Original Article

Method of analysis influences interpretations of sex-related differences in firing rates during prolonged submaximal isometric contractions

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Abstract

Objective: This study examined motor unit (MU) firing rates during a prolonged isometric contraction of the vastus lateralis (VL) for females and males. **Methods**: Surface electromyographic (sEMG) signals were recorded from the VL for eleven females and twelve males during a 45-second isometric trapezoid muscle actions at 40% of maximal voluntary contraction (MVC). For each MU, mean firing rate (MFR) was calculated for the initial and final 10-second epochs of the steady torque segment and regressed against recruitment threshold (RT, expressed as %MVC), as well as time at recruitment (T_{REC}, seconds). MFR was also averaged for each subject. **Results**: Significant differences existed across epochs for the y-intercepts (P=0.009) of the MFR vs. T_{REC} relationship, as well as the grouped MFR analysis (P<0.001); no differences were observed between epochs for the MFR vs. RT relationship. Significant differences existed between sexes for the grouped MFR analysis (P=0.049), but no differences were observed for the MFR vs. T_{REC} or MFR vs. RT relationships. **Conclusion**: Analysis method may impact interpretation of firing rate behavior; increases in MU firing rates across a prolonged isometric contraction were observed in the MFR vs. T_{REC} relationship and the grouped MFR analysis.

Keywords: Electromyography, Motor Unit, Muscle Fatigue, Neuromuscular, Vastus Lateralis

Introduction

Numerous studies have analyzed the activity of motor unit (MU) firing rates during prolonged isometric submaximal contractions. However, there is conflicting evidence regarding the directional change of firing rate behavior during prolonged isometric contractions. Differing methods of analyses may contribute to the lack of consensus on potential changes in firing rates over the course of a prolonged submaximal isometric contraction[1]. Grouped, or pooled, analysis of MU firing rates results in data being collapsed across subjects and, therefore, inter-individual variability is disregarded[2–4]. Changes in MU firing rates over the course of a prolonged contraction quantified with the grouped approach is mixed. For example, decreases in MU firing rates are reported for the first dorsal interosseous (FDI) at 50% maximal voluntary contraction (MVC)[2] and elbow flexors at 3-49% MVC[4], whereas, others report increases in firing rates over time in the biceps brachii at 20% MVC[5], or no change in firing rates for the biceps brachii and brachioradialis at 25% MVC[6].

The grouped analysis discounts the variability in firing rates as a function of recruitment threshold (RT). Whereas, others analyze firing rates separately for individuals and typically account for RTs of each MU[7–17]. Analyzing MUs

separately for individuals and monitoring RTs typically results in the observation of increases in firing rates over the course of a prolonged contraction. This is observed for the vastus lateralis at 20%[18], 30%[19], and 50% MVC[20].

Recently, researchers are applying linear regressions to the MU firing rate vs. RT relationships separately for individuals to quantify changes in firing rates[10,21,22]. However, MUs recruited during the plateau/steady torque phase possess the same RT, which is common when the task requires subjects to perform prolonged contractions using a trapezoidal template. RT is an indirect marker of the physiological properties and, thus, MUs with differing firing rates and twitch forces would be assigned the same RT[23]. Therefore it is common practice to exclude MUs recruited during the steady torque phase of a contraction from the overall analysis[21,24]. The excluded MUs are physiologically relevant and are needed to maintain the task. Therefore, it is necessary to define MUs by a continuous variable that can apply uniformly to all MUs required for maintenance of the task over time. Quantifying the firing rates relative to time at recruitment (T_{REC}) overcomes the limitations of using torque as a marker of recruitment and should be examined.

Literature on sex-related differences in MU firing rates during isometric contractions is also sparse and conflicting. When MUs are pooled across contractions and subjects, some studies report increases in firing rates for females in the tibialis anterior at 20-80% MVC[25] or no differences in the elbow flexors at 15% MVC[26]. When accounting for recruitment thresholds, no differences in firing rates at recruitment were reported for the vastus medialis during a contraction performed to 30% MVC[27]. In addition, Parra et al.[28] reports no differences for firing rates at steady torque in the FDI during a 10% MVC contraction. Nonetheless, it is unclear if there are sex-related differences in firing rates during prolonged isometric contractions of the leg extensors. Potentially, sex-related differences in firing rates may occur when recruitment of additional MUs during steady torque is required to maintain the task. Females have consistently displayed higher tolerance to fatigue in sustained submaximal contractions[29] and, therefore, may utilize different strategies for maintaining torque.

The primary purpose of this study is to understand changes in MU firing rates during a prolonged (45-second) contraction of the leg extensors and investigate potential differences in interpretations of changes in MU firing rates due to methods of analysis. MU firing rates will be examined disregarding inter-individual variability and separately for individuals. For the individual analyses, firing rates will be regressed against RT and T_{REC} to account for MUs recruited during the steady torque segment. A secondary purpose is to examine differences in MU firing rates between males and females during a prolonged contraction. It is hypothesized that the interpretation of changes in MU firing rates will differ among different analysis methods. It is hypothesized that no sex-related differences in MU firing rates will be present at the beginning of the prolonged contraction[27], however, sex-related differences in MU firing rates may occur towards the

end of the prolonged contraction as recruitment of higher-threshold MUs are required to maintain the task[30–32].

Materials and methods

Subjects

Eleven healthy females (mean ± SD, age = 19.82±1.40 years, height = 164.86±6.34 cm, body mass = 63.19±10.64 kg) and 12 healthy males (age = 20.25±1.86 years, height = 179.63±7.13 cm, body mass = 80.79±12.84 kg) participated in this investigation. None of the participants had participated in any form of structured exercise program for the previous 6 months. None of the participants had any history of current or ongoing neuromuscular diseases or musculoskeletal injuries specific to the ankle, knee, or hip joints. This study was approved by the University's institutional review board for human subjects research (approval no. STUDY00002953) and was conducted in accordance with the *Declaration of Helsinki*. Each subject read and signed an informed consent form and completed a health status questionnaire prior to the study beginning.

Testing Timeline

Participants attended two laboratory sessions. Visit 1 included the completion of a health history questionnaire and informed consent form and subjects became familiar with the isometric strength testing measurements, such as, submaximal isometric trapezoidal muscle action, and isometric maximal muscle action. Visit 2 included experimental isometric strength testing of the leg extensor.

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). The thigh of the tested leg (right) was not secured because of the surface EMG electrode, as previously done[33–35]. Isometric leg extensor strength assessment was 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 trial, participants performed three isometric MVCs with strong verbal encouragement for motivation followed by one submaximal isometric trapezoid muscle action at 40% relative to the maximum recorded MVC strength. The highest torque output for visit 2 determined the maximal torque output for each participant and the torque level for the 40% MVC submaximal isometric trapezoid muscle actions for the isometric strength testing. For the isometric trapezoid muscle action, the torque increased at 10% MVC/s to the desired torque level, where it was held during a 45 s plateau and then decreased to baseline at a rate of -10%
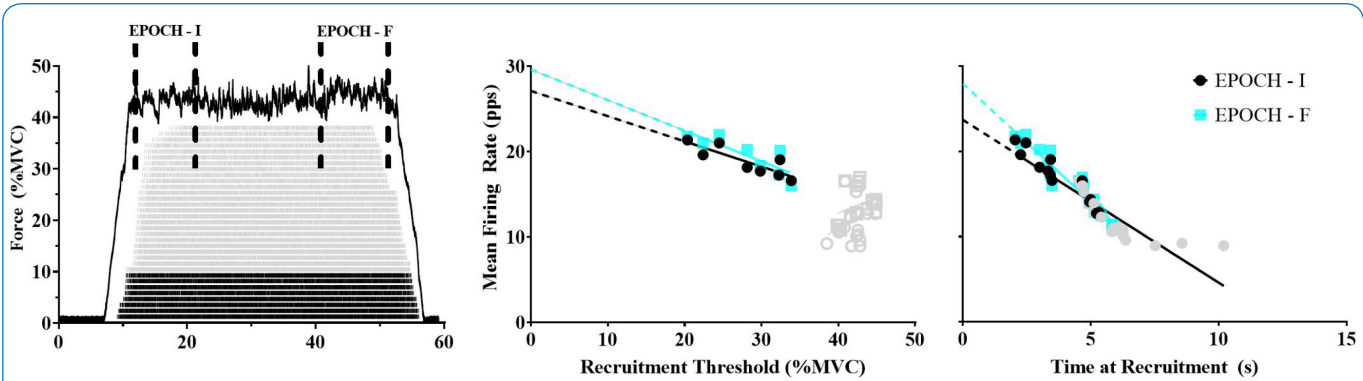
Figure 1. Left: The torque overlaid on the motor unit (MU) firing times (vertical lines) recorded during the prolonged isometric muscle action for one subject. Right: The mean firing rate (MFR, pps) vs. recruitment threshold (RT, expressed as percentage of maximal voluntary contraction [%MVC]) and MFR vs. time at recruitment (T_{REC}, s) for one subject. The light grey symbols represent MUs recruited during steady torque that were not included in the MFR vs. RT relationship but were included in the MFR vs. T_{REC} relationship. Dashed lines represent trendlines plotted to y-axis to demonstrate differences in y-intercepts.

MVC/s (Figure 1). Therefore, the duration of the contraction was 53 seconds. Participants were instructed to maintain their torque output as close as possible to the target torque presented digitally in real time on a computer monitor. Torque outputs were visually inspected for adherence in real time[32]. The participant would perform the contraction again if the torque output was observed to deviate (>3%) from the template in accordance with previous research[36]. However, no subject needed to repeat the 40% MVC. Template adherence for the epochs chosen for MU analyses is presented in the results.

EMG Recording

During the isometric muscle action, sEMG signals were recorded from the VL using a 5-pin surface array sensor (Delsys, Boston, MA). The pins have a diameter of 0.5 mm and were positioned at the corners of a 5 x 5 mm square, with the fifth pin in the center. Prior to sensor placement, the surface of the skin was prepared by shaving, removing superficial dead skin with adhesive tape (3M, St Paul, MN), and sterilizing with an alcohol swab. The sensor was placed over the VL muscle at 50% of the distance between the greater trochanter and the lateral condyle of the femur with adhesive tape. Our sensor location was different from that suggested by Zaheer et al.[37], however, the MU yields from this location tend to be similar or higher[32,36]. 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.[38] and Nawab et

al.[39]. Action potentials (APs) were extracted into firing events of single MUs from the four separate EMG signals via the precision decomposition algorithm as described by De Luca et al.[38]. This algorithm is designed for decomposing EMG signals into their constituent MUAP trains. The PD III algorithm is a valid[38,40,41] method for quantifying firing events. The accuracy of the decomposed firing instances was first tested with the reconstruct-and-test procedure[42]. Only MUs with >90% accuracies were used for further analysis.

Secondarily, a spike trigger average (STA) protocol was performed to validate the firing times and AP waveforms triggered by the PD III algorithm[43–46]. The derived firing times from the PD III algorithm were used to spike trigger average the 4 raw EMG signals. MUs that possessed high correlations (r>0.7) across the 4 channels between the PD III algorithm and the STA-derived AP waveforms, as well as low coefficients of variation (CoV<0.3) for the STA-derived peak-to-peak amplitudes across time were used for analysis[45].

Importantly, it is possible to observe MUAP waveforms from trigger events that appear valid but do not correspond with actual MU discharges[47]. To ensure that all identified MUAP waveforms were valid, we added small amounts of Gaussian noise (1% of the SD of the interspike interval for each MU) to the identified firing times of each MU[43,44,46]. This produced a small, random shift in the firing times to create a set of noise-adjusted MUAP waveforms. Correlations were performed between the noise-adjusted MUAP waveforms created from the raw EMG signals and the MUAP waveforms derived from the firing times triggered by the PD III algorithm. Another correlation was performed between the noise-adjusted MUAP waveforms and the STA waveforms. If the MUAP waveforms triggered by the PD III algorithm are valid, the correlation between the noise-adjusted MUAP waveforms and STA waveforms will decrease significantly[43,46].

Table 1. Motor unit (MU) counts and recruitment ranges as a function of recruitment threshold (expressed as percentage of maximal voluntary contraction [%MVC]) and time (s) for MUs recruited during the initial linear increasing segment of the muscle action. * Indicates significant difference from initial epoch (P<0.05).

Epoch	Sex	MU Count	Recruitment Range (%MVC)	Recruitment Range (s)
I	Females	34.18 ± 8.82	$6.19 \pm 5.72 - 32.39 \pm 5.60$	$0.87 \pm 0.56 - 3.47 \pm 0.41$
	Males	31.92 ± 9.98	$8.15 \pm 6.10 - 33.83 \pm 3.90$	$1.10 \pm 0.77 - 3.81 \pm 0.79$
	Total	33.00 ± 9.30	$7.21 \pm 5.87 - 33.14 \pm 4.74$	$0.99 \pm 0.67 - 3.65 \pm 0.65$
F	Females	32.18 ± 7.68	$6.19 \pm 5.72 - 30.18 \pm 4.73$	$0.87 \pm 0.56 - 3.35 \pm 0.36$
	Males	$28.00 \pm 10.23*$	$8.15 \pm 6.10 - 30.80 \pm 5.64$	$1.10 \pm 0.77 - 3.48 \pm 0.80*$
	Total	$30.00 \pm 9.15*$	$7.21 \pm 5.87 - 30.50 \pm 9.15*$	$0.99 \pm 0.67 - 3.42 \pm 0.62*$

For the prolonged contraction, two epochs were analyzed: (1) the initial 10-second interval during the constant torque segment (~2-12 s) of the contraction (I), and (2) the final 10-second interval during the constant torque segment (~33-43 s) of the contraction (F). For each MU, 4 parameters were extracted from the firing rate data: (1) MFR during the initial epoch (MFR-I, pulses per second [pps]), (2) MFR during the final epoch (MFR-F, pulses per second [pps]), (3) T_{REC} (s), and (4) RT (expressed as %MVC) (Figure 1). MFR was calculated as the average of the instantaneous discharge rates for each designated epoch of steady torque. T_{REC} was calculated as the initial time point at which a MU was recruited, with the start of the ramp segment of the contraction considered zero seconds. An average 0.10 s epoch of force beginning at the first discharge of the MU was selected as the RT for the MU.

Statistical Analysis

For the subjects' demographic data, independent t-tests were used to determine potential differences between sex for body mass, height, age, and isometric MVC strength. For the MU data, linear regressions were performed on the (1) MFR-I vs. T_{REC} and (2) MFR-F vs. T_{REC}, (3) MFR-I vs. RT, and (4) MFR-F vs. RT relationships. For the MFR vs. RT relationships, MUs recruited during the steady torque were excluded from analysis. Slope and y-intercept values were calculated for each linear relationship. Potential significant interactions between sex and epoch for the slopes and y-intercepts of the MFR vs. T_{REC} and MFR vs. RT relationships were analyzed with two-way ANOVAs (sex [male vs. female] x epoch [I vs. F]).

In addition, analyses were performed on MFRs without considering inter-individual variability and recruitment positions similar to previous studies[4,48,49]. Thus, firing rates were grouped or pooled across individuals for each epoch. For this analysis, the MFR was calculated for each subject by averaging the firing rates of all active MUs at each epoch. A two-way ANOVA (sex [male vs. female] x epoch [I vs. F]) was performed on the grouped MFRs.

When appropriate, follow-up analyses for the ANOVA models were performed using independent or dependent samples t-tests with Bonferroni corrections. For all individual relationships, slopes and y-intercepts were calculated using Microsoft Excel version 2016 (Microsoft, Redmond, WA). Additional tests included post-hoc power analysis (1-β) and measures of effect size: partial eta squared (η^2_p) for ANOVAs and Cohen's *d* for t-tests. The level of significance was set at P≤0.05 for the statistical tests. Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, New York).

Results

Subject data

There were no significant differences between sexes for age (females = 19.82±1.40 yrs, males = 20.25±1.86 yrs; P=0.540, *d*=0.260). However, significant differences did exist between sexes for height (females = 164.86±6.34 cm, males = 179.63±7.13 cm; P<0.001, *d*=2.181), body mass (females = 66.19±10.64 kg, males = 80.79±12.84 kg; P=0.002, *d*=1.486), and isometric MVC strength (females= 122.59±28.34 N, males = 218.38±63.30 N; P<0.001, *d*=1.923).

Isometric strength testing

All subjects maintained the required relative torque output throughout the contractions. For females, the mean torque output for epochs 1 and 2 were 41.14 ± 1.28% MVC and 40.34 ± 2.39% MVC, respectively. For males, the mean torque output for epochs 1 and 2 were 40.86 ± 1.24% MVC, and 40.73 ± 1.48% MVC, respectively. There were no significant differences between epochs or sexes.

MU data

MU counts and recruitment ranges are presented in Table 1. All relationships possessed RT ranges >15% MVC. We did not record MUs being recruited during steady torque for most subjects, however, we did for 3 female and 5 male subjects. The T_{REC} ranges were from 3.9±0.2 to 6.7±4.8 s and 4.5±0.7 to 8.4±3.3 s during the steady torque segment for females and males, respectively. The remaining subjects did not have
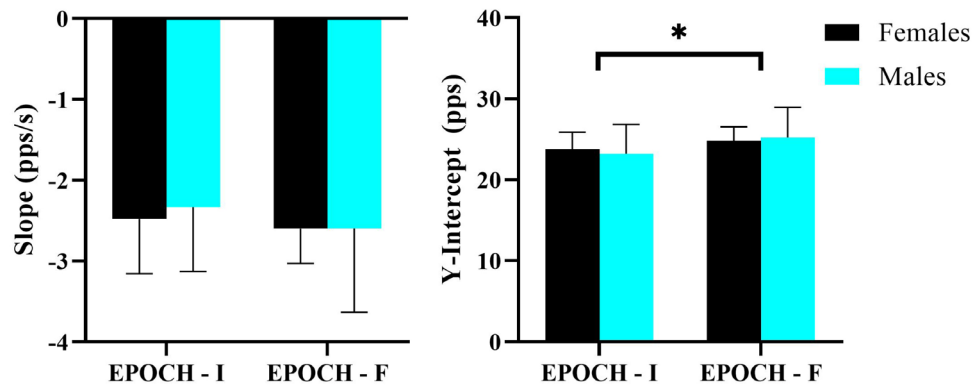
Figure 2. Means and standard deviations for slopes and y-intercepts of the mean firing rate (MFR, pps) vs. time at recruitment (T_{REC}, s) relationship. * Indicates significant differences between epochs (P<0.05)
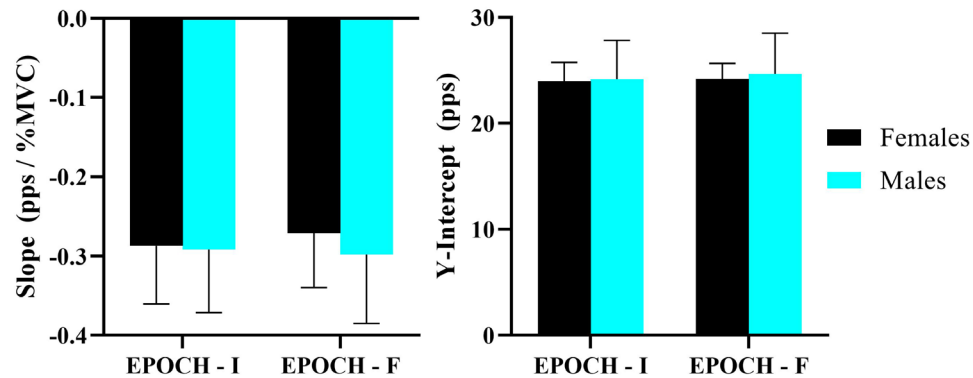

Figure 3. Means and standard deviations for slopes and y-intercepts of the mean firing rate (MFR, pps) vs. recruitment threshold (RT, expressed as percentage of maximal voluntary contraction [%MVC]) relationships.

MUs decomposed that were recruited during the steady torque segment. The MFR vs. T_{REC} relationships for each subject were significant and possessed negative slopes (I: $r=$ -0.94±0.04; F: $r=$-0.91±0.06). The MFR vs. RT relationships for each subject were also significant and possessed negative slopes (I: $r=$-0.95±0.02; F: $r=$-0.92±0.06).

MFR vs. T_{REC} relationship

For the MFR vs. T_{REC} relationships, there were no significant two-way interactions (sex × epoch) or main effects for slope (P=0.409−0.756, 1-β=0.06−0.127, η^2_p=0.005−0.033). There were no significant two-way interactions or main effect for sex for the y-intercepts (P=0.363−0.932, 1-β=0.051−0.144, η^2_p=0−0.04). However, there was a significant main effect for epoch for the y-intercepts (P=0.009, 1-β=0.790, η^2_p=0.286) (Figure 2). The final epoch (25.03±0.6 pps/s) was greater than the initial epoch (23.50±0.6 pps/s) collapsed across sex.

MFR vs. RT relationship

For the MFR vs. RT relationships, there were no significant two-way interactions (sex × epoch) or main effects for slope (P=0.215−0.617, 1-β=0.077−0.231, η^2_p=0.012−0.072) or y-intercepts (P=0.059−0.792, 1-β=0.058−0.478, η^2_p= 0.003−0.160) (Figure 3).

Grouped MFR analysis

For the grouped MFR analysis, there were no significant two-way interaction (sex × epoch; P=0.761, 1-β=0.06, η^2_p=0.004). However, there were main effects for sex (P=0.049, 1-β=0.514, η^2_p=0.172) and epoch (P<0.001, 1-β=0.998, η^2_p=0.547) (Figure 4). Females (18.8±0.5 pps) possessed greater firing rates than males (17.4±0.5 pps)
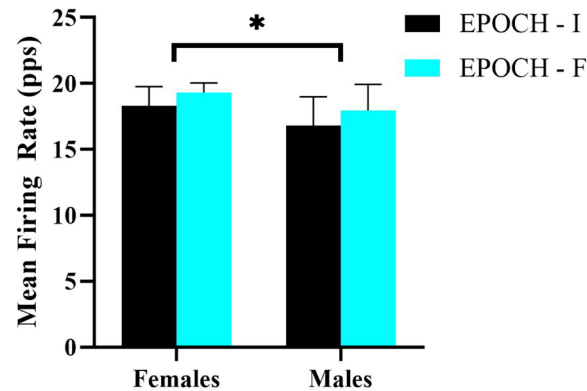
Figure 4. Means and standard deviations for the mean firing rates (MFR, pps) during each epoch for the grouped analysis. * Indicates significant differences between sexes (P<0.05).

collapsed across epochs and the final epoch (18.6±0.3 pps) was greater than the initial epoch (17.5±0.4 pps) collapsed across sex.

Discussion

A major finding of this study was that analysis methods that included MUs recruited during steady torque indicated increases in MU firing rates over the course of the contraction. Additionally, sex-related differences in firing rates were only observed when MUs were grouped across subjects and recruitment thresholds were not considered. Therefore, conclusions regarding underlying physiological mechanisms that are responsible for time-related changes or differences between populations for MU firing rates may be biased as a function of statistical methods.

An orderly pattern of firing rates is evident with strong relationships (r>0.90) when MFRs are regressed against RT separately for individuals and contractions. The earlier-recruited lower-threshold MUs achieved higher firing rates in comparison to the later-recruited higher-threshold MUs during each epoch (Figure 5A). Similarly, strong relationships (r>0.90) were observed when MFRs were regressed against T_{RECS}. Therefore, MUs recruited during the steady torque segment (latest-recruited) possessed lower firing rates than the already recruited MUs (Figure 5B). The MFR vs. T_{REC} relationships demonstrate a clear organized firing rate scheme throughout the contraction despite the recruitment of additional MUs.

The MFR vs. T_{REC} relationships indicated that MU firing rates increased to maintain required torque output during the prolonged contractions. The y-intercepts increased significantly from the beginning of the prolonged contraction to the end (Figure 2), but the slopes were not significantly different, indicating that MU firing rates increased in a relatively uniform manner. These findings support the

previously modeled rightward shift in the operating point of the muscle[50]. However, the main effect for y-intercepts across epoch was driven largely by males (I=23.21±3.65 pps, F=25.22±3.73 pps, P=0.034) (Figure 2). No increases were observed for females (I=23.80±2.08 pps, F=24.83±1.72 pps, P=0.123), which could imply that the task was insufficient to elicit fatigue in females. Evidence for this comes from the observation that females exhibit greater resistance to fatigue during sustained isometric contractions through sex-related neural and muscular mechanisms, as described by Hunter[29].

In contrast, there were no changes observed in the MFR vs. RT relationships during the prolonged contraction, as indicated by the absence of differences in slopes or y-intercepts between epochs. The MFR vs. RT relationships are often used to decipher differences in MU activity as a function of contraction intensity[9,10,51], populations[24,36,51–53], and interventions[33,35,54]. However, in this context the relationship is limited since MUs recruited during steady torque are assigned similar RTs and, therefore, MUs recruited during steady torque must be excluded from analysis. RTs typically provide information about differences in the properties of the MUs, such as, firing rates[55,56]. In this instance, similar RTs would be assigned to MUs recruited well into steady torque that may possess different properties (i.e., lower firing rates)[23,50] (Figure 1). However, while there was no significant change for the y-intercepts across epoch, the effect size would suggest a moderate-to-strong magnitude of increase (η_p^2=0.160) in the y-intercepts from the beginning (24.08±2.86 pps) to the end (24.43±2.91 pps) of the prolonged contraction (Figure 3), unlike for the slopes. Nevertheless, the MFR vs. RT relationships were not sensitive to changes in MU firing rates that were occurring during the prolonged contraction (Figure 3).

A third analysis method uses a grouped approach by pooling all subjects and MUs together[4,48,49]. This allows inclusion of MUs recruited during steady torque and avoids
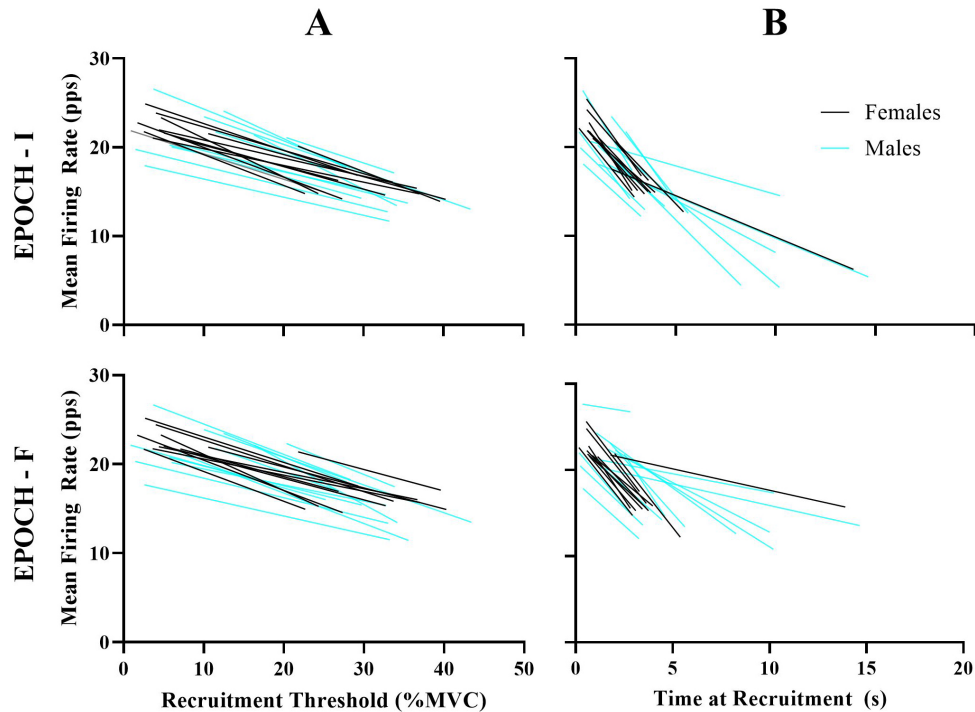
Figure 5. Regression lines representing the (A) mean firing rate (MFR, pps) vs. recruitment threshold (RT, expressed as percentage of maximal voluntary contraction [%MVC]) and (B) MFR vs. time at recruitment (T_{REC}, s) relationships for females and males. The regression lines were plotted within the recorded MU recruitment range for each subject.
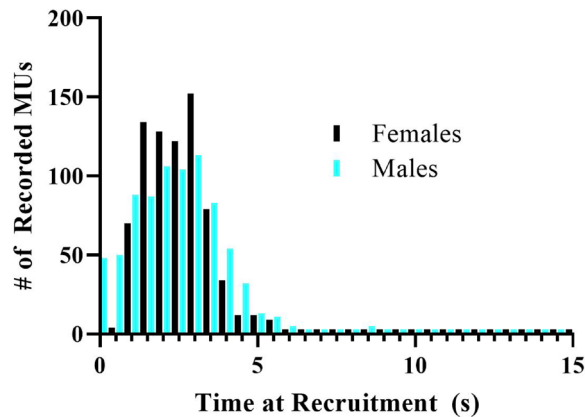

Figure 6. Number of motor units recorded during each time at recruitment (T_{REC}) interval for females and males.

issues with similar RTs. In this study, using this approach resulted in increases in firing rates across the prolonged contraction, similarly to the increase in y-intercepts observed in the MFR vs. T_{REC} relationships (Figure 4). These findings reflect the results reported by Dorfman et al.[5], who demonstrated increases in firing rates from the beginning to

end of prolonged contractions in the biceps brachii at 20% MVC. In contrast, other studies reported decreases[2,57] or no changes[6] in firing rates from the beginning to end of prolonged contractions in the FDI at 50% MVC, rectus femoris at 17-35% MVC, and brachioradialis at 25% MVC, respectively. The grouped analysis method discounts information about

RTs and individuals. Therefore, an individual could have a disproportionately strong influence on the average firing rate based on the range of MUs recorded (RT range= 5 to 25% vs. 25 to 40% MVC). Additionally, using this approach obscures any ability to discern whether changes in firing rates are due to change in recruitment or discharge rates. Thus, the inconsistences among studies could be the result of not accounting for variability as a function of individuals and RTs.

An additional finding of this study was that there were no significant sex-related differences in firing rates via the calculated relationships. However, females had greater firing rates for the MFR grouped analysis. This is similar to previously reported findings by Inglis and Gabriel[25], which found that females demonstrated higher firing rates across submaximal contraction intensities (20-80%) using a grouped analysis. However, this finding contrasts with Harwood et al.[26], which found no significant differences between males and females at 15% MVC while also using a grouped analysis. Additionally, when accounting for recruitment threshold, Parra et al.[28] reported no sex-related differences in the MFR vs. RT relationships for the FDI at 10% MVC, and Peng et al.[31] found no differences in the vastus medialis at 30% MVC. For the present study, the difference between sexes observed in the grouped relationships could potentially be attributed to differences in sampling rather than the physiological characteristics of MUs (Figure 6). Referring to Figure 6, there was a greater likelihood of recording MUs that were recruited earlier for the females (mean T_{REC}=2.27±1.19 seconds) than males (mean T_{REC}=2.72±1.61 seconds). Sixty percent and 47% of MUs for females and males were recruited prior to the group mean of 2.51 seconds and, thus, illustrate the greater propensity to record earlier recruited MUs for the females. MUs activated earlier during the contraction possessed greater firing rates in comparison to the later recruited MUs. Therefore, sampling differences of the recruited MU pool could be the primary explanation for differences between sexes rather than physiological mechanisms.

A limitation of this study was that only one muscle (VL) was studied. However, a previous investigation has noted no differences in the temporal trends in activation between different leg extensor muscles during a prolonged submaximal contraction (50% MVC)[58]. Likewise, Adam and DeLuca[18] found that during prolonged isometric contractions of the knee extensors at 20% MVC, relative force contributions from VL agonists and antagonists remained constant throughout the duration of the contractions.

In summary, differences in MU firing rates were observed across the duration of a prolonged contraction when analysis included MUs recruited during steady torque (i.e. grouped analysis and MFR vs. T_{REC} analysis). In contrast, no changes in MU firing rates were observed when MUs were identified by RT. This represents two novel findings: (1) MU firing rates increase over the course of prolonged contractions and (2) analysis method significantly impacts the interpretation of MU firing rate behavior. We contend that firing rate analysis performed via MFR vs. T_{REC} relationship analysis presents the best option to make detailed observations of firing rate behavior over the course of prolonged contractions. It allows for observations of (1) differences in the properties of individual MUs and (2) the ability to include MUs that are recruited during steady torque. Additionally, sex-related differences were only observed when performing analysis on grouped contractions where sampling bias may be a concern.

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