Exploration of central and peripheral neural properties in young and old humans

Yuxiao Guo

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Exploration of central and peripheral neural properties in young and old humans

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By

Yuxiao Guo

Centre of Metabolism, Ageing & Physiology (COMAP)
Injury, Recovery and Inflammation Sciences
School of Medicine
University of Nottingham

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Abstract

Ageing is associated with declines of skeletal muscle mass and function attributable to neural adaptations at both central and peripheral levels. Voluntary movement is achieved via a complex interplay of motor cortex, spinal circuitry and motoneurons, which may be differentially influenced by the ageing process and sex-specific hormonal changes. However, there is limited data exploring age- and sex-related differences in neural inputs and individual motor unit properties in vastus lateralis, which appears to be highly susceptible to age-related functional impairment.

This thesis introduces the structure of the central and peripheral motor nervous system in humans, along with its contributions to motor unit firing properties and its impacts on force generation and physical performance. It then demonstrates the methodological approaches to investigate the neural inputs to skeletal muscles with a focus on age-related adaptation and sex differences.

In Chapter 2 and 3, data indicates young and older females produced muscle force via different neuromuscular recruitment strategies to males which was characterised by smaller motor units discharging at higher rates. However, similar strategies were employed for force gradation in both sexes, manifested by increased motor unit firing rates and greater motor unit size. Notably, greater motor unit firing rate variability observed in older females may contribute to their greater functional deterioration.

In Chapter 4, it was found that muscle function, motor unit firing rates and common synaptic inputs did not differ bilaterally across limbs, whereas there was a significantly increased variability of motor unit firing rate in the dominant limb in both young and older adults. Additionally, an increased bilateral difference in force steadiness was apparent from early to late elderly, indicating a greater likelihood of unilateral functional weakness contributing to frailty in older age. The results also highlight the age-related reduction in muscle strength may be attributable to the decreased motor unit firing rates. In chapter 5, common synaptic inputs

and persistent inward currents were found to be lower in VL MUs of older individuals, occurring independently of reductions in motor unit firing rates in this cohort. Furthermore, an age-related decline in force control ability was only observed in the ramp contraction rather submaximal sustained contraction, highlighting the role of task complexity in age-related impairments.

To eliminate the influence of progressive disuse in older age, masters athletes were studied in Chapter 6, and used as a model to explore the effects of inherent ageing and circulating sex hormones on neuromuscular function. The results highlight that lifelong power exercise training had more favourable physical characteristics when compared to inactive and endurance trained masters athletes, and a positive association is reported between androgen derivatives and motor unit firing rates, highlighting the potential of hormone administration as a therapeutic intervention strategy in older age.

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道阻且长，行则将至；行而不辍，未来可期。

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致谢，是终点，亦是起点。

愿你我今后都能有前进一寸的勇气，亦有后退一尺的从容。

Declaration

The data in this thesis was collected and analysed by myself unless otherwise acknowledged below.

In Chapter 6, master athlete data was collected by Mathew Piasecki, Jessica Piasecki, Agnieszka Swiecicka and Alex Ireland in Manchester Metropolitan University between 2014 and 2017. These data were analysed by myself.

I declare that this thesis has been constructed by myself and that all the data presented are my own work unless otherwise stated. All published literature within this thesis has been appropriately referenced. None of the data presented in this thesis has been submitted towards the assessment of a higher degrees previously.

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List of Abbreviations

ACh	acetylcholine
AChR	acetylcholine receptors
BMI	body mass index
BDNF	brain-derived neurotrophic factor
CMAP	compound muscle action potential
COP	centre of pressure
CSA	cross-sectional area
CT	computerised tomography
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
DHT	dihydrotestosterone
DQEMG	decomposition-based quantitative electromyography
EMG	electromyography
E2	estradiol
FR	firing rate
FS	force steadiness
GABA	γ-aminobutyric acid
HDsEMG	high-density surface electromyography
iEMG	intramuscular electromyography
MU	motor unit

MUNE	motor unit number estimate
MUP	motor unit potential
MVC	maximal voluntary contraction
MUPT	motor unit potential trains
NFMUP	near-fibre motor unit potential
NMJ	neuromuscular junction
PIC	persistent inward current
sEMG	surface electromyography
sMUP	surface motor unit potential
TA	tibialis anterior
TUG	timed up and go
VL	vastus lateralis

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Chapter 1 - Introduction and Literature Review

1.1 General introduction

The global population is rapidly growing older and the number of people aged over 65 years are the fastest-growing segments of the population; in 2021 there were nearly 12 million people aged 65 and older in the UK (Office for National Statistics, 2021) and this number is expected to increase by more than 40% within 20 years. Ageing is accompanied by the loss of functional reserve capacity. Biologically, ageing is characterised by the accumulation of several molecular and cellular defects over time, which results in a gradual loss of physical and mental capabilities. The loss of muscle mass is one of the most prominent changes, caused by a combination of muscle fibre atrophy and fibre loss. This happens gradually from middle age, with a loss of approximately 1% per year and can reach 50% in the 8th to 9th decade (Mitchell *et al.,* 2012; Wilkinson *et al.,* 2018). As muscle is critical for normal physical function and metabolic homeostasis, its loss of mass and function unsurprisingly results in multiple adverse outcomes including impaired mobility, increased risk of falls, higher probabilities of developing disease, increased insulin resistance, and associated co-morbidities and mortality (Woods *et al.,* 2015; Larsson *et al.,* 2018). It is the leading cause of the loss of independence in old age and places a substantial burden on health and social care services (Cruz-Jentoft *et al.,* 2019).

Age-related decline in muscle mass and strength has been widely documented in humans (Wilkinson *et al.,* 2018), likely attributable to a series of factors including decreased physical activity, altered neural properties, decreased hormones, increased pro-inflammatory cytokines, presence of oxidative stress and impaired mitochondrial function (Cui *et al.,* 2012; Ferrucci & Fabbri, 2018). A critical factor in decreased physical performance with age is the adaptation of the neuromuscular system. However, the majority of studies of neurophysiology have mainly been conducted in males (Woitowich *et al.,* 2020) and the extrapolation of the

findings to the broader population, including females, warrants additional considerations due to the presence of physiological disparities muscles between sexes.

Generally, females tend to exhibit lower levels of physical performance (Hannah *et al.,* 2012) but higher levels of resistance to neuromuscular fatigue during a variety of exercise tasks and muscle groups compared to males (Ansdell *et al.,* 2020). There are several reasons for the apparent disparities in neuromuscular function between males and females, including body composition and fibre type proportion, with females exhibiting a lower quantity of skeletal muscle mass and a greater proportion of type I muscle fibres, and metabolic factors, such as lower VO_{2max} and greater aerobic power in females (Hunter *et al.,* 2023). Alongside the presence of genetic factors, it is crucial to note that the distinct sex hormonal milieu presented between sexes throughout the adult lifespan likely lays the groundwork for sex differences in neuromuscular function, since females typically have higher levels of oestrogen and progesterone, and males typically have higher levels of testosterone. Nevertheless, the sex-based differences of central and peripheral neural properties and how they may be influenced by age have largely been overlooked. This chapter will provide an overarching review of the central and peripheral nervous systems involved in skeletal muscle movements (Zayia & Tadi, 2023).

1.2 Motor nervous system

1.2.1 Motoneuron

A motoneuron is a neuron with its cell body located in the motor cortex, brainstem or the spinal cord and its axon projecting onto effector organs, for example muscles in humans, responsible for transmitting signals away from the central nervous system towards muscle fibres by releasing neurotransmitters to trigger muscle movement (Kandel, 2013). Motoneurons can be classified as upper and lower motoneurons. The upper motoneuron is responsible for integrating all excitatory and inhibitory signals from the cortex via pyramidal tracts and extrapyramidal tracts down to the synapses of the interneurons and lower motoneurons located in the ventral horn of the spinal cord. Action potential propagating along lower motoneurons terminate at the neuromuscular junction (NMJ), initiating the release of acetylcholine across the synaptic cleft, eventually leading to depolarisation of skeletal muscle fibres and the generation of movements.

There are three components that make up the structure of a motoneuron: the soma, dendrites, and axon (Stifani, 2014). The soma is the cell body in which the nucleus is located; the nucleus is responsible for all cell maintenance including protein regulation. The dendrites are the branch-like structures found at the ends of a motoneuron, receiving and processing the incoming excitatory or inhibitory signals to activate a motoneuron or prevent a motoneuron from depolarising. Differing from dendrites, the axon arises from the cell body at a specialised area called axon hillock and tends to stay the same diameter for most of its length, and are covered with myelin, which helps signal transmission rapidly. As it nears its end, the axon divides into numerous branches and forms rounded enlargements called neuromuscular junction, which establish connections with muscle fibres.

Within a single motoneuron pool, further layers of motoneuron diversification can be described. Motoneurons targeting skeletal muscle

can synapse on either extrafusal fibres (alpha-motoneuron), intrafusal fibres (gamma-motoneuron), or both (beta-motoneuron) (Kandel, 2013; Stifani, 2014). The primary drivers of muscle contraction are the alpha motoneurons, which innervate extrafusal muscle fibres and together with them forms the motor unit (MU) (Heckman & Enoka, 2012). Intrafusal fibres surrounding muscle spindles are innervated by gamma motoneurons, whereas beta motoneuron target both intrafusal and extrafusal muscle fibres.

1.2.2 Motor unit recruitment and rate coding

The contraction of skeletal muscles is not controlled by a single MU but instead depends on the regulation and coordination of its neural inputs by concurrent variation in changing the number of active MUs and modulating the discharge frequency of action potentials of the active MUs, known as MU recruitment and rate coding respectively (Enoka & Duchateau, 2017).

As force gradation increases, the smallest MUs are activated first, followed by progressively larger MUs, known as size principle (Henneman, 1957; Henneman *et al.*, 1965), which is an extension of the principle that MU excitability is inversely proportional to its size (Bawa *et al.*, 1984). Larger motoneurons have a larger membrane surface (Clamann & Henneman, 1976; Burke *et al.*, 1982) and a greater number of ion channels. In accordance with Ohm's law, in response to equivalent levels of synaptic input, alterations of membrane potential will be greater in smaller motoneurons than larger motoneuronsl. As such, of those receiving the same input, smaller motoneurons will be activated before larger ones (Henneman *et al.*, 1965). Regardless of the source of excitation and the particular circuits involved in mediating it, a group of MUs should be recruited in the same order if size dictates excitability (Mendell, 2005). According to Enoka and Stuart (Enoka & Stuart, 1984), MU size (the size of the impulses recorded from the axons) has a highly significant correlation with the probability of the MU discharging, suggesting that

smaller MUs are more likely to discharge before larger MUs (De Luca & Erim, 1994).

However, this principle has been challenged recently by studies with the discovery of divergent recruitment orders across MUs within the same motoneuron pool (Menelaou & McLean, 2012; Formento *et al.*, 2021; Marshall *et al.*, 2022). For instance, the recruitment order of MUs innervating human interosseous muscle can exhibit variability depending on the direction of movement (Desnedt & Gidaux, 1981). Moreover, animal models have also demonstrated violations of the size principle, manifested by the absence of firing of the most excitable motoneurons (Menelaou & McLean, 2012) or recruitment of MUs according to behaviours under different mechanical constraints (Marshall *et al.*, 2022). Furthermore, the orderly recruitment of MUs has been reported to provide fewer functional advantages for motor control (Dideriksen & Farina, 2013). Most recently, a new framework has been demonstrated that MUs are recruited in group clusters rather than individually (Hug *et al.*, 2023*a*), although this may still be achieved in a 'size-order' manner. Specifically, the central nervous system does not directly control individual muscles but instead regulates functional clusters of MUs, aiming to achieve muscle contractions in a more flexible and efficient way, which is strongly associated with the levels of common synaptic inputs received. Even though the classical definition of size principle appears to be incompatible with the recruitment functional clusters, these observations can still be explained by altering the scale at which the size principle is applied (Hug *et al.*, 2023*a*).

In addition to the activation of MU functional clusters, maintaining or increasing a force level is also dependent upon rate coding, the rate at which MUs discharge (Milner-Brown *et al.*, 1973; Hatze, 1977; De Luca *et al.*, 1982*a*; Kamen & Du, 1999). It is achieved by increasing the net excitatory synaptic inputs to the motoneuron pool, collectively modulating force output from an individual muscle or groups of muscles. Mechanically, the increasing excitatory inputs cause a faster rate of action potential

propagation, leading to a larger summation of muscle fibre twitches and further generating a greater force output (Thomas *et al.,* 1999). According to Henneman's size principle, larger MUs are normally recruited at later stage of the muscle contraction to generate higher amplitude force twitches during a short period of time, which have a greater excitability threshold to tetanize than smaller lower-threshold MUs (Henneman *et al.,* 1965).

The firing pattern of each MU is believed to match the twitch properties of the muscle fibres it innervated (Kandel, 2013). Although slow-twitch fibres are generally characterised by lower force generation capacity compared to fast-twitch fibres, they are able to maintain the same levels of force for a long period of time, for example maintaining posture, and to generate movements at low levels. When a greater level of excitatory inputs is delivered to the motoneuron pool, higher threshold MUs innervating fast-twitch fibres are recruited and generate greater force (Schiaffino & Reggiani, 2011). However, these fibres are more likely to be prone to fatigue as they contain fewer mitochondria and primarily rely on anaerobic metabolism (Baker *et al.,* 2010). Therefore, we would expect early recruited lower-threshold MUs would discharge more slowly, presumably optimising the metabolic and mechanical properties of the muscle fibres they innervate, and the firing rate modulation would be lower than the modulation in higher threshold MU firing rates (Tansey & Botterman, 1996*a*, 1996*b*; Prather *et al.,* 2002).

The onion skin scheme reflects an inverse hierarchical relationship between MU recruitment threshold and MU firing rate at any input excitation level during a voluntary contraction (De Luca et al., 1982). To elaborate, the analogy refers to the idea that as the force requirement increases, progressively larger higher threshold MUs are recruited, much like the peeling of layers of an onion. This onion skin description (De Luca & Erim, 1994) suggests that at a given force level and even with an increase in excitation level and discharge rates of all activated MUs, the later recruited higher threshold MUs will have and maintain lower

discharge rates than the early recruited lower threshold MUs (De Luca & Forrest, 1973; Tanji & Kato, 1973; Monster & Chan, 1977; De Luca *et al.*, 1982*a*).

This phenomenon could be explained by the alterations in electrical impedance during voluntary contractions, specifically, the membrane electrical impedance of higher threshold MUs could reduce during progressive MU recruitment as the concurrent inhibitory inputs may grow more rapidly than excitatory inputs in higher threshold MUs (Pearcey & Rymer, 2022). Additionally, disruptive inhibitory inputs acting via regional spinal interneurons could be introduced by voluntary command and could influence higher threshold MUs disproportionately, thereby regulating both the magnitude and distribution of excitation across the motoneuron pool (Duque *et al.*, 2017; Pearcey & Rymer, 2022).

The onion-skin control scheme is not always followed, especially during higher force levels. Increasing force levels during low- to mid- level contractions increases the discharge rates of earlier recruited lower-threshold MUs, but they eventually reach saturation at higher force levels. Conversely, high-threshold MUs, once recruited, would not stop increasing their discharge rates until reaching the maximum voluntary force, resulting in higher discharge rates than lower-threshold units recruited earlier (Oya *et al.*, 2009). This control scheme with high levels of the maximal excitation drive (up to 100%) is known as the reversed onion skin phenomenon (Barry *et al.*, 2007; Oya *et al.*, 2009; Inglis & Gabriel, 2021*a*). Overall, it seems that the most convincing conclusions cannot be drawn from the MU firing rates alone. Instead, the magnitude of the common synaptic inputs delivered to the motoneuron pool, and the intrinsic neuronal excitability influenced by the monoamines and persistent inward currents are the key factors and need further exploration.

Individual skeletal muscle is not controlled by a single type of MU exhibiting the same characteristics of the firings but contains a mixture of MU types within each muscle to generate different levels of strength and achieve different movements. Interestingly, the proportion of MU

recruitment and rate coding appears to be muscle specific. There are certain muscles with more recruitment-oriented behaviours, such as elbow flexors and shoulder muscles with higher limits of recruitment occurring up to 85% of MVC (Kukulka & Clamann, 1981). Conversely, the hand muscles are oriented more towards rate coding for fine motor control (De Luca *et al.,* 1982*b*). From the study exploring lower limb muscles, higher threshold MUs in tibialis anterior and soleus muscle are activated at force levels approaching 90% to full MVC (Masakado, 1994; Cutsem *et al.,* 1997), suggesting the utilisation of both recruitment and rate coding strategies over its full range of voluntary contraction rather than only employing rate coding at higher force levels.

1.2.3 Determinants of motoneuron excitability

1.2.3.1 Common synaptic inputs

Within the intricate framework of the motor nervous system, the discharge patterns of individual MUs within motoneuron pool are influenced by a shared command rather than distinct command signals directed to each MU (De Luca & Erim, 1994; Farina & Negro, 2015). The transmission of command information from central nervous system to MUs is achieved through the timing of MU discharging, indicating that information is conveyed through frequency modulation in the nervous system. Despite different discharge rates among MUs in the same pool, it has been observed that the discharge rates of these MUs tend to increase and decrease in unison (De Luca & Mambrito, 1987; De Luca & Erim, 1994). The absolute differences in discharge rates between MUs may be attributable to the organisation of central and peripheral inputs into the given motoneuron pool along with differences in the intrinsic characteristics of MUs.

Theoretically, this common synaptic input represents a convergence of neural signals from various sources that converge onto MUs, propelling

them into action. Mechanistically, this drive arises from a complex interplay of excitatory and inhibitory inputs, emanating from diverse neural circuits distributed throughout the central nervous system (De Luca *et al.,* 1982*a*). This delicate balance of excitatory and inhibitory drive is of utmost importance to the motor nervous system, enabling precise and coordinated motor responses, further determining the neural drive delivered to muscles (Farina *et al.,* 2014). A strong correlation between the force fluctuations during isometric contractions and the low-frequency common component oscillations has been observed in human hand muscles (Negro *et al.,* 2009), whereas the independent components of MU discharge rates appear to play a relatively small role in determining force (Dideriksen *et al.,* 2012).

To estimate the level of common synaptic inputs to motoneurons, coherence, a frequency-domain correlation between cumulative spike trains (CSTs) of MUs, defined as the collection of recorded spike trains from a population of MUs where the spiking activity of each MU is preserved independently, from the same muscle (intramuscular coherence) or from different muscles (intermuscular coherence) was introduced and calculated (Hug *et al.,* 2023*b*). When analysing the spiking activity of motoneurons, the common patterns or synchronised activities could be identified within a specific frequency band (e.g., delta (<5Hz), alpha (5-12Hz), beta (15-30Hz) and low gamma (piper) bands (40-50Hz)), which may correspond to different neuronal circuit functions (Grosse *et al.,* 2002; Dai *et al.,* 2017). Importantly, the majority of the energy in a force signal is concentrated in the lower frequency band, closely associated with shared component of the MU spike trains in the lower-frequency range of force variability (Negro *et al.,* 2009). Conversely, the influence beyond this range is attenuated by the contractile muscle properties and therefore has minimal functional impact on force generation (Negro *et al.,* 2009; Dideriksen *et al.,* 2012), reflecting the common modulation of motoneuron firings rates. Frequency components in the alpha band have been reported to heavily depend on the feedback

from muscle spindles and could possibly derive from rhythmical activities of the spinal reflex loop (Christakos *et al.*, 2006; Erimaki & Christakos, 2008). Data from neurological patients suggest that beta-band coherence may reflect activities from cortical and subcortical structures, including the sensorimotor cortex to control presynaptic inputs (Norton & Gorassini, 2006; Lowery *et al.*, 2007), which is found during isometric contractions of weak to moderate strength. Although low gamma (piper) band is also predominantly driven from the primary motor cortex, it is most evident during strong isometric contractions or during movement (Brown, 2000). The presence of distinct coherence estimates in these frequency bands implies the existence of common or shared inputs to the motoneuron pool within these specific frequency ranges, providing insights into the interpretation of MU firing properties and their relationship to functional performance.

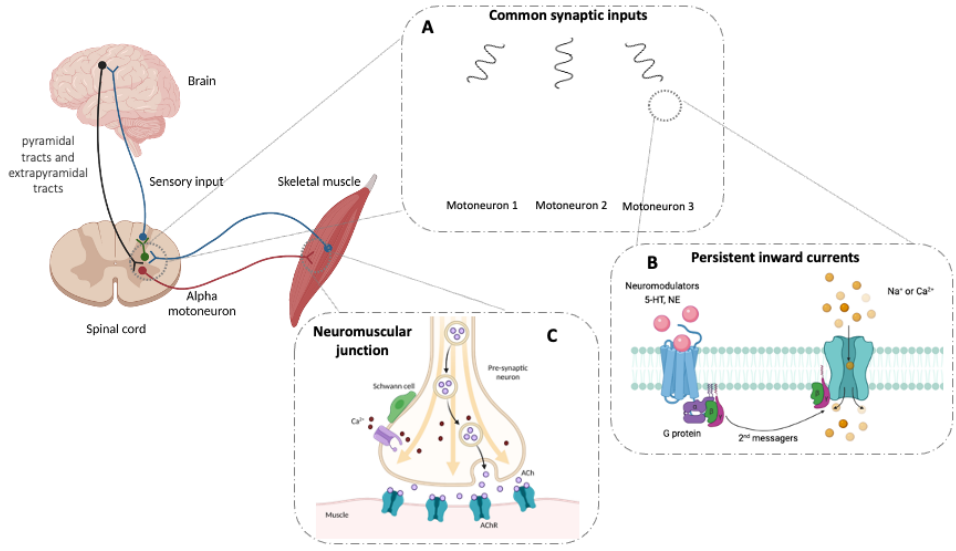

Figure 1.1. Neural input to skeletal muscle. *Central signals travel down descending tracts and synapse to interneurons and lower motoneurons, then to effector skeletal muscles in order to initiate movements. At the level of neuromuscular function, these processes involve; A) common synaptic inputs to motoneurons. B) neuromodulatory inputs to motoneurons. Persistent inward currents play an important role in determining the gain of the motoneurons under conditions of descending monoaminergic drive, such as 5-hydroxytryptamine (5-HT)*

and norepinephrine (NE). This modulation occurs through the binding of G protein coupled receptors and results in prolonged and amplified motoneuron firing. C) neurotransmission at the neuromuscular junction. Action potentials arrive at the pre-synaptic axon terminal and alter the electrical voltage of the membrane, which opens the voltage-gated calcium channels. The influx of calcium triggers the release of acetylcholine (Ach)-containing vesicles into the synaptic cleft.

1.2.3.2 Ionotropic inputs

Motoneurons typically receive common synaptic inputs from two types of receptors: ionotropic and metabotropic. Ionotropic receptors are ion channels triggered by neurotransmitter binding, also known as neurotransmitter-gated or ligand-gated channels. By releasing neurotransmitters into postsynaptic ligand-gated channels within the spinal cord, descending and sensory inputs create synaptic currents to either depolarize or hyperpolarize the motoneuron, change the electrical state of the membrane and generate an electrical signal, known as action potential (Henley, 2021). Ionotropic receptors and neurotransmitters work together like a lock and key and only specific neurotransmitters are able to bind/activate their corresponding receptors. For instance, glutamate, the primary excitatory neurotransmitter in the central nervous system, opens non-selective cation channels, allowing both sodium (Na^+) and potassium (K^+) to cross the membrane (Traynelis *et al.,* 2010). Even though K^+ can leave the cell when the glutamate receptors open, the membrane potential becomes depolarized when the Na+ gradient is stronger than the gradient driving the movement of K^+. Additionally, neurotransmitters such as γ-aminobutyric acid (GABA) and glycine induce inhibitory postsynaptic potentials by opening chloride channels, thereby increasing chloride permeability across the membrane (Lopantsev & Schwartzkroin, 2001; Lynch, 2004).

1.2.3.3 Neuromodulatory inputs

In addition to the release of neurotransmitters into postsynaptic neurons, neuromodulators can enhance excitatory or inhibitory responses by modifying the voltage- and ligand-gated channels of neurons through intracellular signalling pathways and alter a number of neuronal properties, from their excitability to their pattern of discharge in response to a given ionotropic input (Nadim & Bucher, 2014). More specifically, the modulation of this activity occurs through the binding of G protein coupled receptors to ligands, characterized by slow onset and long duration responses that can be distinguished from those induced by inotropic receptors, which are characterized by fast onset and short duration responses (Pavlos & Friedman, 2017). It includes changing the intrinsic firing activity of neurons, increasing, or decreasing voltage-dependent currents, adjusting the synaptic efficacy of neurons, or reconfiguring synaptic connections. Several neuromodulators have been shown to be involved in regulating the excitability of the cortical and spinal motor circuits, such as serotonin (5-hydroxytryptamine, 5-HT) and norepinephrine (NE) (Heckman *et al.,* 2009).

5-HT, as one of the fundamental neuromodulators in the central nervous system, is primarily synthesised in the raphe nuclei of the brainstem, which modulates the intrinsic properties of motoneurons in the ventral horn. A key mechanism by which 5-HT increases neuronal excitability is thought to be the modulation of inward rectifying currents (Takahashi & Berger, 1990; Hsiao *et al.,* 1997), the facilitation of low threshold Ca^{2+} and Na^{+} currents (Berger & Takahashi, 1990), or the suppression of K^{+} leak conductance (Elliott & Wallis, 1992; Perrier *et al.,* 2003). Additionally, 5-HT could enhance discharge rates by reducing the amplitude of slow and medium after-hyperpolarisation phases following the action potential (Kavanagh & Taylor, 2022). Furthermore, although the facilitation is not as strong as in motoneurons, 5-HT also regulates sensory neurons in the spinal cord by influencing the responses of muscle spindle Ia and II afferents and Golgi tendon organ Ib afferents on spinal interneurons.

(Jankowska *et al.,* 2000) The effect of maximum neuromodulation may produce an up to five-fold increase in the amplification of those currents that motoneuron receive from synaptic inputs (Lee & Heckman, 2000).

1.2.3.4 Persistent inward currents

Persistent inward current (Schwindt & Crill, 1980), as an intrinsic property of the neuronal membrane, is a depolarizing current generated by voltage-gated sodium channels and L-type voltage-gated calcium channels on somato-dendritic surfaces of motoneurons (Heckmann *et al.,* 2005). They play an important role in determining the gain of motoneurons under conditions of descending monoaminergic drive. While inward current exceeds PIC threshold, these voltage-sensitive channels remain open. By activating PICs, there is a striking amplification of the depolarizing drive to the motoneuron as well as an acceleration in the firing rate of the motoneuron.

There are four established properties of PICs (Lee & Heckman, 1998, 2000; Heckmann *et al.,* 2005; Heckman *et al.,* 2008). Firstly, induction of self-sustained firing acts to prolong motoneuron firing after a given synaptic input ceases. However, it is not possible to make intracellular recordings from human spinal motoneurons to measure the magnitude of PICs, so alternative methods are employed instead. The rationale for the estimation of PICs is to use steadily firing MU to control the background of synaptic inputs to avoid confusing self-sustained firing with an overall increase in background drive. The second property, onset-offset hysteresis in the frequency-current relationship, indicates that the amount of current required to maintain a motoneuron firing is significantly smaller than the amount of current required to initially recruit the neuron (Heckman *et al.,* 2008). A study using a triangular injected current showed that the difference between discharges that occurred during the ascending phase and those that occurred during the descending phase indicated the PIC contributions to the total current reaching the spike initiation zone (Powers & Heckman, 2015). The third property of PICs is

warm-up, which is a characteristic of L-type Ca^{2+} channels widely present in motoneurons (Bennett *et al.,* 1998; Lipscombe *et al.,* 2004). It has been shown that with repeated stimuli spaced appropriately, the magnitude of PICs increases and in humans, the recruitment threshold can be decreased as well. Amplification followed by attenuation is the fourth fundamental property of PICs (Heckman *et al.,* 2008; Foley & Kalmar, 2019). The acceleration of discharge rate has been observed in humans as a nonlinear relationship between motoneuron firing profiles and the force produced by linearly increasing isometric contractions. Once the synaptic inputs are introduced, activation of the PICs is reflected in the steep increase in discharge rate followed by an attenuated increase, indicating the PIC fully activated.

1.2.4 Nerve-muscle communication

Action potentials from motoneurons communicate with the skeletal muscle fibres via neuromuscular junction (NMJ), the synaptic connection between a motoneuron axon terminal and a muscle fibre. The space between presynaptic and the postsynaptic membrane is known as the synaptic cleft. An action potential propagates down the lower motoneuron to the axon terminal resulting in the opening of the voltage-gated calcium (Ca^{2+}) channels. An influx of Ca^{2+} ions at the presynaptic neuron promotes the fusion of the acetylcholine (ACh)-containing vesicles to the presynaptic membrane. The endplate depolarisation by the released ACh is called an endplate potential, which is accomplished when ACh binds the post-synaptic ACh receptors (AChRs) on the junctional folds of the muscle fibre and further triggers the ligand-gated sodium (Na^{+}) ion channels to open. This influx of Na^{+} generates the depolarisation and triggers an action potential that propagates along the muscle fibre membrane and through the T-tubules (transverse tubules, invaginations of the plasma membrane) within the muscle. This stimulates the opening of voltage gated Ca^{2+} and leads to a release of Ca^{2+} from the sarcoplasmic reticulum of myofibrils (a specialised form of the endoplasmic reticulum responsible for handling Ca^{2+}). When Ca^{2+}

binds to troponin C, this interaction induces a conformational change in tropomyosin, leading it to uncover the myosin binding sites on the actin filaments. Subsequently, tropomyosin repositions itself, allowing myosin heads to attach to their binding sites on the actin molecule. This repositioning is facilitated by the hydrolysis of adenosine triphosphate (ATP) by the myosin ATPase enzyme. Once attached to the actin filament, the resulting products of adenosine diphosphate (ADP) and inorganic phosphate detach from the myosin head, initiating the 'power stroke', which pulls the actin filament towards the centre of the sarcomere, known as the 'M-line'. A new ATP molecule binds to the myosin head, causing it to return to its initial position. Once in a neutral position, the process, referred to as excitation-contraction (E-C) coupling, repeats until the contraction is complete. The cumulative mechanical action of sarcomere shortening throughout the muscle results in muscle contraction (Kress & Mennerick, 2009; Frontera & Ochala, 2015).

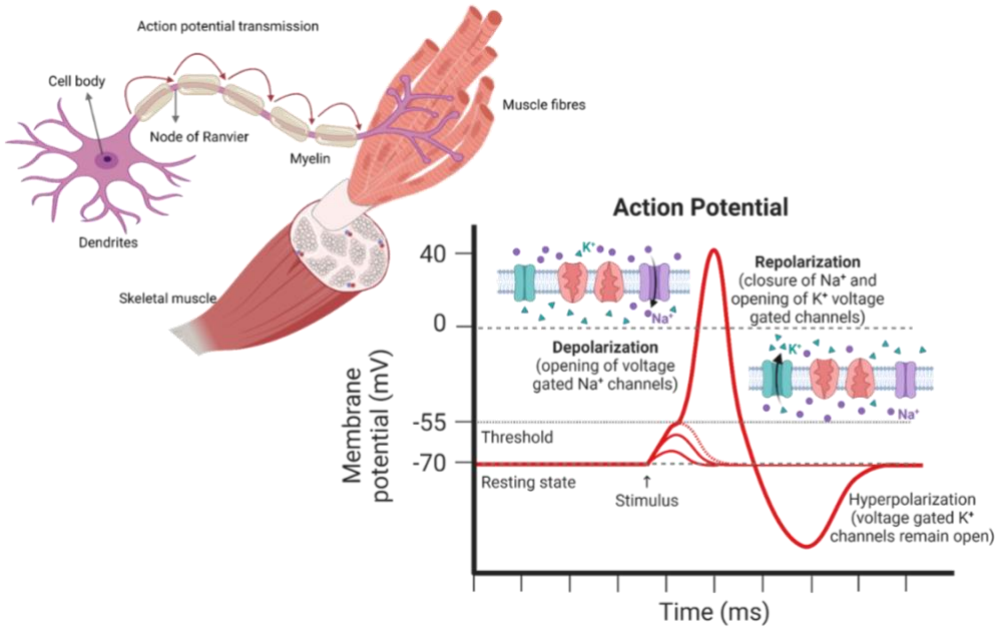

Figure 1.2. Mechanism of the action potential. *Motoneuron excitation results in depolarization of its membrane. If this depolarization reaches activation threshold, it triggers the opening of the voltage-gated sodium channels, allowing an influx of sodium (Na^+) ions into the motoneuron. The action potential propagates along the axon towards the neuromuscular junction. Once the threshold is reached, repolarization takes place, where voltage-gated potassium (K^+) channels open, allowing K^+ ions to transfer to the extracellular space. This rapid outflow restores the initial resting state. Following repolarization, a brief period of hyperpolarization may occur, making the membrane potential more negative than the resting potential due to prolonged potassium channel activity. Once the voltage-gated K^+ channel closes, the membrane will return to the resting potential.*

1.3 Electromyography (EMG)

Activation of motoneurons results in a concurrent activation of all muscle fibres within the MU. With EMG techniques, which range in complexity but largely involve surface based and/or intramuscular electrodes, fluctuations in extracellular voltages can be detected *in vivo* from a contracting muscle over time due to ion exchange across muscle fibre membranes as a result of propagating muscle fibre action potentials. The summated action potential of a single MU is referred to as a MU potential (MUP) and represents the summation of depolarisations from fibres innervated by the same axon, within the recording range of the electrode. It is important to underline that the shape of the MUP depends on the MU properties, including the number of muscle fibres innervated by the motoneuron, the cross-sectional area and type of fibres, as well as the location and orientation of these fibres in relation to the recording electrodes. EMG measurement has been proven essential in the diagnosis and treatment of a number of neuromuscular disorders (de Carvalho *et al.,* 2014) in clinical research, and has also proven useful in non-clinical human research studies with a focus on ageing (McNeil et al., 2005; Piasecki et al., 2018; Hassan et al., 2021), exercise (Piasecki *et al.,* 2019, 2021*a*; Jones *et al.,* 2021) and sex (Parra *et al.,* 2020; Inglis & Gabriel, 2021*b*).

1.3.1 Surface electromyography

Surface EMG (sEMG) as a non-invasive method is a two-dimensional distribution of the voltages lying within the detection volume of the electrodes placed over the skin of the muscle being studied. sEMG can be recorded by a pair of electrodes, known as single channel sEMG or by a more complex array of multiple electrodes, referred to high-density sEMG (HDsEMG) and increasingly favoured in research settings. Compared to single-channel sEMG, HDsEMG with multi-channels provides a larger detection area and accounts for both the spatial and temporal characteristics of the signal, allowing a broader assessment of the muscle electrophysiological activity (Kleine *et al.,* 2007; Holobar *et al.,*

2009; Martinez-Valdes *et al.,* 2016). However, sEMG provides little information about the deeper muscles due to the filtering characteristics of the volume conduction properties of the overlying muscles and other subcutaneous tissues, resulting in signal attenuation. Surface detected signals are also often contaminated by the activity of adjacent muscles, known as crosstalk (Kuiken *et al.,* 2003; Nösslinger *et al.,* 2022). This inability to accurately sample deeper MUs may limit its use in certain cohorts where subcutaneous tissue is greatest (e.g., thigh muscles of the elderly, diabetic or obese), however, these limitations can be overcome with the use of intramuscular EMG (iEMG).

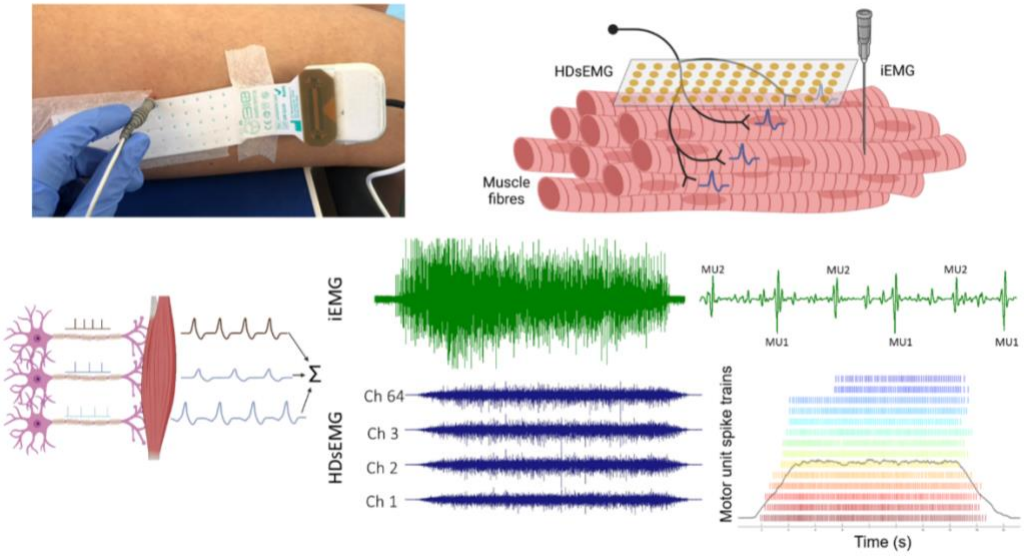

Figure 1.3. Methodological approaches to study neural input to muscle. *Action potentials traveling down the lower motoneuron terminate at the neuromuscular junction and continue propagating along muscle fibres. Action potentials can be recorded simultaneously by both HDsEMG placed over the skin and iEMG inserted into the muscle. Raw EMG signals are decomposed into constituent motor unit action potential trains for further analysis.*

1.3.2 Intramuscular electromyography

Intramuscular EMG (iEMG) is commonly applied using needle or wire electrodes and is viewed as a minimally invasive technique (Enoka & Fuglevand, 2001; Farina & Negro, 2012). Unlike sEMG with a greater detection volume of the muscle, inserting electrodes into muscles allows the detection of MUPs close to the contracting muscle fibres, which is consequently easier to distinguish and identify the MUPs of individual MUs from interference signals. To date, MUP characteristics using iEMG have been reported in healthy volunteers in the upper limb muscles (hand muscles (Boe *et al.,* 2005; Tsang *et al.,* 2020), biceps brachii (Power *et al.,* 2012; Piasecki *et al.,* 2019) and trapezius (Ives & Doherty, 2014)) and lower limb muscles (vastus lateralis (Piasecki *et al.,* 2016*c,* 2019; Swiecicka *et al.,* 2019), tibialis anterior (McNeil *et al.,* 2005; Piasecki *et al.,* 2016*a,* 2021*a*) and soleus).

1.3.3 EMG signal analysis

EMG signal decomposition entails the intricate task of disentangling a complex composite EMG signal into its constituent MUP trains (MUPTs). This decomposition process relies upon two fundamental assumptions for its successful execution: 1) it assumes that the entirety of MUPs generated by the active MUs could be accurately detected; 2) it postulates that the MUPs elicited by an individual MU exhibit a greater degree of resemblance among themselves than to the MUPs generated by distinct MUs. These assumptions form the bedrock of the EMG signal decomposition methodology, facilitating the extraction of meaningful information from the amalgamated EMG signal and enabling a comprehensive analysis of the underlying MU firing properties. In general, an automatic EMG signal decomposition process includes signal acquisition, signal pre-processing, signal segmentation and MUP detection, feature extraction, clustering and supervised classification of detected MUPs, resolving superimposed MUPs, and estimating MU firing properties and MUP templates (Stashuk, 1999a, 1999b).

Though sometimes the MUPTs cannot be reliably extracted due to the factors like signal contamination and/or insufficient signal quality, signal frequency components most likely to be physiologically originating can be amplified with filter settings; a high-pass filter reduces amplification of low frequency signal components, a low-pass filter reduces amplification of high frequency signal components, with bandpass filtering allowing a combination of both (Holobar *et al.,* 2014). In addition, the detected signals are complex superpositions of sinusoidal wave components of mixed frequencies, including those that are non-physiological in origin, such as power supplies (50 or 60 Hz), which are also filtered before decomposition During the decomposition process, all individual MUPs and their corresponding MUPTs are detected, representing the individual activities of concurrently active MUs and providing informative information on neuromuscular system such as regional activation, muscle fibre properties and single MU activity (Dalton *et al.,* 2008; Gallina *et al.,* 2022). For example, the amplitude (positive peak to negative peak) of the MUP waveform can be used to investigate the size of the MU as well as the conduction velocity of the fibres within a MU and the number of phases and turns are measures of MUP complexity, reflecting temporal dispersion across MU muscle fibre potentials as well as the most important feature, MU recruitment and rate modulation, which is highly related to force generation and fluctuations.

1.4 Sex-related differences in neuromuscular system

1.4.1 Muscle type composition

In general, males are considered to possess greater muscle strength than females in both upper and lower extremities, due to the fact that males have larger muscles (Hannah *et al.,* 2012). A growing number of research indicates that sex plays a pivotal role in influencing the relative proportion of muscle fibre types (Landen *et al.,* 2023).

In males, there is generally a a higher proportion and larger cross-section area of fast-twitch type II muscle fibres compared to females. These fast twitch muscle fibres are linked to higher threshold MUs, which have the potential to produce greater action potential amplitudes, resulting in the generation of higher force (Pope *et al.,* 2016). They are also associated with explosive strength and movements (Haizlip *et al.,* 2015). However, females generally have a higher proportion of slow-twitch type I muscle fibres, which are more resistant to neuromuscular fatigue when assessed at a normalised contraction level (Hunter, 2014).

1.4.2 Sex hormones and skeletal muscle function

It appears likely that the contributors for these sex-related differences in skeletal muscles are a consequence of different sex hormonal levels, but the mechanisms behind them are not well understood in humans. Testosterone, more abundant in males, is predominantly produced in the testes and is not only responsible for the development of male secondary sexual characteristics but also for the neuronal synaptic plasticity and musculoskeletal development (Kuwahara *et al.,* 2021). Estrogen and progesterone are primary female sex hormones and are able to cross the blood-brain barrier potentially influencing crucial central nervous system functions (Smith & Woolley, 2004; Sohrabji, 2007). Throughout most of adulthood, females experience a cyclical pattern of sex hormone fluctuations through the menstrual cycle. Estrogen elicits excitatory effects via potentiation of glutamatergic receptors (Smith & Woolley, 2004)

while progesterone increases activity of GABA, causing inhibitory effects (Smith *et al.,* 1989). Both testosterone and estrogen have been reported to influence the expression and activity of brain-derived neurotrophic factor (Delchev & Georgieva, 2017), which are key mediators of synaptic plasticity (Mantilla *et al.,* 2014).

In females, estrogen may maintain skeletal muscle mass by augmenting satellite cell populations (Collins *et al.,* 2019*a*) or increasing the number of cross-bridges (Lowe *et al.,* 2010; Qaisar *et al.,* 2013). Moreover, estrogen has been associated with enhancing mitochondrial function via estrogen receptor signalling, manifested by increased number of mitochondria, improved oxidative capacity and energy production (Ikeda *et al.,* 2019). As well as stimulating antioxidant enzyme expression, estrogen reduces reactive oxygen species production, and helps maintain the integrity of mitochondrial membranes, potentially affecting skeletal muscle alterations according to sex (Ventura-Clapier *et al.,* 2019). However, further research is necessary to fully understand the underlying mechanisms and implications in human skeletal muscle, which can be influenced by factors such as age and exercise training.

1.4.3 Exercise-related hormone changes

Exercise elicits significant hormone changes in both males and females. In males, circulating levels of testosterone typically increase in response to acute bouts of resistance training (Vingren *et al.,* 2010), resulting in the enhanced muscle protein synthesis and further muscle strength and hypertrophy (Hansen *et al.,* 2001; Ahtiainen *et al.,* 2005) due to the increased androgen receptors (Spiering *et al.,* 2009) and subsequent myocellular signalling (Kraemer *et al.,* 2020). Yet the mid- and long-term effects of exercise on circulating testosterone levels remain inconclusive (MacKelvie *et al.,* 2000; Grandys *et al.,* 2009; Riachy *et al.,* 2020). Females, on the other hand, experience hormonal changes related to exercise that differ due to the fluctuations between estrogen and progesterone during

the menstrual cycle, potentially confounding true exercise effects (Cano Sokoloff *et al.,* 2016).

1.4.4 Age-related hormone changes

The decline in estrogen or androgen levels with ageing has been shown to play a crucial role in muscle mass and strength and motor control. Testosterone is an anabolic hormone, and in both sexes, it promotes muscle protein synthesis and muscle fibre growth (Horstman *et al.,* 2012). With ageing, there is a gradual decline in testosterone levels, contributing to the loss of muscle mass and strength. Decreased testosterone also appears to be related to reduced release of neurotransmitters and other neurotrophic agents (Pluchino *et al.,* 2013; Spritzer & Roy, 2020), in turn, affect the partial denervation of muscle fibres and the consequent sarcopenia. The decline in estrogen levels during menopause can also result in muscle loss and decreased muscle function (Collins *et al.,* 2019*b*).

Hormone replacement therapy and exercise training are sometimes used to prevent or mitigate the effects of declining sex hormone levels in ageing individuals (Ari *et al.,* 2004; Hawkins *et al.,* 2008; Phillips *et al.,* 2017). Testosterone or estrogen administration has been associated with improvements in muscle mass, strength and physical performance and has also been reported to exhibit a range of neuroprotective effects in motoneurons, such as dendritic maintenance and axonal sprouting in animal studies (Kurz *et al.,* 1986, 1991; Byers *et al.,* 2012). However, there is limited evidence exploring sex differences in neuromuscular function in lower limb and how ageing would influence the individual MU properties and physical performance in humans.

1.5 Ageing-related neuromuscular adaptations

1.5.1 Adaptations at central nervous system level

There is a vast array of structural and functional alterations that occur in the nervous system during the aging process, which play a significant role in age-related declines in skeletal muscle function. One prominent age-related alteration is the reduction in brain volume. As an individual progress in age, the volume of motor cortex cells tends to diminish (Haug & Eggers, 1991; Salat *et al.,* 2004), particularly affecting specific regions, for example, the prefrontal cortex, potentially contributing to slower gait occurred with aging (Rosano *et al.,* 2012). Moreover, extensive evidence supports the notion that the volume of grey matter decreases with aging (Chen *et al.,* 2015), accompanied by the deterioration of white matter fibres (Marner *et al.,* 2003). Studies of neuron number have yielded controversial results, but a significant reduction in the size of neurons across various regions of the cerebral cortex has been consistently reported (Peters, 2006; Esiri, 2007; Freeman *et al.,* 2008). Additionally, there is an observable decline in the number of synapses in older individuals, particularly in asymmetrical axospinous synapses found in the precentral gyrus, accompanied by an increased length of the postsynaptic contact zone, further highlighting the intricate alterations occurring within the aging brain (Adams, 1987).

Ageing also exerts profound effects on neurotransmission, a fundamental process involving transmission of chemical signals between neurons. One of the neurotransmitters that play a crucial role in the central nervous system is glutamate, which serves as the primary excitatory neurotransmitter (Bukke *et al.,* 2020; Gasiorowska *et al.,* 2021). It has been proposed that dysregulation of glutamate appears to occur with advancing age (Segovia *et al.,* 2001; Brans *et al.,* 2010) accompanied by a reduced expression of glutamate receptors, leading to the perturbations in synaptic function and neuronal excitability (Ménard & Quirion, 2012). Additionally, 5-HT acts as a modulator of neuronal activity, specifically,

inhibiting excessive motoneuron firing, and a reduced serotonergic activity has been widely reported in older age, evidenced by a reduction in 5-HT receptor expression (Morgan *et al.,* 1987; Bigham & Lidow, 1995), resulting in a decreased motoneuron excitability (Andrade, 2011). Furthermore, age-induced impairments of the GABAergic system have been reported in several human studies (Rozycka & Liguz-Lecznar, 2017; Pandya *et al.,* 2019), leading to inhibitory deficits and an imbalance between excitatory and inhibitory neurotransmission, consequently decreasing the responsiveness of motoneurons to external stimuli.

At the spinal cord level, aging is associated with notable structural and functional adaptations (Manini *et al.,* 2013). One prominent alteration is spinal atrophy, which is closely linked to the decline in spinal motoneuron population (Piekarz *et al.,* 2020). This decline is accompanied by a decrease in the density and diameter of both myelinated and unmyelinated axons within the ventral horns of the spinal cord in aged adults (Kawamura *et al.,* 1977; Mittal & Logmani, 1987; Buchman *et al.*, 2019). These morphological changes are accompanied by age-related reductions in conduction velocity and responsiveness of peripheral afferent and efferent neurons, leading to a decrease in spinal motoneuron excitability (Palve & Palve, 2018; Borzuola *et al.,* 2020).

1.5.2 Adaptations at motor unit level

As a result of central plasticity occurs with ageing, the final common element in motor nervous system, MUs which mediates the transduction of neural activity in central nervous system to muscle movement, also undergo considerable adaptations (Lexell, 1997; Valdez, 2019).

1.5.2.1 Motor unit number

Aged muscles have been reported to have a decreased density of active zones in the pre-synaptic regions, leading to the retraction of motoneuron nerve endings from NMJ and the muscle fibres it innervates are no longer

controlled by the nervous system (Valdez, 2019; Iyer *et al.*, 2021; Deschenes *et al.*, 2022). Progressive declines in the number of MUs have been identified in several aged muscles in humans (McNeil *et al.*, 2005; Piasecki *et al.*, 2016*b*). Apart from direct counts (estimates) of motoneurons from spinal cord samples (Tomlinson & Irving, 1977), indirect MU number estimates (MUNE) using surface EMG also points to a progressive loss (Brown *et al.*, 1988; Piasecki *et al.*, 2018*a*). Evidenced by a positive correlation between MU number and muscle strength (Kaya *et al.*, 2013), the loss of MU number has been considered as the major contributor to the loss of muscle mass and further functional impairments observed in the elderly (Piasecki *et al.*, 2016*c*, 2019).

One of the factors affecting the muscle fibre denervation is mitochondrial function, which plays a crucial role in energy production and maintenance of cellular homeostasis (Wallace, 2005). Age-related mitochondria impairment has been demonstrated, including reduced ATP production, increased reactive oxygen species production, and diminished mitochondrial biogenesis (Srivastava, 2017). When mitochondria fail to provide the necessary energy to maintain motoneurons' integrity and function, they become more susceptible to degeneration and denervation (García *et al.*, 2013). Increased oxidative stress and reactive oxygen species can induce increased oxidative damage to cell components, which further aggravates denervation by increasing neuro-inflammation, impairing axonal transport, and triggering apoptosis (Fang *et al.*, 2012; Pizzino *et al.*, 2017; Singh *et al.*, 2019).

1.5.2.2 Motor unit remodelling

The muscle fibre denervation may be accompanied by reinnervation, in which the denervated muscle fibres are reinnervated by adjacent surviving axons (Borzuola *et al.*, 2020; Verschueren *et al.*, 2022). Specifically, this process encompass the sprouting of new axonal growth in surviving neurons, which originates from various anatomical locations, such as nodes of Ranvier, the nerve terminal, or the motor endplate (Tam &

Gordon, 2003) and relies on an increased total length of the nerve terminal branches and the number of terminal branches present at NMJ (Robbins & Fahim, 1985; Prakash & Sieck, 1998; Deschenes *et al.*, 2020). Subsequently, these sprouted growth cones undergo intricate molecular and cellular events leading to the establishment of new connections with denervated muscle fibres (Gordon & Fu, 2021), which plays a pivotal role in functional reintegration of denervated muscle fibres. As a consequence of the remodelling process, aged MUs may be characterised as being fewer in number, and larger in size (innervation ratio).

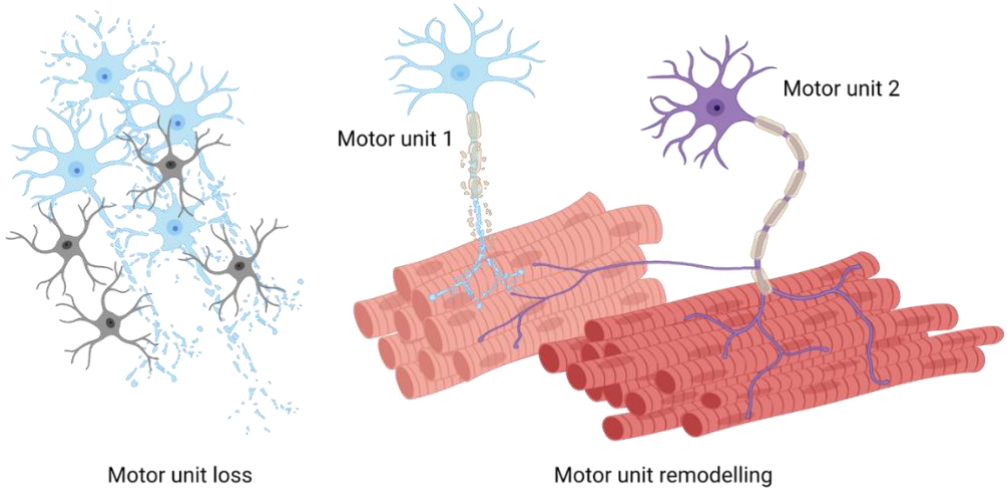

Figure 1.4. Age-related motor unit remodelling. *In response to motor unit loss, some muscle fibres may be reinnervated by adjacent surviving axons, establishing new neuromuscular junctions at the denervated fibre. This compensatory process likely acts to minimise fibre loss and preserve muscle function.*

1.5.2.3 NMJ-related adaptations

A further consequence of MU remodelling, which may also occur independently of it, is adaptation of the NMJ (Willadt *et al.*, 2018; Khosa *et al.*, 2019; Deschenes *et al.*, 2022). Firstly, within each nerve terminal branch, the number of vesicles containing neurotransmitters have also been

reported to reduce with ageing (Deschenes *et al.,* 2022). At the post-synaptic component, an increased fragmentation of the endplate has been demonstrated in aged NMJs, manifested by a decreased but a greater dispersion of AChR (Taetzsch & Valdez, 2018). These morphological alterations consequently contribute to the impairment of neurotransmission between nerve terminals and muscle fibres, and force generation in aged individuals.

As individuals age, there is a chronic increase in the levels of circulating inflammatory markers. For instance, this age-related shift includes a decline in insulin-like growth factor 1 (IGF-1), which likely contributes significantly to the process of motor unit denervation and neuromuscular degeneration(Delbono, 2003). Experimental studies utilizing animal models have shown that enhancing muscle specific IGF-1 can effectively counteract age-related changes observed in nerve terminals and neuromuscular junctions (Messi & Delbono, 2003). This enhancement promotes increased axonal sprouting and reduces post-synaptic endplate fragmentation. By promoting the structural integrity of the neuromuscular junction and facilitating efficient transmission of nerve impulses to muscle fibres, IGF-1 acts as a crucial regulator in preserving neuromuscular connections and alleviating age-related declines in motor unit innervation (Sullivan *et al.,* 2008). However, the ongoing state of chronic inflammation has been found to suppress the production of IGF-1, thereby reducing its biological activity, and further negatively impacting neuromuscular and physical function with ageing (Barbieri *et al.,* 2003).

1.5.3 Adaptations at muscle fibre level

1.5.3.1 Muscle fibre type / number / size

A substantial decrease in muscle fibre size has been widely demonstrated in the elderly (Larsson, 1978*a*) and the reduction in muscle fibre size has been shown to be fibre type specific (Verdijk *et al.,* 2007).

Type I muscle fibre size appears to be largely sustained with ageing, however, type II muscle fibres have been suggested to be more preferential to age-related atrophy (Larsson, 1978*b*; Nilwik *et al.,* 2013; Miljkovic *et al.,* 2015). Muscle atrophy with ageing may partly attributable to a decrease in total muscle fibre number with age alongside an age-related decrease in muscle fibre size (Lexell *et al.,* 1983, 1988). Additionally, considering satellite cells are important to skeletal muscle fibre growth, repair and regeneration, a decrease in satellite cells in type II muscle fibres with no differences seen in type I fibres has been reported in some human studies (Verdijk *et al.,* 2007; Kadi & Ponsot, 2010), indicating a specific type II muscle fibre atrophy as well as increased neuromuscular degeneration during ageing.

1.5.3.2 Muscle contractile function

In addition to the pronounced skeletal muscle fibre loss, the altered fibre quality also contributes to the functional decrements in the elderly, particularly insufficient ability to generate explosive movement (Macaluso & De Vito, 2004; Russ *et al.,* 2012). An increasing body of evidence indicates that the reduction of muscle power output in the elderly is due to the decrease in contractile properties, reduced contractile speed, and decreased maximal shortening velocity of the muscle fibres (Larsson *et al.,* 1997; Krivickas *et al.,* 2001; Bellumori *et al.,* 2013). Alongside the structural and functional changes at skeletal muscle fibres, intermuscular adipose tissue has been found in numerous studies to be more prevalent in aged skeletal muscles, associated with the decrements of physical performance in the elderly.

1.5.3.3 Excitation-contraction coupling

Skeletal muscle contraction and relaxation is regulated by action potentials that travel down the muscle fibre membrane (sarcolemma), and ultimately release calcium ions from the sarcoplasmic reticulum to facilitate actin-myosin binding (Calderón *et al.,* 2014). This process is referred to as excitation-contraction coupling, which could be impaired

with age (Plant & Lynch, 2002). Voltage-gated calcium channels in the sarcolemma have been reported to decline in number and function in aged skeletal muscles (Tieland *et al.,* 2018), accompanied by a decline in the activity of ryanodine receptors in the sarcoplasmic reticulum, resulting in the reduction of Ca^{2+} concentration and further influencing force production (Delbono *et al.,* 1995; Payne *et al.,* 2009; Russ *et al.,* 2011). Additionally, the sarcoplasmic reticulum may become overloaded with calcium ions due to a reduction in the activity of calcium reuptake channels, leading to a variety of downstream effects, including calpain activation and muscle cell apoptosis, further leading to muscle damage and impaired muscle function (Verburg *et al.,* 2009).

Previous studies have also shown the loss of myosin content, which leads to a lower percentage of strongly bound cross-bridges during muscle contraction, contributing to the reduced rates of force production (Miller *et al.,* 2013). Moreover, decreased actin sliding speed, independent of myosin heavy chain expression, has been suggested to be associated with the reduction in maximal shortening velocity and increased myosin attachment times in older individuals (Power *et al.,* 2013).

A dedicate balance between muscle protein synthesis and degradation is essential for maintaining muscle mass, and age-related muscle atrophy is a result of a negative protein balance (Wilkinson *et al.,* 2018), which has been extensively documented in aged muscles (Welle *et al.,* 1993; Breen & Phillips, 2011). However, it becomes increasingly evident that these effects are primarily influenced by factors occurring upstream of the muscle fibres. Notably, neural factors are more likely to be identified as a cause rather than a consequence in this context, given the reduced stimulus experiences by the muscle fibres. This suggess that upstream neural mechanisms play a pivotal role in driving the age-related alterations in muscle function.

1.6 Investigations and Aims

The current knowledge of the human neuromuscular system primarily derives from studies conducted primarily in males. Consequently, it is common practice to extrapolate and expand these findings to females. However, it is crucial to acknowledge the distinguishable physiological disparities between males and females in anatomical and physiological aspects as these differences exert a profound influence on the neuromuscular system and physical performance. Extensive research has reported that females exhibited lower muscle strength, which may arise from the variances in muscle fibre composition and alterations in neural input to muscle.

Several studies have investigated the firing patterns of motor units and force control ability between sexes, revealing that young females exhibit higher motor unit firing rate and present poorer force steadiness. Nevertheless, the sex-based differences of motor unit properties in the substantial muscle groups of the lower extremities, such as quadriceps, and how age will influence these properties have been overlooked and further investigation is warranted. In addition, numerous investigations so far have been routinely conducted unilaterally, which may neglect the potential bilateral differences in neuromuscular function across limbs due to the asymmetry in peripheral and central nervous systems, thereby affecting the effectiveness of unilateral therapeutic intervention. Furthermore, neuromuscular function, particularly motor unit firing properties are largely influenced by the levels of neural inputs received. However, scant scientific evidence exists regarding the level of common synaptic inputs and persistent inward currents occurring in the vastus lateralis muscles. As the age-related functional decline is partly attributable to the absence of physical activity, to better examine the effect of inherent ageing, master athletes serve as an ideal model to explore the influences of circulating sex hormones.

Therefore, this thesis set out to achieve the following aims:

1) To compare individual MU properties and neuromuscular recruitment strategies, as well as MU number estimates in the vastus lateralis of healthy young males and females.
2) To explore the influences of sex and ageing on physical performance and neuromuscular characteristics in vastus lateralis of early to late elderly males and females.
3) To investigate bilateral differences in the vastus lateralis with respect to muscle function and individual MU characteristics in addition to common synaptic inputs in both young and older males, and to explore the influence of advancing age on bilateral differences within older individuals.
4) To explore the differences in physical performance as well as the magnitude of persistent inward currents and common synaptic inputs in the vastus lateralis between healthy young and older males.
5) To investigate the effects of lifelong exercise on circulating sex hormone levels and neuromuscular properties, and to explore whether athletic status influences the associations between circulating sex hormone levels and MU characteristics of the vastus lateralis muscle in older males.

Chapter 2 - Neuromuscular recruitment strategies of the vastus lateralis according to sex

2.1 Abstract

Background

Despite males typically exhibiting greater muscle strength and fatigability than females, it remains unclear if there are sex-based differences in neuromuscular recruitment strategies e.g. recruitment and modulation of motor unit firing rate (MU FR) at normalised forces and during progressive increases in force.

Methods

Twenty-nine healthy male and thirty-one healthy female participants (age range: 18-35 years) were studied. Intramuscular electromyography (iEMG) was used to record individual motor unit potentials (MUPs) and near-fibre MUPs from the vastus lateralis (VL) during 10% and 25% of maximum isometric voluntary contractions (MVC), and spike-triggered averaging was used to obtain motor unit number estimates (MUNE) of the VL.

Results

Males exhibited greater muscle strength (p<0.001) and size (p<0.001) than females, with no difference in force steadiness at 10% or 25% of MVC. Females had 8.4% and 6.5% higher FR at 10% and 25% of MVC, respectively (both p<0.03), while the MUP area was 33% smaller in females at 10% of MVC (p<0.02) and 26% smaller at 25% of MVC (p=0.062). However, both sexes showed similar increases in MU size and FR when moving from low- to mid-level contractions. There were no sex differences in any near-fibre MUP parameters or in MUNE.

Conclusion

In the vastus lateralis, females produce muscle force via different neuromuscular recruitment strategies to males which is characterised by smaller MUs discharging at higher rates. However, similar strategies are employed to increase force production from low- to mid-level contractions. These findings of similar proportional increases between sexes support the use of mixed sex cohorts in studies of this nature.

2.2 Introduction

Skeletal muscle contraction is regulated by the coordinated activation of motoneurons and muscle fibres. The fundamental neuromuscular element regulating muscle contraction is the motor unit (MU), consisting of a motoneuron and the muscle fibres it innervates (Heckman & Enoka, 2012). Increases in muscle force are largely mediated by two neuromuscular recruitment strategies, the recruitment of additional, progressively larger MUs, and an increase in MU firing rate (FR), referred to as MU recruitment and rate modulation, respectively (Enoka & Duchateau, 2017). Several studies have highlighted adaptative remodelling of MUs structure and function in response to exercise training, ageing and disease (Allen *et al.,* 2015; Piasecki *et al.,* 2016*b,* 2019; Del Vecchio *et al.,* 2019), which may influence recruitment strategies, however the majority of data are only available in males.

Males generally possess greater muscle strength than females in upper and lower extremities, which is largely explained by greater muscle size (Hannah *et al.,* 2012). Conversely, although task-specific, females 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 differing fibre type ratios with a 7-23% greater proportion of type I fibres in vastus lateralis (VL) in females(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 exhibits 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) (Drevenšek, 2018), which are key mediators of synaptic plasticity (Mantilla *et al.,* 2014). Acutely, differences in sex hormones partly explain the variability in fatigability in females 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 females are often underrepresented in studies of neuromuscular physiology (Cowley *et al.,* 2021).

Surface electromyography (EMG) has been commonly applied to study sex-based differences of neuromuscular function and recruitment strategies (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 quality signals in females due to the greater subcutaneous tissues (Nösslinger *et al.,* 2022), 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 potential size and complexity (Piasecki *et al.,* 2021*b*). Although we have previously reported the sex-based divergent trajectory of MU FR from middle to older age in long-term trained masters athletes (Piasecki *et al.,* 2021*a*), comparisons of normative values in healthy young males and females at differing contraction levels are unknown.

The aims of the present study were to compare individual MU properties and neuromuscular recruitment strategies, as well as MU number estimates (MUNEs) in the VL of healthy young males and females. We hypothesised that there would be no sex-base differences in motor unit size and firing rate at normalised contraction levels as well as in recruitment strategies employed when moving from a low- to a mid-level contraction.

2.3 Methods

2.3.1 Participants

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.

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 participants 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. Stage of the menstrual cycle, or cycle status, was not assessed in the female participants. Methods or types of birth control were not assessed in the female participants.

2.3.2 Anthropometry

Body mass and height were measured using calibrated scales and a stadiometer, respectively for the calculation of body mass index (BMI). Ultrasound was used to measure the cross-sectional area of the VL 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 medial-to-lateral direction position on the skin, beginning, and ending the image capture at aponeurosis borders using panoramic imaging

(VPAN). 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. CSA was determined by analysing each image three times and taking the average of three images. 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. VL CSA values are available for 23 males and 19 females.

2.3.3 Muscle strength and force steadiness

The maximal isometric voluntary contraction force (MVC) of the right knee extensors 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. Surface adhesive electrodes (detailed below) were placed on the skin. A seat belt was fastened across the pelvis to minimise 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 each trial with maximal effort, with real-time visual feedback and verbal encouragement. This was repeated a further two to three times, with 60 second rest intervals between each, and if the difference between last two attempts was less than 5%, the highest value, in Newtons was accepted as MVC. Peak torque during the selected MVC was also determined by multiplying the selected MVC by the lever arm.

Prior to needle insertion and multiple sustained contractions, a familiarisation trial was performed in which participants were instructed to match the target force at each contraction level for 12-15 seconds. Following the practice trial, the intramuscular needle electrode was inserted into the mid-point of the VL, and participants were instructed to perform between four and six sustained isometric contractions at 10% and 25% of MVC, each lasting 12-15 seconds with a target line displayed on the screen and real time force feedback (Figure 2.1). Participants had 20-30 seconds rest between each contraction.

Force steadiness was quantified as the coefficient of variation of the force [CoV; (SD/mean) × 100]. To avoid corrective actions when reaching the target line, the first two passes of the target (<1s) were excluded from the calculation. The mean CoV at each contraction level was calculated from the middle two contractions.

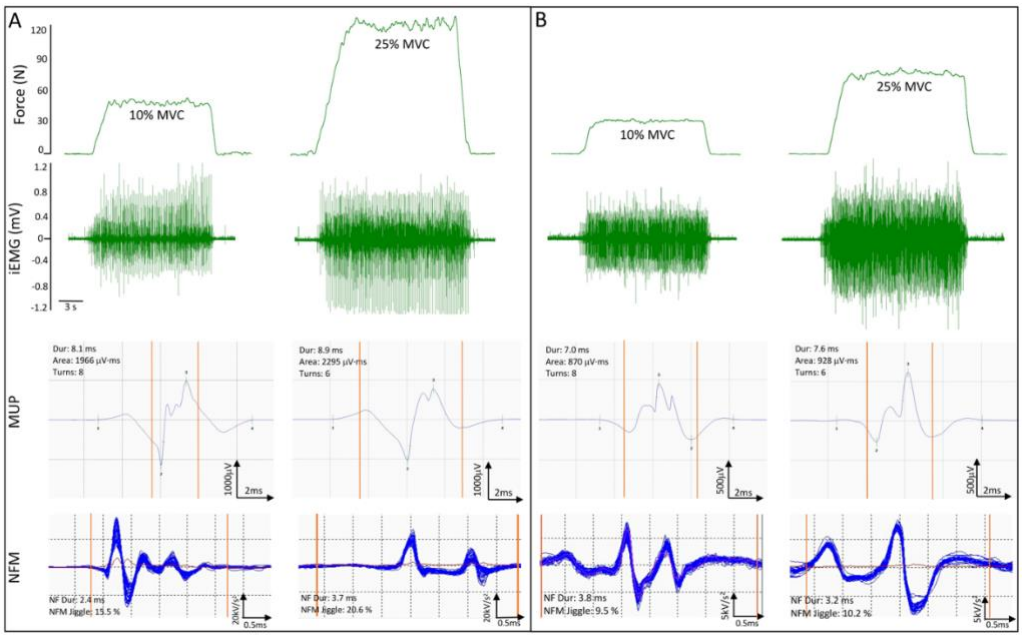

***Figure 2.1**. Representative data from a male **(A)** and a female **(B)** participant. Top panels show knee extensor force traces at 10% and 25% of MVC, and corresponding intramuscular electromyography (iEMG) raw data recorded from the vastus lateralis. A representative MUP template and corresponding NFM shimmer plot isolated from each contraction are shown below each iEMG signal. Vertical orange lines on MUPs and NFMs indicate the start and end time of the NFM. Abbreviations: N, newtons; mV, millivolt; MVC, maximum voluntary contraction; MUP; motor unit potential; Dur, duration; NF, near fibre; NFM, near fibre MUP; kV, kilovolt; µV, microvolt; ms, millisecond.*

2.3.4 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.

2.3.5 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.

2.3.6 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 (Figure 1). After a 10% and 25% of 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).

2.3.7 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 sustained region of the contraction (ramps excluded) and estimate, via ensemble averaging, their corresponding surface MUPs (sMUPs) from the sEMG signals (Stashuk, 1999*c*). 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. 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 FR was assessed as the rate of MUP occurrences within a MUPT, expressed as the number of occurrences per second (Hz). MU FR variability is reported as the coefficient of variation (CoV) for the interspike interval (ISI), displayed as a percentage.

A near fibre MUP (NFM) is defined as the acceleration of its corresponding MUP (Figure 6) 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. All NFMs (and corresponding MUPs) without clear spikes were rejected from analyses. 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.,* 2021*b*).

2.3.8 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% of MVC into the negative peak area of the CMAP (44). 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*a*). MUNE values are available for 15 males and 15 females.

2.3.9 Statistical analysis

All of the statistical analysis was performed using RStudio (Version 1.3.959 for macOS). 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, and CSA). As multiple MUs were recorded from each participant, *multi-level mixed-effect linear regression analysis* was performed to investigate these MU parameters as well as force steadiness with sex and contraction level as factors through the package *lme4* (Version 1.1.23) (Bates *et al.,* 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 contraction 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 *et al.,* 2020) for forest plotting. For data visualisation, individual participant means and group means were shown in box-and-jitter plots. Statistical significance was assumed when $p < 0.05$. Based on the models used, *p* values close to 0.05 were also addressed (Greenland *et al.,* 2016).

2.4 Results

The means and standard deviations for the participants' characteristics are shown in Table 2.1. Significant differences between males and females were detected for weight, height, BMI, peak torque and VL CSA (all $p<0.05$). There was no significant sex difference for age ($p=0.49$). There was a significant interaction between sex and contraction level in force steadiness ($p=0.008$). Both males and females showed a greater improvement in force control from low- to mid-level contractions (both $p<0.001$) with females exhibiting a slightly greater decrease in force fluctuations compared to males. Individual values are shown in Figure 2.2.

Table 2.1. Participant characteristics.

Measure	Males (n=29)	Females (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**
Peak Torque (Nm)	251.65 (67.72)	158.95 (46.69)	**<0.001**
VL CSA (cm^2)	28.35 (6.43)	19.00 (3.90)	**<0.001**

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.

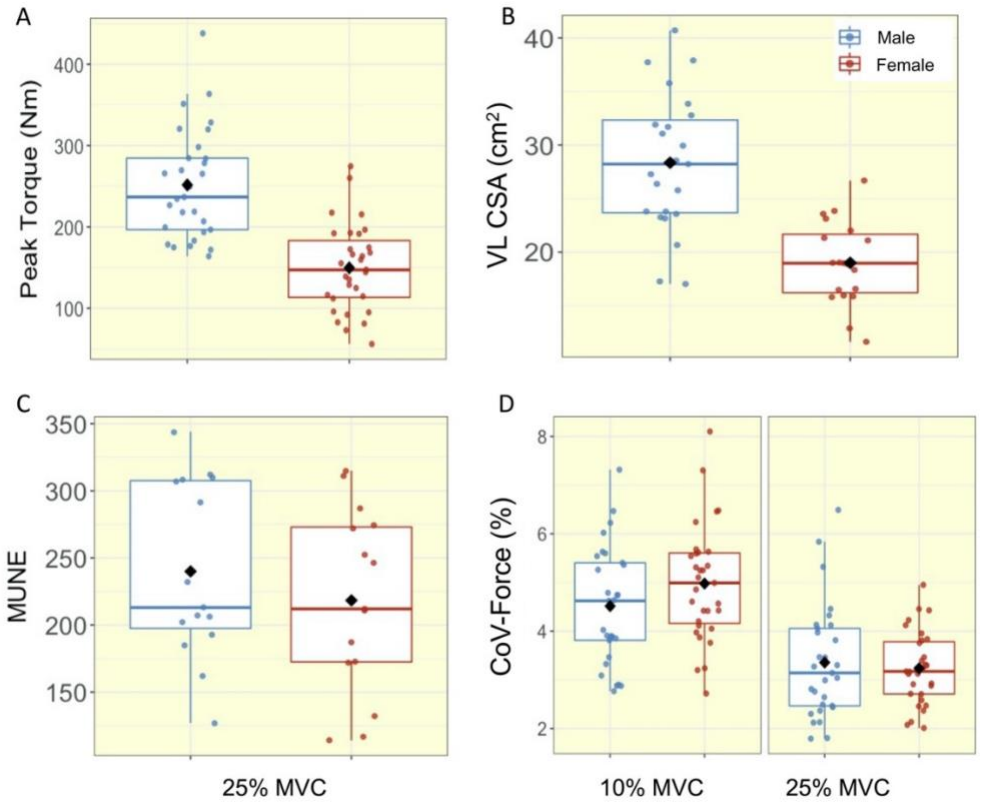

***Figure 2.2**. Box-and-jitter plots of the individual participant means, and group means (black dot) of **(A)** peak torque, **(B)** vastus lateralis cross-sectional area, **(C)** motor unit number estimates (MUNE), and **(D)** force steadiness at 10% and 25% of maximal voluntary contraction (MVC), in males (blue) and females (red). Abbreviations: CoV, coefficient of variation.*

At 10% of MVC, the mean number of MUs isolated per person was 26 in males with a mean of 6 MUs per sampling position, and 17 in females with 4 MUs per sampling position. At 25% of MVC, the mean number of MUs isolated per person was 31 in males with 8 MUs per sampling position and, 22 in females with 6 MUs per sampling position. A total of 1645 MUPs were inspected and included for analysis in males, and 1207 in females. Individual mean values for all functional, MU and NFM parameters are shown in Figures 2.2-2.5. There were no significant interactions between sex and contraction level in any of the MU

parameters. When interactions were removed from the model, multilevel linear regression revealed females had greater MU FR at both 10% (mean; M: 8.08 Hz; F: 8.79 Hz) and 25% (M: 8.62 Hz; F: 9.20 Hz) of MVC (both $p<0.05$, Table 2.2, Figure 2.3A). No sex-based differences ($p>0.10$) were detected in MU FR variability at either contraction level (Table 2.2, Figure 2.3B). MUP area was smaller in females at 10% (M: 741 μV·ms; F: 531 μV·ms) ($p=0.006$), with a non-significant trend at 25% of MVC (M: 1005 μV·ms; F:775 μV·ms) ($p=0.062$). MUP duration was shorter at 10% (M: 8.37 ms; F: 6.61 ms) and 25% of MVC (M: 8.24 ms; F: 6.84 ms) in females when compared to males (both $p<0.01$). There were no significant sex-based differences in any other MU characteristic (Table 2.2, Figure 2.4).

Table 2.2. Motor unit properties in different sexes

Parameter	Level	Beta	95%CI	P value
MU FR (Hz)	10% MVC	0.73	0.14 to 1.32	**0.018**
	25% MVC	0.61	0.07 to 1.14	**0.031**
MU FR Variability (%)	10% MVC	0.26	-0.74 to 1.25	0.617
	25% MVC	0.49	-0.72 to 1.70	0.433
MUP Area (μV·ms)	10% MVC	-210.08	-352.90 to -67.27	**0.006**
	25% MVC	-215.41	-436.82 to 6.00	0.062
MUP Phases	10% MVC	-0.07	-0.46 to 0.31	0.707
	25% MVC	0.05	-0.38 to 0.48	0.823
MUP Amplitude (μV)	10% MVC	-62.83	-151.27 to 25.61	0.170
	25% MVC	-92.01	-206.31 to 22.29	0.120
MUP Turns	10% MVC	-0.19	-0.73 to 0.35	0.500
	25% MVC	-0.10	-0.60 to 0.40	0.691
MUP Duration (ms)	10% MVC	-1.85	-2.93 to -0.77	**0.001**
	25% MVC	-1.35	-2.33 to 0.37	**0.009**
NFM Jiggle (%)	10% MVC	-0.06	-1.84 to 1.72	0.947
	25% MVC	0.09	-1.85 to 2.03	0.928
NFM Duration (ms)	10% MVC	-0.16	-0.60 to 0.29	0.486
	25% MVC	-0.02	-0.40 to 0.36	0.926
NFM Seg Jitter (μs)	10% MVC	-0.22	-4.29 to 3.84	0.915
	25% MVC	0.47	-3.42 to 4.36	0.814
NFM Dispersion (ms)	10% MVC	0.08	-0.22 to 0.38	0.622
	25% MVC	0.03	-0.25 to 0.32	0.825
NFM Area (kV/s^2)	10% MVC	0.001	-0.71 to 0.71	0.998
	25% MVC	-0.22	-0.97 to 0.54	0.575

Beta value and 95% confidence interval (CI) represents the model predicted change per unit from males to females, shown separately for 10 and 25% of maximal voluntary contraction (MVC). 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.

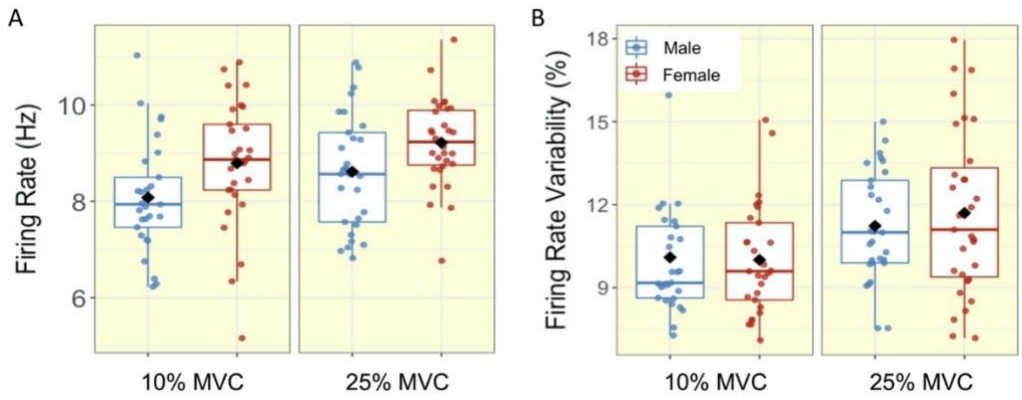

Figure 2.3. *Box-and-jitter plots of the individual participant means, and group means (black dot) of* ***(A)*** *motor unit (MU) firing rate and* ***(B)*** *firing rate variability in males (blue) and females (red) at 10 and 25% of maximal voluntary contraction (MVC).*

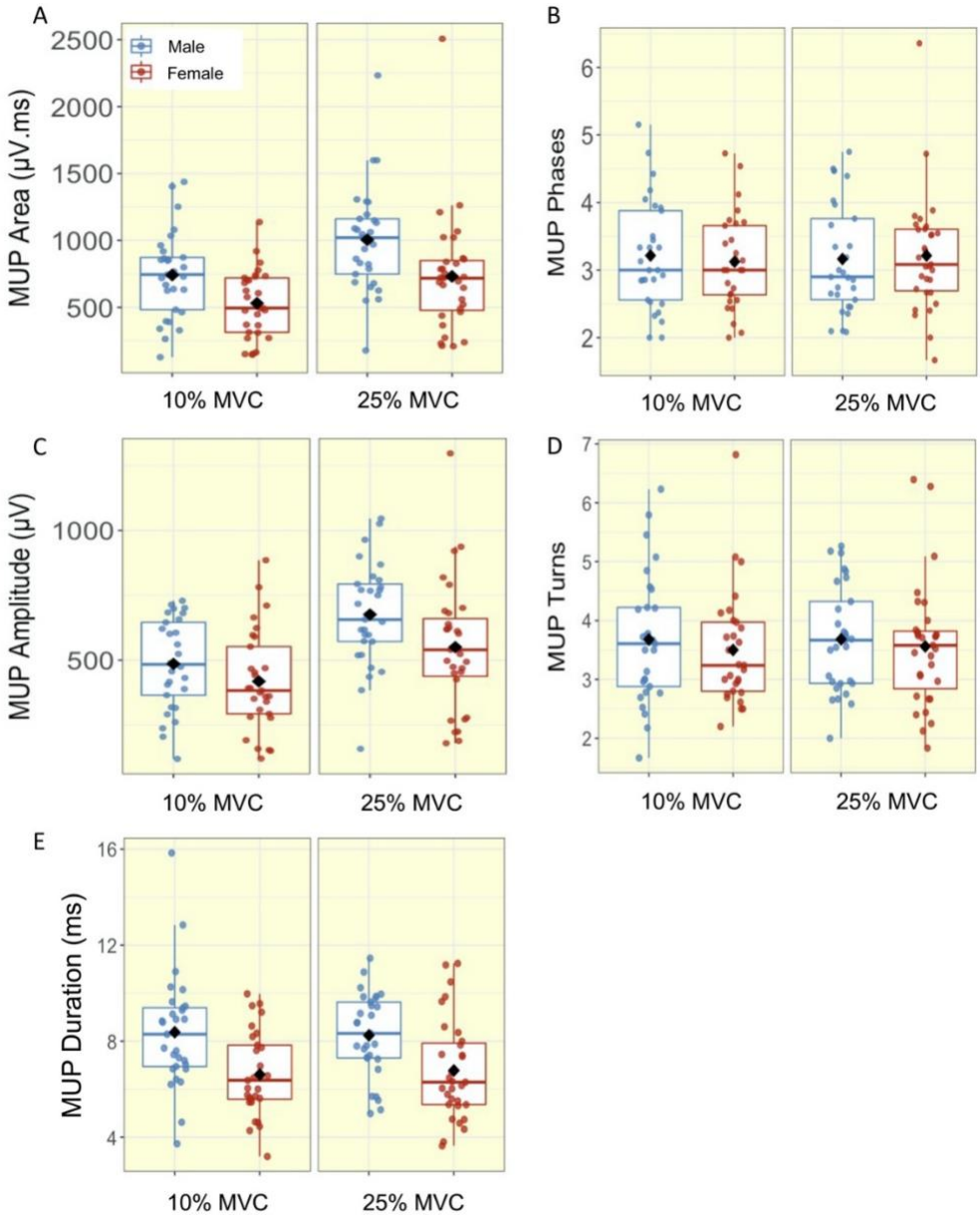

***Figure 2.4**. Box-and-jitter plots of the individual participant means, and group means (black dot) of motor unit potential (MUP) properties in males (blue) and females (red) at 10 and 25% of maximal voluntary contraction (MVC).*

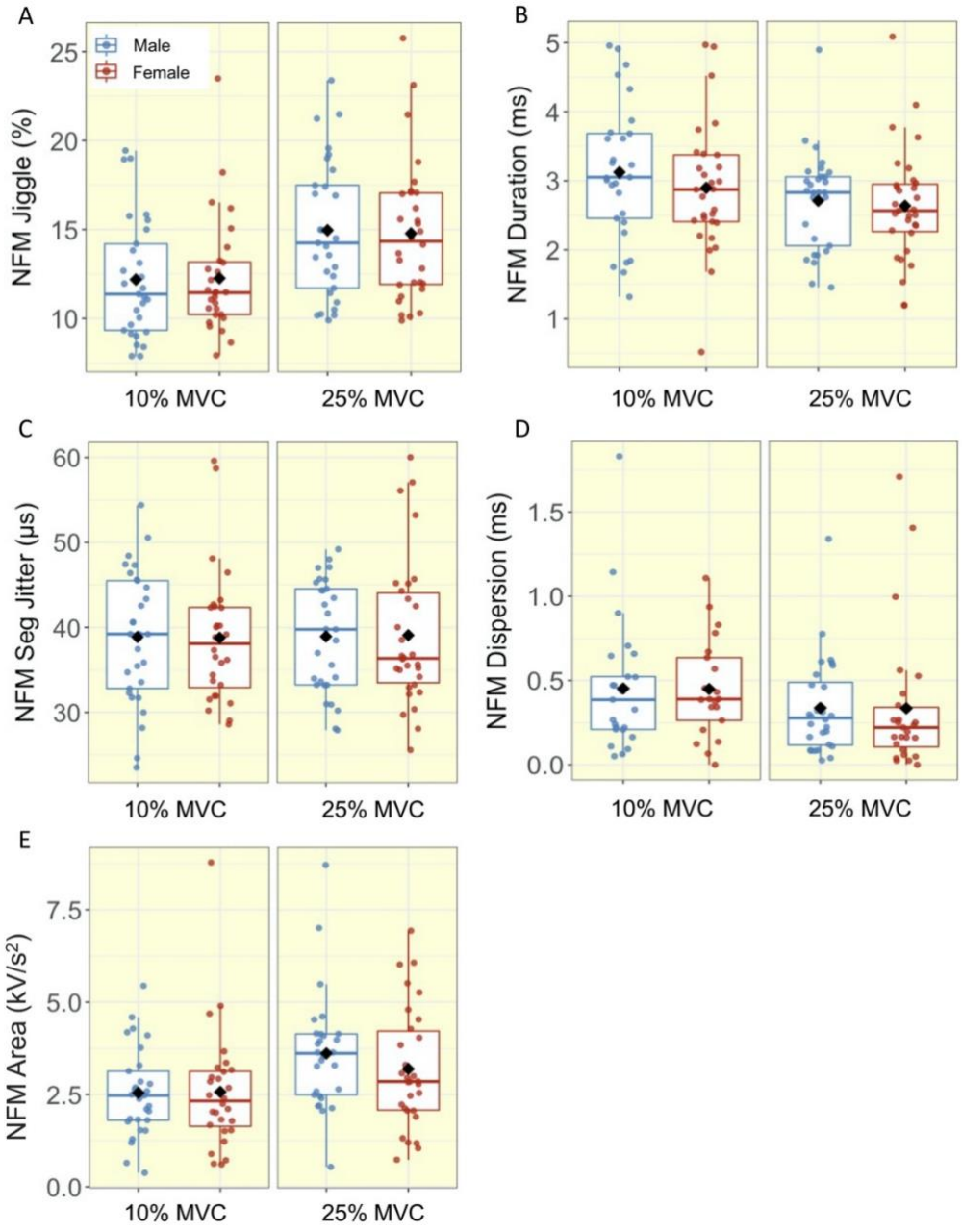

***Figure 2.5**. Box-and-jitter plots of the individual participant means, and group means (black dot) of near fibre motor unit potential (NFM) properties in males (blue) and females (red) at 10 and 25% of maximal voluntary contraction (MVC).*

With increasing contraction level, both males and females exhibited higher MU FR and MU FR variability, as well as greater MUP amplitude, and larger MUP area (all $p \leq 0.001$, Table 2.3, Figures 2.3 and 2.4). NFM area and NFM jiggle were greater, and NFM duration was shorter, with the higher contraction level, differing to a similar extent in males and females (all $p < 0.001$, Table 2.3, Figure 2.6). There were no interactions between sex and contraction level in any of the MU parameters, indicating the difference from 10 to 25% of MVC did not differ between males and females (Figure 2.6).

Table 2.3. Motor unit properties at different contraction levels

Parameter	Sex	Beta	95%CI	*p* value
MU FR (Hz)	Males	0.50	0.28 to 0.71	**<0.001**
	Females	0.43	0.18 to 0.69	**0.001**
MU FR Variability (%)	Males	0.99	0.52 to 1.45	**<0.001**
	Females	1.18	0.63 to 1.72	**<0.001**
MUP Area (μV·ms)	Males	273.62	215.81 to 331.43	**<0.001**
	Females	199.35	148.13 to 250.56	**<0.001**
MUP Phases	Males	-0.05	-0.14 to 0.04	0.302
	Females	0.10	-0.02 to 0.21	0.098
MUP Amplitude (μV)	Males	188.21	153.75 to 222.67	**<0.001**
	Females	142.40	106.27 to 178.53	**<0.001**
MUP Turns	Males	0.05	-0.11 to 0.20	0.565
	Females	0.09	-0.08 to 0.26	0.316
MUP Duration (ms)	Males	-0.16	-0.52 to 0.20	0.381
	Females	0.13	-0.16 to 0.42	0.371
NFM Jiggle (%)	Males	3.08	2.38 to 3.79	**<0.001**
	Females	3.08	2.34 to 3.82	**<0.001**
NFM Duration (ms)	Males	-0.40	-0.54 to -0.26	**<0.001**
	Females	-0.34	-0.50 to -0.18	**<0.001**
NFM Seg Jitter (μs)	Males	0.77	-0.37 to 1.91	0.188
	Females	0.51	-0.85 to 1.87	0.463
NFM Dispersion (ms)	Males	-0.09	-0.23 to 0.05	0.228
	Females	-0.05	-0.28 to 0.18	0.655
NFM Area (kV/s^2)	Males	0.98	0.68 to 1.29	**<0.001**
	Females	0.72	0.39 to 1.05	**<0.001**

Beta value and 95% confidence interval (CI) represents the model predicted change per unit from 10 to 25% of maximal voluntary contraction (MVC), shown separately for males and females. 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.

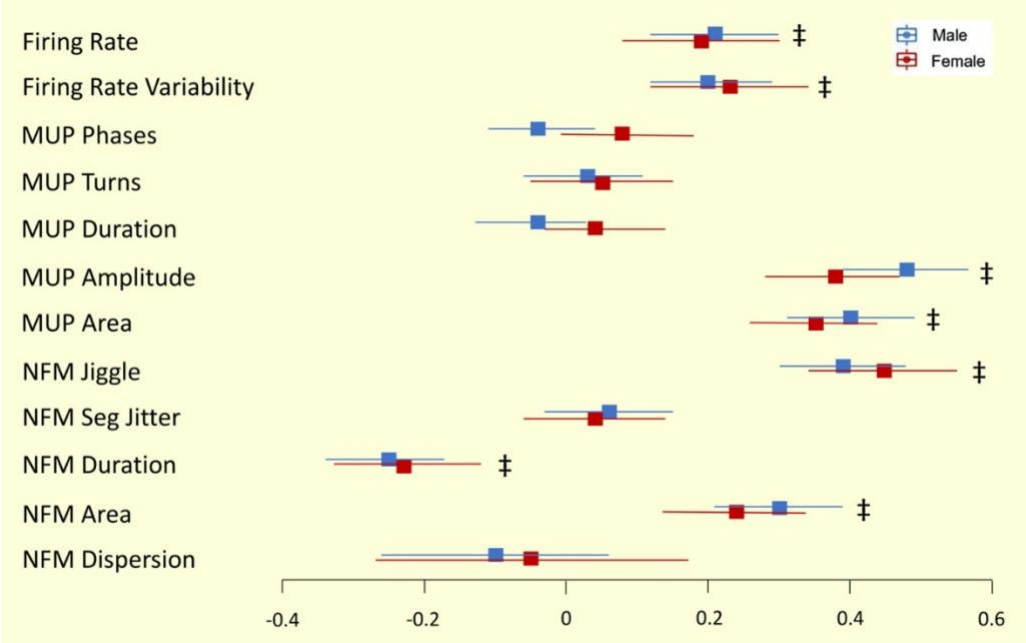

Figure 2.6. *Forest plots of the standardised regression coefficient estimate for associations between motor unit characteristics and contraction levels in male and female models. Beta value and 95% confidence intervals (CI) represents the standardised model predicted change per unit from 10 to 25% of maximal 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.*

2.5 Discussion

This is the first study to compare neuromuscular recruitment strategies and motor unit number estimates of the VL using iEMG techniques in healthy young males and females. Despite males being stronger and having larger muscles, there were no differences in force steadiness at either low- or mid-level contraction levels between sexes. At each contraction level assessed, females displayed smaller markers of MU size and greater MU FR, indicating differing recruitment strategies to achieve a normalised force. When assessing the difference between contraction levels, both males and females exhibited higher MU FR and greater MUP size, which differed to a similar extent in both sexes, indicating a similar recruitment strategy to generate proportional increases in force. In addition, there was no significant sex-based differences in motor unit number estimates of the VL. These data reveal divergent neuromuscular recruitment strategies between sexes to achieve a normalised force, which follow similar trajectories with increasing force.

Consistent with previous studies, females exhibited 39% smaller muscle size (CSA of the VL), which was reflected in a 37% lower strength (Miller *et al.,* 1993; Hannah *et al.,* 2012; Jeon *et al.,* 2019). The greater MU FR of females 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, females exhibited higher MU FR and MU FR variability compared with males 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 & Gabriel, 2021*b*). However, others reported no sex difference in knee extensors during 30% of MVC (Tenan *et al.,* 2013) and significantly greater MU FR at 100% MVC in tibialis anterior in males (Christie & Kamen, 2010; Inglis & Gabriel, 2020). As expected, in the current study MU FR increased with increasing force levels to a similar extent in both males and females, accompanied by greater MU FR variability. Although differing at each contraction level,

the similar proportional increase in MU FR in males and females indicates both sexes follow similar discharge pattern increases from low to mid-level normalised 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 & Gabriel, 2021*b*) found that females had a greater MU FR variability and greater fluctuation in steadiness than males during dorsiflexion in tibias anterior muscles, which may indicate a muscle specific sex difference. In the current study, both males and females exhibited greater force steadiness at 25% of MVC compared 10% of MVC, consistent with Inglis' finding that very high- and low-level force outputs have greater fluctuations compared to mid-level force outputs (Yoon *et al.,* 2014; Inglis & Gabriel, 2021*b*).

The size of a MU can be estimated by the size of the MUP recorded using intramuscular electrodes. As previously mentioned, males typically exhibit larger muscle size than females (Yoon *et al.,* 2014; Haizlip *et al.,* 2015), with increases in force mediated by recruitment of additional larger MUs and increases in MU FR. Here MUP area and duration were smaller in females which reflects smaller MU size. When viewed alongside the greater MU FR in females, it suggests that at the normalised force levels assessed here, females are more reliant on MU FR than on recruitment of larger MUs, when compared to males. As expected, markers of MU size increased with larger contraction levels, as larger MUs are recruited to produce larger forces. Again, the trajectory of each was similar for males and females indicating MU recruitment strategies moving between these force levels do not differ between sexes.

A near fibre MUP (NFM) is derived from a MUP, such that is primarily composed of contributions from MU fibres close to the intramuscular

electrode (Piasecki *et al.,* 2021*b*). Here there were no sex differences in any NFM parameters at either contraction level. When comparing 10% and 25% of MVC contractions, NFM area increased, while NFM duration decreased, albeit to a similar extent in both sexes. These contractions induced alterations may be the result of the activation of larger MU fibres with greater conduction velocity during higher level contractions (Inglis *et al.,* 2017; Inglis & Gabriel, 2021*a*).

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.,* 2016*c,* 2016*a,* 2021*a*) 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% of 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 healthy young males and females. 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 males (240 ± 66) and females (218 ± 68) reported here are similar to those we have previously reported in male cohorts (Piasecki *et al.,* 2016*c,* 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 males and females support minimal sex-based differences in the number of VL MUs. Additionally, differences

in MU size, as reflected by MUP area at 25% of MVC, were minimal compared to differences in total muscle size, therefore the current data support the notion that sex-based differences in total muscle size are largely explained by greater individual fibre size in males (Staron *et al.,* 2000).

Although providing a high level of detail of MU structure and function via MUPs and NFMs sampled in deep and superficial muscle regions, regardless of subcutaneous tissue amount, 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 females naturally occurring during the menstrual cycle nor the use of oral contraceptives, the latter of which may influence vascular tone (Williams & MacDonald, 2021). Thirdly, the limb dominance was not assessed and the right leg was uniformly measured across all the participants. This is a direct comparison of MU features during sustained contractions in young males and females and it was not possible to accurately quantify MU recruitment thresholds, which may bias findings if they differ according to sex in the VL. Further investigations concerning neural drive and influence of hormones on neural drive are still required to further understand the sex-based differences in the motor nervous system.

2.6 Conclusion

In summary, when compared to males, females exhibited smaller VL MUs with higher MU FR, when assessed at a single normalised contraction level. However, both males and females showed similar increases in MU size and MU FR from a low- to a mid-level contraction, indicating a similar neuromuscular recruitment strategy. These results suggest that although sex-based neuromuscular differences are apparent at a single contraction level, relative differences between levels are similar in this widely studied muscle group. These data do not support the notion of excluding females from studies of this nature.

Chapter 3 - Sex disparities in age-related motor unit characteristics and functional decline: unveiling female susceptibility from early to late elderly

3.1 Abstract

Background

Females typically have a longer lifespan than males which is not matched by an improved healthspan, with older females typically having higher rates of frailty, characteristic of a sex specific degradation of the neuromuscular system. Several motor unit (MU) characteristics show sex-specific behaviour during mid-level contractions in healthy younger people, highlighting a potential influence of hormonal differences that may be amplified in older age. The purpose of this study was to investigate sex differences in physical performance and MU features of the aged human vastus lateralis (VL) from early to late elderly.

Methods

This study included 21 healthy older males (mean ± SD, range: 67.2 ± 7.6, 56 – 81 yrs) and 17 healthy older females (69.5 ± 5.2, 60 – 78 yrs). Intramuscular electromyography data were collected from VL during standardised submaximal sustained contractions. Muscle size and physical performance characteristics were also measured. Multiple mixed-effects linear regression models with age considered were conducted and statistical significance was accepted when $p<0.05$.

Results

When compared to males, early to late elderly females had smaller cross-sectional area of VL ($p<0.001$), lower knee extensor torque ($p<0.001$) and poorer force steadiness ($p=0.036$), as well as higher MU firing rate (FR) ($p=0.025$) and greater MU FR variability ($p=0.031$). With

progression from early to late elderly, both sexes showed decreased functional capacity with older females starting and ending lower.

Conclusion

Functional deterioration occurs to a similar extent in both sexes from early to late elderly. Throughout the majority of the elderly period, males demonstrate a greater muscle size and strength, and functional performance. Older females have greater MU FR variability and worse force steadiness than older males. These findings add to the paucity of data in older females and suggest earlier interventions are needed for older females to prevent functional deterioration and reduced the health-sex paradox within ageing.

3.2 Introduction

Sex differences in the ageing process are well established with females typically living longer and having lower biological ages (referring to the decline in tissue/organismal function) than males, yet do so in poorer health (Hägg & Jylhävä, 2021). Though ageing is generally characterized by decreased muscle mass and strength, there is a significant heterogeneity with respects to physical function. Despite living longer, females have been found to have lower levels of physical performance and have a higher frailty index score throughout the lifespan when compared to males, which is exacerbated with increasing age (Gordon *et al.*, 2017), yet there is limited data on neuromuscular function in older females. Thus, there is a growing need to better understand the underlying reasons for these sex differences in aging and their implications for health outcomes to further address age-related health outcomes in a more targeted and effective manner.

Among the most underexplored elements accounting for sex-specific physical performance are alterations of motor unit (MU) properties. Voluntary contraction of muscle relies upon the coordinated activation of individual MUs, sets of muscle fibres innervated by a single motoneuron (Heckman & Enoka, 2012), which can be recruited based on their size and further modulated via the frequency at which they discharge, referred to as MU recruitment and firing rate (FR), respectively (Enoka & Duchateau, 2017). There were ~30% fewer MUs in the quadriceps and tibialis anterior muscles of older males compared to younger males (Piasecki *et al.*, 2016*a*, 2018), which is a major contributing factor to loss of functionality in older age. Following denervation, a muscle fibre may atrophy and eventually be lost, or it may be ‘rescued’ by an adjacent surviving axon, known as MU reinnervation and further completing MU remodelling at neuromuscular junctions (NMJs), resulting in fewer MUs in aged muscles but with larger territory (Jones *et al.*, 2022). These MU adaptations may alter neuromuscular recruitment strategies and/or further influence force control. However, the vast majority of the

knowledge about neuromuscular function in VL are primarily available in males and/or a smaller number of young females, with a distinct lack of direct comparisons.

Sex steroids are key players in the aging process and have been identified as potential contributors to biological sex differences in neuromuscular function (Hunter, 2014). The most common groups of sex steroids include androgens (such as testosterone), which are typically more copious in males. Chapter 6 will specifically explore the association between testosterone and its precursor dehydroepiandrosterone (DHEA), and MU FR in highly active and inactive older males. Estrogens and progestogens are the predominant female sex hormones and have the ability to cross from the blood brain barrier potentially influencing crucial central nervous system function (Del Río *et al.*, 2018). The decline in estrogen and androgen levels with aging has been shown to contribute to the enhanced decline in muscle function, as both exhibit a range of neuroprotective effects in motoneurons, such as dendritic maintenance and axonal sprouting (Hyer *et al.*, 2018). Additionally, the dramatic decline in estrogen levels heightens the risk of osteoporosis for many older females (Sözen *et al.*, 2017), which can have further impacts on physical function and mobility, making fractures from falls much more likely in older females compared to males.

We have previously reported a significant decline in MU FR in tibialis anterior from middle to older highly active females, which was not observed in males (Piasecki *et al.*, 2021*a*), and separately in Chapter 2, a higher MU FR in vastus lateralis (VL) in healthy young females comparatively to males at normalised force levels. This highlights a potential influence of hormonal differences, which may be amplified in older age, yet data on sex differences in neuromuscular function in older adults remains scarce. The quadriceps muscles are highly susceptible to atrophy in aging and are closely related to functional decline (Naruse *et al.*, 2023), therefore, the purpose of the present study was to explore the influences of sex and ageing on physical performance and

neuromuscular characteristics in the VL of early to late elderly males and females. It was hypothesized that females would have lower functional performance than males, as demonstrated by differences in MU firing properties and greater markers of MU remodelling.

3.3 Methods

3.3.1 Participants

This study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (90-0820, 407-1910, 390-1121) and was conducted between 2019-2022 in accordance with the Declaration of Helsinki.

Twenty-one healthy males and seventeen healthy females between the ages of 55 to 85 years were recruited from the local community via advertisement. All recruited participants were recreationally active on a day-to-day basis. Prior to enrolment, all participants completed a comprehensive clinical screening examination based at the School of Medicine, Royal Derby Hospital Centre and subsequently provided written informed consent. Screening procedure allowed exclusion of the participants with musculoskeletal abnormalities, acute cerebrovascular or cardiovascular diseases, uncontrolled hypertension, or metabolic disease.

3.3.2 Anthropometry

Prior to testing, calibrated scales and a stadiometer were used to assess the body mass and height of each participant for calculation of the body mass index. Cross-sectional area (CSA) of the VL was measured using an ultrasound probe (LA532 probe B-mode, frequency range 26-35 Hz and MyLab^{TM}50 scanner, Esaote, Genoa, Italy). To quantify VL CSA, images were captured from the aponeurosis borders of the VL of the right leg in a medial-lateral fashion using panoramic imaging (VPAN) and further analysed with ImageJ software (National Institute of Health, USA) by tracing around the VL following the contour of the aponeurosis. CSA was determined by analysing each image three times and taking the average of three images.

3.3.3 Balance

A Footscan plate (Footscan, 200 Hz, RScan International, Belgium) was used to assess the postural sway during right-leg standing with eyes open. Participants were asked to step on the platform and visually focus on a target point in front of them for the duration of the test (10 seconds). A 5-second countdown was given before instruction to lift up the left leg. Travelled distance and ellipse area of centre of pressuret (COP) were recorded. Each participant was allowed three attempts and the best value was reported. The total distance travelled by COP during the test time was determined as COP travelled distance. COP ellipse area was calculated based on the data in the ellipse set for 95% of the total area covered by the COP trajectory in both anterior-posterior and medial-lateral directions. Smaller area and/or travelled distance indicated a better postural balance.

3.3.4 Grip Strength

A handgrip dynamometer (Grip-D, Takei, Japan) was used to test the grip strength of the dominant hand. When measuring grip strength, the dynamometer was held in a standing position with the pointer facing outward, and the width of the grip was adjusted so that the index finger's interphalangeal joint was bent 90 degrees. The arms were lowered naturally, the feet were hip width apart, and the dynamometer was grasped with maximum force without touching the body or clothing. The maximal value among three measurements was accepted as the grip strength.

3.3.5 Timed Up and Go (TUG)

The TUG test was used to evaluate dynamic balance and functional mobility. TUG test involved getting up from a standard chair without armrests, walking for 3 metres, turning around an obstacle and returning to the original sitting position as quickly as possible without running. The test was initiated with a verbal “go” instruction from the researcher, and

the time taken to complete the test was recorded. After a familiarisation attempt, the participant was asked to have a real go after one minute rest.

3.3.6 Knee Extensor Torque

Participants were placed in a custom-built chair which was altered to ensure the hips and knees were flexed at ~90º. The right lower leg was attached to a force dynamometer with a non-compliant strap (purpose-built calibrated strain gauge, RS125 Components Ltd, Corby, UK) above the medial malleolus. The hips and pelvis were also securely held by a seat belt to reduce movement of the hips and upper trunk during contractions. The distance between the centre of the force strap and the lateral femoral condyle was measured to determine the external knee joint moment arm. With real-time visual feedback and verbal encouragement, participants were instructed to perform each trial with maximal effort as hard and fast as they could following a standardized warm-up of submaximal contractions. During the trial, participants were not allowed to hold onto the side of the chair and were asked to cross their arms across the chest. It was further repeated two to three times with 60 seconds rest intervals between each one. If there was a difference of less than 5% between the two last attempts, the highest value was accepted as the maximal voluntary contraction (MVC). Torque was then determined by multiplying the selected MVC by the lever arm. To quantify force steadiness during 10% and 25% of MVC, the coefficient of variation of the force (CoV) was calculated = (SD/Mean)*100. When calculating CoV, in an attempt to reduce corrective actions, the first two passes of the target (<1s) were excluded from the analysis.

3.3.7 Intramuscular Electromyography (iEMG)

Prior to intramuscular needle electrode insertion, a familiarisation trial was performed in which the participant had an attempt to contract to each submaximal target line at 10% and 25% of MVC observed on a monitor. Following this, a 25-mm or 40mm disposable concentric needle electrode (N53153; Teca, Hawthorne, New York, USA) was inserted into the

muscle belly of VL of the right leg, to a depth of 1.5-2 cm depending on muscle size. A ground electrode was placed over the patella of the same testing leg. To ensure an adequate signal to noise ratio once the needle was positioned, the participants were asked to perform several low-level voluntary contractions. Participants were then asked to perform sustained voluntary isometric contractions at 10 % and 25 % of MVC, 4 times each, with 20-second intervals between contractions. Each time, the needle was repositioned 180 degrees by twisting the bevel edge and withdrawing 5 mm each time to sample from spatially distinct areas (Jones *et al.*, 2021). iEMG signals were recorded in 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.

3.3.8 Intramuscular Electromyography Analysis

Using decomposition-based quantitative electromyography (DQEMG) software, individual motor unit potentials (MUPs) were detected and motor unit potential trains (MUPTs) were extracted from sustained region of the contraction, enabling the evaluation and recording of VL electrophysiological activity during contractions. MUPs were visually inspected and markers corresponding to positive and negative peaks of waveforms were adjusted. MU FR is reported as the number of MUP occurrences per second (Hz) within a MUPT. MU FR variability is reported as the coefficient of variation (CoV) of the interspike interval (ISI), displayed as a percentage. MUP area was determined as the total area within MUP duration (onset to end). A MUP's complexity is determined by the number of phases, which are defined as the number of components above or below the baseline.

The near fibre MUP (NFM) is calculated by applying a low-pass second-order differentiator to the MUP. In this way, only fibre potentials closest to the needle electrode contribute to the NFM and interference from distant active fibres is reduced. All NFMs (and corresponding MUPs) without clear spikes were rejected from analyses. NFM jiggle is a

measure of the shape variability of consecutive NFMs of an MUPT expressed as a percentage of the total NFM area (Piasecki *et al.*, 2021*b*).

3.3.9 Statistical analysis

All statistical analysis was conducted using R Studio (Version 2022.07.1). Descriptive data were generated for all variables. *Multiple linear regression models* with age considered as a covariate were used compare the effects of age and sex on physical characteristics. *Multilevel linear regression mode*ls using the *lme4* package (Version 1.1-27.1) (Bates *et al.*, 2015) with age considered as a co-variate were generated to compare MU parameters between groups across two contraction levels in order to preserve variability between and within participants simultaneously. MUs were regarded as the first level of multilevel models, and participants with clustered MUs were regarded as the second level. The results are displayed as coefficient estimates, 95% confidence intervals and p values. Standardised estimates were calculated through the package *effectsize* (Version 0.8.2) (Ben-Shachar *et al.*, 2020) for forest plotting. Statistical significance was accepted when $p<0.05$.

3.4 Results

Thirty-eight older participants were included in the study, consisting of 21 healthy older males (age range: 56-81 years) and 17 healthy older females (age range: 60-78 years).

Table 3.1. Participant characteristics

Measure		Males (n=21)	Females (n=17)
Age, years		67.2 (7.6)	69.5 (5.2)
Body Mass Index, kg/m^2		26.03 (2.13)	24.02 (4.08)
Physical characteristics			
CSA, cm^2		23.11 (6.68)	13.10 (3.83)
Torque, Nm		158.01 (38.56)	93.68 (22.67)
Grip Strength, kg		42.38 (6.62)	26.08 (4.06)
CoV Force -10%MVC		5.60 (1.04)	8.56 (5.25)
CoV Force -25%MVC		3.42 (0.59)	4.54 (2.09)
Timed Up and Go, s		7.88 (1.35)	9.17 (1.46)
COP Travelled Distance, mm		412.55 (367.23)	379.76 (425.13)
COP Ellipse Area, mm^2		424.65 (1481.25)	385.94 (1272.77)
Motor unit properties			
MU FR, Hz	10% MVC	7.70 (1.23)	8.51 (1.54)
	25% MVC	8.11 (0.97)	8.92 (1.19)
MU FR	10% MVC	8.65 (1.20)	9.48 (1.02)
Variability, %	25% MVC	8.66 (1.45)	9.10 (1.08)
MUP Phases	10% MVC	4.07 (0.89)	4.06 (0.38)
	25% MVC	4.43 (0.70)	4.31 (0.49)
MUP Duration, ms	10% MVC	8.54 (1.66)	8.00 (0.94)
	25% MVC	9.38 (1.69)	8.15 (0.95)
MUP Area, μV·ms	10% MVC	766.04 (303.72)	606.22 (259.95)
	25% MVC	1047.26 (417.73)	824.42 (2.46)
NFM Jiggle, %	10% MVC	14.14 (3.24)	15.24 (3.49)
	25% MVC	17.56 (6.52)	18.49 (3.97)

Data are reported as mean (standard deviation). Abbreviations: CSA, cross-sectional area; CoV, coefficient of variation; MVC, maximal voluntary contraction; COP, centre of pressure; MU, motor unit; FR, firing rate; MUP, motor unit potential; NFM, near fibre motor unit potential.

There were no significant interactions between sex and age detected in functional parameters. When adjusting for sex, for every year increase in age, muscle cross-sectional area decreased by 0.45 cm^2 (95%CI: -0.70 to -0.21; p=0.001) and muscle torque decreased by 1.62 Nm (-3.20 to -0.04; p=0.045). There was no significant difference in grip strength with advancing age (-0.27; -0.58 to 0.04; p=0.084). Though none statistically significant, for every year increase in age, TUG increased by 0.07 s (-0.002 to 0.14; p=0.058). Additionally, force steadiness decreased significantly at both 10% (beta: 0.09; 0.03 to 0.15; p=0.003) and 25% MVC. (0.04; 0.002 to 0.07; p=0.041) and unilateral COP ellipse area increased (7.93; 1.82 to 14.04; p=0.013). However, there was no significant difference in unilateral COP travelled distance (6.54; -2.33 to 15.40; p=0.143).

When adjusting for age, females had 55.3% smaller muscle cross-sectional area (-8.93; -12.23 to -5.62; p<0.001), 48.1% lower knee extensor torque (-60.58; -81.49 to -39.68; p<0.001), 44.7% lower grip strength (-15.60; -19.75 to -11.44; p<0.001), and 15.1% longer TUG times (1.14; 0.23 to 2.04; p=0.015) than males. Females showed 41.8 and 28.1% poorer force steadiness at 10% (1.58; 0.79 to 2.37; p<0.001) and 25% MVC (0.59; 0.13 to 1.06; p=0.014) when compared to males. However, there were no sex differences in unilateral COP travelled distance (-48.05; -161.48 to 65.38; p=0.394) or COP ellipse area (-44.02; -122.67 to 34.63; p=0.263).

Table 3.2. Summary of multiple linear regression analysis for functional parameters.

	Age [a]			Sex [b]		
	beta	95% CI	p value	beta	95% CI	p value
CSA, cm^2	-0.45	-0.70 to -0.21	**0.001**	-8.93	-12.23 to -5.62	**<0.001**
Torque, Nm	-1.62	-3.20 to -0.04	**0.045**	-60.58	-81.49 to -39.68	**<0.001**
Grip Strength, kg	-0.27	-0.58 to 0.04	0.084	-15.60	-19.75 to -11.44	**<0.001**
Timed Up and Go, s	0.07	-0.002 to 0.14	0.058	1.14	0.23 to 2.04	**0.015**
CoV Force- 10% MVC	0.09	0.03 to 0.15	**0.003**	1.58	0.79 to 2.70	**<0.001**
CoV Force - 25% MVC	0.04	0.002 to 0.07	**0.041**	0.59	0.13 to 1.06	**0.014**
COP Travelled Distance, mm	6.54	-2.33 to 15.40	0.143	-48.05	-161.48 to 65.38	0.394
COP Ellipse Area, mm^2	7.93	1.82 to 14.04	**0.013**	-44.02	-122.67 to 34.63	0.263

*[a] Multiple linear regression model with **Age** as dependent variable; [b] multiple linear regression model with **Sex** as dependent variable. Values in bold reflect statistically significant (p<0.05) results. Abbreviations: CI, confidence interval; CSA, cross-sectional area; CoV, coefficient of variation; MVC, maximal voluntary contraction; COP, centre of pressure.*

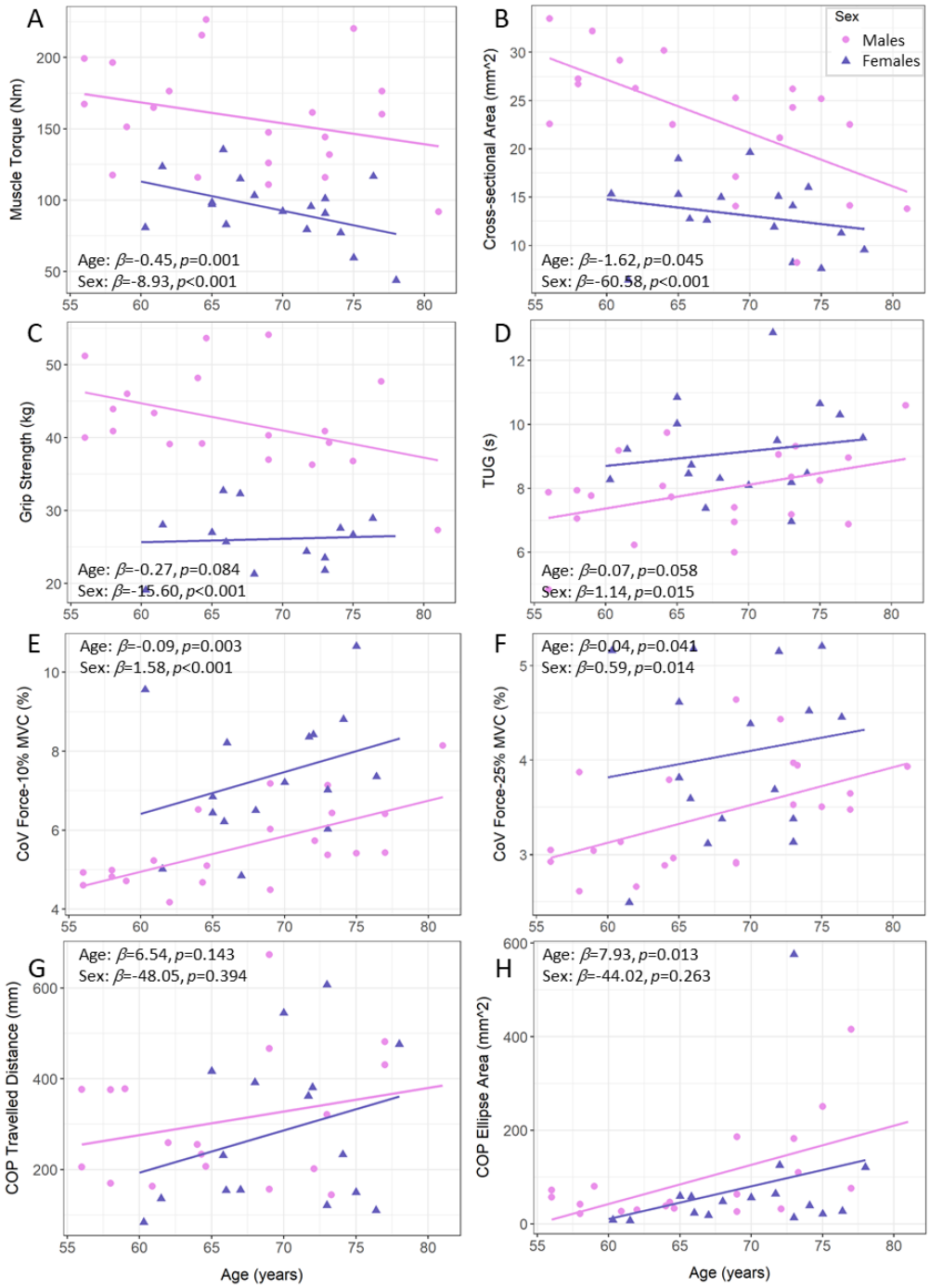

Figure 3.1. *Scatter points of physical parameters in older males (pink) and females (purple). The estimates of the main effect of Age and Sex from multiple linear regression models for physical parameters with no interaction detected are reported in text boxes. For data visualisation, male and female regression lines are displayed separately. Abbreviations: TUG, timed up and go; CoV, coefficient of variation; MVC, maximal voluntary contraction; COP, centre of pressure.*

At 10% MVC, the mean number of MUPs isolated per person was 21 in males with a mean of 5 MUPs per sampling position, and 24 in females with 6 MUPs per sampling position. At 25% MVC, the mean number of MUPs isolated per person was 33 in males with 8 MUPs per sampling position and 38 in females with 9 MUPs per sampling position.

There was no association between age and all MU parameters after adjusting for sex and contraction level (all $p>0.05$). There was no significant sex x contraction level interaction for FR ($p=0.955$) but there was a main effect of sex ($p=0.025$) with females showing a greater FR when compared to males. MU FR increased significantly when moving from low- to mid-level contractions ($p<0.001$) in both sexes. Similarly, there was no significant sex x contraction level interaction for FR variability ($p=0.115$) but there was a main effect of sex ($p=0.031$) with females showing a higher FR variability when compared to males. There was no significant difference in FR variability when moving from low- to mid-level contraction ($p=0.144$).

There was no significant sex x contraction level interaction for MUP phases ($p=0.782$), and there was no statistical difference between sexes ($p=0.731$) whereas there was a main effect of contraction level, with a greater number of MUP phases observed at 25% when compared to 10% MVC contractions ($p=0.001$). A significant sex x contraction level interaction was observed in MUP duration ($p=0.014$) with females exhibiting a shorter MUP duration at mid-level contractions ($p<0.001$). There was no significant sex x contraction interaction for MUP area ($p=0.973$) but there was a main effect of sex ($p=0.042$), with a smaller MUP area in females when compared to males, and both sexes showing greater MUP area when moving from low to mid-level contractions ($p<0.001$).

There was no significant sex and contraction level interaction for NF jiggle ($p=0.371$) and no significant main effect of sex ($p=0.949$) however,

NF jiggle increased significantly when moving from low to mid-level contractions (p=0.004).

Table 3.3. Summary of linear regression analysis for motor unit parameters.

	Sex [a]			Level [b]		
	beta	95% CI	p value	beta	95% CI	p value
MU FR, Hz	0.91	0.15 to 1.68	**0.025**	0.37	0.17 to 0.58	**<0.001**
MU FR Variability, %	0.89	0.10 to 1.68	**0.031**	0.33	-0.11 to 0.77	0.144
MUP Phases	-0.07	-0.48 to 0.33	0.731	0.26	0.10 to 0.42	**0.001**
MUP Duration, ms	-0.76	-1.60 to 0.08	0.084	0.64	0.34 to 0.93	**<0.001**
MUP Area, μV·ms	-217.59	-420.40 to -14.78	**0.042**	246.68	181.63 to 311.73	**<0.001**
NF Jiggle, %	0.10	-3.05 to 3.26	0.949	2.21	0.71 to 3.72	**0.004**

[a] Multiple mixed-effects linear regression model with sex as dependent variable; [b] multiple mixed-effects linear regression model with contraction level as dependent variable. Beta value and 95% confidence interval (CI) represent the model predicted change per unit between sexes and contraction levels with age considered as a covariate. Values in bold reflect statistically significant (p<0.05) results. Abbreviations: MU, motor unit; FR, firing rate; MUP, motor unit potential; NFM, near fibre motor unit potential.

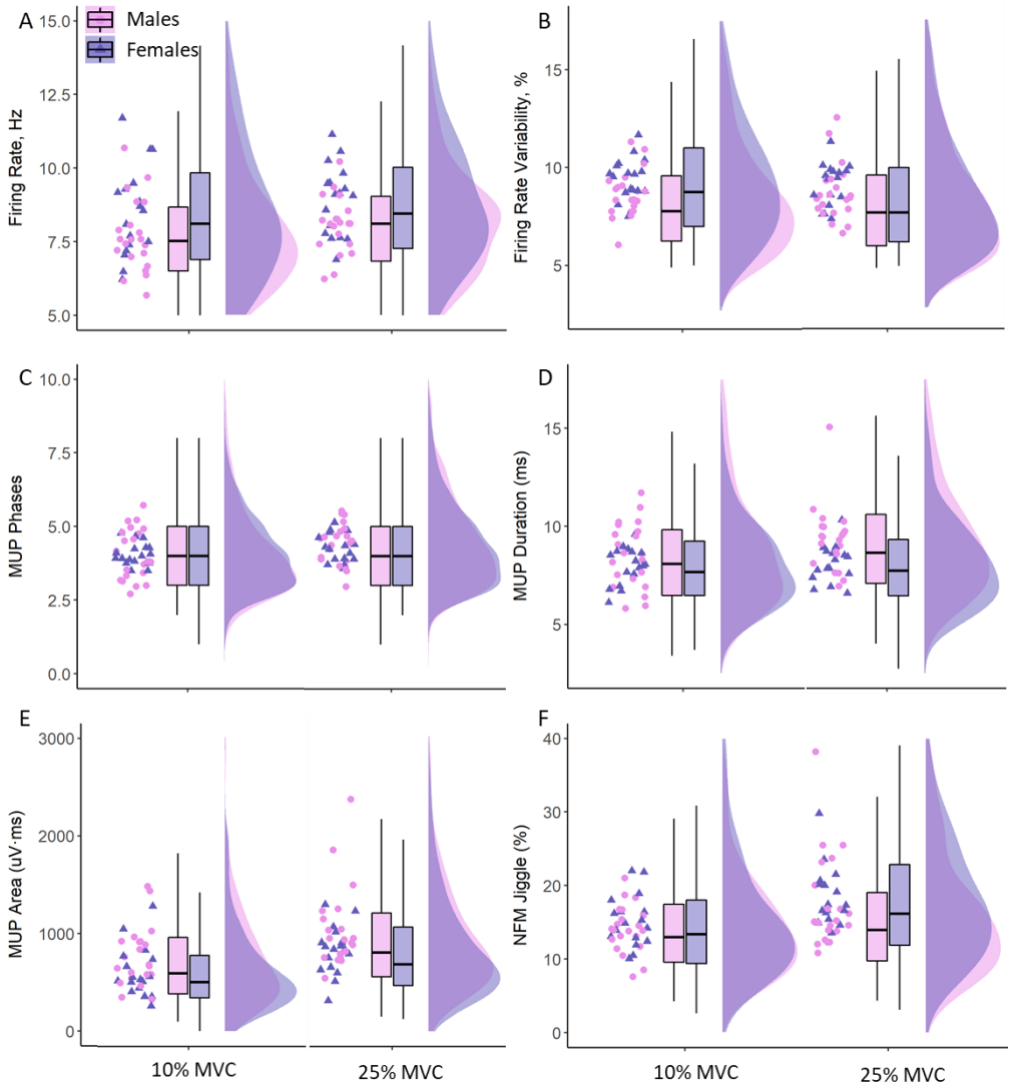

Figure 3.2. *Motor unit properties in males (pink) and females (purple) at 10% and 25% maximal voluntary contraction (MVC). Individual participant means are shown in the left column within each plot with males in rounds and females in triangles. Box plot starts in the first quartile, ends in the third, and represents the 50% of the central data, with a line inside that represent the median. Distributions of each motor unit characteristic are shown in density plots on the right side.*

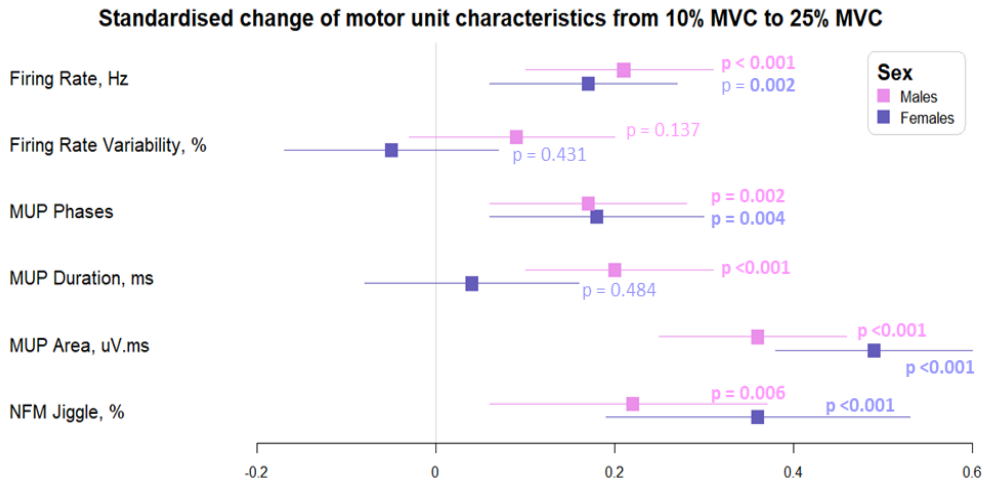

Figure 3.3. *Forest plot of the standardised regression coefficient estimate for associations between motor unit characteristics and contraction levels in older males (pink) and females (purple). Beta and 95% confidence intervals represent the standardised model predicted change per unit from 10% to 25% maximal voluntary contraction (MVC). All statistical analysis was based on multilevel mixed effects linear regression model with age considered as a covariate. Standardised values for each parameter make the comparisons executable between older males and females.*

3.5 Discussion

The present study demonstrated an age-related decrease in physical performance in early to late elderly humans, occurring at a similar rate in males and females, yet with distinct sex differences. In older males and females, muscle size, muscle strength, force steadiness and unilateral balance ability decreased with increasing age. Regardless of the progressive decrease in both sexes, females had a smaller muscle size, lower knee extensor and grip strength, longer TUG time and poorer force steadiness than males. iEMG derived MU data from the VL show females exhibited smaller markers of MU size, higher MU FR and greater MU FR variability. When assessing MU alteration from low- to mid-level contractions, both males and females showed higher MU FR, MUP phases, MUP area and NF jiggle. These data underscore the connection between diminished functionality in older females and a lower baseline observed during early stages of aging, potentially serving as a valuable reference for pinpointing pivotal inflection points along the path towards frailty.

Numerous studies have highlighted the age-related reduction of muscle size and strength (Piasecki *et al.*, 2016*b*; Wilkinson *et al.*, 2018) and the quadriceps appear to be particularly susceptible when compared to other lower extremity muscles such as hamstrings and tibialis anterior (Maden-Wilkinson *et al.*, 2013). Interestingly, the ~55% lower muscle size and ~48% lower strength in older females reported here is greater than the sex-based differences we have previously reported in Chapter 2 when comparing healthy young (~39% and ~37%, respectively), and suggests the sex-based disparities are augmented in older age.

Grip strength has been identified as a robust predictor of frailty and mortality among older individuals. In alignment with large-scale cross-national investigations (Andersen-Ranberg *et al.*, 2009), a considerable lower grip strength has been observed in females when compared to males in their early and late older age. However, deviating from previous reports regarding age-related changes, our multiple regression model did

not exhibit a significant decline in grip strength from early to late elderly. This discrepancy may be attributed to the smaller sample size in this cohort. Additionally, it is widely acknowledged that the sex-health paradox arises from the facts that females, on average, live longer than males but experience a greater proportion of their overall life expectancy in poorer health. However, it is important to note that due to the age range of our participants, older females with diminished functional capacity were inadvertently not recruited. This oversight resulted in underestimation of the impact of age and sex disparities. Furthermore, it has been suggested that females exhibit better preservation of grip strength than males throughout the aging process (Ahrenfeldt *et al.*, 2019), implying that diminished grip strength alone cannot fully predict or elucidate systemic physical frailty (Yeung *et al.*, 2018). Similarly, even though age did not seem to have a statistically significant effect, sex-based differences in TUG scores were observed, with females showing higher scores than males, indicating that females in their early and late older age have poorer physical performance.

Force fluctuations at both low- and mid-level submaximal sustained contractions in both sexes are greater in old compared to young (Enoka *et al.*, 2003; Carville *et al.*, 2007)and here we show a continued decrease in force steadiness from early to late elderly. This age-related loss is likely multifactorial and includes altered synaptic input to MUs, altered MU discharge properties (Farina & Negro, 2015; Castronovo *et al.*, 2018), as well as the loss and subsequent remodelling of MUs (Challis, 2006; Jones *et al.*, 2022). Additionally, considering multiple muscles are involved in the knee extension movements, an increased co-activation of agonist and antagonist muscles in lower limb has been reported with age and may play an important role in regulating force and control ability (Hortobágyi & DeVita, 2006; Krishnan *et al.*, 2011).

The hormonal milieu is one of the most distinguishing features of the two sexes, with the predominant female sex hormone estrogen and progesterone fluctuating across a normal menstrual cycle, whilst the

male sex hormone testosterone remains relatively constant. In crossing the blood brain barrier, estrogen elicits excitatory effects via the potentiation of glutamatergic receptors (Smith & Woolley, 2004) while progesterone increases activity of gamma-aminobutyric acid (GABA), causing inhibitory effects (Smith *et al.*, 1989; Del Río *et al.*, 2018). Following the menopause and the cessation of the menstrual cycle, typically occurring at the age of ~51 yrs (Hall, 2015), estrogen concentrations plummet and generate a severe change in the hormonal milieu that is not apparent in males. The exclusive manifestation of this pronounced change in females only may possibly account for the impaired equilibrium between neuronal excitation and inhibition in advanced age. Alterations in synaptic plasticity have been linked to reduced estrogen levels, albeit in animal models (Dumitriu *et al.*, 2010; Waters *et al.*, 2019), and a significantly lower level of GABA signalling components has also been reported in the cortex of older females (Pandya *et al.*, 2019). This disruption culminates in increased MU FR variability and exacerbates the decline in force steadiness, as observed here in older females during low- and mid-level contractions, setting it apart from our prior findings in Chapter 2 from the same muscle in younger cohorts.

In addition to greater MU FR variability, females also had a higher MU FR than males, accompanied by a smaller MUP area detected, consistent with what we have previously observed in the same muscle in younger cohorts in Chapter 2. A strong correlation between MU size and MU firing properties have been widely reported, with smaller MUs recruited earlier, firing faster than later recruited larger MUs, suggesting a larger proportion of smaller MUs in females than male counterparts. With force gradation increases, we found that recruitment strategies for early and late elderly males and females did not differ, also reported for young cohorts in Chapter 2. Put simply, both sexes employing both rate coding and MU recruitment, as indicated by higher MU FR and larger MUP area, to similar extent.

There was a sex and level interaction for MUP duration, indicating distinct adaptations between males and females when moving from low- to mid-level contractions. Males exhibited an extended MUP duration, whereas no distinct difference was observed among females. This disparity may be attributed to the recruitment of a greater number of muscle fibres within the activated MUs at heightened levels of contraction in males, whereas females possibly rely more on rate coding rather than recruitment of larger MUs. To better understand the characteristics at neuromuscular junction, we explored near fibre jiggle, which is indicative of NMJ transmission instability (Piasecki *et al.*, 2021*b*). There was no significant sex-difference in NF Jiggle, but an increased NF jiggle was detected in both males and females when moving from low- to mid-level contractions, revealing a greater transmission instability at higher contraction levels.

Strengths and Limitations

This is the first study using iEMG techniques to explore VL MU characteristics of early to late older males and females, enabling the avoidance of signal attenuation due to greater subcutaneous tissues in older females. However, we only sampled MU activities at low- and mid-level contractions and these findings cannot be extrapolated to higher contraction intensities or fatiguing contractions. Secondly, the right leg was uniformly assessed across all participants and as such, cannot infer possible bilateral differences.

3.6 Conclusions

Functional deterioration progresses at a similar rate in both sexes from early to late elderly. Males demonstrate a greater muscle size and strength, better motor control and functional performance. Females have greater MU FR variability and worse force steadiness than males, a sex-specific trait that was not apparent in younger cohorts. Moreover, the greater sex-based differences in aged muscle may contribute to greater

functional declines in older females. These findings add to the paucity of data in older females and suggest early interventions are needed for older females to prevent functional deterioration.

Chapter 4 - Bilateral differences in neural aspects of vastus lateralis function in young and old males

4.1 Abstract

Introduction

Muscle strength, a function of muscle size and neural input, is important in tasks of daily living and maintaining physical independence in older age, in addition to being a key outcome measure in many interventional studies. Muscle strength asymmetry may impact muscle function through inter-limb variation in central and/or peripheral features. We therefore investigated if bilateral differences are evident for knee extensors in young and older males, with respect to muscle strength and individual motor unit characteristics.

Methods

Thirteen young healthy males (23 ± 4 years; 25.3 ± 3.2 kg/m^2) and fifteen older healthy males (66 ± 8 years; 26.1 ± 2.4 kg/m^2) were recruited. High-density surface electromyography signals were collected from the vastus lateralis (VL) of each leg at 25% of maximal voluntary isometric contraction (MVC) and decomposed to identify individual motor unit firings. Muscle cross-sectional area (CSA), strength and force steadiness as well as motor unit firing properties and coherence estimates were compared bilaterally using multilevel mixed-effects linear regression models. Statistical significance was accepted at $p<0.05$.

Results

Knee extensor muscle MVC and VL CSA were smaller in older adults when compared to younger adults, with no significant bilateral differences in either group. Force steadiness did not differ between legs, nor did it differ between young and old. Coherence estimates were

indistinguishable bilaterally in both age groups whereas older adults showed a lower level of coherence estimate in Alpha band. Similarly, MU firing rates (FR) were lower in the older group with no significant bilateral difference. MU firing rate variability was lower in older adults and was lower in the dominant limb in both age groups.

Conclusion

In summary, muscle size or maximal strength did not differ between the two sides of legs in both young and older adults, however older adults exhibited an age-related decline in physical properties likely due to age-related decreases in MUFR. Though MUFR variability was lower on the dominant side, there were no bilateral differences in force steadiness which may be explained by similar levels of common synaptic inputs. Interestingly, the bilateral difference in force steadiness becomes more pronounced from early to late elderly, indicating a higher likelihood of unilateral functional weakness as a contributing factor to frailty in older age.

4.2 Introduction

Inter-limb asymmetry refers to imbalances in performance between opposing limbs. This phenomenon results from bilateral asymmetry of motor control circuitry between the right and left hemispheres, seen in both upper and lower limbs (Kapreli *et al.*, 2006). In addition to motor control asymmetries, other factors may also contribute to the development of such imbalances, including biomechanical disparities, previous injuries (Kuenze *et al.*, 2015) and the effects of aging (McGrath *et al.*, 2021) or disease states (Larson *et al.*, 2013). Consequently, such imbalances can give rise to a "dominant" limb that may exhibit greater force production, work performance, and potentially enhanced efficiency compared to the "non-dominant" limb (Newton *et al.*, 2006).

Current evidence pertaining to limb strength asymmetry is conflicting and varies between muscle groups (Rahnama *et al.*, 2005; Carpes *et al.*, 2010*a*; Lanshammar & Ribom, 2011; Lathrop-Lambach *et al.*, 2014), with one study demonstrating significant asymmetry in the knee extensors and flexors, showing 13.5% and 13% greater strength, respectively, during isokinetic contractions in dominant compared to non-dominant. Moreover, a comprehensive review of studies examining the comparative strength of the knee extensors as well as their influence on performance in jump tasks, revealed no significant disparities between the limbs (McGrath *et al.*, 2016).

The presence of such muscle asymmetry can have implications for the interpretation of clinical evaluations of skeletal muscle and for research studies that employ unilateral intervention models. These findings also hold significance for older individuals in terms of their susceptibility to falls and frailty. Furthermore, asymmetrical muscle strength and power have been observed in older women, regardless of their history of falling. However, those who experienced falls exhibited significantly greater bilateral differences (Skelton *et al.*, 2002), highlighting the potential impact of muscle asymmetry on the physical function of older individuals. Importantly, recent research has highlighted the association between

handgrip strength asymmetry, which serves as an increased risk of future falls among older adults (McGrath *et al.*, 2021).

A potential explanation of strength asymmetry may be the accompaniment of neuromuscular asymmetry, in particular variation of common synaptic inputs received from upper motor command and individual motor unit firing properties as voluntary muscle contractions are dependent on both the activity of afferent feedback mechanisms and descending cortical motor pathways, facilitating the transmission of neural signals to motoneurons and neuromuscular junctions, ultimately leading to the activation of motor units responsible for generating force (Heckman & Enoka, 2012). The regulation and coordination of neural inputs play a crucial role in achieving graded increases in muscle force. This involves concurrent variations in the number of active motor units (known as motor unit recruitment) and modulation of the discharge frequency of action potentials within these units (known as rate coding) (Del Vecchio *et al.*, 2019), probably leading to the uniformly distributed muscle strength and motor control ability (Enoka & Farina, 2021) across limbs.

The evidence regarding disparities in neuromuscular function related to laterality is inconclusive. A study investing motor unit discharge properties in a hand muscle during 30% of MVC revealed dissimilar motor unit characteristics between limbs with dominant hand having lower firing rates, firing rate variability and recruitment threshold when compared with non-dominant hand (Adam *et al.*, 1998). Similarly, another investigation demonstrated higher levels of short-term synchronisation among pairs of MUs in the dominant limbs (Schmied *et al.*, 1994). Conversely, a recent study has reported similar levels of motor unit firing rates and firing rate variability between limbs across submaximal isometric contractions at 10, 20, 40 and 60% of MVC in tibialis anterior (Petrovic *et al.*, 2022). Furthermore, the intramuscular common synaptic inputs did not differ between limbs in hand whereas the intermuscular common synaptic inputs were reported to be lower in

dominant hand, allowing a higher flexibility in MU recruitment and mechanical output (Maillet *et al.*, 2022).

However, the existing body of knowledge primarily centres around upper limbs and in young adults, leaving a lack of information regarding the influence of age on limb dominance in older adults and in lower limbs, particularly quadriceps, which constitutes the largest muscle group in the lower body and are the most susceptible to atrophy with ageing. Additionally, it is apparent that both our previous studies (referenced in Chapter 2 and 3) and those of many others have routinely focused only on unilateral limbs. However, the results of these studies have been extrapolated to bilateral limbs, and interventions have been based on these findings obtained from unilateral limbs. Any implications arising from the presence of limb strength asymmetry, as well as central and peripheral single motor unit properties, are yet to be determined.

The purpose of this study was to investigate bilateral differences in the vastus lateralis (VL) with respect to the muscle size and strength, force steadiness and individual motor unit characteristics in addition to common synaptic inputs in both young and older males and to explore the influence of advancing age on bilateral differences within older individuals. We hypothesised that no bilateral differences would present in all outcome measures with each age group and/or with advancing age but clear age-related differences would be detected in both physical and motor unit function.

4.3 Methods

4.3.1 Participants

This study was approved by the University of Nottingham Faculty of Medicine and Health Sciences Research Ethics Committee (160-0121, 90-0820, 390-1121) and was conducted between 2019-2022 in accordance with the Declaration of Helsinki.

Thirteen healthy young (23 ± 4 years; 25.3 ± 3.2 kg/m^2) males and fifteen healthy older (66 ± 8 years; 26.1 ± 2.4 kg/m^2) males were recruited from the local community via advertisement. Prior to enrolment, all recruited participants completed a comprehensive clinical screening examination based at the School of Medicine, Royal Derby Hospital Centre and subsequently provided written informed consent. Participants were excluded if they had BMI< 18.5 or > 35 kg/m^2; were competitive in an athletic discipline at county level or above; had a musculoskeletal disorder; respiratory disease; neurological disorder; metabolic disease; active cardiovascular problems; active inflammatory bowel or renal disease; recent steroid treatment within 6 months or hormone replacement therapy; family history of early (< 55 years) death from cardiovascular disease. The leg dominance was identified by asking which leg they would use to kick a football, which has been reported to have a great agreement with observed leg dominance during bilateral mobilizing and unilateral stabilizing tasks. All experimental techniques (described below) were completed bilaterally.

4.3.2 Anthropometry

Prior to testing, calibrated scales and a stadiometer were used to assess the body mass and height of each participant for calculation of the body mass index. Muscle cross-sectional area (CSA) of the vastus lateralis was assessed bilaterally using ultrasound scans (LA523 probe and MyLab™50 scanner, Esaote, Genoa, Italy). The imaging was conducted at the midpoint between the greater trochanter and the midpoint of the knee. To distinguish the lateral and medial borders of the VL, the

ultrasound probe was positioned vertically, and the borders and midpoint of the muscle were marked on the participant's skin. To quantify muscle CSA, images were captured from the aponeurosis borders of the VL of the right leg in a medial-lateral fashion using panoramic imaging (VPAN) and further analysed using ImageJ software (National Institute of Health, USA) by tracing around the VL following the contour of the aponeurosis. To ensure accuracy, each image was analysed three times, and the average of three images was determined as CSA.

4.3.3 Knee Extensor Strength and Force Steadiness

Participants were seated in a custom-built chair, which was adjusted to ensure approximately 90 degrees of flexion at the hips and knees. The lower leg was secured to a force dynamometer using a non-compliant strap (designed with a calibrated strain gauge, RS125 Components Ltd, Corby, UK) positioned above the medial malleolus. To minimize movement of the hips and upper trunk during contractions, the hips and pelvis were stabilised using a seat belt. The distance between the centre of the force strap and the lateral femoral condyle was measured to determine the external knee joint moment arm. Following a standardized warm-up consisting of submaximal contractions, participants were instructed to exert maximum effort with the aid of real-time visual feedback and verbal encouragement, aiming for forceful and rapid contractions. During the trial, participants were not allowed to hold onto the sides of the chair and were instructed to cross their arms over their chest. It was further repeated two to three times with 60 seconds rest intervals between each one. If there was a difference of less than 5% between the two last attempts, the highest value was accepted as the maximal voluntary contraction (MVC). Muscle torque was then calculated by multiplying the selected MVC by the lever arm.

Offline analysis of the strain gauge signals began with the conversion of the voltage signal to force in Newton and the force signal was then filtered with a zero-lag lowpass Butterworth filter with a cut-off frequency of 20 Hz. To assess force steadiness during contractions at 25% of MVC,

the coefficient of variation of the force (CoV) was calculated as the standard deviation divided by the mean, multiplied by 100.

4.3.4 High-density Surface Electromyography

A semi-disposable high-density surface electromyography (HDsEMG) array (64 electrodes, 13×5, 8mm, I.E.D., GR08MM1305, OT Bioelettronica, Inc., Turin, Italy) was positioned over the muscle belly of the vastus lateralis. Prior to placement, the skin was prepared by shaving, lightly abrading, and cleansing with 70% ethanol. The electrodes were affixed to the skin using flexible tape, and disposable bi-adhesive foam layers (SpesMedica, Bettipaglia, Italy) were used to secure the adhesive grids to the muscle surface. The skin electrode contact was facilitated by filling the cavities of the adhesive layers with conductive paste (AC Cream, SpesMedica). A strap ground electrode (WS2, OTBioelettronica, Turin, Italy) moistened with water was placed around the ankle of the tested leg. The HDsEMG signals were acquired in a monopolar configuration, amplified (x256), and filtered within the range of 10-500 Hz. The signals were then digitally converted at a sampling rate of 2000 Hz using a 16-bit wireless amplifier (Sessantaquattro, OTBioelettronica, Turin, Italy) and transferred to a computer for further offline analysis. The OTBioLab software (OT Bioelecttronica, Turin, Italy) was used to record the HDsEMG signals.

The recorded HDsEMG signals were converted into MatLab files, each representing a single contraction. Participants performed four submaximal contractions at 25% of MVC; however, the third of the four trials was selected for analysis. The monopolar HDsEMG signals were band-pass filtered from 20 to 500 Hz and decomposed offline into MUPTs using a convolutive kernel compensation algorithm. A trained investigator manually inspected the MUPTs and made necessary edits to the discharge patterns of each detected MU. Only MUs with a pulse-to-noise ratio equal to or greater than 30 dB were retained for further analysis. Firing rates of each individual motor units were calculated and mean firing rates below 5 Hz were excluded from the following analysis.

The variability of firing rate was quantified as the coefficient of variation (CoV) of the inter-discharge intervals (IDIs), expressed as a percentage.

To estimate the level of common synaptic input, intramuscular coherence was computed during submaximal contractions at 25% of maximal voluntary contraction (MVC). This measure represents the frequency-domain correlation between cumulative spike trains (CSTs) of the identified MUs. The magnitude-squared coherence was computed using the Welch's averaged periodogram with 50% overlapping Hann windows of 1s duration, across different frequency bands: delta (0-5 Hz), alpha (5-12 Hz), beta (15-30 Hz), and piper (40-50 Hz) (Raethjen *et al.*, 2007; Laine & Valero-Cuevas, 2017). This analysis was repeated for 60 randomly chosen combinations of two equally sized CSTs calculated as the sum of firing times from three MUs randomly selected from the same set of MUs and then averaged. To facilitate statistical comparisons, the Fisher Z-transform was applied to the coherence values, yielding a normally distributed variable (Rosenberg *et al.*, 1989).

4.3.5 Statistical Analysis

Data are presented as mean ± standard deviation unless stated otherwise. *Multilevel mixed-effects linear regression models* were used through the package *lme4* (Version 1.1-27.1) with each individual being regarded as an independent cluster. To explore the bilateral differences in young and older individuals, Leg and AgeGroup were included as categorical variables and their interactions were explored in the models. Further exploratory analysis of the influence of advancing age on bilateral differences within older individuals only, and here Age was applied as a numerical variable with Leg as a categorical variate. The results are displayed as coefficient estimates, 95% confidence intervals (CIs) and p values. All statistical analysis was conducted using RStudio. Statistical significance was accepted when $p<0.05$.

4.4 Results

Twenty-eight healthy participants were included in the study, consisting of 13 young males (age range: 20-36 years) and 15 older males (age range: 56-82 years).

Table 4.1. Participant characteristics

Measures	Young (n=13)		Older (n=15)	
	Dominant	Non-Dominant	Dominant	Non-Dominant
MU Number	18 (11)	15 (9)	18 (5)	20 (8)
CSA (cm^2)	34.30 (7.40)	33.10 (7.70)	25.75 (4.55)	25.43 (4.75)
MVC (N)	662.38 (129.13)	632.15 (178.05)	349.57 (92.98)	351.40 (87.97)
CoV-Force (%)	2.06 (0.70)	1.94 (0.72)	2.19 (0.53)	2.05 (0.61)
MU FR (Hz)	9.03 (1.14)	8.70 (1.24)	7.62 (1.08)	7.62 (0.87)
MU FR Variability (%)	12.06 (3.41)	13.57 (4.00)	10.27 (2.02)	10.06 (1.84)
COH-Delta	0.49 (0.10)	0.53 (0.22)	0.56 (0.15)	0.50 (0.12)
COH-Alpha	0.25 (0.05)	0.26 (0.08)	0.23 (0.04)	0.22 (0.03)
COH-Beta	0.22 (0.02)	0.23 (0.03)	0.22 (0.02)	0.22 (0.02)
COH-Piper	0.24 (0.02)	0.23 (0.04)	0.21 (0.01)	0.22 (0.03)

Data are reported as mean (standard deviation). Abbreviations: CSA, cross-sectional area; MVC, maximal voluntary isometric contraction; CoV, coefficient of variation; MU, motor unit; FR, firing rate; COH, coherence estimate.

There was no significant AgeGroup x Leg interaction detected in CSA, MVC or CoV-Force, nor was there any statistical differences between dominant and non-dominant legs in either young or older groups (all p>0.05). However, there was a main effect of AgeGroup for CSA (p=0.026) and MVC (p<0.001), which were both smaller in older when compared to younger individuals. For CoV-Force, there were no AgeGroup- or Leg-related differences (both p >0.05) (Table 4.2, Fig 4.1A-C).

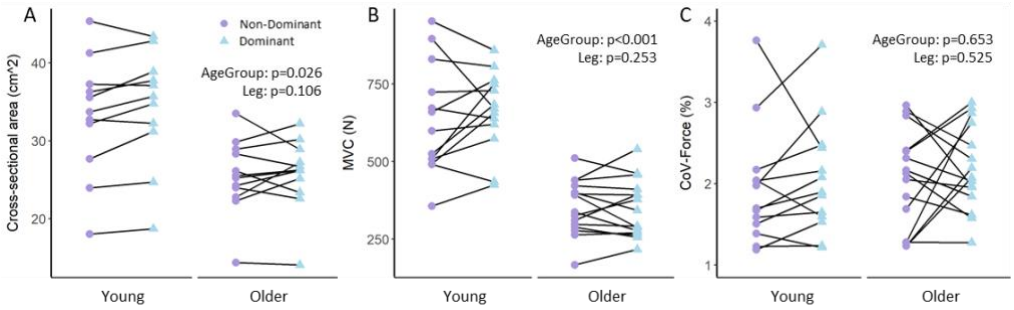

Figure 4.1*. Vastus lateralis cross-sectional area (CSA), maximal isometric voluntary contraction (MVC) of knee extensors and knee extensor force steadiness at a submaximal contraction at 25% of MVC in the dominant (purple round) and non-dominant (blue triangle) legs. Abbreviations: CoV, coefficient of variation.*

There were no significant AgeGroup x Leg interactions for MU FR, but there was a main effect of AgeGroup with older adults showing a lower FR when compared to younger adults (p=0.003) (Fig 4.2A). There were no significant AgeGroup x Leg interactions for MU FR variability, but there was a main effect of Leg with dominant legs showing lower MU FR variability when compared to non-dominant legs (p=0.018). There was also a main effect of AgeGroup with older adults having lower MU FR variability when compared to younger adults (p=0.003) (Fig 4.2B).

Table 4.2. Summary of multilevel linear regression analysis for physical function and motor unit characteristics.

	AgeGroup [a]			Leg [b]		
	Beta	**95% CI**	**p value**	**Beta**	**95% CI**	**p value**
CSA (cm^2)	-6.86	-12.55 to -1.16	**0.026**	1.11	-0.18 to 2.41	0.106
MVC (N)	-280.76	-373.44 to -188.07	**<0.001**	30.23	-20.48 to 80.94	0.253
CoV-Force (%)	0.11	-0.36 to 0.58	0.653	0.12	-0.24 to 0.48	0.525
MU FR (Hz)	-1.21	-1.95 to -0.46	**0.003**	0.14	-0.15 to 0.42	0.344
MU FR Variability (%)	-3.11	-5.01 to -1.22	**0.003**	-1.28	-2.33 to -0.22	**0.018**
COH-Delta	-0.03	-0.14 to 0.09	0.616	-0.04	-0.12 to 0.05	0.401
COH-Alpha	-0.04	-0.08 to -0.01	**0.027**	-0.01	-0.05 to 0.03	0.478
COH-Beta	-0.01	-0.03 to 0.003	0.105	-0.01	-0.03 to 0.003	0.124
COH-Piper	-0.01	-0.03 to 0.01	0.185	0.01	-0.01 to 0.03	0.476

*[a] Multilevel mixed-effects linear regression model with **Leg** as dependent variable. Beta values reflect difference from young to older adults; [b] multilevel mixed-effects linear regression model with **AgeGroup** as dependent variable. Beta values reflect difference from non-dominant leg to dominant leg. Values in bold reflect statistically significant (p<0.05) results. Abbreviations: CI, confidence interval; CSA, cross-sectional area; MVC, maximal voluntary isometric contraction; CoV, coefficient of variation; MU, motor unit; FR, firing rate; COH, coherence estimate.*

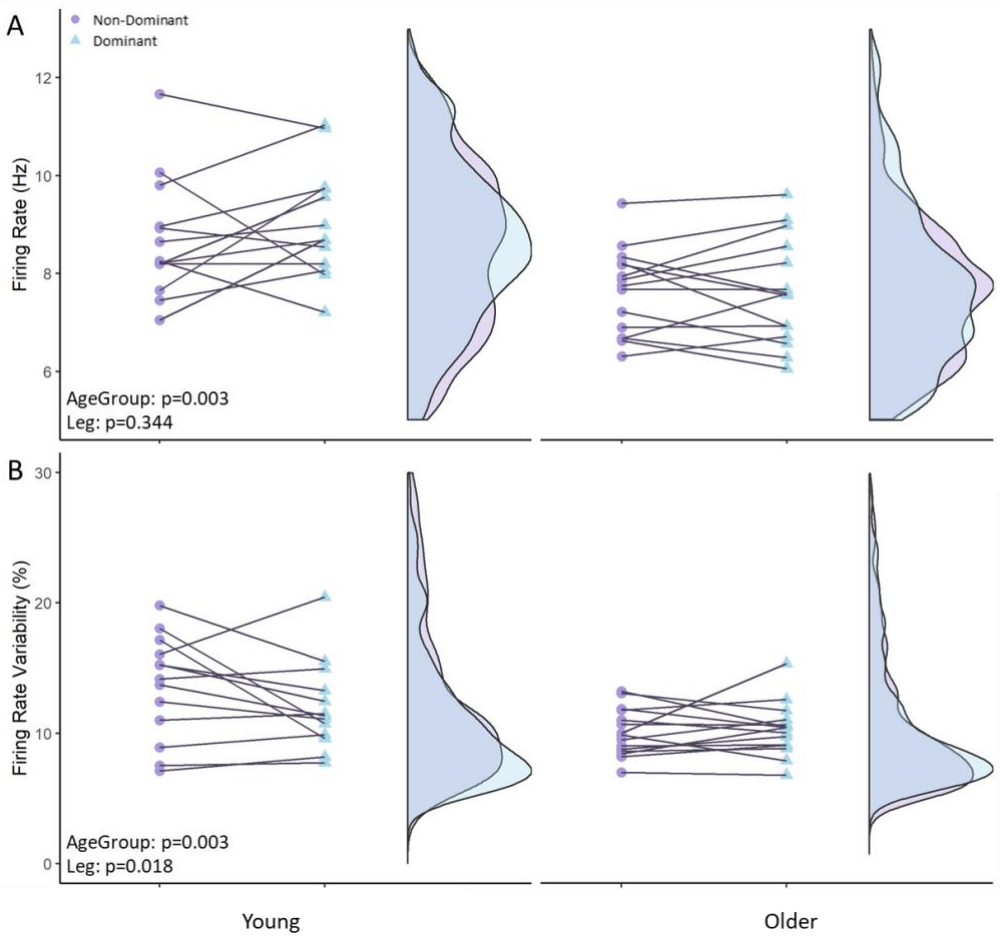

***Figure 4.2**. Motor unit firing features from the vastus lateralis obtained via high-density surface electromyography at a submaximal contraction at 25% of maximal voluntary contraction (MVC). Data points shown are each individual sampled motor unit with calculated mean values for the motor unit feature overlaid, in the dominant (purple round) and non-dominant (blue triangle) legs for visualisation only. Distributions of each motor unit characteristic are shown in density plots on the right side. All analyses were based on multi-level linear regression models.*

There were no significant interactions between AgeGroup x Leg detected in coherence estimates. There was no significant main effect of Leg across all coherence frequency bands (Fig 4.3A-D), but there was a main effect of AgeGroup for COH-Alpha with older adults showing a lower level of coherence estimates in Alpha band (p=0.027) (Fig 4.3B).

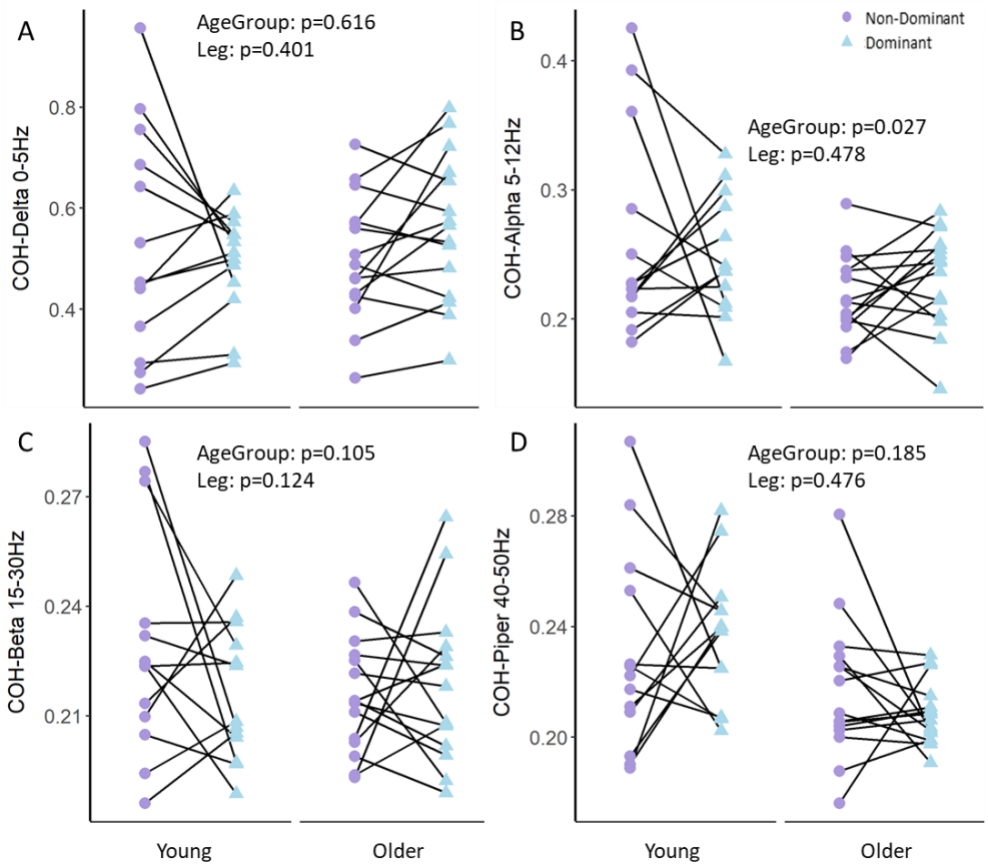

***Figure 4.3**. Individual participant means of intramuscular coherence Z-score at different frequency bands from both dominant (purple round) and non-dominant (blue triangle) legs at a submaximal contraction at 25% of maximal voluntary contraction (MVC) in young and older males.*

Exploratory analysis within older individuals revealed there were no significant interactions between Leg and Age detected in muscle CSA and MVC, but there was a significant main effect of age with MVC decreased by 6.95 N for every year increase in age ($p=0.015$). There was a significant Leg X Age interaction for CoV-Force ($p=0.004$), explained by an increasing difference across legs with increasing age ($p=0.005$). There were no significant Leg x Age interactions detected in MU FR, MU FR variability or common synaptic inputs in any frequency band, nor was there any statistical differences between dominant and non-dominant legs with advancing age (all $p>0.05$).

Table 4.3. Summary of multiple linear regression analysis within older adults.

	Leg [a]			Age [b]		
	Beta	**95% CI**	**p value**	**Beta**	**95% CI**	**p value**
CSA (cm^2)	-9.16	-23.40 to 5.07	0.236	-0.37	-0.77 to 0.02	0.090
MVC (N)	-223.14	-431.31 to -14.97	0.056	-6.95	-11.93 to -1.97	**0.015**
CoV-Force (%)	-3.90	-6.17 to -1.63	**0.005**	-0.02	-0.05 to 0.01	0.202
MU FR (Hz)	-0.48	-3.42 to 2.46	0.753	-0.005	-0.07 to 0.06	0.869
MU FR Variability (%)	-3.23	-11.39 to 4.94	0.452	0.007	-0.12 to 0.13	0.915
COH-Delta	-0.20	-0.70 to 0.31	0.460	-0.002	-0.01 to 0.006	0.596
COH-Alpha	-0.13	-0.30 to 0.04	0.158	-0.000	-0.002 to 0.002	0.969
COH-Beta	0.01	-0.10 to 0.12	0.869	-0.000	-0.001 to 0.001	0.740
COH-Piper	-0.02	-0.14 to 0.10	0.754	-0.001	-0.002 to 0.001	0.406

*[a] Multilevel mixed-effects linear regression model with **Leg** as dependent variable. Beta values reflect difference from non-dominant leg to dominant leg; [b] multilevel mixed-effects linear regression model with **Age** (numerical variable) as dependent variable. Beta values reflect the changes per every year increase in age. Values in bold reflect statistically significant (p<0.05) results. Abbreviations: CI, confidence interval; CSA, cross-sectional area; MVC, maximal voluntary isometric contraction; CoV, coefficient of variation; MU, motor unit; FR, firing rate; COH, coherence estimate.*

4.5 Discussion

The present study aimed to investigate the bilateral differences in physical characteristics and motor unit firing behaviours in young and older adults. This study demonstrated in both young and older healthy males, there were no bilateral differences in muscle size, strength, or force steadiness during isometric voluntary contractions at 25% of MVC. However, older adults had a smaller muscle size and lower muscle strength when compared to younger counterparts. Moreover, the study revealed that older individuals exhibited a significant loss of muscle strength independent of loss of muscle size from early to late elderly and the bilateral difference in force steadiness also increased. Interestingly, there were no bilateral differences in MUFR, but older adults exhibited a lower MUFR. Both young and older adults displayed lower MUFR variability in their dominant legs, and in the older group, this bilateral difference was apparent from early to late elderly. In terms of common synaptic inputs, coherence estimates at any band did not differ between limbs in either group, which remained the same as age increased in old. However, a notable age-related difference was detected in alpha band, with older adults showing lower coherence estimates when compared to young adults. These data contribute comprehensive investigation of bilateral difference in upper and lower neuronal properties in both young and old adults and reveal that perceived limb dominance does not exert a substantial influence on lower limb neuromuscular function, providing valuable insights into the development of assessment and targeted interventions, especially the benefits of unilateral exercise training in elderly.

The existence of bilateral differences in skeletal muscle function has yielded inconsistent findings, with some reporting significant limb asymmetry (Portegijs *et al.*, 2005; Newton *et al.*, 2006) while others failing to observe such disparities (Ditroilo *et al.*, 2010; Carpes *et al.*, 2010*b*). Our data demonstrated no significant bilateral differences in knee extensor muscle strength and muscle size in either young or older

groups, which is consistent with the findings in another lower limb muscle, tibialis anterior, in young cohorts (Petrovic *et al.*, 2022). Mechanically, the absence of bilateral differences may be partly due to a similar level of activation of agonist muscles and/or co-activation of the antagonists involved in knee extension isometric contractions across legs. More importantly, as force generation is highly associated with MU recruitment and firing rate modulation, lack of difference in bilateral muscle strength may partially be attributable to MU firing behaviours besides MU recruitment (Enoka & Duchateau, 2017; Wilkinson *et al.*, 2018).

However, although only assessed at 25% MVC, MU FR did not differ between dominant and non-dominant legs in either group, also in agreement with the findings in TA (Petrovic *et al.*, 2022), indicating limb dominance may have less influence on neuromuscular function in lower limbs when compared to upper limbs (Adam *et al.*, 1998; Maillet *et al.*, 2022). Despite no bilateral differences in both groups, older adults still exhibited a lower MUFR overall and perhaps consequently, a significant lower muscle strength. This age-related difference in strength and size is in alignment with a wealth of ageing literature, as well as the findings observed in unilateral vastus lateralis in Chapter 5. Furthermore, with ageing, the loss of strength typically exceeds the loss of size, which is consistent with what we have reported in the aged cohorts with both sexes in Chapter 2.

We additionally demonstrate no significant bilateral difference in force steadiness at 25% of MVC in either group, highlighting a similar level of neural inputs delivered to both legs. Firstly, the lack of significant disparity in force steadiness between legs was expected in healthy individuals due to equivalent bilateral involvement of legs in motor actions. In fact, motor control ability is regulated by the proportion of independent and common synaptic inputs received by the motoneuron pool from supraspinal and spinal circuitries (Farina *et al.*, 2016; Enoka & Farina, 2021). A significant smaller MUFR variability was observed in dominant legs whereas coherence estimates at any frequency bands did

not differ between legs in both groups. A study exploring relationships between MUFR variability/common synaptic inputs and force steadiness has revealed that MUFR variability contributes more to force steadiness at a lower contraction level (<2.5% MVC), whereas the common synaptic inputs are more dominant at a higher contraction level at 10% of MVC (Duchateau & Enoka, 2022), suggesting that absence of bilateral difference in force steadiness is likely driven by similar level of common synaptic inputs across legs. Furthermore, after adjusting for leg dominance, both MUFR variability and coherence estimate at alpha band were smaller in older group but with no difference in force steadiness. This may be because force steadiness is associated with delta band coherence rather alpha band (Laine & Valero-Cuevas, 2017; Maillet *et al.*, 2022) and the significant difference in MUFR variability had limited contributions to force steadiness during submaximal stable contractions at 25% of MVC.

Nonetheless, such absence of bilateral difference in force steadiness may not hold true when looking at upper-limb muscles (Ireland *et al.*, 2014), where bilateral differences would be much greater. As indicated by the motor cortex activity, lower and upper limb areas exhibit differential movement control (Rothwell *et al.*, 1991) with a greater cortical neuronal connectivity in the upper limbs (Palmer & Ashby, 1992; Menon *et al.*, 2018) compared to the lower limbs due to the finer, more precise movements undertaken by the upper limbs. Unlike muscle strength, after adjusting for leg dominance, force steadiness did not differ between age groups. Consistent with what we have observed in unilateral data, no age-related difference in force control ability during submaximal stable contraction but during a more complex ramp contraction in Chapter 5, highlighting the role of task complexity in age-related impairments (Bootsma *et al.*, 2021).

Interestingly, a clear asymmetry in force steadiness was evident with increasing age within older individuals, though there were no significant bilateral difference in either MUFR variability or levels of common

synaptic inputs. This is consistent with the findings observed in hand muscles, which is within the same muscle the common synaptic inputs showed no bilateral differences but dominant hand exhibited less intermuscular common synaptic inputs between different muscles than non-dominant side (Maillet *et al.*, 2022), allowing for a higher level of flexibility of MU recruitment from the motoneuron pool to achieve better force control. Therefore, the greater bilateral difference in force steadiness with age may be attributable to less intermuscular common synaptic inputs, leading to the unilateral functional weakness in old.

Older adults with a history of falling have been reported to present with greater muscle asymmetry (Skelton *et al.*, 2002), and leg extension asymmetry is reported to impair walking and standing balance in older women (Portegijs *et al.*, 2005). According to a recent study, older adults with asymmetrical handgrip strength are more likely to fall in the future, with odds of falling being 7%, 12%, and 15% greater when demonstrating more than 10%, 20% and 30% asymmetry, respectively. It is likely that reduced skeletal muscle mass and deterioration of the neuromuscular system with advancing age (Wilkinson *et al.*, 2018) underlies such motor control asymmetries in older individuals.

Strengths and limitations

This is the first study to explore the bilateral differences in muscle force across age and this study provides valuable function and mechanistic insight from independent and common synaptic inputs to motoneuron pool of vastus lateralis, allowing for a comprehensive understanding of how these factors may vary across legs and age. However, MUs in the current study were sampled from mid-level contraction intensity only and reveal limited information on later recruited MUs. Secondly, knee extensor movements are controlled by multiple muscles and only investigating vastus lateralis MUs may limit insight into whole muscle group. Thirdly, the current data are available in males only, and although we have highlighted similar neuromuscular recruitment strategies

between sexes, there may be bilateral sex differences in physical performance and MU firing patterns.

4.6 Conclusions

To conclude, muscle size, maximal strength or force steadiness did not differ between the two sides of legs in young and old males. There was a significant age-related decline in MUFR during submaximal isometric contraction, leading to the loss of muscle strength with age in older individuals. Despite lower MUFR variability detected on the dominant side, there were no significant bilateral differences in force steadiness due to the non-different common synaptic inputs. However, the bilateral different in force steadiness increased from early to late elderly, suggesting older individuals are more likely to develop unilateral functional weakness in later life.

Chapter 5 - Common synaptic inputs and persistent inward currents of vastus lateralis motor units are reduced in older age

5.1 Abstract

Background

Although muscle atrophy may partially account for age-related strength decline, it is further influenced by alterations of neural input to muscle. Persistent inward currents and the level of common synaptic inputs to motoneurons influence neuromuscular function. However, these have not yet been described in aged human quadriceps.

Methods

High density surface electromyography (HDsEMG) signals were collected from the vastus lateralis of 15 young (mean±SD, 23 ± 5 y) and 15 older (67 ± 9 y) men during submaximal sustained and 20-s ramped contractions. HDsEMG signals were decomposed to identify individual motor unit discharges, from which delta F and intramuscular coherence were estimated.

Results

Older participants produced significantly lower knee extensor torque (p<0.001) and poorer force tracking ability (p<0.001) than young. Older participants also had lower delta F (p=0.001) and coherence estimates in the alpha frequency band (p<0.001) during ramp contractions when compared to young.

Conclusion

Persistent inward currents and common synaptic inputs are lower in the vastus lateralis of older males when compared to young. These data highlight altered neural input to the clinically and functionally important quadriceps, further underpinning age-related loss of function which may occur independently of the loss of muscle mass.

5.2 Introduction

Human ageing is characterised by a progressive reduction in muscle size and muscle strength, resulting in an impairment of force generation, force control and physical performance (Manini *et al.*, 2013; Wilkinson *et al.*, 2018; Pethick & Piasecki, 2022), all of which can negatively impact quality of life (Daley & Spinks, 2000; Smee *et al.*, 2012). The loss of muscle size is explicable by the combined effects of muscle fibre atrophy and muscle fibre loss (Wilkinson *et al.*, 2018), and although atrophy may partially account for declines in muscle strength, it is further influenced by structural and functional alterations of the nervous system.

The voluntary contraction of muscle fibres occurs via net excitatory signals to the motor units (MUs); groups of muscle fibres innervated by a single motoneuron (Heckman & Enoka, 2012). The central nervous system controls muscle force via two primary strategies; varying the number of MUs recruited and varying the discharge rate of each motor neuron, both of which are susceptible to age-related alterations. Substantial motoneuron loss accelerates in the sixth decade of life, resulting in fewer MUs, and a compensatory process of MU remodelling further results in larger MU innervation ratios (Hepple & Rice, 2016; Piasec*ki et al*>, 201>>). Additionally, although this appears to be muscle specific, an age-related decrease in MU discharge rate has also been documented at normalised contraction levels (Kirk *et al.*, 2021; Orssatto *et al.*, 2022) as well as being associated with circulating hormone levels that are also affected by age (More details in Chapter 6).

In addition to direct neurotransmitter activation onto postsynaptic ligand-gated channels at the spinal cord level via ionotropic mechanisms, activation of the motor nervous system also relies on neuromodulation (Heckmann *et al*., 2005). This is mediated by intracellular second messenger systems that modify the voltage- and ligand-gated channels of neurons and alters several properties from their level of excitability to their pattern of firing in response to a given input (Heckman *et al.*, 2008; Nadim & Bucher, 2014). This process is enhanced by monoamines, e.g.

serotonin and norepinephrine released from the brainstem nuclei by stimulating inward-flowing persistent calcium and sodium currents (Heckmann *et al.*, 2005), known as persistent inward currents (PICs). As long as the membrane potential remains above the activation threshold, these PICs tend to remain activated (Heckmann *et al.*, 2005), combined with neuromodulatory drive amplifying the firing behaviours of motoneurons to synaptic inputs up to five fold (Lee & Heckman, 2000) as well as generating long-lasting plateau potentials which results in self-sustaining firing (Gorassini *et al.*, 2002). Even though PICs appear to decrease with age in upper (Hassan *et al.*, 2021) and lower limb muscles (Orssatto *et al.*, 2021), PICs can be mediated by local ionotropic inhibitory inputs that may be muscle specific in the response to age (Yavuz *et al.*, 2018; Orssatto *et al.*, 2022; Pearcey *et al.*, 2022), particularly those highly susceptible to functional losses such as the vastus lateralis (VL) (Naruse *et al.*, 2023).

Rather than controlling the discharge pattern of individual MUs, the central nervous system controls the excitatory inputs to the MU pool, referred to as common synaptic inputs (Farina *et al.*, 2014) which are defined as the proportion of net synaptic input correlated between MUs. Force fluctuations are largely determined by the alterations in low frequency components of common synaptic inputs to MUs, which is reflected in concurrent fluctuations in MU discharge rates from the same MU pool (Farina & Negro, 2015). Accordingly, a decrease in force steadiness associated with ageing is a consequence of an increase in variance of common synaptic inputs (Castronovo *et al.*, 2018; Feeney *et al.*, 2018). However, the variability of common synaptic inputs in quadriceps has only been explored in healthy young individuals (Avrillon *et al.*, 2020; Rossato *et al.*, 2022), with no available data from older humans.

It is not possible to make intracellular recordings from human spinal motoneurons in order to estimate PICs, so alternative methods are employed which distinguish intrinsic excitability of the MU from its

descending synaptic drive by using the unit-wise MU analysis technique (Hassan *et al.*, 2021). In view of the advances with which MU discharging patterns can be measured, it provides an opportunity to investigate and better understand the physical properties and the underlying mechanisms which may impair motor control in older age. Therefore, the aim of this study was to explore the differences in physical performance as well as the magnitude of persistent inward currents and common synaptic inputs in the vastus lateralis between healthy young and older males. We hypothesized that older participants would exhibit greater physical decrements and lower levels of persistent inward currents and common synaptic inputs.

5.3 Methods

5.3.1 Participants

This study was approved by the University of Nottingham Faculty of Medicine and Healthy Science Research Ethics Committee (90-0820, 199-0221) and conformed to the Declaration of Helsinki.

15 healthy young males between the ages of 18-40 (mean ± SD; age: 23.1 ± 5.2 years; body mass index: 25.0 ± 2.5 kg/m^2) and 15 healthy older males between the ages of 55 to 85 (67.2 ± 8.9 years; 26.1± 2.3 kg/m^2) provided informed consent to take part in the study. All participants were recruited through advertisements in the local community. Prior to enrolment, all participants completed a comprehensive clinical examination and metabolic screening was conducted at the School of Medicine, Royal Derby Hospital Centre. All recruited participants were recreationally active and would be excluded if they display evidence of: BMI < 18.5 or > 35kg/m^2; are competitive in an athletic discipline at county level or above; musculoskeletal disorders; respiratory disease; neurological disorders; metabolic disease; active cardiovascular problems; active inflammatory bowel or renal disease; recent steroid treatment within 6 months or hormone replacement therapy; family history of early (< 55years) death from cardiovascular disease.

5.3.2 Experimental protocol

The measurement of cross-sectional area of VL and maximal voluntary contraction (MVC) of the knee extensors has been described in Chapter 2, Section 2.3.2 and Section 2.3.3.

Following a 60-second rest, participants were then asked to perform four submaximal voluntary isometric contractions at 25% of MVC (~12s) and four triangular shaped contractions (10s up and 10s down) peaking at 20% of MVC, with 30-second intervals between contractions. In each case, a target line was displayed on a screen and participants were

instructed to follow the target as close as possible. Ramp contractions to 20% of MVC have been widely used for estimating persistent inward currents (details below) (Gorassini *et al.*, 2002; Hassan *et al.*, 2020; Afsharipour *et al.*, 2020).

Offline analysis of the strain gauge signals began with the conversion of the voltage signal to force in Newton and the force signal was then filtered with a zero-lag lowpass Butterworth filter with a cut-off frequency of 20 Hz. To quantify force steadiness during 25% of MVC, the coefficient of variation of the force (CoV) was calculated = (SD/Mean)*100. To assess force tracking accuracy, all data were exported to Spike2 (version 9.11, CED Ltd., Cambridge, UK), where a virtual channel was created by subtracting the performed path from the requested (target) path and rectifying it. Area under the curve reflects the level of deviation from the target line reflecting muscle force tracking accuracy, with higher values indicating greater deviation.

5.3.3 High-density surface electromyography (HDsEMG)

HDsEMG signal acquisition and analysis as well as the estimation of coherence (Figure 5.1) have been described in Chapter 4, Section 4.3.3.

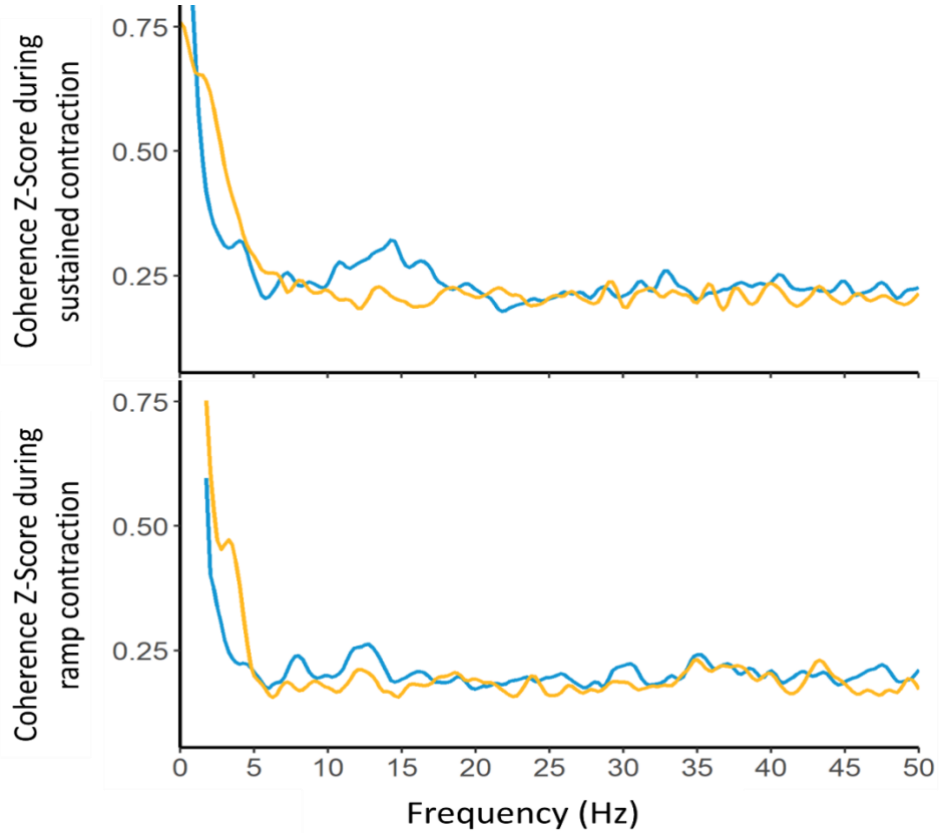

Figure 5.1. *The group mean intramuscular coherence Z-Scores during submaximal contraction at 25% of maximal isometric voluntary contraction (MVC) and ramp contraction at 20% of MVC in young (blue) and older (yellow) participants. The magnitude-squared coherence was calculated from all the combinations (a maximum of 60 random permutations) of two equally sized cumulative spike trains, each composed of three motor units.*

The instantaneous firing rates of both MUs were calculated as the inverse of the interspike intervals of each MU spike train and smoothed by fitting a fifth order polynomial function. An estimate of the PIC magnitude was derived using the unit-wise MU analysis technique (Hassan *et al.*, 2021). The lower threshold unit to be recruited in the ramp contraction is commonly referred to as the “control” unit; the “test” unit is a unit of higher threshold. All MUs from 10 young and 10 older participants were initially isolated but those that met the criteria below were eventually included for ΔF (DeltaF) calculation. ΔF, the contribution of persistent inward currents to self-sustained firing, was calculated as the difference in “control” unit firing rate between the onset and offset of a “test” unit (Gorassini *et al.*, 2002). This technique relies on several assumptions: 1) test and control units share the common synaptic drive; 2) PIC is activated before or at the recruitment; 3) firing rate of the control MU closely reflects the depolarizing input to the parent motoneuron; 4) test and control unit process the synaptic inputs in a similar way. The MU pairs for the delta F calculation were selected based on the following criteria: 1) as a measure of common synaptic modulation, rate-to-rate correlation $r \geq 0.7$; 2) to avoid the high variability in delta F calculation due to the initial acceleration phase of MU firings, test units were recruited at least 1s after the control units; 3) to account for the possibility of control unit saturation leading to an underestimation of delta F, pairs in which rate modulation of the control unit fell within 0.5pps were removed from analysis (Gorassini *et al.*, 2002; Udina *et al.*, 2010; Vandenberk & Kalmar, 2014; Hassan *et al.*, 2020). An average delta F was calculated when individual test units were paired with multiple control units (Figure 5.2).

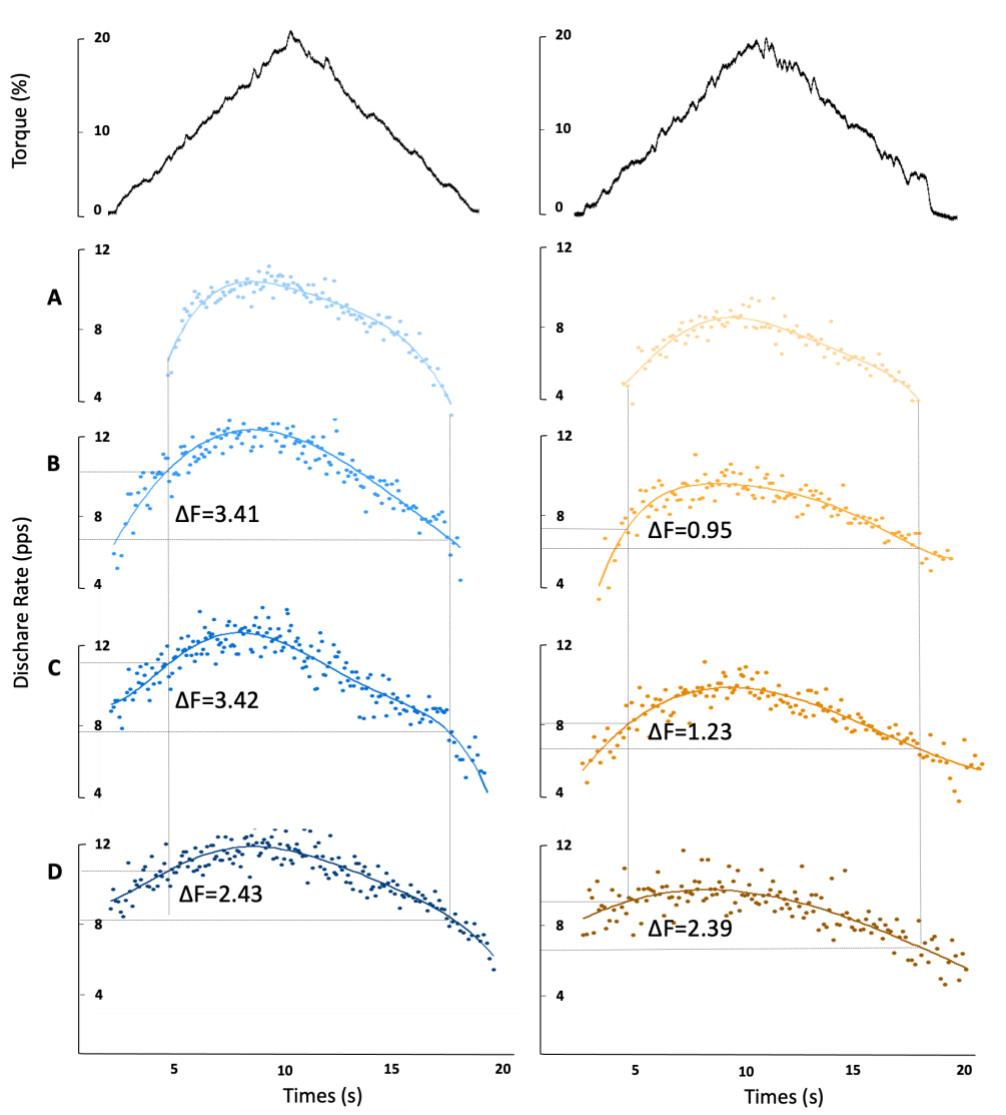

Figure 5.2. *Example data showing delta frequency (ΔF) calculation in vastus lateralis for paired-motor unit technique. Paired motor unit data from a young male is displayed in the left panels (mean ΔF=3.09) and from an older male in the right panels (mean ΔF=1.52). Test units are displayed in panel A and corresponding control units are in B, C and D.*

5.3.4 Statistical analysis

Data management and analysis were performed using RStudio (Version 2022.07.1). Descriptive data were generated for all variables. *An independent t-test* was conducted to test whether any differences existed between young and older males in muscle cross-sectional area, torque, CoV-force, force tracking accuracy and coherence estimates. In order to preserve variability within and across participants simultaneously to the greatest extent, *multilevel linear regression models* were generated to compare mean discharge rate, discharge rate variability, peak discharge rate, delta F, recruitment threshold and derecruitment threshold between groups using *lme4 package* (Version 1.1-27.1) (Bates *et al.*, 2015). In the multilevel models, single MU was regarded as the first level; and individual participant with clustered MUs was considered as the second level. For data visualization, individual participant means are displayed in box-and-jitter plots. A p value <0.05 was considered statistically significant.

5.4 Results

Young males had a larger muscle cross-sectional area (Y v O: 30.59 ± 5.87 v 23.34 ± 7.03 cm^2, p=0.005; Figure 5.3A) and greater muscle torque (241.2 ± 62.69 v 143.3 ± 41.50 Nm, p<0.0001; Figure 5.3B) than older males. There was no difference in force steadiness (CoV-Force) (2.18 ± 0.73 v 2.27 ± 0.65, p=0.735; Figure 5.3C) during sustained contractions. However, there was a significant difference between groups in force tracking accuracy of the ramp contraction, with the younger group performing better than the old (9.41 ± 3.25 v 19.47 ± 8.36 Ns, p<0.001; Figure 5.3D).

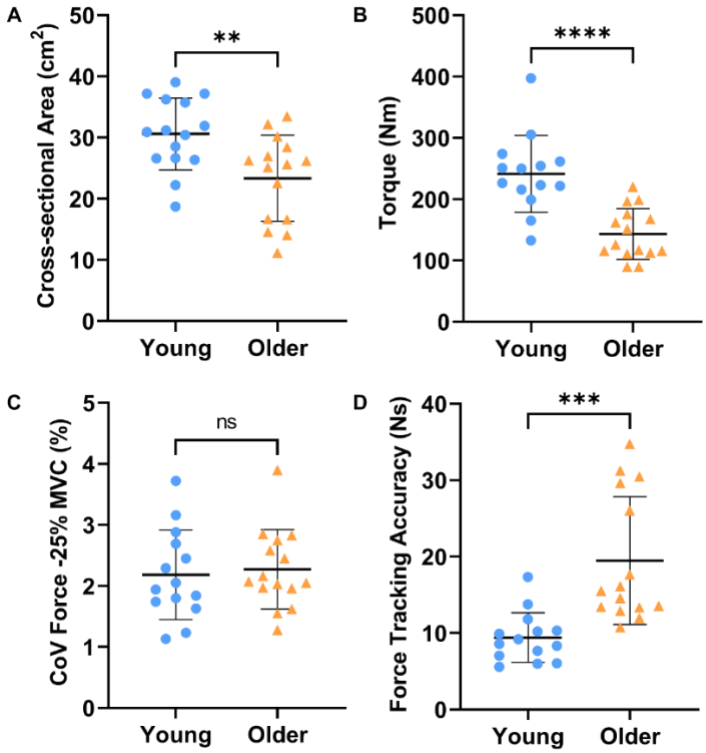

Figure 5.3. *Cross-sectional area (A), torque (B), coefficient of variance of force at 25% maximal voluntary contraction (C) and force tracking accuracy (area under curve) during a single ramp contraction at 20% of maximal voluntary contraction (D) between young (blue) and older (yellow) males. Lines indicate group means and SD. ***p<0.001, **p<0.010, *p<0.05.*

During the submaximal contraction at 25% of MVC, a total of 246 MUs were identified from young males and 282 MUs from older males. There was no statistical difference in mean discharge rates (8.50 ± 2.08 v 7.98 ± 1.64, *p*=0.137, Figure 5.4A) or discharge rate variability (11.34 ± 6.01 v 10.22 ± 5.15, *p*=0.072, Figure 5.4B) between groups.

During the ramp contractions peaking at 20% of MVC, a total of 256 MUs were identified from young males and 262 MUs from older males. There was no statistical difference in peak discharge rates (9.63 ± 2.20 v 9.35 ± 1.94 pps, *p*=0.154; Figure 5.4C). For Delta F calculations, there was an average of 5 (SD: 3.68) pairs of test and control units per person in the young and 6 (4.95) pairs in older group, with the mean number of 4 (1.79) test units per person in young and 3 (1.97) in old. Younger males had significantly greater delta F values when compared to older participants (3.11 ± 0.77 v 1.86 ±0.65 pps, *p*=0.006; Figure 5.4D). There was no significant difference in recruitment (10.60 ± 3.37 v 10.75 ± 3.55 %, *p*=0.604; Figure 5.4E) or derecruitment thresholds (10.14 ± 3.40 v 10.18 ± 3.39 %, *p*=0.866; Figure 5.4F) of the identified MUs between young and older groups.

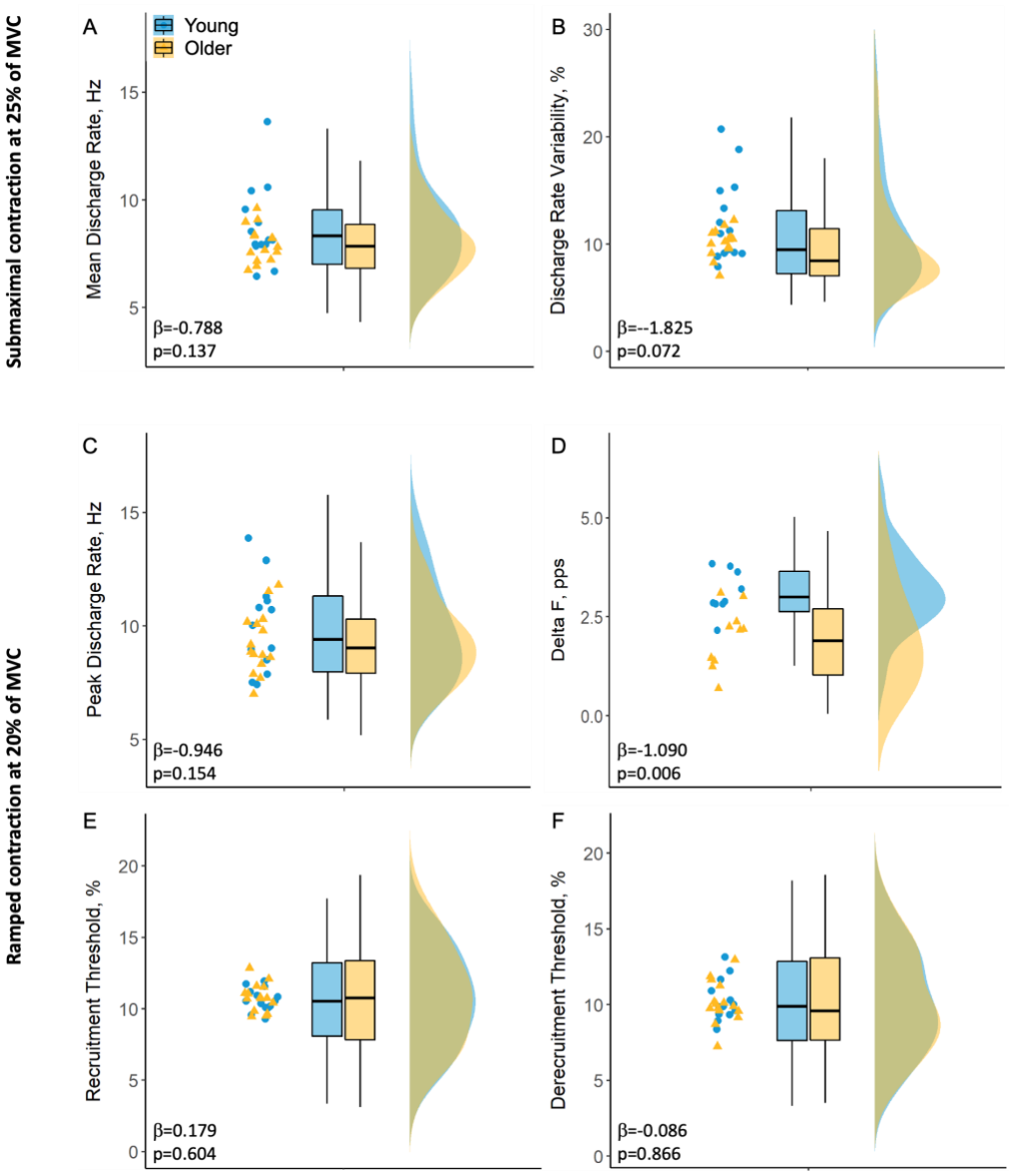

Figure 5.4. *Jitter plots of the individual participant mean values and box and raincloud plots of mean discharge rate (A), discharge rate variability (B), peak discharge rate (C), Delta F (D) and recruitment/decruitment threshold (E&F) in young (blue) and older (yellow) males.*

In the sustained contraction, there was no significant age-related difference in coherence in Delta (0.49 ± 0.14 vs 0.54 ± 0.11, *p*=0.315), Alpha (0.24 ± 0.05 vs 0.23 ± 0.03, *p*=0.462) or Beta (0.22 ± 0.03 vs 0.21 ± 0.02, *p*=0.125) bands, however the young had higher coherence in the Piper band (0.23 ± 0.03 vs 0.21 ± 0.02, *p*=0.026) (Figure 5.5A-D). In the ramped contraction, there were no age-related differences in COH Delta (0.87 ± 0.08 vs 0.92 ± 0.12, *p*=0.182), Beta (0.19 ± 0.02 vs 0.18 ± 0.02, *p*=0.110) or Piper (0.20 ± 0.04 vs 0.18 ± 0.02, *p*=0.156) bands however, young participants had greater coherence estimates in Alpha band (0.21 ± 0.02 vs 0.18 ± 0.01, *p*<0.001) (Figure 5.5E-H).

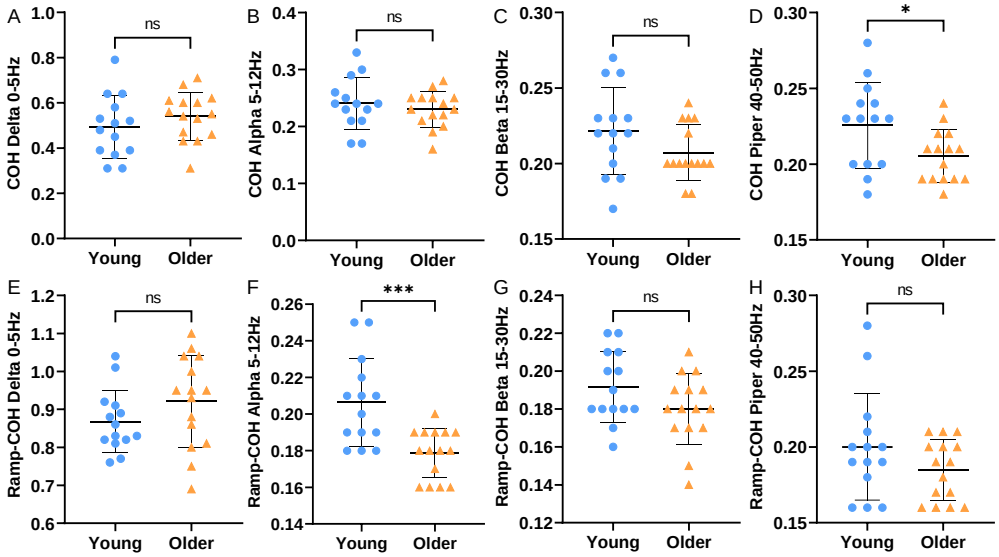

Figure 5.5. *Individual participant means of intramuscular coherence Z-score at different frequency bands during a submaximal contraction at 25% of maximal voluntary contraction (MVC) and a ramp contraction at 20% of MVC in young (blue) and older (yellow) males.*

5.5 Discussion

Here we provide the first evidence for reduced estimates of persistent inward currents combined with reduced common synaptic inputs alongside poorer force control ability in the aged human VL, a muscle with established susceptibility for age-related functional losses. Though no significant age-related difference in MU discharge rates was observed, our findings highlight important aspects of impaired neural input to older muscle that likely contribute to functional decrements.

A smaller muscle size and a lower muscle strength were observed in older participants, which is consistent with the wider ageing literature (Maden-W*ilkinson et al*>, 2013; Piasecki>*et al*>, 201>>). However, no age-related difference in MU discharge rates was detected between groups in both submaximal sustained contraction or ramp contraction, which is not aligned with our previous findings in this muscle using needle EMG recordings (Piasecki *et al.*, 2016*d*). This may reflect differences in the population of MUs sampled, with needle EMG sampling from a greater depth of muscle than surface-based HD EMG used here (Jones *et al.*, 2021).

Age-related differences in force control ability were only observed during the ramp contraction, highlighting the role of task complexity in age-related impairments (Bootsma *et al*., 2021). Numerous factors associated with advancing age may also explain this poorer force control in a more complex contraction, including MU remodelling, reduced excitability of the MUs, decreased muscle spindle sensitivity, less common synaptic inputs, and/or impaired cutaneous afferents (Mynark & Koceja, 2001; Shaffer & Harrison, 2007). Estimates of MU number in the VL are reduced in older age (Piasecki *et al*>, 201>>, 2019), and although direct human evidence is lacking, animal models show large MUs are preferentially lost with ageing and denervated fibres are reinnervated by early recruited MUs (Kadhiresan *et al.*, 1996), resulting in expansion of those earlier recruited MUs (Jones *et al.*, 2022) such as those sampled in the current study (< 20% of MVC). These MUs with a

larger number of fibres, when activated, would have a greater influence on force during their progressive re/derecruitment processes, and may partly explain the age-related differences of force control observed here.

The progressive increase and decrease in force during the targeted isometric ramped contraction used here is partly reliant on afferent neuromodulatory feedback from the site of force application (Koch *et al*., 2018). Several studies have reported a decrease of grey matter and white matter throughout the motor cortex with increasing age (Marner *et al.*, 2003; Salat *et al.*, 2004; McGinnis *et al.*, 2011) and a strong correlation between cortical atrophy and fine control capacity (Kennedy & Raz, 2005). A significant decrease in inhibition has also been observed in aged muscles, as well as a greater level of cortical and subcortical area activity during a targeted motor control task (Shinohara *et al.*, 2003; Marneweck *et al.*, 2011; Plow *et al.*, 2013), all of which may exert greater detrimental effects during a complex task than simple (e.g. ramped vs sustained). Additionally, both animal and human studies have revealed a decreased proprioception in aged muscles (Ferlinc *et al.*, 2019) with the evidence of declined muscle spindle numbers and degeneration of the sensory nerve terminals, leading to the morphological adaptations at a peripheral level and modulation of mechanoreceptor gain at a central level (Winarakwong *et al.*, 2004; Macefield & Knellwolf, 2018). Decreased inhibitory spinal circuits with ageing has also been shown to have influence on Ia afferents from muscle spindles to spinal motoneurons (Kido *et al.*, 2004).

Although not directly assessed, the points made thus far are supported by the intramuscular coherence findings during the ramp contraction. Coherence in alpha band (5-12 Hz) is associated with Ia afferent feedback (Mehrkanoon *et al*., 2014) and when exploring the age-related differences, a significant lower coherence estimate in alpha band was observed in old only during the ramp contraction, corresponding to the poorer force tracking ability. This result supports evidence from previous studies, showing a greater loss of Ia afferent feedback (Scaglioni *et al.*,

2003) and an impaired ability to modulate Ia presynaptic inhibition (Baudry *et al.*, 2010) in older participants. Moreover, it has also been suggested that age-related deterioration of cutaneous afferent inputs may also contribute to reduced force control ability (Peters *et al.*, 2016). Therefore, a decreased ability to regulate the progressive increase and decrease in force may also be a result of the combination of reduced Ia afferent feedback from muscle spindles and reduced cutaneous afferents.

The effect of ageing on MU recruitment thresholds is inconclusive, or more accurately, it appears to be muscle specific. In a single study of the biceps and triceps, MUs were recruited earlier in older triceps, but not the biceps (Hassan *et al*., 2021). This study of VL found no age-related difference in mean MU recruitment or derecruitment threshold, which was approximately 10% of MVC in both young and older participants during contractions up to 20% of MVC; indicating age was probably not the main predictor of MU recruitment/derecruitment threshold of VL.

Compared to younger participants, we report a significantly reduced delta F in the VL of older participants during a ramp contraction, supportive of studies of other muscles in the upper (Hassan *et al*., 2021) and lower (Orssatto *et al.*, 2021) limbs. The current findings in VL showed that the estimates of PICs were lower in old, which is likely attributable to the reduced activity of the monoaminergic system. Serotonin (5-HT) and norepinephrine (NE) appears primarily to be involved in the modulation of motor control and sensory input, partly influencing the magnitude of PICs (Lee & Heckman, 1998). Ageing is associated with decreased levels of 5-HT receptors and transporters in the brain (Karrer *et al.*, 2019; Deza-Araujo *et al.*, 2021) as well as impaired NE synthesis and secretion (Shibata *et al.*, 2006), leading to a reduced monoaminergic neurotransmission. Additionally, the magnitude of PICs may also be influenced by age-related alterations in Na^{+} and L-type Ca^{2+} ion channels, widely distributed on the dendrites of motoneurons. The dysregulation of Ca^{2+} has been reported to have an essential role in the development of ageing and the neurodegeneration process via C. elegans models

(Alvarez *et al.*, 2020), which may also be apparent in human spinal motoneurons. A high sensitivity of PICs to inhibitory input to motoneurons has been reported (Heckmann *et al.*, 2005) and as discussed above, inhibitory inputs significantly decrease with advancing age at the brain and spinal cord levels.

Strengths and limitations

To our knowledge, this is the first study concurrently investigating the upper motor neuron command (common synaptic inputs) and lower motor neuron excitability (persistent inward currents) in young and older VL. Age-related impairments are evident in each. However, there are several limitations. Firstly, the results reported here were from males only which may not directly translate to females, as decreases in estrogen levels may also contribute to the alterations in circulating serotonin and its receptor densities in postmenopausal women (Gonzales & Carrillo, 1993). Secondly, the MU data we report are relevant to those recruited at lower contraction levels (< 25% of MVC) and reveal little of those later recruited MUs. In addition, we used 12s and 20s signal length for calculating coherence estimates during submaximal sustained contraction and ramp contraction respectively, possibly resulting in an underestimated effect of ageing on common synaptic inputs.

5.6 Conclusion

The reduced muscle strength and control ability observed in older males is partially attributable to reduced estimates of persistent inward currents and common synaptic input, which occur independently of reductions in MU discharge rates. These findings have important implications in the field of healthy human ageing and should be considered when applying interventions to mitigate age-related functional decrements.

Chapter 6 - Circulating testosterone and dehydroepiandrosterone are associated with individual motor unit features in untrained and highly active older men

6.1 Abstract

Background

Long-term exercise training has been considered as an effective strategy to counteract age-related hormonal declines and minimise muscle atrophy. However, human data relating circulating hormone levels with motor nerve function are scant. The aims of the study were to explore associations between circulating sex hormone levels and motor unit (MU) characteristics in older men, including masters athletes competing in endurance and power events.

Methods

Forty-three older men (mean ± SD age: 69.9 ± 4.6 years) were studied based on competitive status. The serum concentrations of dehydroepiandrosterone (DHEA), total testosterone (T) and estradiol were quantified using liquid chromatography mass spectrometry. Intramuscular electromyographic signals were recorded from vastus lateralis (VL) during 25% of maximal voluntary isometric contractions and processed to extract MU firing rate (FR), and motor unit potential (MUP) features.

Results

After adjusting for athletic status, MU FR was positively associated with DHEA levels (p=0.019). Higher testosterone and estradiol were associated with lower MUP complexity; these relationships remained

significant after adjusting for athletic status (p=0.006 and p=0.019, respectively).

Conclusions

Circulating DHEA was positively associated with MU firing rate in these older men. Higher testosterone levels were associated with reduced MUP complexity, indicating reduced electrophysiological temporal dispersion, which is related to decreased differences in conduction times along axonal branches and/or MU fibres. Although evident in males only, this work highlights the potential of hormone administration as a therapeutic interventional strategy specifically targeting human motor units in older age.

6.2 Introduction

Ageing of the neuromuscular system is a complex process encompassing numerous pathophysiological conditions which is further compounded by sedentary behaviour (Harridge & Lazarus, 2017). The failure to maintain regular physical exercise while advancing in age not only induces weakness of the extremities (Whitney & Peterson, 2018) but also increases the probability of developing chronic disease (Booth *et al.*, 2012) and associated co-morbidities (Pacifico *et al.*, 2020). As such, masters athletes provide a useful model to examine the effects of inherent ageing disassociated from negative factors such as physical inactivity (Lazarus & Harridge, 2007). Although some aspects of muscle strength may be maintained in masters athletes when compared to age-matched controls (Mckendry *et al.*, 2018), this finding is equivocal (Piasecki *et al.*, 2021*a*) and progressive muscle atrophy demonstrates that lifelong exercise does not completely offset the muscle mass and strength decline caused by ageing (Korhonen *et al.*, 2006). A range of additional factors are involved, such as circulating sex hormones, which is further mediated by levels and/or types of physical activity (Proctor *et al.*, 1998). Collectively, these factors also influence neural adaptations with age.

The final element of the peripheral motor nervous system related to muscle contraction is the motor unit (MU), consisting of an efferent motor neuron and the unique set of muscle fibres it innervates (Liddell & Sherrington, 1997). MUs undergo adaptive responses to external stimuli, most notably a decreased number with advancing age, leaving some fibres denervated (Piasecki *et al.*, 2016*b*, 2016*c*). However, the surviving MUs have the ability to rescue recently denervated adjacent muscle fibres via collateral axonal sprouting and formation of new neuromuscular junctions (NMJ) (Hepple & Rice, 2016). By recording the electrical activity of muscles with intramuscular electrodes during voluntary contractions, a number of parameters relating to the structure and function of MUs can be investigated, including estimates of size,

number and synchronicity of individual fibre activation (Piasecki *et al.*, 2021*b*).

Evidence for the preservation of MU number in human lifelong exercisers is ambiguous, with a study evidencing for (Power *et al.*, 2010) and others against (Power *et al.*, 2012; Piasecki *et al.*, 2016*a*, 2019) this notion. However, there is further evidence indicating a higher level of reinnervation ability in highly active older people. Compared with non-trained age-matched individuals, masters athletes exhibited larger motor unit potentials (MUPs), fewer denervated muscle fibres, and increased fibre type grouping (Power *et al.*, 2010, 2016; McPhee *et al.*, 2018; Sonjak *et al.*, 2019). Thus, there are established benefits of exercise for the ageing neuromuscular system, yet the interactions with circulating sex hormones in these highly active older individuals are unclear.

Testosterone (T) is the primary androgenic hormone and a precursor to estrogen synthesis (Wood & Stanton, 2012), and has an anabolic impact on skeletal muscle (Bhasin *et al.*, 2010). Dehydroepiandrosterone (DHEA), the precursors of T and its 3-sulfooxy derivative (DHEAS), as well as the dihydrotestosterone (DHT) synthesised from T, have been reported to progressively decrease with ageing in men (Orentreich *et al.*, 1984; Nafziger *et al.*, 1998; Villareal & Holloszy, 2006; Yeap *et al.*, 2007). Estrogens are primarily produced by the ovaries in women but can also be synthesised in men through aromatization of T to estradiol (E2) in brain and adipose tissue (Cooke *et al.*, 2017), which also contributes to maintenance of muscle via estradiol receptors (Dieli-Conwright *et al.*, 2009; Enns & Tiidus, 2010).

To counteract age-related hormonal declines, exercise training (Kraemer *et al.*, 1991; Ari *et al.*, 2004; Hawkins *et al.*, 2008; Phillips *et al.*, 2017) and exogenous hormone administration have been employed in several studies as an interventional strategy in older men and women (Urban *et al.*, 1995; Morales *et al.*, 1998; Gharahdaghi *et al.*, 2019). Resistance exercise training acutely elevates T concentrations (Gharahdaghi *et al.*, 2019), and a recent study combining middle-aged (mean age 51 years)

endurance and power athletes found them to have higher T than age-matched inactive subjects (Barbosa *et al.*, 2021), suggesting athletic status directly influenced hormone levels in this age group. However, young individuals who regularly undergo endurance training have been reported to have a lower level of sex hormones compared to age-matched sedentary controls (Wheeler *et al.*, 1984; Hooper *et al.*, 2018). *In vitro* models demonstrate that T treatment plays a neuroprotective role against the deprivation-mediated apoptosis of human neurons (Hammond *et al.*, 2001), and animal studies have shown that the atrophy of motoneuron dendrites could be attenuated or even reversed by T administration (Kurz *et al.*, 1986, 1991; Byers *et al.*, 2012). Both pre- and post-synaptic elements of the NMJ have been improved after T administration, independent of muscle fibre atrophy/hypertrophy, suggesting that it may largely contribute to the enhancement of NMJ transmission stability (Blanco *et al.*, 2001; Sieck & Mantilla, 2004). Additionally, exogenous E2 administration has been reported to increase axonal regeneration (Tanzer & Jones, 1997; Sharma *et al.*, 2009; Acosta *et al.*, 2017).

We have previously highlighted the potential role of androgens in peripheral neuroplasticity via associations between circulating sex hormones and electrophysiological markers of MU function in frail elderly men (Swiecicka *et al.*, 2020), and separately, the effects of long-term athletic training on MU remodelling, specifically, the improved capacity to reinnervate denervated fibres in older age (Piasecki *et al.*, 2019). There is, however, limited data describing the influence of athletic status on relationship between hormones and MU function. The aims of the present study were therefore to investigate the effects of different lifelong exercise on circulating sex hormone levels and neuromuscular properties, and to explore whether athletic status influences the associations between circulating sex hormone levels and MU characteristics of the vastus lateralis (VL) muscle in older males. We hypothesised that higher concentrations of circulating sex hormones

would be observed in masters athletes and the athletic status would influence the associations between hormones and MU properties.

6.3 Methods

6.3.1 Participants

This study was approved by Manchester Metropolitan University Research Ethics Committee and the National Research Ethics Service Committee Northwest (15/NW/4026) in accordance with the Declaration of Helsinki.

A total of 43 males aged between 60-85 years were recruited from the Greater Manchester area between 2014 and 2017. This included 18 untrained controls (CON), 14 endurance masters athletes (EMA) and 11 power masters athletes (PMA). The controls, defined as recreationally active, did not take part in any form of regular and/or intensive exercise training and were recruited from the local communities. The athletes were recruited from running clubs and national masters athletic competitions, as well as through an advertisement in a national athletics magazine. At the time of testing, all masters athletes were regularly competing within their discipline and were completing more than 5 hours of specified training per week. Power athletes were defined as those that were competing and training in running events less than 800 m along with throw and jump events. Endurance athletes were defined as those competing in running events greater than or equal to 800 m in distance. Mean age-graded performance (AGP) was determined by taking the athlete's highest ranked performance within the last 2 years and expressing it as a percentage of the world record for that age and distance. The AGP was 79 ± 10% for EMA and 85 ± 10% for PMA, indicating a high level of performance relative to respective age group records. All masters athletes had been training and competing specifically within their discipline since adulthood (>18 years), and the median training years for all masters athletes was 49.8 years. All participants provided written informed consent. Prior to enrolment, all participants completed a comprehensive clinical examination and metabolic screening. All recruited participants would be excluded if they display evidence of: BMI < 18.5 or > 35kg/m^2; musculoskeletal disorders;

respiratory disease; neurological disorders; metabolic disease; active cardiovascular problems; active inflammatory bowel or renal disease; recent steroid treatment within 6 months or hormone replacement therapy; family history of early (< 55years) death from cardiovascular disease.

6.3.2 Anthropometry

Total body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, version EnCore 10.50.086; GE Healthcare, Little Chalfont, United Kingdom) with arms and legs fully extended in the supine position. The cross-sectional area (CSA) of the quadriceps muscles was obtained using magnetic resonance imaging (MRI) at the muscle motor point, around mid-muscle belly. A T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoa, Italy) with participants lying supine was used. 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 quadriceps muscles following the contour of the aponeurosis. The highest CSA was recorded as peak quadriceps CSA (PQCSA) (Maden-Wilkinso*n et al*., 2014).

6.3.3 Physical function

The measurement of maximal isometric voluntary contraction (MVC) of knee extensors has been described in Chapter 2, Section 2.3.3.

Hand grip strength was measured using a handheld dynamometer (Jamar, Sammons Preston Inc., Bolingbrook, IL, US). After adjusting the width of the dynamometer for each participant, participants were instructed to squeeze against the handle as hard as possible for approximately three seconds. This process was repeated twice for each hand with 30 seconds rest intervals between each. The maximum contraction force was recorded in kilograms to the nearest 0.1 kg.

A Leonardo Jump Mechanography Platform (Leonardo Software version 4.2: Novotiec Medical GmbH, Pforzheim, Germany) was used to assess lower limb power from a countermovement vertical jump (Hannam *et al.*, 2017). Participants were instructed to flex the knee joint with feet approximately 30cm apart (slightly narrower than shoulder width) and to jump as high and forcefully as possible with hands placed on the waist. Each participant repeated the jump sequence three times with approximately 30 seconds rest in between each; the highest value for relative jump power (W/kg) was recorded for further analysis.

A “Timed Up and Go (TUG)” test required participants to stand from a seated position, walk a distance of three metres (10 feet), turn around a cone, return to the chair and sit down again as quickly as possible. Time started with the command “GO” and stopped when the participants returned to their original seated position.

6.3.4 Hormone quantification

Following an overnight fast, a 10ml venous blood sample was collected from each participant at ~0900h. Samples were immediately centrifuged at 3200 rpm, for 20 min at 4 ◦C, carefully aliquoted and frozen at -80◦C for future analysis. The serum concentrations of dehydroepiandrosterone (DHEA), DHEA sulphate (DHEAS), total testosterone (T), dihydrotestosterone (DHT), and total estradiol (E2) were obtained and analysed using a liquid chromatography mass spectrometry high resolution system.

6.3.5 Intramuscular electromyography (iEMG)

iEMG technique has been described in Chapter 2, section 2.3.6. Here, the iEMG data were collected during a sustained voluntary isometric contraction lasting 12-15 seconds at 25 % MVC only. To avoid repeat sampling of the same MUs, after each contraction, the needle electrode was repositioned by rotating 180 degrees and withdrawing by approximately 10-25mm mm to obtain a minimum of 6 recordings from spatially distinct areas (from deep to superficial portions) (Jones *et al.*,

2021). The iEMG signal analysis has also been described in Chapter 2, section 2.3.7. Here we report MU firing rate (FR), MU action potential (MUP) duration, and MU complexity (number of turns) (Figure 6.1) (Piasecki *et al.*, 2016*a*).

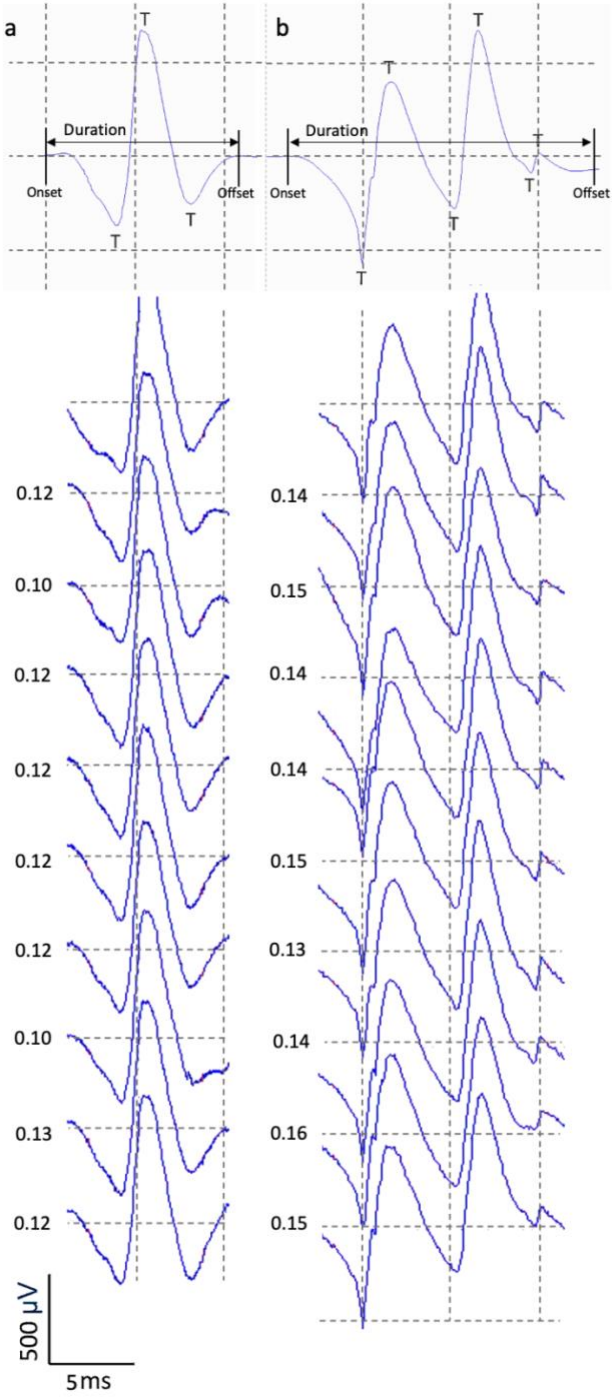

Figure 6.1. *Example MUP templates (top) and 10 consecutive observations of the same MUP (bottom, raster plot) used to determine MUP duration, complexity (number of turns(T)) and firing rate. Inter discharge intervals (IDIs, seconds) are shown to left of each MUP in the raster plot, corresponding to a firing rate of approximately 10.1 Hz (a) and 8.8 Hz (b).*

6.3.6 Statistical analysis

Descriptive statistics in men by athletic status are presented as *mean ± standard deviation (SD)*. Between-group differences in functional measures and circulating hormones were assessed using *one-way ANOVA* followed by *Tukey's post hoc* analysis. As multiple MUs were recorded from each participant, *multi-level linear regression models* were used to investigate the associations between hormones and MU properties, with each individual being regarded as an independent cluster and athletics status as a covariate. These results are displayed as coefficients estimate (beta and 95% confidence intervals) *and p*-values. Significance was assumed when $p<0.05$. All statistical analyses were performed using STATA-version 16 SE software (StataCorp, College Station, Texas) and the figures 6.2-4 were created in RStudio version 4.0.2.

6.4 Results

Forty-three men were included in the analyses, consisting of 18 elderly controls (mean ± SD age: 70.7 ± 3.7 years), 14 masters endurance athletes (68.6 ± 3.6), and 11 masters power athletes (70.5 ± 6.8) (Table 6.1).

Table 6.1. Participant characteristics by athletic status

	Control (CON)	Masters Endurance Athletes (EMA)	Masters Power Athletes (PMA)
No.	18	14	11
Age, y	70.7 ± 3.7	68.6 ± 3.6	70.5 ± 6.8
Physical properties			
Lean Mass, kg	54.95 ± 5.3	54.48 ± 5.9	58.48 ± 4.0
Fat Mass, kg	**16.87 ± 4.7[b]**	7.92 ± 3.3	**13.80 ± 5.3[b]**
PQCSA, cm2	62.2 ± 7.4	64.8 ± 11.0	**75.8 ± 12.4[ab]**
Grip Strength, N	43.2 ± 6.3	41.0 ± 4.9	44.3 ± 5.2
Jump Power, W/kg	2.47 ± 0.37	2.29 ± 0.31	**2.79 ± 0.56[b]**
TUG, s	5.91 ± 0.43	**5.41 ± 0.42[a]**	**5.36 ± 0.66[a]**
MUP features			
Complexity (No. of Turns)	4.23 ± 0.81	4.51 ± 0.93	4.25 ± 1.1
Duration, ms	16.3 ± 1.87	16.15 ± 3.01	15.72 ± 2.07
Firing Rate, Hz	**9.11 ± 1.19[b]**	8.30 ± 1.01	**9.61 ± 1.69[b]**

Data are mean ± standard deviation. . The values in bold in the tables reflect statistically significant (p<.05) differences between groups. [a]Significant difference to CON; [b]Significant difference to EMA. All MUP features were recorded at 25% of maximal isometric voluntary contractions (MVC). Abbreviations: PQCSA, peak quadriceps cross-sectional area; TUG, Timed Up and Go; MUP, motor unit potential.

Power masters athletes had greater muscle size than endurance and controls (both p<0.05). There was no difference in lean mass or grip strength between groups. Power athletes exhibited greater jump power than endurance (p=0.014), with no difference compared to controls

(p=0.134). Both endurance and power masters athletes had better TUG performance (p<0.05) than their age-matched controls. Endurance athletes had lower fat mass and MU FR compared to controls and power athletes (p<0.001). There were no significant differences in MUP duration or complexity between the groups (Table 6.1).

Lower levels of E2 were observed in endurance masters athletes when compared to controls (p=0.016) and power athletes (p=0.036). There were no differences in serum concentrations of DHEAS, DHEA, T, or DHT between the three groups (Figure 6.2).

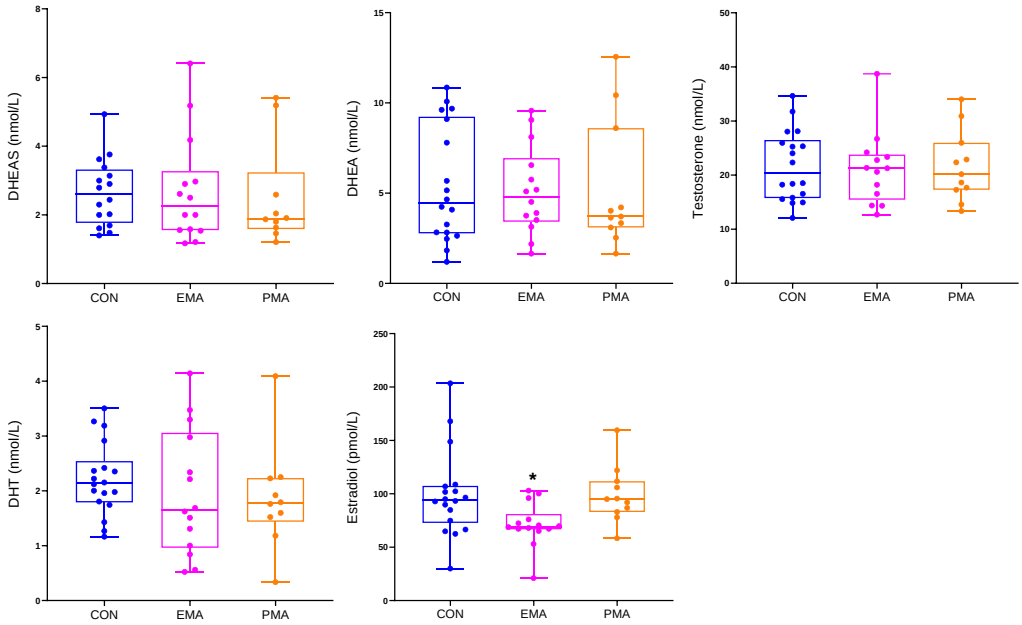

Figure 6.2. *Circulating sex hormone levels among controls (CON), endurance masters athletes (EMA) and power masters athletes (PMA). *=p<0.05. vs. CON and PMA.*

After adjusting for athletic status, for every unit increase in DHEAS, PQCSA increased by 4.07 cm^2 (95% CI=1.93 to 6.20, p<0.001) (Figure 6.3c). Similarly, for every unit increased in E2, jump power increased by 0.005 W/kg on average (95% CI=0.001 to 0.01, p=0.020) (Figure 6.3d). Moreover, E2 was positively related to fat mass (β=0.06, 95% CI=0.01 to 0.11, p=0.019) (Figure 6.3b), becoming non-significant in adjusted models (p=0.346). There were no significant relationships between any circulating sex hormones and lean mass (Figure 6.3a), grip strength (Figure 6.3e) or TUG (Figure 6.3f) after adjustment for athletic status.

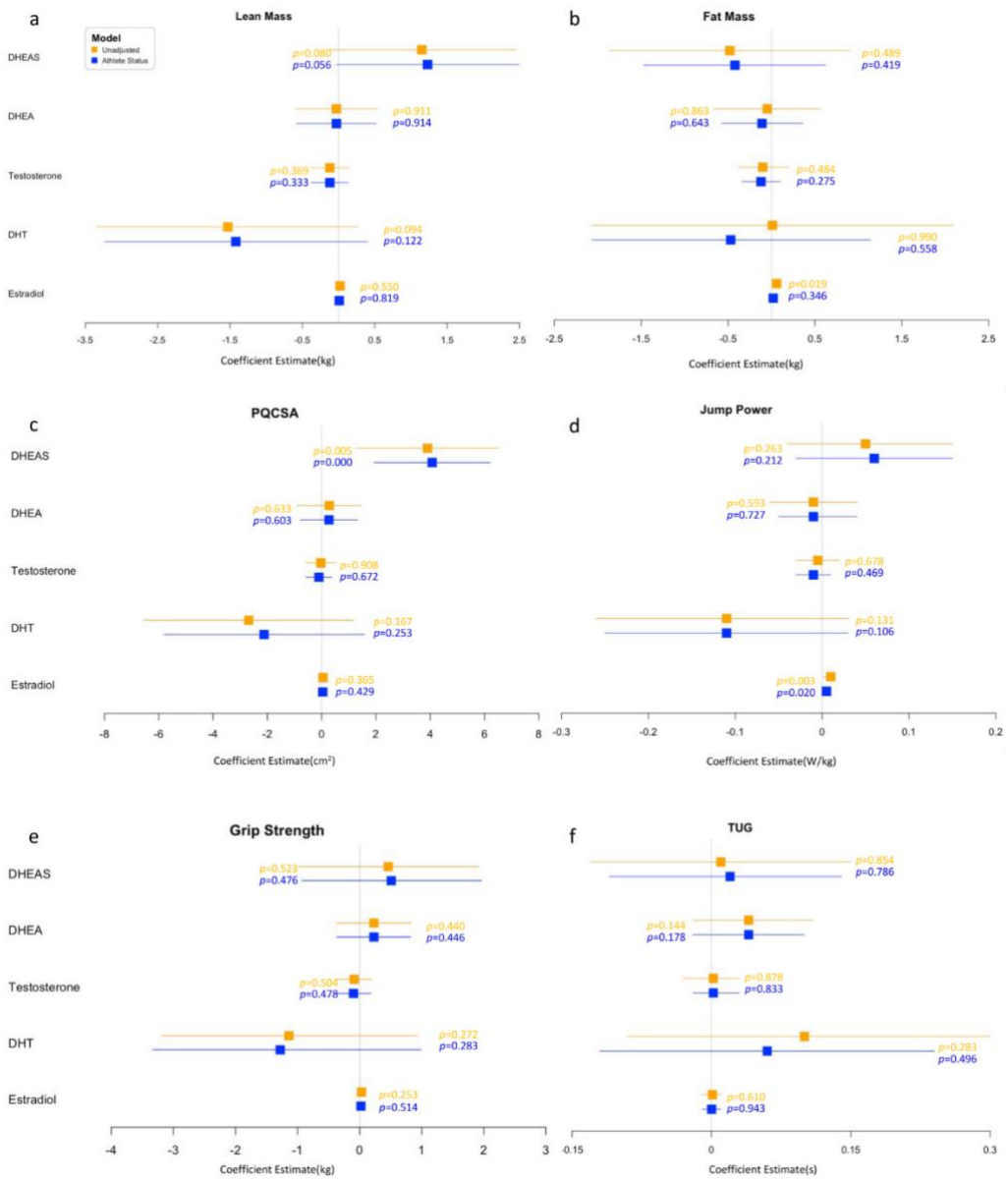

Figure 6.3. *Forest plot for regression coefficient estimate (beta and 95% confidence interval) for unadjusted (orange) and adjusted (+athletic status, blue) associations between hormone levels and physical function parameters in trained and untrained older adults. Beta represents the difference in outcome for 1-unit change in predictor (endocrine parameters). Abbreviations: PQCSA, peak quadriceps cross-sectional area; TUG, Timed Up and Go; DHEAS, dehydroepiandrosterone sulphate; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.*

In both unadjusted and adjusted (for athletic status) analysis, DHEA showed a positive association with MU FR (β=0.15, 95% CI=0.02 to 0.27, p=0.019) (Figure 6.4c), and negative associations with MUP duration (β=-0.24, 95% CI=-0.46 to -0.01, p=0.040) (Figure 6.4b). Both T (β=-0.05, 95% CI=-0.09 to -0.002, p= 0.006) and E2 (β=-0.01, 95% CI=-0.02 to -0.002, p= 0.019) were negatively associated with MUP complexity (Figure 6.4a). No significant relationships were observed between DHT and any MUP features.

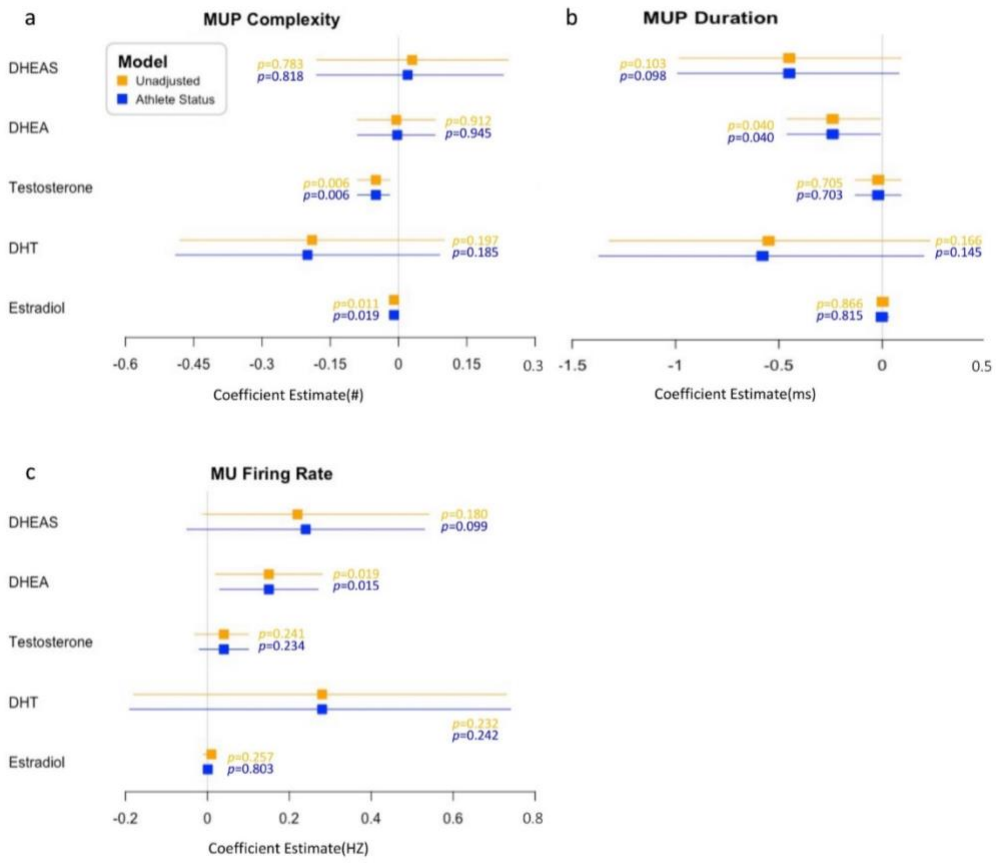

Figure 6.4. *Forest plot for regression coefficient estimate (beta and 95% confidence interval) for unadjusted (orange) and adjusted (+athletic status, blue) associations between hormone levels and motor unit (MU) features in trained and untrained older adults. Beta represents the difference in outcome for 1-unit change in predictor (endocrine parameters). Abbreviations: MUP, motor unit potential; MUP Complexity is defined as the number of turns; DHEAS, dehydroepiandrosterone sulphate; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone.*

6.5 Discussion

To our knowledge, this is the first study using combinations of MRI, DXA, intramuscular EMG and mass spectrometry techniques to explore associations between circulating sex hormone levels and MU characteristics in elite masters athletes. Although there was no difference in androgen concentrations across our groups, we show that power masters athletes generally had more favourable physical characteristics. We demonstrate that DHEA has a positive association with MU FR in elderly men. Additionally, the identification of an association between T levels and reduced MUP complexity suggests decreased electrophysiological temporal dispersion (increased activation synchronicity of MU fibres) in those with higher T levels. We also demonstrate that estrogen levels are positively associated with muscle power in both untrained and highly active older men.

Both longitudinal and cross-sectional studies have reported a downregulation of DHEA and its sulphate with ageing (Orentreich *et al.*, 1984; Villareal & Holloszy, 2006; Yeap *et al.*, 2007; Sato *et al.*, 2014), which has been suggested to be an independent predictor of muscle strength, muscle mass or muscle quality in elderly men and women (Valenti *et al.*, 2004; Pöllänen *et al.*, 2011). Chronic resistance exercise training has the benefit of elevating plasma and/or muscle levels of DHEA and T, and concurrently induces muscle size in older men (Kraemer *et al.*, 1991; Sato *et al.*, 2014). However, although a twelve-week resistance exercise training regime appeared to attenuate age-related hormone reductions, there was no significant correlation between hormone levels and muscle strength or muscle mass (Sato *et al.*, 2014). Somewhat contrary to this, our study did show a positive association between DHEAS and quadriceps muscle size in old controls and elite athletes, which was independent of athletic specialism. Although observational, our findings further support previously reported associations between androgenic hormones and muscle size in older males (Valenti *et al.*, 2004).

In addition to its positive effects on cognition (Maggio *et al.*, 2015), notable evidence to date demonstrates that DHEA acts as a neurosteroid, regulating the motility and/or growth of neocortical neurons in the central nervous system (Baulieu, 1997). DHEA is also known to influence neuronal excitability via the modulation of neurotransmitter receptors, such as N-methyl-D-aspartate (NMDA), gamma-aminobutyric acid type A ($GABA_A$), and sigma receptors (Holsboer *et al.*, 1994; Compagnone & Mellon, 1998). Additionally, DHEA also contributes to neurogenesis and neuroprotection by mediating brain-derived neurotrophic factor (BDNF) (Pluchino *et al.*, 2013; Sakr *et al.*, 2014), which further regulates axonal regeneration, neuromuscular connections and ultimately, muscle force production. Increases in generation of force relies, in part, on MU FR, which responds differently to ageing and exercise training (Duchateau & Baudry, 2014; Del Vecchio *et al*>, 201>>). Several studies have reported an apparent age-related decrease in MU FR, negatively influencing force production (Connelly *et al.*, 1999; Klass *et al.*, 2008; Watanabe *et al.*, 2016; Piasecki *et al.*, 2016*c*, 2016*a*), and MU FR can be altered in response to exercise in young (Vila-Chã *et al.*, 2010; Martinez-Valdes *et al.*, 2017) and older (Kamen & Knight, 2004) people. The relationships between DHEA and MU FR with muscle strength have been established separately in humans (Valenti *et al.*, 2004; Kamen & Knight, 2004), and the positive associations between DHEA and MU FR during a submaximal contraction shown here highlight DHEA as a potential therapeutic intervention to increase MU FR, known to decrease with age and a probable factor in limiting neuromuscular function (Piasecki *et al.*, 2016*c*).

The number of MUP turns reflects the level of complexity of the MUP; greater turns indicate greater electrophysiological temporal dispersion. Notably, higher DHEA levels were associated with shorter MUP durations, also a measure of temporal dispersion. The negative associations between androgens and MUP temporal dispersion may be explained by greater MU fibre activation synchronicity or smaller conduction time differences along axonal branches and/or MU fibres,

which is partly attributable to fibre conduction velocity (Stålberg *et al.*, 1996; Rodríguez-Carreño *et al.*, 2012; Soares *et al.*, 2015). Animal studies have demonstrated that androgens positively influence neural plasticity and axonal regeneration following nerve injury (Amy Yu, 1982; Yu & Yu, 1983; Tetzlaff *et al.*, 2006), and the potential ability of androgens to accelerate MU remodelling relies on the existence of androgen receptors (AR) (Gharahdaghi *et al.*, 2020), and androgen/AR signalling may improve neural transmission, motoneuron soma and dendrite size, and nerve regeneration (Brooks *et al.*, 1998). Importantly, ARs are expressed in both motoneurons and muscle fibres, and may influence the release of synaptic vesicles and neurotransmitters at pre- and post-synaptic elements of NMJs directly or indirectly (Sieck & Mantilla, 2004). Androgen administration in animal models significantly expanded the pre- and post-synaptic elements of NMJs in fast twitch fibres, resulting in the improvement of neuromuscular transmission (Blanco *et al.*, 2001). Although we did not directly quantify parameters related to androgen/AR signalling in these older males, long-term physically trained athletes exhibited similar levels of circulating androgens to untrained controls.

Circulating levels of E2 are primarily dependent on testosterone in males, converted via aromatisation partly occurring in adipose tissue. Although there were no differences in T concentrations, levels of E2 were lower in the endurance group when compared to controls and power athletes, which suggests an altered T:E2 ratio in the endurance athletes. Furthermore, the significant association between E2 and fat mass was not apparent when adjusting for athletic status, indicating the form of training influenced this relationship and this may be attributable to the lower fat mass in endurance athletes (Vermeulen *et al.*, 2002; Gates *et al.*, 2013) and potentially, their lower levels of aromatase activity (Greising *et al.*, 2009; Finkelstein S *et al.*, 2013).

Previous studies of older females reported a greater improvement in muscle strength and power in those receiving estrogen hormone therapy

(Moran *et al.*, 2007; Ronkainen *et al.*, 2009), and here we report a similar association in older men. Moreover, these associations remained significant in follow-up analyses when adjusting for T, the precursor of E2, indicating total T concentrations do not influence this association. Mechanistic insight from animal models highlights marked improvements in myosin binding with estrogen hormone therapy (Horstman *et al.*, 2012), which may also extend to humans. For example, when estrogens were diminished, significant decrements in force generation were observed, and restored by hormone replacement (Mann *et al.*, 2020). Although predominantly associated with female neuromuscular health, E2 has several functions via both alpha and beta E2 receptors. Activation of both ERs promote a beneficial effect on bone health as well as playing an important role in regulating metabolic pathways and adipose tissue functions (Horstman *et al.*, 2012). Moreover, ER-beta knockout mice models highlighted the importance of this receptor in the regulation of skeletal muscle growth and regeneration (Velders *et al.*, 2012). We have previously reported that both masters power and endurance athletes exhibited larger MUP size compared to age-matched controls, indicating a greater level of MU expansion (Piasecki *et al.*, 2019), with no difference between endurance and power athletes, and that exercise has a range of established benefits on neuromuscular health. Other than E2, the current data shows long-term exercise training has minimal effects on circulating hormone levels in this age-group. Taken together, these data suggest aspects of MU remodelling occurring in response to lifelong exercise do so independently of changes in circulating hormones.

Strengths and limitations

This is the first study to investigate the relationship between hormone levels and MU characteristics in elite endurance and power masters athletes who were current competitors within their respective disciplines. As there were multiple MUs sampled during each muscle contraction, we used a multi-level mixed-effect linear regression model, allowing MU parameters to be clustered to an individual and overcome within-

individual variability. However, the sample size is limited in this rare elite athlete cohort. It should be noted that only males were recruited into our study, and there is a lack of convincing evidence to explain the underlying mechanism of hormones on MU characteristics in females. Our study cannot provide evidence for causality between circulating sex hormone levels and neuromuscular characteristics.

6.6 Conclusions

This study highlights the associations between circulating sex hormones and MU properties in older men. DHEA was positively associated with MU FR in these older men, a key component of muscle force generating capacity. Higher T levels were associated with reduced MUP complexity, indicating reduced electrophysiological temporal dispersion, which is related to the reduced differences in conduction times along axonal branches and/or MU fibres. Although evident in males only, this work highlights the potential of hormone administration as a therapeutic interventional strategy specifically targeting the human neuromuscular system in older age.

Chapter 7 - General discussion

7.1 Overview of aims

This thesis has explored the influence of age, sex, and lifelong exercise on MU properties. It has successfully identified key similarities and differences between males and females that are augmented in older age, and provided a basis for the increased inclusivity of females in studies of this nature. In older males, the bilateral difference across lower limbs appears to be minimal, and this is preserved in older age. The methods applied here have characterised multiple points in the motor pathway that are impaired in older age, and this contributes to reduced neuromuscular function. Finally, the association of circulating sex hormones and motor function in older athletes offers the opportunity to further explore exogenous hormones as a therapeutic intervention specifically targeting motoneurons in humans.

Females are underrepresented in studies of human physiology, which is often intended to simplify experimental protocols by eliminating established sex-based differences. Females generally exhibit an extended lifespan when compared to males; however, this longevity advantage is not accompanied by an enhanced healthspan, as females typically spend more time in poorer health in older age. Although sex hormones have been proposed as an obvious contributor to the biological sex-based differences of the neuromuscular system, there are limited data on their influence on individual MU properties between sexes and across the lifespan. The neural inputs to skeletal muscles are considered as key determinants of neuromuscular function, more specifically, MU firing properties in older age. There is, however, limited scientific evidence regarding the adaptations in neuromuscular function associated with aging, particularly in the functionally relevant quadriceps muscle group. Therefore, the underlying hierarchical reasons and mechanisms contributing to these observed alterations in MU firing

characteristics have yet to be fully explored. With these aspects in mind, this thesis set out to achieve the following aims:

1) To compare individual MU properties and neuromuscular recruitment strategies, as well as MU number estimates in the vastus lateralis of healthy young males and females.
2) To explore the influences of sex and ageing on physical performance and neuromuscular characteristics in vastus lateralis of early to late elderly males and females.
3) To investigate bilateral differences in the vastus lateralis with respect to the muscle function and individual MU characteristics in addition to common synaptic inputs in both young and older males and to explore the influence of advancing age on bilateral differences within older individuals.
4) To explore the differences in physical performance as well as the magnitude of persistent inward currents and common synaptic inputs in the vastus lateralis between healthy young and older males.
5) To investigate the effects of different lifelong exercise on circulating sex hormone levels and neuromuscular properties, and to explore whether athletic status influences the associations between circulating sex hormone levels and MU characteristics of the vastus lateralis muscle in older males.

7.2 Summary of key findings

There were a number of key findings throughout the thesis, and these will be summarised in order of chapter.

As Chapter 2 highlights, males exhibited greater muscle strength and size than females, yet at normalised contraction intensities females had smaller MUs and higher firing rates. However, both sexes showed similar increases in MU size and firing rate when moving from low- to mid-level contractions. The results also highlight similar estimates of the number of MUs in vastus lateralis and are the first to do so in females. Additionally, this chapter also provides normative values for a range of electrophysiological measures in healthy young males and females to be used as a future reference point. Collectively, these data highlight that females generate muscle force via different neuromuscular recruitment strategies to males, yet display similar strategies from low- to mid-level contraction levels.

To further investigate the sex differences with ageing and how increasing age would influence the sex differences in MU properties, here in Chapter 3, we used the same technique, intramuscular electromyography recorded at low- and mid-level contractions to explore the individual MU properties between sexes in early to later elderly.

We show a progressive decrease in numerous functional tasks from early to late elderly in both sexes. Similar to the sex differences reported in Chapter 2, older females had smaller muscle size and strength, and these sex disparities are augmented in older age. Similar neuromuscular recruitment strategies from low- to mid-level contractions between sexes were maintained with age, with both sexes showing higher MU firing rate and greater MU potential size. However, older females also exhibited poorer force steadiness than older males, and interestingly this was also accompanied by a greater MU firing rate variability in older females. These findings highlight distinguishable sex disparities in physical performance and MU properties and suggest the early interventions are

needed for females to prevent the functional deterioration to reduce the health-sex paradox within ageing. By shedding light on these sex-specific differences in neuromuscular characteristics, our study contributes to the limited body of knowledge concerning the aging female population.

The above findings were based on the studies investigating unilateral MU properties of vastus lateralis, while ignoring potential differences between dominant and non-dominant limbs, which is particularly crucial for the motor control. Therefore, Chapter 4 was designed to explore the bilateral differences in vastus lateralis muscle function. Here we applied HDsEMG to concurrently explore the upper motor neuron command and individual MU properties in young and older vastus lateralis. The primary findings showed no bilateral differences in muscle size, strength, force steadiness, MU firing rates or common synaptic inputs in both young and older healthy males. However, both young and older adults displayed lower MU firing rate variability in their dominant legs, and this bilateral difference was apparent in the older group from early to late elderly, accompanied by increased bilateral difference in force steadiness. The age-related reduction in muscle strength may be attributable to the decreased MU firing rates in older adults. These data contribute comprehensive investigation of bilateral difference in upper and lower neuronal properties in both young and old adults and reveal that perceived limb dominance does not exert a substantial influence on lower limb neuromuscular function, providing valuable insights into the development of assessment and targeted interventions, especially the benefits of unilateral exercise training in elderly.

As previous chapters highlight, distinguishable differences in muscle strength and control ability may be largely associated with neural inputs to motoneuron pool. Therefore, in Chapter 5 we applied both submaximal sustained contractions and ramp contractions to further explore the underlying mechanisms contributing to these alterations in MU firing patterns. Age-related decline in force control ability was only observed in

ramp contractions, highlighting the role of task complexity in age-related impairments. This chapter also highlights that the reduced muscle strength and control ability observed in older males is partially attributable to reduced estimates of persistent inward currents and common synaptic inputs, which occur independently of reductions in MU firing rates. These findings have important implications in the field of healthy human ageing and should be considered when applying interventions to mitigate age-related functional decrements.

The decline in neuromuscular function occurred with age is multifactorial, one of which is the decrease in physical activity. To better examine the effects of inherent ageing, Chapter 6 involved the study of masters athletes, often used as a model of ageing that is dissociated from progressive disuse, to explore associations between individual MU features and circulating sex hormones. The results highlight that the testosterone derivative, dehydroepiandrosterone (DHEA), has a positive association with MU firing rates in all elderly males. Additionally, the identification of an association between testosterone levels and reduced MU potential complexity suggests decreased electrophysiological temporal dispersion in those with higher testosterone levels, an important factor in neuromuscular performance. Collectively, these data highlight the role of hormones in MU structure and function in older age and support the use of pharmaceutical intervention in future research.

7.3 Concluding remarks and future work

This thesis has achieved its aim of exploring central and peripheral neural properties according to sex and across age. Through both non-invasive high-density surface electromyography and invasive intramuscular electrography, the determinants of motoneuron excitability and individual MU properties have been studied specifically. The key summaries of each study are presented above, however, the overall conclusion is that though females consistently showed poorer neuromuscular function compared to males and used different recruitment strategies to achieve a specific targeted force level, it is evident that both males and females employed similar strategies when moving from low- to mid-level contractions and exhibited similar functional deterioration with age, supporting the use of mixed sex cohorts in neuromuscular physiology in the future and suggesting early interventions could be particularly beneficial for older females. Furthermore, this body of work has established that the decline in physical performance and MU characteristics with age was influenced by alterations in both common and independent synaptic inputs, accompanied by decreased persistent inward currents. The observed associations between sex hormones and MU properties also highlight the potential of hormone administration as a therapeutic intervention strategy for targeting the human neuromuscular system in older age. Overall, these findings contribute to our understanding of age-related adaptations in neuromuscular function and offer valuable implications for future research and clinical interventions.

However, there are a number of fundamental questions that remain unresolved and should be the focus of future work. Firstly, we only determined the common synaptic inputs to the motoneuron pool, however it would be of interest to explore the neural drive to skeletal muscles which is generated by the transformation of the synaptic input to the motoneurons into output spike trains, which is essential for estimating the strength of common synaptic input to populations of

motoneurons. Secondly, we did not measure the circulating levels of sex hormones in all populations and did not determine the effects of sex hormones on motoneuron excitability between sexes. This is particularly important given the potential excitatory and inhibitory effects of estrogen and progesterone. Thirdly, hormonal changes that occur with age can negatively impact the neuromuscular function, leading to the functional decline and increased susceptibility to age-related disorders. Older individuals recruited here did not receive any hormone replacement therapy, thus it would be of interest to investigate the neuromuscular function in older individuals with hormone replacement therapy and further understand how these hormonal administration influences individual MU properties and functional outcomes. In addition, as our findings highlight, leg dominant did not exert substantial influence on neuromuscular function at a normalised contraction level, there is however lack of MU specific evidence of the bilateral responses to unilateral interventions. Thus, investigating the effects of unilateral exercise training on bilateral neuromuscular function could provide valuable insights into neural adaptations and inter-limb coordination patterns, thereby contributing the development of comprehensive training programs and rehabilitation protocols.

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