 1 minute rest period every 5 mins. EMG was recorded in 10 sec epochs with concentric needle electrodes and MUAPs were extracted using an automated decomposition method to determine MUAP sizes, MU firing rates, and coefficient of interspike-interval variability (CIV). A total of 4551 MUAP were analyzed for this study. Following 15 minutes of exercise for the 30% MVC, mean MU firing rate increased and continued to rise until fatigue (pre = 13.4 ± 1.7 Hz; post = 16.0 ± 1.9 Hz). In addition, MUAP size was significantly greater at the end of the exercise (605 ± 134 μ V) compared to baseline (514 ± 137 μ V). The authors reported a triphasic behavior in response to prolonged, submaximal exercise, such as: (1) a brief decrease and stabilization of the MU firing rates of recruitment, followed by (2) a slow and continual increase, and then (3) the recruitment of larger, higher-threshold MUs. The increase in firing rates and the recruitment of larger, higher-threshold MUs is likely a compensation for the loss in MU twitch forces, which would explain the increases in the surface EMG signal that is commonly associated with fatigue in other research studies.

Carpentier, Duchateau and Hainaut (2001)

The authors investigated MU behavior and contractile changes during fatigue for the FDI. Eight healthy participants (4 male and 4 female) were tested on multiple occasions (3 to 11

visits) with at least 1 week between visits. Surface and intramuscular EMG was recorded for the FDI and single MU mechanical behavior was determined with a spike triggered averaging. Participants completed intermittent contractions at 50% MVC which included a 3 sec linearly increasing segment, a 10 second plateau at targeted force, followed by a 3 sec return to baseline. There was a 4 sec rest period between contractions and this was repeated 3 times per minute until inability to maintain the targeted force for three, consecutive contractions. Investigated MU variables included recruitment and derecruitment thresholds, and mean discharge frequency for each MU during the steady force plateau. The mean endurance time was 8.38 ± 3.55 mins (range = 6 – 16 mins). As participants completed successive contractions, additional MUs were recruited and there were changes in recruitment thresholds and discharge rates with a 31% increases in the surface EMG signal. Recruitment order was unchanged during the protocol, however, there were non-systematic changes in recruitment thresholds. Lower-threshold MUs either maintained or increased recruitment forces, whereas recruitment thresholds were decreased for the higher-threshold MUs. However, derecruitment thresholds increased for the majority of the observed MUs. Lower- and higher-threshold MUs showed a systematic and progressive decrease in firing rates but the change was only significant for the lower-threshold (<25% MVC) MUs. Alterations of contractile forces differed as a function of recruitment threshold. For example, MUs recruited below 25% MVC increased contractile forces whereas the converse was true for MUs recruited above 25% MVC. The authors hypothesized the decline in discharge rates for the lower-threshold MUs was due to a slowing of their mechanical responses. However, the same adaptation during the task did not occur in the higher-threshold MUs that exhibited an increase in their mechanical time course with only a slight increase in

discharge rate. Lastly, it appears that for the FDI, lower-threshold MUs (<25% MVC) are fatigue resistant whereas the same cannot be said for the higher-threshold MUs (>25% MVC).

Adam and De Luca (2003)

In this study, five healthy men (21.4 ± 0.9 yrs) performed three isometric MVCs for the knee extensors followed by repeated contractions at 20% MVC. The template for the repeated contractions included a 5 sec ramp up to 50% MVC, followed by a brief hold and then a decrease to 20% MVC for a 50 sec constant hold. The surface EMG signal was recorded for the VL, VM, rectus femoris, and the biceps femoris and a quadrifilar fine wire electrode was inserted into the VL to observe individual MU behavior. Three subjects also completed the fatiguing protocol on a separate occasion and received transcutaneous electrical stimulation during a 6 sec rest period between each contraction. Results indicated that surface EMG recorded for the leg extensors increased from the first to the last contraction. Individual firing rate behavior indicated more MUs were recruited for the linearly increasing segment as contraction number increased, more MUs were continuously active in successive contractions when torque was decreased from 50% to 20% MVC, and a greater number of MUs were active during the 20% torque plateau. In addition, recruitment thresholds of all MUs decreased and more MUs were recruited, while MU recruitment order was maintained. It was suggested the loss in MU twitch forces due to fatigue resulted in an increase in excitatory drive to match the targeted forces.

Adam and De Luca (2005)

The authors of this study investigated MU firing rates for the VL during fatiguing contractions. Five healthy males performed three isometric MVCs for the knee extensors

followed repeated contractions at 20% of maximal strength. The template for the repeated contractions included a 5 sec ramp up to 50% MVC, followed by a brief hold and then a decrease to 20% MVC for a 50 sec constant hold. The surface EMG signal was recorded for the VL, VM, rectus femoris, and the biceps femoris and a quadrifilar fine wire electrode was inserted into the VL to observe individual MU behavior. Three subjects also completed the fatiguing protocol on a separate occasion and received transcutaneous electrical stimulation during a 6 sec rest period between each contraction. Normalized EMG amplitude indicated the relative force contribution for all investigated muscles remained constant throughout the fatiguing protocol. MU firing rates for the VL decreased during the first 10-20% of the work time during the fatiguing contractions. However, as the contraction time increased, there was an increase in firing rates and recruitment of additional MUs as force was maintained. In addition, electrical stimulation between fatiguing contractions indicated increases in whole muscle twitch forces later followed by a decrease. The authors stated the short-term MU potentiation (increased MU twitches forces) at the onset of the fatiguing protocol resulted in a reduced net excitatory drive that reduced firing rates. As the force twitches decreased during the fatiguing task, net excitatory drive increases to increase firing rates and recruit additional MUs to compensate for a reduction in twitch forces. Furthermore, the hierarchical arrangement between MU firing rates and recruitment thresholds remained intact during fatigue.

Contessa, De Luca, and Kline (2016)

This study investigated the relationship between force and MU firing rates during fatigue. Five healthy participants (3 men and 2 women; 24 – 33 yrs) performed MVCs of the dominant leg extensors followed by repetitive isometric trapezoidal muscle actions that included a linearly

increasing segment at a rate of 10% MVC/s to 30% MVC, a 48 sec steady force segment, followed by a linear decreasing segment at a rate of 10% MVC/s to baseline. Participants repeated the task until the average force decrease by more than 5% from the 30% targeted force. All contractions had a 6 sec rest interval for baseline noise calculation. Surface EMG was recorded for the VL and the surface EMG signal was decomposed to extract the firing events of their constituent MUAP trains. The following was identified for each MU: (1) MUAP size, (2) recruitment threshold, and (3) mean firing rate during between sec 35 and 45 during the steady force segment. During the protocol, the mean firing rates for MUs with similar amplitudes increased across contractions while the 30% MVC was maintained. In addition, higher-threshold MUs were recruited during the linearly increasing and steady force segment during subsequent contractions. Furthermore, the recruitment thresholds for MUs with similar MUAP amplitudes decreased across contractions. Lastly, the surface EMG signals for the VL, VMO, and rectus femoris similarly increased and, thus, the observed MU changes were not a result of differences in the activation patterns of the leg extensors. The inverse relationship between MU recruitment threshold and firing rate remained, however, the adaptations observed during fatigue were hypothesized to be a compensation for changes in the mechanical properties (force twitches) of the MU.

Skeletal Muscle Cross-Sectional Area and Quality Measures via Ultrasonography

Ahtiainen, Hoffren, Hulmi, Pietikainen, Mero, Avela, and Hakkinen (2010)

This study investigated the reliability and validity of US to detect changes in muscle CSA after a heavy resistance training program or control period by comparing results between US and

magnetic resonance imaging (MRI). Twenty-seven untrained, healthy males volunteered for this investigation. Twenty males were randomized to the resistance training group and 7 to the non-training group. mCSA measurements were taken on the right leg pre- and post-study with US and MRI. The resistance training program designed for muscular hypertrophy was performed twice a week. Both the US and MRI indicated significant increases in VL CSA following training while no changes were observed in the control group. A high repeatability and validity was reported between subsequent US measurements and US and MRI, respectively. Although MRI measurements CSA values were systematically higher than the US, both methods displayed a high agreement in identifying muscle CSA changes. Thus, US provides measures of muscle CSA that are repeatable even though MRI consistently produced larger CSA values.

Rosenberg, Ryan, Sobolewski, Scharville, Thompson, King (2014)

In this study, the authors investigated the test-retest reliability of panoramic US imaging to measure muscle size and quality. Sixteen men had US images taken of their medial gastrocnemius on two occasions separated by 2-7 days. For CSA measurements, the region of interest was “as much of the muscle as possible without any surrounding fascia.” Muscle quality was determined by the echo intensity values (EI) (gray-scale analysis of individual pixels within the US image) by a computer-aided gray scale analysis. The same area used for the CSA measurement was used for the mean EI value. EI values can range from 0 to 255 arbitrary units (0 = black, 255 = white). Between testing visits, there was acceptable reliability for CSA and EI. Therefore, US panoramic imaging is a reliable method for determining muscle size and quality.

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**THE INFLUENCE OF SEX AND MUSCLE QUALITY ON MOTOR UNIT
RECRUITMENT PATTERNS OF THE VASTUS LATERALIS**

ABSTRACT

Strong relationships have been reported between the increase in muscle cross-sectional area (mCSA) and motor unit action potentials (MUAP) sizes for higher-threshold MUs for the vastus lateralis (VL) of males. However, it is unknown if sex-related differences in muscle size and quality are correlated with the slopes and y-intercepts for the MUAP size vs. recruitment threshold (RT) relationships. Ten males (21.10 ± 1.97 yrs; 81.08 ± 13.75 kg) and ten females (23.70 ± 6.27 yrs; 61.26 ± 14.23 kg) volunteered for this investigation. Ultrasonography was used to examine mCSA, echo intensity (mEI), subcutaneous fat (sFAT), and muscle quality (mQT [mCSA/mEI]) for the VL. Surface electromyographic decomposition techniques were applied to assess MUAP sizes in relation to RT of the VL during a 40% and 70% submaximal isometric contraction. The males had greater mCSA ($P = 0.002$), mQT ($P = 0.001$), and slopes for the MUAP size vs. RT relationships ($P = 0.001$), whereas the females had greater sFAT ($P = 0.003$) and mEI ($P = 0.001$) for the VL. In addition, all relationships between ultrasound parameters and the slopes for the 40% and 70% MUAP size vs. RT were significant ($P < 0.001 - 0.020$), with the greatest amount of variance explained by mQT ($r = 0.707 - 0.778$). The sex-related differences in mCSA, mQT, and MUAP sizes in relation to RT suggests greater muscle fiber sizes of the higher-threshold MUs for the males.

INTRODUCTION

Increases in excitatory synaptic input to the motor unit (MU) pool (1) results in the orderly recruitment of MUs that are not only greater in size (2-4), but are also characterized by greater MU action potential (MUAP) amplitudes (2, 4, 5) as measured via intramuscular or surface electromyographic (sEMG) electrodes (6). Recent technological advancements in the automated algorithms used to decompose the sEMG signal from multichannel electrode arrays now allow the discrimination of MUAP waveforms from large populations of MUs (7, 8). Previous studies have reported strong positive relationships between the MUAP size vs. recruitment threshold (RT) relationships (2-4, 9). Therefore, the examination of the size principle (10) is now possible across large force ranges via the decomposition of the sEMG signal (11).

Previously, it has been reported that there is a strong correlation between the increase in muscle cross-sectional area (mCSA) from ultrasound (US) images and MUAP sizes of the higher-threshold MUs as a result of high intensity resistance training (12). Thus, increased contractile area (muscle fiber growth) that comprised the higher-threshold MUs resulted in an increase in MUAP sizes and, subsequently, changes in muscle size were associated with the change in slopes from the MUAP size vs. RT relationships. In theory, an individual that has greater muscle fiber area and greater mCSAs would possess larger y-intercepts and slopes from the MUAP size vs. RT relationships, however, this has yet to be tested.

In addition, echo intensity (mEI), via the US images, reflects intramuscular non-contractile tissue and has been correlated with intramuscular fat (13-16). Thus, normalizing mCSA to mEI provides an estimation of muscle quality (mQT [(mCSA/mEI)]. It is hypothesized that the y-intercepts and/or slopes from the MUAP size vs. RT relationships might have a stronger correlation to mQT since it reflects both the contractile and non-contractile area.

Previously, it has been reported that untrained young adult men possess greater area of type I (19%), IIA (59%), and IIB (66%) muscle fiber (17) and lower mEI than women for the vastus lateralis (VL) (18). It is understood that fibers that comprise a MU do not strictly express one fiber type (I vs. II), but rather co-express type I and II characteristics (19, 20). However, it is believed that fibers that express primarily type II characteristics tend to comprise higher-threshold more so than lower-threshold MUs (21-23). Given that the type I and II fibers are reported to be 19% and 59 to 66% larger for untrained men than women, it is speculative that there would be greater differences in the MUAP sizes of higher- than lower-threshold MUs between the men and women.

MUAP size vs. RT relationships can also provide insight on MU recruitment patterns, such as, it has been reported that larger sized MUs were being recruited to achieve the same absolute targeted force during repetitive fatiguing contractions (24). The MUAP size vs. RT relationship, however, has yet to be investigated as a function of contraction intensities. It is unclear if MU recruitment patterns are similar for moderate- and high-targeted force levels. Therefore, the purposes of this study were to examine potential sex-related differences in the vastus lateralis (VL) for the mCSA, mEI, and mQT and the possible correlations of these parameters with the MUAP size vs. RT relationships. In addition, a secondary aim of this project was to indicate if MU recruitment patterns differed between moderate- and high-intensity targeted forces via the MUAP sizes vs. RT relationships.

METHODS

Subjects

Ten healthy men (mean \pm SD age = 21.10 \pm 1.97 yrs, height = 180.60 \pm 3.41 cm, weight = 81.08 \pm 13.75) and ten healthy women (age = 23.70 \pm 6.27 yrs, height = 165.00 \pm 6.43 cm, weight = 61.26 \pm 14.23) who reported no participation in any form of structured exercise programs for the previous 3 years volunteered for this investigation. None of the participants reported 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 and signed an informed consent form and completed a pre-exercise health status questionnaire.

Ultrasound Imaging

US was used to measure anatomical mCSA, mEI, 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 laid in a supine position for 10 minutes 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 (4.5 cm) to optimized image quality and was 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-made probe support composed of high-density foam padding was positioned perpendicular to the longitudinal axis of the thigh and fastened with an adjustable Velcro strap to ensure US probe movement in the transverse plane. The panoramic function was used to obtain a single mCSA image of the VL. All US 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 the straight-line function. For mCSA, the muscle was outlined using the polygon function, with care taken to exclude the surrounding fascia. Muscle quality was evaluated using mEI from the same selected region to determine mCSA. The raw mEI was calculated via gray-scale analysis using the standard histogram function and corrected for sFAT (15). sFAT was quantified as the distance between the skin and the superficial aponeurosis of the muscle. mCSA was then normalized to EI ($mCSA/mEI$) to provide an estimate of muscle quality, such as, the ratio of contractile area vs non-contractile area (mQT).

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 submaximal

isometric trapezoid muscle actions at 40% and 70% MVC. The highest torque output for the MVCs determined the maximal torque output for each participant and the force level for the submaximal isometric trapezoid muscle actions. For all isometric trapezoid muscle actions, the force was increased at 10% MVC/s to the desired force level, where it was held during 12 s plateau and then decreased to baseline at a rate of 10% MVC/s. Therefore, the duration of each contraction lasted 20 and 26 s, respectively. Three to five minutes of rest was given between each muscle action. 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. Participants were given a second attempt if unable to maintain the targeted force during the initial trial.

Electromyographic Recording

During the trapezoid 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 are 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. A 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. (7) and Nawab et al. (8). Action potentials were extracted into firing events of single MUs from the four separate EMG signals via the Precision Decomposition III algorithm as described by De Luca et al. (7). 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 (8). Only MUs with > 90% accuracies were used for further analysis. For each MU, 3 parameters were extracted from the firing rate data: 1) the recruitment threshold (RT) [expressed relative to MVC (%MVC)], 2) motor unit action potential size (MUAP_{SIZE}) [expressed in millivolts (mV)], and 3) MUAP duration (MUAP_{DUR}) [expressed in milliseconds (ms)]. 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 (mV) and the duration (ms) from each of the four action potential waveforms were used to calculate MUAP_{SIZE} and MUAP_{DUR}, respectively.

Statistical Analysis

For each subject and contraction, linear regressions were performed on the MUAP_{SIZE} vs. RT relationships. Separate two-way mixed factorial ANOVAs (sex [male vs. female] x intensity [40% vs. 70%]) were used to examine possible differences in the y-intercepts and slopes from the MUAP_{SIZE} vs. RT relationships. In addition, separate two-way mixed factorial ANOVAs (sex [male vs. female] x intensity [40% vs. 70%]) was used to examine the differences in and the y-intercepts and slopes for the MUAP_{DUR} vs. RT relationships. Post-hoc follow-up tests included independent and paired samples *t*-tests with Bonferroni corrections. In addition, independent samples *t*-tests were used to examine differences in mCSA, mEI, mQT, and sFAT between sexes. Furthermore, Pearson's product moment correlation coefficients were calculated

comparing mCSA, sFAT, mEI, and mQT of the VL with the slopes and y-intercepts from the 40% and 70% MVCs. The slopes and y-intercepts were calculated using Microsoft Excel version 2013 (Microsoft, Redmond, WA). The level of significance was set at $P \leq 0.05$, and all statistical analyses were performed with SPSS 22 (IBM, Armonk, NY).

RESULTS

Table 1 indicates the number of MUs recorded per subject, RTs and MUAP_{SIZE} ranges, slopes, y-intercepts, and r values from the linear regressions. The MUAP_{SIZE} vs. RT relationships for each subject and contraction were significant ($P \leq 0.05$, males: $r = 0.81 \pm 0.09$, females: $r = 0.75 \pm 0.11$) with the slopes being positive.

MUAP_{SIZE} vs. RT Relationships

For the slopes, the analysis indicated no significant two-way interaction (sex x intensity; $P = 0.066$), however, there were significant main effects for sex ($P = 0.001$) and intensity ($P = 0.018$). The slopes for the 70% MVC (0.00430 ± 0.00256 mV/%MVC) were significantly greater than the 40% MVC (0.00343 ± 0.00185 mV/%MVC) when collapsed across sex. In addition, the slopes for the males (0.00525 ± 0.00224 mV/%MVC) were significantly greater than the females (0.00241 ± 0.00102 mV/%MVC) when collapsed across intensity (Figure 1A). The greater slopes indicate there was a greater relative growth in MUAP_{SIZES} in relation to RT for the males than females. The differences in slopes between the 70% and 40% MVCs was a function of observed RT ranges, such as, relative growth of MUAP_{SIZES} of the higher-threshold MUs (26.5 – 57.7% MVC) from the 70% MVC was much greater than for the lower-threshold MUs (3.4 – 22.0% MVC) from the 40% MVC.

Of note, 15 of the 20 linear regression models (75%) produced a negative y-intercept value from the 70% MVC. The negative y-intercepts were the result of the rapid rise in MUAP_{SIZES} for the higher-threshold MUs in conjunction with few recorded lower-threshold MUs recruited $< 26.5 \pm 8.1\%$ MVC (Table 1). The negative y-intercepts is not within

physiological expectations and, thus, considering the RT ranges when interpreting these relationships from the 70% MVC is necessary.

Nonetheless, the statistical analysis indicated a significant two-way interaction (sex x intensity; $P = 0.005$). The y-intercepts for the males from the 70% MVC were significantly less than the females ($P = 0.004$; males = -0.108 ± 0.074 mV/%MVC, females = -0.017 ± 0.074 mV/%MVC), the y-intercepts for the males was significantly greater during the 40% MVC compared to the 70% MVC ($P = 0.001$; 40% = 0.0174 ± 0.0100 mV/%MVC, 70% = -0.108 ± 0.074 mV/%MVC), and the y-intercepts for the females during the 40% MVC was greater compared to the 70% MVC ($P = 0.019$; 40% = 0.0194 , 70% = -0.0172 ± 0.0460 mV/%MVC).

There was not an overlap of RTs ranges between the 40% and 70% MVC (Table 1) and, therefore, a thorough examination of differences in recruitment patterns was not possible. However, to explore the possible intensity-related differences on MU recruitment patterns, predicted MUAP_{SIZES} for MUs recruited at 26% MVC were calculated from the linear regression equations for each subject from the MUAP_{SIZE} vs. RT relationships for the 40% and 70% MVCs and was analyzed with a mixed factorial ANOVA (sex x contraction). The RT of 26% MVC was chosen as it was the average minimum RT recorded during the 70% MVCs (Table 1). To avoid bias as a result of the range of RTs, subjects with no MUs recorded < 35% MVC for the 70% MVC (4 subjects) or had a negative predicted MUAP_{SIZE} at 26% MVC (1 subject) were not included in the analysis. The analysis indicated no significant two-way interaction ($P = 0.051$), however, there were main effects for sex ($P = 0.012$) and intensity ($P = 0.003$). The predicted MUAP_{SIZES} for the 40% MVC (0.107 ± 0.055 mV) were greater than the 70% MVC (0.056 ± 0.020 mV) when collapsed across sex. In addition, 14 of 15 individuals had a smaller predicted MUAP_{SIZE} at 26% MVC from the 70% than the 40% MVC. The predicted MUAP_{SIZES} for males

(0.100 ± 0.062 mV) were greater than the females (0.066 ± 0.025 mV) when collapsed across intensity.

Ultrasound measurements

The analysis indicated significantly greater mCSA ($P = 0.002$) (Figure 2A) and mQT ($P = 0.001$) (Figure 2B) for the males (25.21 ± 6.82 cm², 0.33 ± 0.13 AU/cm²) than the females (15.61 ± 4.64 cm², 0.12 ± 0.04 AU/cm²) and significantly greater mEI ($P = 0.001$) (Figure 2C) and sFAT ($P = 0.003$) (Figure 2D) for the females (135.38 ± 32.54 AU, 1.49 ± 0.57 cm) than the males (83.02 ± 32.54 AU, 0.66 ± 0.53 cm).

Correlations

Table 2 contains the Pearson's product moment correlations among mCSA, mEI, mQT, sFAT, and the slopes and y-intercepts from the MUAP_{SIZE} vs. RT relationships for the 40% and 70% MVCs. There were many significant correlations and, of importance, the slopes from the 40% and 70% MVCs were significantly correlated ($P \leq 0.001 - 0.020$) with mCSA ($r = 0.631$, $r = 0.516$) and mQT (Figures 3A, 3B) ($r = 0.778$, $r = 0.707$) (Figures 3C, 3D).

Noteworthy, there were slightly stronger correlations between mCSA and mQT with the slopes from the 40% than 70% MVC. There are two possible non-physiological explanations for the stronger correlations between the ultrasound and MUAP_{SIZE} data from the 40% MVC. First, more MUs on average were recorded during the 40% (26.0 ± 6.4) than 70% MVC (19.7 ± 5.7) and, thus, the ratio of recorded vs. active MUs during the 40% MVC would have been greater and may lead to a better characterizing of the MU pool. Second, there was a greater commonality of RT ranges among subjects during the 40% than 70% MVC (Table 1).

When separated by sex, there was no longer correlations between mCSA ($r = 0.293$, $P = 0.412$) and mQT ($r = 0.581$, $P = 0.078$) with the slopes from the MUAP_{SIZE} vs. RT relationships for the men, unlike for the women (Table 2). A possible explanation for lack of significance was the recruitment range for two of the men. For two men, there were no recorded MUs with RTs > 14% MVC. Thus, these two men possessed the lowest RTs of the last observed recruited MUs during the 40% MVC of all the subjects, which was well below the average RT for the men and women (22.0% MVC). When those two men were removed from the analysis, the correlations among the slopes and mCSA ($P = 0.038$, $r = 0.736$) and mQT ($P = 0.004$, $r = 0.884$) were significant (denoted in Figure 3). Therefore, the RT ranges of recorded MUs are important for the characterizing MUAP_{SIZES} in relation to RT and should be closely examined prior to interpretation.

MUAP_{DUR} vs. RT Relationships

Five and seven of the 40 relationships were significant ($P < 0.05$; $r = 0.379 - 0.610$) for the 40% and 70% MVC with two subjects having significant relationships for both MVCs. No statistical comparisons could be made with only two subjects possessing significant relationships for both MVCs. Overall, the depth of recorded MUs (i.e., the positive slope) may have slightly increased with increments in RTs for a few of the subjects, however, there were no sex-related differences (Figure 4). Therefore, the sex-related differences in the MUAP_{SIZE} vs. RT relationships was not a function of depth within the muscle of the recorded MUs (11, 25).

DISCUSSION

The main findings from this study was there were sex-related differences in mCSA, mQT, and slopes from the MUAP_{SIZES} vs. RT relationships and there were significant correlations among mCSA and mQT with the slopes from the MUAP_{SIZE} vs. RT relationship for the 40% and 70% MVC. The y-intercepts from the 70% MVC was also correlated, however, the majority (75%) of the y-intercepts from the individual relationships were negative due to rapid increases in MUAP_{SIZES} for the higher-threshold MUs in conjunction with a lack of observed lower-threshold MUs. There was not an overlap of RTs ranges between the 40% and 70% MVC and, therefore, a comprehensive examination of differences in recruitment patterns was not possible. However, there is limited evidence that MUs recruited from 20% to 30% MVC during the 40% MVC were larger than at the same relative forces during the 70% MVC (Figures 5C, 5D).

Previous research has reported greater muscle thickness and lower mEI for males than females for multiple muscles (18). In support, the results of the current study indicated males had greater mCSA for the VL than the females. In addition, females displayed greater mEI for the VL, likely indicating greater amounts of intramuscular fat (15, 26). Subsequently, when normalizing mCSA to mEI to provide an assessment of mQT, the males exhibited larger values and, thus, had greater amounts of contractile tissue (i.e. lean mass) of the VL compared to the females. Despite the similar activity levels, women displayed lower mQT, which might help explain the greater age-related impairments in function for women than men (18).

It was hypothesized that sex-related differences in mCSA and mQT would be reflected in the MUAP_{SIZE} vs. RT relationships. Indeed, the findings via the US were supported by the

MUAP_{SIZE} vs. RT relationships. For the 40% MVC, there were no sex-related differences for the y-intercepts and the observed MUAP_{SIZES} for the lowest-threshold MUs were not different between groups (Figure 5A, 5B). The slopes, however, for the 40% and 70% MVC were greater for the men and, thus, indicating a greater increase in MUAP_{SIZES} with increments in RT. Therefore, the men possessed larger MUAP_{SIZES} of the higher-threshold MUs (Figure 5A, 5B). It is possible the sex-related differences in contractile area might primarily be the result of differences in the muscle fibers that comprise the higher-threshold MUs as evident in Figure 5B. Previously, Staron et al. (17) reported that the CSAs of type I and II fibers were larger for untrained men in comparison to untrained women. In addition, only the type II fibers for the men were larger in comparison to the type I fibers, unlike for the women where the type II fibers were of similar size to the type I fibers (17). Overall, the mean type I, IIA, and IIB fiber sizes were 19%, 59%, and 66% greater for the men than women. Although speculative, the primary differences in MUAP_{SIZES} might be the result of the larger type II muscle fibers that comprise more so the larger higher- than smaller lower-threshold MUs. Nonetheless, the differences in contractile area between untrained men and women might primarily be the result of muscle fiber sizes of the higher-threshold MUs.

The anatomical mCSA measurement includes contractile and non-contractile tissue and, thus, mCSA might be arbitrarily increased by fat and collagen (*pseudo hypertrophy*). To account for non-contractile tissue of the VL, a measurement of mQT (mCSA/mEI) was utilized to investigate if this measure provided stronger relationships than mCSA with the slopes from the MUAP_{SIZE} vs. RT relationships. mCSA was significantly correlated with the slopes for the MUAP_{SIZES} vs. RT threshold relationships for the 40% and 70% MVC ($P \leq 0.001 - 0.020$; $R^2 = 0.27 - 0.40$), however, a greater amount of the variance was explained by mQT ($P \leq 0.001$; $R^2 =$

0.50 - 0.61). For example, individuals with greater mQT displayed larger increases in MUAP_{SIZES} in relation to RT. Therefore, accounting for non-contractile tissue with a measurement of mQT may provide more insight regarding the contractile area of the muscle.

A secondary aim of the current study was to investigate if MU recruitment patterns, via the MUAP_{SIZE} vs. RT relationship, are altered as a function of the targeted force. For the current investigation, the force increased at the same rate (10% MVC/s) during the 40% and 70% MVCs and, thus, insight on the influence of the targeted force level on recruitment patterns could be provided by comparing MUAP_{SIZES} among common RT ranges for the 40% and 70% MVC. However, only a few subjects had MUs recruited in common ranges for both the 40% and 70% MVC. Therefore, predicted MUAP_{SIZES} for MUs recruited at 26% MVC were calculated from the individual regression equations for each subject from the MUAP_{SIZE} vs. RT relationship for the 40% and 70% MVC as it was the average minimum RT recorded for the 70% MVC. The analysis indicated the predicted MUAP_{SIZE} at 26% MVC was greater for the 40% MVC compared to the 70% MVC with 14 of the 15 individuals included in the analysis displaying larger predicted MUAP_{SIZES} at a RT of 26% MVC for the 40% MVC (Figure 5C, 5D). In addition, close examination of figure 5 also provides evidence, particularly for the men, that MUs with amplitudes of 0.06 to 0.10 mV and 0.04 to 0.06 mV were recruited at higher forces during the 70% than 40% MVC for the men and women, respectively. However, these finding should be interpreted with caution due to the lack of observed MUs with common RT ranges between the two MVCs. Future research should investigate the influence of contraction intensities on the MU recruitment patterns by utilizing contraction intensities with closer targeted forces (i.e. 40% and 60% MVC) to increase the observed MUs with common RT ranges.

It is worth noting that sFAT, an anatomical factor, may be a confounding variable for the differences in MUAP_{SIZES} between the males and females. For example, it has been reported that MUAP_{SIZES} decrease with increases in skinfold thickness (4 to 18 mm) for superficially located MUs detected with surface EMG electrodes (27) and for the current study, there were greater amounts of sFAT for the females (1.49 ± 0.57 cm) than the males (0.66 ± 0.53 cm). Investigating the slopes from the MUAP_{SIZE} vs. RT relationship allows the relative increase in MUAP_{SIZES} throughout the RT spectrum to be statistically compared among individuals. Therefore, sFAT may reduce overall amplitudes of the MUs, however, the slopes provide insight on the rate of increase in MUAP_{SIZES} with increments in RT. The greater slopes for the males compared to the females and the strong correlations with mCSA and mQT, despite differences in sFAT between groups, provide evidence that the males had greater relative increases in AP_{SIZES} for the higher-threshold MUs than females.

In summary, there were sex-related differences in mCSA and mQT that were associated with the slopes from the MUAP_{SIZES} vs. RT relationships for the VL. The results tentatively indicated that the sex-related differences in the contractile area of the muscle was primarily the result of smaller muscle fibers that comprised the higher-threshold MUs for the females.

GRANTS

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Table 1. Sex (expressed as male [M] or female [F]), number of motor units (MUs), recruitment threshold (RT) ranges (expressed as % of maximal voluntary contraction [MVC]), MU action potential size (MUAP) ranges (expressed as millivolts), slope, y-intercept (y -Int), and Pearson correlation coefficient (r) values for the MUAP vs. RT relationships for the 40% and 70% MVC.

Subject	Sex	40% MVC						70% MVC					
		MUAP vs. RT						MUAP vs. RT					
		MUs	RT Range	MUAP Range	Slope	y -Int	r	MUs	RT Range	MUAP Range	Slope	y -Int	r
1	M	18	1.31-13.01	0.0212-0.0697	0.00307	0.0175	0.825	16	21.89-49.58	0.0569-0.1844	0.00298	-0.0189	0.751
2	M	22	4.04-30.25	0.0414-0.1859	0.00530	0.0172	0.783	23	29.82-56.38	0.0902-0.3402	0.00636	-0.1340	0.832
3	M	28	4.42-24.12	0.0374-0.1064	0.00274	0.0268	0.789	21	26.98-51.66	0.0771-0.2053	0.00488	-0.0759	0.902
4	M	28	1.05-17.89	0.0263-0.1329	0.00401	0.0253	0.607	14	19.87-62.93	0.0497-0.2053	0.00333	-0.0221	0.627
5	M	24	1.22-16.90	0.0298-0.1261	0.00411	0.0206	0.630	18	32.59-61.82	0.0898-0.3644	0.00763	-0.2045	0.753
6	M	22	4.19-18.31	0.0211-0.0876	0.00250	0.0124	0.818	20	35.40-68.90	0.0409-0.1599	0.00299	-0.0608	0.769
7	M	15	3.67-13.65	0.0382-0.1299	0.00616	0.0030	0.753	14	40.12-64.73	0.1330-0.3762	0.00644	-0.1120	0.774
8	M	20	3.87-31.48	0.0365-0.1713	0.00370	0.0088	0.821	22	23.78-58.89	0.0816-0.3232	0.00692	-0.1111	0.805
9	M	24	0.76-20.24	0.0341-0.2287	0.00884	0.0356	0.831	23	27.96-50.25	0.0893-0.3422	0.01053	-0.2499	0.816
10	M	20	5.45-21.08	0.0383-0.1287	0.00510	0.0072	0.716	19	21.42-43.44	0.0804-0.2504	0.00744	-0.0875	0.716
11	F	15	1.42-16.23	0.0342-0.1102	0.00345	0.0324	0.938	21	11.42-42.82	0.0421-0.0978	0.00130	0.0351	0.671
12	F	24	2.97-31.08	0.0113-0.0368	0.00074	0.0052	0.836	9	25.91-52.89	0.0507-0.1279	0.00173	0.0034	0.689
13	F	25	8.10-38.03	0.0436-0.0996	0.00160	0.0418	0.870	24	18.89-53.97	0.0575-0.2141	0.00265	0.0052	0.831
14	F	30	4.00-21.06	0.0296-0.0772	0.00183	0.0218	0.774	30	18.62-69.34	0.0474-0.2014	0.00236	-0.0042	0.869
15	F	28	1.26-16.24	0.0282-0.0932	0.00239	0.0295	0.665	27	22.04-57.67	0.0436-0.1366	0.00210	0.0039	0.634
16	F	36	1.87-21.14	0.0156-0.0543	0.00137	0.0126	0.922	25	16.12-45.53	0.0273-0.1305	0.00233	-0.0199	0.860
17	F	34	5.73-34.20	0.0343-0.1241	0.00275	0.0204	0.907	14	35.07-58.95	0.0826-0.2179	0.00457	-0.0802	0.886
18	F	23	2.22-17.10	0.0209-0.0927	0.00319	0.0128	0.710	10	25.54-72.47	0.0386-0.1323	0.00161	-0.0003	0.576
19	F	39	4.70-17.38	0.0234-0.0965	0.00250	0.0174	0.778	27	34.26-68.53	0.0541-0.1349	0.00185	0.0031	0.815
20	F	24	4.98-21.17	0.0242-0.0702	0.00327	0.0006	0.839	17	41.41-63.12	0.0716-0.1695	0.00459	-0.1180	0.859
Mean		24.95	3.36-22.03	0.0294-0.1111	0.00343	0.0184	0.791	19.70	26.46-57.69	0.0652-0.2156	0.00423	-0.0624	0.772
SD		6.39	1.96-7.17	0.0088-0.0456	0.00185	0.0111	0.091	5.71	8.10-8.86	0.0253-0.0881	0.00256	0.0758	0.093
Mean	M	22.10	3.00-20.69	0.0324-0.1367	0.00455	0.0174	0.757	19.00	27.98-56.86	0.0789-0.2748	0.00595	-0.1077	0.774
SD	M	4.12	1.72-6.29	0.0074-0.0473	0.00191	0.0100	0.082	3.43	6.60-8.00	0.0260-0.0827	0.00242	0.0739	0.073
Mean	F	27.80	3.72-23.36	0.0265-0.0855	0.00231	0.0194	0.824	20.40	24.94-58.53	0.0515-0.1563	0.00251	-0.0172	0.769
SD	F	7.15	2.2-8.05	0.0096-0.0262	0.00090	0.0126	0.091	7.49	9.48-10.01	0.0161-0.0417	0.00116	0.0461	0.114

Table 2. Pearson correlation coefficients (r) among muscle cross sectional area (mCSA), subcutaneous fat (sFAT), echo intensity (mEI), muscle quality (mQT), slopes and y-intercepts (y -Int) from the motor unit action potential size (MUAP) vs. recruitment threshold (RT) relationships for 40% and 70% maximal voluntary contraction.

Variable	All Subjects				Males Only				Females Only			
	CSA	sFAT	mEI	mQT	CSA	sFAT	mEI	mQT	CSA	sFAT	mEI	mQT
CSA	1				1				1			
sFAT	-0.441	1			-0.128	1			0.035	1		
mEI	-0.500 *	0.977 **	1		-0.256	0.979 **	1		0.102	0.981 **	1	
mQT	0.896 **	-0.696 **	-0.737 **	1	0.864 **	-0.542	-0.655 *	1	0.832 **	-0.496	-0.448	1
40% Slope	0.631 **	-0.676 **	-0.687 **	0.778 **	0.293	-0.482	-0.518	0.581	0.661 *	-0.564	-0.521	0.888 **
40% y -Int	0.036	-0.293	-0.264	0.172	0.503	-0.254	-0.365	0.613	-0.296	-0.596	-0.519	-0.048
70% Slope	0.516 *	-0.643 **	-0.657 **	0.707 **	0.080	-0.595	-0.559	0.427	0.230	-0.039	-0.136	0.266
70% y -Int	-0.558 *	0.505 *	0.552 *	-0.691 **	-0.219	0.413	0.444	-0.488	-0.365	-0.097	0.006	-0.336

Notes: ** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed)

Figure 1. The mean (\pm SD) slope (graph A) and y-intercept (graph B) values for the motor unit action potential size vs. recruitment threshold relationships from the 40% and 70% maximal voluntary contractions for the males and females. *The slopes were greater for the males than the females collapsed across contractions. **The slopes were greater for the 70% MVC than the 40% MVC collapsed across sex. †The y-intercepts were greater for the males during the 40% MVC than the 70% MVC. ††The y-intercepts were greater for the females during the 40% MVC than the 70% MVC. #The y-intercepts for the females during the 70% MVC was greater than the males.

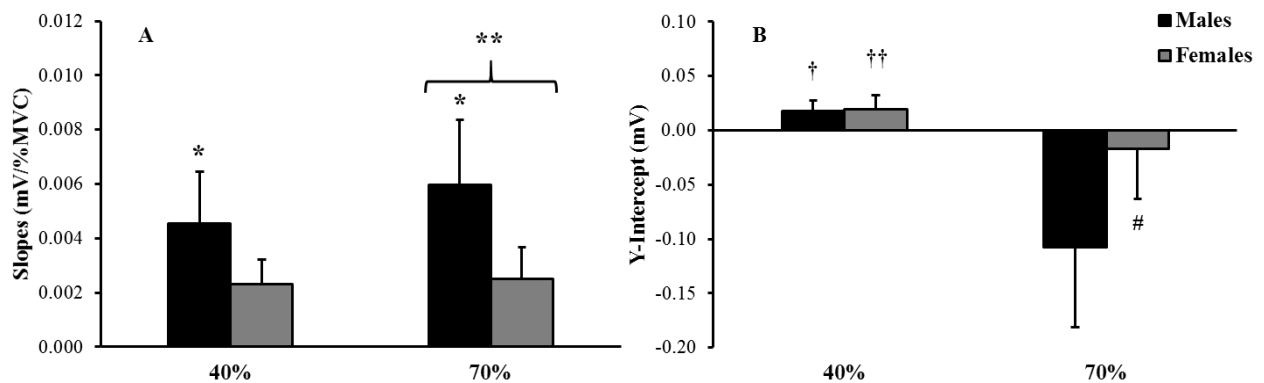


Figure 2. Muscle cross sectional area (A), muscle quality (B), echo intensity (C), and subcutaneous fat (D) for male and female participants. The echo intensity values were corrected for subcutaneous fat thickness. Data are presented as means \pm SD and * indicates a significant difference between groups ($P < 0.05$).

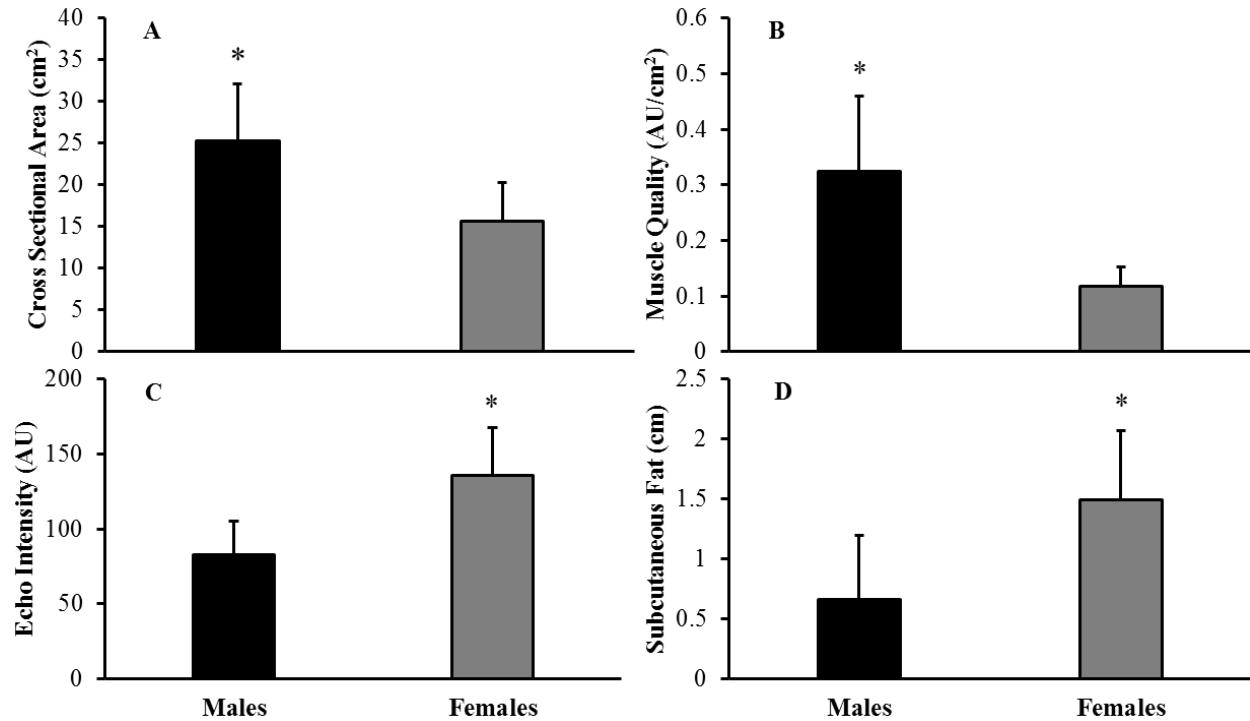


Figure 3. The plotted relationships between the slopes for the motor unit action potential size (millivolts [mV]) vs. recruitment threshold (expressed relative to percent maximal voluntary contraction [%MVC]) relationships and muscle quality (top graphs) and cross-sectional area (CSA) (bottom graphs) for the males (black circular markers) and females (grey circular markers) from the 40% (A, C) and 70% (B, D) MVCs. Plots with horizontal line (A, C) indicate two individuals for the 40% MVC with maximal recruitment thresholds outside the group mean \pm SD and bottom Pearson r values represent correlations with these individuals removed.

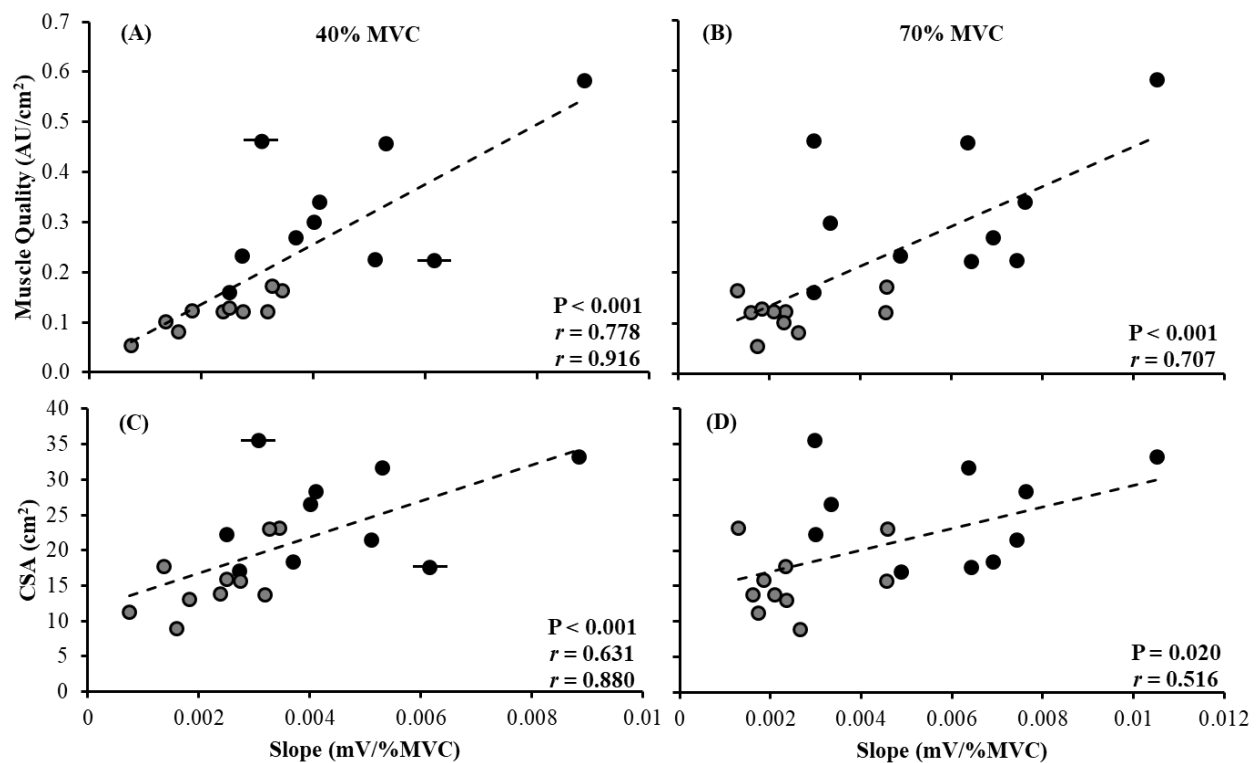


Figure 4. Plotted motor unit action potential (MUAP) duration (milliseconds [ms]) vs. recruitment threshold relationships (% maximal voluntary contraction [%MVC]) with linear regressions applied to the relationships for males (black markers and black solid regression lines) and females (grey markers and grey dashed regression lines) for the 40% (circular markers) and 70% MVC (triangular markers) with the coefficient of determination (R^2) displayed for each relationship.

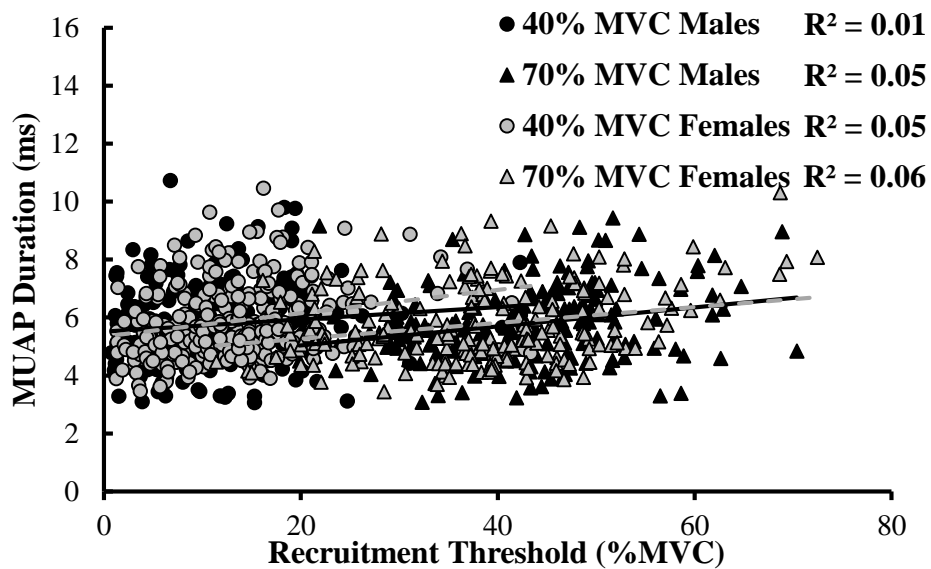


Figure 5. Plotted predicted motor unit action potential (MUAP) (millivolts [mV]) sizes and standard deviations vs. recruitment threshold relationships (% maximal voluntary contraction [%MVC]) with linear regressions applied to the relationships from the 40% MVC (A; solid regression lines) and 70% MVC (B; dashed regression lines) and for the males (C; black markers) females (D; grey markers). Dashed lines (C) represent MUAP sizes across contractions.

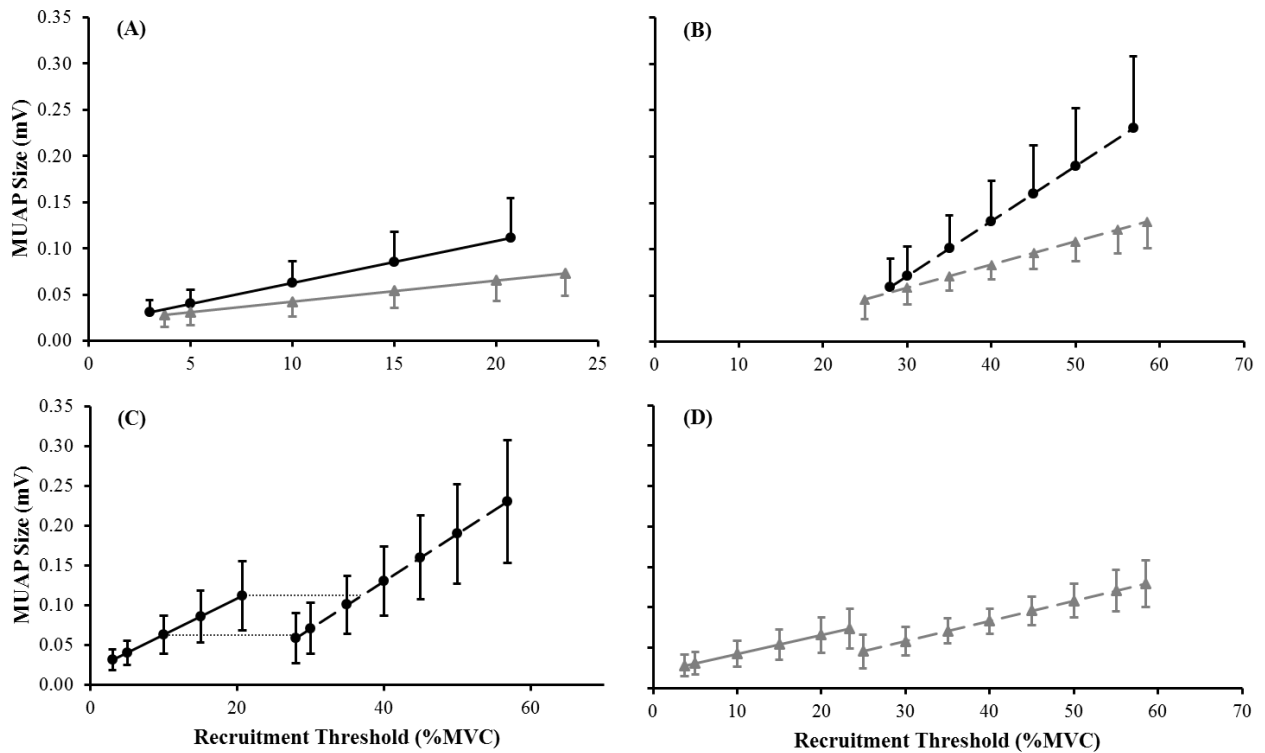
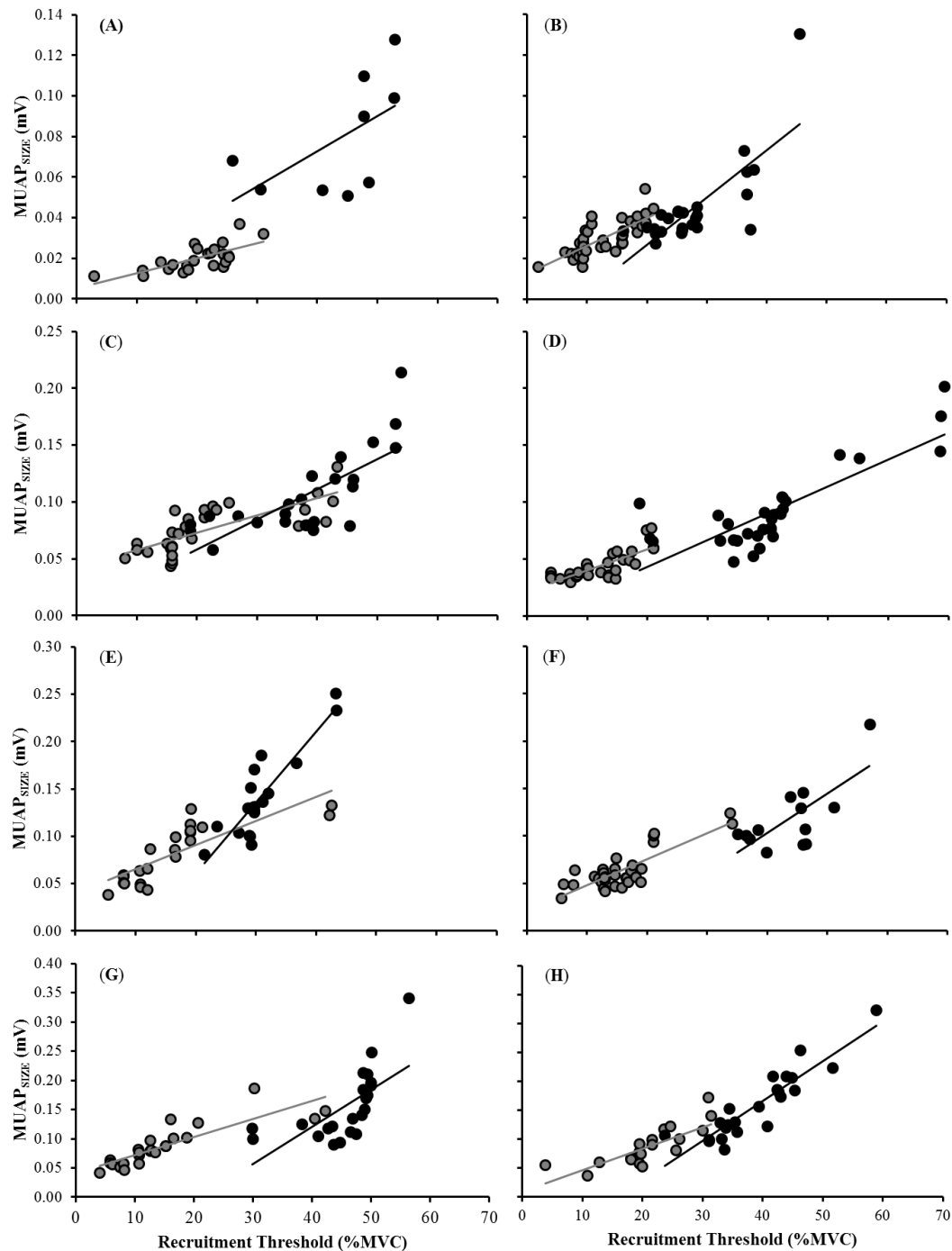


Figure 6. Plotted individual motor unit action potential (MUAP) (millivolts [mV]) sizes vs. recruitment (% maximal voluntary contraction [%MVC]) threshold relationships for the 40% (black markers) and 70% (grey markers) MVC with linear (solid regression lines) regression models applied for subjects 12 (A), 16 (B), 13 (C), 14 (D), 2 (E), 17 (F), 10 (G), and 8 (H).



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THE EFFECTS OF 10 WEEKS OF CONTINUOUS CYCLING ON MOTOR UNIT BEHAVIOR OF THE VASTUS LATERALIS

ABSTRACT

This study investigated the effects of 10 weeks of continuous cycling on motor unit (MU) behavior for the vastus lateralis (VL). Ten sedentary males and 13 sedentary females completed 40 continuous cycling training sessions over 10 weeks. Pre-, mid-, and post-training, participants performed maximal isometric voluntary contractions (MVC) followed by a 40% MVC (relative to pre-training) isometric trapezoidal muscle actions of the leg extensors. Surface electromyography (EMG) was recorded for the VL and decomposed for the 40% MVC to extract action potentials and firing events of single MUs. Recruitment thresholds (RT) and mean firing rates (MFR) were determined for each MU and the slopes and y-intercepts were calculated for the MFR vs. RT relationships. EMG amplitude (EMG_{RMS}) for the 40% MVC was normalized to the MVC for the respective visit. MVC of the leg extensors significantly decreased after post- ($P=0.006$) but not mid-testing ($P=0.059$). The y-intercepts and slopes significantly increased ($P = 0.006$) and decreased ($P=0.017$) at mid-, and returned to baseline at post-testing. Normalized EMG_{RMS} was significantly increased at mid- ($P=0.001$) and post- ($P<0.001$) in comparison to pre-testing. Ten weeks of continuous cycling resulted in decreases for maximal strength for the leg extensors. In addition, firing rates of the lower- and higher-threshold MUs increased and decreased at mid- and returned to baseline at post-testing. Greater normalized EMG_{RMS} likely indicated increases MU recruitment to match pre-training absolute torques. In summary, continuous cycling resulted in alterations for MU firing rate and recruitment patterns for the VL.

INTRODUCTION

Endurance training consists of repetitive movements performed for long durations. It is well understood that improvements in aerobic performance are a result of a variety of adaptations to metabolic and morphological responses/adaptations in skeletal muscle (28, 29). However, less is known regarding the neural adaptations to endurance training. Cross-sectional investigations have reported differences in motor unit (MU) firing rate behavior as a result of chronic training, such as, greater firing rates for Olympic caliber swimmers compared to Olympic caliber lifters and healthy controls (30) and chronic aerobically- compared to resistance-trained individuals (31-33) for the deltoid and vastus lateralis (VL) muscles, respectively. In addition, similar MU behavior has been reported between resistance-trained and sedentary individuals (31, 32). Therefore, it is plausible that endurance training elicits alterations in the overall MU control scheme, unlike resistance training (34-40).

However, few studies have longitudinally investigated the effects of endurance training on MU firing rate behavior for the VL (41-43). Vila-Cha et al. (42) reported decreases in MU firing rates and increases in muscle activation at a targeted force of 30% maximal voluntary contraction (MVC), whereas Martinez-Valdes et al. (43) reported no change in electromyographic amplitude (EMG_{RMS}) with increments in targeted forces (10%, 30%, 70%, and 90% MVC). The endurance training protocols utilized by Vila-Cha et al. (42) and Martinez-Valdes et al. (43) were performed at a low intensity (60% of heart rate reserve for a large portion of the study) or a shorter duration (2 weeks). In addition, the indwelling EMG and high-density surface EMG decomposition techniques used by Vila-Cha et al. (42) and Martinez-Valdes et al. (43) yielded limited observable MU counts per experimental session. Furthermore, Vila-Cha et

al. (42) did not account for recruitment threshold. Thus, it remains unclear when alterations in MU firing rate characteristics occur as a result of endurance training.

It is well understood that MU firing rates at a steady force vary as a function of recruitment threshold (44-46). Therefore, investigating the effects of endurance training on the mean firing rate vs. recruitment threshold relationships may provide insight on changes in MU firing rates relative to recruitment position. Previous studies have averaged observed MUs to produce a single firing rate value for experimental time points while disregarding recruitment (42, 43). In addition, the addition of normalized EMG_{RMS} may provide further insight on alterations in muscle activation to achieve the targeted force as a result of endurance training. Therefore, the purpose of this investigation was to examine the influence of 10 weeks of continuous cycling on mean firing rate vs. recruitment threshold relationships and normalized EMG_{RMS} of the VL for previously sedentary individuals.

METHODS

Subjects

Ten healthy men (mean \pm SD age = 20.20 ± 1.87 yr, height = 179.50 ± 4.95 cm, weight 77.50 ± 8.66 kg) and 13 healthy females (21.85 ± 5.54 yr, height = 164.00 ± 5.31 cm, weight 63.02 ± 13.23 kg) volunteered for this investigation. A subset of 5 subjects (2 males, 22.50 ± 3.54 yrs, 181.00 ± 0.00 cm, 84.75 ± 4.45 kg; 3 females, 20.33 ± 4.04 yrs, 169.00 ± 4.58 cm, 70.40 ± 14.93 kg) served as controls for the experimental testing procedures but did not participate in the continuous cycling training protocol. None of the participants reported participation in any form of structured exercise program for the previous 3 years. None of the participants reported 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 pre-exercise health status questionnaire.

Testing Time Line

Participants attended three laboratory sessions prior (visits 1 – 3), at the midpoint (visits 4 – 6), and after the completion (visits 7 – 9) of a 10 week continuous cycling training program for a total of nine experimental testing visits. Participants also performed 10 weeks of continuous cycling training at a frequency of 4 sessions per week for a total of 40 training sessions. Therefore, participants were required to make 49 total visits for this study. Visit one included the completion of a health history questionnaire and informed consent form and to become familiar with the isometric strength testing measurements, such as, isometric maximal muscle actions and

submaximal isometric trapezoidal muscle actions. Visits 4 and 7 included a re-familiarization of isometric strength testing measurements after 5 and 10 weeks of the continuous cycling training programs. Visits 2, 5, and 8 included a maximal aerobic capacity (VO_{2MAX}) test on a cycle ergometer to determine cardiovascular fitness and maximal heart rate. The maximal heart rate values from visits 2 and 5 were used to calculate the target heart rates for the subsequent 5 weeks of the continuous cycling training program. Visits 3, 6, and 9 included experimental isometric strength testing of the leg extensors (Figure 1).

VO_{2MAX} Testing

Testing was completed on an electronically-braked cycle ergometer (Lode, Groningen, Netherlands). Prior to any bike tests, participant seat height was measured and recorded for consistency among trials. Participants stood next to the bike to estimate proper seat height (greater trochanter), then examined while mounted on the bike to ensure there was a slight bend at the knee at the bottom of the pedal stroke, not full hyperextension. Once the seat height was set and comfortable, the foot straps were secured and were used to prevent the feet of the participants from slipping off the pedals during the test. After a two minute warm-up at 25 W, the workload was increased an additional 25 W every minute. Participants were encouraged to maintain 70 rpm, but the test was terminated when the participant could no longer maintain 60 rpm (volitional exhaustion). A true VO_{2MAX} was determined if participants meet three of the five indicators according to the American College of Sports Medicine Guidelines (47). During testing, heart rates were recorded with a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA) to determine maximal heart rate.

Respiratory gases were collected and monitored using a metabolic cart (Parvo Medics

TrueOne® 2400 Metabolic Measurement System, Sandy, Utah). The metabolic cart was calibrated prior to each test with room air and standard gases of known volume and concentration for the O_2 and CO_2 analyzers. Flowmeter calibration was performed prior to each graded exercise test. Respiratory gases were collected by use of a two-way rebreathing valve (Hans Rudolph Inc., Shawnee, Kansas) and mouthpiece attached to headgear, which held them in place. Participants wore a nose clip to ensure that breathing will occur entirely through the mouth. O_2 and CO_2 was analyzed through a sampling line after the gases are passed through a heated pneumotach and mixing chamber. The metabolic cart software reported the values as ventilated oxygen and carbon dioxide (V_{O_2} and V_{CO_2} , respectively) and calculate $V_{O_{2MAX}}$ automatically.

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 submaximal isometric trapezoid muscle actions at 40% relative to visit 3 (pre-training) 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 all subsequent isometric strength testing visits. For all isometric trapezoid muscle actions, the force increased at 10% MVC/s to the desired torque level, where it was held during a 12 s plateau and then decreased to baseline at a rate of 10% MVC/s. Therefore, the duration of each contraction was 20 s. Three to five minutes of rest was given between each muscle action. Participants were instructed to maintain their torque output as close as possible to the target force presented digitally in real time on a computer monitor. Participants were given a second attempt if unable to maintain the targeted torque during the initial trial.

Electromyographic Recording

During the trapezoid 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 are 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 the 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. (7) and Nawab et al. (8). Action potentials were extracted into firing events of single MUs from the four separate EMG signals via the Precision Decomposition III algorithm as described by De Luca et al. (7). 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 (8) and 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 (44, 48, 49). For each MU, 3 parameters were extracted from the firing rate data: 1) recruitment threshold (RT) expressed as %MVC, 2) mean firing rate (MFR) at the targeted contraction level (pulses per second [pps]), and 3) motor unit action potential duration ($MUAP_{DUR}$, ms). The MFR will be calculated as the average value of the MFR trajectory during the entire steady force (45, 50). An average 0.10-ms epoch of force that began at the first discharge of the MU was selected as the recruitment threshold for the MU, respectively. For $MUAP_{DUR}$, durations from peak-to-peak of each of the four unique action potential waveform templates were averaged using a custom-written software program (LabVIEW 2016, National Instruments, Austin, TX, USA).

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 entire steady force segment of the trapezoidal muscle contraction 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,

and EMG_{RMS} for the steady force segment of the isometric muscle actions was normalized to EMG_{RMS} that corresponded to the highest 0.25 s peak force epoch during the current visit MVCs. Offline processing was performed with custom-written LabVIEW software.

Continuous Cycling Training Protocol:

Participants performed 10 weeks of continuous cycling training on Life Fitness Upright Bikes (Model CLSC, Rosemont, IL, USA) at a frequency of 4 sessions per week for a total of 40 training sessions (Figure 1). Exercise intensity was prescribed based on the upper limits of heart rate reserve (HRR). Target heart rates (THR) were calculated with the Karvonen method $[(\text{maximal heart rate} - \text{resting heart rate}) \times \% \text{intensity} + \text{resting heart rate}]$ (51). The use of % HRR has been recommended for prescribing exercise intensity in cycling activities (52) because it provides accurate target workloads for individuals with a low fitness level (53).

Weeks 1 – 3 consisted of 30 minutes of cycling at 70% of the HRR, whereas weeks 4 – 6 and 7 – 10 were 40 minutes at 75% and 80% of HRR. Heart rates were monitored with a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA). All training sessions were supervised and subject heart rates were recorded every 3 minutes to ensure participants were maintaining the exercise intensity within the required THR.

Statistical Analysis

For each subject, linear regressions were performed on the MFR (pps) vs. RT (%MVC), and $MUAP_{DUR}$ (ms) vs. RT (%MVC) relationships. Slopes and y-intercepts were calculated for each linear regression model.

Eight separate two-way mixed factorial analysis of variances (ANOVAs) (sex [male vs. female] x time [pre vs. mid vs. post]) were used to examine possible differences in body mass, VO_{2MAX} , MVC strength, and normalized EMG_{RMS} , as well as the slopes and y-intercepts for the MFR vs. RT, and $MUAP_{DUR}$ vs. RT relationships. For the control subjects, similar statistical procedures were performed, however, sex was not included as a factor in the analyses. When appropriate, follow-up analyses included lower-order ANOVA models and independent samples *t*-tests with Bonferroni corrections. For all individual relationships, slopes and y-intercepts were calculated using Microsoft Excel version 2013 (Microsoft, Redmond, WA). The level of significance was set at $P \leq 0.05$, and all statistical analyses were performed with SPSS 22 (IBM, Armonk, NY).

RESULTS

Control Participants

Results (mean \pm SD) from the control participants are presented in table 1. There were no significant one-way interactions (time) for maximal strength ($P = 0.739$), maximal aerobic capacity ($P = 0.860$), normalized EMG_{RMS} ($P = 0.859$), and body mass ($P = 0.978$). For the MFR vs. RT relationships, there were no significant one-way interaction (time) for the slopes ($P = 0.770$) or y-intercepts ($P = 0.242$). For the MUAP_{DUR} vs. RT relationships, 6 of the 15 relationships (40%) were significant ($P < 0.05$; $r = 0.381 - 0.521$) for the 40% MVC with none of the subjects having significant relationships across the three visits. Therefore, no statistical comparisons could be made with none of the subjects possessing significant relationships for all testing visits.

Experimental Participants

Body Mass

The analysis indicated no significant two-way interaction (sex x time, $P = 0.780$) or main effects for time ($P = 0.696$) and sex ($P = 0.604$).

Maximal Aerobic Capacity

The analysis indicated no significant two-way interaction (sex x time, $P = 0.230$). There were main effects for sex ($P < 0.001$) and time ($P < 0.001$). VO_{2MAX} was significantly greater for the males (3.575 ± 0.490 L/min) in comparison to the females (2.231 ± 0.436 L/min) when collapsed across time. In addition, pre-VO_{2MAX} (2.645 ± 0.818 L/min) was significantly less in

comparison to mid- $\text{VO}_{2\text{MAX}}$ (2.795 ± 0.800 L/min, $P < 0.001$) and post- $\text{VO}_{2\text{MAX}}$ (3.005 ± 0.850 L/min, $P < 0.001$), and mid- $\text{VO}_{2\text{MAX}}$ was significantly less in comparison to post-testing $\text{VO}_{2\text{MAX}}$ ($P < 0.001$) when collapsed across sex (Figure 2).

MVC Strength

The analysis indicated no significant two-way interaction (sex x time, $P = 0.411$). There were main effects for sex ($P < 0.001$) and time ($P = 0.011$). MVC strength was significantly greater for males (217.33 ± 44.44 Nm) in comparison to females (124.16 ± 32.83 Nm) when collapsed across time. In addition, MVC strength was significantly less post- (167.45 ± 39.61 Nm) in comparison to pre-testing (175.93 ± 37.34 Nm, $P = 0.006$) when collapsed across sex (Figure 3). Despite similar values at mid- compared to post-testing, there were no differences between mid- (168.81 ± 41.10 Nm) and post-MVC strength (167.45 ± 39.61 Nm).

Normalized EMGRMS

The analysis indicated no significant two-way interaction (sex x time, $P = 0.819$) or main effects for sex (0.647). However, there were main effects for time ($P < 0.001$). Normalized EMGRMS was significantly less at pre- (34.854 ± 9.218 % max) in comparison to mid- (45.984 ± 13.141 %, $P = 0.001$) and post-testing (45.369 ± 9.256 %, $P < 0.001$) when collapsed across sex (Figure 4).

MU Data

Decomposition of surface EMG signals yielded 1,601 MUs. Observed MUs, RT ranges, and slopes and y-intercepts for the MFR vs. RT relationships per individual are presented in table 2.

MFR vs. RT Relationships

For all individuals, the MFR vs. RT ($r = -0.89 \pm 0.06$) relationships were significant. For the slopes, the analysis indicated no significant two-way interaction (sex x time, $P = 0.875$) or main effect for sex ($P = 0.121$). However, there was a main effect for time ($P = 0.008$). The slopes at mid- (-0.449 ± 0.144 pps/%MVC) were significantly less in comparison to pre- (-0.344 ± 0.082 pps/%MVC, $P = 0.017$), but not post-testing (-0.392 ± 0.108 pps/%MVC) when collapsed across sex.

For the y-intercepts, there was no significant two-way interaction (sex x time, $P = 0.304$) or main effect for sex ($P = 0.162$). However, there was a main effect for time ($P = 0.003$). The y-intercepts were significantly greater at mid- (25.285 ± 3.904 pps) in comparison to pre- (22.808 ± 2.854 pps), but not post-testing (23.894 ± 2.325 pps) when collapsed across sex ($P = 0.006$) (Figure 5).

MUAP_{DUR} vs. RT Relationships

Ten of the 69 relationships (14%) were significant ($P < 0.05$; $r = 0.368 - 0.699$) for the 40% MVC with none of the subjects having significant relationships across all time points. No statistical comparisons could be made with zero of the subjects possessing significant relationships for all testing visits. Of importance, figure 6 indicates that there was no systemic

differences in MUAP_{DUR} among pre-, mid-, and post-testing and, therefore, MUs were recorded from similar depths within the muscle across all visits.

DISCUSSION

As expected, maximal aerobic capacity increased following 5 and 10 weeks of continuous cycling training. Significant findings as a result of the continuous cycling training include the following: (1) isometric maximal strength of the leg extensors decreased after 5 (not significant) and 10 weeks, (2) 5 weeks of continuous cycling training was associated with increases and decreases in firing rate behavior of the lower- and higher-threshold MUs, respectively, whereas firing rate behavior returned to baseline at 10 weeks, and (3) normalized EMG_{RMS} was elevated at 5 and 10 weeks and, thus, indicating increased muscle activation to match pre-training forces.

The results from the current study indicated VO_{2MAX} significantly increased after 5 and 10 weeks of continuous cycling. These findings are in agreement with Hoppeller et al. (54) and Howald et al. (29), as well as Konopka et al. (55), that reported significant increases in VO_{2MAX} following 6 and 12 weeks of cycle ergometer training for young and older individuals, respectively. It has been reported that a number of sex-related morphological and physiological differences in blood volume, red blood cells, hemoglobin, heart size, resting and submaximal heart rate, stroke volume, and oxygen pulse result in lower VO_{2MAX} for females (56). In support, VO_{2MAX} values were greater for the males than the females when collapsed across time. However, there were no differences in the sex-related responses to training for the current study ($P = 0.394$), which has previously been reported (57-60).

Few studies have examined the influence of endurance training on MU behavior (42, 43, 61). Vila Cha et al. (42) reported a decrease in MU firing rates at a 10% and 30% MVC with an increase in EMG_{RMS} for the VL following 6 weeks of endurance bicycle ergometer training with no significant changes in maximal strength. Since increases in EMG_{RMS} reflects an increase in

net MU activity (62-64), the authors suggested the decrease in MU firing rates likely resulted in the recruitment of more, higher-threshold MUs to achieve the same relative forces. In addition, Mettler and Griffin (61) reported that 4 weeks of isometric thumb abduction contractions at 20% MVC yielded increased endurance times but no significant changes in maximal strength or MU firing rates for the adductor pollicis during sustained contractions. More recently, Martinez-Valdes et al. (43) longitudinally tracked MUs following two weeks of long duration continuous cycling and reported no changes for maximal strength, EMG_{RMS} , recruitment thresholds, or firing rates at 10%, 30%, 50% and 70% MVC despite increases in VO_{2MAX} . Vila-Cha (42) and Mettler and Griffin (61) did not report RTs of the MUs and, thus, it is unknown if firing rate behavior, relative to recruitment position, was altered following endurance training.

In the current study, MU firing rate behavior and likely recruitment patterns were altered following 5 weeks of continuous cycling. Such as, there were increases in the firing rates of the lowest-threshold MUs (increased y-intercepts of the MFR vs RT relationships) with decreases in the firing rates of the highest-threshold MUs (decreased slopes of the MFR vs RT relationships) (Figure 7). Greater y-intercepts have been reported for higher- in comparison to lower-intensity contractions due to increases in excitation to achieve higher targeted forces (44, 65). In the present study, normalized EMG_{RMS} , a measure of muscle activation, was greater at mid- and post-testing and, together with greater y-intercepts from the MFR vs RT relationships, likely indicates that greater excitation was needed to achieve the same absolute force tasks following 5 and 10 weeks of continuous cycling.

It has previously been theorized that the operating point of excitation to the MU pool is altered to compensate for changes in force twitches (24, 48, 50, 66). In the present study, torque was lower following 5 (-4.05%) and 10 (-4.79%) weeks, albeit only significant lower after 10

weeks of cycling. In addition, previous research has reported reduced force twitches of MUs following endurance training, such as the type I and IIa fibers of chronic runners produced 15% less peak force compared to age-matched sedentary controls (67). Subsequently, it is likely that MU twitch forces were depressed for the routinely activated lower-threshold MUs following the endurance cycling training in the present study. Therefore, the significant increases in normalized EMG_{RMS} and the firing rates of the lowest-threshold MUs suggests a rightward shift in the operating point (greater excitation) to achieve the same submaximal torques following 5 weeks of continuous cycling (24, 48, 50, 66). The resulting increases in MU firing rates simultaneously occurs with the recruitment of higher-threshold MUs that possess lower firing rates (3, 44, 48, 68, 69). Thus, the recruitment of additional higher-threshold MUs, which possess lower firing rates, to match the same absolute torques would result in the slopes becoming more negative. These findings support Vila-Cha et al. (42) as the increases in normalized EMG_{RMS} and y-intercepts in conjunction with the decrease in the slopes for the MFR vs. RT relationships suggest greater excitation and the recruitment of additional higher-threshold MUs that could potentially lead to lower firing rates when disregarding RTs.

Following 10 weeks of the continuous endurance cycling, firing rate behavior returned to baseline, however, normalized EMG_{RMS} remained elevated. In addition, MVC strength of the leg extensors remained depressed and was significantly lower in comparison to pre-testing. The decrease in firing rates for the lowest-threshold MUs following 10 weeks of continuous cycling may be due to alterations in the membrane potentials of motoneurons. Animal studies investigating endurance training have reported greater hyperpolarized resting membrane potentials, voltage thresholds, afterhyperpolarization amplitudes, and decreased antidromic action potential rise time of the lower-threshold MUs following 12 and 16 weeks of endurance

training (70, 71). Although the implications of these adaptations are not fully understood (72), a decrease in spike rise time and a greater afterhyperpolarization would decrease MU firing rates (73), which was reported for the lower-threshold MUs in the current study from 5 to 10 weeks of continuous cycling.

Another possible explanation for the decrease in firing rates for the lowest-threshold MUs was a change in MU twitch relaxation rates. Majerczak et al. (74) reported 5 weeks of endurance cycling in humans resulted in a downregulation for sarco(endo)plasmic reticulum calcium ATPase isoform (SERCA) pumps, resulting in a prolonged muscle relaxation rate. It has been suggested that a reduction in calcium ATPase activity may result in prolonged twitch durations, leading to tetanus of MUs at lower firing rates following training (42). Furthermore, differences in sarcoplasmic reticulum characteristics have been reported as a function of fiber type (75). For example, muscle fibers that possess fast-twitch characteristics exhibit greater calcium release and uptake by the sarcoplasmic reticulum (76) due to greater sarcomplasmic reticulum surface area, as well as greater SERCA isoform kinetics (75, 77). Previously, Konopka et al. (55) reported increases in myosin heavy chain isoform content of the VL following 12 weeks of continuous cycling. Therefore, it is plausible that downregulation of SERCA for the current study occurred as a function of continuous cycling and changes to the area of myosin heavy chain isoform content of the muscle.

The increases in firing rates for the higher-threshold MUs following weeks 10 of continuous cycling may be a function of lengthened twitch durations coupled with decreased twitch forces for the lower-threshold MUs. It has been proposed that MUs with greater twitch amplitudes have lower firing rates at a targeted force (46). However, the significant increase for normalized EMG_{RMS} suggests the decrease in firing rates for the lower-threshold MUs was more

likely a function of increases in twitch durations and not twitch amplitudes as muscle activation remained elevated to achieve the targeted torques. In addition, if twitch durations were elongated for the lower-threshold MUs, there would be no benefit to increasing their firing rates as tetanus would occur at lower frequencies (78). Thus, a greater reliance would be placed on the recruitment and modulation of firing rates of the higher-threshold MUs to match the targeted force compared to mid-testing, which was supported by significantly greater normalized EMG_{RMS} and the increase in the slopes for the MFR vs. RT relationships to pre-testing values.

In summary, 5 and 10 weeks of continuous cycling resulted in increases in muscle activation to match pre-training absolute forces. The non-uniformed changes in firing rate behavior suggests a decrease in MU twitch forces (week 5) and/or alterations to motoneurone intrinsic properties (week 10). Consequently, this is the first study to report continuous cycling changes recruitment patterns for the VL. Future research should investigate the influence of concurrent training on MU behavior to achieve pre-training absolute forces.

GRANTS

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Table 1. Sex, number of motor units (MUs), recruitment threshold (RT) ranges (expressed as % MVC), and slope and y -intercept (y -Int) values for mean firing rate (MFR) vs. RT relationships for pre-, mid-, and post-training.

Subject	Sex	Pre-Testing					Mid-Testing					Post-Testing				
		MUs	RT Range	MFR vs. RT			MUs	RT Range	MFR vs. RT			MUs	RT Range	MFR vs. RT		
				Slope	y -Int	r			Slope	y -Int	r			Slope	y -Int	r
1	F	24	2.97-31.08	-0.247	19.50	-0.85	23	8.95-26.92	-0.288	22.47	-0.94	26	3.57-24.32	-0.256	20.15	-0.91
2	F	25	8.10-38.03	-0.218	23.80	-0.89	23	18.45-40.56	-0.419	29.23	-0.93	26	16.82-40.31	-0.311	27.35	-0.85
3	F	16	9.45-37.11	-0.289	24.87	-0.76	25	9.39-37.72	-0.523	33.65	-0.87	20	1.59-31.38	-0.318	26.01	-0.91
4	F	16	6.55-22.23	-0.305	22.36	-0.81	31	1.38-19.21	-0.346	20.77	-0.94	23	3.09-18.60	-0.474	23.90	-0.91
5	F	30	4.00-21.06	-0.314	21.55	-0.94	27	5.12-23.52	-0.379	22.17	-0.92	32	0.79-24.16	-0.208	19.73	-0.97
6	F	36	1.87-21.14	-0.357	20.79	-0.92	11	3.10-12.71	-0.560	23.76	-0.83	20	2.21-11.47	-0.568	23.66	-0.92
7	F	18	3.63-12.81	-0.349	22.38	-0.73	26	0.27-9.83	-0.639	22.37	-0.89	22	1.58-19.45	-0.373	23.20	-0.93
8	F	34	5.73-34.20	-0.250	19.52	-0.82	20	6.41-22.89	-0.304	20.50	-0.85	28	4.30-16.13	-0.497	23.82	-0.90
9	F	36	5.45-21.98	-0.340	22.69	-0.92	25	2.92-21.14	-0.489	23.48	-0.96	15	2.67-26.12	-0.258	21.26	-0.87
10	F	39	4.70-17.38	-0.359	20.42	-0.90	24	4.95-20.27	-0.296	19.40	-0.93	24	2.33-18.84	-0.390	25.25	-0.91
11	F	27	10.62-28.39	-0.335	23.56	-0.92	34	8.59-23.29	-0.446	24.68	-0.92	32	3.78-24.38	-0.372	22.11	-0.92
12	F	22	5.51-26.83	-0.502	24.55	-0.95	14	10.12-38.79	-0.373	24.52	-0.93	17	6.33-21.12	-0.537	26.24	-0.82
13	F	24	4.98-21.17	-0.424	23.86	-0.85	25	3.74-18.39	-0.437	24.05	-0.96	19	11.77-38.37	-0.204	23.56	-0.90
14	M	22	4.04-30.25	-0.224	18.77	-0.93	18	8.01-26.61	-0.414	24.38	-0.87	24	11.82-40.48	-0.341	23.50	-0.96
15	M	16	16.76-39.03	-0.471	29.42	-0.94	25	5.56-39.35	-0.269	23.68	-0.88	16	8.19-32.01	-0.348	23.77	-0.92
16	M	18	5.53-19.82	-0.387	19.19	-0.73	24	3.80-27.21	-0.263	19.98	-0.83	14	4.55-13.85	-0.564	21.26	-0.78
17	M	28	1.05-17.89	-0.418	24.44	-0.89	28	3.03-14.27	-0.559	26.64	-0.91	24	2.10-19.37	-0.466	22.82	-0.91
18	M	24	7.39-30.36	-0.378	27.67	-0.73	14	12.13-28.79	-0.615	30.88	-0.99	27	11.17-34.10	-0.424	26.54	-0.97
19	M	24	10.61-35.16	-0.341	25.77	-0.96	20	17.88-39.66	-0.592	32.34	-0.96	19	15.43-35.70	-0.402	27.77	-0.89
20	M	15	3.67-13.65	-0.442	24.26	-0.72	12	7.28-19.48	-0.734	31.67	-0.95	21	0.07-11.21	-0.465	23.04	-0.93
21	M	20	3.87-31.48	-0.344	24.13	-0.96	28	11.98-40.62	-0.372	25.65	-0.97	28	6.18-32.30	-0.510	28.29	-0.92
22	M	24	0.76-20.24	-0.359	19.52	-0.89	18	16.09-37.52	-0.283	24.21	-0.86	26	2.73-39.91	-0.319	24.10	-0.94
23	M	20	5.45-21.08	-0.214	20.01	-0.77	18	5.09-15.19	-0.657	26.94	-0.91	27	6.26-24.09	-0.420	22.24	-0.91
Mean		24.26	5.81-25.96	-0.342	22.74	-0.86	22.30	7.75-26.62	-0.446*	25.10†	-0.91	23.04	5.34-25.42	-0.392	23.89	-0.91
SD		6.94	3.65-7.95	0.079	2.81	0.08	5.98	5.08-9.97	0.140	4.02	0.05	5.04	4.64-9.18	0.108	2.33	0.04
Mean	F	26.69	5.66-25.65	-0.330	22.30	-0.87	23.69	6.41-24.25	-0.423	23.93	-0.91	23.38	4.68-24.20	-0.367	23.56	-0.90
SD	F	7.81	2.51-7.72	0.076	1.83	0.07	6.13	4.79-9.56	0.108	3.80	0.04	5.32	4.61-9.34	0.123	2.32	0.04
Mean	M	21.10	5.91-25.90	-0.358	23.32	-0.85	20.50	9.09-28.87	-0.476	26.64	-0.91	22.60	6.85-28.30	-0.426	24.33	-0.91
SD	M	4.07	4.77-8.40	0.084	3.77	0.10	5.56	5.18-10.20	0.176	3.96	0.05	4.90	4.83-10.54	0.078	2.38	0.05

* Indicates the slopes at mid- were significantly less in comparison to pre-, but not post-training when collapsed across sex.

† Indicates the y -intercepts at mid- were significantly greater in comparison to pre-, but not post-training when collapsed across sex.

Table 2. Mean \pm SD for body mass (kg), maximal aerobic capacity (Liters/minute [L/min]), leg extensor maximal voluntary contraction (MVC) (Newton meters [Nm]), electromyographic amplitude (EMG_{RMS}) normalized to MVC EMG (% max), slopes and y-intercepts (Y-Int) for the mean firing rate (MFR) (pulses per second [pps]) vs. recruitment threshold (RT) (%MVC).

Measurement		Pre	Mid	Post
Body Mass (kg)		76.14 \pm 13.35	76.56 \pm 14.14	76.18 \pm 13.21
Maximal Aerobic Capacity (L/min)		2.78 \pm 0.85	2.79 \pm 0.84	2.81 \pm 0.78
Leg Extensor MVC (Nm)		177.20 \pm 67.28	171.66 \pm 53.96	174.70 \pm 69.31
Normalized EMG _{RMS} (% max)		34.40 \pm 4.96	35.76 \pm 4.36	39.95 \pm 16.21
MFR vs. RT	Slopes (pps/%MVC)	-0.413 \pm 0.092	-0.455 \pm 0.151	-0.427 \pm 0.060
	Y-Int (pps)	22.46 \pm 2.10	23.67 \pm 2.37	23.20 \pm 1.28

Figure 1. Timeline of testing and training. Fam = familiarization; Exp = experimental, HRR = heart rate reserve.

Pre-Testing			Continuous Cycling		Mid-Testing			Continuous Cycling		Post-Testing		
Visit 1	Visit 2	Visit 3	Weeks 1 - 3	Weeks 4 - 5	Visit 4	Visit 5	Visit 6	Week 6	Weeks 7 - 10	Visit 7	Visit 8	Visit 9
Strength Testing (Fam)	Maximal Aerobic Testing	Strength Testing (Exp)	30 mins 4x's/week 70% HRR	40 mins 4x's/week 75% HRR	Strength Testing (Fam)	Maximal Aerobic Testing	Strength Testing (Exp)	40 mins 4x's/week 75% HRR	40 mins 4x's/week 80% HRR	Strength Testing (Fam)	Maximal Aerobic Testing	Strength Testing (Exp)

Figure 2. The mean (\pm SD) absolute maximal aerobic capacity (VO_{2MAX}) (L/min) from pre-, mid, and post-training for the males and the females. # VO_{2MAX} was significantly greater for the males in comparison to the females collapsed across time ($P < 0.001$). * VO_{2MAX} was significantly greater at mid- in comparison to pre-training ($P < 0.001$) when collapsed across sex. ** VO_{2MAX} was significantly greater at post- in comparison to pre- ($P < 0.001$) and mid-training ($P < 0.001$) when collapsed across sex.

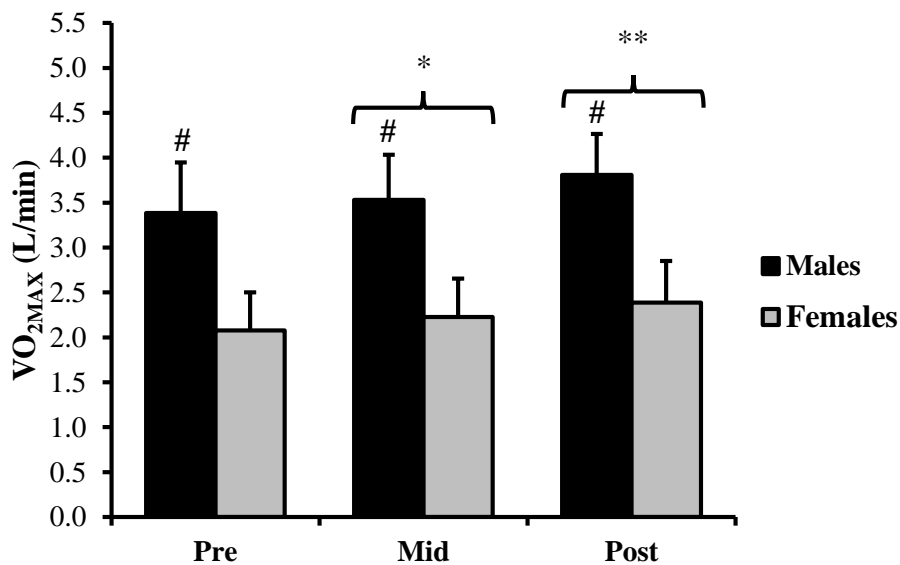


Figure 3. The mean (\pm SD) maximal voluntary contraction for pre-, mid, and post-training for the males and females. # Maximal strength was significantly greater for the males in comparison to the females when collapsed across time ($P < 0.001$). * Maximal strength was less for post- in comparison to pre-training when collapsed across sex ($P = 0.006$).

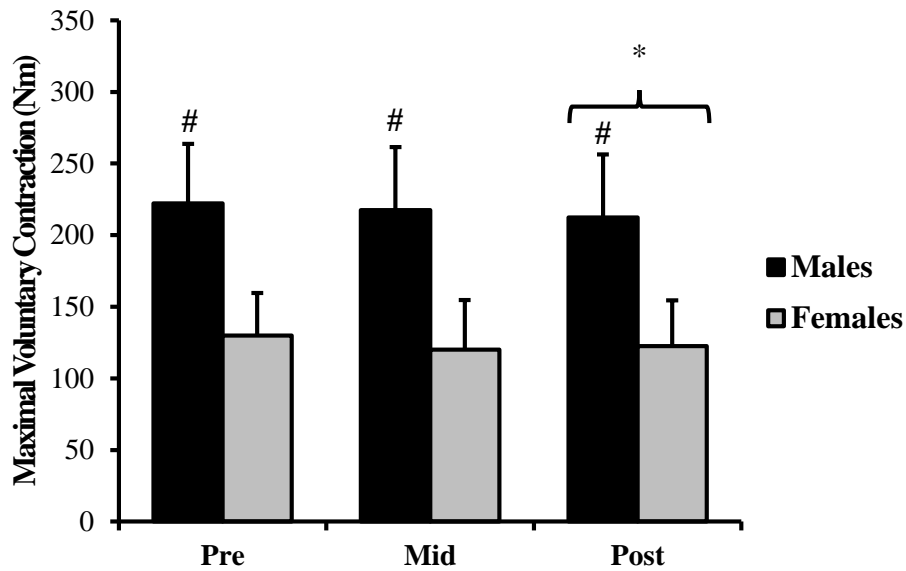


Figure 4. The mean (\pm SD) normalized (% max) electromyographic amplitude (EMG_{RMS}) for pre-, mid-, and post-training from the 40% maximal voluntary contraction for the males and females.

* Normalized EMG_{RMS} was greater at mid- in comparison to pre-training ($P = 0.001$) when

collapsed across sex. ** Normalized EMG_{RMS} was greater at post- in comparison to pre-training

($P < 0.001$) when collapsed across sex.

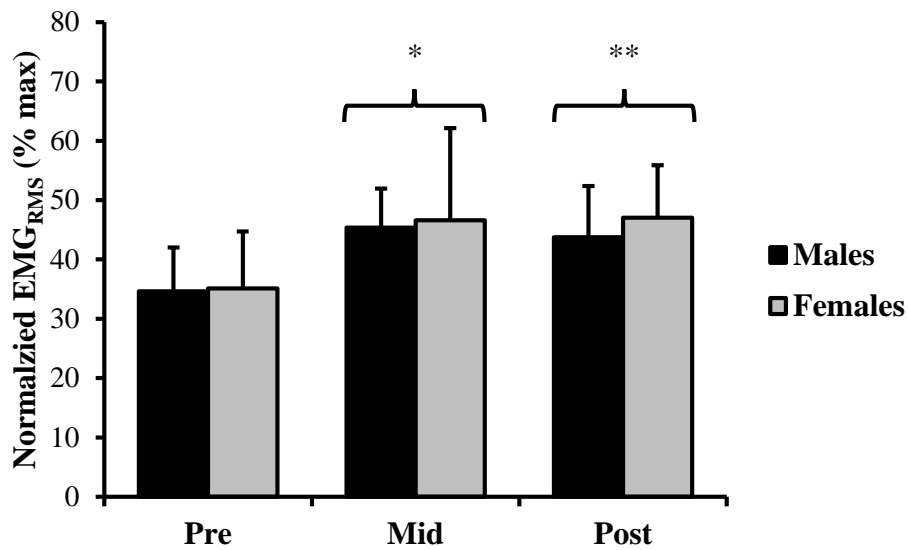


Figure 5. The mean (\pm SD) slopes (top graph) and y-intercept (bottom graph) values from the mean firing rate (pulses per second [pps]) vs. recruitment threshold (expressed as percent of maximal voluntary contraction [%MVC]) relationships for the males and females for pre-, mid, and post-training for the 40% MVC. * The slopes were significantly less at mid- in comparison to pre-training ($P = 0.017$) when collapsed across sex. ** The y-intercepts were significantly greater at mid- in comparison to pre-training ($P < 0.006$) when collapsed across sex.

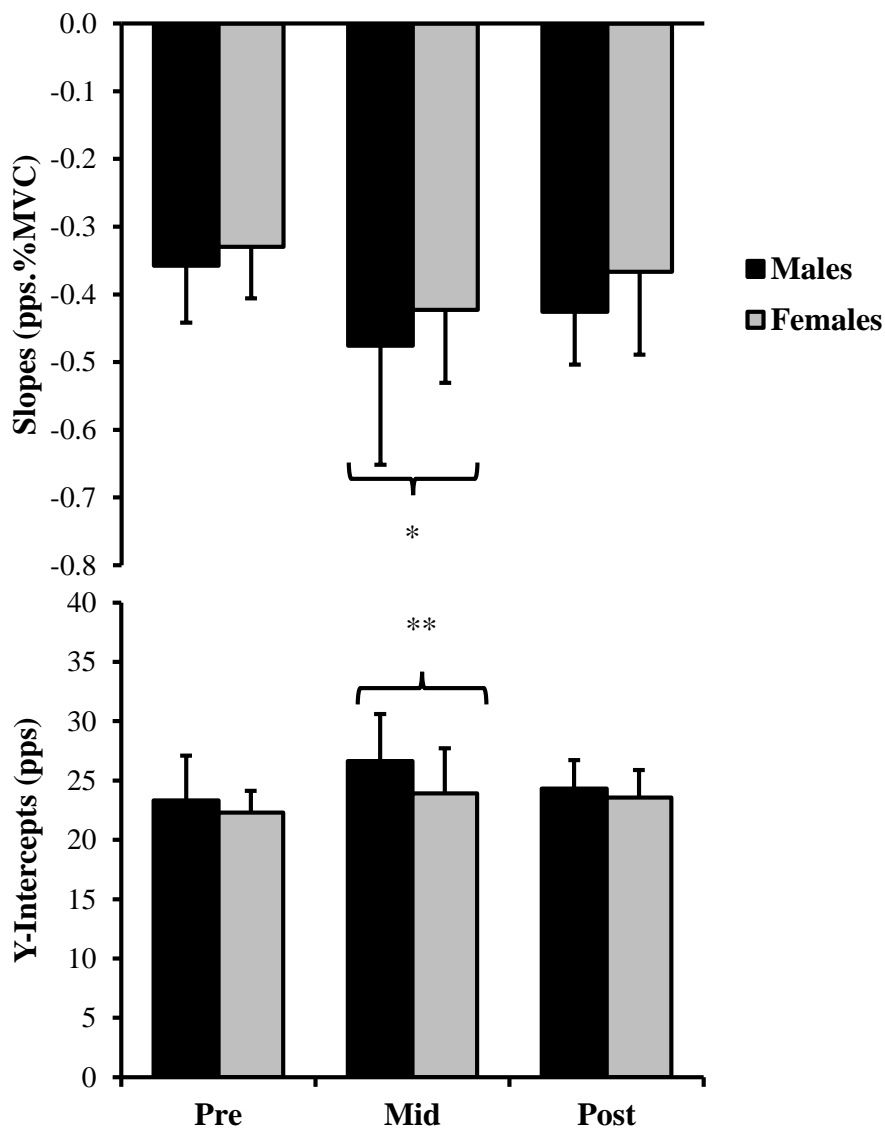


Figure 6. Plotted motor unit action potential duration ($MUAP_{DUR}$) (milliseconds [ms]) vs. recruitment threshold relationships (% maximal voluntary contraction [%MVC]) with linear regressions applied to the relationships for pre- (circles with black fill and black solid regression line), mid- (circles with grey fill and grey solid regression line) and post-training (triangles with white fill and black dashed regression line) with the coefficient of determination (R^2) displayed for each relationship.

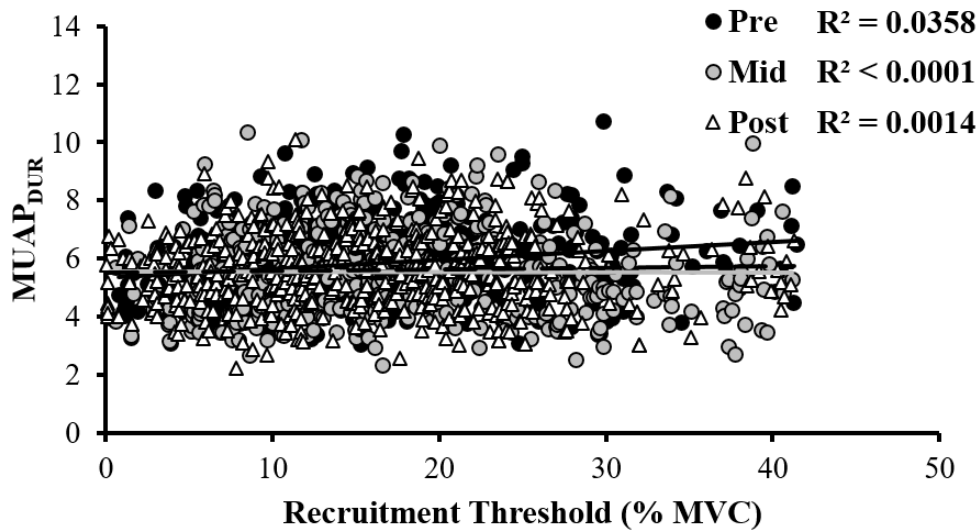
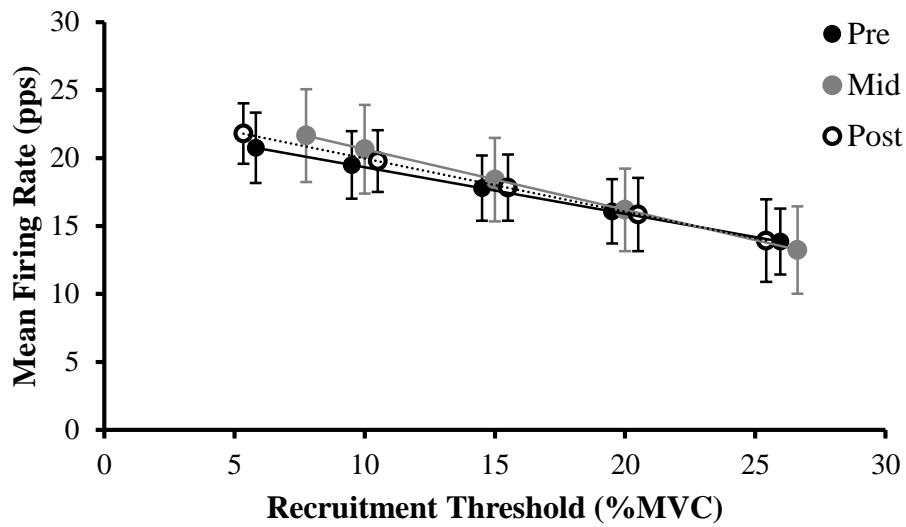


Figure 7. Plotted predicted mean firing rate (pulses per second [pps]) and standard deviations vs. recruitment threshold relationships (% maximal voluntary contraction [%MVC]) with linear regressions applied to the relationships for pre- (black markers with solid fill, black solid regression line), mid- (grey markers, grey regression line), and post-training (black markers with empty fill, black dashed regression line).



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THE EFFECTS OF CONTINUOUS CYCLING ON MOTOR UNIT FIRING RATES AND MUSCLE ACTIVATION DURING CONSECUTIVE CONTRACTIONS

ABSTRACT

This study investigated the effects of continuous cycling on motor unit (MU) behavior for the vastus lateralis (VL). Twenty-five sedentary participants completed 20 training sessions over 5 weeks. Pre- and post-training included maximal voluntary contractions (MVC) followed by two consecutive 40% relative to pre-training MVCs of the leg extensors with a 45 s steady torque segment. Surface electromyographic amplitude (EMG_{RMS}) was recorded from the VL and decomposed for the 40% MVCs to extract action potentials and firing events of single MUs. Recruitment thresholds (RT), mean firing rates (MFR), and MU action potential sizes ($MUAP_{SIZE}$) were determined for each MU with slopes and y-intercepts calculated for the MFR vs. RT and $MUAP_{SIZE}$ vs. RT relationships. EMG_{RMS} for the 40% MVC steady torque segment was normalized to MVC for the respective visit. MVC of the leg extensors significantly decreased for post-training ($P=0.005$) whereas normalized EMG_{RMS} was increased for post-training ($P<0.001$) and repetition two for pre- and post-training ($P=0.001$). For the MFR vs. RT relationships, the y-intercepts for post-training repetition two were less than post-training repetition one ($P=0.017$) and pre-training repetition two ($P=0.036$). In summary, continuous cycling decreased maximal strength for the leg extensors. The firing rates of the lowest-threshold MUs for repetition two of post-training were less than pre-training. In addition, the firing rates for the lowest-threshold MUs for repetition two of post-training were less than repetition one, unlike for pre-training. Furthermore, MUs exhibited a decrease in RT for the second repetition in

comparison to the first repetition post-training. Greater normalized EMG_{RMS} suggests increased muscle activation to match pre-training absolute torques. Five weeks of continuous cycling resulted in training and repetition dependent changes in MU firing rate and recruitment patterns for the VL.

INTRODUCTION

Numerous studies have investigated the presence of fatigue on motor unit (MU) behavior (24, 41, 61, 79-82). As fatigue increases, alterations in MU firing rate and recruitment patterns are observed to maintain a constant force (24, 79-81). It has been suggested these changes occur to compensate for changes in MU twitch forces (24, 79, 83-85). However, there have been discrepancies in the findings from previous studies. For example, during repeated or sustained submaximal contractions, the accumulation of fatigue has been associated with increases in the neural drive to the MU pool, resulting in MU firing rate increases and the recruitment of additional MUs to maintain a constant force (24, 61, 79, 85). Conversely, it has also been reported that MUs decrease their firing rates while additional MUs are recruited despite increases in excitation to the MU pool during fatigue (41, 86-89). It has been suggested these discrepancies are due to methodological issues, such as grouping MU data (41, 88, 89), low MU yields from intramuscular electromyographic (EMG) signals (86, 87, 89), and decreased MU recruitment thresholds (84, 88) during fatigue.

It is well understood that endurance training improves fatigue resistance due to adaptations such as decreased production (90) and increased clearance (90, 91) of metabolites during muscle contractions. However, few studies have investigated the effects of endurance training on firing rates of single MUs. Vila-Cha et al. (41) reported six weeks of cycle ergometer training resulted in an increase in (~ 30%) time to task failure for the leg extensors without alterations in overall MU firing rate behavior for the vastus lateralis during a 30% maximal voluntary contraction. Mettler and Griffin et al. (61) recently reported 4 weeks of endurance training for the adductor pollicis increased endurance time (~ 45%) without changes to the average MU firing rates during a fatigue task at 20% MVC. The overall MU firing rate patterns

reported by Vila Cha et al. (41) and Mettler and Griffin (61) included an initial decline in MU firing rates followed by an increase to values that were less than the onset of the contraction. Therefore, the pattern of MU firing rate changes occurred over a longer duration following endurance training (41, 61).

Despite the findings by Vila Cha et al. (41) and Mettler and Griffin (61), questions remain regarding endurance training and MU behavior. For example, the indwelling techniques utilized in these studies resulted in low MU counts (2.7 and 1.2) per contraction. Normalized EMG amplitude (EMG_{RMS}) and recruitment thresholds were also not reported and, thus, it is unknown if changes in muscle activation and firing rates relative to recruitment threshold occur as a result of endurance training. Therefore, the purpose of this investigation was to investigate the effects of 5 weeks of continuous cycling on MU behavior of the VL for previously sedentary individuals utilizing a surface EMG signal decomposition system (7, 8) that yields large MU counts to increase the sample size and resolution of the data. Pre- and post-training included the examination of the mean firing rate vs. recruitment threshold relationships and normalized EMG_{RMS} . In addition, during pre- and post-training participants performed consecutive submaximal muscle actions to provide insight on the effects of endurance training during a repeated fatigue protocol, which has yet to be investigated. Lastly, the motor unit action potential size vs. recruitment threshold relationships and the mean firing rate vs. motor unit action potential size relationships were also investigated to account for decreases in MU recruitment thresholds that have been reported during fatigue (81, 84, 88) and can introduce bias into the interpretation of the data (24).

METHODS

Subjects

Twenty-five healthy participants (mean \pm SD age = 20.44 ± 2.27 , height = 169.88 ± 8.62 cm, body mass = 69.09 ± 14.36) volunteered for this investigation. A subset of 5 subjects (age = 18.40 ± 0.89 yrs, height = 173.20 ± 7.19 , body mass = 68.18 ± 13.71 kg) served as controls for the experimental testing procedures but did not participate in the continuous cycling training protocol. None of the participants reported participation in any form of structured exercise program for the previous 3 years. None of the participants reported 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 and signed an informed consent form and completed a preexercise health status questionnaire.

Testing Time Line

Participants attended three laboratory sessions prior (visits 1 – 3) and after the completion (visits 4 – 6) of a 5 week continuous cycling training program for a total of six experimental testing visits. Participants performed 5 weeks of continuous cycling training at a frequency of 4 sessions per week for a total of 20 training sessions. Therefore, participants were required to make 26 total visits for this study. Visit one included the completion of a health history questionnaire and informed consent form and to become familiar with the isometric strength testing measurements, such as submaximal isometric trapezoidal muscle actions, and isometric maximal muscle actions. Visits 4 included a re-familiarization of isometric strength testing

measurements after 5 weeks of the continuous cycling training programs. Visits 2 and 5 included a maximal aerobic capacity (VO_{2MAX}) test on a cycle ergometer to determine cardiovascular fitness and maximal heart rate. The maximal heart rate values from visit 2 was used to calculate the target heart rates for the 5 week continuous cycling training program. Visits 3 and 6 included experimental isometric strength testing of the leg extensors.

VO_{2MAX} Testing

Testing was completed on an electronically-braked cycle ergometer (Lode, Groningen, Netherlands). Prior to any bike tests, participant seat height was measured and recorded for consistency among trials. Participants stood next to the bike to estimate proper seat height (greater trochanter), then examined while mounted on the bike to ensure there was a slight bend at the knee at the bottom of the pedal stroke, not full hyperextension. Once the seat height was set and comfortable, the foot straps were secured and were used to prevent the feet of the participants from slipping off the pedals during the test. After a two minute warm-up at 25 W, the workload was increased an additional 25 W every minute. Participants were encouraged to maintain 70 rpm, but the test was terminated when the participant could no longer maintain 60 rpm (volitional exhaustion). A true VO_{2MAX} was determined if participants meet three of the five indicators according to the American College of Sports Medicine Guidelines (92). During testing, heart rates were recorded with a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA) to determine maximal heart rate.

Respiratory gases were collected and monitored using a metabolic cart (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah). The metabolic cart was calibrated prior to each test with room air and standard gases of known volume and

concentration for the O_2 and CO_2 analyzers. Flowmeter calibration was performed prior to each graded exercise test. Respiratory gases were collected by use of a two-way rebreathing valve (Hans Rudolph Inc., Shawnee, Kansas) and mouthpiece attached to headgear, which held them in place. Participants wore a nose clip to ensure that breathing will occur entirely through the mouth. O_2 and CO_2 was analyzed through a sampling line after the gases are passed through a heated pneumotach and mixing chamber. The metabolic cart software reported the values as ventilated oxygen and carbon dioxide ($\dot{V}O_2$ and $\dot{V}CO_2$, respectively) and calculate $\dot{V}O_{2MAX}$ automatically.

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 visit 3 (pre-training) 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 both isometric strength testing visits. For all isometric trapezoid muscle

actions, the force increased at 10% MVC/s to the desired torque level, where it will be held during a 45 s plateau and then decreased to baseline at a rate of 10% MVC/s. Therefore, the duration of each contraction was 53 s and participants were given a 6 s rest interval between repetitions for the 3 s quiescent 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 are 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. (7) and Nawab et al. (8). 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.

(7). 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 (8). 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 (44, 48, 49). 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_{SIZE}, 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_{SIZE} and MUAP_{DUR}, respectively.

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, and EMG_{RMS} for each steady force segment of the plateau for the isometric muscle actions was normalized to EMG_{RMS} that corresponded to the highest 0.25 s peak force epoch during the

current visit MVC (% max). Offline processing was performed with custom-written LabVIEW version 16 software (National Instruments).

Continuous Cycling Training Protocol:

Participants performed 5 weeks of continuous cycling training on a Life Fitness Upright Bike (Model CLSC, Rosemont, IL, USA) at a frequency of 4 sessions per week for a total of 20 training sessions. Exercise intensity was prescribed based on the upper limits of heart rate reserve (HRR). Target heart rates (THR) were calculated with the Karvonen method [(maximal heart rate – resting heart rate) x %intensity + resting heart rate] (51). The use of % HRR has been recommended for prescribing exercise intensity in cycling activities (52) because it provides accurate target workloads for individuals with a low fitness level (53).

Weeks 1 – 3 consisted of 30 minutes of cycling at 70% of the HRR, whereas weeks 4 – 5 were 40 minutes at 75% HRR. Heart rates were monitored with a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA). All training sessions were supervised and subject heart rates were recorded every 3 minutes to ensure participants were maintaining the exercise intensity within the required THR. Training sessions took place in the Robinson Center on the University of Kansas, Lawrence campus.

Statistical Analysis

For the MU data, linear regressions were performed on the MFR vs. RT, MUAP_{SIZE} vs. RT, and MUAP_{DUR} vs. RT relationships, whereas, exponential regressions were performed on the MFR vs. MUAP_{SIZE} relationships (24). Slope and y-intercept values were calculated for each linear relationship, while A and B terms were calculated for the exponential relationship. Two

separate paired samples *t*-tests (time [pre- vs. post-training]) were used to examine differences in VO_{2MAX} and MVC. Nine separate two-way repeated measures ANOVAs (visit [pre- vs. post-training] x repetition [one vs. two]) were used to examine differences in the normalized EMG_{RMS} , the A and B terms for the MFR vs. $MUAP_{SIZE}$ relationships, and among the slopes and y-intercepts for the MFR vs. RT, $MUAP_{SIZE}$ vs. RT, and $MUAP_{DUR}$ vs RT relationships. For the control subjects, similar statistical procedures were performed. 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 22 (IBM Corp., Armonk, New York).

RESULTS

Control Participants

Results (mean \pm SD) for the control participants are presented in table 1. Paired samples *t*-tests indicated no differences in body mass, MVC, and VO_{2MAX} (P = 0.618 – 0.740). In addition, there were no significant two-way interactions (P = 0.204 – 0.998; visit x repetition) or main effects (P = 0.083 – 0.728; visit and repetition) for normalized EMG_{RMS}, the slopes and the y-intercepts for the MFR vs. RT relationships, and A and B terms for the MFR vs. MUAP_{SIZE} relationships.

For the MUAP_{SIZE} vs. RT relationships, there was no significant two-way interaction (visit x repetition; P = 0.099) or main effect for visit (P = 0.407). There was, however, a main effect for repetition. The slopes for repetition two (0.00273 \pm 0.00055 mV/%MVC) were significantly greater (P = 0.013) in comparison to repetition one (0.00201 \pm 0.00050 mV/%MVC) when collapsed across visit.

Experimental Participants

VO_{2MAX} and MVC Strength

A paired samples *t*-test indicated VO_{2MAX} significantly increased (P < 0.001) following 5 weeks of continuous cycling (Pre = 2.622 \pm 0.819 L/min, Post = 2.798 \pm 0.806 L/min) (Figure 1). A paired samples *t*-test indicated maximal strength was significantly less (P = 0.005) following 5 weeks of continuous cycling (Pre = 173.45 \pm 51.64 Nm, Post = 156.92 \pm 55.37 Nm) (Figure 2).

Normalized EMG_{RMS}

The analysis indicated no significant two-way interaction (visit x repetition, $P = 0.786$). However, there were main effects for visit ($P < 0.001$) and repetition ($P = 0.001$). Normalized EMG_{RMS} was greater for post- ($49.45 \pm 16.54\%$) in comparison to pre-training ($38.16 \pm 10.77\%$) when collapsed across repetition. Normalized EMG_{RMS} was greater for repetition two ($45.56 \pm 13.35\%$) in comparison to repetition one ($42.06 \pm 11.99\%$) when collapsed across visit (Figure 3).

MU Characteristics

Decomposition of surface EMG signals yielded a total of 3705 MUs, with 1863 and 1842 MUs recorded for visits 1 and 2. The average number of MUs recorded for each subject per contraction was 37.05 ± 10.75 and the observed RT ranges (%MVC) were $11.94 \pm 7.71 - 39.40 \pm 5.28$ (visit 1) and $8.72 \pm 6.07 - 36.90 \pm 8.21$ (visit 2).

MFR vs. RT Relationships

For all contractions, the MFR vs. RT ($r = -0.94 \pm 0.04$) relationships were significant. For the slopes, the analysis indicated no significant two-way interaction (visit x repetition, $P = 0.441$) or main effect for visit ($P = 0.606$). However, there was a main effect for repetition ($P = 0.013$). The slopes for repetition one (-0.319 ± 0.129 pps/%MVC) were significantly less negative in comparison to repetition two (-0.356 ± 0.129 pps/%MVC) when collapsed across visit.

For the y-intercepts, the analysis indicated a significant two-way interaction (visit x repetition, $P = 0.042$). The y-intercepts for pre-training repetition two (26.06 ± 6.00 pps) were significantly greater ($P = 0.036$) in comparison to post-training repetition two (24.40 ± 4.46 pps).

The y-intercepts for post-training repetition one (25.36 ± 4.84 pps) were significantly greater ($P = 0.017$) in comparison to post-training repetition two (Figure 4).

MUAP_{SIZE} vs. RT Relationships

For the MUAP_{SIZE} vs. RT relationships, 99 of the 100 individual relationships were significant ($r = 0.746 \pm 0.14$). For the slopes, the analysis indicated no significant two-way interaction (visit x repetition, $P = 0.430$) or main effects for visit ($P = 0.953$) and repetition ($P = 0.144$). For the y-intercepts, the analysis indicated no significant two-way interaction (visit x repetition, $P = 0.706$), or main effects for visit ($P = 0.107$) and repetition ($P = 0.719$) (Figure 5).

MFR vs. MUAP_{SIZE} Relationships

For the MFR vs. MUAP_{SIZE} relationships, 99 of the 100 relationships were significant ($r = 0.81 \pm 0.10$). For the A terms, the analysis indicated a significant two-way interaction (visit x repetition, $P = 0.044$). The A terms for the pre-training repetition one (22.91 ± 2.59 pps) were significantly less ($P = 0.012$) in comparison to the post-training repetition one (24.43 ± 2.73 pps). The A terms for post-training repetition one were significantly greater ($P = 0.015$) in comparison to post-training repetition two (23.44 ± 2.54 pps). For the B terms, the analysis indicated no significant two-way interaction (visit x repetition, $P = 0.569$) or main effects for visit ($P = 0.898$) and repetition ($P = 0.519$) (Figure 6).

MUAP_{DUR} vs. RT Relationships

Fifty-eight of the 100 individual relationships were significant (y-intercepts = 4.20 ± 1.05 ms, slopes = 0.058 ± 0.040 ms/%MVC). However, only 6 of the 25 participants had significant

relationships all repetitions and visits and, thus, an ANOVA model could not be performed. The positive slopes may suggest the depth of the recorded MUs might have slightly increased with increments in RT for a few of the subjects (Figure 7). Of importance, there does not appear to be differences in pre- and post-training and, therefore, recorded MUs were from similar depths and is not a confounding factor.

DISCUSSION

As anticipated, 5 weeks of continuous cycling training significantly increased maximal aerobic capacity. Significant findings as a result of the continuous cycling training included the following: (1) decreases in maximal strength of the leg extensors, (2) time and repetition dependent decreases in firing rates of the lowest-threshold MUs for post-training, (3) time dependent increases in firing rates relative to MU size for post-training, and (4) increases in muscle activation to match pre-training absolute torques.

The results from the current study indicated VO_{2MAX} was significantly increased following 5 weeks of continuous cycling. These findings are in agreement with Hoppeller et al. (54) and Howald et al. (29) that reported increases in VO_{2MAX} for males and females following 6 weeks of cycle ergometer training. In contrast to VO_{2MAX} , participants for the current study exhibited a significant decrease in MVC for the leg extensors. It has been suggested that increases in type I fiber type proportions resulting from aerobic training (29, 55, 67) negatively affect strength capabilities (93). Howald et al (29) reported an increase of 12%, decreases of 8% (not significant), and 24% in the percentage of type I, IIA, and IIX fibers following 6 weeks of continuous cycling. Thus, it is plausible the reductions in MVC for the current study post-training may be a result of shifts towards a greater percentage of type I fiber composition.

Numerous researchers have investigated individual MU firing rate behavior during muscle fatigue (24, 41, 61, 79-86, 88, 89), however, few studies have longitudinally examined the effect of an endurance training protocol on MU behavior. Vila-Cha et al. (41) reported 6 weeks of cycle ergometer training increased time to task failure for the leg extensors during a 30% MVC. However, there were no training-related effects on MVC or MU firing rates, such as,

there were lower firing rates of the VL and vastus medialis during a time period of 60 – 70 s compared to the first 10 s for pre- and post-training. Mettler and Griffin (61) reported 4 weeks of muscular endurance training for the abductor pollicis improved endurance time for a 20% MVC. The authors reported an initial decrease in MU firing rates followed by an increase later during the contraction and similar to Vila-Cha et al. (41), there were no training-related changes. Therefore, the participants for these studies (41, 61) were able to maintain force for a longer duration without training alterations in MU firing rate behavior.

For the current study, alterations in MU firing rates and likely recruitment patterns as a result of continuous cycling training were repetition and time dependent. For example, the firing rates of the lowest-threshold MUs for the second repetition at post-training were significantly less in comparison to the first repetition at post-training. These MU firing rate patterns are in agreement with the findings of Vila-Cha et al. (41). The decrease in the firing rates of the lowest-threshold MUs for the second repetition was also associated with an increase in normalized EMGRMS, indicating an increase in muscle activation (62-64). Therefore, it is likely a greater amount of higher-threshold MUs were recruited to achieve and maintain the targeted force during repetition two. The recruitment of additional higher-threshold MUs, which possess lower firing rates, to match the same absolute torques would result in the slopes for the MFR vs. RT becoming more negative, which was supported in the current study.

The firing rates for the lowest-threshold MUs for the second repetition post-training were also significantly less compared to the second repetition at pre-training. These findings may be a result of the decreases in maximal strength. Therefore, the targeted torques for post-training were at a greater relative % MVC compared to pre-training. It has been reported that Group III and IV small diameter afferents are activated with increases in metabolite production (94, 95) leading to

cortical motoneurone inhibition (96) and decreases in firing rates (97). Since firing rates of the lowest-threshold MUs were decreased and normalized EMG_{RMS} was elevated for the second repetition post- (50.49 %) compared to pre-training (40.10 %), additional higher-threshold MUs were likely recruited to a greater degree to match the targeted torque level during the 45 second plateau for repetition one and the linearly increasing segment and plateau for repetition two. It would be expected that higher-threshold MUs would possess a greater percentage of fast-twitch type characteristics, such as, greater amounts of glycolytic and lower amounts of aerobic enzymes (98, 99). Thus, increased recruitment and sustained activation of higher-threshold MUs would result in a greater accumulation of metabolites that can activate Group III and IV afferents and, subsequently, reduce firing rates (100) for the lowest-threshold MUs.

The $MUAP_{SIZE}$ vs. RT relationship can provide insight in MU recruitment patterns. For example, Contessa et al. (24) reported that larger MUs were being recruited to achieve the same absolute targeted force during repetitive long duration contractions at 30% MVC for the leg extensors (not statistically tested). For the current study, there were no effects for repetition and training on the size of $MUAP_{SIZES}$ for the lowest- (y-intercepts) and highest-threshold (slopes for the $MUAP_{SIZE}$ vs. RT relationships) MUs. However, a closer examination suggests RTs of MU decreased for the second repetition in comparison to the first repetition post-training (Figure 8), which has been reported during fatiguing contractions (24, 81, 84, 88, 101, 102). This would support greater recruitment of larger higher-threshold MUs was necessary to achieve the targeted absolute torques due to a decrease in the overall contribution of force (reduced MU force twitches and firing rates) from the lower-threshold MUs and the accumulation of metabolites associated with fatigue. In support, larger $MUAP_{SIZES}$ were observed for the second repetition for post-training (Figure 9).

The MFR vs. MUAP_{SIZE} relationships were also investigated to account for possible decreases in RT during the repetitive muscle actions, which may induce bias in the interpretation of MU firing rates (24). The greater A terms for the first repetition at post-training compared to the first repetition pre-training indicated that the smallest-sized MUs had greater firing rates after the continuous cycling protocol. This is likely a result of decreases in strength as fatigue would not be a factor for the first repetition of either testing visit. It is well understood that the level of relative excitation to the MU pool is the main determinant of the number of recruited MUs and their firing rates. In addition, greater firing rates of the lowest-threshold MUs, which are smallest in size (4, 6, 10, 11), have been reported during increments in targeted forces (44, 103). Therefore, the increase in the firing rates of the smallest sized MUs can be explained by the decrease in strength, which required greater muscle activation at post- compared to pre-training to achieve and maintain the targeted torques. The A terms for the first repetition for post-training were greater than the second repetition. Thus, continuous cycling resulted in the smallest MUs having reduced MU firing rates for the second repetition despite greater overall muscle activation. This may be due to the activation of small diameter afferents by the accumulating metabolites, as discussed earlier. These findings are in agreement with Carpentier et al. (81), who reported increases in muscle activation (EMG_{RMS}) and significant decreases in firing rates for lower-threshold MUs, whereas firing rates of higher-threshold MUs were not different across trapezoidal contractions at 50% MVC.

For the current investigation, participants completed two repetitive, long duration contractions at 40% MVC relative to pre-training before and after 5 weeks of continuous cycling. In summary, 5 weeks of training resulted in decreased maximal strength for the leg extensors and, subsequently, increases in muscle activation to match pre-training absolute torque levels.

For post-training, there were increases in the firing rates of the lowest-threshold MUs for the first repetition compared to pre-training, which suggests twitch forces were smaller post-training. For the second repetition post-training, there was a decrease in the firing rates of the lowest-threshold MUs compared to repetition one. This was likely due to the inhibition of motoneurons via the activation of small diameter afferents, which resulted in the recruitment of additional higher-threshold MUs to achieve and maintain targeted torque levels.

GRANTS

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Table 1. Mean \pm SD for body mass (kilograms [kg]), maximal aerobic capacity (Liters/minute [L/min]), leg extensor maximal voluntary contraction (MVC; Newton meters [Nm]), and the slopes and y-intercepts for the mean firing rate (MFR; pulses per second [pps]) vs. recruitment threshold (RT; expressed as %MVC), motor unit action potential size (MUAP_{SIZE}; millivolts [mV]) vs. RT, MFR vs. MUAP_{SIZE} relationships, electromyographic amplitude (EMG_{RMS}) normalized to MVC EMG (%), from repetition (rep) one and two for the pre- and post-testing 40% MVC.

Measurement		Pre-Testing	Post-Testing		
Body Mass (kg)		68.18 \pm 13.71	68.60 \pm 13.33		
Maximal Aerobic Capacity (L/min)		2.33 \pm 0.99	2.36 \pm 0.91		
Leg Extensor MVC (Nm)		157.51 \pm 47.66	159.04 \pm 41.82		
		Rep 1	Rep 2	Rep 1	Rep 2
MFR vs. RT	Slopes (pps/%MVC)	-0.314 \pm 0.067	-0.338 \pm 0.094	-0.330 \pm 0.205	-0.428 \pm 0.202
	Y-Int (pps)	25.46 \pm 3.33	26.01 \pm 3.30	28.26 \pm 8.98	28.81 \pm 6.00
MUAP _{SIZE} vs. RT	Slopes (pps/%MVC)	0.00213 \pm 0.00102	0.00217 \pm 0.00045*	0.00189 \pm 0.00079	0.00329 \pm 0.00091*
	Y-Int (pps)	0.0142 \pm 0.0183	0.0159 \pm 0.0141	0.0167 \pm 0.0212	0.00276 \pm 0.0250
MFR vs. MUAP _{SIZE}	A Terms (pps)	24.027 \pm 3.039	24.991 \pm 2.511	25.965 \pm 2.515	28.097 \pm 8.904
	B Terms (pps/mV)	-5.223 \pm 1.165	-5.696 \pm 1.000	-5.111 \pm 1.501	-4.979 \pm 1.922
Normalized EMG _{RMS} (%)		41.85 \pm 5.48	44.80 \pm 4.68	45.96 \pm 9.18	55.80 \pm 14.49

* Indicates the slopes for rep 2 were significantly greater for post- in comparison to pre-testing when collapsed across visit.

Figure 1. The mean (\pm SD) absolute maximal aerobic capacity (VO_{2MAX}) (L/min) from pre- and post-training. * VO_{2MAX} was significantly greater ($P < 0.001$) at post- (2.798 ± 0.801 L/min) in comparison to pre-training (2.622 ± 0.819 L/min).

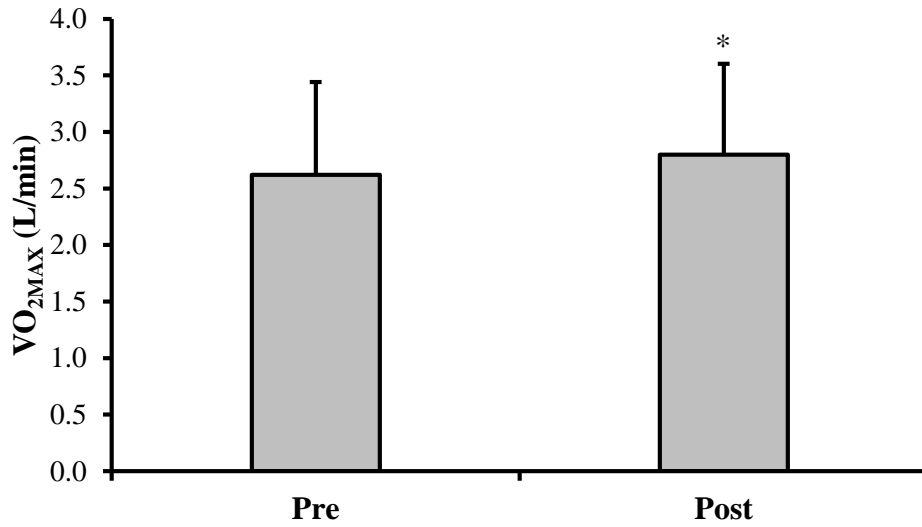


Figure 2. The mean (\pm SD) maximal voluntary contraction for pre- and post-training maximal strength. * Maximal strength was less ($P = 0.005$) for post- (165.059 ± 64.777 Nm) in comparison to pre-training (173.494 ± 64.327 Nm).

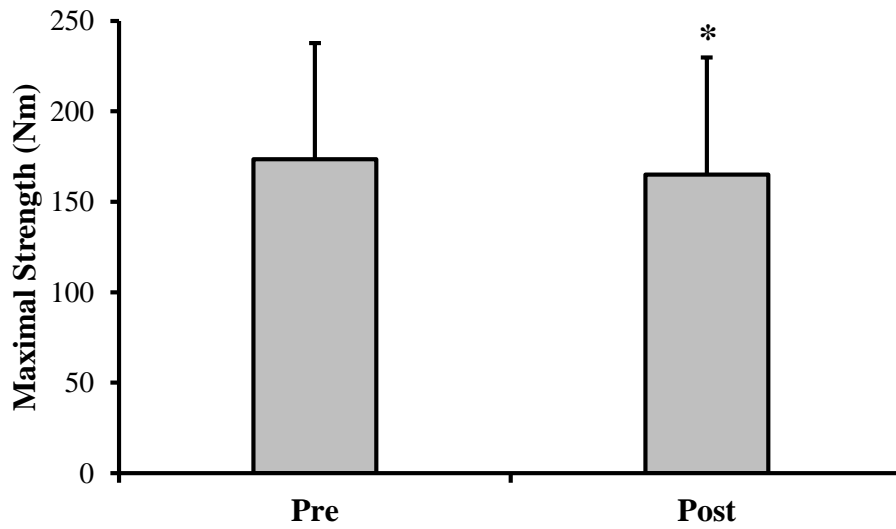


Figure 3. The mean (\pm SD) normalized (% max) electromyographic amplitude (EMG_{RMS}) for the first and second repetition (rep) for pre- and post-training for the 40% MVC. # Normalized EMG_{RMS} was greater for rep two (45.56 ± 13.35 % max) in comparison to rep one (42.06 ± 11.99 % max) when collapsed across visit. * Normalized EMG_{RMS} was greater for post- (49.45 ± 16.54 % max) in comparison to pre-training (38.16 ± 10.77 % max) when collapsed across rep.

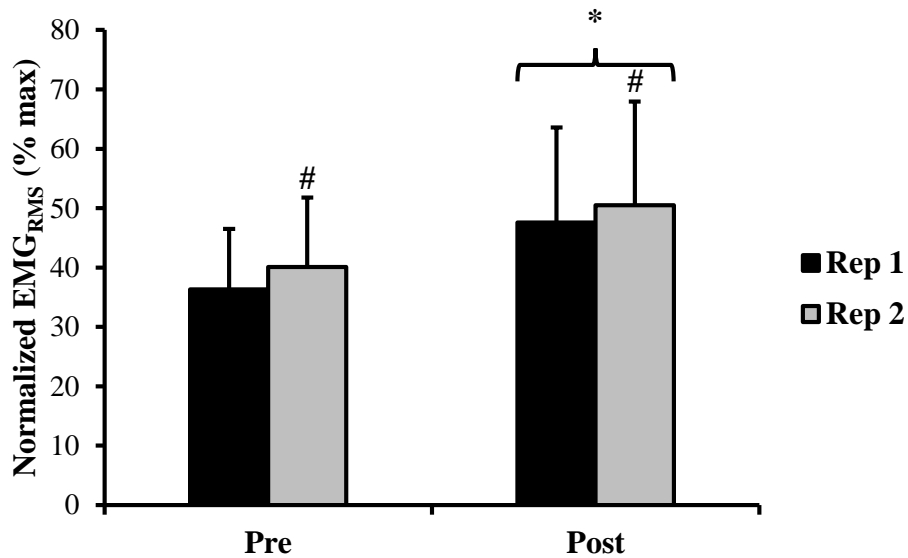


Figure 4. The mean (\pm SD) slope (top graph) and y-intercept (bottom graph) values from the mean firing rate (pulses per second [pps]) vs. recruitment threshold (expressed as percent of maximal voluntary contraction (%MVC) relationship for the first and second repetition (rep) for pre- and post-training for the 40% MVC. # The slopes were significantly greater ($P = 0.013$) for rep one in comparison to rep two when collapsed across visit. * The y-intercepts were significantly greater ($P = 0.036$) for the pre- rep two in comparison to post-training rep two. ** The y-intercepts were significantly greater ($P = 0.017$) for the post- rep one in comparison to the post-training rep two.

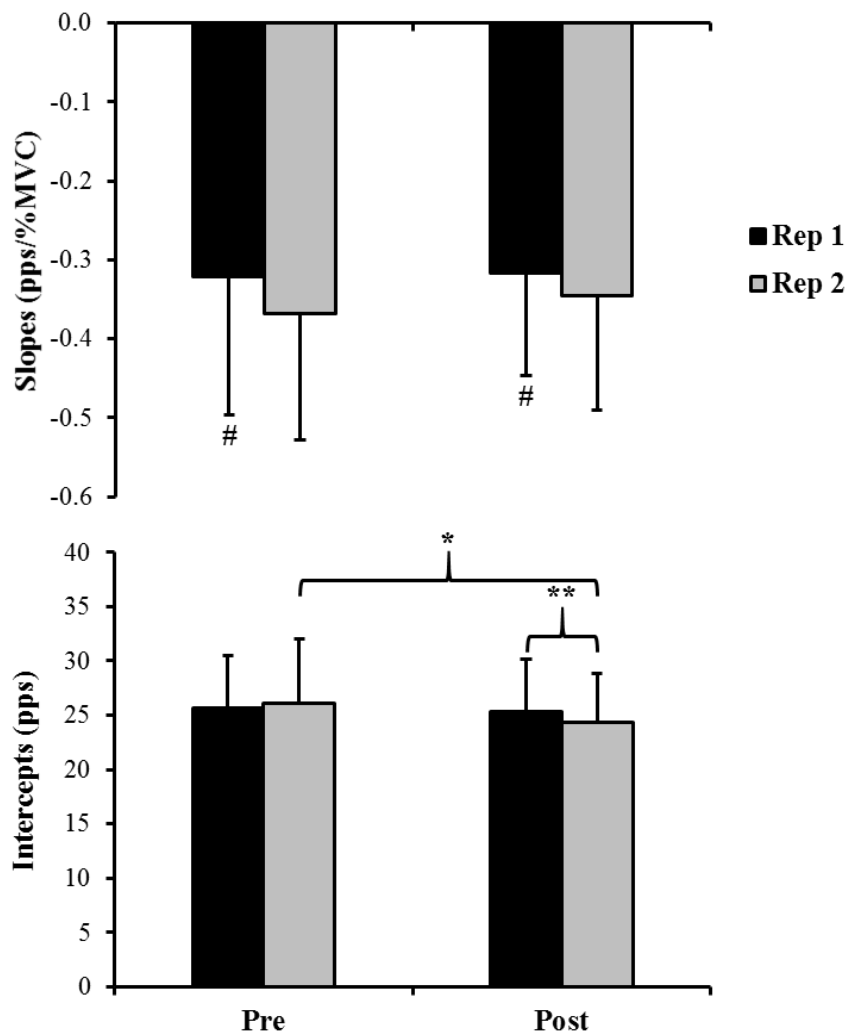


Figure 5. The mean (\pm SD) slope (top graph) and y-intercept (bottom graph) values from the motor unit action potential size vs. recruitment threshold (expressed as percent of maximal voluntary contraction (%MVC) relationship for the first and second repetition (rep) for pre- and post-training for the 40% MVC.

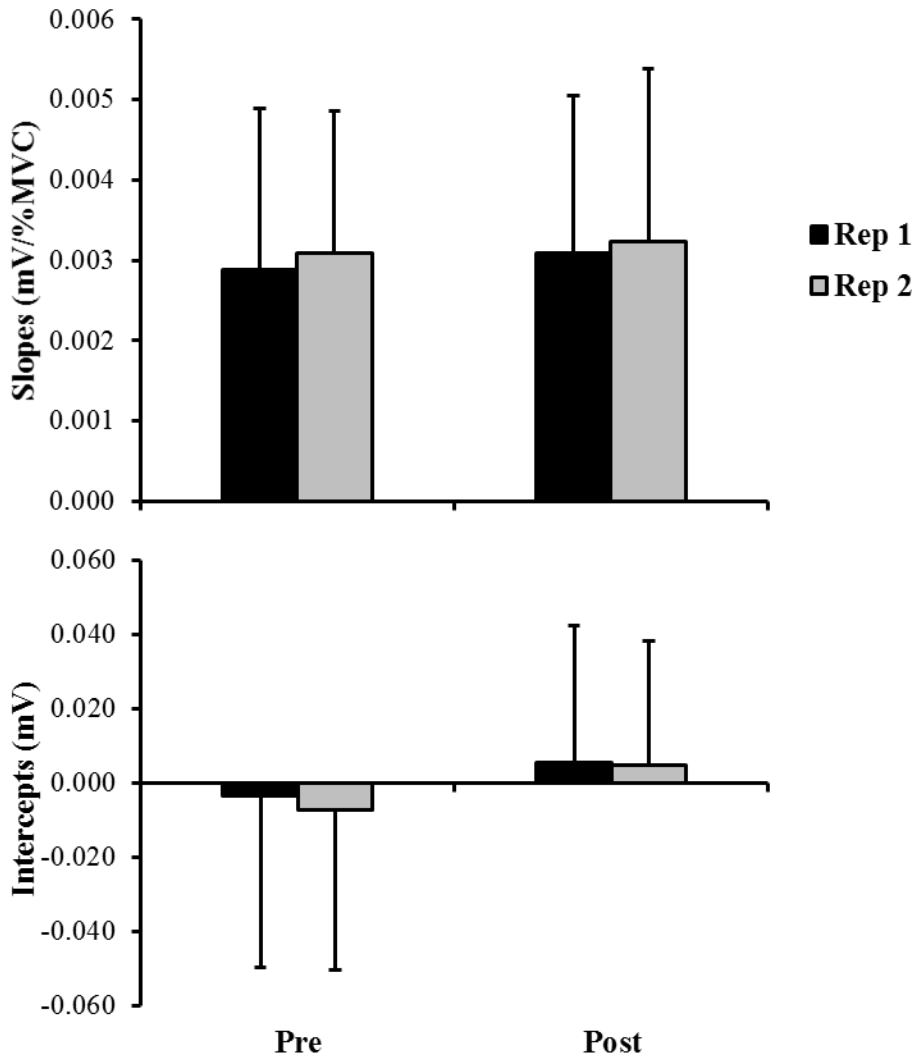


Figure 6. The mean (\pm SD) B term (top graph) and A Term (bottom graph) values from the mean firing rate (pulses per second [pps]) vs. motor unit action potential size (mV) relationships for the first and second repetition (rep) for pre- and post-training for the 40% MVC. * The A terms pre-rep one (22.91 ± 2.59 pps) were significantly less ($P = 0.012$) in comparison to the post-training rep one (24.43 ± 2.73 pps). ** The A terms for the post-rep one were significantly greater ($P = 0.015$) in comparison to the post-training rep two (23.44 ± 2.54 pps).

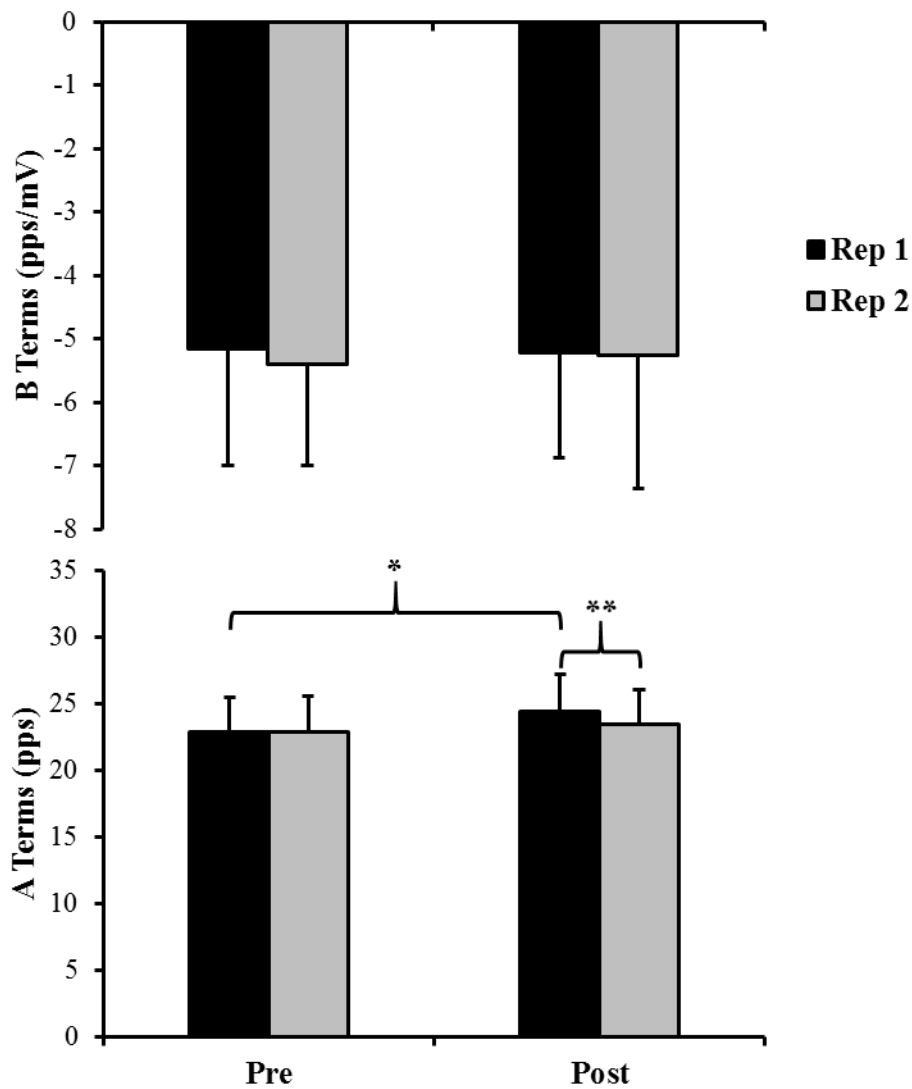


Figure 7. Plotted motor unit action potential (MUAP) duration (milliseconds [ms]) vs. recruitment threshold relationships (% maximal voluntary contraction [%MVC]) with linear regressions applied to the relationships for pre- (black markers and black solid regression lines) and post-training (grey markers and grey dashed regression lines) for repetition (rep) one (circular markers) and rep two (triangular markers) with the coefficient of determination (R^2) displayed for each relationship.

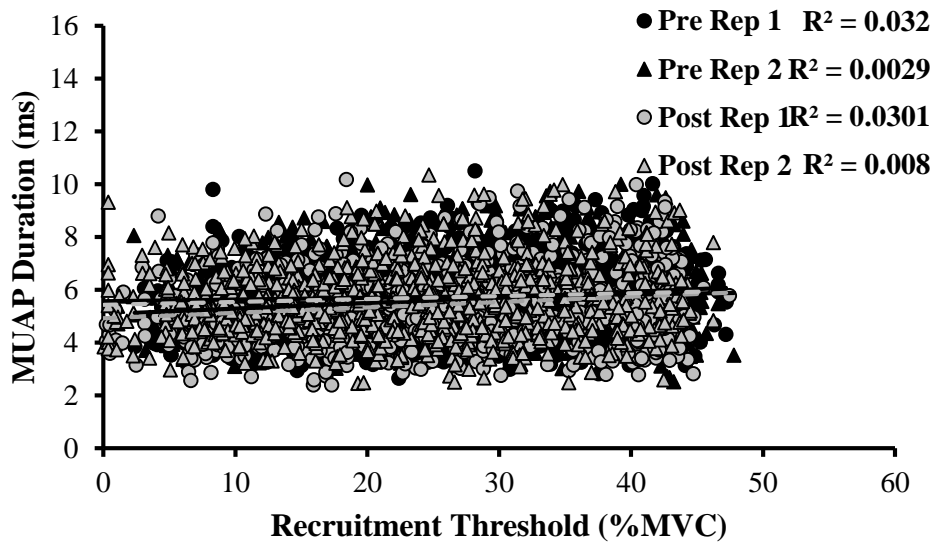


Figure 8. The mean (\pm SD) predicted motor unit action potential (MUAP) (millivolts [mV]) sizes from the $MUAP_{SIZE}$ vs. recruitment threshold (% maximal voluntary contraction [%MVC]) for the first repetition (rep) for pre- (black fill), the second rep for pre- (grey fill), the first rep for post- (grey fill), and the second rep for post-training (black pattern fill). The observed recruitment range across contractions was $10.33 \pm 2.00 - 38.15 \pm 1.51$ %MVC and, therefore, 0 – 10% MVC increments were not included in the figure.

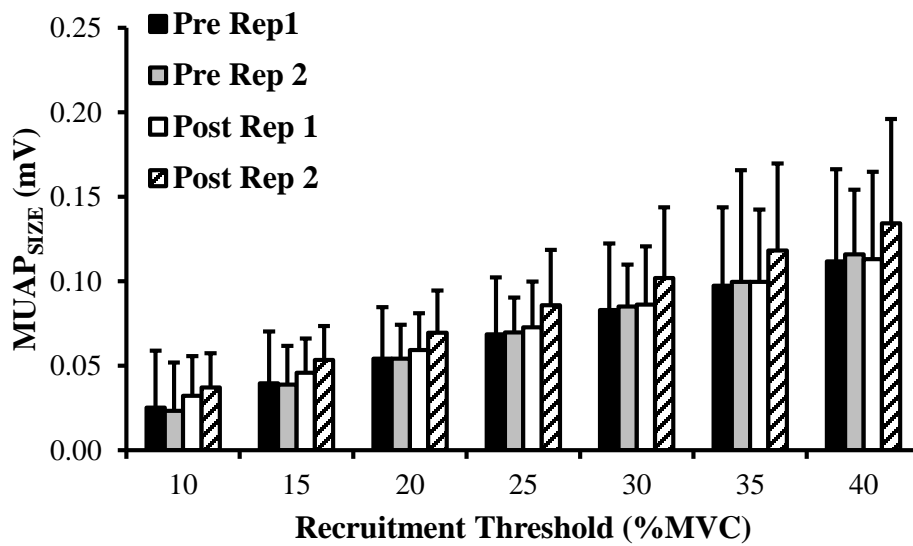
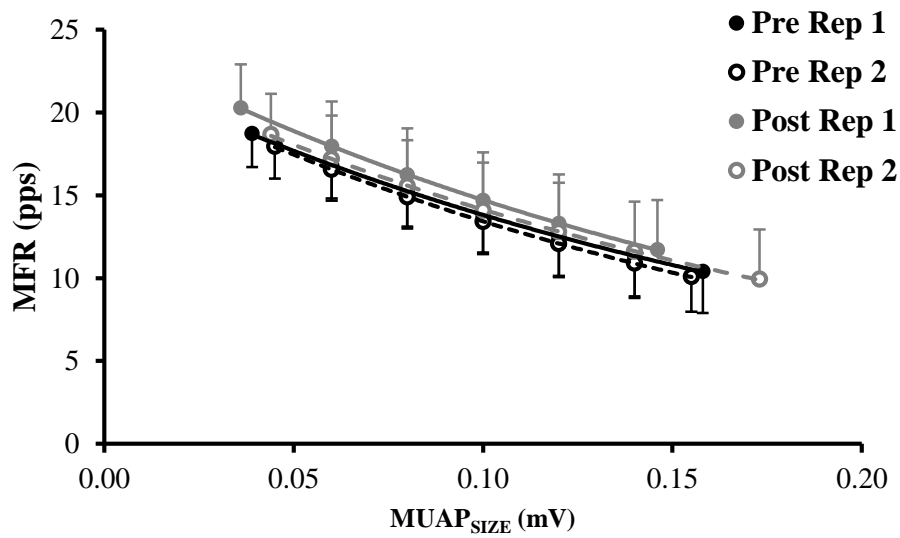


Figure 9. Plotted predicted mean firing rate (MFR; pulses per second [pps]) and standard deviations vs. motor unit action potential (MUAP) (millivolts [mV]) sizes with linear regressions applied to the relationships from the first repetition (rep) for pre- (black markers, solid fill; solid black regression line), the second rep for pre- (black markers, empty fill, dashed black regression line), the first rep for post- (grey markers, solid fill; solid grey regression line), and the second rep for post-training (grey marker, empty fill; dashed grey regression line).



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