MOTOR UNIT CHARACTERISTICS IN YOUNG AND OLD SKELETAL MUSCLE

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Abstract

The neuromuscular changes associated with increasing age include a reduction in the number of motor units (MU) and an increase in the size of those that remain. Electromyography (EMG) has been used to investigate these changes, however the available literature is largely concerned with small peripheral muscles and little is known of the large leg muscles, in particular the vastus lateralis (VL) which is particularly susceptible to age-related atrophy.

In Chapter 2 we highlight the variations in MU structure across a single large muscle, the VL. Motor unit properties differed according to location, recording depth and contraction intensity. These findings were applied in Chapter 3 to study differences in MU properties of the VL between Young and healthy Old participants. It was noted that the Old males had fewer, but larger MUs than the young. The Old MUs also showed signs of greater instability in neuromuscular transmission and lower firing rates.

It has been suggested that lifelong exercise may have beneficial effects on neuromuscular changes, in Chapter 4 this was investigated in the VL and tibialis anterior (TA) of competitive Masters Athletes (MA). It was found the MA were no different to the Old, and showed significant signs of neuromuscular decline and MU remodelling.

The MU remodelling in VL and TA was further assessed in Chapter 5, where it was found the effects of age on MU characteristics were greater in the TA when compared to the VL. The age-related differences also differed from low to moderate contraction intensities, and indicated a more homogenous MU pool in Old participants.

Older people show clear differences in MU properties than Young, and lifelong exercise does not appear to have any beneficial effects on these changes. The MU remodelling process likely has consequences beyond the loss of muscle fibres, and the lack of heterogeneity in the MU pool may well cause functional limitations.

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For Mam and Dad.

"To move things is all that mankind can do, for such the sole executant is muscle, whether in whispering a syllable or in felling a forest."

Charles Sherrington, 1924

"Onwards and upwards"

Paul Piasecki, 2013

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List of abbreviations

BB: biceps brachi

CMAP: compound muscle action potential

DQEMG: decomposition –based quantitative electromyography

EDB: extensor digitorum brevis

EMG: electromyography

FDI: first dorsal interosseous

iEMG: intramuscular electromyography

iMUNE: intramuscular motor unit number estimate

MA: Masters athlete

MU: motor unit

MUNE: motor unit number estimate

MUP: motor unit potential

MVC: maximal voluntary contraction

NF: near fibre

sEMG: surface electromyography

sMUP: surface recorded motor unit potential

STA: spike triggered averaging

TA: tibialis anterior

VI: vastus intermedius

VL: vastus lateralis

VM: vastus medialis

Chapter 1

Introduction and Literature Review

1.1 General introduction

The ageing process is associated with a general reduction of skeletal muscle mass resulting in weakness which is closely associated with, if not the direct cause of, reduced mobility, poor balance and motor control (Doherty, 2003; Goodpaster *et al.*, 2006). The consequences of this for the elderly are a reduced quality of life, increased social isolation, frailty and increased morbidity (Jette & Jette, 1997). In an ageing population this is becoming an issue of increasing importance.

The age-related loss of muscle mass is termed sarcopenia for which there are two primary causes. Firstly, that of disuse atrophy which is a direct result of reduced activity, commonly seen in elderly people. It may be caused by a lack of desire to exercise or be active, or as a consequence of another age-related disorder such as cardiovascular or musculoskeletal disease. Secondly, there are a number of factors that directly influence skeletal muscle mass, such as altered hormonal status, reduced protein intake and problems with systemic amino acid distribution and uptake, inflammation affecting muscle fibres and motor neurons, and an age-related loss of spinal motor neurons resulting in muscle fibre denervation and fibre loss (reviewed by (Edström *et al.*, 2007; Narici & Maffulli, 2010).

Loss of muscle does not appear to be evenly spread, with the leg muscles undergoing a greater loss with age than those in the upper body (Janssen *et al.*, 2000), and the quadriceps femoris group seems to be most affected (Abe *et al.*, 2011; Maden-Wilkinson *et al.*, 2013; Abe *et al.*, 2014). Furthermore, an age-related reduction in fibre pennation angle has also been reported in VL and gastrocnemius (Kubo *et al.*, 2003; Morse *et al.*, 2005). This reduced pennation angle indicates fewer sarcomeres in parallel and thus, smaller muscle fibre cross sectional areas which in turn gives a lower physiological cross sectional area of whole muscle and lower muscle strength (Narici, 1999).

Most attention has focused on ways of maintaining muscle size and strength by exercise, diet and hormone replacement but without appropriate neural innervation, muscle cannot function and there is increasing evidence that deficits in neural control make a major contribution to the problems of old age.

1.2 Motor unit organisation

The motor unit (MU), as described by Sherrington (1925) is the 'common final pathway of the motor system and comprises a motor neuron in the ventral horn of the spinal cord, its axon, and the muscle fibres that the axon innervates.' It is the smallest functional component of the neuromuscular system. The muscle fibres within a healthy MU are believed to have the same phenotypic characteristics (i.e. slow or fast, type I or type II) and are activated together. Motor unit territories are distributed over several cm of the longitudinal muscle axis (Vieira *et al.*, 2011; Gallina & Vieira, 2015; Héroux *et al.*, 2015) and around 5-10 mm muscle depth in large human limb muscles (BUCHTHAL *et al.*, 1959). The dispersal of fibres belonging to individual MUs across relatively large volumes of muscle gives the characteristic mosaic, or chequer board, pattern when muscle is cut in cross sections and stained for different fibre types. Buchthal (1959) estimated that around two-dozen individual MUs with innervation ratios of 500-2000 fibres can span a muscle cross-section of around 8 mm in large human muscles. Later experiments estimated innervation ratios to be lower at around 100-300 fibres per MU in human limb muscles (Gath & Stålberg, 1981). Nevertheless, fibres of the same MU are rarely located immediately adjacent to one another. This ensures a more even distribution of forces across relatively large areas of muscle and repetitive extracellular depolarisations are less likely to be intense in any one area upon activation (Edström & Larsson, 1987).

Cadaveric studies have shown the first dorsal interosseous (FDI), a small hand muscle, to have around 120 motor units and 40,000 muscle fibres (FEINSTEIN *et al.*, 1955) and the thenar is estimated to average around 160 MUs (Neto *et al.*, 2004), while larger limb muscles can each contain many hundreds or thousands of MUs of varying sizes innervating over a million muscle fibres per muscle (e.g. see (Tomlinson & Irving, 1977; Lexell *et al.*, 1988). The precise innervation ratios of motor units are difficult to estimate and vary considerably between MUs within a single muscle (Enoka & Fuglevand, 2001), the variation in size being almost entirely responsible for the variation in the force generated by individual

MUs (Burke & Rymer, 1976; Kanda & Hashizume, 1992; Tötösy de Zepetnek *et al.*, 1992). In humans, innervation ratios in the biceps brachii are estimated to be between 70 – 750 (BUCHTHAL *et al.*, 1959; Gath & Stålberg, 1981), the tibialis anterior (TA) averages around 125- 562 (FEINSTEIN *et al.*, 1955; Gath & Stålberg, 1981), the thenar around 90 fibres (Neto *et al.*, 2004) and the deltoid may have only 70 fibres per MU (Gath & Stålberg, 1981). By estimating number of motor neurons and number of muscle fibres, Feinstein (1955) estimated average innervation ratios at around 340, 410 and 1934 in FDI, brachialis and medial gastrocnemius, respectively.

The body of knowledge that exists on MU physiology in humans has largely come from cadaveric studies, and more recently electromyography (EMG).

1.3 Electromyography

When a muscle fibre receives an impulse from a nerve the permeability of the fibre membrane to sodium is temporarily increased, which reverses the membrane potential of the muscle fibre. Muscle fibres of the same MU receive the stimulus virtually simultaneously, and as the action potentials propagate along fibres an electrical signal, the MU potential (MUP), can be detected with surface or intramuscular electrodes and an appropriate amplifier. Electrodes can be inserted into the muscle to record action potentials within a relatively small volume (intramuscular or iEMG), or on the surface of the skin, to record electrical activity from a larger proportion of muscle (surface or sEMG).

Motor unit number estimate (MUNE)

McComas (1971) first reported an EMG-based technique for estimating the number of MUs in a muscle, now known as the motor unit number estimate (MUNE). It was based upon the principle that if it is possible to measure the size of a single MUP, and also possible to measure the summation of all MUPs generated by the whole muscle, then the former may be divided into the latter

to obtain an estimate of the number of MUs present. The nerve branch innervating the extensor digitorum brevis (EDB) in the foot was stimulated percutaneously, with each consecutive stimulation being slightly higher intensity than the previous: when the recruitment thresholds of individual MUs was reached, an increase in MUP size was seen. The MUP amplitude for the individual MUs was calculated and taken as representative of the area of the individual MU. The MUNE was derived by calculating the 'average' MUP size and dividing it into a maximal CMAP, recorded after supramaximal stimulation of the motor neuron branch. This method was known as incremental stimulation MUNE. A problem with this technique is *alternation*, which occurs when MUs with similar activation thresholds discharge individually, or together as one. Thus there are three possible recording outcomes from two axons in response to a stimulus that is very close to the activation threshold of both axons; the first axon may discharge alone, the second axon may discharge alone, or they may discharge together and be mistakenly recorded as one (McComas *et al.*, 1993).

Since the development of the original MUNE method, there have been several variations and improvements, which have been extensively reviewed (Daube, 2006; Bromberg, 2007; Gooch *et al.*, 2014). They largely differ in the way the average surface representation of the MUP, or the sMUP, is obtained. Current methods calculate a MUNE by dividing a component of the sMUP into a component of the CMAP. The component commonly used is the negative peak area or the negative peak amplitude, as it is believed this best represents the level of muscle activity that can be recorded from the surface (the skin overlying the muscle).

The problem of alternation has been largely overcome by recording MUPs during low and moderate intensity voluntary contractions (Brown *et al.*, 1988) and using intramuscular EMG to record from a small proportion of muscle fibres within individual MUs. The MUPs are then used to 'trigger' the recording of the corresponding surface motor unit potential (sMUP), the technique being known as 'spike triggered averaging' (STA). An average sMUP is generated from around 20 sMUPs; this is then divided into the maximal CMAP to derive a MUNE value.

The sEMG needs to be recorded over the motor point, which is a point along the superficial region of the muscle belly that is particularly excitable with percutaneous electrical stimulation, evidenced as a relatively large muscle twitches from a very low-intensity electrical stimulus, presumably due to clustering of motor neuron axonal branches and neuromuscular junctions. This ensures EMG signals are recorded where the concentrated cluster of motor axons gives fast and reliable rise-times for the CMAP and sMUPs, and the MUPs and sMUPs are more likely to be time-locked as they are 'seen' by both electrodes at the same time (Figure 1.1) (Brown *et al.*, 1988). The use of an indwelling electrode (needle or fine-wire) makes the STA technique invasive, but this is only a minor inconvenience for most adults because the needles used (around 26 gauge) are often smaller than those used to collect routine blood samples.

The complex iEMG and sEMG signals from voluntary contractions need to be separated into the constituent MUPs from individual MUs and automated signal decomposition software has been developed which removes most of what was previously subjective and laborious analysis. Decomposition based Quantitative Electromyography (DQEMG) is one such decomposition program that is able to extract individual MUPs, their corresponding sMUPs, and calculate MUNE. Briefly, algorithms are used to first resolve an intramuscular EMG signal into individual MUPs using shape and firing pattern analysis. The individual MUPs 'trigger' the sEMG signal to obtain a surface representation of each MU. All MUPs and sMUPs are presented to the operator for final inclusion or exclusion. Full details on the engineering and computer coding can be found in previous publications (Stashuk, 1999a, b). The DQEMG analysis program has been used and validated in a number of muscles (Boe *et al.*, 2004, 2006; Hourigan *et al.*, 2015).

The surface EMG signal is a composite of potentials arising from possibly a large number of recruited MUs, and is increasingly complex with higher intensity contractions, causing difficulties when decomposing into individual MUs. Thus, most studies of MU number or characteristics make use of low or moderate

intensity contractions. According to the Henneman size principle (HENNEMAN *et al.*, 1965; Milner-Brown *et al.*, 1973); it is the smaller MUs that are recruited during low-force contractions. The STA-MUNE will, therefore, disproportionately sample from the early-recruited, smaller MUs and subsequently give excessively high MUNE values. In this respect, differences in the MUNE values with contraction intensity have been demonstrated in the soleus (Dalton *et al.*, 2008) and the TA (McNeil *et al.*, 2005a), and it can be speculated that higher contraction intensities would identify larger sMUPs and therefore lead to the calculation of even lower MUNE values, at least in muscles that principally increase external force by MU recruitment rather than rate coding.

There are, however, several limitations with the available MUNE techniques. The first is that a CMAP can only be performed on muscles that have a major nerve branch accessible superficially to be stimulated percutaneously. Secondly, it is not entirely clear what proportion of muscle is captured by the CMAP. The reported CMAP values for a variety of small muscles are surprisingly similar to those reported for larger muscles (Galea, 1996; McNeil *et al.*, 2005b; Dalton *et al.*, 2008; Power *et al.*, 2010; Power *et al.*, 2012). In large muscles the CMAP is smaller than would be expected if it were the summation of the electrical activity of all motor units within the muscle. This suggests that the CMAP, and by extension the MUNE values, of the larger muscles is not an estimate of the number of MUs within the whole muscle but is, instead, a representation of MUs in the volume of muscle 'seen' by the surface electrode. No previous studies have taken account of this point to correct the MUNE value for muscle size. Thirdly, techniques that rely on sEMG signals face the problem of cross contamination from other muscles and attenuation of the signal by fat, skin and, possibly, connective tissue within the muscle. One study estimated that in the small abductor digiti minimi, more than 60% of the sMUPs originated from other nearby hand muscles (Kawamura *et al.*, 2013), thereby complicating the interpretation of the sMUPs and MUNE values. This may happen during voluntary contractions, and also following nerve stimulation if nearby muscles share the main nerve pathway.

No previous studies have derived MUNE values for the large thigh muscles, such as VL, but these muscles are of interest in studies of ageing since they are particularly susceptible to age-related weakness. When recording EMG from larger muscles, the surface electrode will preferentially sample from more superficial areas of the muscle, due to the limited recording volume of the electrode which is estimated to have a radius of around 2 cm (Barkhaus & Nandedkar, 1994). Therefore, MUs located deeper within large muscles such as VL are subject to greater attenuation of the EMG signal and contribute minimally to the sEMG (Muceli *et al.*, 2015) and presumably, the CMAP. The VL has a distinct fascicular arrangement with a pennate structure and is compartmentalized with separate innervating nerve branches (Patil *et al.*, 2007; Becker *et al.*, 2010) and multiple motor points, raising the question of which motor points should be sampled to derive representative MUPs, sMUPs and CMAPs. Furthermore it appears that MUs may not be randomly distributed throughout a muscle, although the literature is mixed. Lexell (1991) reported larger fibre CSA in deeper regions of the VL and although this is not a direct indication of MU size, it may be inferred that the larger fibres are part of MUs that have different properties compared with those comprised of smaller fibres, while Knight (2005) using EMG reported that superficial MUs were larger than deep MUs.

The possible organisation of MUs within the VL has implications for both iEMG and sEMG. The surface EMG signal will be biased towards MUs located closest to the surface while for iEMG the position of the needle will influence the type of MU that is sampled. To accurately study how MU properties of a large muscle can change with disease or ageing, it is clearly important to first understand how MU size and properties vary throughout the muscle.

The MUPs extracted from the iEMG signal display a variety of shapes, and these can offer further information on MU characteristics in addition to what can be learned from the sEMG signal. A MUP is a summation of the action potentials from fibres within the recording area of the electrode belonging to a single MU. These can be categorized into two features; global and detailed (Zalewska &

Hausmanowa-Petrusewicz, 1999). The global feature is represented by the envelope of the MUP, such as its amplitude, area or duration and is indicative of the total fibre cross sectional area (CSA) contributing to the MUP. The detailed features are evident as the number of turns and phases of the MUP, and reflect the extent of synchronised firing of individual fibres within the MU (Zalewska & Hausmanowa-Petrusewicz, 1999) (Figure 1.2).

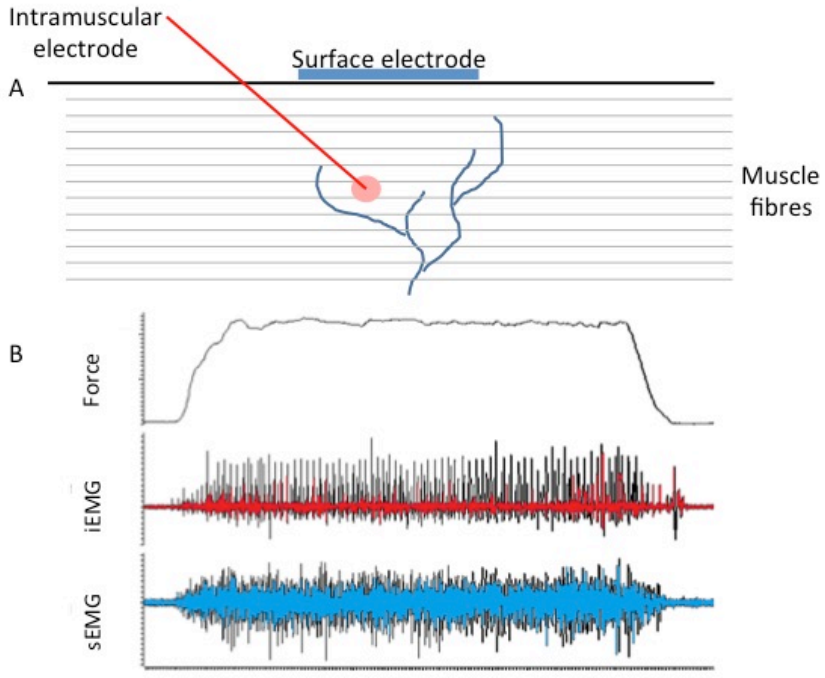

Figure 1.1. Surface and intramuscular EMG recording. A) Schematic of indwelling needle and nearby surface electrode over the motor point; B) Raw data recorded from the vastus lateralis of a healthy older man showing force and intramuscular and surface EMG signals.

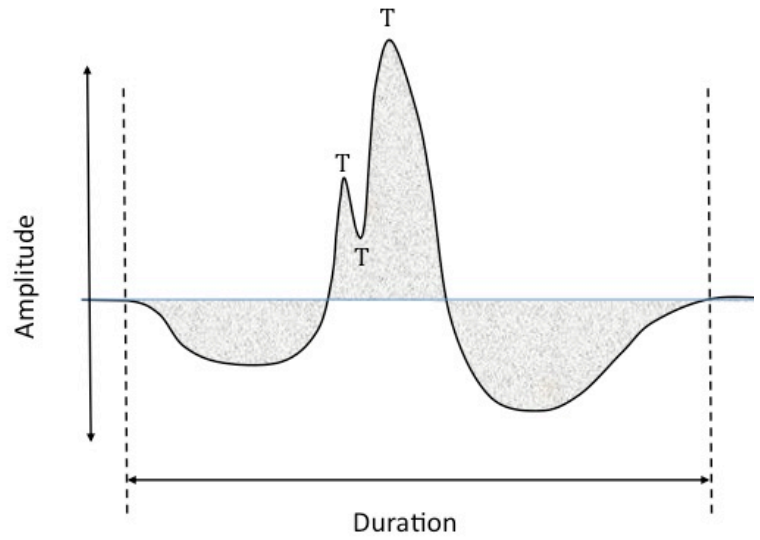

Figure 1.2. Global and detailed features of a MUP. The summation of the signals from muscle fibre action potentials forms the motor unit potential (MUP), showing duration, amplitude, area (combined area of all phases), turns (T), and phases (shaded area).

1.4 Age-related motor unit remodelling

Denervation and reinnervation

If a muscle fibre becomes denervated, either as a result of peripheral nerve damage or through the loss of the motor neuron there are two possible outcomes. The first is that fibre may be reinnervated by a nearby functioning axon, termed sprouting or collateral reinnervation. This results in a larger MU, or more specifically a MU with more fibres and a greater innervation ratio; whether the territory of the MU will increase depends on whether the newly innervated fibre is on the periphery of the existing MU territory. Secondly, the denervated muscle fibre may atrophy due to a lack of contractile activity and eventually disappear, presumably as a consequence of apoptosis. If the process of reinnervation is unable to keep up with the rate of denervation, the fibre loss will contribute to an overall loss of muscle mass and it is suggested that this is a significant mechanism underlying age-related sarcopenia (Aagaard *et al.*, 2010).

Motor neuron loss with age

Post mortem anatomical studies indicate neurological changes within the spinal cord with ageing. Tomlinson and Irving (1977) examined the lumbosacral spinal cord of 47 cadavers, aged 13 to 95 years. Motor neuron cell body numbers remained relatively constant until the age of around 60, after which they declined progressively such that specimens from those aged around 75 had approximately 30% fewer motor neurons supplying the lower limbs (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987). Although it is difficult to differentiate between sensory and motor neurons an age-related decline is still clearly evident.

EMG estimates of MU number and effects of age

The first study to demonstrate MU loss during healthy ageing using EMG was by Campbell et al (1973). They used the incremental stimulation technique to study the EDB in 94 subjects aged 3-96 years. In agreement with the anatomical counts of spinal motor neurons made by Tomlinson and Irving (1977), MUNE remained relatively constant (mean = 197±58) up to the age of around 60 yrs, but a progressive decline was noted thereafter. People aged over 75 yrs had fewer than 50% of the MUs of the Young subjects and some of the very oldest subjects apparently had fewer than 10% of their MUs remaining. A similar pattern of age-related reduction in MUNE has since been shown in the tibialis anterior (TA) (McNeil *et al.*, 2005b; Hourigan *et al.*, 2015), and the thenar (Doherty & Brown, 1993; Galea, 1996), but not in the soleus (Dalton *et al.*, 2008), and the biceps has shown mixed results (Galea, 1996; Power *et al.*, 2012) (Figure 1.3). These EMG techniques indicate much greater loss of MUs than was indicated by the motor neuron counts of Tomlinson and Irving (1977).

Compound Muscle Action Potential

An integral part of the MUNE calculation is the size, of the surface recorded CMAP, generally the area of the negative phase. A consistent finding is that the CMAPs recorded from older subjects are smaller than those from young (Figure

1.3). This might be thought to simply reflect the smaller size of the older muscles but this is unlikely because disuse atrophy in young subjects did not change CMAP amplitude in the soleus (Clark *et al.*, 2006) or the FDI (Fuglevand *et al.*, 1995). In addition is the observation that the CMAP is very similar for a range of muscles of varying size (see below) which indicates that it reflects the electrical activity of a volume of muscle lying beneath the active electrode and is thus relatively independent of the muscle size. In this case it is surprising that older muscle should have smaller CMAPs than young muscle.

Electrical signals originating within the muscle and recorded at the surface will inevitably be attenuated by the skin, subcutaneous fat and connective tissue as well as the overlying muscle tissue itself. Older muscle may attenuate electrical signals to a greater extent than young as a consequence of increased intramuscular fat (Hogrel *et al.*, 2015) and connective tissue (Serrano & Muñoz-Cánoves, 2010). The extent of attenuation may differ between old and young or, indeed, between individuals due to differences in muscle and subcutaneous tissue composition, but the attenuation presumably affects both CMAP and sMUP to a similar extent and consequently MUNE values (which normalize sMUPs to the CMAP) are probably not affected.

As muscle atrophy is a common feature of ageing, the relationship between muscle size and MUNE requires further comment. The reported CMAP values for a variety of small muscles are surprisingly similar to those reported for larger muscles (Galea, 1996; McNeil *et al.*, 2005b; Dalton *et al.*, 2008; Power *et al.*, 2010; Power *et al.*, 2012). This is also apparent when comparing MUNE values reported for individual muscles against anatomical counts of spinal motor neurons. MUNE values derived from leg muscles (albeit, mainly smaller muscles) are often around 200 – 400 and even these are likely to be on the high side as a result of preferential sampling of smaller units during STA-MUNE. However, they are clearly some way short of the anatomical counts of Tomlinson and Irving (1977) who estimated around 60,000 motor neuron cell bodies in the lumbo-sacral region (innervating the legs) of the spinal cord of young men and 40,000 in older men.

It is not possible to know precisely what volume of muscle is captured by the surface electrode, but one study estimated it to be from a depth of around 2 cm (Barkhaus & Nandedkar, 1994). If this is a radius of a hemisphere of muscle (~16cm^3), it is a very small proportion (0.008) of the total volume of large muscles such as the quadriceps (~2000 cm^3). If a nominal MUNE of 300 is adjusted for this proportion, the total number of MUs is around 37,500. This value is high, most likely due to preferential sampling of smaller MUs during MUNE procedures.

Motor Unit Potential size

Using the incremental stimulation MUNE technique, Campbell et al (1973) noted that older subjects tended to have larger sMUPs compared with the young. Other studies using iEMG and voluntary contractions similarly reported larger MUPs in old during low and moderate force contractions. Figure 1.3 shows MUPs reported in various healthy, older muscles when expressed as a percentage of MUPs from young subjects. Extending the observation of larger sMUPs in the EDB of older people made by Campbell et al (1973), the incremental stimulation technique was later used to show larger sMUPs in the thenar and EDB of older subjects. However, sMUPs in the biceps brachii (BB) were around 25% smaller in old compared with young (Galea, 1996). A macro EMG technique was developed to more accurately reflect the MU size from iEMG-recorded MUPs. Similar to STA, signals from the cannula of the needle are triggered by intramuscular recordings, thereby increasing the recording area making it more likely to capture an entire MU (Stålberg, 2011). This technique showed almost two-fold larger MUPs in older TA and VL compared with young, while the MUs of the BB were increased by around 30-50% (Stalberg & Fawcett, 1982). Data from concentric needle electrodes show larger MUPs in older soleus across a range of voluntary contraction intensities (Dalton *et al.*, 2008), and larger MUPs have also been reported in TA and vastus medialis with this technique (Hourigan *et al.*, 2015).

It is clear there are some inconsistencies in measures of MU size relating to age. In particular, a large MUP does not always correspond to a large sMUP. This was

demonstrated in the soleus by Dalton et al (2008) where the older group had larger MUPs than young, but smaller sMUPs. The higher levels of signal attenuation in the old may explain this, as previously discussed (Section 1.3).

In one of the few studies to investigate MU properties of a large anterior thigh muscle, Hourigan et al (2015) showed higher near fibre (NF) jiggle, which is a measure of variability in the size of consecutive MUPs from a MUP train, in the TA and vastus medialis (VM) of nine older compared with nine younger men. Higher jiggle is thought to occur due to increased transmission variability from unstable neuromuscular junctions within individual MUs (Stålberg & Sonoo, 1994). This may occur alongside the remodeling process of denervation and reinnervation, however MUNE values were not reported in the study.

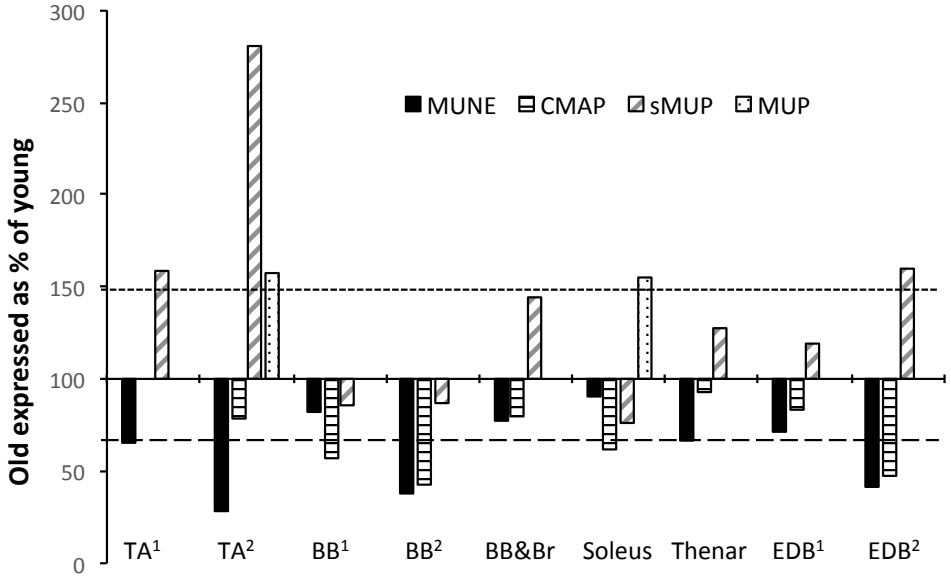

Figure 1.3. MUNE, CMAP, sMUP and MUP values in old compared with young. The dashed horizontal line indicates the median MUNE in old and the dotted horizontal line indicates the median MUP or sMUP in old expressed as % of young from the various studies. Where multiple older age groups or contraction intensities were reported in a single study, the age range 60-80 yrs and closest intensity to 25% MVC were used in this figure. TA: tibialis anterior; BB: biceps brachii; BB&Br: biceps brachii and brachialis; EDB: extensor digitorum brevis. Data are from: TA[1] (Power *et al.*, 2010); TA[2] (Hourigan *et al.*, 2015); BB[1] (Galea, 1996); BB[2] (Power *et al.*, 2012); BB&Br (Brown *et al.*, 1988); Soleus (Dalton *et al.*, 2008); Thenar (Galea, 1996); EDB[1] (Galea, 1996) and EDB[2] (Campbell *et al.*, 1973).

1.5 Motor unit remodelling and muscle function

Figure 1.4 provides a summary of muscular changes during healthy ageing. Dual-energy x-ray absorptiometry is most commonly used to assess muscle (or 'lean') mass, but it underestimates the extent of muscle loss during ageing and therefore more accurate measures of age-related muscle changes are acquired from magnetic resonance (Maden-Wilkinson *et al.*, 2013; Maden-Wilkinson *et al.*, 2014).

Motor unit remodelling contributes to the muscle losses, but it remains unclear whether any particular MUs, small or large, are most affected, or which are more engaged in the reinnervation processes during normal ageing. Motor neuron loss leaves the muscle fibres within the MU denervated, but some are 'rescued' by sprouting of nearby neuron branches. The reinnervation process leads to enlarged MUPs, increased fibre density (Stalberg & Thiele, 1975; Stalberg & Fawcett, 1982; McComas *et al.*, 1993; Luff, 1998) and fibre-type grouping (Lexell & Downham, 1991) with healthy older age.

The rescue of denervated fibres is a compensatory mechanism that probably helps to preserve total muscle mass and maximal force generating capacity in the face of extensive motor neuron losses. Therefore, MU loss must precede clinically-relevant muscle losses such as sarcopenia or dynapenia, but longitudinal data are not available to confirm this. The reinnervation process is, however, limited, as post-mortem studies showed that around 30-40% of fibres in VL are lost by age around 75 yrs (Lexell *et al.*, 1988; Lexell & Downham, 1991), with both type I and type II fibre losses being the major cause of muscle atrophy with healthy ageing, rather than atrophy of individual fibres (Lexell *et al.*, 1988). In a further study using MUNE, MU loss was stated as a cause of muscle weakness in older BB (Doherty *et al.*, 1993).

Although fewer alpha motor neurons have been found in spinal sections of older people (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987), it may not be the case that this is where the problem occurs. It has been

suggested that degeneration of the neuromuscular junction (NMJ) occurs first and progresses in a retrograde manner resulting in the loss of the cell body in the spinal cord (reviewed by (Deschenes, 2011; Nishimune *et al.*, 2014). However, human studies have revealed age-related structural alterations in both the motor endplate, evident as spreading of acetylcholine receptors on the fibre and elongation of the motor endplate, and atrophy as well as irregular shaped muscle fibres that have low specific tension and higher rates of oxidized proteins within muscle fibres, so it is not clear whether fibre denervation is a cause or a consequence of motor neuron losses (reviewed by (Tintignac *et al.*, 2015).

Coordinated movements require not only the proper involvement of different muscles, but also the correct recruitment and activation of MUs in the individual muscles. Motor unit size may be described relative to the innervation ratio or the axonal diameter, and in young healthy muscles the two are likely to correlate well, however older muscle tends to have a larger innervation ratio due to remodelling but without necessarily the increased axonal diameter. This is likely to result in larger MUs with lower recruitment thresholds, but also grouping of muscle fibres and slowing of firing rates. This neuromuscular remodelling is expected to have implications for fine motor control. It is notable in this respect that increased tremor, slower walking speeds and poor balance are common complaints of the elderly. It is not a straightforward task to link poor mobility and balance directly to MU remodelling, since so many other factors contribute to mobility including eyesight, vestibular function and proprioception (Luu *et al.*, 2012), which also deteriorate with ageing.

To date there is little evidence to evaluate the extent of MU remodelling in a large locomotor muscle during healthy ageing. As previously mentioned the quadriceps muscle appears to be susceptible to a more severe level of age-related atrophy than other muscles, and a greater knowledge of the MU changes in this muscle may offer an insight as to what extent this atrophy is due to neurological changes.

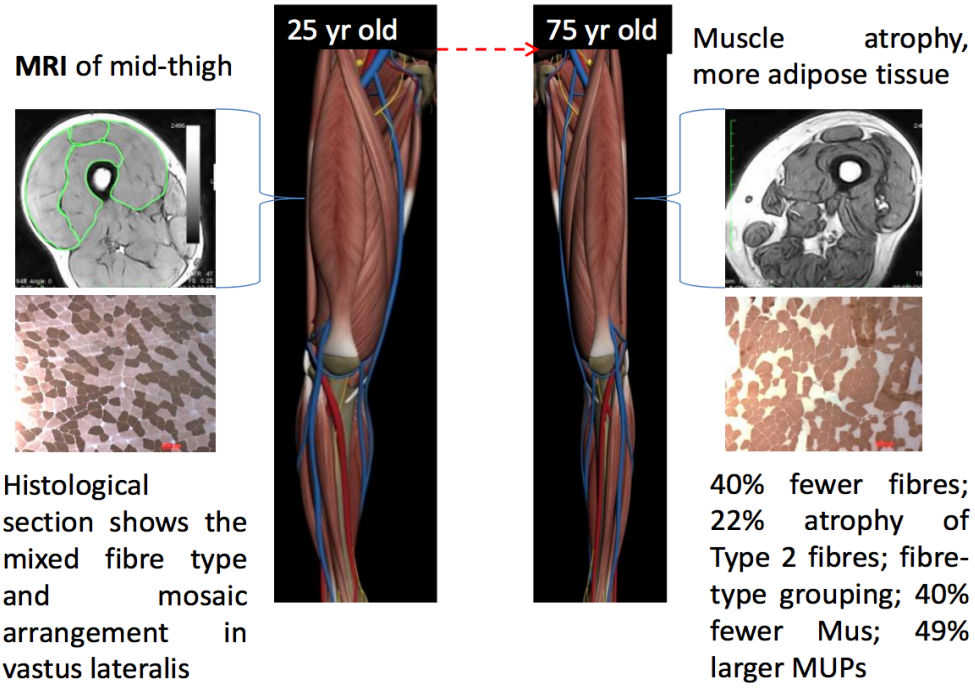

Figure 1.4. Atrophic muscles in older age. Compared with young, a typical healthy 75 yr old man has around 15% lower appendicular lean mass (McPhee *et al.*, 2013); 30% smaller knee extensor muscles (Maden-Wilkinson *et al.*, 2014); 35% lower knee extension strength (McPhee *et al.*, 2013); and 35% lower leg power (Stenroth *et al.*, 2015); 20-40% fewer muscle fibres in the VL, fibre-type grouping and small, angular fibres (Lexell *et al.*, 1988; Lexell & Downham, 1991)

1.6 Exercise as a possible intervention strategy

Since motor neurons are terminally differentiated, the large numbers that are lost during healthy ageing can never be recovered. It is, therefore, important from the public health perspective to find ways to prevent the losses from occurring, or to find ways to help older people cope with any deficits in motor unit numbers and function. There are no known pharmacological agents that act to preserve motor neuron numbers or stabilize neuromuscular junctions, but an intriguing possibility is that lifestyle, or exercise, might be effective.

In mice, maintaining exercise through middle and older age helps to preserve neuromuscular function, with evidence in mice that lifelong exercise maintains the stability of neuromuscular junctions (reviewed by (Deschenes, 2011;

Nishimune *et al.*, 2014). There is very little evidence of such effects in humans, although histological and electron microscopic examination of human VL muscle biopsy samples revealed fibre type grouping in older athletes, which differs from the usual mosaic fibre-type arrangements seen in the VL muscle and is thought to occur as a consequence of MU remodelling as axons sprout to reinnervate neighbouring fibres that were denervated, possibly following MU losses. While this may well reflect neuromuscular changes even in the athletic old, the more frequent grouping in athletic older muscle was taken as evidence of improved ability to reinnervate denervated fibres (Mosole *et al.*, 2014; Zampieri *et al.*, 2015).

A study using STA-MUNE showed that 10 older endurance runners aged around 64 yrs had similar MU numbers to young adults in their TA, while non-athletic older adults aged around 66 yrs had fewer MUs than the young; however, when the BB was examined, the masters athletes had similarly low MU numbers as the old (Power *et al.*, 2010; Power *et al.*, 2012). This was taken as evidence of exercise-related preservation of the specific MUs within the muscles most often used during exercise. This notion of "use it or lose it" is very appealing, but the small sample size and cross-sectional study design mean that additional studies are needed.

1.7 Which MUs are preferentially lost during healthy ageing?

The reduction in MU numbers, together with an increase in average MU size, could occur as a result of the smaller units being lost or, a loss of motor neurons supplying the larger MUs and reinnervation of the denervated fibres by sprouting of axons from surviving motor neurons that originally innervated small MUs. There is little indication either way from the EMG data. A modest reduction in MU discharge rates was reported in healthy older subjects in the TA (Connelly *et al.*, 1999; Patten *et al.*, 2001; Klass *et al.*, 2008), FDI (Kamen *et al.*, 1995) and soleus (Dalton *et al.*, 2008), but others reported no difference between young and old in TA, BB or VM in MU discharge rates (Roos *et al.*, 1999; Power *et al.*, 2010; Power *et al.*, 2012). Slower discharge rates may be due to the recruitment

of fewer, but larger slow-phenotype MUs during moderate intensity contractions. If the motor neurons with low innervation ratios do indeed have a greater propensity for reinnervation of orphaned fibres, then their MUPs should be proportionately larger than the later-recruited higher threshold MUs. One of the few studies to report MUPs, sMUPs and MUNE at a range of contraction intensities was by Dalton et al (2008). They showed that older muscles had around 60% larger MUPs during all measurements collected from a range of intensities including very low through to 30% MVC in soleus. This indicates that older people have larger MUs compared with young across a broad range of recruitment thresholds, and thus all MUs are involved in the remodelling.

There is a skewed distribution of motor unit types in muscles such as VL or FDI that have around 50% type I and 50% type II in the overall muscle cross section. Enoka & Fuglevand (2001) explained that 84% of MUs were type I, despite the type I fibre area being just 50% of the overall FDI muscle area. Thus, loss of just one of the fast MUs with high innervation ratio would have little impact on MU numbers but potentially impacts greatly on fibre losses. Lexell (1988) suggested similar numbers of type I and type II fibres are lost with ageing, so assuming random reinnervation of the different fibre types, this would indicate substantially greater losses of smaller MUs compared with large. The MUs lost, large or small, might affect the success of reinnervation, with the fewer fibres of smaller MUs possibly more easily being accommodated into other units, but very little human evidence exists.

1.8 Summary of the available research

The work reviewed here suggests that healthy older people have around 30-40% lower MUNE values compared with young, but there is limited evidence in large muscles. The surviving low- and moderate-threshold motor units are around 20-50% enlarged and the little evidence that is available suggests increased MU complexity and fibre density as well as instability of the neuromuscular junction transmissions. There is considerable inter-individual variability in motor unit characteristics at all ages and this may lead to sampling bias in the majority of

the published work that invariably included small numbers of participants.

1.9 Unresolved issues

It remains unclear:

- Whether the existing MUNE techniques can be applied to large leg muscles, such as VL;
- how the MU characteristics of the large leg muscles, such as VL, are affected during ageing;
- whether MU losses contribute to the loss of muscle mass with ageing;
- whether exercise can help to preserve MU numbers, promote the reinnervation process, or maintain stability of neuromuscular junction transmission;
- which, if any, MUs (large or small) are preferentially lost or altered during the process of remodelling;
- whether different muscles experience similar MU losses and show differences in ability to reinnervate denervated fibres.

1.10 Thesis Aims and Objectives

The overall aim of the work presented in this thesis was to compare MU structural and functional properties of VL and TA leg muscles between young and older men in order to characterise the changes that occur with ageing.

The Objectives were:

1) to establish the techniques needed to estimate motor unit numbers and sizes in the VL, a large leg muscle, and to characterize functional properties such as firing rates and stability of neuromuscular junction transmission.

2) to use the newly developed techniques to compare VL MU characteristics between Young and Old men.

3) to investigate whether lifelong activity protects against MU changes associated with ageing.

4) to investigate whether differences exist between VL and TA muscles in the extent of age-related changes to MU characteristics.

The following four chapters present novel research data to address each of the Objectives in turn.

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Chapter 2

Motor unit structure and function in the human vastus lateralis: effects of recording depth, location and contraction intensity

2.1 Abstract

Introduction: Motor unit (MU) number, size, recruitment and firing frequency are key aspects of motor control yet little is known of these MU characteristics in large human locomotor muscles. In the present study, MU characteristics of the vastus lateralis (VL) were compared between 1) proximal and distal locations; 2) low and moderate intensity voluntary contractions and 3) deep and superficial locations.

Methods: Intramuscular and surface electromyography signals were recorded from spatially localized regions at the proximal and distal motor points of the VL in 10 healthy Young men (27±5yrs). Deep and superficial MUs were sampled during sustained 10% and 25% maximal voluntary contractions (MVC). Individual MU potential (MUP) size, phases, turns and discharge rates were recorded and near fibre jiggle and fibre count determined. Average surface-recorded MUP (sMUP) size was divided into the electrically evoked maximal compound muscle action potential to derive a motor unit number estimate (MUNE) at proximal and distal sites.

Results: MUPs were larger during higher intensity contractions and larger at the proximal compared with the distal locations. Near-fibre jiggle and fibre count were similar when recorded at proximal and distal locations. MUPs were larger in deep compared with superficial regions, and had a higher number of phases and turns as well as higher near fibre jiggle. The sMUP was larger during higher compared with lower intensity contractions. Consequently, MUNEs were lower during 25% MVC compared with 10% MVC, and also lower at the distal location compared with proximal.

Conclusion: MU characteristics of the VL vary between proximal and distal locations and with depth within the muscle. MUNE values vary with the intensity of contraction while estimates of MU number based on a comparison of MUP area and muscle cross-sectional area (CSA) are less affected by some of the technical problems associated with MUNE methodology.

2.2 Introduction

The proper control of voluntary muscle contraction requires the appropriate recruitment and activation of motor units (MU) (for review see (Enoka & Fuglevand, 2001). However, little is known about the characteristics of individual MUs of large locomotor muscles that provide much of the power needed for daily activities. A knowledge of these characteristics is a prerequisite for understanding basic motor control mechanisms and associated changes that may occur with ageing or disease and adversely affect function and mobility.

Surface electromyography (sEMG) signals reflect gross muscle activation, but offer limited information about individual motor units or those located deeper in the muscle (Muceli *et al.*, 2015). Indwelling needle or fine-wire electromyography (iEMG) signals can record individual MU potential (MUP) size, discharge rates, complexity and stability from small muscle regions, typically up to 2 mm radius (Nandedkar *et al.*, 1988b). Recording from single locations using iEMG in a small muscle such as the FDI which is estimated to have around 140 MUs (Boe *et al.*, 2006), may be representative of the entire muscle but this is not necessarily the case for the larger proximal muscles such as the vastus lateralis (VL) in the anterior thigh.

The VL in a typical Young man is around 40 cm long, 2.5 cm in thickness at the muscle belly and has a volume of around 1.2 l (Maden-Wilkinson *et al.*, 2013). Fascicles are pennate and around 10 cm in length (Erskine *et al.*, 2010; Wakahara *et al.*, 2015). Autopsy data estimate around 600,000 fibres within a single mid-muscle belly cross-sectional section in Young men and indicate that the type 2 fibres were more abundant in superficial compared with deep locations (Lexell *et al.*, 1988). Electromyographic (EMG) recordings of VL showed larger MUs in the superficial regions (Knight & Kamen, 2005). This suggests spatial organisation of MUs with respect to depth from the surface of the VL but there is no information as to whether proximal and distal locations differ. While there is some information about MU sizes, data concerning other features such as MU firing frequency or stability of the MU potentials is missing.

The VL muscle origin is on the base of the femur greater trochanter and it inserts via the patella tendon to the anterior tibial tuberosity. And its main function is knee extension. Autopsy studies have revealed that the VL is compartmentalized into four distinct compartments, the central, superficial proximal, deep proximal and deep distal partitions. All compartments differ in fibre pennation angle, with the distal region having around 25% greater angle than the proximal. There are also separate distinct innervating nerve branches, separating from the femoral nerve into individual branches in proximal and distal regions, with the proximal further separating into 2 secondary nerve branches resulting in three motor points (Patil *et al.*, 2007; Becker *et al.*, 2010; Botter *et al.*, 2011). This emphasizes the possibility of spatial variation in MU characteristics.

In addition to the size and functional characteristics of MUs, there is interest in determining the numbers of MUs within muscles. Estimates of MU numbers usually require EMG signals to be recorded at the surface over the motor point during voluntary contractions. The mean surface-recorded MU potential (sMUP) is measured and its amplitude or area divided into the amplitude or the area of the electrically-evoked maximal compound muscle action potential (CMAP) to achieve the motor unit number estimate (MUNE) (Daube, 2006; Gooch *et al.*, 2014). Possible problems with the surface-recordings include cross-contamination of electrical signals from nearby muscles (Kawamura *et al.*, 2013), particularly in smaller muscles as the surface electrodes record from around 2 cm depth (Barkhaus & Nandedkar, 1994). In larger muscles, the deeper motor units may not be fully represented in surface recordings (Muceli *et al.*, 2015) since attenuation of the electrical signal occurs as they pass through muscle, subcutaneous tissue and skin (Nordander *et al.*, 2003). An additional issue with large muscles, such as the VL, is that they can have more than one motor point (Botter *et al.*, 2011) around which structure and function of MUs may differ and thus, may give different values for motor unit number estimates.

The aim of the present study was to determine individual motor unit characteristics recorded at different sites within the VL muscle of healthy Young men. Surface motor unit potentials, CMAP and MUNE were compared between

proximal and distal VL motor points. Intramuscular EMG measurements were recorded from deep and superficial regions at the two motor points and were compared for MUP size, discharge rates, near fibre (NF) density, number of turns and phases and NF jiggle (Stashuk, 1999a). These measurements were collected during voluntary sustained contractions held at 10% and 25% MVC and, in a separate group (n=4), measurements were also collected at 40% and 50% MVC at the proximal motor point.

2.3 Methods

Participants and recruitment

Ethical approval was granted by the University Research Ethics Committee and informed consent was provided in writing by all participants. Volunteers were included if they were male, habitually physically active and aged between 18-35 years. For the additional analyses that also included contractions at 40% and 50% MVC, the participants were all healthy, recreationally active men aged 30, 31, 36 and 72 yrs. Volunteers were excluded if they were sedentary or competing in sports at a regional level or above, or if they had any history of femur fracture or metabolic, cardiovascular or neuromuscular diseases.

Anthropometry measures

Magnetic resonance imaging (MRI) was used to measure peak quadriceps cross-sectional area and VL muscle thickness at proximal and distal motor points using a T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoe, Italy). The scanning coil was positioned over the thigh to cover the proximal and distal VL motor points, and contiguous transverse-plane slices of 6 mm thickness were collected with the participant lying rested and supine. Images were analysed off-line using Osirix imaging software (OsiriX medical imaging, OsiriX, Atlanta, USA) and the slice with the highest quadriceps cross-sectional area (CSA) was recorded as the peak CSA (example images shown in Figure 2.1) (Maden-Wilkinson *et al.*, 2014).

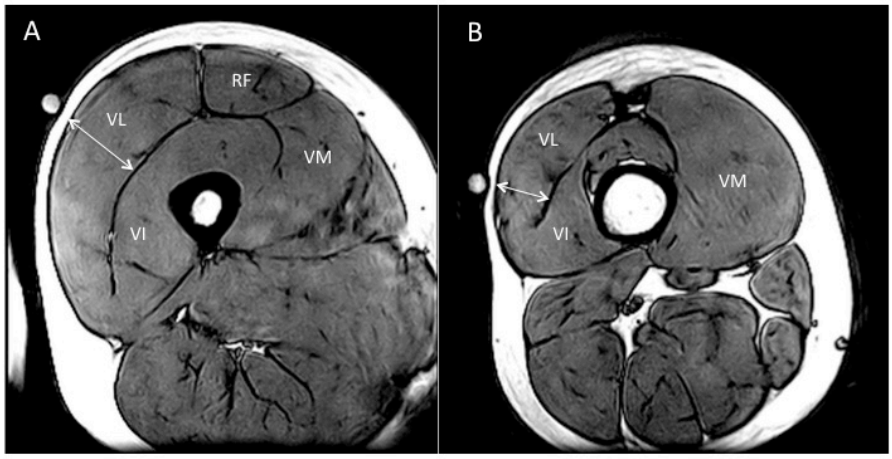

Figure 2.1. Transverse section of mid thigh taken by MRI. Arrows indicate VL thickness at the point of testing at A) proximal motor point, and B) distal motor point.

Knee extensor strength

Participants were seated with knees and hips flexed at approximately 90° and the right leg securely fastened to a force transducer 30 cm below the centre of the knee joint. Participants performed four moderate intensity warm-up contractions and were then instructed to perform a maximal isometric knee extension. This was repeated three times with short rest intervals between each contraction. The highest value from the three attempts was taken as the maximum voluntary contraction force (MVC).

EMG acquisition

The proximal and distal VL motor points were identified by low intensity percutaneous electrical stimulations (Botter *et al.*, 2011) and the skin over each motor point was prepared by shaving and cleansing with an alcohol swab. The distal motor point was located along the central line of the VL around 90 mm from the lateral femoral condyle. The proximal motor point was located around 220 mm from the lateral femoral condyle. The active recording sEMG electrode was placed over the motor point with a reference electrode placed over the patella tendon (disposable self-adhering Ag-AgCl electrodes; 95 mm^2, Ambu Neuroline, Baltorpbakken, Ballerup, Denmark). Intramuscular EMG signals were

recorded using disposable concentric needle electrodes with a recording area at the bevel of 0.07 mm^2 (Model N53153; Teca, Hawthorne, NY). A shared ground electrode for surface and intramuscular EMG signals was placed over the patella. Two CED 1902 amplifiers (Cambridge Electronics Design Ltd, Cambridge, UK) were used to bandpass sEMG and iEMG signals at 5 Hz to 5 KHz at 10 Hz to 10 KHz, respectively. Signals were digitized with a CED Micro 1401 data acquisition unit (Cambridge Electronic Design). The sEMG signals were sampled at 10 KHz and the iEMG signals at 25 KHz. Both EMG signals and the force signal were recorded and displayed in real-time via Spike2 software (v8.01). Data were stored for off-line analysis.

Experimental procedures

Percutaneous stimulation of the femoral nerve (approximately half way between the anterior superior iliac spine and the pubic tubercle, proximal to the groin crease, but distal to the inguinal nerve) was used to acquire the maximal compound muscle action potential (CMAP, or maximum M-wave) using a manually triggered stimulator (model DS7A; Digitimer, Welwyn Garden City, Hertfordshire, UK). The anode was placed at the top of the right gluteus and the cathode in the crease of the hip. The recording sEMG electrode was positioned to ensure the fastest rise-time of the CMAP. The current was increased incrementally with each stimulation until the M-wave no longer increased in size, generally at between 100-200mA. The current was then increased by 30 mA to ensure supra-maximal stimulation.

The participant was asked to relax the quadriceps and the concentric needle was inserted at an angle to ensure the tip of the needle electrode was beneath the active sEMG electrode at a depth of 1.0 to 2.5 cm. The participant was instructed to perform a voluntary contraction equivalent to 10% or 25% MVC lasting 15 sec with real time visual feedback (Figure 2.2). The needle was positioned to ensure sampled MUPs with sharp rise-times. Between four and six contractions were performed at each contraction intensity with the subject resting for about 30 sec

between contractions. During the rest intervals the concentric needle was repositioned by combinations of rotating the needle 180° and withdrawing it by around 5 mm to ensure recordings were from spatially distinct areas. This was performed at each motor point.

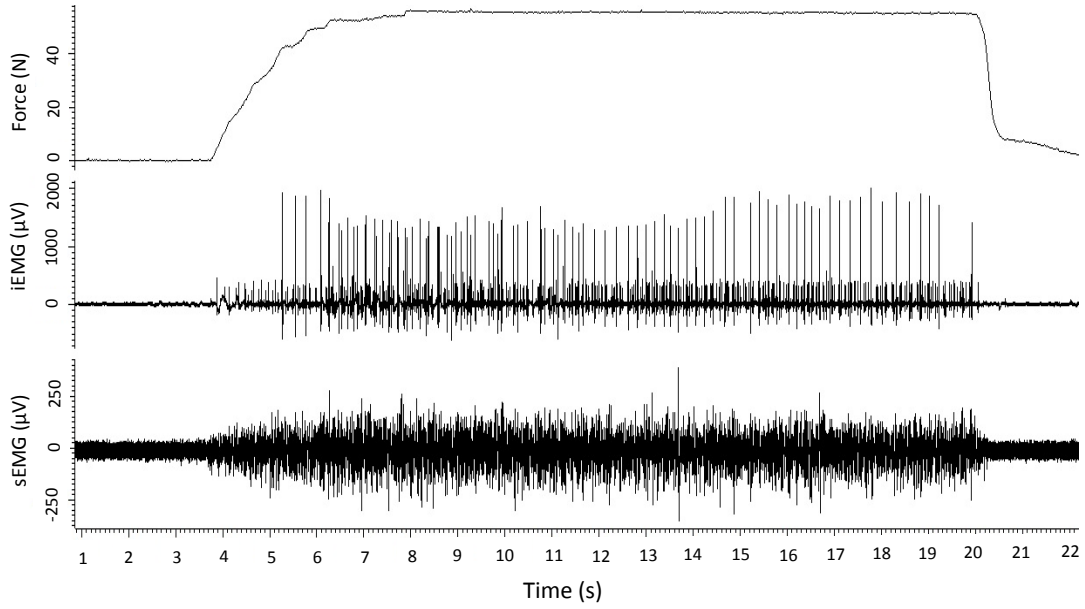

Figure 2.2. Force and EMG recordings. Raw signals from an isometric voluntary contraction held at 10% MVC (top trace), with simultaneously recorded intramuscular (middle trace) and surface (bottom trace) EMG signals.

EMG signal decomposition and analysis

Intramuscular and surface EMG signals were decomposed and MUNE values calculated using decomposition based quantitative electromyography (DQEMG) software, which has been previously described and validated with test-retest correlations of r = 0.72 – 0.99 (Stashuk, 1999a; Boe *et al.*, 2004, 2006). There are several variations of the MUNE method (Daube, 2006; Gooch *et al.*, 2014), which largely differ in the way the average sMUP is obtained. A series of consecutive MUPs generated by the same MU is known as a MUP train (MUPT). DQEMG calculates a MUNE using spike-triggered averaging (STA), in which the MUPs of MUPTs extracted from iEMG signals are used to 'trigger' an associated sEMG

signal, allowing representations of surface-detected MUPs (sMUPs) to be estimated. This avoids the problem of 'alternation' which is associated with incremental stimulation techniques where MUs with similar thresholds may be recorded as one (Brown *et al.*, 1988). For each extracted MUPT, DQEMG provides an averaged estimate of the component sMUP. Those sMUPs of the individually sampled MUs were then aligned and ensemble-averaged to estimate a mean sMUP. This averaged sMUP is representative of an average surface based MUP. The negative peak area of this mean sMUP was then divided into the negative peak area of the electrically evoked maximal CMAP to obtain a MUNE. Motor unit potential trains were not included in the iEMG analysis if they contained fewer than 40 MUPs or appeared to be composed of MUPs from two or more MUs (see Figure 2.3 for an example MUPT). All MUP and sMUP templates were visually inspected and where required the markers were adjusted to correspond to the onset, onset of negative phase (sMUP only), end, and positive and negative peaks. To be included as a sMUP the onset had to occur within 10 ms of the triggering MUP.

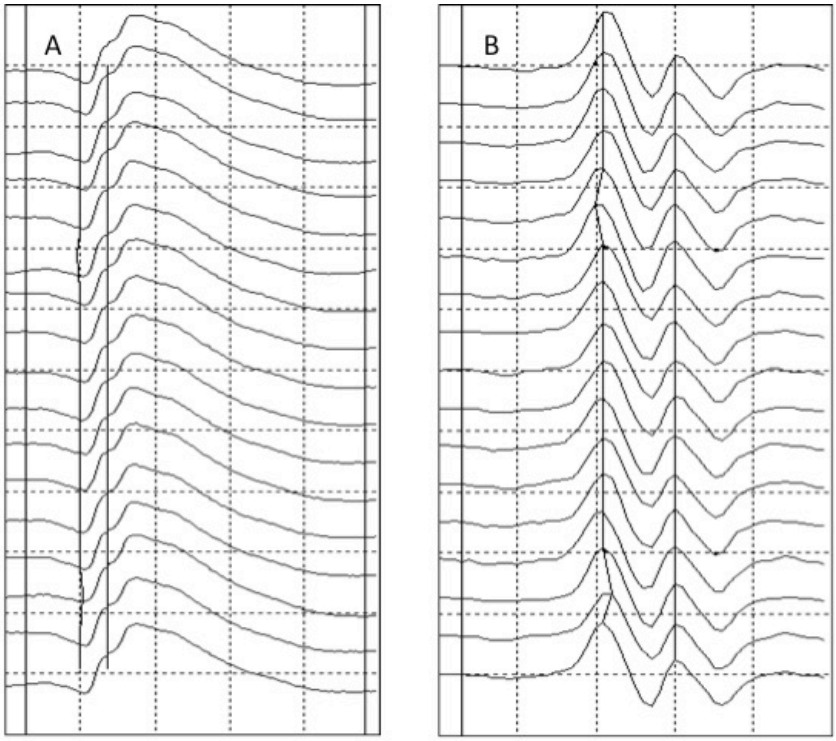

Figure 2.3 MUP and near fibre MUP raster plots. A) MUPT showing 17 consecutive firings of the same MU. B) The acceleration of the same MUPT showing the 2 peaks representing two fibres that significantly contribute to the MUP. The vertical lines over the MUP correspond to the two peaks in the NF MUP. Some variation in the timing and shape to the potentials is evident and this is quantified as NF jiggle, the variability in shape/area of the NF MUPs expressed as a percentage of the total shape area. NF jiggle for this MU = 19.8%.

Motor unit characteristics: firing rates and near fibre analysis

For firing rate analysis a MUPT was included if it had a minimum of 40 recorded potentials. The MUP amplitude was measured from the maximal positive and negative peaks, and the area was the total area within the MUP duration (onset to end). The number of phases of the template MUP was defined as the number of components above or below the baseline. A turn was defined as a change in direction of the waveform of at least 25 µV (Figure 2.4). DQEMG calculates a near fibre MUP (NF MUP) based on the acceleration of a MUP. This was identified by applying a second order low-pass differentiator to the MUP, thereby effectively creating a 350 µm radius, semi-circular recording area. This

ensures only the 'near' fibres significantly contribute to the NF MUP (Stashuk, 1999b). The NF fibre count is a measure of fibre density and was obtained from the NF MUP template (Figure 2.5). NF jiggle is a measure of the variability of consecutive NF MUPs, and thus can be an indication of NMJ transmission stability. It is expressed as a percentage of the total template NF MUP area (Figure 2.3).

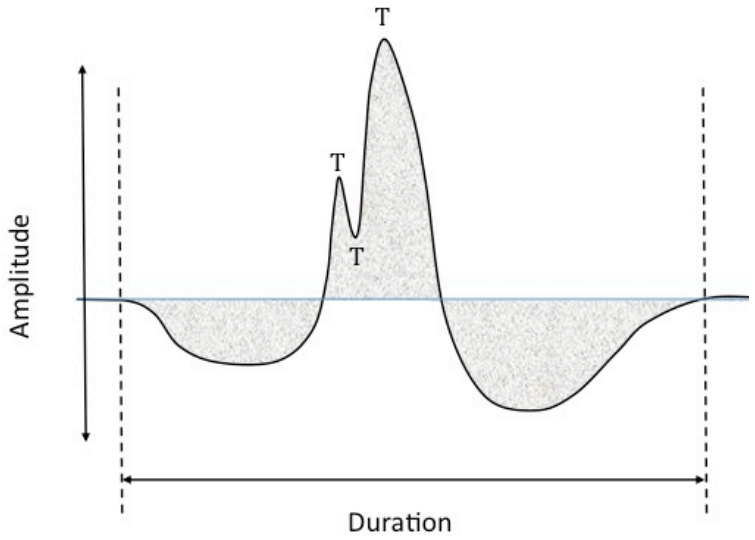

Figure 2.4 Motor unit potential. The summation of the muscle fibre potentials forms the motor unit potential (MUP), showing duration, amplitude, area (combined area of all phases), turns (T), and phases (shaded area).

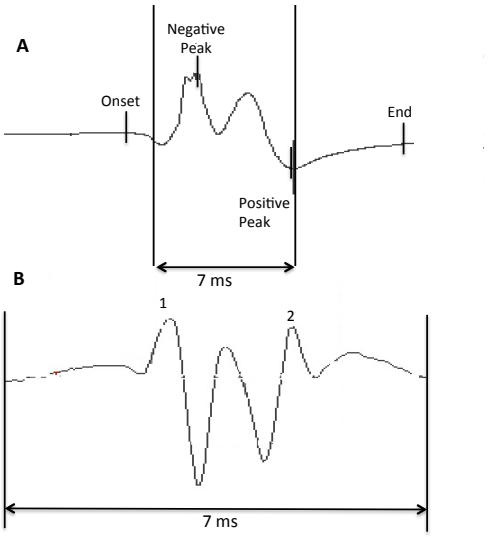

Figure 2.5. A MUP and corresponding NF MUP detected by iEMG. A MUP is high pass filtered in DQEMG using a second order low-pass differentiator to generate a NF MUP. The MUP (A) shows onset, positive peak, negative peak and end. The corresponding NF MUP (B) shows contributions from those fibres closest to the recording electrode.

Statistical analysis

MRI measurements were compared between locations using paired-samples t-tests. Linear mixed models were used to assess effects of contraction intensity, recording location and (for iEMG results only) needle depth on sEMG and iEMG results: these factors were fixed effects, with participant as a random effect. Data are presented as mean (SD) or median (IQR), unless otherwise stated. Statistical significance was accepted at $p<0.05$.

2.4 Results

General participant characteristics and MRI measurements of quadriceps and VL are shown in Table 2.1. The skin and subcutaneous tissue thickness did not differ between the proximal and distal locations. VL thickness and CSA were significantly larger at the proximal compared with distal location (all $p<0.0005$).

Table 2.1. Participant characteristics

Age (yrs)	27.3 (5.2)	
Height (cm)	176 (5.3)	
Weight (Kg)	73.2 (10.8)	
Peak quadriceps CSA (cm^2)	86.6 (14.9)	
MRI	Proximal	Distal
Fat + skin thickness (cm)	0.39 (0.07)	0.38 (0.06)
VL thickness (cm)	2.65 (0.23)	1.99 (0.17)***
VL CSA (cm^2)	29.84 (2.55)	14.25 (1.24)***

Data are shown as mean (SD). Peak quadriceps CSA was defined as the MRI slice that gave the largest value of quadriceps CSA. Significant differences between proximal and distal locations are shown as: ***p<0.0005. CSA: cross sectional area; MRI: magnetic resonance imaging; VL: vastus lateralis.

iEMG measurements

The mean number of MUs detected per person at 10% MVC was 13 ± 4 at the proximal site and 10 ± 3 at the distal site. This increased ($p=0.002$) at 25% MVC to 22 ± 6 MUs at the proximal site and 17 ± 7 at the distal site.

Motor units with larger MUPs, higher firing rates and greater NF jiggle were recorded during moderate intensity contractions (25% MVC) compared with contractions held at low intensity (10% MVC), (Table 2.2).

Motor units sampled around the proximal motor point had larger MUPs compared with those sampled distally (Table 2.2).

Differences between deep and superficial recordings existed for most of the iEMG measurements. Compared with superficial, the deeper sampled MUs had a

6% higher firing frequency (p=0.002) and MUPs with 10% larger area (p=0.065), 15% greater amplitude (p=0.009), 10% more phases (p<0.0005), 11% more turns (p=0.001) and 14% greater NF fibre count (p=0.007)(Figure 2.6).

Table 2.2. Motor unit potential characteristics recorded from intramuscular EMG signals

	10% MVC		25% MVC		Main effects	
	Proximal (n=134)	Distal (n=101)	Proximal (n=215)	Distal (n=170)	Contraction intensity	Location
MUP amplitude (μV)	469 (305-725)	469 (311-654)	605 (408-976)	520 (399-721)	**	***
MUP area (μV.ms)	1020 (700-1411)	893 (607-1416)	1141 (688-1796)	995 (666-1646)	**	***
Phases	3.06 (0.31)	2.90 (0.39)	2.96 (0.25)	3.26 (0.38)		
Turns	4.11 (0.33)	3.64 (0.87)	3.66 (0.38)	3.71 (0.51)		
NF count	1.82 (0.44)	1.63 (0.45)	1.69 (0.37)	1.61 (0.38)		
NF Jiggle (%)	22.1 (18.9-25.7)	21.6 (17.2-25.2)	23.5 (19-29.2)	22.8 (19.6-27.8)	*	
Mean firing rate (Hz)	8.7 (7.2-10)	8.5 (6.8-9.7)	9.7 (8.4-11.4	9.5 (8.3-11.2)	***	

Recordings were made around the proximal and distal motor points of VL during contractions held at 10% and 25% MVC. Linear mixed models were used to assess effects of contraction intensity, recording location and needle depth on iEMG results. Data shown as mean (SD) or, if not normally distributed, as median (IQR). MUPT: motor unit potential train; MUP: motor unit potential; NF: near fibre. Significant effects are shown as: *p<0.05; **p<0.01 ***p<0.0005.

sEMG measurements

There were no significant differences in CMAP or sMUP size between proximal and distal locations. The sMUP area was larger in the moderate compared with lower intensity contractions at both locations (p<0.0005).

The MUNE values were lower at 25% compared with 10% MVC contractions. Despite there being no significant differences in CMAP or sMUP size between proximal and distal locations, the small differences that did exist combined to give MUNE values that were significantly greater at the proximal compared to the distal location (Table 2.3).

A separate group of four participants completed additional contractions at 10%, 25%, 40% and 50% MVC. As contraction intensity increased, the MUP and sMUP areas significantly increased and since the CMAP is independent of voluntary

muscle activation, the MUNE values decreased significantly with increasing contraction intensity. The MUPs had more turns, higher NF jiggle and MUs had higher firing rates as contraction intensity increased (Table 2.4).

Table 2.3. Surface EMG parameters and MUNE

	Proximal		Distal		Contraction intensity	Location
CMAP Negative Peak Area (μV.ms)	92160 (26425)		88228 (16773)			
	10% MVC		25% MVC			
	Proximal	Distal	Proximal	Distal		
sMUP Negative Peak Area (μV.ms)	290 (112)	379 (128)	499 (239)	653 (265)	***	
MUNE	327 (96)	243 (74)	208 (87)	146 (47)	***	**

Data are shown as mean (SD). Significant effects are shown as: **p<0.01 ***p<0.0005. CMAP: compound muscle action potential; sMUP: surface motor unit potential; MUNE: motor unit number estimate.

Table 2.4. Individual motor unit complexity, stability and firing rates as a function of contraction intensity.

	10% MVC	25% MVC	40% MVC	50% MVC	Main effect
Number of MUs	n = 46	n=63	n=55	n=51	
MUP area (μV.ms)	818 (589-1738)	1183 (636-1804)	1314 (931-2388)	1388 (825-2316)	**
NF Jiggle (%)	20.9 (15.8-26.6)	24.3 (19.5-31.8)	25.5 (20.1-33.8)	26.8 (20.9-37.4)	*
Mean firing rate (Hz)	8.1 (7.3-9.4)	9.2 (8.6-10.9)	10.3 (9.4-12.2)	10.9 (10.3-13.6)	***
Phases	3 (0.76)	2.94 (0.76)	2.84 (0.86)	3.14 (1.13)	
Turns	3.33 (1.45)	3.67 (1.55)	3.18 (1.32)	4.31 (2.34)	**

Data are from three Young (aged 32 (2) years) and one Older man (aged 72 years) and were collected around the proximal motor point. Data are shown as mean (SD), or if not normally distributed as median (IQR). Main effects are shown as *p<0.05 **p<0.01 ***p<0.0005. MU: motor unit, MUP: motor unit potential, NF: near fibre

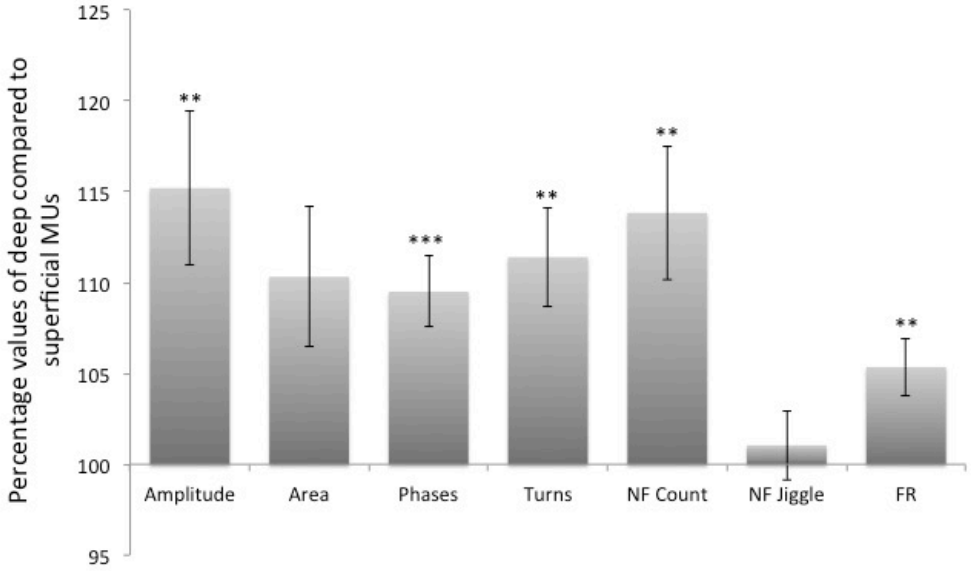

Figure 2.6. iEMG measurements from deep compared with superficial MUs in vastus lateralis. Variables are expressed as a percentage of the mean values from superficial recordings. As there was no location*depth interaction, proximal and distal locations are combined. NF: near fibre; MUP: motor unit potential; FR: firing rate. Data shown as mean ± SEM. Significant effects are shown as: **p<0.01 ***p<0.0005.

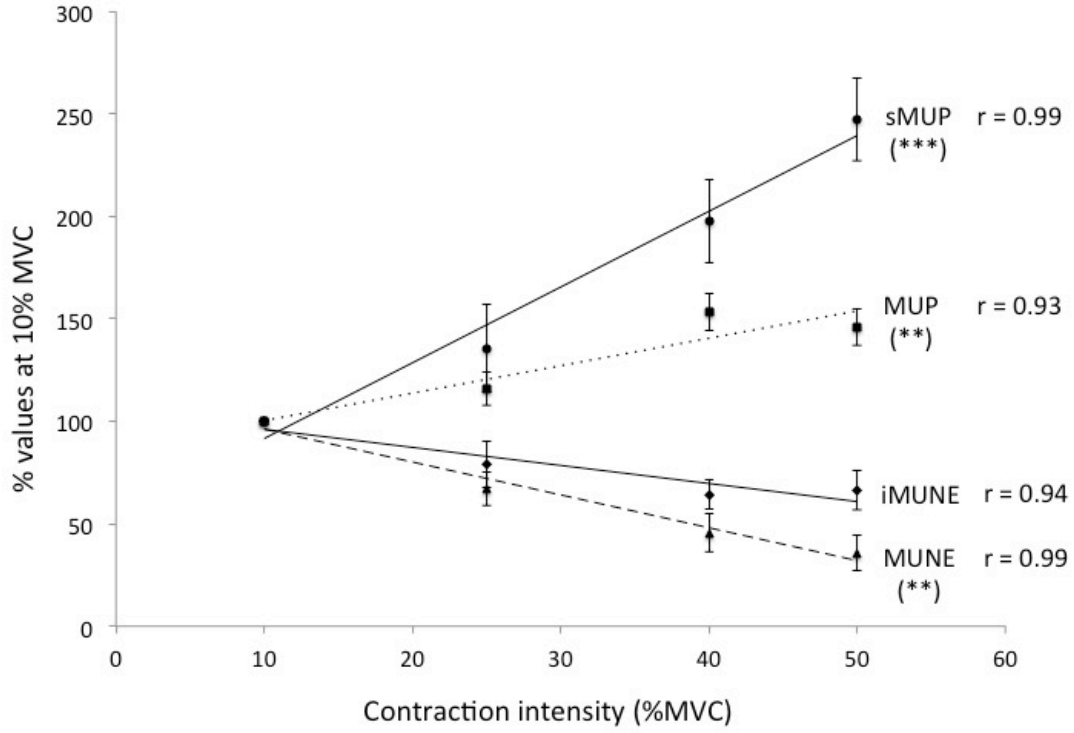

Figure 2.7. Motor unit number estimates, MUP area and sMUP negative peak area as a function of contraction intensity. sMUP: surface motor unit potential; MUP: motor unit potential; iMUNE intramuscular motor unit number estimate; MUNE: motor unit number estimate. Data are shown as mean (SEM). Main effects are shown as **p<0.01 ***p<0.0005. The main effect of contraction intensity on iMUNE was p = 0.056.

2.5 Discussion

In this study intramuscular and surface EMG signals of the VL were used to determine MUP size, firing frequencies and stability together with a MUNE value, as a function of contraction intensity, recording location and recording depth. The main finding was that MUs sampled in the deeper region of VL had, on average, higher firing rates and larger, more complex MUPs compared with superficially sampled MUPs. Individual MUPs recorded at the proximal motor point tended to be larger compared to those recorded at the distal motor point, but measures of complexity and stability were similar at the two sites. These results reveal the extent of variability in MU organisation within a single, large proximal muscle. The results have implications for studies designed to compare MU characteristics and numbers between people or to track changes over time, indicating the care that needs to be taken to standardise the location of recording sites.

The VL is a large knee extensor muscle that contributes to the power needed for activities such as standing from a chair, stair negotiation and cycling. Although the VL muscle architecture, function and fibre type composition are well characterised, very little is known about the MU organisation and recruitment.

Autopsy and physiological examinations have revealed multiple motor points in VL spread along the longitudinal axis (Botter *et al.*, 2011) which create distinct regions, each with separate nerve branches (Becker *et al.*, 2010). We chose to sample from the proximal (mid-muscle belly) and distal motor points because they have the largest physical separation and were easily identified using percutaneous electrical stimulations.

Effects of recording location and contraction intensity

As expected from the 'size-principle' of MU recruitment (HENNEMAN *et al.*, 1965; Milner-Brown *et al.*, 1973), larger MUPs, indicating larger MUs, were recruited at 25% compared to 10% MVC (Table 2.2) and the data in Figure 2.7 and Table 2.4 suggest a further increase in size of recruited MUs to 40% MVC,

but no difference in MUP size between 40 and 50% MVC contractions. There were higher mean firing frequencies at 25% compared to 10% MVC, which is similar to previous reports in vastus medialis (VM) (Conwit *et al.*, 1999; Roos *et al.*, 1999) and is consistent with larger and faster MUs being recruited. The MUPs recorded during higher intensity contractions had more complexity (higher number of turns), which is indicative of asynchronous firing of, or asynchronous detection of fibres that are part of the same MU (Zalewska & Hausmanowa-Petrusewicz, 1999; Zalewska *et al.*, 2004).

Jiggle is a measure of the shape variability of an individual MUP over successive firing (Stålberg & Sonoo, 1994) which is used for clinical assessments of neuropathy or myopathy. Calculating the jiggle from a small recording volume, which has been termed NF jiggle, minimizes the level of contamination from more distant fibres which may artificially increase the variation in MUP shape (Hourigan *et al.*, 2015). Higher values for NF jiggle at higher contraction intensities were previously reported in the VM (Hourigan *et al.*, 2015) and the tibialis anterior muscles (Allen *et al.*, 2015; Hourigan *et al.*, 2015). The combined data from these studies indicate that MUs in healthy muscle experience around 20-30% NF jiggle.

We report here for the first time that MUPs were larger at the proximal motor point compared with the distal location, independently of contraction force. We are not aware of any previous studies comparing electrophysiological or muscle fibre type and morphological characteristics at these two locations in VL. The larger MUPs could originate from larger fibre cross-sectional area due to larger diameter fibres giving a higher electrical potential, or more fibres within the MU (Zalewska & Hausmanowa-Petrusewicz, 1999). Either way, the total area occupied by fibres within individual MUs was larger in the mid-belly compared with distal regions, but measures of MU firing frequency, complexity and stability were similar at proximal and distal locations.

Effects of recording depth

A notable finding of the present study was the extent of the differences in electrophysiological characteristics between the superficial and deep motor units. The deeper MUs had higher firing rates and MUPs tended to be larger and more complex compared with superficial MUPs at both sites. This further supports the idea of a level of MU organization within the VL, as larger MUs contain more fibres, and are therefore more likely to occupy a greater territory of which individual fibre contributions may be detected at slightly different times, increasing the number of phases and/or turns. This also reflects the previously reported differences in fibre cross-sectional area, where deep fibres were larger than superficial in VL (Lexell & Taylor, 1991). Our results conflict with those previously reported by Knight and Kamen (2005), who found larger MUPs in superficial compared with deep VL. However, these authors described inserting the concentric needle electrode 4.7 cm into the muscle, but the MRI images (Figure 2.1) clearly show that the VL muscle thickness was, on average, only 2.7 cm at the mid-muscle belly, so it is not clear whereabouts in the vasti muscles these authors sampled. It is also possible that when recording from deeper regions of VL there may be a level of crosstalk from vastus intermedius (Figure 2.8), but such effects are expected to be minor due to attenuation of the signal through VI and VL aponeuroses and muscle tissues.

Our data indicate that the MU organisation in VL differs between the superficial and deep regions when measured in a transverse plane. It is not clear how this relates to the pennate fascicle architecture of VL (Figure 2.8) (Fukunaga *et al.*, 1997). One possibility is that fibres may not necessarily span the entire length of the fascicles in large muscles, as was shown in animals (for review see, (Trotter, 1993; Monti *et al.*, 1999).

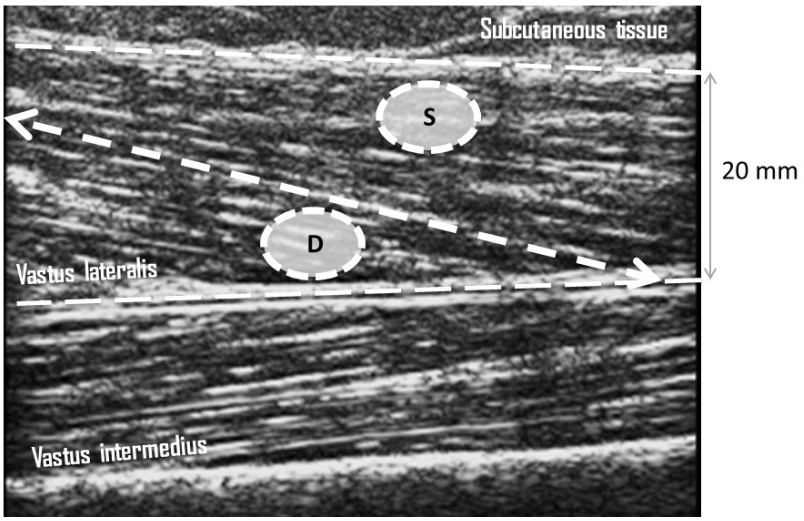

Figure 2.8. Ultrasound image showing vastus lateralis and intermedius around the proximal VL motor point. The superficial and deep VL aponeuroses and a fascicle have been highlighted as white dashed lines. The superficial (S) and deep (D) iEMG target-sampling areas are indicated as pale shaded areas.

sEMG and motor unit number estimates

The negative peak area of the CMAP elicited by supramaximal stimulation of the femoral nerve was similar at the proximal and distal motor points. The CMAP reflects the amount of excitable tissue within range of the surface recording electrodes (Wee, 2006; Severinsen & Andersen, 2007) and it might therefore be expected that the CMAP would be larger at the proximal site where the muscle has its largest thickness (Table 2.1). However, the effective recording depth of surface electrodes is around 2 cm (Barkhaus & Nandedkar, 1994) and most of the surface-recorded signal is composed of potentials originating from the superficial MUs due to substantial attenuation of the signals from deeper MUs (Muceli *et al.*, 2015). Consequently, it is not surprising that CMAPs were not significantly different between the two locations.

A sMUP is a surface representation of the potentials created by a single MU. The negative peak areas of the sMUPs were similar at proximal and distal motor points (Table 2.3), which contrasts with the fact that larger intramuscular MUPs were recorded at the proximal site (Table 2.2). The size of a sMUP in relation to the intramuscular MUP will depend on the distance of the MU from the surface (Muceli *et al.*, 2015) and given the tendency for larger MUs to be located deeper

in the muscle at the proximal site (Figure 2.6), it is likely that the larger units may be underrepresented in the surface-recorded sMUP. This highlights a limitation with the standard technique to derive MUNE values for large muscles.

The MUNE values are derived by dividing the CMAP negative peak size by the mean sMUP negative peak size (McComas *et al.*, 1971; Brown *et al.*, 1988). For a large muscle such as the VL, MUNE is not an absolute value of the number of motor units in the muscle, but rather an estimate of the numbers within the recording volume of the surface-electrodes. Two MUNE values are reported here, and it is recommended they are combined to produce an estimate of the entire muscle. The values also differed between the proximal and distal locations, and higher intensity contractions resulted in progressively larger sMUPs (Figure 2.7) and a corresponding decline in MUNE so that for a 50% MVC MUNE values were only half those obtained at 10% MVC.

In large muscles, an alternative approach may be more appropriate to derive a MU number estimate that does not depend on any measurements taken from the surface. Assuming that MUP area is proportional to the size of the MU, dividing muscle cross-sectional area by mean MUP area provides a value which is proportional to the total number of MUs in the muscle cross-section, an intramuscular recorded MUNE (iMUNE). When using data collected during 10% MVC, iMUNE gives values of 29.3 and 15.9 cm^2/mV.ms, for proximal and distal sites, respectively and for a 25% MVC the values were 26.1 and 14.3 cm^2/mV.ms for proximal and distal sites, respectively, the difference between the two sites being primarily due to differences in muscle CSA. The iMUNE values for the four subjects for whom data were collected up to 50% MVC are shown in Fig 2.7 and it is evident that the iMUNE values are less affected by contraction force than the surface recorded MUNE.

Limitations

The electrophysiological data presented here were collected from two distinct locations along the lateral, longitudinal axis of VL, but this still represents only a small proportion of the large locomotor muscle. A range of low, moderate and higher intensity voluntary contractions were assessed up to 50% MVC (in a

separate group of four subjects). Holding the brief contractions at 50% MVC repeatedly may have caused fatigue that affected MU recruitment, although rest periods of at least 30 sec were provided between efforts and participants tolerated it well. During the higher intensity contractions (40% and 50% MVC) it was increasingly difficult to decompose the iEMG signals to identify individual MUPTs due to the overall high levels of electrical activity and this limited our efforts to collect data during even higher intensity contractions.

Conclusion

The VL muscle shows distinct spatial organisation of motor units. Proximally recorded MUPs are larger than those recorded distally, but complexity and stability were similar at the two locations. Deeper recorded MUPs were larger than superficially recorded MUPs, more complex and had increased fibre density. MUNE values vary dependent on the contraction force and are limited when derived for large muscles due to attenuation of surface-recorded potentials. For practical purposes, therefore, it is very important to standardize both the location and depth of intramuscular recordings and to specify the contraction force. An alternative estimate of MU number based on intramuscular recorded MUPs and muscle CSA may be a more reliable method to be used with large muscles.

Chapter 3

Age-related neuromuscular changes affecting human vastus lateralis

Results presented in this chapter are based on an article submitted to and accepted by The Journal of Physiology.

Authors:

Piasecki M; Ireland A; Stashuk D; Hamilton-Wright A; Jones DA and McPhee JS

3.1 Abstract

Introduction: The anterior thigh muscles are particularly susceptible to muscle loss and weakness during ageing, but it is unclear how this is associated with changes to neuromuscular structure and function in terms of motor unit (MU) number, size and MU potential (MUP) stability.

Methods: Intramuscular and surface electromyography (EMG) signals were recorded from the vastus lateralis (VL) during voluntary contractions held at 25% maximal knee extensor strength in 22 Young (25.3±4.8yrs) and 20 physically active Older men (71.4±6.2yrs). MUP size, firing rates, phases, turns and near fibre (NF) jiggle were determined and motor unit number estimates (MUNE) were made by comparing average surface MUP with maximal electrically-evoked compound muscle action potentials. Quadriceps cross-sectional area was measured by magnetic resonance imaging.

Results: 379 individual MUs were sampled in Young and 346 in Older men. Compared with Young, the Older MUs had 8% lower firing rates and larger MUP size (~+25%) as well as increased complexity evidenced as, phases (+13%), turns (+20%) and NF jiggle (+11%) (all $p<0.0005$). The MUNE values (derived from the area of muscle in range of the surface-electrode) in Older subjects were around 70% of the Young ($p<0.05$). Taking into consideration the 30% smaller cross-sectional area of the VL, the total number of MUs in the Older muscles was between 50-60% lower than in the young ($p<0.0005$).

Conclusion: A large portion of the VL MU pool is lost in older men and those recruited during moderate intensity contractions were enlarged and less stable. These MU changes were evident before clinically relevant changes to muscle function were apparent; nevertheless, the changes in MU number and size are likely to be a prelude to future movement problems.

3.2 Introduction

A decrease in mobility and a loss of independence are common experiences of ageing and while there are many factors contributing to this, a loss of muscle mass and function are central to the process. Muscle can only function in tandem with the nervous system. Neural control of muscle is organized through motor units (MU) which consist of a single alpha motor neuron and all of the muscle fibres it innervates (Sherrington, 1925), and in large limb muscles there are many thousands of MUs (e.g. see (Tomlinson & Irving, 1977). In considering the reasons for age-related loss of muscle mass and function and the factors that might influence any changes, for better or worse, it is clearly essential to take into account any alterations in the organization and control of the motor units.

Post mortem anatomical studies have shown that compared with young adults, those aged around 75 yrs have approximately 30% fewer motor neurons supplying muscles of the lower limbs (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987), while Lexell (1988) reported 40% fewer fibres in the vastus lateralis of older quadriceps muscles. Such studies clearly indicate the probable importance of neurological changes affecting muscle function, but methods that can be used with living human subjects are required for more detailed investigations.

Campbell et al (1973), recorded evoked potentials and compared these with a maximal CMAP to estimate there to be around 50% fewer MUs in the extensor digitorum brevis of people over 60 years of age. However, there is some uncertainty about this technique; the main problem being *alternation*, occurring when MUs with similar depolarisation thresholds fire individually, or together, to produce EMG signals that reflect either the single or the simultaneous firing of the different MUs (McComas *et al.*, 1993). These complications are largely overcome by intramuscular recordings from single MUs during voluntary contractions and using 'spike triggered averaging' to extract MU potentials (MUP) from the surface recorded signal (Brown *et al.*, 1988). Subsequent

developments in signal decomposition enhancement have removed most of the subjective interpretations of complex electromyography (EMG) signals (for review see (Gooch *et al.*, 2014). By comparing the average size (either area or amplitude) of the surface MUP (sMUP) with that of a CMAP elicited by supramaximal stimulation of the motor nerve, it is possible to estimate the number of MU in the muscle, providing a motor unit number estimate (MUNE) value.

Motor unit number estimate values determined using signal decomposition and spike triggered averaging have shown older people to have fewer MUs in peripheral limb muscles, such as the tibialis anterior and soleus (Galea, 1996; McNeil *et al.*, 2005b; Dalton *et al.*, 2008; Power *et al.*, 2010; Hourigan *et al.*, 2015). However, no MUNE data are available for the anterior thigh muscles which provide the power needed for daily locomotion, such as stair negotiation or rising from a seated position. It is important to obtain MU data on the thigh muscles since not only are they functionally relevant, but they also appear to be particularly susceptible to the effects of ageing. For instance, the leg muscles undergo greater loss of mass with ageing than those in the upper body (Janssen *et al.*, 2000) and even the muscles of the legs do not deteriorate equally during ageing. Cross-sectional studies show the size of the hamstrings, triceps surae and tibialis anterior muscles to be relatively well preserved and the quadriceps most affected by age-related losses (Abe *et al.*, 2011; Maden-Wilkinson *et al.*, 2013; Abe *et al.*, 2014). Furthermore, a longitudinal study of 12 older people investigated at ~71 years and again at ~80 years found a reduction in the cross sectional area of the anterior thigh muscles, but no such reduction in the posterior thigh muscles (Frontera *et al.*, 2008). It is possible that the higher proportion of fast type 2 fibres in the VL makes it more susceptible to atrophy, as animal models have shown type 2 fibres to be more susceptible to loss (Kadhiresan *et al.*, 1996). Together, these studies indicate the quadriceps are more susceptible to age-related atrophy and are therefore of particular interest in studies of neuromuscular decline.

In addition to MUNE values, intramuscular EMG recordings provide details of MU firing rates as well as shape and stability of the MUPs, which are associated with MU remodelling. Specifically, these are evidenced in terms of MUP area, number of turns, number of phases and jiggle, the latter statistic reflecting variations in individual MUP shapes (Stashuk, 1999b; Abdelmaseeh *et al.*, 2014). A novel "near fibre" method has recently been developed which allows examination of contributions only from fibres located very close to the recording needle electrode, thereby reducing artefact or attenuation as signals pass through muscle, fat and connective tissues (Allen *et al.*, 2015).

Hourigan et al (2015) recently identified larger MUPs and, for the first time in healthy ageing, found increased near-fibre jiggle in the vastus medialis (VM) of 9 Older compared with 9 Younger men, indicative of larger MUs due to reinnervation and increased NMJ transmission variability (Stålberg & Sonoo, 1994). Ling et al (2009) detected surface MUPs from intramuscular recordings in vastus medialis to show that Older people recruited larger MUs than Young during low and moderate intensity contractions. However, MUNE values and muscle size were not obtained in either of these studies (Ling *et al.*, 2009; Hourigan *et al.*, 2015).

The purpose of the present study was to apply the range of recently developed techniques for studying MU number and organization to the question of age-related changes in the quadriceps muscle, a muscle that is functionally important for mobility but is also one that appears to be particularly susceptible to the effects of ageing.

3.3 Methods

Participants and recruitment

The study was approved by the University Research Ethics Committee and all participants provided written informed consent. Volunteers were included if they were male, aged between 18-35 years or 65-90 years and habitually physically active. Volunteers were excluded if they were sedentary or competing in sports at a regional level or above, had a recent history of bone fracture or neuromuscular, metabolic or cardiovascular diseases. All older participants were asked to self-rate the amount of time they had performed physical activities for health, fitness or leisure purposes (not including usual domestic activities) in the previous 7 days, and if the previous 7 days was representative of a 'normal' week.

Anthropometry and general mobility

Body mass (kg) and height were measured and total body composition assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, version EnCore 10.50.086) with the participant lying supine with legs and arms fully extended. Details of fat mass and lean mass of the whole body and of the body segments on the left and right sides were recorded. Sarcopenia (clinically-relevant loss of muscle mass) was defined according to the original criteria as appendicular lean mass divided by height-squared (ALM/h^2) (Baumgartner *et al.*, 1998). An ALM/h^2 of <6.76 was used to identify older participants as sarcopenic, indicating a value below two standard deviations of the mean of a Young, male European reference population (Bijlsma *et al.*, 2013).

The sarcopenia definitions that also include assessments of grip strength and poor mobility were additionally used to characterise the participants (Cruz-Jentoft *et al.*, 2010). Grip strength was assessed using a Jamar hand grip dynamometer. The right hand and left hand were tested separately, three times each, with the participant standing and arms extended by their side (McPhee *et al.*, 2013). Weakness was defined as having a grip strength below 32 kg, which is two standard deviations below the average of a young, male European reference

population (Dodds *et al.*, 2014). The Short Physical Performance Battery (SPPB) (Guralnik *et al.*, 1994) was used to identify clinically-relevant limitations to balance, walking speed and ability to stand from a seated position. A maximum of 12 points indicates no mobility limitations, while less than 8 has been used to indicate sarcopenia or frailty (Bauer *et al.*, 2015). Balance was assessed in three ways with increasing difficulty, and a maximum of 4 points could be gained. The participant was required to stand upright, with arms beside their body and to stand for 10 sec without needing support or taking a step, first with feet side-by-side as close together as possible (one point); secondly, with feet in a semi-tandem position (one point) and finally with the feet fully tandem (two points for achieving 10 sec, one point for achieving between 4-9.9 sec). During tests of 'normal' gait speed, four points were awarded for completing the 4 m track in less than 4.8 sec; three points for 4.8-6.2 sec; two points for 6.2-8.7 sec; one point for >8.7 sec and no points for failure to walk 4 m. The five-times-chair-rise test required participants to stand-up and sit-down five times as quickly as possible. Four points were awarded for completing the task in <11.2 sec; three points for 11.2-13.6 sec; two points for 13.7-16.6 sec; one point for >16.6 sec and no points for failure to complete the test.

Knee extensor size

Magnetic resonance imaging (MRI) was used to measure peak quadriceps and vastus lateralis muscle anatomical cross sectional areas at the proximal motor point using a T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoe, Italy). Contiguous transverse-plane slices of 6 mm thickness were collected. Images were analysed off-line using Osirix imaging software (Osirix medical imaging, OsiriX, Atlanta, USA) and the slice with the highest quadriceps cross-sectional area as well as the cross-sectional area of the vastus lateralis at the proximal motor point were recorded.

Experimental procedures, EMG acquisition and analysis in VL

The experimental procedures for knee extensor MVC, EMG acquisition and EMG signal decomposition and analysis have been described in Chapter 2, Section 2.3. In the current study the experimental procedures apply to the proximal motor point of the VL. Additionally the iEMG signal intensity is reported here in Young and Old, described as the number of pulses per second (pps). Signal intensity is the average, maximum number of peaks/s across 500 ms windows within the iEMG signal, and is a reflection of the level of muscle activity within the recording area of the needle electrode. As well as the MUNE value described in Chapter 2, an additional value was calculated by dividing the negative peak amplitude of the CMAP by the negative peak amplitude of the averaged sMUP, rather that the negative peak areas (Figure 3.2b).

Statistical analysis

Motor unit potential parameters and MUNE values from Young and Old were not normally distributed and were compared using a Mann-Whitney test. The CMAPs and sMUPs were compared between Young and Old using an Independent Samples t-test. All correlations were done using the Spearman's correlation coefficient (r). Data are presented as mean (SD) or median (IQR), unless otherwise stated. Statistical significance was accepted at $p<0.05$.

3.3 Results

Participant characteristics are shown in Table 3.1. The older participants reported physical activities for health, fitness or leisure purposes exceeding 4 hours in total over the previous 7 days, and it was representative of their usual activity levels.

Young and Old were similar in height, weight and BMI. The Old had higher total body fat percentage than Young and all but one Older man achieved full marks in the SPPB. Young were stronger than the Older men and had larger quadriceps muscles. Anterior thigh fat thickness and femur bone CSA did not differ between young and old.

Table 3.1 – Participant characteristics

	Young (n=22)	**Old (n=20)**
Age (yrs)	25.3 (4.8)	71.4 (6.2)***
Physical activity level (hrs/wk)	-	6.9 (1.7)
Height (cm)	177 (6)	173 (7)
Weight (Kg)	75.8 (12.5)	75.9 (11)
BMI (kg/m^2)	23.9 (3.7)	25.3 (3.7)
Body fat (%)	14.8 (7.2)	23.8 (7.9)**
ALM/h^2	9.58 (1.17)	8.57 (0.59)**
SPPB score	12 (0)	11.8 (0.4)
Grip strength right hand (kg)	50.4 (8.6)	40.3 (7.1)**
Grip strength left hand (kg)	47.4 (6.9)	38.4 (7.5)**
Knee extension MVC (N)	500 (158)	341 (109)**
Anterior thigh fat thickness (cm)	0.36 (0.16)	0.36 (0.14)
Quadriceps CSA (cm^2)	89.2 (14.9)	59.8 (7.5)***
VL CSA (cm^2)	29.2 (6.2)	18.2 (4.3)***
Femur CSA (cm^2)	6.28 (0.44)	6.27 (0.67)

Data are shown as mean (SD). Significant differences between young and old are shown as: *p<0.05 **p<0.01 ***p=<0.0005. BMI: Body mass index; SPPB: short physical performance battery; MVC: maximal voluntary contraction; CSA: cross sectional area; VL: vastus lateralis.

Motor unit potential characteristics

All participants were able to perform voluntary knee extensions with the concentric needle in position without reporting any adverse reactions.

The iEMG data are shown in Table 2. A mean of 20±5 individual MUs (capturing both MUPs and sMUPs) were sampled per younger man and 17±5 per older man. This gave a total of 379 MUs analysed in the Young and 346 in the Old.

Compared with Young, the Old MUs had larger MUPs with more phases and turns as well as increased fibre count and NF jiggle, which is associated with MU remodelling via reinnervation with nascent NMJs.

Figure 3.1 shows a clear rightward shift in the MUP size distribution in Old men as they recruited a higher proportion of larger MUs than Young in order to generate the same relative force. The MU discharge rate was slower in Old compared with Young.

Table 3.2 – Motor unit potential characteristics

	Young (n=379 MUs)	**Old (n=346 MUs)**
MUP amplitude (μV)	540 (359-856)	664 (455-1052)***
MUP area (μV·ms)	1050 (589-1619)	1353 (914-1982)***
Number of Phases	2.97 (0.94)	3.36 (1.13)***
Number of Turns	3.69 (1.64)	4.41 (2.25)***
Average intensity (pps)	53.1 (19.4)	37.8 (2.0)*
NF count	1.66 (0.98)	1.97 (1.25)***
NF Jiggle (%)	23.7 (19.3-29.5)	26.2 (4.92-32.5)***
NF MUP area (kV/s^2)	2.83 (1.72-5.08)	3.88 (2.26-6.21)***
NF MUP duration (ms)	2.16 (1.56-2.92)	2.84 (2.12-3.96)***
Discharge rate (Hz)	9.56 (8.29-11.3)	8.81 (7.65-10.49)***

Motor Unit (MU) recordings were collected from six spatially distinct regions around the proximal vastus lateralis (VL) motor point during voluntary contractions held at 25% maximal voluntary strength. Data are shown as mean (SD), or if not normally distributed as median (IQR). Significant differences between young and old are shown as: *p<0.05; ***p<0.0005. MUP: motor unit potential; NF: near fibre.

Spike-triggered averaging determination of surface motor unit potentials and MUNE

Table 3.3 shows the MUP characteristics collected from sEMG. The CMAP was significantly larger in the young compared with old. Although the MUPs were larger in Old compared with Young (Figure 3.1), the representations from surface-recorded ensemble-averaged sMUPs did not differ between young and old (Table 3.3). Figure 3.2a shows example raw traces for CMAP and mean sMUP potentials. Figure 3.2b shows the MUNE values in young and old, the median values being 195 for the Young and 139 for the Old subjects, when calculated based on area of the CMAP and mean sMUP, and 111 and 78 for Young and Old, respectively, when calculated based on amplitude. The MUNE values from older men were 71% and 70% of the values for the Young when based on area (p=0.008) and amplitude (p=0.018), respectively.

There was a strong correlation between all MUNE values derived from area and amplitude (r=0.808; p<0.0005).

Table 3.3 – Surface EMG parameters

	Young (n=22)	Old (n=20)
CMAP Area (μV·ms)	95125 (20594)	60371 (21336)***
CMAP Amplitude (μV)	11260 (2832)	7690 (2360)***
Mean sMUP Area (μV·ms)	515 (251)	415 (130)
Mean sMUP Amplitude (μV)	96.7 (39.2)	87 (39.8)

Surface EMG recordings were made over the motor point of VL during contractions held at 25% maximal voluntary strength. Data are shown as mean (SD). Significant differences between young and old are shown as ***p<0.0005. CMAP: compound muscle action potential; mean sMUP: surface motor unit potential.

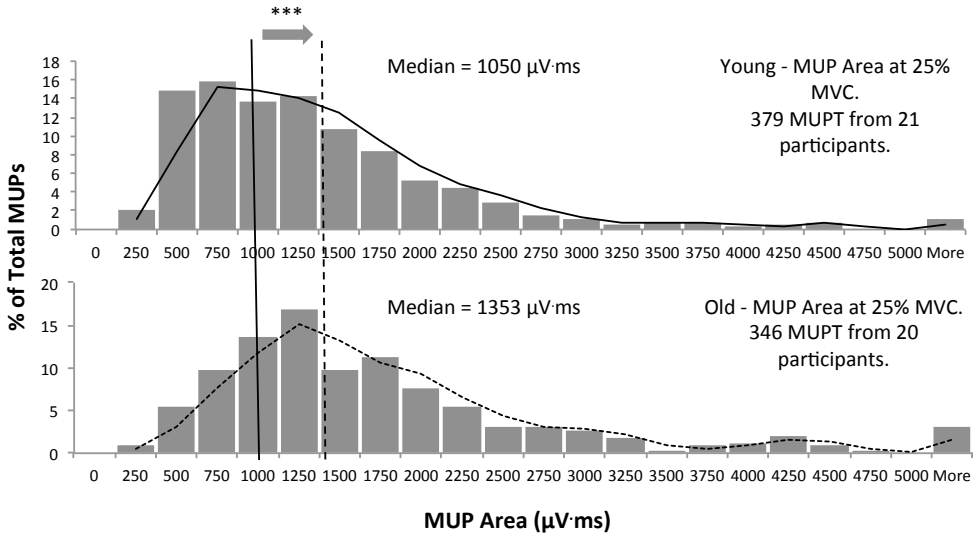

Figure 3.1. Frequency distribution of MUP areas detected by iEMG. Data were collected using concentric needle electrodes in young (top) and old (bottom) from six spatially distinct regions around the proximal vastus lateralis motor point during voluntary contractions held at 25% maximal voluntary strength. The vertical lines indicate the median motor unit potential (MUP) area for young (solid) and old (dashed). The significant difference between young and old is shown as: ***p<0.0005. MUP: motor unit potential.

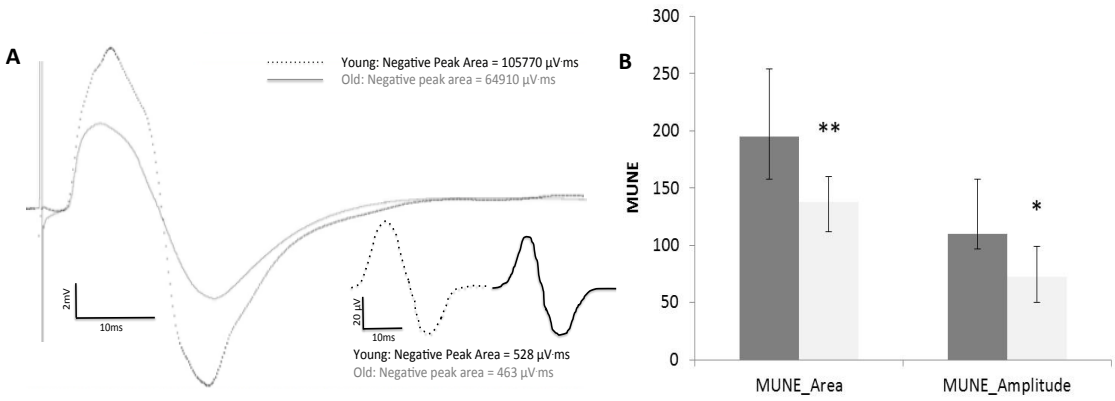

Figure 3.2. MUNE values in Young and Old men. A. Example raw traces for CMAP and mean sMUPs (inset). B**.** MUNE values derived from calculations based on area or amplitude of the mean sMUP and the CMAP. Data are median IQR. Dashed lines are young, solid lines are older men. Significant difference between young and old represented as *p<0.05; **p<0.01.

3.4 Discussion

The aim of this study was to investigate the nature and extent of MU changes in the vastus lateralis of healthy, independent living, physically active Old men. Compared with Young, the Old men had fewer MUs and smaller quadriceps muscles. The MUs that remained in the older participants were enlarged with less stable NMJ transmissions and slowed firing rates. Consequently, the Old men recruited larger, and presumably fewer, MUs in order to maintain the same relative external force as Young. The extent of motor unit loss is considerable even in relatively healthy Old men and would be expected to have consequences for motor control.

Participants

The participants recruited for this study were living independently and exceeded the recommended minimum physical activity levels (DOH, 2011). None of the older participants could be classified as having muscle weakness according to cut-off values based on grip strength (Dodds *et al.*, 2014) and all but one gained maximum marks in the SPPB: a test used to identify clinically significant mobility limitations (Guralnik *et al.*, 1994). Furthermore, none of the older men were sarcopenic according to the original definition of muscle loss with ageing (Baumgartner *et al.*, 1998) or the more recent version that combines lean mass, grip strength and mobility (Cruz-Jentoft *et al.*, 2010). The lower MU number and function in older men therefore occurred prior to the onset of clinically-relevant muscle wasting, weakness and mobility impairments.

Knee extensor size and strength

Even though the older participants were relatively healthy, physically active, non-obese and similar in stature to Young, their quadriceps cross-sectional area was 67% that of the Young and their MVC was proportionately reduced at 68%. These muscle changes are similar in magnitude to those previously reported in otherwise healthy older men and women (Maden-Wilkinson *et al.*, 2013) of this

age and show the extent of thigh muscle loss and weakness in what would be otherwise known as 'non-sarcopenic' older men.

The vastus lateralis is representative of the general quadriceps in terms of atrophy with age (Maden-Wilkinson *et al.*, 2013), and is a superficial muscle accessible for intramuscular and surface EMG and the femoral nerve can be easily stimulated to record a CMAP. The VL is a muscle that is frequently biopsied so the fibre morphology and physiology have been extensively studied, and it has a large cross sectional area which minimises EMG cross-talk from nearby muscles.

Motor unit potentials:

The iEMG recordings in the Old men showed higher values for MUP size (area, amplitude), complexity (number of phases, number of turns) and fibre density (NF count), along with slower discharge rates when compared to the Young (Table 3.2). The VL MUP amplitudes and areas were 23% and 29%, respectively, larger in Old compared to Young. Area and amplitude of the MUPs correlated strongly ($r=0.871$; $p<0.0005$).

Larger MUPs are generally taken to indicate MUs with a greater number of muscle fibres, giving a higher innervation ratio, and this is consistent with the increased near-fibre count in older subjects (Table 2). The process of collateral reinnervation of orphaned fibres that increases the number of fibres per MU as a compensatory mechanism (McComas *et al.*, 1993; Luff, 1998) serves to preserve total muscle force generating capacity against the backdrop of motor neuron losses. Reinnervation is not completely successful, however, as around 40% of fibres in VL are lost by age around 75 yrs (Lexell *et al.*, 1988) although our own estimates based on unpublished data for differences in muscle fibre and quadriceps size suggest a figure closer to 20% loss in healthy ageing. Other changes possibly also affecting MU function in the Old are increased NF jiggle, number of turns and phases (Table 2) indicating discharge variability and

asynchronous action potential transmission within the same MU (Nandedkar *et al.*, 1988a).

The MU discharge rates were, on average, around 10 Hz in young and 9 Hz in old, with ranges from 5-18 Hz in young and 5-15 Hz in old. This modest reduction in the older subjects is similar to reports of the TA (Connelly *et al.*, 1999; Patten *et al.*, 2001; Klass *et al.*, 2008) and FDI (Kamen *et al.*, 1995), although a previous study in VM showed no difference between young and old MU discharge rates (Roos *et al.*, 1999). The slightly lower discharge rate in the older subjects may be related to altered central drive (Klass *et al.*, 2008), but it is also likely that the older participants achieved the required external force (25% MVC) by recruiting fewer MUs (i.e. a greater reliance on early recruited MUs that have lower firing rates than higher threshold MUs (Conwit *et al.*, 1999)), which were contributing a higher force per MU compared with the young (Ling *et al.*, 2009).

The finding of larger MUPs in healthy older people was first highlighted in the extensor digitorum brevis by Campbell et al (1973), but has since been shown across a range of limb muscles. The technique of incremental nerve stimulation has been used to reveal larger MUP area in old compared with young in the extensor digitorum brevis and thenar muscles (Galea, 1996). The MUP amplitude was larger in older soleus compared with young across a range of voluntary contraction intensities (Dalton *et al.*, 2008). More recently, MUP area was found to be higher in vastus medialis of old compared with young subjects when holding a voluntary contraction matched for 'average pps intensity' (Hourigan *et al.*, 2015). Matching pps may not be a good way of comparing young and old since we found pps to be lower in old compared with young when working at the same relative external force (Table 3.2). The increased NF jiggle in older men reported by Hourigan (2015) was similar to the values reported here for VL muscle (Table 3.2). The higher NF jiggle is taken to indicate variability in muscle fibre potential transmission along fibres within the MU possibly due to the increased transmission variability of nascent NMJs of newly reinnervated fibres (discussed in detail by Hourigan *et al.* (2015)).

In contrast to the present observations, Galea (1996) found MUP area to be around 25% smaller in older biceps brachii, which may indicate that motor axon 'sprouting' could be less successful in the biceps brachii. However, studies of age-related changes to MUs in the biceps brachii have provided mixed results (Brown *et al.*, 1988; Doherty *et al.*, 1993; McComas *et al.*, 1993; Power *et al.*, 2012).

Motor Unit Number Estimation

The VL MUNE values were positively associated with VL muscle size, indicating that loss of motor neurons contributes to loss of muscle mass with ageing, although significance of this relationship was lost when limiting the correlation to only the smaller group of older men. Previous studies did not report muscle size in relation to MUNE values, although Kaya et al (2013) used a non-invasive index of motor unit numbers to show low values were associated with muscle weakness.

The MUNE values depend on two measurements: the area or amplitude of the surface recorded CMAP, and the area or amplitude of a mean sMUP. On average, the sMUP areas of the Old were 81% of those of the Young while the older CMAPs were 68% of the Young. Area-based MUNE values for the Old subjects were 71% of those of the Young, while the amplitude-based MUNE values were 70% of those of the Young (Figure 3.2b). Although MUNE values are a widely used representation of motor unit numbers, there are aspects of this methodology that require some comment, particularly relating to the CMAP.

The CMAP is obtained by maximally stimulating the motor nerve and the expectation is that all motor units in the muscle will have been activated and thus the CMAP is the summation of the electrical activity of all the motor units in the muscle. This depends, however, on the volume of muscle "seen" by the recoding electrodes. For a small muscle such as the FDI or Thenar, the surface electrode's recording area will likely encompass the whole muscle. Several studies have been based on the assumption that the CMAP is related to the total

number of axons, and thus, presumably MUs, within the muscle (Kurokawa *et al.*, 1999; Wee, 2006; Severinsen & Andersen, 2007), although the occurrence of cross-contamination from other muscles may be a problem. For a large muscle such as the VL it is unlikely that the CMAP represents the firing of all units. The MUNE values we find for the VL (Figure 3.2b) are similar to those reported for other smaller muscles (Galea, 1996; McNeil *et al.*, 2005b; Dalton *et al.*, 2008; Power *et al.*, 2010; Power *et al.*, 2012). This suggests that the MUNE values presented here for the VL are an estimate of the number of MUs within a volume of muscle rather than the whole muscle.

Barkhaus & Nandedkar (1994) estimate that the CMAP is recoded from a depth of about 2 cm, which is a small proportion of the total VL in Young and Old subjects. The CMAP recorded from the Older subjects was consistently smaller than that from the Young, an observation also made by others (Dalton *et al.*, 2008; Power *et al.*, 2012). The lower CMAP in the Older subjects is unlikely to be due to fibre atrophy alone, since two studies of disuse atrophy in Young subjects showed that CMAP amplitude did not change in the soleus (Clark *et al.*, 2006) or the FDI (Fuglevand *et al.*, 1995). The reduced CMAP in the old may be the result of relatively fewer muscle fibres within the recording area of the electrodes as well as the interference effects of intramuscular fat and connective tissue, which increase in healthy aged muscle (Lexell *et al.*, 1988; Hogrel *et al.*, 2015), causing diminution of signals recorded at the surface.

The same factors causing attenuation of the CMAP will also affect the sMUPs, since both were recorded by the same sEMG electrode. The sMUPs were identified after being 'triggered' from the intramuscular-recorded MUPs (Brown *et al.*, 1988; Stashuk, 1999a) and despite the MUPs being larger in Old than Young, the sMUPs were (non-significantly) smaller in Old than in Young, which was previously reported in studies of MUNE (Dalton *et al.*, 2008). Whatever the causes of attenuation of the CMAPs and sMUPs of the Young and Old, we can expect that when recording from a specific muscle (of a Young or Old subject)

the CMAP and sMUP will be similarly affected by the electro-physiological characteristics of the muscle and overlying tissue.

If MUNE values represent the number of MUs in the same cross-sectional area of muscle in Young and Old it is possible to estimate the difference between Young and Old with respect to the total number of MUs in the whole muscle, knowing the differences in cross-sectional area of the VL. From Table 3.1 the cross sectional areas are 29.2 and 18.2 cm^2, respectively. In this case, the total number of MUs will be proportional to MUNE value multiplied by CSA (5694 and 2530 for Young and Old, respectively, based on MUNE area, and 3241 and 1420, based on amplitude). Thus, the total number of MUs in the VL of the older subjects is either 44 or 42% of the younger value depending on whether area or amplitude based MUNE values are considered.

There is an alternative approach to estimating the effects of age on MU number that does not depend on any assumption about the volume of the muscle represented by a MUNE value. This alternative makes only the assumption that MUP area or amplitude is indicative of the size of the MU that generated the MUP. Thus, dividing VL muscle cross-sectional area by mean MUP area provides a value which is proportional to the total number of MUs in the VL muscle. This gives values of 27.8 and 13.5 cm^2/mV.ms, for Young and Old, respectively, showing the Old to be 49% of the Young. Based on MUP amplitude, the Old have 50% of the MU number of the Young. These conclusions are remarkably similar to the older subjects having 44 and 42% of the younger MUNE values derived from the CMAP and mean sMUP described above. These values are somewhat greater than the ~30% motor neuron loss estimated from cadaveric specimens (Tomlinson & Irving, 1977).

It is evident, therefore, that the major change in old muscle is loss of motor units which is partly offset by reinnervation of denervated fibres and enlargement of surviving MUs. Fibre atrophy plays a lesser role in the age-related loss of the VL muscle, a conclusion also reached from examination of cadaveric specimens (Tomlinson & Irving, 1977).

Limitations

The values we have presented here are based on MUs activated at 25% of MVC and recorded from in the mid-muscle belly. Based on the size order of recruitment (Henneman *et al.*, 1965) it is possible we are sampling from the smaller and medium MUs within the entire pool. With an increase in contraction level we would expect an increase in MU size, reflected in the mean sMUP, which once divided into the CMAP would result in a smaller MUNE. In practice, however, it is more difficult to extract MUPTs created by individual larger MUs during high force voluntary contractions.

Loss of motor neurons and the associated remodelling of surviving MUs places restrictions on the central nervous system in terms of programmed motor pathways. This is highly likely to affect motor control in old age, but to date the effects of MU remodelling on the control of movements remains largely unknown.

Conclusion

The present study has described, for the first time, MU remodelling in the vastus lateralis as a result of healthy ageing, in terms of: a reduction in the total number of MUs; an increase in the size of the surviving MUs; an increased instability in NMJ transmission, and a decrease in surviving MU firing rates. These changes have a significant impact on overall muscle loss. Further studies are required to ascertain the effects of this MU remodelling on muscle function and mobility.

Chapter 4

The effects of lifelong exercise on age-related neuromuscular changes in the vastus lateralis and tibialis anterior

4.1 Abstract

Introduction: Extensive loss of motor units (MU), the remodelling of surviving units and instability of neuromuscular junction transmission is evident in the vastus lateralis of healthy Old people. In the present study, it was hypothesised that regular, intense exercise throughout middle- and older-age would attenuate the age-related neuromuscular remodelling.

Methods: Using a combination of surface and intramuscular EMG, motor unit number estimates (MUNE) were calculated for proximal and distal motor points of vastus lateralis (VL) together with intramuscular electromyography (iEMG) parameters thought to be indicative of denervation and reinnervation, in 3 male groups; 22 Young (~25 yrs), 26 physically active Old (~71 yrs), and 15 competitive Master Athletes (MA, ~69 yrs). Body composition and quadriceps size were measured by DEXA scanning and magnetic resonance imaging. Intramuscular EMG parameters were also investigated in the tibialis anterior (TA) in a smaller sub group.

Results: A total of 1739 individual MUs were sampled in the VL, and 657 in the TA. The MA athletes were weaker than the Young, and had significantly larger and fewer MUs. The MUs from the MA were significantly larger than those of the Old. In the TA both Old and MA showed evidence of considerable MU remodelling compared with the Young.

Conclusion: Lifelong exercise does not attenuate MU loss in the male VL, in fact the MA showed a greater extent of remodelling than the Old subjects. The TA also displays significant MU remodelling in Old and MA. These results indicate little or no protection of life long exercise against the effects of ageing on skeletal muscle.

4.2 Introduction

Loss of motor neurons during ageing (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977) results in denervation and, potentially, loss of muscle fibres. However reinnervation of denervated fibres by axonal branches of surviving motor neurons helps to preserve muscle mass and strength in spite of motor unit loss, but the process appears to be limited in older age (Luff, 1998), resulting in significant fibre loss and muscle atrophy (Lexell *et al.*, 1988).

In a recent examination of the human vastus lateralis (VL) it was found that around half the number of motor units were lost by age ~75 yrs (Chapter 2) and the declining numbers of peripheral motor neurons was the principal feature of age-related loss of muscle mass. The surviving MUs that were recruited during moderate intensity contractions were enlarged compared with young adults, were more complex, had slower firing rates and higher jiggle, which is characteristic of unstable NMJ transmission and asynchronous firing of fibres belonging to the same MU (Chapter 3).

The value of any intervention that could preserve MU numbers and function into older age is clear but there are no known pharmacological agents that preserve MU numbers or function in ageing humans (Sepulveda *et al.*, 2015). However, a cross-sectional study by (Power *et al.*, 2010), reported higher MU numbers for older athletes compared with non-athletic controls in the tibialis anterior, which is loaded during exercise, but not in the biceps brachii, which remained unloaded in the older runners (Power *et al.*, 2012). However, histological and electron microscopic examination of VL muscle biopsy samples suggested fibre-type grouping in athletic older people, which was taken as evidence of sprouting to reinnervate previously denervated fibres and is consistent with loss of MUs (Mosole *et al.*, 2014; Zampieri *et al.*, 2015).

There are currently no data showing MU function or stability of NMJ transmissions in athletic older people, but research in rodent models points

towards probable beneficial effects from long-term exercise in humans. In mice (reviewed by (Deschenes, 2011; Gonzalez-Freire *et al.*, 2014)), the ageing NMJ shows marked alterations in structure and function. These age-related changes at the NMJ were less apparent in endurance-trained mice, although exercise showed no effect in preserving motor neuron number (Valdez *et al.*, 2012).

The aim of the present study was, first, to estimate motor unit numbers and size and, secondly, to determine MU firing frequencies and NMJ transmission stability in limb muscles of healthy young and old and compare these with a group of athletic older people (Masters Athletes: MA) who had been training and competing for the majority of their adult lives. It was hypothesised that MU numbers and function of the MA would be more similar to those of the young subjects than those of the age-matched older subjects.

4.3 Methods

Participants and recruitment

The study was approved by the University Research Ethics Committee and all participants provided written informed consent. Twenty-two young men, 20 Old men and 15 male MA took part in the study. The Young and Old participants were recruited from the university population and the local community. They were excluded if they were sedentary or competing in sports at a regional level or above.

Masters Athletes

Four out of the 15 MA were classed as sprinters (<400m), and the remainder were distance runners (>3000m). All MA had trained for athletics since they were around 18 years of age and details of their training history are given in Table 4.1. All MA were competing at the time of testing and had achieved the standards of the British Masters Athletics Federation (BMAF) in their respective distances and age groups at least once within the last 2 years.

Volunteers would have been excluded if they had a recent history of bone fracture or neuromuscular, metabolic or cardiovascular diseases, or if they reported any problems with mobility.

Table 4.1. Masters athletes training. Number of training hours per week at different ages.

	Up to 18 yrs	18 – 29 yrs	30 -49 yrs	Since 50 yrs
Estimated hours per week				
0 – 1 hrs	4	4	1	0
2- 3 hrs	5	4	3	3
4- 7 hrs	5	4	5	10
Over 7 hrs	1	3	6	2

The Masters athletes were asked to estimate the number of hours per week they had specifically trained for athletics throughout their lifetime.

Anthropometric measures

Body mass (kg) and height were measured and total body composition assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, version EnCore 10.50.086) with the participant lying supine with legs and arms fully extended. Details of fat mass were recorded. The peak quadriceps cross-sectional area (CSA), outer femur CSA, the VL CSA at each of the 2 motor points and the TA CSA were measured with magnetic resonance imaging (MRI) using a T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoe, Italy). The scanning coil was positioned over each location and contiguous transverse-plane slices of 6 mm thickness were collected with the participant lying supine. Images were exported and analysed off-line using Osirix imaging software (OsiriX medical imaging, OsiriX, Atlanta, USA) and the slice with the highest quadriceps cross-sectional area was used to make measurements of muscle cross sectional area and femur area (Maden-Wilkinson *et al.*, 2014).

Knee extensor and dorsiflexor strength assessments

Knee extensor strength was assessed as described in Chapter 2, Section 2.3. Maximal dorsiflexor strength was assessed with the participant sat upright on a bed with legs straight. The right foot was placed in a custom built dynamometer with the metatarsus securely fastened to the force transducer (Jones *et al.*, 2009). The following procedure was applied to both muscles. After a standardised warm up, participants were instructed to perform a maximal isometric knee extension, with verbal encouragement from the investigators. This was repeated a further two times with short rest intervals and the highest value was taken as maximum voluntary contraction force (MVC).

Experimental procedures and EMG acquisition and analysis in VL

The experimental procedures for VL EMG acquisition and signal decomposition and analysis have been described in Chapter 2, Section 2.3.

Experimental procedures and EMG acquisition and analysis in TA

The TA motor point was identified by low intensity percutaneous electrical

stimulations, with the anode placed over the patella tendon and the cathode probe over the tibial notch.

With the participant sitting fully relaxed, the skin immediately adjacent to the active sEMG electrode on the TA was pulled taut and the concentric needle inserted to a depth of 1-1.5 cm. The participant performed a voluntary, low intensity contraction and the needle was positioned to obtain MUPs with rise times of > 5 kV/s^2. The participant then performed a voluntary contraction lasting 15 sec, keeping as close as possible to a target set at 25% MVC with real-time visual feedback. The needle electrode was then repositioned by combinations of twisting the bevel 180° and withdrawing it by around 5-10 mm. The procedure of needle positioning, voluntary contraction and signal recording was repeated until a minimum of six recordings from spatially distinct regions at varying depths had been obtained at the TA motor point. The participant rested for 15-30 sec between contractions.

In VL, the mean number of acceptable MUs analysed per Young subject was 34 ± 9, for the Old subjects it was 28 ± 9, and for the MA, 27 ± 9. The total number of MUs analysed was 1739, with 698 MUs from the Young, 632 MUs from the Old and 414 MUs from the MA. In a sub group of ten Young (28 ± 4.8 yrs), nine Old (72 ± 3.5 yrs) and six MA (67.7 ± 2.8 yrs), a total of 657 MUs were identified in the TA muscle during contractions held at 25% MVC.

Motor unit number estimate based on intramuscular MUP size

An alternative estimate of motor unit number was also used to estimate MU numbers in the VL and TA. The intramuscular MUNE (iMUNE) does not rely on surface based EMG measurements that may be susceptible to signal attenuation or crosstalk, but makes only one assumption that MUP area is indicative of the physical size of the MU that generated the MUP. Thus, dividing muscle cross-sectional area by mean MUP area provides a value which is proportional to the total number of MUs in the muscle (the units being cm^2 divided by mV.ms).

Statistical analysis

A univariate analysis of variance (ANOVA) was used to identify differences in physical characteristics between groups. Linear mixed models were used to assess effects of group and recording location – these factors were fixed effects, with participant as random effect. When a significant main effect was observed a Bonferroni post-hoc test and correction to identify where significant differences existed Data are presented as mean (SD) or median (IQR) unless otherwise stated. Statistical significance was accepted at $p<0.05$.

4.4 Results

Participant characteristics are shown in Table 4.2. The Old had around 65% higher body fat than the Young and the MA. Quadriceps size and strength of the Old and MA were not significantly different but were significantly less than for the Young and since femur CSA did not differ between groups, this was also the case when muscle size was normalised to femur CSA. Muscle size and strength of the TA did not differ between Young, Old and MA groups.

Table 4.2. Participant characteristics

	Young (n=22)		Old (n=26)		MA (n=15)	
Age (yrs)	25 (4.7)		71 (6.2)		69.5 (4.9)	
Height (cm)	178 (5.8)		173 (7.2)		172 (5.0)*	
Weight (Kg)	76.2 (9.1)		75.0 (11.5)		63.1 (7.8)** †	
BMI (kg/m^2)	24.1 (3.6)		25.3 (3.7)		22.1 (2.8)†	
Body fat (%)	14.9 (7.2)		24.4 (7.9)***		14.5 (6.1)†††	
Knee extensors						
Knee extension MVC (N)	501 (154)		334 (109)**		375 (117)*	
Quadriceps CSA (cm^2)	89.2 (14.9)		61.3 (10.1)***		65.3 (12.6)***	
Outer femur CSA (cm^2)	6.12 (0.62)		6.43 (0.79)		6.12 (0.82)	
Muscle to Bone Ratio	14.3 (2.2)		9.7 (1.5)***		10.5 (1.9)***	
	Proximal	Distal	Proximal	Distal	Proximal	Distal
VL CSA (cm^2)	29.2 (6.2)	14.2 (3.9)	18.6 (4.6)***	8.9 (2.4)***	21.5 (5.5)**	10.5 (3.6)*
Ankle dorsiflexors	Young (n=10)		Old (n=9)		MA (n=7)	
Tibialis anterior MVC	362 (92)		247 (58)		290 (67)	
Tibialis anterior CSA (cm^2)	8.0 (1.8)		7.8 (1.8)		7.2(1.2)	

Data are shown as mean (SD). Significant differences from Young are shown as: *p<0.05; **p<0.01 ***p<0.0005. Significant differences between old and MA are shown as: †p<0.05; †††p<0.0005. BMI: body mass index; MVC; maximum voluntary contraction; VL; vastus lateralis; CSA: cross sectional area; TA: tibialis anterior.

Motor unit size and number estimates

Measurements indicating MU size and MUNE values are shown in Table 4.3 for the VL and TA. Vastus lateralis MUPs of the Old were significantly larger than those of the Young while MUPs of the MA were significantly larger than both Young and Old. MUPs of the TA were on average 70% larger in Old than Young and 59% larger in MA compare with the Young.

The range of MUP values detected during contractions held at 25% MVC are shown in Figure 4.1, which shows a rightward shift in the distribution of MUP

areas for the Old and MA, compared with the Young. For the VL the proportion of smaller MUs (<1000 μV·ms) was greatest in the Young, and the proportion of larger MUs (>3000 μV·ms) was greatest in the MA. The pattern was similar in TA, with the Old and MA having a smaller proportion of smaller MUPs (<1000 μV·ms). It is notable that the MUPs of TA in the Young were smaller than in the VL.

The CMAPs of the VL were largest in the Young, followed by the MA and were smallest in the Old. The Old subjects also had the smallest sMUP area but the median area of the sMUPs for the MA was slightly larger than those of the Young subjects. Calculating MUNE from these values of CMAP and sMUP showed both Old and MA to have significantly lower values than the Young, while there were no significant differences between Old and MA. Using the intramuscular recorded MUP area and muscle CSA to calculate iMUNE values gave a similar result with the Old having 49% lower MU numbers than the Young while the MAs had 53% lower values (Table 4.3).

CMAP and MUNE data are not reported for the TA due to difficulties in obtaining a reliable CMAP. Nevertheless, iMUNE values showed the Old and MA to have significantly lower values than the Young, with no difference between Old and MA. Thus, the Old had iMUNE values that were 59% of the Young and for the MA it was 53% of the Young (Table 4.3).

Table 4.3. Motor unit size and number estimates in VL and TA

	Young (n=698 MUs in 22 subjects)	Old (n=627 MUs in 26 subjects)	Master Athletes (n=414 MUs in 15 subjects)	Group effect Post-hoc comparisons		
VASTUS LATERALIS				**YvO**	**YvMA**	**OvMA**
CMAP	94835 (18537)	61883 (19807)	75309 (16999)	***	***	*
Mean sMUP Area (μV·ms)	608 (275)	470 (173)	657 (235)	*		**
MUP area (μV·ms)	1107 (687-1657)	1329 (943 - 1993)	1659 (1113-2595)	***	***	***
MUNE $_{(CMAP/sMUP)}$	184 (87)	142 (73)	127 (58)	*	**	
i MUNE ($cm^2/mV \cdot ms$)	74.6 (19.6)	38.2 (16.4)	35.2 (14.7)	***	***	
TIBIALIS ANTERIOR	**Young (n=263 MUs in 10 subjects)**	**Old (n=241 MUs in 9 subjects)**	**Master Athletes (n=153 MUs in 7 subjects)**	**YvO**	**YvMA**	**OvMA**
MUP area (μV·ms)	862 (476-1349)	1407 (849 - 1957)	1262 (833-1961)	***	***	
i MUNE ($cm^2/mV \cdot ms$)	9.7 (3.7)	5.7 (2.5)	5.1(1.4)	*	*	

For VL, the sEMG data (CMAP, sMUP and MUNE) are shown as mean of the values from proximal and distal. The VL MUP and iMUNE are calculated from MUPs sampled from both sites. Data are shown as mean (SD), or if not normally distributed as median (IQR). Significant differences are shown as: *p<0.05; **p<0.01 ***p<0.0005. CMAP: compound muscle action potential, sMUP: surface motor unit potential, MUP: motor unit potential; iMUNE: intramuscular motor unit number estimate.

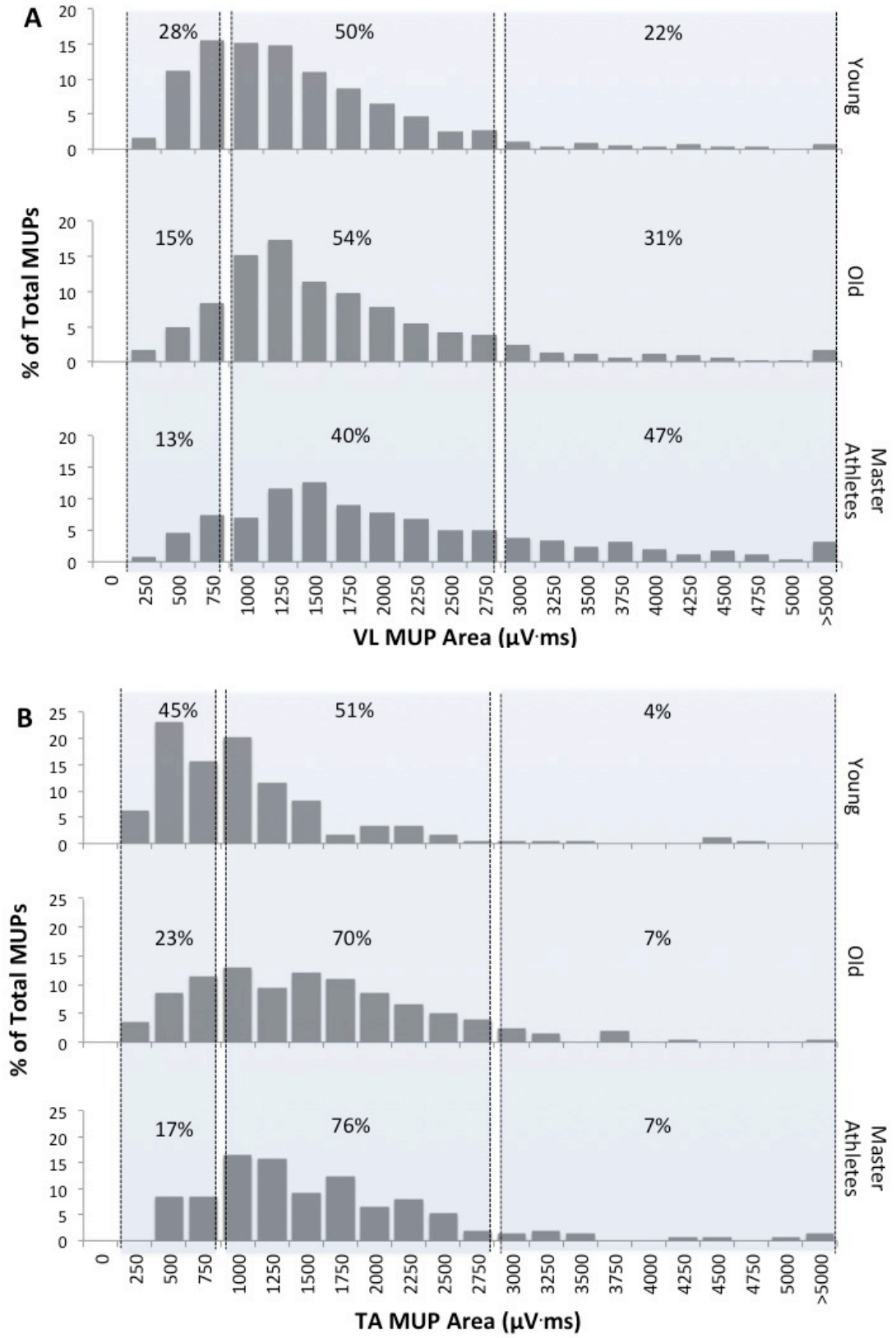

Figure 4.1. Frequency distribution of MU areas recorded during contractions held at 25% MVC, in A) VL and B) TA. Shaded areas and percentage values show the proportion of MUs within that size range for each group. In VL MU areas from proximal and distal motor points are combined.

4.5 Discussion

There are many benefits of regular physical activity and the idea that neuromuscular structure and function can be preserved into old age by maintaining regular and intense exercise is appealing. However, the results suggest that neural deterioration in older age likely affects everyone, irrespective of habitual activity levels, and helps to explain why physical performance declines sharply with increasing age even amongst Masters Athletes (Rittweger *et al.*, 2009).

The Masters athletes tested here were all runners who had met the British Masters Athletic Federation standards within the past two years, had been training at high intensity for 6±2 hrs per week and competing several times per year for the previous 19 ± 5 yrs. The MA were lighter than both Young and Old subjects, principally because a lower body fat content, indicative of very good metabolic health. Nevertheless quadriceps muscle size and strength of the Old and MA were similar and both significantly less than for the Young. It might be thought that because the MA were of a slightly smaller stature than the Old subjects they might have had somewhat smaller muscles when young and so the fact that at the age of around 70 years, their muscles were of a similar size to the untrained Old group, would suggest they had experienced less age-related muscle loss. However, this is unlikely since when muscle CSA was normalised to femur CSA, which allows for differences in body size (Maden-Wilkinson *et al.*, 2014), there were no differences between Old and MA groups. While the VL was significantly smaller in Old and MA compared with Young this was not the case with the TA where there was no difference in size between the two older groups and the younger subjects (Table 4.2). Abe et al (2014) have reported that the more peripheral muscles of the lower limb are relatively protected against age related atrophy. In the TA, strength normalised to muscle size was slightly, although not significantly, higher in the MA compared to the old. This is an indication that the specific force of the muscle might be higher in the MA than non-athletic old, possibly due to higher levels of voluntary activation or to higher

specific tension of single muscle fibres. This is in contrast to the findings reported by Power et al (2010), where the MA produced a lower torque than the Old. The contradictory findings might be due to differences in participant exercise history or due to the small sample sizes used in both the present study and that of Power et al (2010).

The data presented in Table 4.3 clearly show that both Old and MA have larger MUs than Young, there being a shift in the distribution of MUP size towards larger units (Figure 4.1). Large MUPs are taken to indicate larger MU and could come about due to a loss of small units or an increase in size of either the small, or of all units. If only small units were lost it would be expected that the distribution would become more restricted, possibly within the middle band shown in Figure 4.1. In practice there was a considerable increase in the proportion of large MUPs which would suggest that there had been a general increase in size of all MUs of the VL. This effect was particularly evident for the MA where both the mean MUP size and the proportion of large MUPs was greater than with Old subjects.

A further notable difference between the Old and MA is the CMAP and sMUP, which were larger in the MA. This may be explained by the lower body fat or intramuscular connective tissue in the MA, resulting in a reduced level of attenuation of the EMG signal (as discussed in section 1.3).

The situation is slightly different with the TA where the main difference between the two older groups and the Young was that in the former there was a marked decrease in the proportion of small units and an increase in those of a medium size but with little evidence of an increased proportion of large units. This is similar to what would happen if, as a result of ageing, small units were either lost or converted into larger units.

Whatever the difference between the two muscles, the similarity is that MUPs, and it is assumed MUs, of the two older groups were larger than those of the Young subjects. Since it is most unlikely that the number of fibres was greater in either muscle in the older subjects, the only way in which MUs can increase in

size is if there is a decrease in the number of units and this is what was observed when estimating MU numbers with MUNE, based on CMAP and average sMUP size, in the case of VL, or iMUNE, calculated from intramuscular recorded MUP and muscle CSA for both VL and TA. The two methods give slightly different values but the overall indication is that both Old and MA have between 30 and 50% fewer MUs than the Young with no substantial difference between MA and Old. This conclusion is not what was hypothesised, nor does it agree with the conclusion of Power et al (2010) who reported that MU number of the TA was preserved in Masters Athletes. The reason for the difference between our findings and those of Power et al are not obvious. The MA in the current study were slightly older (~5yrs), and it has been reported that the decline of TA MU numbers becomes more rapid with increasing age (McNeil *et al.*, 2005b), however such a large difference would not be expected.

Whether the MU changes occur throughout life or only after a certain age remains to be determined but Campbell et al (1973) found MU numbers to be preserved until age around 60 yrs, after which they declined and increasingly rapidly after the of age 75 yrs. This was, however, a small peripheral muscle and it remains to be seen whether the same is true for a large proximal locomotor muscle such as the VL.

The apparent percentage decrease in MU number in the VL, estimated as iMUNE, for the older subjects when compared with the Young was greater than the decrease in muscle CSA. It is evident therefore, that the increase in MU size compensated for the loss of MU number but this was only a partial compensation as the muscle was 30% smaller than in the Young. For the TA, however, while MU numbers were lower in the older subjects the muscle size was the same in the older subjects and the Young. The increase in MU size in the TA of the Old subjects completely compensated for the decrease in MU number. The reasons for the differences between these two muscles is intriguing and may reflect differences in the ability of motor neurons supplying the two muscles to reinnervate denervated fibres; the increase in MUP size for the VL was approximately 135% while for the TA it was 165%. It is notable that the MUPs of

the TA in the Young subjects were appreciably smaller than for the VL (Table 4.3, Figure 4.1) and this may reflect the fibre type composition of the muscle. The TA is reputed to consist mainly of slow fibres which would be expected to be organised into relatively small MUs, in contrast to the VL which is a mixed muscle and might be expected to contain a wider range of MUs, as appears to be the case comparing MUP size of VL and TA in Figure 4.1. There may, therefore, be differences in the ageing characteristics of fast and slow MUs. It could also be that there is a maximum size to which a MU can grow and when this is reached there is no further chance of reinnervating denervated fibres and consequently they are lost and muscle wasting occurs. The VL, with larger MUs in Young may reach the critical point where muscle wasting occurs sooner than the TA which starts with smaller MUs in the Young.

It can be concluded that life-long high levels of physical activity, whatever they may do for cardiovascular health, do not prevent the loss of quadriceps muscle bulk or the loss and remodelling of MUs. It is possible, however, that there is a benefit from physical activity and that the modest levels undertaken by our Old subjects were sufficient to obtain the maximum benefit and that higher levels of activity provide no additional benefit. It would be instructive therefore to study a truly sedentary population.

Chapter 5

Age-related motor unit remodeling in human leg muscles is muscle and contraction level specific

5.1 Abstract

Introduction: Older age is associated with loss of motor neurons, with some orphaned fibres being reinnervated by surviving neurons resulting in higher innervation ratios in older motor units (MUs). The present study examined whether age-related MU remodelling is consistent across different muscle and contraction levels.

Methods: Twenty-five Young and twenty-seven Old, healthy men performed voluntary isometric contractions of the vastus lateralis (VL) and the tibialis anterior (TA) at 10% and 25% of maximum voluntary contraction (MVC). Intramuscular electromyography (iEMG) was used to sample individual motor unit potentials (MUPs).

Results: MUP size was greater at higher contraction levels and in older people in both muscles (all $p < 0.05$), in accordance with orderly recruitment and consistent with age-related collateral reinnervation of MUs. Effects of age on MUP size were 60-77% greater in TA than VL dependent on contraction level ($p < 0.05$). Effects of contraction level on MUP size were smaller in old males in TA, but not VL.

Conclusion: These data suggest a reduced heterogeneity of MU size in older males, supportive of more pronounced remodelling of early-recruited units in line with findings in animal models. Greater effects of MU remodelling in TA than VL may explain preservation of TA muscle size with ageing.

5.2 Introduction

Loss of muscle fibres in older VL was identified as the principle cause of muscle wasting following autopsy examinations (Lexell *et al.*, 1988; Lexell, 1995), most likely as a result of a substantial loss of motor neurons (Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987). The electrophysiological assessments conducted on healthy older and young men reported in Chapter 3 support these findings. However, not all of the 'orphaned' (denervated) fibres are degraded, some are reinnervated by axonal sprouting from neighbouring neurons, termed collateral innervation (for review see (Luff, 1998)(Chpaters 3 and 4)). Consequently, older people were found to have around 50% fewer VL motor units (MUs) than young, but those that remained were around 25% larger, with higher innervation ratios.

The preliminary findings presented in Chapter 4 indicate that the reinnervation process may be more effective in TA than VL, since the TA showed around 70% increase in MU size and very little loss of muscle mass, despite around 50% loss of MUs. Variation between muscles in their responses to ageing has been reported several times. For instance, lower limb muscle mass decreases more than upper limb muscles (Janssen *et al.*, 2000), and even within the legs, the quadriceps atrophy considerably more than other thigh and lower limb muscles, as shown in a small number of participants in Chapter 4, and previous studies (Maden-Wilkinson *et al.*, 2013; Abe *et al.*, 2014). The variations between muscles is likely to reflect differences in the extent of MU losses, fibre losses and fibre atrophy: the VL loses MUs, muscle fibres and muscle mass (Chapter 3), soleus MU number and muscle size are little affected by age (Dalton *et al.*, 2008), while TA shows loss of MUs (McNeil *et al.*, 2005b), but does not atrophy substantially with age (Abe *et al.*, 2014) (Chapter 4), suggesting a dissociation between MU losses and muscle wasting with ageing in this muscle.

Muscle follows an orderly recruitment of motor units (MU) based on size (innervation ratio) (HENNEMAN *et al.*, 1965): the smaller, slower, more fatigue-

resistant units are recruited during low-intensity contractions, with larger, faster, fatigable units recruited at higher contraction intensities. The MU losses are likely to affect the central nervous system control of movements and in this respect, an increase in the size of the early recruited units in a small hand muscle was shown to influence the ability to maintain a steady force (Dideriksen *et al.*, 2012).

It is not clear how the age-related MU remodelling of human leg muscles impacts on MU recruitment during low and moderate intensity voluntary contractions. In Chapter 4 it was noted that the change in distribution of MUP size with age for the VL was consistent with a general increase in size of all MUs, while for the TA the pattern was similar to what might be expected if the changes were limited to the small units, possibly their loss, or more likely their conversion into larger units. If this were the case then we might expect to see a hierarchy of recruitment preserved in the ageing VL while there would be less evidence of this in the TA. Therefore VL and TA muscles in young and older men were examined using intramuscular EMG at low and medium contraction intensities. It was hypothesised that young adults would show the characteristic recruitment of small, followed by larger MUs with increasing external force. Conversely, the older subjects were expected to show less variation in MU size between low and moderate intensity contractions due to the enlargement of the low-threshold MUs.

5.3 Methods

Participants and recruitment

The study was approved by the University Research Ethics Committee and all participants provided written informed consent. Volunteers were included if they were male, aged between 18-35 years or 65-90 years and habitually physically active. Volunteers were excluded if they were sedentary or competing in sports at a regional level or above, had a recent history of bone fracture or neuromuscular, metabolic or cardiovascular diseases.

Anthropometric measures

Body mass (kg) and height were measured and total body composition assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance, version EnCore 10.50.086) with the participant lying supine with legs and arms fully extended. Details of fat mass were recorded. The peak quadriceps cross-sectional area (CSA), outer femur CSA, the VL CSA at each of the 2 motor points and the TA CSA were measured with magnetic resonance imaging (MRI) using a T1-weighted turbo 3D sequence on a 0.25-T G-Scan (Esaote, Genoe, Italy). The scanning coil was positioned over each location and contiguous transverse-plane slices of 6 mm thickness were collected with the participant lying supine. Images were exported and analysed off-line using Osirix imaging software (OsiriX medical imaging, OsiriX, Atlanta, USA) and the slice with the highest quadriceps cross-sectional area was used to make measurements of muscle cross sectional area and femur area. (Maden-Wilkinson *et al.*, 2014).

Knee extensor and dorsiflexor strength assessments

Knee extensor strength was assessed as described in Chapter 2, Section 2.3. Dorsiflexor strength was assessed as described in Chapter 4, Section 4.3.

Experimental procedures and EMG acquisition and analysis in VL and TA

All motor points were identified with low intensity percutaneous electrical stimulation, with a cathode pen and separate anode. In the VL the anode was placed at the top of the right gluteus and the cathode placed in the crease of the hip to stimulate the femoral nerve. In the TA the anode was placed over the patella tendon and the cathode pen placed over the tibial tuberosity to stimulate the tibial nerve, as previously described (Section 2.3 and 4.3).

With the participant sitting fully relaxed, the skin around the motor point was pulled taut and the concentric needle was inserted to a depth of 1.5-2 cm in VL and 1-1.5 cm in TA. All iEMG acquisition and analysis in VL and TA has been previously described in Section 2.3.

Statistical Analysis

A univariate analysis of variance (ANOVA) was used to identify differences in physical characteristics between groups. Linear mixed models were used to assess effects of group, contraction intensity and muscle, which were considered as fixed effects, with participant as random effect. In addition, the group*muscle interaction was examined. Data are presented as mean (SD) or median (IQR) unless otherwise stated. Statistical significance was accepted at $p<0.05$.

5.4 Results

Participant characteristics are shown in Table 5.1. Maximum ankle dorsiflexion and knee extension forces were 26% and 36%, respectively, lower in Old men compared with Young (both $p < 0.01$). Vastus lateralis CSA was 34% lower in Old men, but TA CSA did not differ between Young and Old.

Table 5.1. Participant characteristics

	Young (n=25)	**Old (n=27)**
Age (yrs)	25.9 (4.8)	71.8 (5.1)***
Height (cm)	178 (6)	173 (7)*
Weight (Kg)	80.6 (12.5)	78.1 (11.2)
BMI (kg/m^2)	25.3 (5.3)	26.1 (3.7)
Body fat (%)	18.1 (9.8)	26.7 (7.5)**
Knee extensors		
Knee extension MVC (N)	546 (175)	348 (112)**
VL CSA (cm^2)	28.4 (8.6)	18.8 (5.1)***
Ankle dorsiflexors		
Ankle dorsiflexion MVC (N)	368 (134)	271 (58)**
TA CSA (cm2)	7.6 (1.4)	7.2 (1.6)

Data are shown as mean (SD). Significant differences between Young and Old are shown as: *p<0.05; **p<0.01 ***p<0.0005. BMI: body mass index; MVC; maximum voluntary contraction; VL; vastus lateralis; CSA: cross sectional area; TA: tibialis anterior.

Motor unit recruitment during low and moderate intensity contractions

MUP area was greater in VL than TA ($p < 0.001$, Table 5.2). In the TA muscle, the older men had 46% larger MUPs than young during 10% contractions and 32% larger MUPs during 25% contractions. In the VL muscle, the older men had 19% larger MUPs than young during 10% contractions and 23% larger MUPs during 25% contractions. Thus, the age-dependent increase in TA MUPs was significantly greater than that seen in VL. This was reflected in the significant age*muscle interaction (Table 5.2).

Table 5.2. Intramuscular motor unit potentials.

	Young (n=25)		Old (n=27)		Main Effects			Interaction
	10% MVC	25% MVC	10% MVC	25% MVC	Age	Contraction Level	Muscle	Age*Muscle
VL MUP area (μV.ms)	943 (627-1292)	1132 (675-1685)	1203 (818-1725)	1367 (930-2038)	*	***	***	*
TA MUP area (μV.ms)	806 (513-1140)	974 (562-1517	1199 (707-1903)	1297 (819-1963)				

MU size separated by age group, muscle and contraction level. Data are shown as median (IQR). Significant differences are shown as: *p<0.05; **p<0.01 ***p<0.0005. VL; vastus lateralis; MUP: motor unit potential; TA: tibialis anterior.

MUP size was greater at 25% than 10% MVC (Table 5.2). Given the greater effect of age on TA MU size, group differences in contraction level effects were examined separately for each muscle (Figure 5.1). In TA only, effects of contraction level were smaller in Old than Young. In the Young TA there was a 23% increase in MUP area between contractions at 10 and 25% MVC. In the Older men, there was a 12% increase in MUP area between contractions at 10 and 25% MVC. In the VL, both the Young and Old showed similar (16%) increases in MUP area between contractions at 10 and 25% MVC (Figure 5.1).

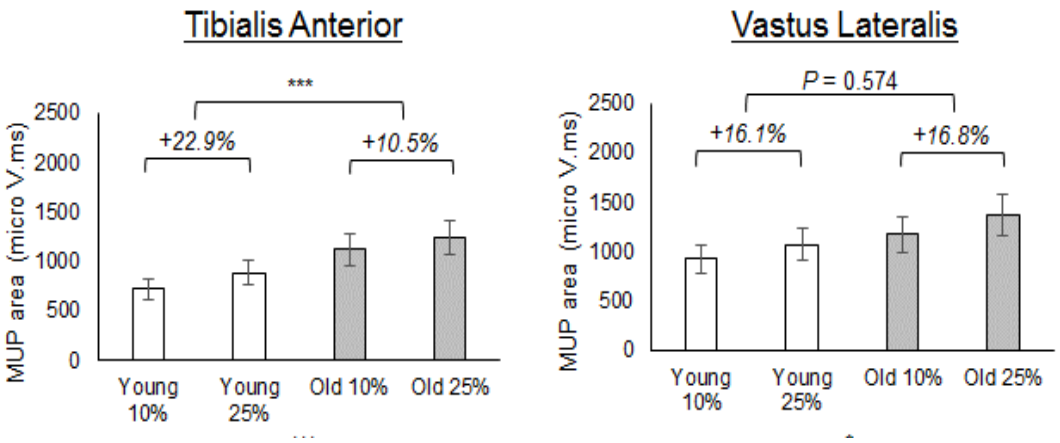

Figure 5.1. Young and Old MUPs. Motor unit potential area separated by age group and contraction level. Data are mean (SEM). Italicised values indicate percentage change from 10% MVC mean to 25% MVC mean for each group. p values and asterisks indicate significance of group*contraction level interaction. *** p<0.0005

5.5 Discussion

The aim of this study was to investigate effects of contraction level on MU recruitment in Young and Old men in the expectation that 1) TA muscle would show greater increases in MU size than VL during ageing, 2) older muscle would not follow the same size-order of recruitment as young.

The premise of this study was based on the findings reported in Chapter 4 showing that the distribution of MU sizes in VL had retained heterogeneity, while the TA became more homogeneous due to more substantial enlargement of the low-threshold MUs. The results presented in this chapter largely support those suggestions. Age-related differences in MUP size were more pronounced in TA than VL. Conversely, effects of contraction level on MUP size were less pronounced in older males in TA but not VL. The VL muscle followed the characteristic hierarchical recruitment in both Young and Old, although the magnitude of increase in MU sizes (between 10% and 25% contractions) was greater in the young. Conversely, in the TA, the Old no longer clearly followed the size-principle, unlike the Young whose increase in MU sizes between 10% to 25% contractions were two fold greater than the increases seen in the Old. The main reason for the failure of the Old to recruit substantially larger MUs at 25% compared with 10% dorsiflexion contractions was that the MUs that were already active at 10% MVC were enlarged. In fact, judging by the MUPs, the old MUs in both TA and VL during 10% contractions were larger than those recruited by Young during the 25% contractions. This finding indicates that the low-threshold MUs are more involved in the MU remodelling, either being more susceptible to losses, or being more involved in the reinnervation of denervated fibres and thus increasing in size. The success of TA muscle to reinnervate fibres had the benefit of preserving total TA muscle CSA, although it was notable that dorsiflexor MVC decreased, indicative of either altered voluntary activation or specific force of the muscle. It may be that increased connective tissue and/or fatty infiltration of the muscle has occurred leading to a decrease in volume of contractile material without measurable changes in whole muscle size.

The hierarchical recruitment of MUs dictates that larger MUPs should be observed at higher contraction intensities compared with low (HENNEMAN *et al.*, 1965). This had previously been observed in young soleus and TA muscles (McNeil *et al.*, 2005a; Dalton *et al.*, 2008). However, it should not be assumed that all muscles respond similarly to ageing, even those in the same limb segment, since the TA has been shown to lose MUs in several studies while the soleus preserves MU numbers with ageing (McNeil *et al.*, 2005a; Dalton *et al.*, 2008). These differences are unlikely to be due to fibre type differences, with the soleus being a slow, postural muscle and the TA estimated to be composed of ~75% type 1 fibres (Jaworowski *et al.*, 2002).

Heterogeneity of MU size has clear functional advantages in that small units can be used for small contractions and gradations of force are likewise small when recruiting additional units for tasks requiring fine control. The increased homogeneity of MU size in early recruited units seen with advancing age could also result in reduced ability to complete fine motor control tasks, due to greater twitch force of individual MUs (Galganski *et al.*, 1993). In contrast, stability or decrease in size of later-recruited units would imply that control of tasks involving forceful contractions is less affected. This is supported by contraction-level specific effects of age on the ability to produced steady force, whereby differences between Old and Young are more pronounced at low contraction levels (Laidlaw *et al.*, 2000).

It remains unclear whether during ageing the processes of MU loss and of denervation and reinnervation affects all MUs, or whether particular MU types or sizes are most involved. Studies in rats have revealed substantial increases in innervation ratio in type I fibre MUs in older age, but unchanged, or even smaller, MU size of type II units (Kadhiresan *et al.*, 1996). This is consistent with observations in humans that older people tend to show greater decrements in power events requiring recruitment of faster MUs (Caspersen *et al.*, 2000). However, it is difficult to draw any firm conclusions in humans because MU sizes

are not easily measured across the entire MU pool. This is primarily a methodological constraint. Autopsy studies only estimate an average MU size (FEINSTEIN *et al.*, 1955; CHRISTENSEN, 1959), and *in vivo* glycogen depletion studies used to estimate MU territory (Burke & Tsairis, 1973; Bodine-Fowler *et al.*, 1990), offer little information on MU remodelling during ageing and disease. EMG studies capture highly complex signals that are increasingly difficult to decompose into individual MUs above contraction intensities of around 50% MVC (Chapter 2). Nevertheless, the results of the current study offer the first evidence in humans to partially support findings of previous animal studies, whereby the innervation ratio of small, early recruited MUs was three times greater in older than young rats (Kadhiresan *et al.*, 1996). However, it cannot be assumed that humans respond similarly to rodents in respect to age-dependent MU remodelling. The first and obvious complication is that human muscles do not all change in the same way with advancing age. Secondly, detailed studies of the VL showed similar loss of type I and II fibres in older muscle (Lexell & Taylor, 1991). Given that type II fibre MUs have much higher innervation ratios than type I, this suggests a greater absolute loss of type I MUs, which conflicts with the results of rodent models (Kadhiresan *et al.*, 1996).

In conclusion, the low-threshold MUs of TA and VL are more clearly involved in age-related remodelling. Differences exist between muscles, with the TA showing signs of more successful reinnervation of denervated fibres which has the advantage of preserving muscle size. The motor unit remodelling disrupts the usual hierarchical recruitment of MUs with increasing contraction intensity and this is likely to have implications for the control of movements in older age.

Chapter 6

General discussion

6.1 Aims and Objectives

The overall aim of the work presented in this thesis was to compare MU structural and functional characteristics of VL and TA leg muscles between young and older men in order to identify age-related differences.

The Objectives were:

1) to establish the techniques needed to estimate motor unit numbers and sizes in the VL, a large leg muscle, and to characterize functional properties such as firing rates and stability of neuromuscular junction transmission.

2) to use the newly developed techniques to compare VL MU characteristics between Young and Old men.

3) to investigate whether lifelong activity protects against MU changes associated with ageing.

4) to investigate whether differences exist between VL and TA muscles in the extent of age-related changes to MU characteristics.

6.2 Novel research findings

Chapters 2-5 present the results from the studies designed to address each of the Objectives in turn.

The existing techniques to determine MUNE values in vivo in humans were previously applied to relatively small muscles controlling movements of the hands or feet. The most commonly used technique ('spike-triggered averaging') first uses intramuscular recordings to identify individual MUPs which are subsequently used to 'trigger' their corresponding sMUPs from surface-recordings. The sMUPs are then normalised to the CMAP (a maximally stimulated potential). The focus of the present work was on larger muscles of the legs, so the first task was to determine whether the same MUNE methodology could be used with larger muscles. In Chapter 2, the EMG decomposition algorithm named 'DQEMG' was used to identify individual MUPs and their corresponding sMUPs in the VL, as well as MU firing rates, complexity and jiggle. The results showed that MU characteristics varied across different regions of the

VL in healthy Young men. Most notably, the MUP sizes were larger and more complex in deeper than superficial regions as well as with higher contraction intensities, and the MUNE values differed when recorded at different motor points of the same muscle and with different contraction intensities. Another important point that was often overlooked in previous studies of MU remodelling (in ageing and disease), is that needle- and surface- EMG electrodes capture potentials from finite muscle areas (around 2 mm and 2 cm, respectively). Therefore, the MUNE values are representative only of the muscle within the recording volume. Since a major change with ageing is a loss of muscle mass (at least in VL muscle), then it is necessary to normalise the MUNE value to muscle size. A further method of estimating MU numbers was proposed, which does not rely on surface measurements that may be susceptible to signal attenuation, and also accounts for differences in muscle size. The intramuscular motor unit number estimate (iMUNE) makes only the assumption that MUP size is representative of MU size, therefore dividing muscle cross sectional area by mean MUP area provides a value that is proportional to the number of MUs in the muscle. There was also the added benefit that the iMUNE value varied little with changes in contraction level.

Applying these techniques to Young and Older men (Chapter 3) revealed that older VL had larger MUPs, increased MUP complexity and jiggle, decreased firing rates and lower MUNE and iMUNE values. The conventional MUNE value showed older muscle to have around 30% fewer MUs than young, but after correcting for the significantly smaller VL muscle size in Old compared with Young, it was estimated using the iMUNE that older men had less than 50% of the MUs compared with Young. This was the first study to report MUNE and iMUNE values in large thigh muscles and the work suggested that loss of MUs was the principal cause of muscle wasting and weakness in older VL. However, none of the participants were classed as sarcopenic by any definition of the term, nor did they report any problems of mobility. This indicates the loss of MUs precedes any clinically relevant symptoms of muscle loss.

If motor unit loss is causally implicated in muscle weakness and sarcopenia, then it is important to identify interventions aimed at preserving MU numbers. A previous study had reported 'preservation' of MU numbers in TA of athletic older runners compared with controls, raising the possibility that life-long exercise may attenuate MU losses. However, the results presented in Chapter 4 do not support this notion. The Masters Athletes (MA) had trained and competed in running events for the majority of their adults lives and yet their VL muscle size and MUNE values were similar to those of non-athletic Old men, which were considerably lower than the Young men. The MA had around 50% larger MUPs than the Young and 25% larger than the Old. Their MUPs also had fewer phases and turns than the old, suggesting more synchronous firing of fibres within a MU. The TA also showed loss of MU numbers of similar magnitude to the losses in VL, but muscle mass was seemingly preserved in the TA of older men (both athletic and non-athletic). A closer look at the TA MUPs (representative of MU size) revealed that they were even more enlarged in older muscle than was the case in VL. This is consistent with more successful reinnervation of denervated fibres in the TA compared with the VL which may help to preserve muscle mass. These results show that life-long exercise does not attenuate MU losses, but might promote neuromuscular environments that favour reinnervation in TA, but less-so in VL.

Chapter 5 was designed to investigate in more detail the differences between VL and TA muscles in the extent of age-related neuromuscular changes. Compared with the Young, Old men recruited considerably larger MUs (and presumably fewer) in order to sustain contractions at 10% MVC. When increasing intensity to 25% MVC, the Young recruited MUs that were much larger than those identified at 10% MVC, while the Old recruited MUs of similar size to those already active at 10% MVC. These results indicate altered recruitment strategies in older age as a consequence of MU remodelling (giving a more homogeneous MU pool) in order to meet the task requirements. Supporting the work of the previous chapter, the larger sample size also provided evidence of greater reinnervation of denervated fibres in the TA than the VL. These results offer the intriguing

possibility that the low-threshold MUs are more involved in the MU remodelling process.

6.3 Unresolved issues and directions for future research

The work presented in this thesis raises awareness of the extent of MU remodelling in large leg muscles. The losses seemingly affect everyone, even those who continue to be active throughout their lives (dispelling the notion of 'use-it or lose-it'). However, there are a number of fundamental questions that remain unresolved and should be the focus of future work.

First, the work presented here cannot identify which, if any, MUs are most susceptible to loss with ageing. Secondly, it remains unclear why the VL and the TA muscles would respond differently for some aspects of neuromuscular remodelling with ageing. Thirdly, the participants studied were all healthy and living independently, it remains unknown whether sarcopenic or frail older people would be more affected. Finally, the consequences of MU remodelling for motor control remain unknown. The following section briefly covers each of these points in turn.

The conventional wisdom suggests that the low-threshold, early-recruited MUs are smallest (with the lowest innervation ratio) and composed of type 1 fibres, while the high-threshold MUs are largest (highest innervation ration), composed of type 2 or 2x fibres and are last to be recruited. The older men recruited larger MUs than young both at 10% and at 25% MVC. It may be the case that the older men had lost their smaller MUs, and must therefore recruit the slightly larger MUs. This is partly supported by the autopsy studies of Lexell (1988, 1991) that report similar loss of type 1 and type 2 fibres in VL muscles of previously healthy older people. Therefore, much larger numbers of type 1 MUs must be lost compared with type 2 MU because the type 2 MUs have a much larger innervation ratio than type 1 in mixed fibre-type muscles (Enoka & Fuglevand, 2001). However, it seems unlikely that entire low-threshold MUs were lost (at

least in the TA) because losing the smaller MUs completely would also lead to loss of muscle mass, and the results showed that TA muscle mass was preserved in older age. Another possible explanation has arisen from work undertaken using animal models. Aged rats increased their innervation ratio of smaller MUs (Kadhiresan *et al.*, 1996) as a consequence of the reinnervation of denervated fibres from the loss of larger MUs. Irrespective of which MUs are lost, the low-threshold MUs are heavily involved in the remodelling, either directly being lost, or contributing to the reinnervation of denervated fibres.

It is not clear why both the TA and VL show substantial loss and remodelling of MUs with age, yet only the VL shows muscle atrophy. It is possible that the TA is also losing contractile tissue, and becoming replaced with connective tissue and intramuscular fat which maintains overall muscle volume. However a similar effect may be expected in VL, which was not observed. The distribution of MUP areas in Figure 4.1 offers another possibility. Those in the Young TA are smaller than the Young VL. If there is a maximum innervation ratio a MU can sustain following axonal sprouting, then a MU in the TA would have a greater capacity for growth, and thus preservation of muscle size. It is possible that a MU may be lost once it has exceeded that capacity, which may occur sooner in the VL.

Chapter 4 revealed that lifelong exercise does not prevent MU remodelling in the TA or VL. The MU characteristics were no different to the active Old men. It may be the case that exercise does have a beneficial effect, but not beyond being active, and being highly active to the extent of a competitive runner confers no additional benefits. Applying the same methods to an elderly sedentary or frail population would likely reveal more.

The MU remodelling process likely has consequences beyond the loss of muscle fibres and its contribution to overall muscle atrophy. The lack of heterogeneity in the Older MU pool may well cause functional limitations, particularly those requiring fine motor control, and possibly balance. The extent of these functional limitations associated with MU remodelling is yet to be explored.

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