




# Influence of sex on the age-related adaptations of neuromuscular function and motor unit properties in elite masters athletes

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## Key points

- Masters athletes maintain high levels of activity into older age and allow an examination of the effects of aging dissociated from the effects of increased sedentary behaviour.
- Evidence suggests masters athletes are more successful at motor unit remodelling, the reinnervation of denervated fibres acting to preserve muscle fibre number, but little data are available in females.
- Here we used intramuscular electromyography to demonstrate that motor units sampled from the tibialis anterior show indications of remodelling from middle into older age and which does not differ between males and females.
- The age-related trajectory of motor unit discharge characteristic differs according to sex, with female athletes progressing to a slower firing pattern that was not observed in males.
- Our findings indicate motor unit remodelling from middle to older age occurs to a similar extent in male and female athletes, with discharge rates progressively slowing in females only.

**Abstract** Motor unit (MU) remodelling acts to minimise loss of muscle fibres following denervation in older age, which may be more successful in masters athletes. Evidence suggests performance and neuromuscular function decline with age in this population, although the majority of studies have focused on males, with little available data on female athletes. Functional assessments of strength, balance and motor control were performed in 30 masters athletes (16

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male) aged 44–83 years. Intramuscular needle electrodes were used to sample individual motor unit potentials (MUPs) and near-fibre MUPs in the tibialis anterior (TA) during isometric contractions at 25% maximum voluntary contraction, and used to determine discharge characteristics (firing rate, variability) and biomarkers of peripheral MU remodelling (MUP size, complexity, stability). Multilevel mixed-effects linear regression models examined effects of age and sex. All aspects of neuromuscular function deteriorated with age ( $P < 0.05$ ) with no age  $\times$  sex interactions, although males were stronger ( $P < 0.001$ ). Indicators of MU remodelling also progressively increased with age to a similar extent in both sexes ( $P < 0.05$ ), whilst MU firing rate progressively decreased with age in females ( $p = 0.029$ ), with a non-significant increase in males ( $p = 0.092$ ). Masters athletes exhibit age-related declines in neuromuscular function that are largely equal across males and females. Notably, they also display features of MU remodelling with advancing age, probably acting to reduce muscle fibre loss. The age trajectory of MU firing rate assessed at a single contraction level differed between sexes, which may reflect a greater tendency for females to develop a slower muscle phenotype.

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## Introduction

Master athletes (MAs) provide an attractive model within aging research, offering examination of the effects of aging independent of the commonly observed associated sedentary behaviour (Lazarus & Harridge, 2017). Regular exercise from middle to older age may minimise the musculoskeletal decline reported to begin from around the age of 40 years (Mitchell *et al.* 2012), with a wealth of literature clearly demonstrating the detrimental effects of the loss of muscle mass and function into older age (Larsson *et al.* 2019). Encompassing recent definitions of sarcopenia (Cruz-Jentoft *et al.* 2019) this potentially avoidable condition is explicable by both the atrophy and loss of individual muscle fibres, and associated motor unit (MU) remodelling (Piasecki *et al.* 2016b; Wilkinson *et al.* 2018), with a multitude of underlying factors. This general musculoskeletal decline with age may contribute to the increased incidence of falls (Yeung *et al.* 2019) and associated co-morbidities (Pacifico *et al.* 2020).

Studies involving MAs have demonstrated the benefits of lifelong exercise on minimising loss of strength (McKendry *et al.* 2018), improved immunological function (Duggal *et al.* 2018) and greater aerobic capacity when compared to age-matched controls (McKendry *et al.* 2020). Furthermore, regular exercise that is maintained from middle into older age helps to maintain lower body fat percentage, and possibly enhanced lean mass and skeletal muscle strength into later life (Piasecki *et al.* 2019a; Crossland *et al.* 2020). Understandably, MAs are not entirely resistant to the effects of age and notable declines have been observed within older competitive athletes in overall performance (Lazarus & Harridge, 2017; Bagley *et al.* 2019). Data at the single fibre level are less clear, suggesting older athletes have preserved

contractile properties in the vastus lateralis, yet of type II fibres only (Power *et al.* 2016b; Gries *et al.* 2019). Moreover, as individual fibre power normalised to size appears to improve with age (Grosicki *et al.* 2016), the neural input to muscle warrants greater research interest.

Evidence of preservation of MU number in older athletes is equivocal; it was shown to be preserved in the tibialis anterior (TA) of one study (Power *et al.* 2010) but not another (Piasecki *et al.* 2016a). Nor is it preserved in the biceps (Power *et al.* 2012) or vastus lateralis of lifelong runners (Piasecki *et al.* 2019b), the latter including both power and endurance disciplines. Expansion of the MU is believed to partly compensate for MU loss, acting to reinnervate locally denervated fibres in aging muscle (Hepple & Rice, 2016). There is mounting evidence to suggest that life-long exercise exerts beneficial effects within the peripheral motor system, attenuating the denervation of muscle fibres and/or improving rates of reinnervation, as demonstrated by fewer markers of denervation in older athletes (Sonjak *et al.* 2019), larger MU potentials (MUPs) recorded from indwelling needle electrodes (Piasecki *et al.* 2019b), less neuromuscular junction (NMJ) transmission instability (Power *et al.* 2016a) and increased fibre-type grouping compared to that expected during normal aging (Mosole *et al.* 2014). However, the last of these has been disputed (Messa *et al.* 2020) and may highlight methodological limitations of histology when assessing MU remodelling.

Contraction of skeletal muscle relies upon successful synaptic input from descending cortical motor pathways to lower motor neurons and their associated NMJs, and the rate at which they discharge (the firing rate) is dictated by this descending drive in addition to neuromodulatory afferent feedback (Heckman & Enoka, 2012). MU firing

rate (FR) is a key component of muscle strength (Del Vecchio *et al.* 2019) and decreases observed with age (Watanabe *et al.* 2016; Piasecki *et al.* 2016c) undoubtedly contribute to associated strength reductions. Although complete mechanisms for a general reduction in FR with older age are unclear, they are probably multifactorial and encompass hormonal fluctuations and environmental factors such as activity level (Hunter *et al.* 2016).

The majority of data relating neural adaptations to age and/or activity are based entirely on males, which is largely generalisable across human physiological research (O'Halloran, 2020). The lack of female data represents a significant shortfall of translational research when considering the male–female disability paradox; women tend to live longer than men but with greater disability (Kingston *et al.* 2014). However, recent histological findings from the vastus lateralis (VL) showed *female* MAs (~81 years) also display greater reinnervation capacity than their inactive age-matched counterparts, based on morphological markers from muscle biopsies (Sonjak *et al.* 2019). Furthermore, few sex differences were noted in MU parameters of the TA in older athletes (Power *et al.* 2016a), indicating peripheral MU adaptations may not be sex-specific. Further knowledge gaps exist in the ages of the populations studied, with a focus on comparisons between the young (<40 years) and old (>65 years) populations, often omitting the middle ages where functionality begins to decline (Mitchell *et al.* 2012).

The aims of the present study were to investigate the age-related trajectory of neuromuscular function and MU adaptations in male and female elite MAs, from middle to older age. It was hypothesised that functional performance and all MU characteristics would decline with increasing age and do so to a similar extent in both sexes.

## Methods

### Ethical approval

The study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. The study was approved by the local ethics committee at Nottingham Trent University (ethics number 619), and all participants provided written informed consent. Participant recruitment and data collection took place at the British Masters Athletics federation National track and field championships, 10–11 August 2019, Birmingham, UK.

### Participant recruitment

A total of 30 MAs (16 males) took part in the study (age range 44–83 years). Of those tested, five competed at 1500–5000 m (two male), 22 competed at 800 m and

below (11 male), and three took part in field events only (jumping events; two male). Participants had been training and competing for an average of  $32 \pm 18.1$  years, training between 5 and 7 h per week for the majority of their training years and at the time of recruitment. The age-graded performance (AGP) of an athlete enables a direct comparison to the current world record, within the athletes specific age group and discipline, and is expressed as a percentage of that world record. The mean AGP for this cohort was  $83.8 \pm 7.37\%$ , indicating a high level of performance relative to respective age group records and favoured event. For example, a marathon of 3 h and 30 min as a 70-year-old male gives an AGP of 80%.

### Strength and force steadiness assessments

TA strength of the right leg was assessed with participants seated with the foot secured to an isometric force dynamometer (purpose-built calibrated strain gauge, RS Components Ltd, Corby, UK) with the knee and ankle bent at approximately 90 degrees. Participants were familiarised to dorsal flexion by performing three isometric contractions lasting 2–3 s at around 80% of maximal effort. Next, isometric maximum voluntary contraction (MVC) of the TA was assessed three times with 60 s rest between efforts. The highest value was accepted as MVC. Force was recorded at 100 Hz and displayed in real-time using Spike2 software (v8.01). Force steadiness was quantified as the coefficient of variation [CoV;  $(SD/mean) \times 100$ ] of force set at target lines of 10, 25 and 40% MVC. Participants were given a single familiarisation trial at each contraction intensity, before performing six contractions at 10%, six contractions at 25% and two contractions at 40% MVC, with each lasting approximately 12–15 s and a rest of 30 s in between contractions. The mean CoV at each contraction level was calculated.

### Balance and jump mechanography

Limb dominance was assessed by asking which limb would be used to kick a ball. All participants were right-leg dominant. An RS Foot scan (Gait and Motion Technology Ltd, Bury St Edmunds, UK) pressure sensor plate was used to assess balance of all participants. The centre of pressure (COP), or postural sway, of the vertical plane was measured throughout assessment and is expressed as total distanced moved in millimetres (mm). This was assessed for 30 s with participants standing in the centre of the sensor plate, on the right leg only. Following this, a G-walk (a small inertial sensor placed at the base of the spine, secured with a Velcro waist belt; Gait and Motion Technology Ltd) was used to assess power (kW) from a series of counter movement jumps. Participants were instructed to jump as high as possible, with hands

remaining on their waist with a trained assistant present and in reach of the participants in case of a fall or falter (Piasecki *et al.* 2018). Each participant repeated the jump sequence three times, with approximately 30 s rest between jumps, and the highest value was recorded.

### Intramuscular EMG

After establishing the MVC, a concentric needle electrode (Model N53153; Teca, Hawthorne, NY, USA) was inserted at the muscle belly of the TA, to a depth of 0.5–1.5 cm. The intramuscular electromyography (iEMG) signals were bandpass filtered from 10 to 10 kHz and sampled at 50 kHz. iEMG signals were displayed in real-time using Spike2 software (v8.01) and data were stored for off-line analysis.

### Sampling of individual MUs during voluntary contractions

Each participant performed a voluntary isometric contraction at 25% MVC. Needle position was initially adjusted, where needed, to obtain intramuscular MUPs with peak second derivative values  $>5 \text{ kV/s}^2$ , to ensure the recording needle electrode was close to depolarizing fibres (Stashuk, 1999a). Each voluntary contraction lasted 12–15 s, keeping as close as possible to a force target line shown on the computer monitor that was set at 25% MVC with real-time visual feedback. After each contraction the needle electrode was repositioned by combinations of rotating the bevel 180 degrees and withdrawing by approximately 5–10 mm. Participants had 30 s rest between each contraction. Needle re-positioning, voluntary contraction and signal recording was repeated until between four and six recordings from varying depths and perspectives had been obtained.

### iEMG signal analysis

iEMG data are available for 16 males and 12 females. The mean (SD) number of individual MUs sampled was 26 (10) for males and 29 (8) for females. iEMG signals were analysed as previously described (Piasecki *et al.* 2016a) using decomposition-based quantitative electromyography (DQEMG) (Stashuk, 1999b). Extracted motor unit potential trains (MUPTs) with fewer than 40 MUPs were excluded. All MUP templates were visually inspected and their markers adjusted, where required, to correspond to the onset, end, and positive and negative peaks of the waveforms. The MUP area is the integral of absolute values of MUP between the onset and end and is expressed as  $\mu\text{V}\cdot\text{ms}$ . MUP duration (ms) is measured from the onset to the end of the MUP. 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  $25 \mu\text{V}$  (turns). A near fibre MUP (NFM) is the ‘acceleration’ of an MUP and is identified by applying a second-order low-pass differentiator to the MUP. This ensures only potentials from fibres close to the needle electrode significantly contribute to the recorded NFM. NFM segment jitter (NFM SJ) 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 (Fig. 1). NFM jiggle is a measure of the shape variability of consecutive NFMs of an MUPT expressed as a percentage of the total NFM area (Allen *et al.* 2015; Piasecki *et al.* 2016a). Firing rate was assessed as the rate of consecutive observations of the same MUP, expressed as number of observations per second (Hz). MU FR variability was measured using FR mean absolute consecutive difference (FR:MACD) across the train measured in hertz [ $\text{FR:MACD} = \text{mean}(\text{abs}(\text{IFR}_{i+1} - \text{IFR}_i))$ ]; here mean represents the mean across the MUPT and  $\text{IFR}_i$  is the local ‘instantaneous’ FR at the time of the  $i$ th sampled MU firing. The local ‘instantaneous’ FR, at each sampled MU firing time, is the inverse of the Hamming-weighted mean interdischarge interval (IDI) of the previous five and following five consistent IDIs. A consistent IDI is one that is within  $\pm 3$  standard deviations of the mean IDI of the MUPT.

### Statistical analysis

All statistical analysis was completed using STATA (v.15). The age-graded performance of male and female athletes was compared using an unpaired  $t$  test. Neuromuscular functional parameters were assessed across age and sex using multiple linear regression, first examining interaction effects. Where none existed, interactions were removed from the model and effects of age and sex were investigated, adjusting for each other. Individual athletes have multiple values for MU parameters therefore multilevel mixed effects linear regression models were used to investigate these parameters with Age and Sex as factors. Interactions were first examined, where not present they were removed from the model, Sex and Age were explored individually, and mutually adjusted. Significance was accepted at  $P < 0.05$ .

### Results

The age range of the female athletes was 45–82 years, and 44–83 years for male athletes. The AGP was  $81.8 \pm 6.8$

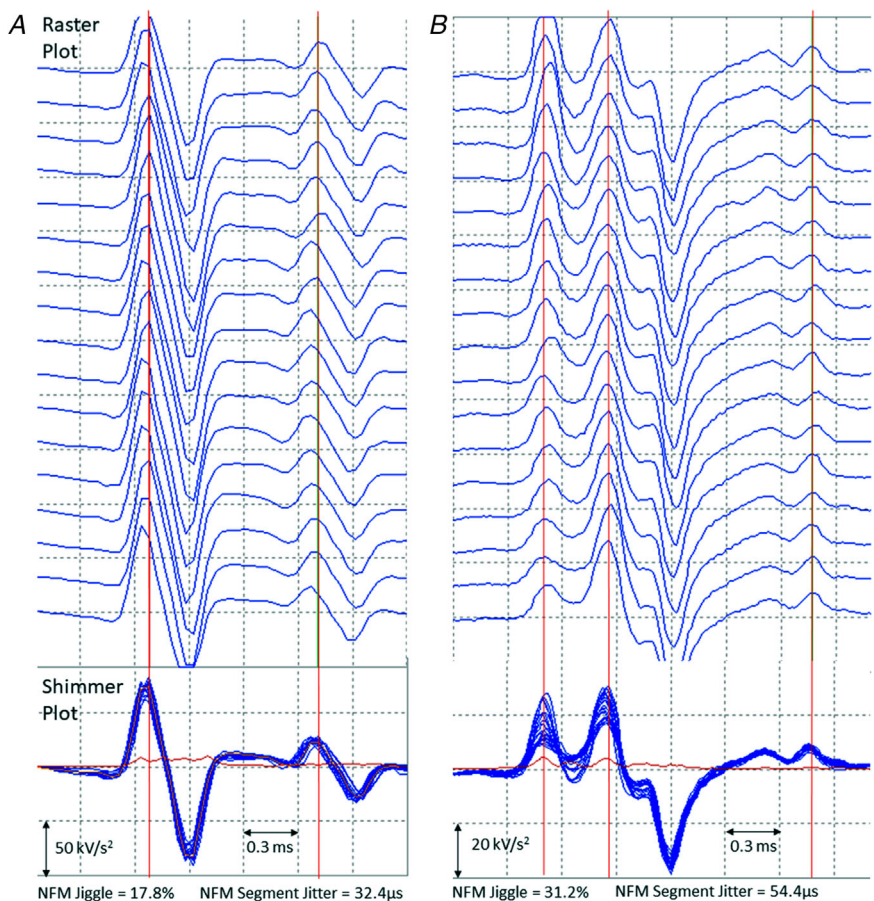


for males and  $85.8 \pm 7.5$  for females and did not differ between sexes ( $P > 0.1$ ).

Multiple linear regression revealed no Age  $\times$  Sex interactions in any of the functional parameters. MVC decreased progressively with older age ( $\beta = -1.66$ ; 95% CI =  $-2.62$  to  $-0.69$ ;  $p = 0.002$ ). When adjusting for age, MVC was greater in males than females ( $\beta = -53.89$ ; 95% CI =  $-76.14$  to  $-31.64$ ;  $P < 0.001$ , Fig. 2A, Table 1). Similarly, jump power decreased with age ( $\beta = -0.024$ ; 95% CI =  $-0.040$  to  $-0.006$ ;  $p = 0.011$ ) but was higher in males than females ( $\beta = -0.810$ ; 95% CI =  $-1.208$  to  $-0.413$ ;  $P < 0.001$ , Fig. 2B, Table 1). Balance, as assessed by distance travelled during postural sway whilst standing on the right leg, increased with older age ( $\beta = 22.49$ ; 95% CI =  $2.25$ – $42.74$ ;  $p = 0.031$ ) with no differences between sexes ( $\beta = -288.11$ ; 95% CI =  $-667.85$  to  $91.62$ ;  $p = 0.131$ , Fig. 3A, Table 1). Force steadiness was assessed at three intensities and decreased with age at 10% ( $\beta = 0.118$ ; 95% CI =  $-0.047$  to  $0.189$ ;  $p = 0.002$ , Fig. 3B), 25% ( $\beta = 0.073$ ; 95% CI =  $-0.015$  to  $0.132$ ;  $p = 0.016$ , Fig. 3C), and 40% MVC ( $\beta = 0.075$ ; 95% CI =  $-0.043$  to  $0.134$ ;  $p = 0.014$ , Fig. 3D). Males performed better than females at 10% MVC only ( $\beta = 2.744$ ; 95% CI =  $1.126$ – $4.361$ ;  $p = 0.002$ , Fig. 3A).

There were no Age  $\times$  Sex interactions in any peripheral related MU features. When interaction effects were removed from the model, there were no associations of any peripheral related MU parameters with sex (Table 2). The area ( $\beta = 24.31$ ; 95% CI =  $10.84$ – $37.70$ ;  $P < 0.001$ ) and duration ( $\beta = 0.093$ ; 95% CI =  $0.058$ – $0.129$ ;  $P < 0.001$ ) of MUPs were progressively larger with increasing age. The complexity of MU potentials increased with age, with a greater number of phases ( $\beta = 0.016$ ; 95% CI =  $0.004$ – $0.029$ ;  $p = 0.010$ ), and a non-significant increase in the number of turns with older age ( $\beta = 0.023$ ; 95% CI =  $-0.001$  to  $0.047$ ;  $p = 0.060$ ). NFM area ( $\beta = 0.075$ ; 95% CI =  $0.009$ – $0.149$ ;  $p = 0.047$ ) and NFM duration ( $\beta = 0.045$ ; 95% CI =  $0.016$ – $0.070$ ;  $p = 0.003$ ) were also progressively greater with age. Older age was also associated with greater NMJ transmission instability, as assessed by NFM jiggle ( $\beta = 0.213$ ; 95% CI =  $0.009$ – $0.417$ ;  $p = 0.040$ ) and NFM segment jitter ( $\beta = 0.285$ ; 95% CI =  $0.077$ – $0.494$ ;  $p = 0.007$ ) (Fig. 4, Table 2).

There was a significant Age  $\times$  Sex interaction of MU FR (interaction  $\beta = -0.096$ ; 95% CI =  $-0.165$  to  $-0.027$ ;  $p = 0.006$ ), explained by the age-related decrease in FR with age in females ( $\beta = -0.054$ ; 95% CI =  $-0.102$  to  $-0.005$ ;  $p = 0.029$ ), which was not observed in males



### Figure 1. Near fibre motor unit potentials

Near fibre MUPs (NFMs) from a 67-year-old male (A) and a 75-year-old female (B). In each case 17 consecutive NFMs are displayed in a raster plot and overlaid in respective shimmer plots. NFM jiggle tracks changes in consecutive NFM shapes and is expressed as a percentage of the total NFM area. NFM segment jitter tracks shifts in segments of consecutive NFMs over the duration of the NFM template, across the NFMs of an MUPT, and is expressed in microseconds. Higher values indicate increased neuromuscular junction transmission instability.

**Table 1. Linear regression analysis summary for parameters of neuromuscular function**

	Age			Sex		
	Adjusted $\beta$	95% CI	<i>P</i>	Adjusted $\beta$	95% CI	<i>P</i>
MVC	<b>-1.66</b>	<b>-2.62 to -0.69</b>	<b>0.002</b>	<b>-53.89</b>	<b>-76.14 to -31.64</b>	<b>&lt;0.001</b>
Power	<b>-0.024</b>	<b>-0.040 to -0.006</b>	<b>0.011</b>	<b>-0.810</b>	<b>-1.208 to -0.413</b>	<b>&lt;0.001</b>
Postural sway (right leg)	<b>22.49</b>	<b>2.25-42.74</b>	<b>0.031</b>	-288.11	-667.85 to 91.62	0.131
Force steadiness 10% MVC	<b>0.118</b>	<b>0.047-0.189</b>	<b>0.002</b>	<b>2.744</b>	<b>1.126-4.361</b>	<b>0.002</b>
Force steadiness 25% MVC	<b>0.073</b>	<b>0.015-0.132</b>	<b>0.016</b>	1.253	-0.078 to 2.584	0.058
Force steadiness 40% MVC	<b>0.075</b>	<b>0.043-0.134</b>	<b>0.014</b>	0.681	-0.653 to 2.015	0.304

The linear regression model was mutually adjusted for covariates Age and Sex. B, unstandardized adjusted beta coefficient; CI, confidence interval. MVC, maximum voluntary contraction. *P*-value < 0.05 highlighted in bold. Sex = adjusted mean difference; adjusted  $\beta$  represents the difference of females compared to males.

( $\beta = 0.042$ ; 95% CI =  $-0.007$  to  $0.092$ ;  $p = 0.092$ ) (Fig. 5A, Table 2). The variability in firing rate did not differ according to age ( $\beta = -0.0006$ ; 95% CI =  $-0.0005$  to  $0.0018$ ;  $p = 0.206$ ) or sex ( $\beta = -0.033$ ; 95% CI =  $-0.0763$  to  $0.1433$ ;  $p = 0.550$ , Fig. 5B, Table 2).

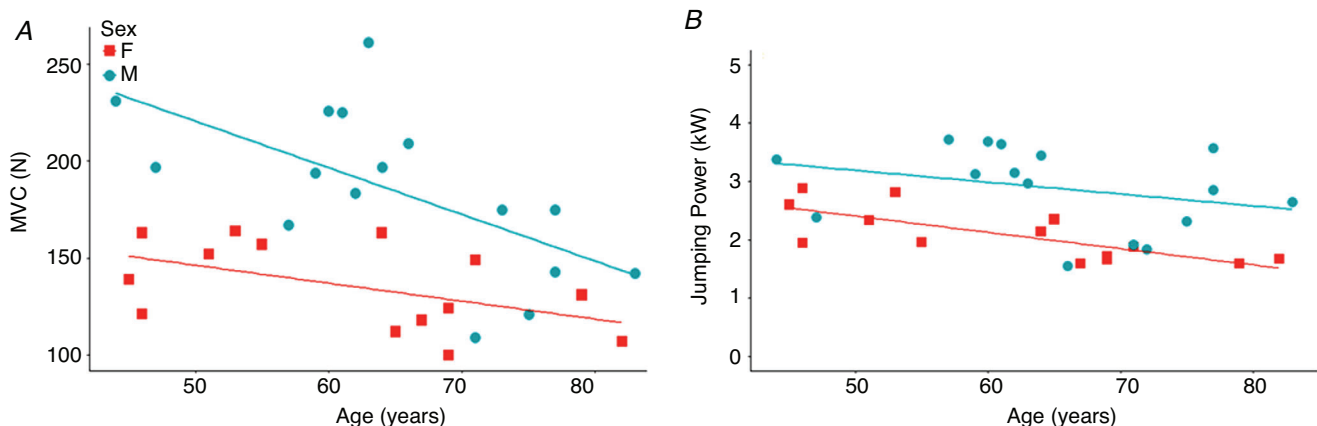
## Discussion

To our knowledge these findings are the first to identify, using intramuscular techniques supported by functionality data, neuromuscular alterations from middle to older age in male and female competitive MAs. The data herein show there is no sex-based difference in the age-related decline of strength or control in the TA muscle, with a similar pattern in peripheral MU adaptations across age. Yet there is disparity between the sexes in MU firing rate, with females demonstrating a reduction over the life-span that was not observed in males.

Despite the MAs demonstrating exceptionally high ability as evidenced by their high AGP and regular physical activity (our cohort trained for an average

of 5–7 h per week), functionality still declined into older age. These age-related decrements of strength (Wilkinson *et al.* 2018) and control (Castronovo *et al.* 2018; Mani *et al.* 2018) have been well described, and lifelong exercisers are no exception to this, although it is probable they have attenuated balance declines when compared to age-matched controls (Leightley *et al.* 2017). Comparisons of detailed muscle function may not be logically extrapolated to athletic performance, but the current data do support previous observed reductions in competitive performance (Ganse *et al.* 2018), also with negligible sex differences in an/aerobic power (Bagley *et al.* 2019) or from world record performances (Gava & Ravara, 2019).

MUPs recorded from indwelling electrodes during voluntary contractions allow a detailed overview of individual MU structure and electrophysiological function, with NFMs consisting of significant contributions from only a few local MU fibres. We have previously shown power and endurance MAs have larger MUPs than age-matched controls (Piasecki *et al.*

**Figure 2. Isometric dorsiflexion strength and maximum jump power**

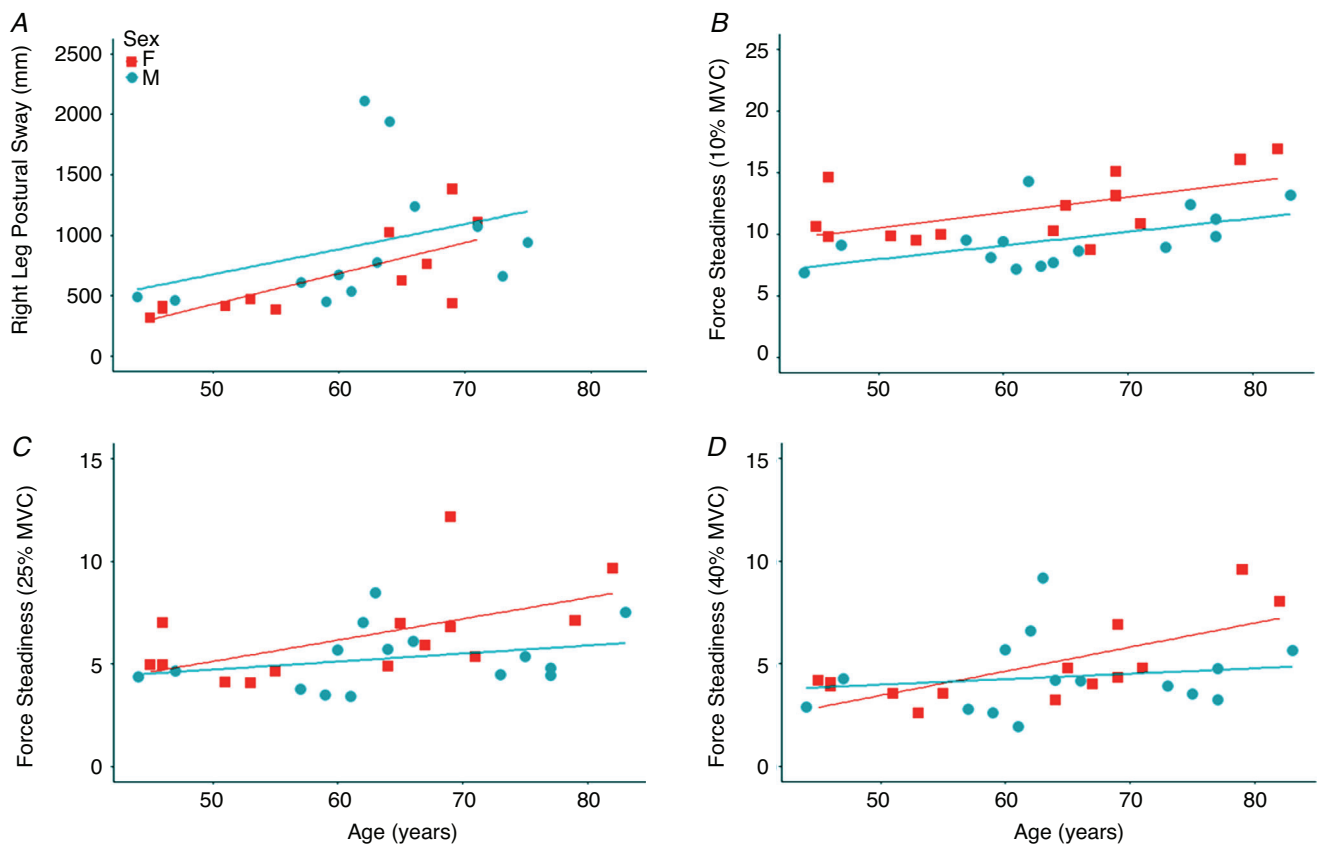
Isometric dorsiflexion strength (A) and maximum jump power (B) in male and female masters athletes. Results of related linear regression analyses are shown in Table 1. MVC, maximum voluntary contraction.

2019b), possibly as a result of larger MUs from greater reinnervation-based MU expansion, and in line with this we again observed a progressive increase in MUP area and duration with older age. Notably, this occurred to a similar extent in males and females, further supporting the notion that older female athletes also have a greater capacity for reinnervation-based MU expansion (Sonjak *et al.* 2019). MUP complexity also increased with age without a sex difference, in line with increases in MUP size here and in previous studies of the VL of non-athletes (Piasecki *et al.* 2016c). The complexity of an MUP is related to the temporal dispersion of its comprising individual fibre potentials, and reinnervation can cause increased MUP complexity.

The increases in NFM jiggle and NFM segment jitter are indicative of greater NMJ transmission instability, and all follow the same age-related pattern of an increase, with lack of a sex difference. Although there are no available data on NFM segment jitter, previous investigations of NFM jiggle in this muscle found no age-related differences between young (~26 years) and

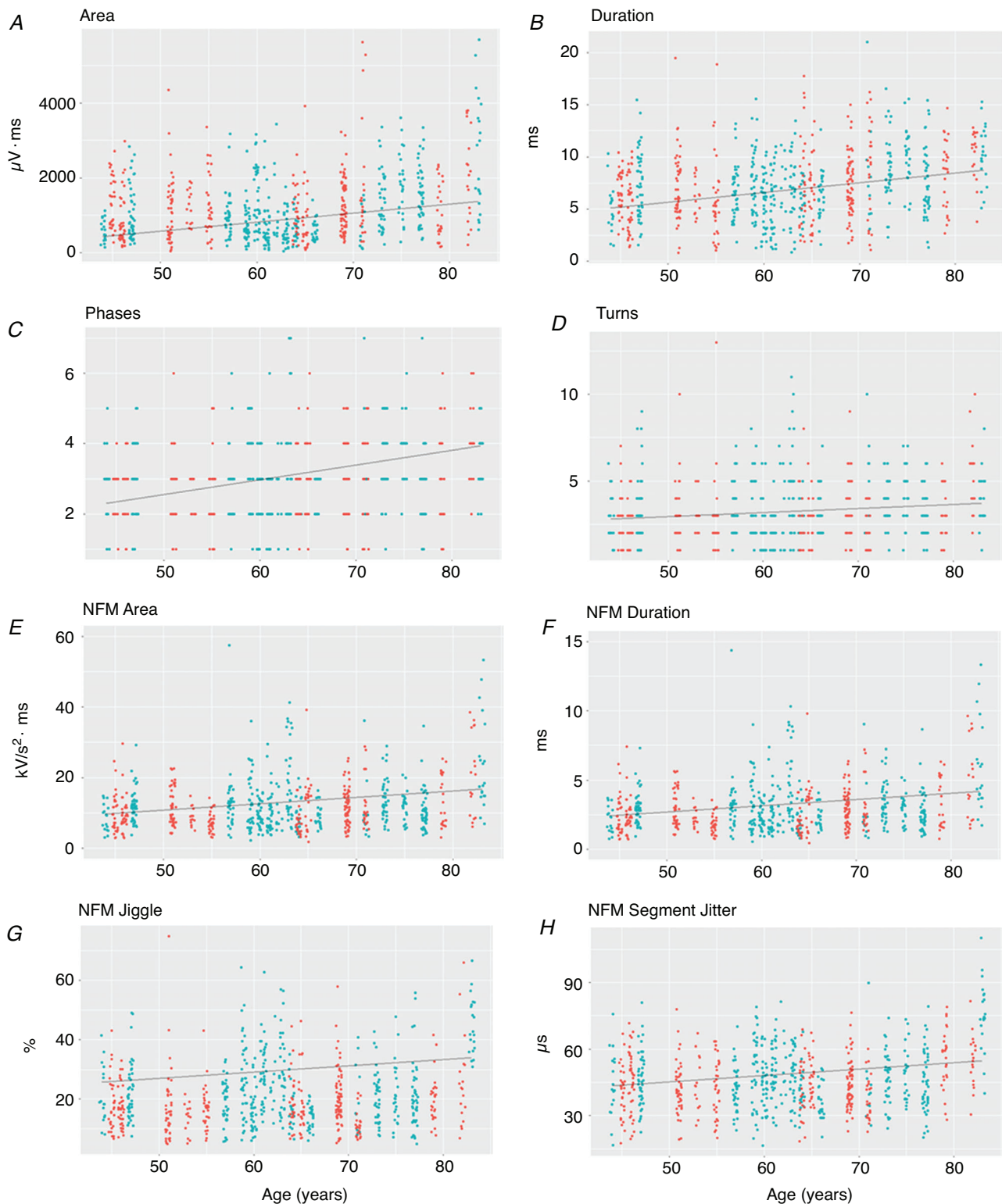
old (~71 years) non-athletic men, compared to a slightly greater instability in male MAs (~69 years) (Piasecki *et al.* 2016a). Similar methods deduced very old (~80 years) MAs had lower instability than their age-matched counterparts (Power *et al.* 2016a), highlighting the potential impact of prolonged exercise. The plasticity of the NMJ in response to age and exercise has been well described via histological markers (Soendenbroe *et al.* 2020), but with regard to *in vivo* electrophysiological measures such as those applied here, it is less clear what a favourable outcome would be as newly formed NMJs from axonal sprouts (i.e. 'rescued' fibres) are likely to demonstrate instability (Balice-Gordon, 1997).

The outcome of the MU discharge parameters yielded a slightly different finding; FR decreased with age for females but not for males. The firing rate of an MU is contraction-level- and contraction-type-specific (Duchateau & Baudry, 2014), and is closely related to the MU/fibre type (i.e. slow or fast). Here we assessed FR during an isometric contraction at a force normalised to individual maximum (25% MVC) to help mitigate these



**Figure 3. Neuromuscular control**

Standing balance represents the total distance moved of the centre of pressure during 30 s of standing on the right leg only. Dorsiflexion force steadiness was assessed at three separate contraction intensities normalised to individual MVC. Results of related linear regression models are shown in Table 2. Three male and two female participants were unable to perform single leg balance (A). Force steadiness data from one male participant are not available (B–D). MVC, maximum voluntary contraction.



**Figure 4. Peripheral features of motor unit structure and electrophysiological function in male ( $n = 16$ ; green) and female ( $n = 12$ ; red) elite masters athletes aged 44–83 years**

Grey lines indicate the  $\beta$  coefficient from multi-level mixed effects linear regression where there was no age  $\times$  sex interaction. *A*, motor unit potential area; *B*, motor unit potential duration; *C*, number of phases; *D*, number of turns; *E*, near fibre motor unit potential area; *F*, near fibre motor unit potential duration; *G*, near fibre jiggle; *H*, near fibre motor unit potential segment jitter.  $n = 756$  MUs scattered  $\pm 1$  year for data visualisation. All statistical analyses were based on multi-level mixed effects linear regression.



**Table 2. Multi-level linear regression analysis summary for peripheral and discharge properties of motor units**

	Age			Sex		
	Adjusted $\beta$	95% CI	<i>P</i>	Adjusted $\beta$	95% CI	<i>P</i>
<b>Peripheral properties</b>						
Area ( $\mu\text{V}\cdot\text{ms}$ )	<b>24.31</b>	<b>10.84–37.79</b>	<b>&lt;0.001</b>	236.30	–64.74 to 537.35	0.124
Duration (ms)	<b>0.093</b>	<b>0.058–0.129</b>	<b>&lt;0.001</b>	0.176	–0.834 to 1.187	0.732
Phases	<b>0.016</b>	<b>0.004–0.029</b>	<b>0.010</b>	–0.077	–0.357 to 0.203	0.591
Turns	0.023	–0.001 to 0.047	0.060	–0.256	0.0792–0.279	0.349
NFM area ( $\text{kV}/\text{s}^2\cdot\text{ms}$ )	<b>0.075</b>	<b>0.009–0.149</b>	<b>0.047</b>	0.050	–1.636 to 1.739	0.953
NFM duration (ms)	<b>0.045</b>	<b>0.016–0.070</b>	<b>0.003</b>	–0.223	–0.811 to 0.365	0.456
NFM jiggle	<b>0.213</b>	<b>0.009–0.417</b>	<b>0.040</b>	–4.149	–8.70 to 0.406	0.094
NFM segment jitter ( $\mu\text{s}$ )	<b>0.285</b>	<b>0.077–0.494</b>	<b>0.007</b>	–2.320	–6.919 to 2.278	0.323
<b>Discharge properties</b>						
FR variability (Hz)	–0.0006	–0.0005 to 0.0018	0.206	0.0334	–0.0763 to 0.1433	0.550
<b>Males</b>						
Firing rate (Hz)*	0.042	–0.007 to 0.092	0.092	<b>Females</b>		
				<b>–0.054</b>	<b>–0.102 to –0.005</b>	<b>0.029</b>

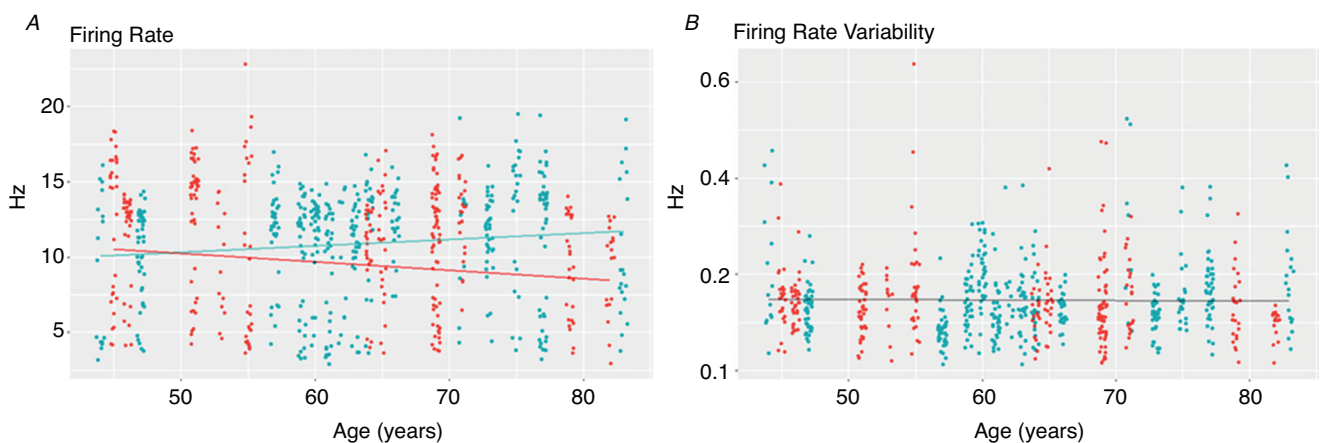
The multi-level mixed effects linear regression model was adjusted for covariates Age and Sex, mutually adjusted. B, unstandardized adjusted beta coefficient; CI, confidence interval. *P*-value < 0.05 are highlighted in bold. Sex = adjusted mean difference; adjusted  $\beta$  represents the difference of females compared to males.

\*Firing rate coefficients were calculated from a significant age  $\times$  sex interaction coefficient ( $p = 0.006$ ), so effects of age are shown independently of effects of sex for this parameter only.

contributing factors. The TA muscle is predominantly composed of type 1 fibres ( $\sim 70\%$ ) (Henriksson-Larsén *et al.* 1985; Nakagawa *et al.* 2005) and in young individuals this composition is not affected by sex (Porter *et al.* 2002). Therefore, it is possible the female athletes display a more prominent shift to a slower muscle phenotype with increasing age when compared to males, although muscle biopsies (particularly difficult in this cohort and muscle group) would be required to confirm this. Moreover, evidence emerging from cross-innervation and electrical

stimulation studies has demonstrated fibre phenotype is governed by its innervation status (reviewed by Blaauw *et al.* 2013), so sex-based differences in fibre composition in these athletes would be a consequence of their differing FR, and not a cause.

Alterations to MU FR have shown a high level of plasticity even in response to relatively short (2 weeks) exercise interventions (Martinez-Valdes *et al.* 2017), and are exercise type-specific with a reported decrease from endurance training and increase from resistance training,



**Figure 5. Motor unit discharge properties in male ( $n = 16$ ; green) and female ( $n = 12$ ; red) elite masters athletes aged 44–83 years**

A, firing rate. Separate  $\beta$  coefficients are indicated for males (green line) and females (red line). B, firing rate variability; the grey line indicates the  $\beta$  coefficient from multi-level mixed effects linear regression where there was no age  $\times$  sex interaction.  $n = 756$  MUs scattered  $\pm 1$  year for data visualisation. All statistical analyses were based on multi-level mixed effects linear regression.

following a 6-week intervention (Vila-Chã *et al.* 2010). Therefore, the competitive discipline and training regime of an athlete could influence MU FR. However, given the even distribution across short (<800 m) and longer (>1500 m) event specialism of males and females in this current study, exercise discipline is unlikely to explain this age by sex difference. The ability to voluntarily maximally contract the TA muscle does not differ between sexes (Russ & Kent-Braun, 2003) and differs little into older age in other leg muscle groups (McPhee *et al.* 2018), so, when near maximal muscle contractions are considered, there appears little sex-based influence of neural drive.

The large age range of these athletes offers insight into the often-overlooked neuromuscular health trajectory from middle to older age. However, it also presents a number of challenges such as the associated change in the hormonal milieu, specifically the decrease in sex hormones known to influence neuromuscular function (Sipilä *et al.* 2013; Swiecicka *et al.* 2020) and regulate skeletal muscle signalling (Laakkonen *et al.* 2017). This is particularly important with regard to the transition from pre- to post-menopausal state as performance effects may be minimal in pre-menopausal (McNulty *et al.* 2020). Animal models have demonstrated a neuroprotective effect of oestrogens and androgens centrally (Spence & Voskuhl, 2012), and in humans some aspects of centrally mediated function alter concordantly with hormonal fluctuations (Smith *et al.* 2002; Ansdell *et al.* 2019). Therefore, the normal age-related decreases in sex hormones, of which highly active individuals are not spared, may exert a greater central influence on females than on males. Further research of the hormonal influence on neuromuscular deterioration in aging males and females is warranted.

### Strengths and limitations

This is the first study, to our knowledge, to demonstrate a detailed trajectory of functionality and associated MU properties from middle to older age, within a particularly valuable cohort. Applying iEMG at a range of muscle depths ensures we have sampled from a range of active MUs, and the inclusion of NFMs allowed a detailed view of MU remodelling at the single MU level. Our findings demonstrate that despite high levels of physical activity, MAs experience declines in functionality but also demonstrate features of peripheral MU remodelling. Importantly, these changes occur to a similar extent between sexes. The MU FR reduced in females but not males, indicating the importance of the CNS and afferent feedback in aging neuromuscular function. There are several limitations within this study that are important to acknowledge; in our assessment of functionality, we focused on postural sway taken from one footed balance, which can be improved with targeted balance training

(Kiss *et al.* 2018), and bilateral differences between dominant and non-dominant limbs were not explored. Whilst we have identified some interesting sex-based differences, these data are related to MUs active during mid-level contractions and reveal nothing of the level of remodelling of higher recruitment threshold MUs. The hormonal milieu between sexes differs, particularly in relation to sex hormones and we are unable to describe their possible influence on MU function.

### Conclusion

Highly active competitive MAs exhibit an age-related decrease in neuromuscular function from middle to older age, and this is associated with increased MU remodelling. These changes occur to a similar extent in males and females. Discharge properties of MUs differ in the age response, with firing rate decreasing in females but not in males, possibly reflecting a greater tendency towards a phenotypically slower muscle in the female athlete population.

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## Additional information

### Data availability statement

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

### Competing interests

The authors have no competing interests to declare.



### Author contributions

All authors contributed to the conception and design of the work. J.P., T.B.I., J.J.B., R.S. and M.P. acquired the data. J.P., T.B.I., J.J.B., D.S. and M.P. analysed the data. J.P., D.S., B.E.P., P.J.A. and M.P. drafted the manuscript and prepared the figures. All authors contributed to the interpretation of the results and in the revision of the manuscript. 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.

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### Keywords

electromyography, master athlete, motor unit, neuromuscular function

### Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

### Statistical Summary Document