Motor unit recruitment strategies of the vastus lateralis according to sex

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Key points

- Increases in muscle force production are mediated by motor unit (MU) recruitment and firing rate.
- Women are underrepresented in studies of human neuromuscular research and markedly differ to men in a number of aspects of neuromuscular function, yet little is known of the recruitment strategies of each.
- Here we show with increasing contraction levels, men and women exhibited higher MU firing rates, and larger and more variable MU potentials. The magnitude of increase did not differ according to sex, nor did motor unit number estimates (MUNE).
- Minimal sex-based differences of muscle recruitment strategies, MU features and MUNE highlight key similarities in neuromuscular function across sex and support greater inclusion of women in controlled studies of this nature.

Abstract

Despite men exhibiting greater muscle strength than women it remains unclear if there are sex-based differences in muscle recruitment strategies e.g. motor unit (MU) recruitment and modulation of firing rate with increasing force. These differences may explain underrepresentation of women and mixed-sex cohorts in studies of neuromuscular function. Twenty-nine healthy male and thirty-one healthy female participants (18-35 years) were studied. Intramuscular electromyography was used to record individual motor unit potentials (MUPs) and near fibre MUPs from the vastus lateralis (VL) during 10% and 25% maximum isometric voluntary contractions (MVC), and spike-triggered averaging was used to obtain motor unit number estimates (MUNE) of the VL. Multilevel mixed-effects linear regression models were used to investigate the effects of sex and contraction levels. Men exhibited greater muscle strength ($p<0.001$) and size ($p<0.001$) than women, with no difference in force steadiness at 10% or 25% MVC. Although women showed higher firing rate (FR) ($p=0.014$) and FR variability ($p=0.044$), both sexes showed similar increasing trajectories from low- to mid-level contractions with no sex x contraction level interactions. With increasing contraction level, both men and women exhibited greater MUP size ($p<0.001$) and neuromuscular junction transmission instability ($p<0.001$). There were no sex differences in MUNE calculated at 25% MVC. Although some sex-based neuromuscular differences are apparent, similar MU recruitment strategies are employed to increase force production during low to moderate contractions. These findings of notable similarities between sexes support the use of mixed sex cohorts in studies of this nature.

Keywords

Sex differences, motor unit, neuromuscular junction, vastus lateralis

Introduction

Skeletal muscle contraction is regulated by central and peripheral motor and sensory nerve function and excitation-contraction coupling of muscle fibres. The fundamental neuromuscular element regulating muscle contraction is the motor unit (MU), consisting of a motor neuron and the muscle fibres it innervates (Heckman and Enoka, 2012). Progressive increases in muscle force are largely mediated by recruitment of additional MUs and an increase in MU firing rate, referred to as recruitment and rate modulation (Enoka and Duchateau, 2017). Several studies have highlighted adaptative remodelling of MUs in response to exercise training, ageing and disease (Miller et al., 2014, Piasecki et al., 2016b, Piasecki et al., 2019), with the majority of data only available in men.

Men generally possess greater muscle strength than women in upper and lower extremities, which is largely explained by greater muscle size (Hannah et al., 2012). Conversely, although task-specific, women are generally more resistant to neuromuscular fatigue when assessed at a normalised contraction level (Hunter, 2014), which in the knee-extensors, is likely explained by fibre type ratios with a 7-23% greater proportion of type I fibres in vastus lateralis (VL) in women (Haizlip et al., 2015, Ansdell et al., 2020). Sex differences of the hormonal milieu also influence neuromuscular function; testosterone and estrogen are the major sex hormones in males and females, respectively, and each exhibites a range of neuroprotective effects in motoneurons, such as dendritic maintenance and axonal sprouting (Hyer et al., 2018). Furthermore, hormonal metabolites are associated with the release of brain-derived neurotrophic factors (BDNF) (Georgieva, 2018), which are key mediators of synaptic plasiticity (Mantilla et al., 2014). Acutely, differences in sex hormones partly explain the variability in fatigability in women across phases of the menstrual cycle (Ansdell et al., 2019). Such differences in the hormonal milieu are difficult to experimentally control for and may explain why women are often excluded from and underrepresented in neuromuscular physiology (Cowley et al., 2021).

Surface electromyography (EMG) has been commonly applied to study sex-based differences of neuromuscular function (Clark et al., 2005, Bolgla et al., 2014). However, such approaches are limited by the distance between activated MUs and recording electrodes (Jones et al., 2021), offering poor MU yield in women, and in some cases, being influenced by adjacent muscles (Christie et al., 2009). These limitations can be overcome with the use of intramuscular EMG (iEMG), which also has the added benefit of revealing further electrophysiological parameters relevant to MU size and complexity (Piasecki et al., 2021b). Although we have previously reported the influence of sex on MU features in long-term trained master athletes (Piasecki et

al., 2021a), comparisons of normative values in healthy young men and women at differing contraction levels are unknown. The aims of the present study were to compare individual MU properties and muscle recruitment strategies, as well as the MU number estimates (MUNEs) in the VL of healthy young men and women. We hypothesised several parameters would differ in accordance with differences in MU size, structure and MUNE, with no sex-based differences in proportional increases when moving from a low- to a mid-level contraction.

Materials and Methods

Ethics approval

This research was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (C16122016, 160-0121, 186-1812, 103-1809, 302-1903) and was conducted between 2019 and 2021 in accordance with the Declaration of Helsinki.

Participants

Twenty-nine healthy male and thirty-one healthy female participants, aged 18-35 years, were recruited via advertisement posters in the local community. All the participants volunteered to take part in the studies and provided written informed consent. Prior to enrolment, all participants completed a comprehensive clinical examination and metabolic screening were conducted at the School of Medicine, Royal Derby Hospital Centre. All articipants were recreationally active. Participants with metabolic disease, lower limb musculoskeletal abnormalities, acute cerebrovascular or cardiovascular disease, active malignancy, uncontrolled hypertension, or those on medications that impact muscle protein metabolism or modulate vascular tone were excluded.

Anthropometry

Body mass and height were measured using calibrated scales and a stadiometery, respectively for the calculation of body mass index (BMI). Ultrasound was used to measure the cross-sectional area of the vastus lateralis (VL) muscle using an ultrasound probe ((LA523 probe, B-mode, frequency range 26–32 Hz, and MyLabTM50 scanner, Esaote, Genoa, Italy) at the anatomical mid-point of the muscle which was identified between the greater trochanter and the mid-point of the patella with participants lying supine. Ultrasound images were acquired aligning the superior edge of the probe following a middle-to-lateral direction position on the skin, beginning, and ending the image capture at aponeurosis borders. A water-based

conductive gel was applied on the surface of the ultrasound probe to enhance the fidelity of the image without causing excessive contact pressure on the skin during the acquisition of the images. Images were subsequently analysed using ImageJ software (National Institutes of Health, USA) to quantify CSA measurements. The mean area of three images was taken as CSA. The CSA of eight female participants was measured using magnetic resonance imaging (MRI) with a T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoa, Italy). Continuous transversal images with a 6-mm slice were acquired and analysed by using Osirix imaging software (Osirix medical imaging, Osirix, Atlanta, GA, United States) through tracing around the VL following the contour of the aponeurosis.

Muscle strength and force steadiness

The maximum isometric voluntary contraction force (MVC) of the right knee extensor was assessed with the participants sitting in a custom-built chair with hips and knees flexed at ~90 degrees. The lower leg was securely attached to a force dynamometer with non-compliant straps (purpose-built calibrated strain gauge, RS125 Components Ltd, Corby, UK) slightly above the medial malleolus. A seat belt was fastened across the pelvis to avoid superfluous movement of the upper trunk during the test. To obtain the external knee joint moment arm, the distance from centre of the force strap to the lateral femoral condyle was measured. After a standardised warm-up of submaximal contractions, participants were instructed to perform a maximal isometric contraction with real-time visual feedback and verbal encouragement. This was repeated a further two times, with 60 second rest intervals between each, and the highest value was accepted as MVC. Peak torque during the selected MVC was also determined.

After determination of the MVC, participants were instructed to perform between four and six sustained isometric contractions at 10% and 25% MVC, respectively, each lasting 12-15 seconds with a target line displayed on the screen in front of the participants (with iEMG, below). Participants had ~30 seconds rest between each contraction. Prior to the assessment, participants were allowed a single familiarisation practice at each contraction level. Force steadiness was quantified as the coefficient of variation of the force [CoV; (SD/mean) × 100]. The mean CoV at each contraction level was calculated from the middle two contractions.

Surface electromyography (sEMG)

An active recording sEMG electrode (disposable self- adhering Ag-AgCl electrodes; 95 mm^2, Ambu Neuroline, Baltorpbakken, Ballerup, Denmark) was placed over the motor point located around the mid-point of the VL, identified using a cathode probe (Medserve, Daventry, UK)

to apply percutaneous electrical stimulation at 400 V, pulse width of 50 μs and current of around 8 mA (DS7A Digitimer, Welwyn Garden City, Hertfordshire, UK) with a self-adhesive anode electrode (Dermatrode, Farmadomo, NL) placed over the right gluteus. A reference electrode was placed over the patella tendon and a common ground electrode placed over the patella. The common ground electrode served for both sEMG and iEMG measurements. sEMG signals were sampled at 10kHz, and bandpass filtered between 5 and 5 kHz (1902 amplifier, Cambridge Electronics Design Ltd., Cambridge, UK) and digitized with a CED Micro 1401 data acquisition unit (Cambridge Electronic Design) for offline analysis.

Compound muscle action potential (CMAP)

The CMAP of the VL was evoked by a manually triggered stimulator (model DS7A; Digitimer) using percutaneous stimulation (Medserve, Daventry, UK) of the proximal femoral nerve (approximately halfway between the anterior superior iliac spine and the pubic tubercle) with a carbon-rubber anode electrode (Dermatrode self-adhering electrode, 5.08 cm in diameter; Farmadomo Linde Homecare Benelux Bv, Leiden, The Netherlands) placed over the skin overlying the gluteus muscle. The stimulator voltage was fixed at 400 V and the pulse width at 50 μS, with the current increased incrementally until the M-wave amplitude plateaued. At this point, the current was increased again by ~30 mA to ensure supramaximal stimulation, ensuring a sharp rise time of the negative peak of the m-wave.

Intramuscular electromyography (iEMG)

A 25-mm disposable concentric needle electrode (N53153; Teca, Hawthorne, New York, USA) was inserted at the muscle belly of VL, adjacent to the recording surface electrode over the motor point, to a depth of 1.5-2 cm depending on the muscle size. The iEMG shared the same ground electrode as the sEMG, which was placed over the patella. iEMG signals were recorded using Spike2 (Version 9.06), sampled at 50 kHz and bandpass filtered at 10 Hz to 10 kHz (1902 amplifier; Cambridge Electronic Design Ltd, Cambridge, UK) and stored for future off-line analysis.

Prior to EMG and CMAP assessments, participants performed a series of voluntary, low-level contractions once the needle was positioned to ensure adequate signal to noise ratio, thus ensuring the recording needle electrode was close to depolarizing fibres. Each participant then performed the sustained voluntary isometric contractions as detailed above. After a 10% and 25% MVC contraction, to avoid repeat sampling of the same MUs, the needle electrode was repositioned by the combinations of twisting the bevel edge 180 degrees and withdrawing by

~5 mm. This process was repeated until four to six contractions from spatially distinct areas (from deep to superficial portions) were recorded (Jones et al., 2021). Participants had ~30 seconds rest between each contraction.

EMG analysis

Decomposition-based quantitative electromyography (DQEMG) software was used to detect motor unit potentials (MUPs), extract motor unit potential trains (MUPTs) generated by individual MUs from the iEMG signals and estimate, via ensemble averaging, their corresponding surface MUPs (sMUPs) from the sEMG signals (Stashuk, 1999). MUPTs that were composed of MUPs from more than one MU or had fewer than 40 MUPs were excluded. The occurrence times of individual MUPs within a MUPT were used to trigger and align sEMG signal epochs for ensemble-averaging to produce an estimate of their corresponding sMUPs. The sMUPs estimated from signals recorded during 25% MVC contractions were use to represent MU size. All MUP and sMUP templates were visually inspected and their markers adjusted, where required, to correspond to the onset, onset of negative phase (sMUP only), end, and positive and negative peaks of the waveforms.

MUP amplitude was measured from the maximal positive and negative peaks and the MUP area was taken as the total area within the MUP duration (onset to end) and is indicative of MU size. The number of phases and turns are measures of MUP complexity and are classified as the number of components above or below the baseline (phases) and a change in waveform direction of at least 25 μV (turns), which indicates the level of temporal dispersion across individual muscle fibre contributions to a single MUP. MU firing rate (FR) was assessed as the rate of MUP occurrences within a MUPT, expressed as the number of occurrences per second (Hz).

A near fibre MUP (NFM) is defined as the acceleration of its corresponding MUP and calculated by applying a second-order, low-pass differentiator to the MUP which effectively reduces the recording area of the needle electrode to within ∼350 μm, thereby ensuring only potentials from fibres closest to the needle electrode significantly contribute to the NFM and reducing interference from distant active fibres of other MUs. NFM jiggle is a measure of the shape variability of consecutive NFMs of an MUPT expressed as a percentage of the total NFM area. NFM segment jitter is a measure of the temporal variability of individual fibre contributions to the NFMs of a MUPT. It is calculated as a weighted average of the absolute values of the temporal offsets between matched NFM segments of consecutive isolated (i.e., not contaminated by the activity of other MUs) NFMs across an MUPT expressed in

microseconds. NFM dispersion is the time, in ms, between the first and last MU fibre contributions (Piasecki et al., 2021b).

Motor unit number estimates (MUNE)

The MUNE value was derived by dividing the negative peak area of the ensemble averaged mean surface MUP (msMUP) from 25% MVC into the negative peak area of the CMAP (Brown et al., 1988). A msMUP is an ensemble average of the negative-peak onset aligned, sMUPs of the MUs sampled from a muscle. The negative peak area of the msMUP was divided into the negative peak area of the electrically evoked CMAP (Piasecki et al., 2018). MUNE values are available for 15 men and 15 women.

Statistical analysis

All of the statistical analysis was performed using RStudio (Version 1.3.959 for macOS) (RStudioTeam, 2020). Descriptive statistics of participant characteristics are presented as *mean ± standard deviation (SD)*. *Student's unpaired t-test* was used to compare physical parameters (age, BMI, MVC, CSA, and force steadiness). As multiple MUs were recorded from each participant, *multi-level mixed-effect linear regression analysis* was performed to investigate these MU parameters with sex and contraction level as factors through the package *lme4* (Version 1.1.23) (Bates D, 2015). In the linear mixed models, the first level was single motor unit; single motor units were clustered according to each participant to form the second level, which was defined as the participant level. This linear mixed-effect modelling framework is suitable for data of this nature as it: i) incorporates the whole sample of extracted MUs not just the mean values obtained from each participant, which preserves variability within and across participants simultaneously to the greatest extent; ii) handles missing data better than an *analysis of variances (ANOVA)* framework as the removal of a single missing observation has a much smaller effect in the mixed model; and iii) provides coefficient estimates that indicate the magnitude and direction of the effects of interest (Brown, 2021). Interactions were first examined and where not present they were removed from the model, sex and level were explored individually. The results are displayed as coefficient estimates, 95% confidence intervals, and *p*-values. Standardized estimates were calculated through the package *effectsize* (Version 0.4.5) (Ben-Shachar MS, 2020) for forest plotting. Significance was assumed when $p < 0.05$.

Results

The means and standard deviations for the participant's characteristics are shown in Table 1. Significant differences between men and women were detected for weight, height, BMI, peak torque and VL CSA (all $p<0.05$). There were no significant sex differences for age and force steadiness (both $p>0.05$). Individual values are shown in Figure 1.

Table 1. Participant characteristics.

Measure	Men (n=29)	Women (n=31)	P value
Age (years)	23.7(5.0)	22.9(3.6)	0.490
Weight (kg)	81.2(11.6)	63.1(10.1)	**<0.001**
Height (cm)	180.3(7.5)	165.8(6.8)	**<0.001**
BMI (kg/m^2)	25.0(2.9)	23.0(3.2)	**0.012**
VL CSA (cm^2)	28.35(6.43)	19.00(3.90)	**<0.001**
Peak Torque (Nm)	251.65(67.72)	158.95(46.69)	**<0.001**
Force CoV-10%	4.51(1.21)	4.98(1.18)	0.139
Force CoV-25%	3.36(1.17)	3.24(0.74)	0.638

Data are reported as mean (standard deviation). Values in bold reflect statistically significant ($p<0.05$) results. Abbreviations: BMI, body mass index; VL CSA, vastus lateralis cross-sectional area.

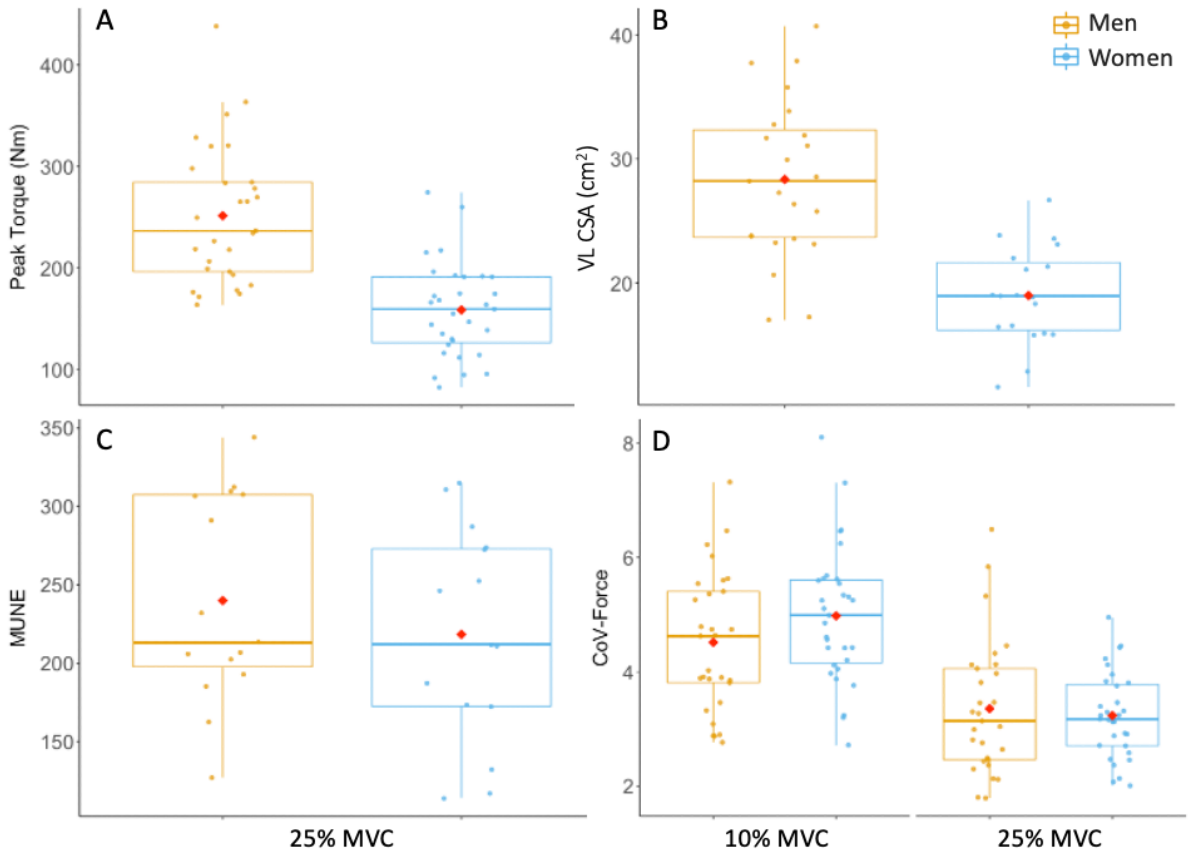

Figure 1. Box-and-jitter plots of the individual participant means, and group means (red dot) of **(A)** peak torque, **(B)** vastus lateralis cross-sectional area, **(C)** motor unit number estimates (MUNE), and **(D)** force steadiness at 10% and 25% maximum voluntary contraction (MVC), in men (yellow) and women (blue). Abbreviations: CoV, coefficient of variation.

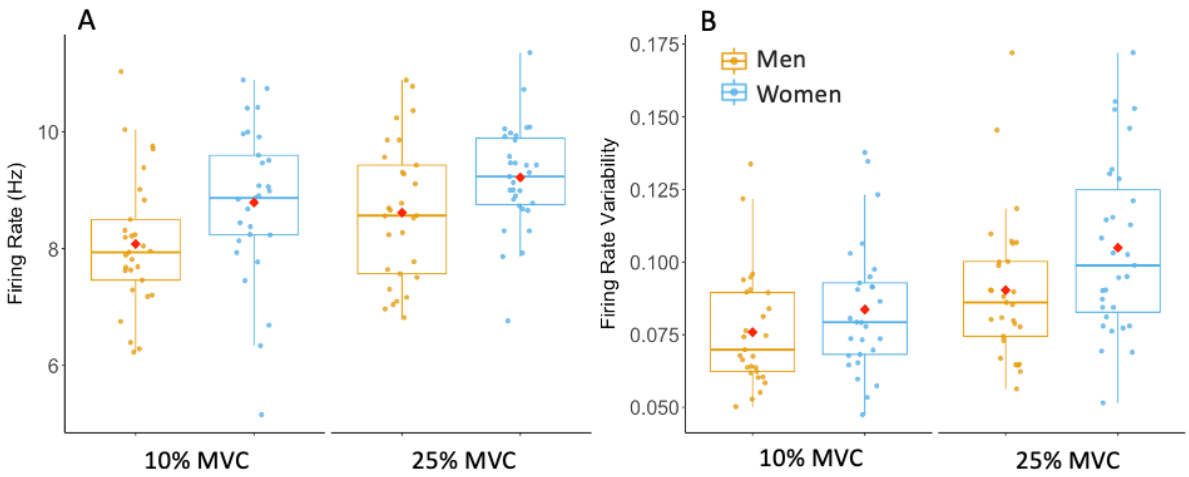

Figure 2. Box-and-jitter plots of the individual participant means, and group means (red dot) of **(A)**motor unit (MU) firing rate and **(B)** firing rate variability in men (yellow) and women (blue) at 10 and 25% maximum voluntary contraction (MVC).

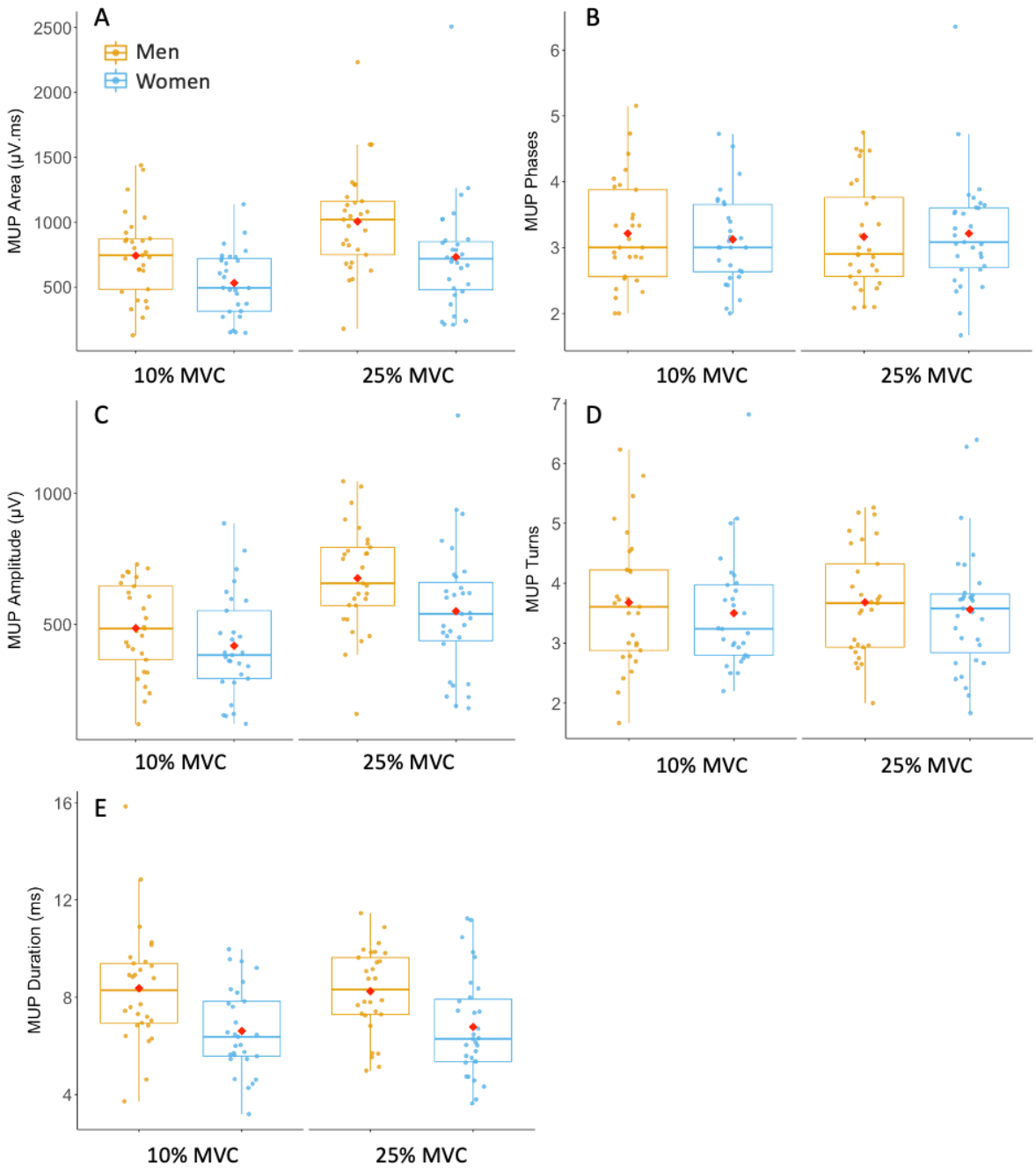

Figure 3. Box-and-jitter plots of the individual participant means, and group means (red dot) of motor unit potential (MUP) properties in men (yellow) and women (blue) at 10 and 25% maximum voluntary contraction (MVC).

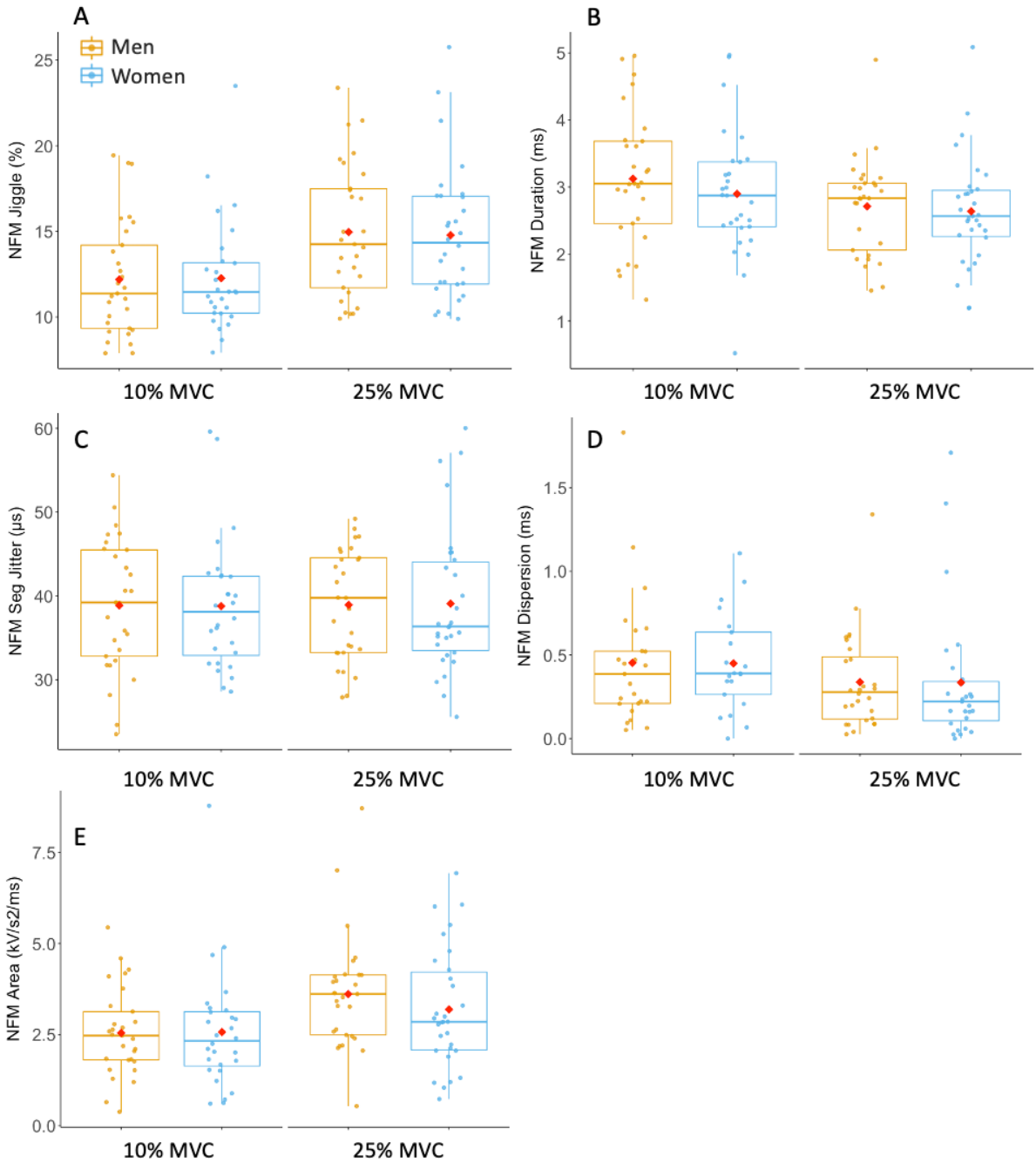

Figure 4. Box-and-jitter plots of the individual participant means, and group means (red dot) of near fibre motor unit potential (NFM) properties in men (yellow) and women (blue) at 10 and 25% maximum voluntary contraction (MVC).

There were no interactions between sex and contraction level in any of the motor unit parameters, indicating the difference from 10 to 25% MVC does not differ between men and women. When interactions were removed from the model, multi-level linear regression revealed a significant difference between sexes for MUP duration, MU FR and CoV-FR. MUP

duration was longer, and MU FR and MU FR variability were lower in men at both 10 and 25% MVC. There were no significant sex-based differences in any other related MU characteristic (Table 2).

Table 2. Adjuested sex difference of motor unit properties

Parameter	Beta	95%CI	P values
MU FR	0.64	0.14 to 1.13	**0.014**
MU FR Variability	0.01	0.00 to 0.02	**0.044**
MUP Phases	-0.03	-0.42 to 0.37	0.898
MUP Turns	-0.17	-0.65 to 0.31	0.484
MUP Duration	-1.57	-2.53 to -0.61	**0.002**
MUP Amplitude	-75.98	-178.81 to 26.85	0.153
MUP Area	-194.53	-389.62 to 0.57	0.056
NFM Jiggle	0.05	-1.61 to 1.71	0.955
NFM Seg Jitter	0.64	-2.94 to 4.22	0.728
NFM Area	-0.18	-0.86 to 0.50	0.614
NFM Duration	-0.08	-0.45 to 0.28	0.659
NFM Dispersion	0.02	-0.23 to 0.27	0.880

Beta value and 95% confidence interval (CI) represents the model predicted change per unit from men to women. The values in bold reflect statistically significant ($p<0.05$) results. All statistical analysis was based on multi-level mixed effect linear regression models with each subject as an independent cluster. Abbreviations: MU, motor unit; MUP, motor unit potential; FR, firing rate; NFM, near fibre motor unit potential; Seg, segment.

With increasing contraction level, both men and women exhibited higher MU FR and MU FR variability, as well as greater MUP amplitude, and larger MUP area (Table 3). NFM segment jitter, NFM duration and NFM area were also larger with the higher contraction level, increasing to a similar extent in men and women (Figure 2 and Figure 5).

Table 3. Motor unit properties at different contraction levels

Parameter	Sex	Beta	95%CI	P value
MU FR	Men	0.50	0.28 to 0.71	**<0.001**
	Women	0.43	0.18 to 0.69	**0.001**
MU FR Variability	Men	0.02	0.01 to 0.02	**<0.001**
	Women	0.02	0.01 to 0.02	**<0.001**
MUP Phases	Men	-0.05	-0.14 to 0.04	0.302
	Women	0.10	-0.02 to 0.21	0.098
MUP Turns	Men	0.05	-0.11 to 0.20	0.565
	Women	0.09	-0.08 to 0.26	0.316
MUP Duration	Men	-0.16	-0.52 to 0.20	0.381
	Women	0.13	-0.16 to 0.42	0.371
MUP Amplitude	Men	188.21	153.75 to 222.67	**<0.001**
	Women	142.40	106.27 to 178.53	**<0.001**
MUP Area	Men	273.62	215.81 to 331.43	**<0.001**
	Women	199.35	148.13 to 250.56	**<0.001**

NFM Jiggle	Men	3.08	2.38 to 3.79	**<0.001**
	Women	3.08	2.34 to 3.82	**<0.001**
NFM Seg Jitter	Men	0.77	-0.37 to 1.91	0.188
	Women	0.51	-0.85 to 1.87	0.463
NFM Area	Men	0.98	0.68 to 1.29	**<0.001**
	Women	0.72	0.39 to 1.05	**<0.001**
NFM Duration	Men	-0.40	-0.54 to -0.26	**<0.001**
	Women	-0.34	-0.50 to -0.18	**<0.001**
NFM Dispersion	Men	-0.09	-0.23 to 0.05	0.228
	Women	-0.05	-0.28 to 0.18	0.655

Beta value and 95% confidence interval (CI) represents the model predicted change per unit from 10 to 25% maximum voluntary contraction (MVC), shown separately for men and women. All statistical analysis was based on multilevel mixed effect linear regression models with each subject as an independent cluster. The values in bold reflect statistically significant ($p<0.05$) results. Abbreviations: MU, motor unit; MUP, motor unit potential; FR, firing rate; NFM, near fibre motor unit potential; Seg, segment.

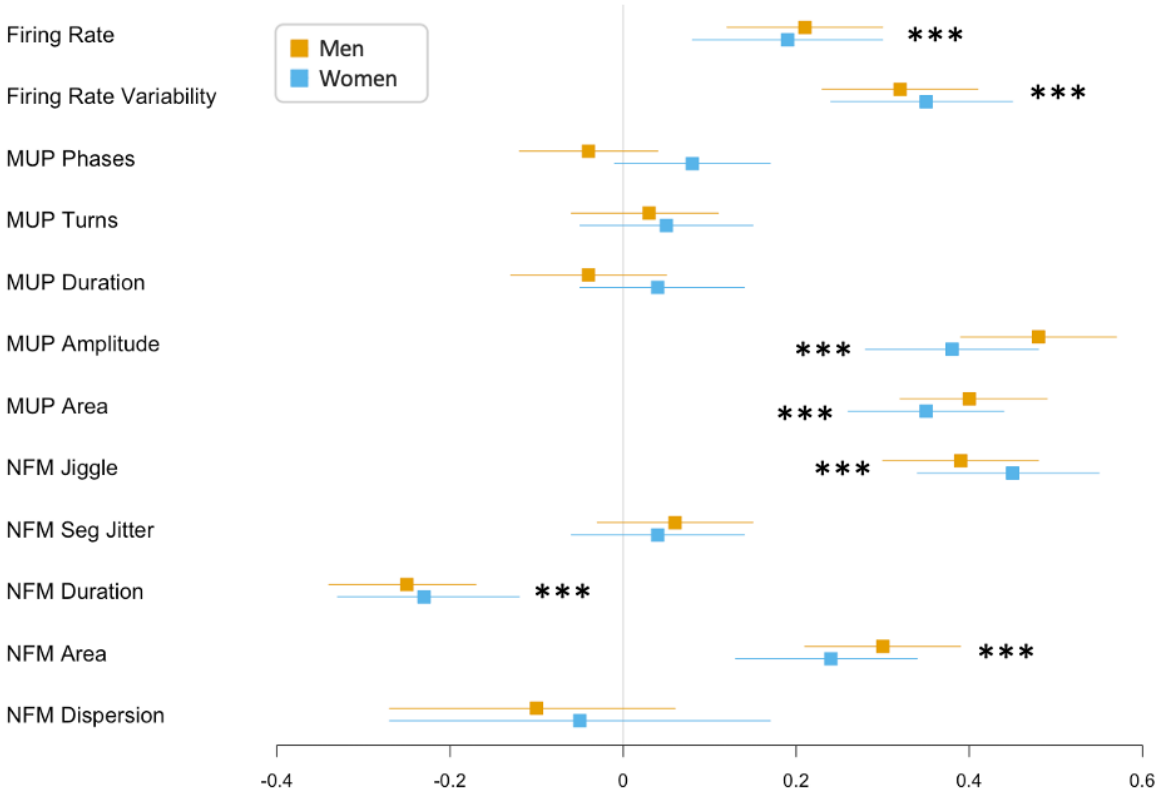

Figure 5. Forest plots of the standardised regression coefficient estimate for associations between motor unit characteristics and contraction levels in men and women models. Beta value and 95% confidence intervals (CI) represents the standardised model predicted change per unit from 10 to 25% maximum voluntary contraction (MVC). All statistical analysis was based on multilevel mixed effect linear regression models with each subject as an independent cluster, largely maintaining the motor unit variability within each subject. Standardised values of each parameter make the comparisons executable between men and women. *** =$p<0.001$.

Discussion

This is the first study to compare muscle recruitment strategies and motor unit number estimates of the VL using intramuscular electromyography techniques in healthy young men and women. Despite men having stronger and larger muscles, there were no differences in force steadiness at either lower level or moderate contraction levels between sexes. Alongside shorter MUP duration, women, compared with men, had higher MU FR and greater MU FR variability. With increasing contraction levels, both men and women exhibited higher MU FR and greater MUP size, with no sex x contraction level interactions, indicating a similar MU recruitment strategy during voluntary isometric contractions between sexes. In addition, there was no significant sex-based difference in motor unit number estimates of the VL. These data reveal very few sex differences in the MU characteristics after adjusting for contraction intensity.

Consistent with previous studies (Miller et al., 1993, Hannah et al., 2012, Jeon et al., 2019), women exhibited 33% smaller muscle size (CSA of the VL), which was reflected in a 31% lower strength. The greater MU FR of women shown here in VL is in an agreement with some but not all previously published data and again highlights probable muscle specific confounders. For instance, women exhibited higher MU FR and MU FR variability compared with men in elbow flexors, flexor digitorum indicis, biceps, knee extensors and tibialis anterior (Taylor et al., 2003, Harwood et al., 2014, Peng et al., 2018, Inglis and Gabriel, 2021). However, others reported no sex difference in knee extensors during 30% MVC (Tenan et al., 2013) and significantly greater MU FR at 100% MVC in tibialis anterior in men (Christie and Kamen, 2010, Inglis and Gabriel, 2020). These differences in intrinsic motoneuron excitability may be explained by sex-specific levels of persistent inward currents, which amplify and prolong synaptic input to MUs and affected by the levels of central monoanmines (Heckman et al., 2008), which are reported to be higher in women (Nishizawa et al., 1997). As expected, in the current study MU FR increased with increasing force levels in both men and women, accompanied by greater MU FR variability. Recruitment threshold dominates at lower force levels, whereas MU FR is more significant at higher forces (De Luca et al., 1982, Contessa and De Luca, 2013), which may explain the increased variability with increased force. The similar trajectory of increases in MU FR in men and women indicates both sexes follow similar discharge pattern changes across low to mid-level contractions.

Despite large differences in muscle strength, force steadiness - representing the ability to hold a constant force, which is also influenced by MU FR and its variability (Yao et al., 2000, Enoka et al., 2003, Taylor et al., 2003, Farina et al., 2014), did not differ between sexes at either contraction level. Differing from the current findings, Inglis (Inglis and Gabriel, 2021) found that women had a greater discharge variability and greater fluctuation in steadiness than men during dorsiflexion in tibiais anterior muscles, which may indicate a muscle specific sex difference. In the current study, both men and women exhibited greater force steadiness at 25% MVC compared 10% MVC, consistent with Inglis's finding that very high and low force outputs have greater fluctuations compared to moderate force outputs (Yoon et al., 2014, Inglis and Gabriel, 2021).

The size of a MU can be estimated by the size of the MUP recorded using intramuscular electrodes. As previously mentioned, men typically exhibit larger muscle size than women, (Staron et al., 2000, Haizlip et al., 2015). However, MUP area and amplitude did not differ according to sex, indicating similar MU sizes. As expected, markers of MU size increased at larger contraction levels, as larger MUs are recruited to produce larger forces. Again, the trajectory of each was similar for men and women indicating MU recruitment strategies moving between these force levels do not differ between sexes. When making direct sex comparisons, the shorter MUP duration observed in women may reflect differences in predominant fibre types recruited at a given force level; it is possible that women recruited a greater proportion of type II fibres with greater conduction velocity (Kupa et al., 1995, Jeon et al., 2019).

A NFM is derived from a MUP, such that is primarily composed of contributions from MU fibres close to the intramuscular electrode (Piasecki et al., 2021b). Here the NFM area increased, while NFM duration decreased with higher contraction level albeit to a similar extent in both sexes. These contraction induced alterations may be the result of the activation of larger MU fibres with greater conduction velocity during higher level contractions. NFM dispersion was not affected by sex or contraction level. Increases in NFM instability, as measured by NFM jiggle or NFM segment jitter can reflect increases in neuromuscular junction (NMJ) transmission instability with age (Hourigan et al., 2015, Piasecki et al., 2016a, Piasecki et al., 2016c, Piasecki et al., 2021a) and in diabetic neuropathy (Allen et al., 2015). In the current study NFM instability, as measured by NFM jiggle, increased with contraction level for both sexes, and to similar extents. NFM jiggle is based on variability in the amplitudes of NFM shapes, and although these amplitude changes are normalized by the size of the NFM, it is possible that these increases with contraction level may be due to the recruitment of larger MUs

with more MU fibres contributing to larger NFMs at 25% MVC. Combined with the lack of a sex difference in NFM segment jitter, it is clear NMJ transmission instability in the VL is sensitive to contraction level and is similar in heathy young men and women. However, there were no statistically significant contraction-based differences in NFM segment jitter, which is based on variability in the occurrence times of NFM segments and is not affected by NFM size, indicating it is less sensitive to the influences of contraction level.

The mean values of MUNE in men (240 ± 66) and women (218 ± 68) reported here are similar to those we have previously reported in male cohorts (Piasecki et al., 2016c, Piasecki et al., 2019), and highlights the repeatability of this method in this muscle group when applying identical experimental procedures. Although the MUNE should be viewed as an index relative to the number of MUs within a muscle and not a true anatomical count, the similar values reported here in men and women support minimal sex-based differences. Combined with the lack of statistical difference in MU size, the current data support the notion that sex-based differences in total muscle size are largely explained by greater individual fibre size in men (Staron et al., 2000).

Although providing a high level of detail of MU structure and function via MUPs and NFMs, iEMG is sensitive to contraction level and reliably identifying individual MU activity at high levels in this muscle can be problematic. Therefore, data presented here were obtained during low and mid-level contractions only. Secondly, we did not control for hormonal fluctuations in women naturally occurring during the menstrual cycle, and although there is limited evidence to confirm this, these fluctuations may influence several parameters included here. This is a direct comparison of MU features in young men and women, and further investigations concerning neural drive and influence of hormones or neural drive are still required to further understand the sex-based differences in the motor nervous system.

In summary, women exhibited higher MU FR and greater MU FR variability than men in the VL, which had no effect on force steadiness at normalised contraction intensities. With increasing contraction levels, both men and women exhibited higher MU FR and MUP size, indicating a similar MU recruitment strategy during voluntary isometric contractions. These results suggest that although some sex-based neuromuscular differences are apparent, similar MU recruitment strategies are employed to increase force production in this widely studied muscle group. These data do not support the notion of excluding women from studies of this nature.

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Author contributions

All authors contributed to the conception and design of the work. Y.G., E.J.J., T.B.I, I.A.E., J.P., and M.P. contributed to the acquisition and analysis of the data. YG analysed the data and drafted the manuscript. B.E.P, P.J.A., D.J.W, K.S., D.W.S, and M.P. provided comments. All authors have approved the final version of the submitted manuscript for publication and are accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Conflict of interest

The authors have no conflict of interest to declare.

Data availability statement

The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Reference

ALLEN, M. D., STASHUK, D. W., KIMPINSKI, K., DOHERTY, T. J., HOURIGAN, M. L. & RICE, C. L. 2015. Increased neuromuscular transmission instability and motor unit remodelling with diabetic neuropathy as assessed using novel near fibre motor unit potential parameters. *Clin Neurophysiol,* 126**,** 794-802.

ANSDELL, P., BROWNSTEIN, C. G., ŠKARABOT, J., HICKS, K. M., SIMOES, D. C. M., THOMAS, K., HOWATSON, G., HUNTER, S. K. & GOODALL, S. 2019. Menstrual cycle-associated modulations in neuromuscular function and fatigability of the knee extensors in eumenorrheic women. *Journal of Applied Physiology,* 126**,** 1701-1712.

ANSDELL, P., THOMAS, K., HICKS, K. M., HUNTER, S. K., HOWATSON, G. & GOODALL, S. 2020. Physiological sex differences affect the integrative response to exercise: acute and chronic implications. *Experimental Physiology,* 105**,** 2007-2021.

BATES D, M. M., BOLKER B, WALKER S 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software,* 67**,** 1-48.

BEN-SHACHAR MS, L. D., MAKOWSKI D 2020. effectsize: Estimation of Effect Size Indices and Standardized Parameters. *Journal of Open Source Software,* 5**,** 2815.

BOLGLA, L., COOK, N., HOGARTH, K., SCOTT, J. & WEST, C. 2014. Trunk and hip electromyographic activity during single leg squat exercises do sex differences exist? *Int J Sports Phys Ther,* 9**,** 756-64.

BROWN, V. A. 2021. An Introduction to Linear Mixed-Effects Modeling in R. *Advances in Methods and Practices in Psychological Science,* 4**,** 2515245920960351.

BROWN, W. F., STRONG, M. J. & SNOW, R. 1988. Methods for estimating numbers of motor units in biceps-brachialis muscles and losses of motor units with aging. *Muscle Nerve,* 11**,** 423-32.

CHRISTIE, A., GREIG INGLIS, J., KAMEN, G. & GABRIEL, D. A. 2009. Relationships between surface EMG variables and motor unit firing rates. *European Journal of Applied Physiology,* 107**,** 177-185.

CHRISTIE, A. & KAMEN, G. 2010. Short-term training adaptations in maximal motor unit firing rates and afterhyperpolarization duration. *Muscle Nerve,* 41**,** 651-60.

CLARK, B. C., COLLIER, S. R., MANINI, T. M. & PLOUTZ-SNYDER, L. L. 2005. Sex differences in muscle fatigability and activation patterns of the human quadriceps femoris. *European Journal of Applied Physiology,* 94**,** 196-206.

CONTESSA, P. & DE LUCA, C. J. 2013. Neural control of muscle force: indications from a simulation model. *J Neurophysiol,* 109**,** 1548-70.

COWLEY, E. S., OLENICK, A. A., MCNULTY, K. L. & ROSS, E. Z. 2021. "Invisible Sportswomen": The Sex Data Gap in Sport and Exercise Science Research. *Women in Sport and Physical Activity Journal***,** 1-6.

DE LUCA, C. J., LEFEVER, R. S., MCCUE, M. P. & XENAKIS, A. P. 1982. Control scheme governing concurrently active human motor units during voluntary contractions. *J Physiol,* 329**,** 129-42.

ENOKA, R. M., CHRISTOU, E. A., HUNTER, S. K., KORNATZ, K. W., SEMMLER, J. G., TAYLOR, A. M. & TRACY, B. L. 2003. Mechanisms that contribute to differences in motor performance between young and old adults. *Journal of Electromyography and Kinesiology,* 13**,** 1-12.

ENOKA, R. M. & DUCHATEAU, J. 2017. Rate Coding and the Control of Muscle Force. *Cold Spring Harb Perspect Med,* 7.

FARINA, D., NEGRO, F. & DIDERIKSEN, J. L. 2014. The effective neural drive to muscles is the common synaptic input to motor neurons. *The Journal of Physiology,* 592**,** 3427-3441.

GEORGIEVA, S. D. A. K. 2018. Sex Hormones in Neurodegenerative Processes and Diseases. *Cellular and Molecular Mechanisms of the Effects of Sex Hormones on the Nervous System.* InTech.

HAIZLIP, K. M., HARRISON, B. C. & LEINWAND, L. A. 2015. Sex-based differences in skeletal muscle kinetics and fiber-type composition. *Physiology (Bethesda),* 30**,** 30-9.

HANNAH, R., MINSHULL, C., BUCKTHORPE, M. W. & FOLLAND, J. P. 2012. Explosive neuromuscular performance of males versus females. *Experimental Physiology,* 97**,** 618-629.

HARWOOD, B., CORNETT, K. M. D., EDWARDS, D. L., BROWN, R. E. & JAKOBI, J. M. 2014. The effect of tendon vibration on motor unit activity, intermuscular coherence and force steadiness in the elbow flexors of males and females. *Acta Physiologica,* 211**,** 597-608.

HECKMAN, C. J. & ENOKA, R. M. 2012. Motor Unit. *Comprehensive Physiology.*

HECKMAN, C. J., JOHNSON, M., MOTTRAM, C. & SCHUSTER, J. 2008. Persistent inward currents in spinal motoneurons and their influence on human motoneuron firing patterns. *Neuroscientist,* 14**,** 264-75.

HOURIGAN, M. L., MCKINNON, N. B., JOHNSON, M., RICE, C. L., STASHUK, D. W. & DOHERTY, T. J. 2015. Increased motor unit potential shape variability across consecutive motor unit discharges in the tibialis anterior and vastus medialis muscles of healthy older subjects. *Clin Neurophysiol,* 126**,** 2381-9.

HUNTER, S. K. 2014. Sex differences in human fatigability: mechanisms and insight to physiological responses. *Acta Physiol (Oxf),* 210**,** 768-89.

HYER, M. M., PHILLIPS, L. L. & NEIGH, G. N. 2018. Sex Differences in Synaptic Plasticity: Hormones and Beyond. *Front Mol Neurosci,* 11**,** 266.

INGLIS, J. G. & GABRIEL, D. 2020. Sex Differences in Motor Unit Discharge Rates at Maximal and Submaximal Levels of Force Output. *Applied Physiology, Nutrition, and Metabolism,* 45.

INGLIS, J. G. & GABRIEL, D. A. 2021. Sex differences in the modulation of the motor unit discharge rate leads to reduced force steadiness. *Appl Physiol Nutr Metab,* 46**,** 1065-1072.

JEON, Y., CHOI, J., KIM, H. J., LEE, H., LIM, J. Y. & CHOI, S. J. 2019. Sex- and fiber-type-related contractile properties in human single muscle fiber. *J Exerc Rehabil,* 15**,** 537-545.

JONES, E. J., PIASECKI, J., IRELAND, A., STASHUK, D. W., ATHERTON, P. J., PHILLIPS, B. E., MCPHEE, J. S. & PIASECKI, M. 2021. Lifelong exercise is associated with more homogeneous motor unit potential features across deep and superficial areas of vastus lateralis. *Geroscience*.

KUPA, E. J., ROY, S. H., KANDARIAN, S. C. & DE LUCA, C. J. 1995. Effects of muscle fiber type and size on EMG median frequency and conduction velocity. *J Appl Physiol (1985),* 79**,** 23-32.

MANTILLA, C. B., STOWE, J. M., SIECK, D. C., ERMILOV, L. G., GREISING, S. M., ZHANG, C., SHOKAT, K. M. & SIECK, G. C. 2014. TrkB kinase activity maintains synaptic function and structural integrity at adult neuromuscular junctions. *Journal of Applied Physiology,* 117**,** 910-920.

MILLER, A. E. J., MACDOUGALL, J. D., TARNOPOLSKY, M. A. & SALE, D. G. 1993. Gender differences in strength and muscle fiber characteristics. *European Journal of Applied Physiology and Occupational Physiology,* 66**,** 254-262.

MILLER, M. S., CALLAHAN, D. M. & TOTH, M. J. 2014. Skeletal muscle myofilament adaptations to aging, disease, and disuse and their effects on whole muscle performance in older adult humans. *Front Physiol,* 5**,** 369.

NISHIZAWA, S., BENKELFAT, C., YOUNG, S. N., LEYTON, M., MZENGEZA, S., DE MONTIGNY, C., BLIER, P. & DIKSIC, M. 1997. Differences between males and females in rates of serotonin synthesis in human brain. *Proceedings of the National Academy of Sciences,* 94**,** 5308.

PENG, Y.-L., TENAN, M. S. & GRIFFIN, L. 2018. Hip position and sex differences in motor unit firing patterns of the vastus medialis and vastus medialis oblique in healthy individuals. *Journal of Applied Physiology,* 124**,** 1438-1446.

PIASECKI, J., INNS, T. B., BASS, J. J., SCOTT, R., STASHUK, D. W., PHILLIPS, B. E., ATHERTON, P. J. & PIASECKI, M. 2021a. Influence of sex on the age-related adaptations of neuromuscular function and motor unit properties in elite masters athletes. *The Journal of Physiology,* 599**,** 193-205.

PIASECKI, M., GARNÉS-CAMARENA, O. & STASHUK, D. W. 2021b. Near-fiber electromyography. *Clinical Neurophysiology,* 132**,** 1089-1104.

PIASECKI, M., IRELAND, A., COULSON, J., STASHUK, D. W., HAMILTON-WRIGHT, A., SWIECICKA, A., RUTTER, M. K., MCPHEE, J. S. & JONES, D. A. 2016a. Motor unit number estimates and neuromuscular transmission in the tibialis anterior of master athletes: evidence that athletic older people are not spared from age-related motor unit remodeling. *Physiological Reports,* 4**,** e12987.

PIASECKI, M., IRELAND, A., JONES, D. A. & MCPHEE, J. S. 2016b. Age-dependent motor unit remodelling in human limb muscles. *Biogerontology,* 17**,** 485-96.

PIASECKI, M., IRELAND, A., PIASECKI, J., DEGENS, H., STASHUK, D. W., SWIECICKA, A., RUTTER, M. K., JONES, D. A. & MCPHEE, J. S. 2019. Long-Term Endurance and Power Training May Facilitate Motor Unit Size Expansion to Compensate for Declining Motor Unit Numbers in Older Age. *Frontiers in Physiology,* 10.

PIASECKI, M., IRELAND, A., PIASECKI, J., STASHUK, D. W., MCPHEE, J. S. & JONES, D. A. 2018. The reliability of methods to estimate the number and size of human motor units and their use with large limb muscles. *European Journal of Applied Physiology,* 118**,** 767-775.

PIASECKI, M., IRELAND, A., STASHUK, D., HAMILTON-WRIGHT, A., JONES, D. A. & MCPHEE, J. S. 2016c. Age-related neuromuscular changes affecting human vastus lateralis. *J Physiol,* 594**,** 4525-36.

RSTUDIOTEAM 2020. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA.

STARON, R. S., HAGERMAN, F. C., HIKIDA, R. S., MURRAY, T. F., HOSTLER, D. P., CRILL, M. T., RAGG, K. E. & TOMA, K. 2000. Fiber type composition of the vastus lateralis muscle of young men and women. *J Histochem Cytochem,* 48**,** 623-9.

STASHUK, D. W. 1999. Decomposition and quantitative analysis of clinical electromyographic signals. *Med Eng Phys,* 21**,** 389-404.

TAYLOR, A. M., CHRISTOU, E. A. & ENOKA, R. M. 2003. Multiple Features of Motor-Unit Activity Influence Force Fluctuations During Isometric Contractions. *Journal of Neurophysiology,* 90**,** 1350-1361.

TENAN, M. S., PENG, Y.-L., HACKNEY, A. C. & GRIFFIN, L. 2013. Menstrual Cycle Mediates Vastus Medialis and Vastus Medialis Oblique Muscle Activity. *Medicine & Science in Sports & Exercise,* 45.

YAO, W., FUGLEVAND, R. J. & ENOKA, R. M. 2000. Motor-Unit Synchronization Increases EMG Amplitude and Decreases Force Steadiness of Simulated Contractions. *Journal of Neurophysiology,* 83**,** 441-452.

YOON, T., VANDEN NOVEN, M. L., NIELSON, K. A. & HUNTER, S. K. 2014. Brain areas associated with force steadiness and intensity during isometric ankle dorsiflexion in men and women. *Experimental Brain Research,* 232**,** 3133-3145.