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ORIGINAL ARTICLE

Endurance training alters motor unit activation strategies for the vastus lateralis, yet sex-related differences and relationships with muscle size remain

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Abstract

Purpose To examine the effects of 10 weeks of endurance cycling training on mechanomyographic amplitude (MMG_{RMS})–torque relationships and muscle cross-sectional area (mCSA) of the vastus lateralis (VL) for 10 sedentary males (Age ± SD; 20.2 ± 1.9 years) and 14 sedentary females (21.9 ± 5.3 years).

Methods Participants performed maximal voluntary contractions (MVCs) and an isometric ramp up muscle action to 70% MVC of the knee extensors before (PRE) and after training at the same absolute pre-treatment submaximal torque ($POST_{ABS}$). MMG was recorded from the VL and *b* terms were calculated from the natural log-transformed MMG_{RMS}–torque relationships for each subject. mCSA was determined with ultrasonography.

Results Cycling decreased MVCs from pre- (168.10 ± 58.49 Nm) to post-training (160.78 ± 58.39 Nm; $p = 0.005$) without changes in mCSA. The *b* terms were greater for $POST_{ABS}$ (0.623 ± 0.204) than PRE (0.540 ± 0.226; $p = 0.012$) and for males (0.717 ± 0.171) than females (0.484 ± 0.168; $p = 0.003$). mCSA was correlated with the *b* terms for PRE ($p < 0.001$, $r = 0.674$) and $POST_{ABS}$ ($p = 0.020$, $r = 0.471$).

Conclusion The decrease in MVC and increase in MMG_{RMS} (*b* terms) post-training suggests increased motor unit (MU) recruitment to match pre-training torques. The greater acceleration in the *b* terms by males may reflect sex-related differences in fiber-type area. MMG_{RMS}–torque relationships during a high-intensity contraction provided insight on MU activation strategies following endurance training and between sexes. Furthermore, the findings suggest a relationship between MMG_{RMS} and muscle size.

Keywords Endurance training · Log-transform model · Mechanomyography · Motor unit activation strategies · Vastus lateralis

Abbreviations

ANOVA	Analysis of variance
HRR	Heart rate reserve
mCSA	Muscle cross sectional area

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MHC	Myosin heavy chain
MMG_{RMS}	Mechanomyographic amplitude
MVC	Maximal voluntary contraction
MU	Motor unit
$POST_{ABS}$	Post absolute torque level
sFAT	Subcutaneous fat
VL	Vastus lateralis
VO_{2MAX}	Maximal aerobic capacity

Introduction

It is well understood that short-term and chronic training elicit-specific adaptations relative to exercise mode (Kraemer et al. 1995). Although numerous studies have investigated the effects of endurance training on aerobic capacity, skeletal muscle structure and fiber type of the vastus lateralis (VL), few have longitudinally examined the effects of endurance training on neuromuscular adaptations despite differences in motor unit (MU) control strategies among chronic training statuses (De Luca et al. 1982; Herda et al. 2010; Trevino and Herda 2016). It is hypothesized that these differences are partially due to training induced changes in the physical properties (% myosin heavy chain [MHC] expression) of the MU pool (De Luca et al. 1982). Research elucidating the effects of endurance training on neuromuscular adaptations would be beneficial for health fitness professionals, coaches, and clinicians, and could result in improved exercise programming.

Recent studies investigating the effects of short-term (2–6 weeks) endurance training on neuromuscular behavior for the VL have reported mixed results, such as decreases (Vila-Chã et al. 2010) or no change (Martinez-Valdes et al. 2017; Vila-Cha et al. 2012) in MU firing rates. However, the training programs were primarily low-intensity (Vila-Cha et al. 2012; Vila-Chã et al. 2010), or short duration (2 weeks) (Martinez-Valdes et al. 2017). These studies also largely examined low-intensity contractions at a steady force (10–30% maximal voluntary contraction [MVC]) due to MU signal processing requirements (Vila-Cha et al. 2012; Vila-Chã et al. 2010). Thus, the effects of a high-intensity continuous cycling program on MU activation strategies to a high targeted torque remain unclear.

Surface mechanomyography (MMG) is defined as the recording of low-frequency lateral oscillations of muscle fibers during a muscle action (Orizio 1993). MMG is described as the mechanical counterpart to neural activation and the amplitude of the signal can be influenced by active stiffness modulated by MU recruitment and the firing rates of the active MUs (Coburn et al. 2005). For example, during linearly increasing muscle actions, it is suggested that MMG amplitude (MMG_{RMS}) increases due to rapid MU recruitment. However, the amplitude of the MMG signal will plateau, or even decrease, when force/torque is primarily being modulated by MU firing rates to due to a mechanical fusion of MU twitches (Orizio 1993; Orizio et al. 1996). MMG_{RMS}–torque relationships may provide a unique depiction of MU recruitment and firing rates and have previously differentiated MU control strategies as a function of chronic training status and MHC expression for the VL. For example, Herda et al. (2010) and Trevino and Herda (2015) reported MMG_{RMS} plateaued earlier for chronic aerobically compared to chronic resistance-trained and sedentary individuals during high-intensity linearly increasing isometric muscle actions up to 50% and 90% MVC and suggested the greater amounts of type I% MHC expression for the aerobically trained (Herda et al. 2010) may have resulted in the earlier reliance on rate coding to modulate force or greater overall firing rates (Herda et al. 2015). In addition, the influence of the physical properties on the mechanical behavior of muscle was further supported as individuals exhibiting greater type I% MHC expression displayed lower slope values ($r = -0.804$) for MMG_{RMS}–torque relationships during a moderate intensity (40% MVC) linearly increasing muscle action (Trevino et al. 2016a). Thus, it is plausible MMG_{RMS}–torque relationships may be able to track alterations in MU behavior due to endurance training and its effect on MHC expression. However, this is yet to be investigated.

Recently, sex-related differences in muscle cross-sectional area (mCSA), type II %MHC expression, and the relative increase in MU size for the VL were reported between sedentary, college-aged males, and females (Trevino et al. 2019). It was suggested males displayed greater values for all variables due to larger type II fiber diameters as muscle fiber number (Miller et al. 1993; Schantz et al. 1983, 1981) and fiber-type distribution (Staron et al. 2000) does not differ between sexes. Consequently, it is possible that males would display greater acceleration in MMG_{RMS}–torque relationships during a linearly increasing muscle action due to larger amounts of type II %MHC expression (Trevino et al. 2019; Staron et al. 2000). Although sex-related differences in MU activation strategies have been reported for the biceps brachii (Nonaka et al. 2006), this has yet to be investigated for the VL of young adults. In addition, mCSA and total type II %MHC expression were correlated ($r = 0.617–0.836$) with the relative size of higher in comparison to lower threshold MUs for college-aged males and females and it was suggested the larger MU sizes were due to greater diameters for fibers that primarily expressed type II characteristics (Trevino et al. 2019). Therefore, it is plausible that individuals with larger mCSA would possess muscles composed of greater type II fiber diameters, which may suggest a relationship between muscle size and the mechanical behavior of muscle. However, no study has correlated MMG_{RMS} recorded during a linearly increasing muscle action with mCSA for the VL.

Due to the relatively large amount of variability reported among individuals for MMG_{RMS}–torque relationships during linearly increasing muscle actions (Ryan et al. 2008), it has been suggested these relationships should be examined on a subject-by-subject basis (Farina et al. 2004; Orizio et al. 1989; Zwarts and Keidel 1991) as polynomial regression and ANOVA model analyses of composite MMG_{RMS} values at distinct %MVC levels may not accurately describe the individual patterns of response (Ryan et al. 2008). Consequently, Herda et al. (2009) suggested applying a natural log-transformation to the MMG_{RMS} and torque values for each contraction and calculating slope values (*b* terms) to examine possible changes in the individual patterns. Furthermore, the 95% confidence intervals (CIs) constructed around the *b* terms provide additional information regarding the linearity of the original relationships. For example, if the *b* term equals 1 or its 95% CI include 1, the relationship is linear between MMG_{RMS} and torque. If the *b* term is less than 1 and its 95% CI do not include 1, this reflects a downward deceleration in the relationship as the rate of change is greater in the *X* variable (torque) than the *Y* variable (MMG_{RMS}). During linearly increasing muscle actions, the *b* term from this model has successfully differentiated MMG_{RMS}–torque relationships between chronic training statuses for the VL (Herda et al. 2010; Trevino and Herda 2015). Although *b* terms during a linearly increasing muscle action display similar reliability to the surface electromyographic signal (Herda et al. 2008) and exhibit good day-to-day reliability (ICC = 0.896, 95% CI = 0.770–0.960) (Herda et al. 2009), no study has used the *b* terms to investigate the influence of a continuous cycling program on MU activation strategies for the VL.

Therefore, the purpose of this study was to examine the effects of a short-term (10 week) continuous endurance cycling protocol on MU activation strategies for the VL during an isometric ramp muscle action at a 70% pre-training MVC. For a 70% MVC of the knee extensors, it is estimated that 90% of the MU pool is recruited for the VL (De Luca and Kline 2011) and, thus, the effects of endurance training could be investigated on MUs composed of muscle fibers that express type I and/or type II characteristics (Burke et al. 1973; Bottinelli et al. 1996; Canepari et al. 2010) at this contraction intensity. An absolute torque level was investigated as endurance training is reported to increase the percentage of type I % fibers (Howald et al. 1985), which may decrease twitch forces of MUs (Garnett et al. 1979) and require increased muscle activation to match pre-training targeted torques. Consequently, we hypothesized a decrease in knee extensor MVC, resulting in greater slopes (*b* terms) from the MMG_{RMS}–torque relationships post-training due to increased MU recruitment. In addition, we hypothesized women would exhibit smaller *b* terms compared to men due to sex-related differences in % MHC expression for the VL (Trevino et al. 2019; Staron et al. 2000). Lastly, we hypothesized a positive relationship would exist between the *b* terms and mCSA due to previous reports of greater type II fiber diameters for larger muscles (Trevino et al. 2019).

Methods

Subjects

Ten healthy males (mean ± SD; age: 20.2 ± 1.9 years; height: 179.5 ± 4.9 cm; body mass: 77.5 ± 8.7 kg) and 14 healthy females (age: 21.9 ± 5.3 years; height: 163.9 ± 5.1 cm; body mass: 63.7 ± 13.0 kg) completed this investigation. None of the subjects reported structured exercise for the previous 3 years or 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. Written informed consent was obtained from all participants before their participation.

Experimental approach

To determine the effects of continuous cycling on MMG_{RMS}–torque relationships, all subjects attended three laboratory sessions before (visits 1–3) and after (visits 4–6) 10 weeks of cycling. Visits 1 and 4 consisted of ultrasound imaging of the VL to determine mCSA and subcutaneous fat (sFAT) followed by a maximal aerobic capacity (VO_{2MAX}) test. Visits 2 and 5 were familiarization visits, where subjects practiced isometric MVCs and submaximal isometric ramp up muscle actions of the knee extensors from 0 to 70% MVC. During visits 3 and 6, subjects performed MVCs and submaximal isometric ramp up muscle actions from 0 to 70% MVC. The MVCs were used to determine maximal strength for the current visit and the MVC value from visit 3 (PRE) was used to determine the torque level (Nm) for the submaximal contractions at pre- (PRE) and post- ($POST_{ABS}$) training. To avoid fatigue, there was at least 48 h among all testing visits and between visit 4 and the last training session. All participants were asked to refrain from all physical activity outside of study protocols. Participants were to refrain from all supplements, including caffeine, throughout the study duration. All experimental testing visits were scheduled at the same time of day (± 1 h) from their original testing visit.

Continuous cycling training

All subjects were asked to complete 10 weeks of continuous cycling on Life Fitness Upright Bikes (Model CLSC, Rosemont, IL, USA) 4 times per week for a total of 40 cycling sessions. Exercise intensity was prescribed on the upper limits of heart rate reserve (HRR). Target heart rates were calculated with the Karvonen method [(maximal heart rate−resting heart rate) × %intensity + resting heart rate)] (Karvonen 1957). The use of percent HRR has been recommended to prescribe cycling exercise intensities (Lounana et al. 2007) for low fitness populations (Swain 2000).

Weeks 1–3 consisted of 30 min of cycling at 70–75% of HRR, whereas weeks 4–6 and 7–10 were 40 min at 75–80% and 80–90% of HRR, respectively. Heart rates were monitored with Polar FT7 Heart Rate Monitors (Polar Electro, INC, Lake Success, NY, USA). Every subject completed the entire duration of all 40 training sessions. All training sessions were supervised by a research assistant and heart rate was recorded every 3 min to ensure exercise intensity compliance.

Ultrasound imaging

Ultrasound imaging was used to measure anatomical mCSA and 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. Prior to imaging, the participants laid in a supine position for 10 min to allow fluid shifts to occur. A portable brightness mode (B-mode) ultrasound imaging device with a multifrequency 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. The scan depth was set to 4.5 cm, gain was 68 dB, and transducer frequency was 10 MHz to optimize 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 ultrasound probe movement in the transverse plane. The panoramic function was used to obtain a single mCSA image of the VL. All ultrasound imaging analyses were 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 polygon function was used to outline the muscle with care taken to exclude the surrounding fascia. sFAT was quantified as the distance between the skin and the superficial aponeurosis of the muscle. Data from cadaver (Cartwright et al. 2013) and magnetic resonance imaging studies (Scott et al. 2012) have supported ultrasound to be a valid and reliable tool for assessing mCSA. Data from our laboratory have indicated excellent reliability (ICC = 0.948, $p = 0.491$) for mCSA of the VL, which is in agreement with Scott et al. (2012).

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. Subjects stood next to the bike to estimate proper seat height using the greater trochanter, then were examined while mounted on the bike to confirm there was a slight bend at the knee at the bottom of the pedal stroke, not full extension or 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. Following 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 met three of the five indicators according to the American College of Sports Medicine Guidelines (Amstrong et al. 2005; Medicine ACoS ACSM's guidelines for exercise testing and prescription 2005). During testing, heart rates were recorded with a Polar FT7 Heart Rate Monitor (Polar Electro, INC, Lake Success, NY, USA), and the maximal heart rate was used to calculate the target heart for the continuous cycling training.

Respiratory gasses were collected and monitored using a metabolic cart (Parvo Medics TrueOne® 2400 Metabolic Measurement System, Sandy, Utah, USA). The metabolic cart was calibrated prior to each test with room air and standard gasses of known volume and concentration for the O_2 and CO_2 analyzers. Flowmeter calibration was performed prior to each graded exercise test. Respiratory gasses were collected by use of a two-way rebreathing valve (Hans Rudolph Inc., Shawnee, Kansas, USA) and mouthpiece attached to headgear, which held them in place. Participants wore a nose clip to ensure that breathing occurred entirely through the mouth. O_2 and CO_2 were analyzed through a sampling line after the gasses were passed through a heated pneumotach and mixing chamber. The metabolic cart software (Parvo Medics TrueOne 2400, Sandy, UT, USA) reported the values as ventilated oxygen and carbon dioxide (VO_2 and VCO_2, respectively) and calculated relative VO_{2MAX}.

Isometric strength testing

Each subject 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). Prior to any testing, all Biodex settings were measured and recorded for consistency among trials. All isometric knee extensor strength assessments were performed on the right leg at a flexion of 90°. Isometric strength for the right knee extensor muscles was measured using the torque signal from the Biodex System 3 isokinetic dynamometer.

During experimental trials, subjects performed three, three-second MVCs of the knee extensors with strong verbal encouragement for motivation. The highest torque output determined the torque level for the subsequent submaximal contractions. For pre-training testing, subjects performed an isometric ramp up muscle action at 70% of MVC (PRE) (Fig. 1). For post-training testing, subjects performed an isometric ramp up muscle action at the same absolute torque as pre-treatment MVC ($POST_{ABS}$). For all isometric ramp up muscle actions, the torque increased at 10% MVC/s to the targeted torque level; therefore, each ramp lasted 7 s. Subjects were instructed to maintain their torque output as close as possible to the target torque presented digitally in real time on a computer monitor. Subjects were given a second attempt following a five-minute period if unable to maintain the targeted torque during the initial trial.

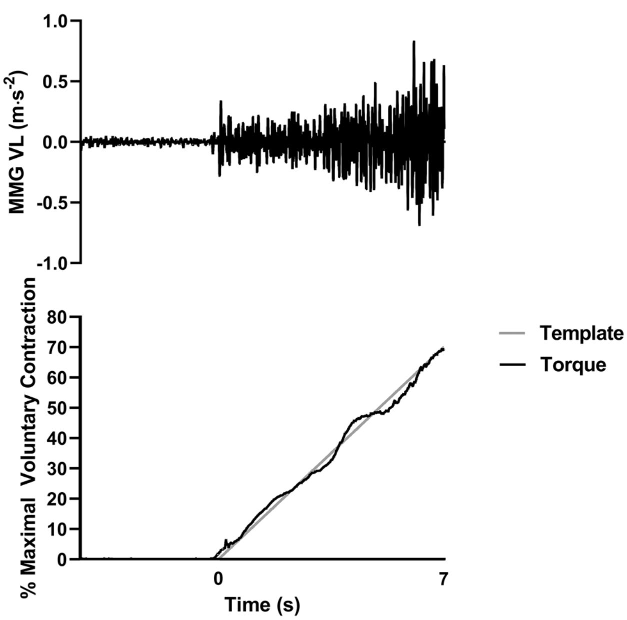


Fig. 1 An example of the mechanomyographic (MMG) signal from the vastus lateralis (VL) during a 70% isometric ramp up muscle action for one participant (top). The torque signal (bottom) is overlaid on the ramp up template as it appeared for the participant during the trial

Mechanomyographic and signal processing

During the ramp up muscle actions, surface MMG signals were recorded from the VL using an active miniature accelerometer (model 352A24, bandwidth = 10–8000 Hz, sensitivity of 98.8 mV m/s^2, PCB Piezotronics, Inc, Depaw, NY) that was placed over the VL on the lateral/anterior portion of the muscle at 50% of the distance between the greater trochanter and the lateral condyle of the femur. Prior to sensor placement, the surface of the skin was shaved and a permanent marker (Sharpie®, Atlanta. GA) was used to mark the location of the accelerometer on the skin and subjects were instructed to remark the location when necessary. The position of the accelerometer was also measured and recorded for consistency using 50% of the distance between the anterior superior iliac spine and the lateral portion of the patella as a reference to confirm similar placement between experimental sessions. In addition, the measurements and sensor placement were performed by the same researcher. Double-sided tape was used to affix the accelerometer to the skin.

The MMG (m/s^2) and torque (Nm) signals were simultaneously sampled at 2 kHz with a National Instruments data acquisition system (NI cDAQ-9174). MMG signals were bandpass filtered (fourth-order Butterworth) at 5–100 Hz. During the isometric ramp up muscle action, consecutive, non-overlapping 0.25 s epochs were analyzed for the torque and MMG signals. Root mean square (RMS) was used to calculate the amplitude of the MMG signal. All subsequent signals were stored and processed off-line with custom-written software (LabVIEW, version 11; National Instruments, Austin, TX).

Statistical analysis

Simple linear regression models were applied to the log-transformed MMG_{RMS}–torque relationships (Herda et al. 2010) for the linearly increasing ramp up muscle action. The equations were represented as:

$$\ln[Y] = b(\ln[X]) + \ln[a] \tag{1}$$

where $\ln[Y]$ = the natural log of the MMG_{RMS} values, $\ln[X]$ = the natural log of the torque values, b = slope, and $\ln[a]$ = the natural log of the y-intercept. After the antilog transformation, this can also be expressed as an exponential equation:

$$Y = aX^b \tag{2}$$

where Y = the predicted MMG_{RMS} values, X = torque, b = slope of Eq. (1), and a = the antilog of the y-intercept from Eq. (1).

Six separate two-way mixed factorial repeated measures ANOVA (time [pre vs. post] × sex [men vs. women]) were used to examine differences in the *b* terms from the log-transformed MMG_{RMS}–torque relationships during the linearly increasing ramp up muscle actions, MVC, VO_{2max}, mCSA, sFAT, and body mass. Additionally, four separate Pearson's product moment correlation coefficients were calculated comparing mCSA and sFAT with the *b* terms during the isometric ramp up muscle actions at the PRE and $POST_{ABS}$ torque levels.

sFAT may low-pass filter the MMG signal (Cooper et al. 2014), thus, a subset of subjects from each sex were matched for sFAT to further examine its effects on MMG_{RMS}–torque relationships and mCSA. Four males (sFAT = 0.862 ± 0.137 cm) and four females (sFAT = 0.952 ± 0.066 cm) were selected for this secondary analysis. Three separate two-way mixed factorial repeated measures ANOVA (sex [men vs. women] x time [pre vs. post]) were performed between the groups for sFAT, mCSA, and *b* terms. In addition, two separate (pre and post) Pearson's product moment correlation coefficients were calculated comparing mCSA with the *b* terms. The level of significance was set at $p \le 0.05$ and all statistical analyses were performed using SPSS 25 (IBM Corporation, Armonk, NY, USA).

Results

Ultrasound and body mass data

There were no two-way interactions (sex × time; $p = 0.623$–0.785) or main effects for time ($p = 0.303$–0.933) for body mass, mCSA, or sFAT. There were main effects for body mass ($p = 0.007$), mCSA ($p < 0.001$), and sFAT ($p < 0.001$) for sex when collapsed across time. Body mass was significantly greater for males (77.51 ± 8.77 kg) compared to females (63.53 ± 12.99 kg). Males had significantly greater mCSA (24.80 ± 4.94 cm^2) compared to females (16.64 ± 3.61 cm^2) and females had greater sFAT (1.59 ± 0.651 cm) compared to males (0.534 ± 0.327 cm).

Maximal aerobic capacity

There was no two-way interaction (sex × time; $p = 0.710$). There were significant main effects for time ($p < 0.001$) and sex ($p < 0.001$). VO_{2MAX} increased from PRE (37.51 ± 7.97 mL/kg min) to POST (42.96 ± 8.11 mL/kg min) when collapsed across sex, and was greater for males (46.67 ± 5.76 mL/kg min) than females (35.64 ± 5.70 mL/kg min) when collapsed across time.

Maximal strength

There was no two-way interaction (sex × time; $p = 0.434$). There were significant main effects for sex ($p < 0.001$) and time ($p = 0.005$). MVC decreased from PRE (168.10 ± 58.49 Nm) to POST (160.78 ± 58.39 Nm) when collapsed across sex and was greater for males (217.24 ± 44.35 Nm) than females (126.73 ± 30.70 Nm) when collapsed across time (Fig. 2).

Isometric ramp up MMG_{RMS}–torque relationships

The MMG_{RMS}–torque relationships were significant ($r = 0.401$–0.957; $p \le 0.05$) for 47 of 48 contractions. There was no significant two-way interaction (sex × time; $p = 0.959$); however, there were significant main effects for time ($p = 0.012$) and sex ($p = 0.003$). The *b* terms were greater $POST_{ABS}$ (0.623 ± 0.204) compared to PRE (0.540 ± 0.226) when collapsed across sex, and for males (0.717 ± 0.171) than females (0.484 ± 0.168) when collapsed across time (Fig. 3). The 95% CIs were < 1 for PRE (0.449–0.630) and $POST_{ABS}$ (0.541–0.704) when collapsed across sex, and for males (0.611–0.823) and females (0.396–0.572) when collapsed across time. Thus, the relationships were nonlinear with a greater rate of change in the *X* variable (torque) than the *Y* variable (MMG_{RMS}) for both time points and sexes. Figure 4 illustrates the MMG_{RMS} patterns during the linearly increasing muscle action the PRE and $POST_{ABS}$ torque levels.

To further determine the influence of sex on MMG_{RMS}–torque relationships, *b* terms were normalized to mCSA to account for muscle size. A repeated measures

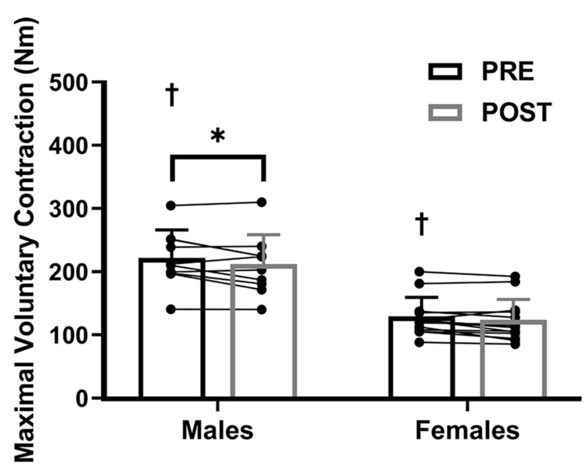


Fig. 2 Plotted individual values (black markers) and means (bars) ± SD for maximal voluntary contraction (MVC) for the males and females pre- (PRE) and post- (POST) cycling training. †Indicates greater MVCs for PRE than POST when collapsed across sex ($p < 0.001$). * Indicates greater MVCs for males than females when collapsed across time ($p = 0.005$)

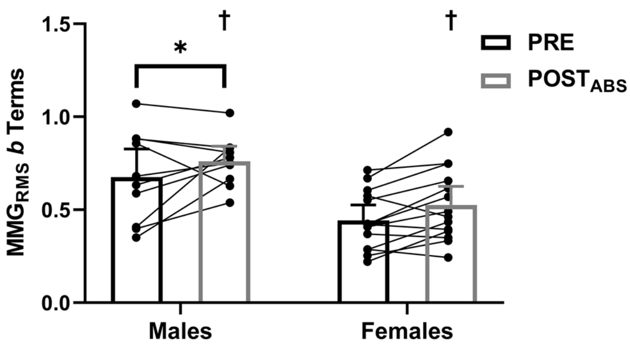


Fig. 3 Plotted individual values (black markers) and means (bars) ± 95% confidence intervals for b terms from the mechanomyographic amplitude (MMG_{RMS}) vs. torque relationships for the males and females from the pre- (PRE) and post-training absolute ($POST_{ABS}$) torque levels. †Indicates greater b terms $POST_{ABS}$ than PRE when collapsed across sex ($p = 0.003$). *Indicates greater b terms for the males than females when collapsed across time ($p = 0.012$)

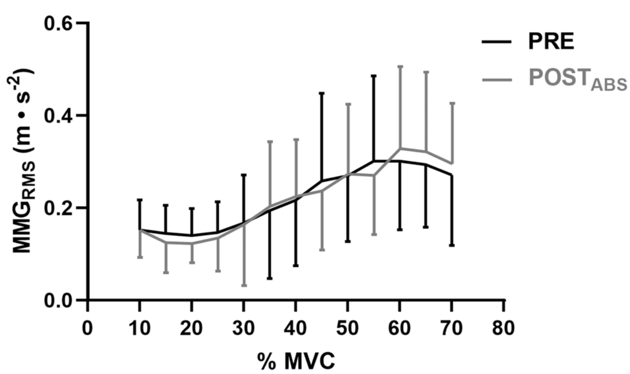


Fig. 4 Plotted means ± SD for pre- (PRE) (black line) and post-training absolute ($POST_{ABS}$) (gray line) torque levels during the isometric ramp muscle action of the mechanomyographic amplitude (MMG_{RMS})-torque relationship

ANOVA indicated no significant difference ($p = 0.863$) between males (0.029 ± 0.007 slope/mCSA) and females (0.030 ± 0.010 slope/mCSA) when collapsed across time. The nonsignificant differences between sexes provides further evidence that the relationship between b terms and mCSA is similar for males and females, and thus the significantly larger b terms were the result of larger VL muscle for males.

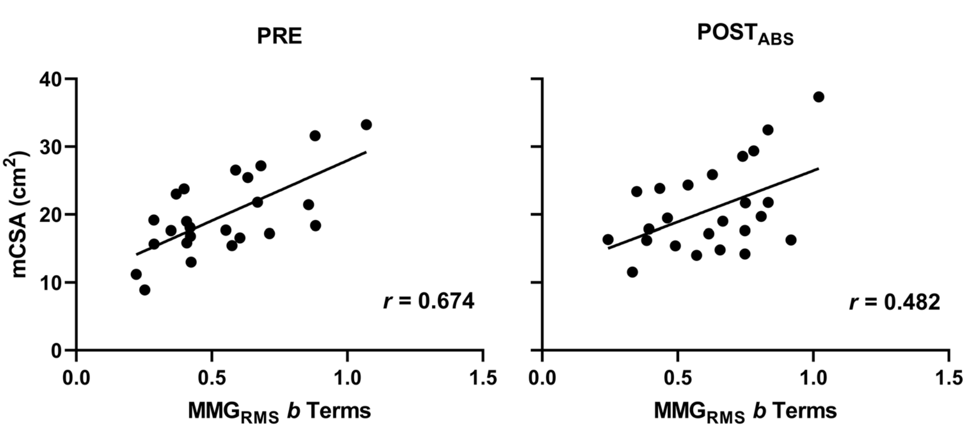
Correlations

Pearson's product moment correlations were significant between mCSA and the b terms from the MMG_{RMS}–torque relationships from the 70% MVC for PRE ($p < 0.001$, $r = 0.674$) and $POST_{ABS}$ ($p = 0.020$, $r = 0.471$) contractions (Fig. 5). Conversely, sFAT measurements at pre- and post-training were not correlated with the b terms for the PRE ($p = 0.091$) and $POST_{ABS}$ torque levels ($p = 0.219$), which aligns with previous investigations (Cooper et al. 2014; Herda et al. 2010; Trevino and Herda 2015; Trevino et al. 2016a).

sFAT matched subset analyses

For the sFAT matched groups, there was no significant two-way interaction (sex × time) for sFAT ($p = 0.935$), and no main effects for time ($p = 0.981$) or sex ($p = 0.468$). For the b terms, there was no two-way interaction (sex × time; $p = 0.625$) and no main effect for time ($p = 0.268$), but there was a main effect for sex ($p = 0.027$). The b terms for the males (0.707 ± 0.033 m/s^2) were significantly greater than the females (0.468 ± 0.162 m/s^2) when collapsed across time. For mCSA, there was no two-way interaction (sex × time; $p = 0.829$) or main effect for time ($p = 0.135$). However, there was a main effect for sex ($p = 0.002$). mCSA was significantly greater for the males (25.86 ± 2.23 cm^2) compared to females (14.38 ± 3.74 cm^2) when collapsed across time. mCSA and the b terms were significantly correlated PRE ($p = 0.035$, $r = 0.742$) and $POST_{ABS}$ ($p = 0.048$, $r = 0.712$)

Fig. 5 Plotted relationships between muscle cross-sectional area (mCSA) and the b terms from the mechanomyographic amplitude (MMG_{RMS}) vs. torque relationships for the pre- (PRE) and post-training absolute ($POST_{ABS}$) torque levels



cycling intervention. The secondary analysis in conjunction with the nonsignificant correlations between sFAT and the b terms for the PRE and $POST_{ABS}$ torque levels provides evidence that differences in sFAT were not the primary contributor to the differences in the MMG_{RMS}–torque relationships between sexes and the strength of the correlations.

Discussion

As expected, VO_{2MAX} significantly increased following 10 weeks of continuous cycling training and is in agreement with previous studies (Howald et al. 1985; Martinez-Valdes et al. 2017). Significant findings in the present study include: (1) a decrease in MVC of the knee extensors despite no change in mCSA, (2) greater b terms for the MMG_{RMS}-torque relationships post-cycling training during an isometric ramp up muscle action performed at the same absolute torque, (3) greater b terms for the males in comparison to females, and (4) significant correlations for mCSA with the b terms for pre- and post-training.

In the present study, 10 weeks of continuous cycling training significantly decreased (4.35%) MVC of the knee extensors in previously sedentary subjects. Conversely, no change in knee extensor MVC has been reported following continuous cycling (Martinez-Valdes et al. 2017; Vila-Cha et al. 2012; Vila-Chã et al. 2010). The differences in findings are likely due to the subject populations and cycling protocols. The previous studies investigated sedentary- and recreationally trained college-aged males, whereas the subjects for the current study were sedentary, college-aged males and females. In addition, the training programs were much shorter with lower exercise intensities for Martinez-Valdes et al. (2017) (2 weeks at 65% VO_{2PEAK}) and Vila-Cha et al. (2012) and Vila-Chã et al. (2010) (6 weeks at 50–75% HRR). Consequently, the subjects in the present study trained at greater exercise intensities (70–90% HRR) and overall duration (1480 min) compared to the aforementioned studies (540–720 min) (Martinez-Valdes et al. 2017; Vila-Cha et al. 2012; Vila-Chã et al. 2010). Interestingly, the decrease in knee extensor MVC for the current study was associated with no change in mCSA. It has been reported that endurance training can elicit fiber-type shifting without changes in the mean diameters of all fiber types (Howald et al. 1985). In addition, Harber et al. (2004) reported markedly lower normalized twitch forces for type I and II muscle fibers in chronically trained endurance runners compared to untrained individuals. Therefore, the greater intensity and total training time for the current study may have resulted in greater fiber-type shifts and negatively affected the force generating capabilities for the knee extensors to a larger extent than previous studies and reduced MVC without changes in mCSA.

The b terms (slopes) indicate the linearity for the log-transformed MMG_{RMS}–torque relationships. Herda et al. (2009) reported 95% CIs constructed around the b terms that do not include and are < 1, indicate a downward deceleration (plateau) in the relationship with greater increments in X variable (torque) than the Y variable (MMG_{RMS}). In addition, the relative position of the deflection point is reflected by the magnitude of the b terms. Thus, a smaller b term decelerates earlier in the torque spectrum, whereas the converse is true for a larger b term (Herda et al. 2010). Therefore, changes in b terms during an isometric ramp up muscle action following a short-term endurance training program may provide insight on alterations in MU activation strategies when matching a pre-training absolute torque.

For the current study, the 95% CIs constructed around the b terms were < 1 for PRE and $POST_{ABS,}$ and thus the patterns were nonlinear with deceleration throughout the torque spectrum. However, there were significant increases in the b terms following training, indicating a later deflection point in the relationship when matching the $POST_{ABS}$ torque (Fig. 6). It has been reported that a muscle relying primarily on MU recruitment would have a larger b term compared to a muscle relying primarily on MU firing rate increases to reach peak torque (Cooper and Herda 2014). Following training, participants in the current study exhibited a significant decrease in MVC and, consequently, a higher relative torque was necessary (74% MVC) to match the $POST_{ABS}$ torque compared to pre-training (70% MVC). The VL continues to recruit MUs up to 95% MVC (De Luca and Hostage 2010) and MMG_{RMS} values continue to increase with relative torque values up to 95% MVC (Coburn et al. 2005). Thus, the larger b terms following the continuous cycling

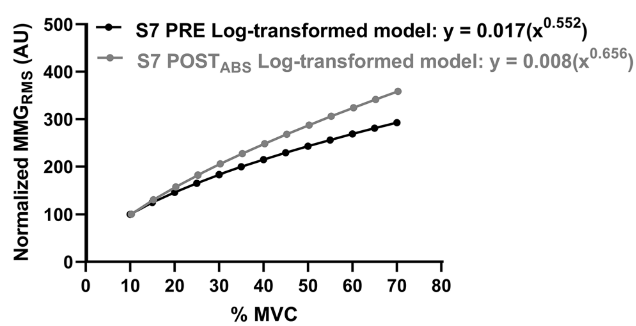


Fig. 6 Plotted normalized mechanomyographic amplitude (MMG_{RMS})–torque relationships from 10 to 70% maximal voluntary contraction (MVC) at the pre- (PRE) and post-training absolute ($POST_{ABS}$) torque levels during the isometric ramp muscle action at 70% MVC for one subject (S7) with b terms similar to the group means. The original relationships are fitted with the exponential equation ($Y = aX^b$) from the natural log-transformed model. MMG_{RMS} relationships were normalized to arbitrary units (AU) to allow for the overlay of the relationships from the two time points

protocol suggest an increase in MU recruitment (Trevino et al. 2016a) to match the $POST_{ABS}$ torque. It should be noted that the greater *b* terms post-training may be a function of completing a contraction to an absolute torque level in conjunction with decreases in MVC. Therefore, it is possible that muscle activation could be similar if the same percent of MVC was investigated at pre- and post-training. Future research should investigate the effects of endurance training on the *b* terms for the VL during an isometric ramp up muscle action to the same relative intensity.

There were also sex-related differences in MU activation strategies for the current study. For example, males exhibited greater *b* terms independent of training (Fig. 7). It is suggested the central nervous system provides the entire MU pool with a common synaptic input (De Luca and Erim 1994); thus, MU firing rates are determined by the physical properties (% MHC expression) of the MU pool (Trevino et al. 2016b; De Luca et al. 1982). In support, Trevino et al. (2016b) reported higher firing rates of the VL for individuals expressing greater amounts of type I% MHC expression. Although the MMG_{RMS}–torque relationship reflects MU recruitment (Orizio 1993), the degree it plateaus or decreases from active muscle stiffness or MU twitch fusion due to higher firing rates (Akataki et al. 2003) is likely influenced by the % MHC expression of the muscle. In support, individuals expressing a greater percentage of type I% MHC have displayed smaller *b* terms (Trevino et al. 2016a). Therefore, the lower *b* terms for the females in the current study may be attributable to greater type I% MHC expression previously reported for the VL (Trevino et al. 2019; Staron et al. 2000).

The *b* terms and mCSA were significantly correlated at the PRE ($r = 0.67$) and $POST_{ABS}$ ($r = 0.47$) torque level; thus,

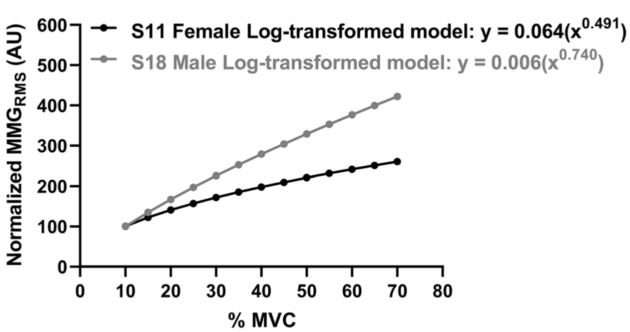


Fig. 7 Plotted normalized mechanomyographic amplitude (MMG_{RMS})–torque relationships from 10 to 70% maximal voluntary contraction (MVC) during the isometric ramp muscle action at 70% MVC for a female (S11) and male (S18) subject with *b* terms that were similar to the group means. The original relationships are fitted with the exponential equation ($Y = aX^b$) from the natural log-transformed model. MMG_{RMS} relationships were normalized to arbitrary units (AU) to allow for the overlay of the relationships from the two subjects

individuals with larger mCSA exhibited greater *b* terms during the isometric ramp up muscle actions. Recently, positive correlations ($r = 0.596$–0.877) were reported among mCSA, the relative size of higher in relation to lower threshold MUs, and type II% MHC expression of the VL for sedentary college-aged males and females (Trevino et al. 2019). Therefore, it is possible that individuals exhibiting larger mCSA values in the current study had greater sized fibers expressing type II characteristics as similar quantities of muscle fibers and a nearly identical fiber-type distribution (ratio of fast- and slow-twitch fibers) have been reported between sexes (Miller et al. 1993; Schantz et al. 1983, 1981; Staron et al. 2000). Consequently, individuals with larger mCSA are likely expressing a greater type II fiber area (Trevino et al. 2019) and would possess a lower proportion of the MUs that would fuse and cause earlier deceleration in the MMG_{RMS}-torque relationship (Trevino et al. 2016a). This would be supported for the current study as individuals with greater mCSA exhibited larger *b* terms regardless of training status and sex (Fig. 5). In addition, when the *b* terms were normalized to mCSA, there were no significant differences between sexes ($p = 0.863$), further supporting the influence of muscle size on the *b* terms. Post-training, the weaker correlations may be a function of individuals completing a contraction intensity based off pre-training strength; therefore, not all individuals were performing a contraction at the same relative torque.

It should be acknowledged that sFAT may be a confounding variable for the sex-related differences in the *b* terms as sFAT can low pass filter the MMG_{RMS} signal (Cooper and Herda 2014). For the current study, there were significant differences ($p < 0.001$) in sFAT between the males (0.53 ± 0.33 cm) and females (1.59 ± 0.65 cm). However, the lack of relationships between sFAT and the *b* terms for the PRE and $POST_{ABS}$ isometric ramp up muscle actions for the current study and previous investigations (Cooper et al. 2014; Herda et al. 2010; Trevino and Herda 2015; Trevino et al. 2016a) in conjunction with the results from the subset of participants (males $= 0.86 \pm 0.14$ cm; females $= 0.95 \pm 0.07$ cm) provides further evidence that sFAT was not a contributing factor for the larger *b* terms observed for the males and the positive correlations between mCSA and the *b* terms. For the sFAT-matched subgroup, the sex-related differences for the *b* terms and the correlations between mCSA and the *b* terms remained significant.

There are some limitations for this study that should be acknowledged. First, there was no control group. However, ICC values from our laboratory have indicated excellent reliability for mCSA of the VL and are in agreement with values reported from other laboratories (Scott et al. 2012). In addition, previous research has indicated good day-to-day reliability for the *b* terms from the log-transformed MMG_{RMS}–torque relationships across multiple testing days during numerous contraction intensities (Herda et al. 2009). Second, we do not have MHC expression values for the males and females. Although previous research has reported sex-related differences in MHC expression in the VL in similar populations (Trevino et al. 2019; Staron et al. 2000), we cannot say with total certainty that differences existed between the males and females in our study.

In summary, this is the first study to report MMG_{RMS}–torque relationships were altered following a short-term (10 week) endurance training program, differences in MU activation strategies for the VL as a function of sex, and positive relationships between muscle size and MMG_{RMS}–torque relationships. The greater acceleration in the *b* terms post-training suggests increased recruitment to match pre-training torque levels, whereas larger values for the males may be due sex-related differences in the MHC expression of the VL. In addition, the positive correlations between muscle size and *b* terms may be due to individuals with larger type II muscle fibers relying less on firing rates to modulate torque. MMG_{RMS} may provide insight on MU activation strategies following endurance training and between sexes, and reflect muscle size. Of note, continuous cycling training significantly improved maximal aerobic capacity, but decreased maximal strength of the knee extensors. Thus, strength and conditioning coaches may want to use caution when prescribing continuous cycling training. In addition, practitioners and personal trainers should include resistance training when prescribing weight loss interventions as a means to improve strength.

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Author contributions MAT and TJH designed the experiments. SAS, MAT, AJS, JDM, MEP, HLD, and JAD conducted the experiments. SAS, MAT, TJH, AJS, JDM, MEP, HLD, and JAD analyzed the data. SAS and MAT wrote the manuscript. SAS and MAT edited and revised the manuscript. All authors approved the final version of the manuscript submitted for publication and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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Compliance with ethical standards

Conflict of interest The authors declare they have no competing interests.

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