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Examining motor unit stability of first dorsal interosseous (FDI) and biceps brachii (BB) muscles in healthy and older adults using decomposition-based quantitative electromyography (DQEMG)

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

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Abstract

Aging of the human neuromuscular system is associated with gradual decline in motor unit (MU) number leading to denervation of muscle fibers and subsequent compensatory reinnervation from surviving MUs. Lower limb muscles exhibit age-related increased MU instability (measured electrophysiologically), however not much is known regarding MU stability of aging upper limb muscles. The purpose of this study was to examine age-related MU loss in upper limb muscles (first dorsal interosseous [FDI] and biceps brachii [BB]) and the impact on MU stability in younger and older healthy subjects using electrophysiological near fiber analysis from decomposition-based quantitative electromyography (DQEMG). FDI and BB muscles from older (74 ± 5 years) and younger (31 ± 13 years) healthy subjects were examined through surface and intramuscular collection of EMG signals during volitional contractions, which were analyzed with DQEMG. Older subjects showed significantly larger MUs associated with greater instability in the form of near fiber (NF) jiggle and NF jitter in the FDI and BB muscles when compared to younger controls. These results suggest that age-dependent MU remodeling and progressive reduction in FDI and BB MU pools are associated with greater transmission instability at the neuromuscular junction.

Keywords

Motor unit, near fiber, jiggle, jitter, electromyography, neuromuscular junction

Acknowledgments

I would like to thank my supervisor, Timothy Doherty, for his invaluable guidance and unwavering support in the progress and completion of this project. It has truly been a pleasure sharing my graduate years with his enduring charisma and expertise that any student could only appreciate.

My utmost gratitude is expressed as well for my advisory committee members, Charles Rice and Brian Allman, who have provided sensible feedback and practical suggestions for presenting my thesis and achieving overall academic fulfilment.

I am grateful for fellow lab members, Neal McKinnon and Shannon Davidson, for their useful insight and cooperative participation in the research environment.

Much thanks to Kevin Gilmore for his help in elucidating data analysis procedures used in this project.

I am highly appreciative of all subjects who consented to be involved in this study for their participation, interest and patience in helping me fulfil this academic endeavor.

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List of Abbreviations

AAR:	Area to amplitude ratio
ACh:	Acetylcholine
ALS:	Amyotrophic lateral sclerosis
BB:	Biceps brachii
CMAP:	Compound muscle action potential
CNS:	Central nervous system
DE-STA:	Decomposition-enhanced spike-triggered averaging
DQEMG:	Decomposition-based quantitative electromyography
EMG:	Electromyography
FDI:	First dorsal interosseous
IDI:	Inter-discharge interval
MFAP:	Muscle fiber action potential
MN:	Motor neuron
MRC:	Medical Research Council
MU:	Motor unit
MUNE:	Motor unit number estimate
MUP:	Motor unit potential
MUPT:	Motor unit potential train
MuSK:	Muscle-specific kinase
mS-MUP:	mean surface motor unit potential
NF:	Near fiber
NMJ:	Neuromuscular junction
PNS:	Peripheral nervous system
S-MUP:	Surface motor unit potential
STA:	Spike-triggered averaging

Chapter 1

1 Literature review

1.1 Overview

Neuromuscular function depends upon the excitation of motor neurons and the skeletal muscle fibers they innervate in order to initiate appropriate muscle contraction during voluntary and reflex movement. The motor neuron, its myelinated axon, and the group of muscle fibers innervated by that axon via the neuromuscular junction comprise the motor unit (MU). The MU is the basic functional component of the motor system and determines the efficacy of whole muscle contraction based on their firing characteristics when activated as well as their organization within the muscle.

As a result of the normal aging process, there is marked loss of motor units caused by age-dependent variables such as accumulation of reactive oxygen species (that stress the mitochondria of energetically-demanding motor neurons) resulting in denervation of muscle fibers, many of which degenerate as a result of the loss of their parent motor neuron. However, a compensatory motor unit remodeling process of reinnervating remaining denervated muscle fibers (through their incorporation into surviving motor units by sprouting collateral axons) attempts to mitigate the consequential decline in muscle mass and strength often referred to as sarcopenia. Electrophysiological measures of motor unit and muscle fiber potential characteristics, and in particular, those that examine transmission stability (jiggle and jitter) aid in assessing the viability of remodeled motor units with normal aging and further the understanding of the

mechanisms and impact of sarcopenia in various muscle groups. This overview will highlight the basics of motor unit and neuromuscular junction physiology, biological consequences of normal aging in humans and animal studies, and electrophysiological parameters and techniques for analyzing the underlying features associated with aging of the MU.

1.2 The motor unit

1.2.1 Motor unit anatomy

Neurons are highly specialized cells involved in electrochemical signaling for information transmission throughout the human body. These electrically excitable, fundamental processing units of the nervous system receive ion gradient impulses in their branched, antenna-like structures known as dendrites. In the peripheral nervous system (PNS), specialized efferent neurons will then transfer this electrical signal down long processes called axons (usually grouped into mixed nerves along with afferent sensory nerve axons) for the release of chemical neurotransmitters into the surrounding extracellular space termed the synaptic cleft. In motor neurons (MNs), which initiate body movement, this cleft is known as the neuromuscular junction (NMJ), where acetylcholine (ACh) is the neurotransmitter that binds to the post-synaptic receptors on target muscle endplates. This triggers muscle fiber excitation and subsequent muscle contraction through the interaction of myosin heads attaching to actin filaments of the sarcomere contractile apparatus. This process is true of all lower MNs, which are situated in the anterior horn of the spinal cord and brainstem and are activated by upper MNs of the central nervous system (CNS), other descending pathways (such as the cerebrospinal

and rubrospinal tracts, both of which are important for distal fine motor control) or from afferent input from the periphery. These specialized cells project their axons in the PNS to the muscle fibers they innervate to effect voluntary motion through skeletal muscle contraction. Therefore, the MU is a functional unit comprised of the motor neuron, its myelinated axon and varying numbers of innervated muscle fibers and is designated as the final common pathway (Sherrington, 1929) underlying all voluntary and reflex muscle contraction.

1.2.2 Motor unit physiology

The motor unit is composed of the motor neuron, motor axon, and the population of muscle fibers supplied by the axon. During voluntary muscle contraction, it was noted in classical early studies that motor units of smaller size tended to be recruited earlier, while larger motor units, with more muscle fibers, entered contraction later as intensity increased (Denny-Brown and Pennybacker, 1938). This pattern of motor unit recruitment was later established as a physiological phenomenon in mammalian neuromuscular function, termed Henneman's size principle of orderly MU recruitment, whereby activation of additional motor neurons during increasing contractile intensity was based on their somatic and dendritic size (Henneman, 1957). Additionally, MUs can be activated with increasing impulse frequency in a phenomenon termed rate coding. In humans, initial sustained discharge frequencies of MUs are between 7-10 Hz. During ramp contractions of increasing force, the discharge frequencies of these earliest recruited MUs increase resulting in increased force contribution. Subsequent increases in force result from a combination of recruitment and rate coding with human MUs firing at upwards of 40 Hz during maximal contractions (Doherty, Chan and Brown, 2002).

Further studies examining mean innervation ratios (number of muscle fibers per motor unit) revealed that relative motor unit size was in part dependent upon the size of the muscle it comprises, since MU innervation ratios tend to be much higher on average in larger muscles like the gastrocnemius than in comparatively smaller muscles (e.g. extraocular muscles) (Feinstein et al., 1955). It is suggested that this disparity in the number of muscle fibers innervated per motor unit across muscles of varying size may be related to requirement of fine motor control in smaller muscles, such as intrinsic hand muscles, whereby a higher number of MUs is observed relative to their mass, in larger muscles where power generation or control of posture are more important functionally (Doherty et al., 1995).

In general, motor units are classified according to their metabolic strategy as well as by their contraction speed. Type I MUs with constituent slow-twitch fibers express a significant quantity of oxidative enzymes (Buchthal and Schmalbruch, 1980), contracting under aerobic conditions for a longer period of time than fast-twitch muscle fibers comprising type II MUs, which contract under anaerobic conditions revealing a significant proportion of glycolytic enzymes (Burke et al., 1973). Intermediate type IIA MUs express high levels of both aerobic and anaerobic enzymes. Typically, human muscles tend to exhibit heterogeneous populations of both motor unit subtypes and fiber type distribution, with some exception such as the type I fiber-dominant soleus and type II fiber-dominant orbicularis muscles (Johnson et al., 1973).

1.2.3 The neuromuscular junction

Also known as the neuromuscular synapse, the neuromuscular junction is the site of release of quanta of acetylcholine from the motor neuron terminal to its corresponding acetylcholine receptor on a muscle fiber endplate within the muscle fibers of a given motor unit (Dale et al., 1936). Transmission at the neuromuscular junction, from axonal electrochemical signaling to muscular excitation allowing for muscle contraction and movement, is initiated by the arrival of a motor neuron action potential at the axon terminal, causing an increased local intracellular concentration of calcium ions (Baker, 1977). This chemical gradient causes acetylcholine vesicle release from the presynaptic terminal for diffusion into the neuromuscular synaptic cleft (Silinsky, 1985).

Acetylcholine receptors expressed on the muscle fiber membrane or endplate have two binding sites for the ACh neurotransmitter, which when bound successfully, generates a local sub-threshold depolarization known as an end-plate potential at the muscle membrane (Fatt and Katz, 1951). Once the end-plate potential exceeds threshold and produces an action potential for propagation along the muscle fiber, the active contraction of muscle tissue occurs as a result of the release of calcium from the sarcoplasmic reticulum initiating coupling of actin and myosin. However, for successive transmission and subsequent contraction to occur via the neuromuscular junction, unbound acetylcholine within the synapse is catabolized by the synaptic enzyme acetylcholinesterase to acetyl coenzyme A and choline (the latter of which is actively transported back into the presynaptic nerve terminal), in addition to reuptake of ACh transmitter by the nerve terminal through passive diffusion (Marnay and Nachmansohn,

1938; Taylor, 1991). This process of excitation-contraction coupling across the ACh-mediated neuromuscular junction provides the basis for effective MU function.

1.3 Aging of the motor unit

1.3.1 Sarcopenia

Sarcopenia describes the process by which skeletal muscle shows a decline in both mass and strength as a consequence of the normal aging process (Rosenberg 2011; Doherty, 2003). Progressive decreases in muscle mass and strength beginning as early as the 4th decade (Marcell, 2003) as well as reduced power output eventually result in reduced mobility and loss of functional independence (McKinnon et al., 2017). Muscular atrophy and frailty associated with sarcopenia is influenced by changes in hormone secretion, inadequate nutrition, inflammatory mediators and in particular, changes in innervation of the peripheral and central nervous systems (Doherty, 2003). Although the exact mechanisms that contribute to the development of sarcopenia remain to be identified, it has been well-established that skeletal muscle fiber loss (Lexell, Taylor and Sjöström, 1988) as well as decreased cross-sectional area of surviving muscle fibers (Aniansson et al., 1986) occurs. Several possible biological scenarios are likely to yield the functional consequences of sarcopenia, which include increasing sedentary lifestyle, downregulation in protein production, altered hormonal expression, increased neuromuscular denervation rate, in addition to greater turnover of apoptotic cells (Pollack and Leeuwenburgh, 2001). Secondary to decreased muscle mass, strength loss due to aging can occur by as much as 20-40% in various upper and lower limb muscles, while

total cross-sectional area of muscle from 20 to 60 years of age shows a roughly 40% decline (Doherty, 2003).

In addition to losses in muscle mass, aging has been associated with gradually more ineffective elimination of faulty proteins within muscle tissue, possibly accounting for the observed greater reduction in strength than losses of muscle mass alone can account for; so called decrease in muscle quality. For example, it has been discovered that expression of the autocrine factor myostatin, which is involved in inhibition of muscle development (McPherron, Lawler and Lee, 1997), is up-regulated in type II glycolytic skeletal muscle fibers (Lexell and Downham, 1992), which are thought to be preferentially targeted by age-related muscle loss.

Despite these multiple potential mechanisms, survival of muscle fibers in response to aging is thought to be largely dependent upon their regular activation by corresponding motor neurons, whose number has been shown to decline significantly beyond the 7th decade. This progressive loss of MUs results in progressive cycles of denervation and reinnervation, and eventually loss of muscle fibers as reinnervation cannot keep pace with denervation (McKinnon et al., 2017). Additionally, lower motor neuron loss in older adults is associated with decreased myelination of mainly fast-conducting axons through Schwann cell reduction, which contributes to significantly diminished motor nerve conduction velocity (Doherty et al., 1993; Rivner et al., 2001) in part related to increased intermodal distance (Delbono, 2003; Jang and Van Remmen, 2011). Muscle biopsy studies provide evidence for neural factors due to MU loss as a major contributor for the development of sarcopenia in older individuals. Increased

numbers of angulated muscle fiber morphology (typical of denervation) and greater fiber type grouping (secondary to collateral reinnervation) have been observed in multiple studies (Brown and Hasser, 1996; Lexell and Downham, 1992). Such age-associated MU remodeling has the most pronounced impact on type II muscle fibers, with greater loss of type II fibers and more substantial decreases in cross-sectional area, in comparison to type I fibers. (Lexell, Taylor and Sjöström, 1988; Grimby et al., 1982; Larsson, Grimby, and Karlson, 1979).

Aged skeletal muscle has also been shown to exhibit higher rates of mitochondrial DNA mutation in individual muscle fibers of both rats and nonhuman primates (Bua et al., 2002; Lopez et al., 2000), with older human subjects also displaying higher genetic alterations when compared with younger individuals (Wang, Michikada and Mallidis, 2001). This observation could describe why long-term accumulation of mitochondrial reactive oxygen species throughout life may increase vulnerability of energetically-demanding motor neurons and fast-twitch muscle fibers to apoptosis. In response to aging, muscle fibers exhibit marked histological changes in their morphology which contribute to gradual decline in neuromuscular function. Such features as variation in the size of fibers in addition to grouping of neighbouring fibers of the same type are increased in both aged humans as well as older mice (Hepple and Rice, 2016; Andersen, 2003; Kanda and Hashizume, 1989; Rowan et al., 2011). Further indication of sarcopenia involves co-expression of various myosin heavy chain isoforms (Andersen et al., 1999; Patterson et al., 2006; Rowan et al., 2012), which combined with modified fiber size and type distribution denote effects from MU remodeling driven by recurring fiber denervation-reinnervation.

1.3.2 Animal studies of aging

The process of aging has a prominent impact upon neuromuscular junction physiology in not only older men and women, but these observations are also present on aged animals as well. Organization of contiguous ACh receptor expression at the mouse motor endplate indicates rapid turnover. Additionally, a shifting toward disjointed populations of post-synaptic receptors in conjunction with formation of proximal nerve terminal varicosities contribute to weakened maintenance of the synapse (Li, Lee and Thompson, 2011). Following age-associated disturbance of the mouse neuromuscular junction, there is denervation-dependent muscle fiber necrosis and reinnervation-driven synapse modification as evidence by centrally-positioned nuclei in fibers comprising affected synapses (Li, Lee and Thompson, 2011). Though age-related injury to fibers produces irreversible changes in mice that become exacerbated with age, presence of central nuclei in reinnervated fibers along with corresponding muscle fiber necrosis occurred only briefly and rarely (Li, Lee and Thompson, 2011).

Histochemical examination of aged mice using transmission electron microscopy revealed prominent signs of neuromuscular junction alteration in the fibularis longus muscle when compared with younger controls (Boaro, Soares and König Jr, 1998). Much of this modification observed in aged mice was thought to be the result of declining motor neuron function as their degenerating terminal axons displayed mitochondrial decay, aberrant Schwann cell activity and reduced synaptic vesicle production. Most notably, simultaneous accumulation of lysosomal waste material was observed in the

muscle fiber endplate of aged mice, implying severe morphological and functional consequences for synapses affected by retreating motor neurons (Boaro, Soares and Konig Jr, 1998)

Similar to studies of human muscle, studies on aging rats have provided evidence of MU remodeling compromising neuromuscular junction configuration over time. Greater degree of poly-innervated synapses, axon growth and decay, reinnervation of orphaned muscle fibers, and deprivation of acetylcholinesterase sites on denervated muscle fibers were all observed in aged rats when compared with younger controls (Kawabuchi et al., 1995). Specifically, older rats exhibited extensive terminal axon sprouting including neural growth even towards innervated muscle fibers, suggesting a parallel process of neuromuscular synapse formation and collapse in rats prior to advanced age that could trigger the adaptive process of MU remodeling and increasing size through collateral reinnervation of the remaining MUs (Kawabuchi et al., 1995).

Examination of neuromuscular aging in *Drosophila* flies with fluorescence and electron microscopy has also demonstrated similar change to the structure of neuromuscular synapses. In response to aging, there is evidence of impeded release and sequestering of synaptic vesicles since increased terminal axon size, area of endplate receptor distribution and number of active zones present in the neuromuscular synapse may compensate for presynaptic vesicular accumulation seen in aging flies (Wagner et al., 2015).

Research on the effect of aging on MU properties in rat medial gastrocnemius muscle demonstrated several notable observations. Utilizing methods to examine functional components of individual gastrocnemius MUs in old and middle-aged rats, aged animals had high proportion of slow MUs (with proportionally less fast MUs) with generalized grouping by fiber-type, higher amount of type I muscle fibers (with corresponding decrease in type II fibers), and more angular fiber appearance (Kanda and Hashizume, 1989). Furthermore, slow-twitch MUs in older rats produced overall higher force output when compared to those in middle-aged rats due to expanded innervation ratio observed as a result of age-related MU remodeling whereby additional muscle fibers that have been denervated are integrated into surviving MUs (Kanda and Hashizume, 1989). Thus, evidence for age-related changes to MUs has been established in animals, including MU loss, selective denervation of type II fibers, and modified neuromuscular junction transmission.

1.3.3 Human aging and motor unit loss

One of the more characteristic features that illustrate the extent to which human neuromuscular aging has occurred involves the gradual reduction in MU number, rendering a decreased MU pool of predominantly larger remodeled MUs with altered function (Doherty, 2003). Electrophysiological studies of both proximal and distal upper and lower limb muscles have revealed significant decrease in MU number in older adults that may lead greatly to progressive loss of muscle mass and decline in strength associated with aging (Boe et al., 2004; Doherty et al., 1993; Hourigan et al., 2015;

McKinnon et al., 2015; McNeil et al., 2005; Power et al., 2010; Vandervoort, 2002). Prior to significant MU loss, muscle fibers undergo denervation, which is brought about through motor axon damage by excessive oxidative stress with age (Jang and Van Remmen, 2011; Manini et al., 2013; Misgeld, 2011). Type II motor axons undergo reduced expression of oxidative enzymes, which when coupled with higher metabolic demand observed in type II axons (Gordon et al., 2004), could make them susceptible to long-term aging effects, resulting in greater type II axon loss and subsequent fiber denervation. It is possible that these metabolic factors may account for selective degeneration of type II fibers and observed predominance of type I muscle fibers, since type I motor neurons may increasingly reinnervate remaining type II fibers. These combined processes result in expansion of MU size in the MU pool of aging muscle (Doherty, 2003). Therefore older muscles are comprised of larger MUs with increased numbers of type I fibers.

Age-related changes secondary to MU loss that impact normal innervation of muscle fibers directly influence muscle function and subsequently result in loss of muscle contractile strength (Doherty et al., 1993). Previous research has demonstrated that individuals on average begin to lose approximately 10-15% of muscle strength capacity each decade after 30 years of age (Vandervoort and McComas, 1986), which only tends to have a pronounced effect on function around 60 years of age, when up to 50% of strength has been lost (Doherty et. al., 1993). These physiological signs of sarcopenia, including significantly decreased muscle mass (Doherty, 2003), are thought to be secondary to a substantial decline in motor unit number in old age, as a primary

underlying mechanism for muscle fiber degeneration for both distal and proximal muscle groups, with an approximate 50% reduction in the estimated number of MUs beyond the 6th decade in humans (Doherty et al, 1993; Lexell, Taylor and Sjoström, 1988). This significant loss of MUs has been demonstrated based on various electrophysiological motor unit number estimate (MUNE) techniques for the thenar (Brown, 1972; Sica et al., 1974; Doherty and Brown, 1993), extensor digitorum brevis (Campbell et al., 1973), hypothenar (Sica et al., 1974), and biceps/brachialis (Doherty et al., 1993), and tibialis anterior (McNeil et al., 2005) muscles of older individuals. The tibialis anterior in particular, was shown to exhibit further significant MU loss corresponding to significant reduction in strength in individuals beyond the 8th decade in life when compared to older adults of around 65 years of age. This observation may suggest that accelerated losses of strength occur when a critical number of MUs are lost at which time reinnervation cannot further effectively compensate (McNeil et al., 2005). Evidence of such age-related alterations associated with MU loss preceding significant loss in muscle mass and decline in strength demonstrates the importance of estimating motor unit number in older subjects.

Though it has been also previously noted that aged (71 ± 4 years) subjects exhibit about 45% less motor units (in the tibialis anterior) when compared with healthy young (26 ± 5 years) controls (Piasecki et al., 2016), age-related motor unit remodeling (as a consequence of MU loss) yields MUs that are increased in size, with greater muscle fiber density per MU (Stalberg et al., 1989). Remodeled motor units that remain are recruited mainly at low to moderate thresholds and reveal motor unit potentials of increased size, complexity and greater firing variability at the neuromuscular junction (Stalberg and

Fawcett, 1982). Recently, lifelong exercise has been shown to diminish neural effects of aging in the tibialis anterior muscle of master's athletes beyond the 8th decade in life, with significantly less reduction in the MU pool and less MU instability when compared with non-trained, healthy older subjects (Power et al., 2016; Power et al., 2010).

1.3.4 Neuromuscular junction aging and stability

The integrity of the neuromuscular junction and indeed the stability with which muscle fibers are activated is dependent upon a biochemical signaling cascade that effectively works to concentrate ACh receptor expression on the muscle endplate directly where synapses are formed by nerve terminals (Hepple and Rice, 2016). This is accomplished through the expression of agrin by motor axon nerve terminals, which acts upon muscle-specific kinase (MuSK) of muscle fibers to coordinate other signaling proteins that help facilitate the coupling of ACh receptors to the muscle cell membrane (Hepple and Rice, 2016).

It has been documented that processed agrin components in high quantity were discovered in vivo for certain aged subjects with excessive muscle loss (Hettwer et. al., 2013). A possible explanation for this occurrence could involve age-related overexpression of neurotrypsin, the agrin protease which quickly triggers MU collapse. The effect that such ageing processes may have on perisynaptic Schwann cells, which promote effective reinnervation and maintain neuromuscular junction configuration (Sugiura and Lin, 2011), remains to be studied. Alternatively, decreased levels of MuSK protein at the endplate may also be relevant in neuromuscular aging. Decreased agrin has

been observed in elderly rats (Gouspillou et al., 2013) in addition to other rodent models that exhibit disruption in normal recycling of ACh receptors (Carnio et al., 2014).

Further investigations are necessary for identifying the presence of diminished MuSK in aged rodents as an underlying cause of neuromuscular decline or as a response to neural mechanisms.

In humans, the extent of sarcopenia and loss of strength in older subjects (of relatively similar age and number of MUs) has been shown to be greatly influenced by MU stability (Gilmore et al., 2017b). Older subjects with severe sarcopenia displayed reduced maximal volitional strength in comparison to sarcopenic and pre-sarcopenic older subjects of similar age, while also showing increased electrophysiological measures of neuromuscular junction instability when compared with pre-sarcopenia individuals (Gilmore et al., 2017b). Recent studies on proximal and more distal lower limb muscles (vastus medialis and tibialis anterior, respectively) have revealed the correlation between MU loss and increased MU instability with normal aging in comparison with younger controls (Hourigan et al., 2015). Average near fiber (NF) jiggle from the tibialis anterior muscle was reported in young controls as 26 % compared to 36 % for older individuals. A similar significant increase in mean NF changes for the vastus medialis with older men showing 31 % NF jiggle in relation to younger men with 23 % (Hourigan et al., 2015). Electrophysiological measures of instability (NF jiggle and jitter) have also been used successfully for assessing variability in the MU in other disorders involving denervation and MU loss, including diabetic polyneuropathy (Allen et al., 2015) and chronic inflammatory demyelinating polyneuropathy (Gilmore et al., 2017a). Examination of neuromuscular stability has been revealing in research on older masters athletes, which

demonstrated significant mitigation of age-related increase in MU instability from lifelong exercise when compared to age-matched controls (Power et al., 2016). These results might suggest the importance of examining the stability of the neuromuscular junction when assessing progressive loss of strength with aging, once reduction of MU number has become functionally relevant.

1.4 Quantitative electromyography

1.4.1 MUP analysis

In order to visually represent the physiology of individual MUs for clinical analysis and diagnosis of neuromuscular disorders, needle electromyography (EMG) involves the intramuscular recording of motor unit potentials using an inserted needle electrode during voluntary muscle contractions. Important features of individual MUPs for analysis include their amplitude, duration and complexity of their waveforms (phases and turns). Muscle fiber loss or necrosis (as in myopathies) may be conveyed by MUP amplitude and duration reduction whereas collateral reinnervation secondary to MU loss and subsequent reinnervation yields MUPs of increased amplitude and longer durations in neuropathic disorders such as nerve injury (Stashuk, 1999b). In the typical clinical setting, an experienced electromyographer makes a subjective determination of MUP size, shape and recruitment to indicate the presence or absence of diseases affecting the nerve and muscle. However, in the research setting it is often necessary to obtain a representative sample of MUPs to make a statistical determination of MUP size and shape. To do so, during a sub-maximal muscle contraction, a composite signal representing successively activated motor unit potentials (MUPs) indicative of recorded

MU firing activity, is displayed and recorded. In order to produce MUP templates that convey electrophysiological information of recruited individual MUs, the initial EMG signal must be processed through digital filtering that accentuates constituent MUP peaks as spikes for more reliable classification into templates. From this composite signal of detected MUs, periodic spikes can be organized into valid spike trains through recognition of significant spike detections and examination of consistency in shape (often through pattern recognition algorithms) and the interval between spikes, respectively (Stashuk, 1999b). From these MUP trains, a representative MUP is extracted, and calculated variables such as amplitude, duration, phases, turns, and firing rate, for each detected motor unit are determined (Stashuk, 1999b).

One method of obtaining a sample of MUPs for further analysis is decomposition-based quantitative EMG (DQEMG) and decomposition based spike triggered averaging (DE-STA). DE-STA is a quantitative needle EMG method whereby intramuscular signals and surface EMG are obtained to extract simultaneously-discharging needle MUPs and their surface detected-MUPs (S-MUPs) (Doherty, Stashuk, and Boe, 2009; Nandedkar, Stålberg, and Sanders, 2002). This is achieved through automated spatial and temporal pattern recognition algorithms that decompose, sort and then match intramuscular templates as MUP trains displaying the firing activity of distinctly sampled motor units. From the intramuscular needle-detected MUP train a representative MUP template is produced, and the associated S-MUP from the surface EMG signal is extracted through spike-triggered averaging. Standard quantitative measures of MUP size (amplitude, duration) and shape (phases, turns) are calculated from the MUP template, whereas an average MU size is calculated from the sample of corresponding S-MUPs (usually based

on 20 or more MUPs from 4 to 6 contractions). The advantage of simultaneously recording S-MUPs with non-selective surface electrodes and MUPs with selective intramuscular needle electrodes is seen in the former providing information regarding motor unit size and spatial distribution of muscle fibers (Stashuk, 1999b), while the latter yields information concerning the spatial and temporal properties of the individual muscle fibers (Stashuk, 1999b).

1.4.2 MUNE analysis

One approach to examining the state of all motor units in a particular muscle is the motor unit number estimate (MUNE). The various MUNE methods, usually obtained through electromyographic measures, approximate the number of functional motor units in a muscle or muscle group, but also provide important information about motor unit remodeling/collateral reinnervation by determination of mean MU size (Nandedkar, Stålberg, and Sanders, 2002).

All electrophysiological methods of MUNE share a similar basic concept, that is, the number of functioning MUs in a muscle or muscle group can be estimated by dividing the mean size of a sample of individual MUs (mean S-MUP size) into the maximum compound muscle action potential size (representing the sum of all constituent S-MUPs). The methods vary only based on the way in which the sample of individual S-MUPs is obtained.

Several electromyographic techniques have been developed to estimate motor unit number through variation of the method used to acquire an average S-MUP value, which

when divided into the compound muscle action potential (a maximal evoked response representing the sum total of a muscle's MUPs), yields the MUNE. Manual incremental stimulation (McComas et al., 1971) is a MUNE technique that relies on successive nerve stimulation of increasing intensity, whereby individual response increments to the CMAP are thought to represent the additional individual MUs and their S-MUPs. This method has the advantage of being collected at a single stimulation site with minimal invasiveness. However, this incremental MU estimation method is limited by it being inapplicable to proximal muscles (as their motor nerves are less accessible) and tendency to overestimate MU number through the phenomenon of alternation (error derived from considerable overlap in MU thresholds causing various combinations of previously activated MUs with successive increments) (Milner-Brown and Brown, 1976). Multiple-point stimulation (Doherty and Brown, 1993) was developed to avoid alternation completely by stimulating at multiple points along the motor nerve, obtaining only a single S-MUP at each site to provide a sample of S-MUPs to derive the mean S-MUP size. While this method avoids alternation, it has been contested whether or not this technique provides a valid mean S-MUP size, given that smaller axons with lower threshold may be those that are preferentially excited along the nerve (Doherty and Brown, 1993). Furthermore, the estimation strategy is restricted to distal muscles and requires significant skill and practice on the part of the operator to reliably stimulate single axons. Spike-triggered averaging (Brown et al., 1988) is a MUNE technique that relies upon simultaneous recording of intramuscular and surface electromyography (via needle and macro surface electrodes) during low-intensity volitional contractions in order to isolate single recruited MUs in both proximal and distal muscles. Needle-electrode

detected MUP spikes are used as triggers to ensemble-average surface EMG signals from their corresponding MU, thus providing a sample of S-MUPs to calculate average S-MUP size (Brown et al., 1988). However, it becomes apparent that with greater contraction force, the more complex MUP interference pattern can distort the triggering of surface detection, which limits spike collection to the lowest threshold MUs recruited. These low threshold MUs are typically of smaller size (Milner-Brown et al., 1973), resulting in a mean S-MUP that might not be physiologically representative. The addition of MU signal decomposition to spike-triggered averaging (Stashuk, 1999b; Gooch et al., 2014), allows for automated identification and clustering of MUP trains to assist in obtaining a sample of MUPs from at least moderate intensity contractions and more rapid extraction of their associated S-MUPs. DE-STA achieves reliable automated identification and ordering of MUP signals through initial first-order filtering of the raw EMG composite signal, which is subsequently scanned for detection of individual MUPs. (Stashuk, 1999b). Then, unsupervised classification (or clustering) of MUP data can be grouped according to a shape and temporal-based pattern recognition algorithm for the goal of estimating the number of active MUs as well as their corresponding MUP waveform template (Stashuk, 1999b). Finally, supervised classification of clustered MUPs uses a certainty algorithm which compares candidate MUPs to prospective MUP templates for the purpose of generating MUP trains that accurately reflect the activity of distinct MUs (Stashuk, 1999b).

DE-STA has previously been used to obtain reliable MUNE in studies of aging, amyotrophic lateral sclerosis, diabetic neuropathy, and chronic inflammatory demyelinating polyneuropathy (Hourigan et al., 2015; Boe et al., 2007; Allen et al., 2015;

Gilmore et al., 2017a). This MUNE strategy has yielded S-MUPs which represent a greater diversity of MU size from stronger contractions (Conwit et al., 1997) and provides useful information regarding individual MU firing and waveform characteristics (Conwit et al., 1999).

1.4.3 Jiggle and jitter analysis

An important variable for examining motor unit firing characteristics is jiggle. Jiggle is the variability in MUP shape according to changes in output of individual fiber contributions to the MUP, between consecutive discharges of their motor unit (Stålberg and Sonoo, 1994; Figure 1). This jiggle parameter, or mean consecutive difference in MUP shape between repetitive firings, has shown to be useful in denoting the presence and time course of collateral reinnervation in response to age-related MU decline (Stålberg and Sonoo, 1994). Within a given active motor unit, muscle fiber action potential (MFAP) contributions to the MUAP vary in the intervals between their discharges. This mean temporal variation between consecutive MFAPs is termed jitter and in addition to jiggle, comprise statistics used for measurement of MU stability of neuromuscular junction transmission, with greater jitter and jiggle indicative of greater instability (Stålberg, 2012; Stålberg and Sonoo, 1994). Increases in jitter and jiggle have been observed with age-related MU-remodeling as well as in amyotrophic lateral sclerosis (ALS) and myasthenia gravis patients (Campos et al., 2000; Stålberg, 2012). Typical values for these stability parameters in normal healthy adults (under the age of 60) has been observed within a range of about 0 - 28 % for jiggle (Stålberg and Sonoo, 1994) and within approximately 30 - 55 ms for jitter (Bromberg et al., 1994). Jitter values vary depending on the muscle studied and age of subject. Recent studies on aging have

shown significantly increased jiggle and jitter statistical measures of MUP stability in healthy older adults (Power et al., 2012; Hourigan et al., 2015, Power et al., 2016).

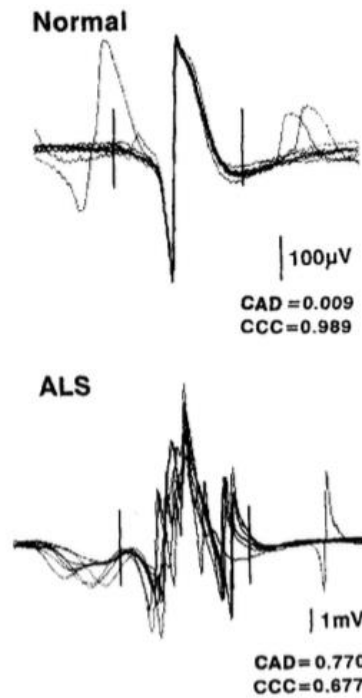


Figure 1: Schematic representing the significantly increased shape variability or jiggle in an ALS patient. (as quantified by higher consecutive amplitude difference between successive MUPs), relative to a normal subject (reproduced with permission from Stålberg and Sonoo, 1994).

1.4.4 Near fiber analysis

Muscle fiber contributions to recorded MUs detected mainly within approximately 350 μm of the concentric needle electrode are referred to as near fibers (NFs) and are derived from the NF MUP template (Allen et al., 2015). This template is achieved through high-pass acceleration filtering of the automated DQEMG-derived MUP template and a second order differentiator for low pass signals. This so-called “acceleration filtering” helps to accentuate detected fibers with spatial proximity close to the needle electrode when creating the NF-MUP template (Stashuk, 1999a). Thus, NF contributions accentuate temporal interval features, which are representative of individual NF conduction times toward the needle electrode detection surface by single MFAPs (Allen et al., 2015; Stashuk, 1999a). Given that MU fibers share comparable conduction velocities, recorded NF temporal differences convey spatial distance of MFAP and axon conduction, and by inferring similar muscle fiber conduction velocity within an MU, temporal variance in NFs (jiggle and jitter) indicate the amount of nerve collateral sprouting in aged reinnervated MUs (Allen et al., 2015; Stashuk, 1999a).

Several NF characteristics have been defined that describe relationships between muscle fibers within a MU that contribute significantly to the NF MUP, and pertain to specific underlying electrophysiological processes. NF count is suggestive of approximate muscle fiber density in a MU since it measures the quantity of distinct, detected NF contributions, distinguished as positive turns showing adequate amplitude and symmetry (Stashuk, 1999a; Allen et al., 2015). Maximum NF interval is defined as the maximum time measured between NF contributions detected consecutively, in which increased values denote the presence of lengthening nerve terminals as part of collateral

reinnervation (Stashuk, 1999a; Allen et al., 2015). NF dispersion is the time interval from the first to last recorded NF MUP contribution and also provides evidence of reinnervation taking place. Analogous to the MUP parameter, NF duration is a temporal measure reflecting the whole duration of the NF MUP (Stashuk, 1999a; Allen et al., 2015). NF jiggle is a measure of NF MUP variability (akin to traditional MUP jiggle), which is the mean consecutive difference in NF shape (expressed as a percentage), from one individual NF MUP in a train to the next. Similarly, NF jitter measures temporal variation between consecutive muscle fiber pairs detected in the NF MUP (Stashuk, 1999a; Allen et al., 2015) and is analogous to traditional jitter values acquired from either a single fiber or concentric needle electrode. These NF methods of measuring MU stability have been used successfully in studies in previous studies on human aging (Hourigan et al., 2015), chronic inflammatory demyelinating polyneuropathy (Gilmore et al., 2017a) and diabetic neuropathy (Allen et al., 2015), with significantly increased NF instability reported in these aging and clinical subject groups.

1.5 The first dorsal interosseous and biceps brachii muscles

The ulnar nerve derives its axons from primarily the eighth cervical and first thoracic roots that eventually form a collection of nerves that enter the lower trunk and medial cord of the brachial plexus (Stewart and Bourque, 2002). The ulnar nerve is derived from the medial cord. Beginning in the axilla, the nerve begins to run underneath the intramuscular septum of the upper arm, along the medial triceps brachii muscle, only to reach the elbow. The nerve then innervates the flexor carpi ulnaris and part of the flexor digitorum profundus (of the anterior forearm), before reaching the wrist. At the

wrist, the nerve passes through Guyon's canal, also known as the ulnar tunnel, this canal is important as the site of ulnar branching into deep and superficial pathways, whereby the latter innervates primarily the palmaris brevis muscle and the former extends across the hand to innervate the hypothenar, lumbricals (3rd and 4th), interosseous (palmar and dorsal) muscles, the adductor pollicis, and often a component of flexor pollicis brevis (Kincaid and Campbell, 2002). Ulnar innervation is present in certain intrinsic muscles of the hand, such as the interosseous, hypothenar and some thenar muscles, and is responsible for contributing to fine motor control movements such as pinch and grip strength (Kozin et al., 1999). The first dorsal interosseous (FDI) muscle, situated superficially on the back of the hand between the thumb and index finger, is responsible for abducting the latter digit exclusively, since dorsal interossei and lumbricals of the hand primarily function in spreading the fingers away from the hand's midline. The FDI along with the other dorsal interossei are also involved in assisting finger flexion at the metacarpophalangeal joints during grip and pinch contractions of the hand (Kozin et al., 1999).

The musculocutaneous nerve originates in the lateral cord of the brachial plexus and is derived from the fifth to seventh cervical roots, extending to supply the biceps brachii (BB) and brachialis muscles of the upper arm (Stewart and Bourque, 2002). Lateral to the BB tendon, the musculocutaneous nerve continues across the elbow to become the lateral cutaneous nerve, innervating the lateral forearm to the level of the wrist (Stewart and Bourque, 2002). One of the BB muscle's functions during voluntary movement is flexion of the elbow, however it is also involved in supination of the forearm at the radioulnar joint. The FDI and BB muscles vary to a large extent not only in

their relative size, but also in that the latter muscle's constituent MUs occupy a larger territory with greater innervation ratio of muscle fibers incorporated in each MU and a significantly larger MU pool in general. Specifically, the FDI reveals on average a total of 119 α -motor axons, 40,500 muscle fibers and an innervation ratio per MU to be 340, compared with the BB, which is estimated to have around 774 α -motor axons, 580,000 muscle fibers and an innervation ratio of about 750 (Feinstein et al., 1955; Doherty et al., 2002). The FDI also differs from the proximal BB in fiber type percentage, with more than half of muscle fibers (around 57%) in the former identified as type I, while the BB exhibits a predominance of type II fibers or about 62% of total MUs (Johnson et al., 1973; Brooke and Kaiser, 1970). The various anatomical and physiological differences between these upper limb muscles make them ideal for studying the impact of aging where muscle undergoes MU remodeling involving recurring cycles of denervation and nerve collateral reinnervation of muscle fibers.

1.6 Study objectives

The primary aim of this study is to examine MU loss and remodeling, and age-related MU instability via electrophysiological measures by comparing a distal (FDI) and proximal (BB) muscle in young and older healthy subjects. DQEMG methods have been previously applied to study MU loss (Boe, Stashuk and Doherty, 2006; McNeil et al., 2005; McKinnon et al., 2017) and NF jiggle and NF jitter parameters have been used to examine age-related MU stability for proximal and distal lower limb muscles (Hourigan et al., 2015). However, MU stability has not been examined to date in proximal and distal upper limb muscles. It is hypothesized that both muscles of interest in older

individuals will show significant decline in MU number, associated increase in MU size, as well as increased MU instability as measured by NF jitter and jiggle, when compared with younger controls.

Chapter 2

2 Introduction

The process of normal neuromuscular aging (referred to as sarcopenia) is marked by gradual deterioration in skeletal muscle mass and quality with significant loss in contractile strength, which culminates in reduced mobility and inevitable loss of functional independence (Doherty, 2003). Underlying this process is evidence of muscle fiber loss (particularly of type II fibers) as a result of chronic alpha-motor neuron denervation (and subsequent motor unit loss) due to excessive oxidation damage over time associated with the highly metabolic environment of motor axons (Manini et al., 2013).

Since sprouting nerve terminals incorporate more muscle fibers in remodeled MUs, this contributes to increased MU size, as exemplified by higher amplitude, longer duration needle MUPs and larger surface MUPs (S-MUPs). These neuromuscular features of normal aging can be extracted through the use of decomposition-based quantitative EMG (DQEMG), which through simultaneous recording of needle-detected intramuscular and macroelectrode-detected surface signals, can sample a MU pool assisted by MU recognition and sorting algorithms to assess presence of reinnervation as well as MU stability (Stashuk, 1999a; Stashuk, 1999b). These electrophysiological measures using DQEMG have demonstrated MU remodeling due to aging, with characteristic increased MUP and S-MUP amplitude as well as decreased MUNE (Power et al., 2010). Additionally, electrophysiological MU number estimation techniques have

demonstrated reduced MU numbers for upper and lower limb muscles that approach fifty percent by the 7th decade and may accelerate thereafter (Doherty, 2003; McNeil et al., 2005).

The significant decline in MU number, mainly as a result of increased denervation and collateral reinnervation in response to aging, yields larger surviving MUs of fewer number. These remodeled MUs exhibit physiological changes at the neuromuscular junction, which impact their transmission stability during muscle activation. Such increased transmission variability (or decreased firing stability) at the neuromuscular junction is observed with animal as well as human studies on aging (Boaro, Soares and Konig Jr, 1998; Kawabuchi et al., 1995; Li, Lee and Thompson, 2011; Gousspillou et al., 2013; Gilmore et al., 2017a; Gilmore et al., 2017b; Hourigan et al., 2015). The integrity of the neuromuscular junction can be examined using MUP stability parameters, jiggle and jitter (Stålberg and Sonoo, 1994). Where jitter represents the average variability in time interval between consecutively firing muscle fiber contributions to a single MU, jiggle describes variability in MUP shape between successive discharges of one MU (Stålberg and Sonoo, 1994).

Novel near fiber (NF) parameters of MUP stability (NF jiggle and NF jitter), through the use of decomposition-based quantitative electromyography (DQEMG), have shown evidence of MU instability in previous studies of aging and neuropathic disorders (Hourigan et al., 2015; Allen et al., 2014; Gilmore et al., 2017). Significantly increased NF jiggle (measuring shape variability of successive NF MUPs in a MUP train) and NF

jitter (measuring mean consecutive difference in time interval between pairs of contributions to a set of isolated NF MUPs) have been observed in in lower limb muscles of healthy older adults when compared with younger control subjects (Hourigan et al., 2015; Power et al., 2012; Power et al., 2016). Although MU loss due to aging has already been reported for proximal upper limb muscles such as the biceps brachii (Power et al., 2010), the NF MUP stability of upper limb muscles in older adults has not yet been studied. Due to functional differences between proximal and distal muscles of the upper and lower limbs, it remains unclear which muscle groups would be impacted more greatly due to aging mechanisms of denervation, MU loss and decline in muscle mass.

The purpose of the current study was to compare MU loss and age-related MU stability between a distal (FDI) and proximal (BB) upper limb muscle in healthy young and older adults with the specific objective of using NF parameters from DQEMG to assess any age-related changes in stability for these muscles. It is hypothesized that both muscles of interest in older individuals will show significant decline in MU number, associated increase in MU size, as well as increased MU instability as measured by NF jitter and jiggle, when compared with younger controls.

Chapter 3

3 Methods

3.1 Participants

This study compared thirteen younger (31 ± 13 years; 5 males and 8 females) to eleven older adults (74 ± 5 years; 5 males and 6 females). All young and older subjects were free of known neuromuscular disorders. Specifically, no participant had any known history of generalized polyneuropathy, ulnar nerve injury, or cervical radiculopathy. We did not attempt to screen out subclinical disease and screening electromyography was not specifically performed to rule out asymptomatic ulnar neuropathy or cervical radiculopathy. Young subjects were recreationally active but not highly trained and the older subjects took part in regular physical activity through the Retirement Research Association and Ladies Retirement Research Association programs at the University of Western Ontario.

For every participant tested, data from both their first dorsal interosseous (FDI) and biceps brachii (BB) muscles were obtained. All subjects gave informed consent and the study was approved by The University of Western Ontario Health Sciences Research Ethics Board.

3.2 Muscle strength grading

FDI and BB strength were tested using the Medical Research Council (MRC) scale for muscle strength, where participants were asked to perform index finger abduction and elbow flexion for their dominant limb against manual resistance from the examiner. Contractions of full, normal strength were ranked a maximum score of 5, while weaker contractions were graded lower on the positive integer scale, the lowest possible score being 0 (indicating no movement). All participants also completed grip strength testing with a Jamar hand-grip dynamometer (JAMAR® 5030J1, Chicago, IL) and pinch-grip dynamometer (JAMAR® 749805, Chicago, IL). For both grip and pinch tests, three trials were performed whereby the highest force measurement in kilograms was recorded while subjects carefully rested their dominant forearm on a table while comfortably seated. In this position, each subject squeezed the hand-grip dynamometer by making a fist on the apparatus and applied force to the pinch-grip dynamometer by pushing their thumb against their medial index finger with the pinching apparatus in between. Subjects were verbally encouraged to give their maximal performance. In addition to force output and MRC score, age, weight and height were recorded for each subject.

3.3 Needle EMG data acquisition

The FDI and BB were studied in all subjects. Both surface and intramuscular EMG signals were detected simultaneously and analyzed using commercial software on the Viking EMG system (Natus Medical Incorporated, San Carlos, California). Surface EMG signals were acquired through the use of self-adhesive Silver Mactrode© electrodes (GE Medical Systems, Milwaukee, Wisconsin) and intramuscular EMG with 25 mm 30 gauge concentric needle electrodes (TECA Elite, CareFusion, Middleton, Wisconsin).

For each subject, surface potentials were detected using a bandpass of 5 Hz – 5 kHz, while for intramuscular signals, 10 Hz – 10 kHz was used. Sampling rates for surface and intramuscular potentials were at 4.8 Hz and 48 KHz, respectively.

Subjects were comfortably seated with their dominant forearm outstretched and pronated on a table. Prior to EMG examination of the FDI muscle, the skin surface overlying the muscle's belly, MCP joint and distal ulnar styloid process (at the wrist) were cleansed using wipes containing 70% isopropyl alcohol. Following this, surface electrodes were cut into thirds (0.66 cm x 3.5 cm). The active electrode was placed over the mid-belly of the FDI, the reference electrode over the MCP joint, and ground electrode over the ulnar styloid. For the BB, the skin over the mid-belly of the muscle was cleansed with alcohol. Full sized electrodes (2 cm x 3.5 cm) were placed over the mid-belly of the muscle (active) and the distal tendon (reference), with the ground electrode over the olecranon process.

In order to obtain a maximum compound muscle action potential for both muscles of interest, a manual bipolar stimulator was used to apply stimuli to the ulnar nerve at the wrist for the FDI muscle, and to the musculocutaneous nerve high in the axilla for the BB. Stimulation intensity was gradually increased for each muscle until a maximal potential was observed with no further increase in negative peak amplitude. The active electrode was repositioned as necessary to minimize the rise time and maximize the negative peak amplitude, at which point the surface electrodes were secured with adhesive tape to prevent movement during subsequent data collection. Based on initial testing it became clear that valid maximum CMAPs were unable to be obtained for the

majority of the older subjects due to adiposity, and the close proximity of the musculocutaneous nerve to the median and ulnar nerves in the upper arm. As a result valid CMAPs were obtained for the FDI, the majority of young subjects BB, but not older subjects BB. Therefore, MUNE were not calculated for BB.

After collection of the maximal CMAPs (where indicated), careful insertion of a concentric needle electrode in both muscles, proximal to the active surface electrode, was performed in each subject. All voluntary contractions performed by the subjects were ensured to be isometric for both muscles by carefully stabilizing the limb and the examiner providing manual feedback.

Voluntary isometric contractions were elicited by the subject, involving abduction of the index finger for FDI activation and elbow flexion for BB movement, for a duration of 30 seconds and recorded at submaximal intensities. The intensity of contractions was controlled such that they generated between 40 – 60 pulses per second of the aggregate needle EMG signal which was displayed in real time by the software and monitored by the investigator to maintain appropriate levels of contraction intensity (Figure 2). This intensity has been established to provide efficient detection of MUPTs previously by DQEMG studies and approximates 25-30% of maximum voluntary contraction (McNeil, 2005; McKinnon, 2017; Hourigan et al., 2015).

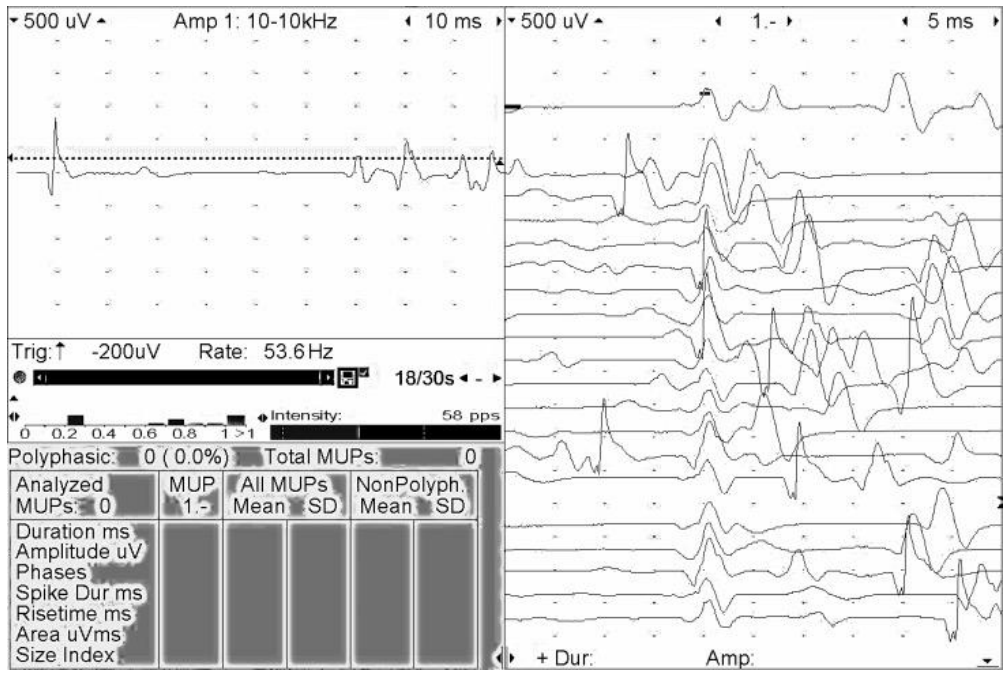


Figure 2: Intramuscular recording of FDI muscle from a younger subject during a submaximal voluntary contraction. Intramuscular recording of FDI muscle screen depicting aggregate of concurrently firing MUs (upper left panel) sampled via concentric needle electrode at monitored contraction intensity for a single 30 second voluntary contraction. The panel on the right is a raster plot of successive traces.

Following each contraction, a summary of concurrently acquired intramuscular and surface EMG data was displayed and were reviewed for suitability for analysis (Figure 3). Sixty seconds of rest was provided between contractions. Contractions were repeated for approximately 6 – 8 trials, with the inserted needle electrode being repositioned for each trial to sample different motor units. All subjects were instructed to maintain relatively consistent intensity once motor units were activated and to refrain from movement during each isometric contraction. Data collection for each muscle was concluded when at least 20 or more individual MUP trains that met specific analysis inclusion criteria (see below) were recorded from each muscle studied.

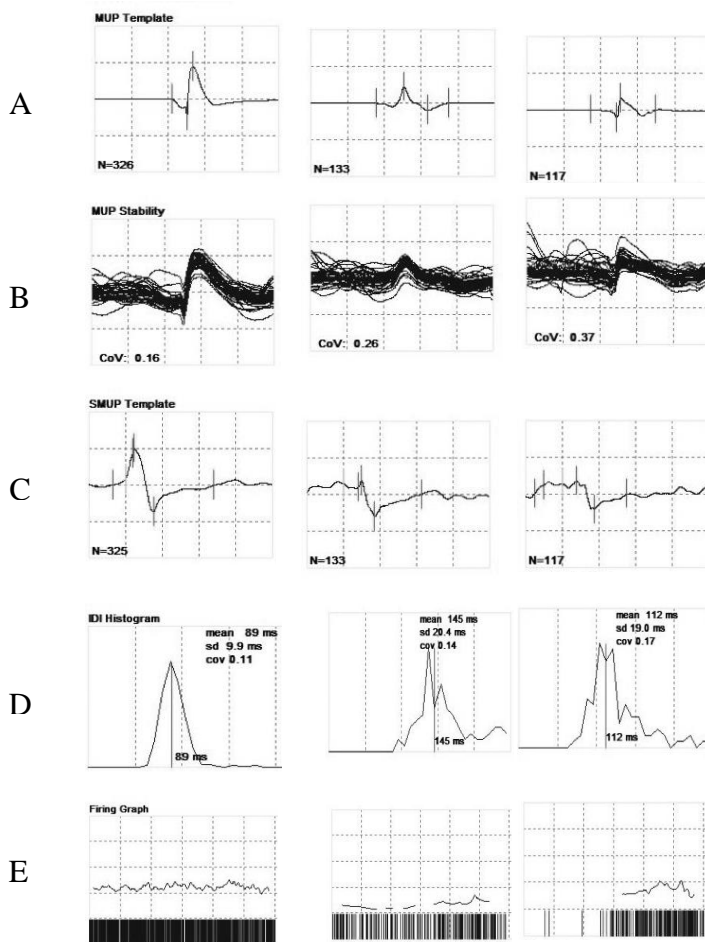


Figure 3: Summary screen displaying MUP template (A), shimmer plot (B), S-MUP template (C), IDI histogram (D), and firing graph (E) from sampled MUs in an FDI contraction. Three distinct MUP trains are represented by their MUP template (A), which accurately represents overall shape and duration for 51 traces of the MUP train, as displayed by its shimmer plot (B). S-MUP templates corresponding to each MU are also shown (C), along with the inter-discharge interval (IDI) histogram for each train (D) and instantaneous firing rate plot for all detected MUPs in the MUP train.

3.4 EMG data signal analysis

Throughout data acquisition, the D-QEMG software automatically saved and organized all recorded intramuscular and surface EMG data signals. This was accomplished by the software's specific decomposition algorithms comparing temporal and spatial similarities from the EMG needle recording that can be clustered and sorted into their derivative MUP trains (Figure 2) (Doherty and Stashuk, 2003). First, detection of suitable MUPs (Figure 3) for extraction was based on defined criteria regarding amplitude and slope parameters (amplitude of at least 50 μV and 0.3 V/s, respectively). These detected MUPs were initially clustered algorithmically into MUP trains first according to similar potential waveform shape (unsupervised classification) and subsequently by classifying unidentified MUPs through comparisons to the newly-created groupings (supervised classification, Stashuk, 1999b). Automated analysis of clustered MUPs concluded with confirming their attribution to assigned, distinct MUs by examining their firing rates and looking for instances where MUPs never fired in unison (indicating MUPs as belonging to the same MU) and MUPs that fired simultaneously on occasion (belonging to different MUs) (Stashuk, 1999b). Through this process, distinct MUPTs were derived that represented the firings of distinct MUs. For each MUPT, all MUPs collected by the intramuscular needle were time-locked to the surface EMG signal, allowing the constituent S-MUPs to be extracted for each associated MUP through spike-triggered averaging (Doherty and Stashuk, 2003) (Figure 5). For each detected MUPT, this information was necessary to yield ensemble averaged needle MUP template (which were based on the median filtered data of 51 isolated contributions) and their associated SMUP templates based on the number of spike-triggered averages.

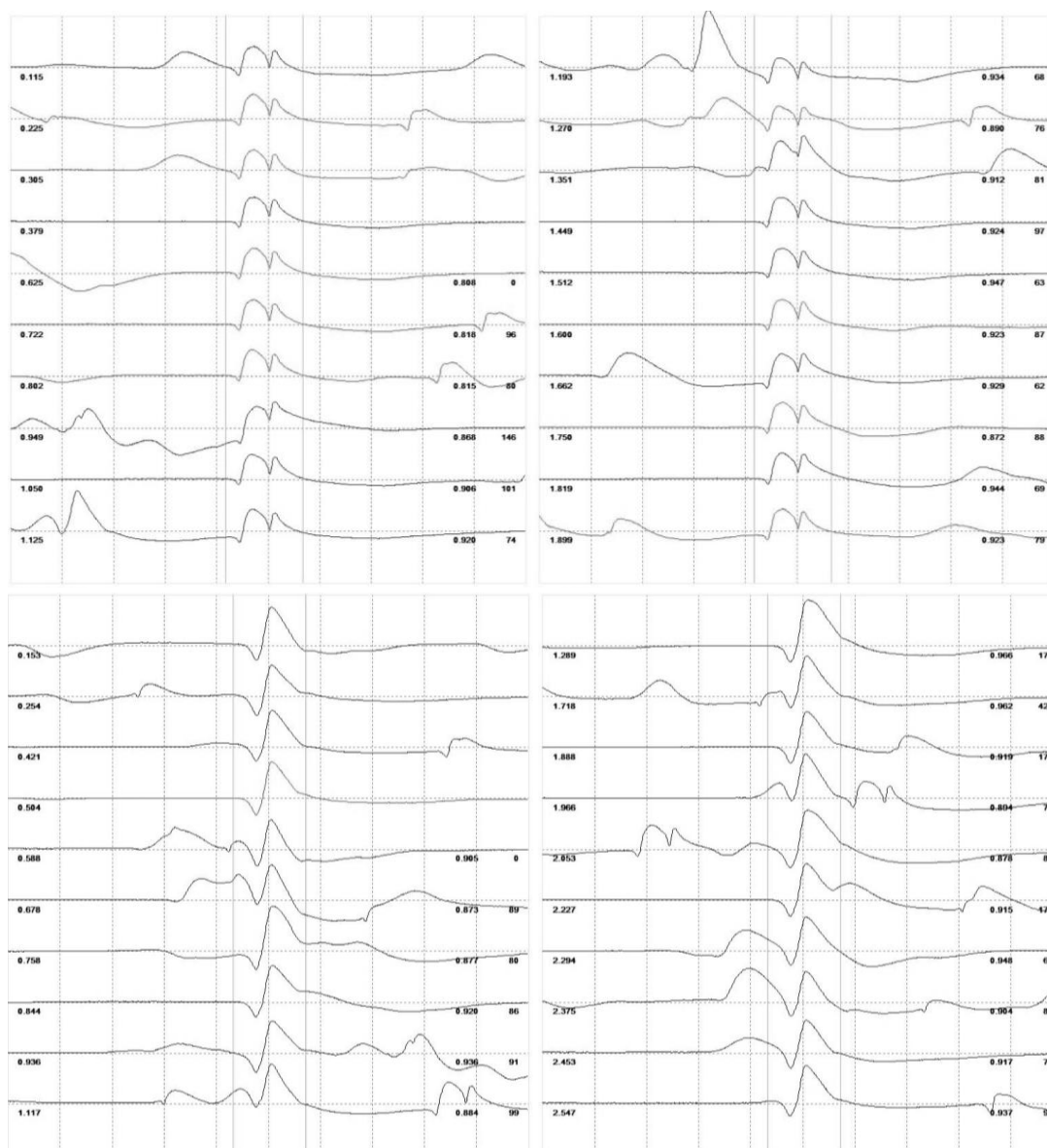


Figure 4: Raster plots for two MUP trains algorithmically classified as distinct MUs from one voluntary FDI contraction. Each sweep represents the observed physiological variation in a given MUP contributing to its respective motor unit potential train (MUPT). Times of occurrence (in seconds) in addition to inter-discharge intervals (in milliseconds) for each MUP contribution are shown.

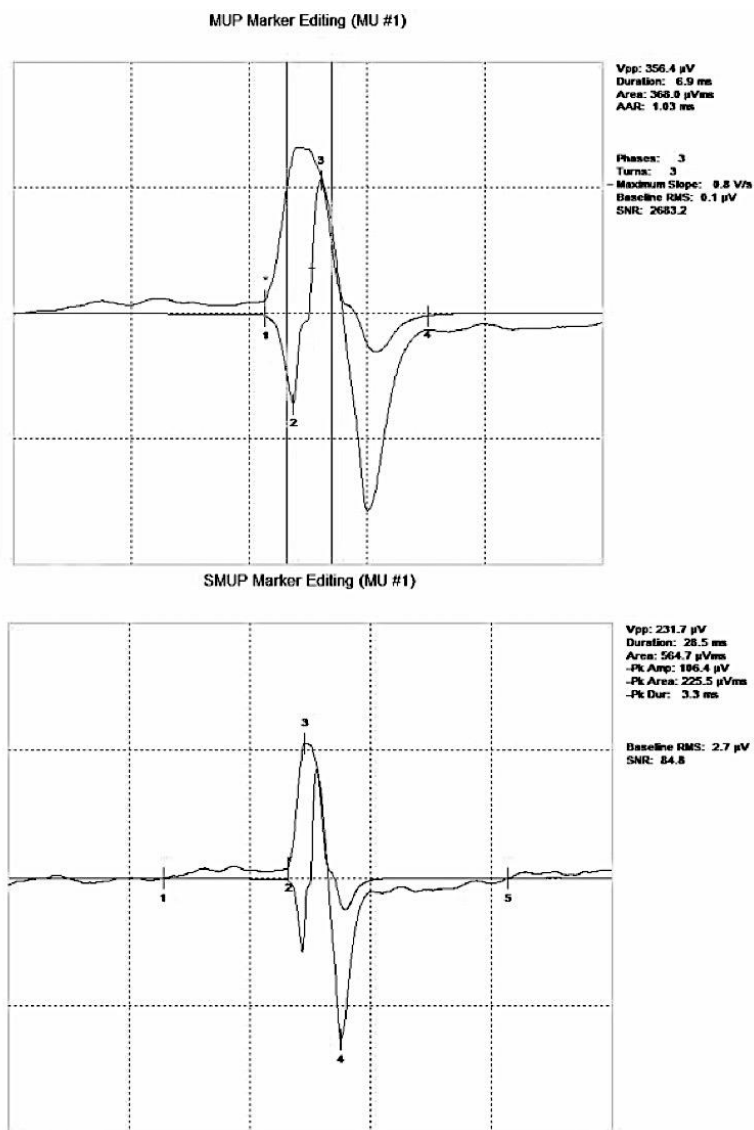


Figure 5: Motor unit potential (MUP) template and corresponding spike-triggered surface motor unit potential (S-MUP) sampled from the FDI. Each MUP template (top panel) and its corresponding spike-triggered S-MUP (bottom panel) are examined for onset and end marker positions (which quantify the duration of a template waveform) as well as the negative and positive peak positions, which give a measure of the peak-to-peak voltage (Vpp) for the MUP or negative peak amplitude for the S-MUP.

All MUPs either met the inclusion criteria and were suitable for analysis or failed to meet the criteria and were discarded from calculations. For MUPs to be considered valid, their trains needed to contain at least 51 firings, exhibit a normal distribution of the main peak in the inter-discharge interval (IDI) histogram (which represents a physiologic firing rate), and have a coefficient of variation less than 0.3 (Hourigan et al., 2015; Stashuk, 1999). Examination of the plotted firing rate for train discharge consistency was necessary in establishing if MUPs were within physiological firing pattern parameters. Exclusion of candidate MUP trains was necessary for MUP templates displaying largely positive amplitudes (known as cannula potentials), but their associated SMUP templates were preserved as these were adequate for STA of the SMUP (Hourigan et al., 2015). Close review of MUP train pairs identified as disparate was required for distinguishing whether or not the trains derived from the same MU, in which case there was exclusion of the MUP template with fewer discharges (Figure 4). For valid MUPs and their coupled SMUP templates that were recorded within 10 ms of each other, marker positions for negative and positive peaks (as well as potential onset and termination) were adjusted if needed (Figure 5) (Hourigan et al., 2015). After having adjusted the markers for both the MUPs and S-MUPS, mean quantitative values for all size related and other parameters were calculated (Figure 5).

For FDI in older and younger subjects mean S-MUP templates were calculated on a data point by data point basis from all suitable S-MUPS. For each subject the Maximum C-MAP amplitude was divided by the mean S-MUP to derive the MUNE (Figure 6).

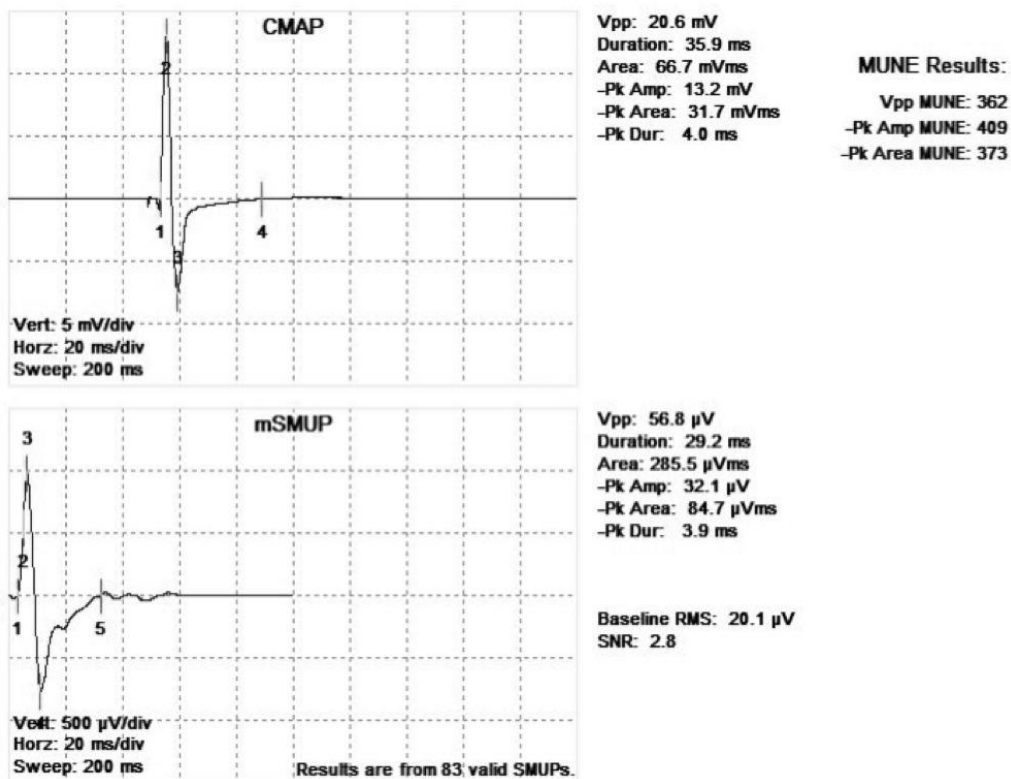


Figure 6: Compound muscle action potential (CMAP) and motor unit number estimate (MUNE) for the first dorsal interosseus (FDI) of a younger subject. Motor unit number estimates (MUNE) for each subject were automatically calculated (by DE-STA) through division of their compound muscle action potential (CMAP) by the mean S-MUP (mS-MUP). The negative peak amplitude for the CMAP is a total summation of contributing S-MUPs in the FDI muscle from graded stimulation. The mS-MUP displays a representative sample of collected S-MUPs during volitional contractions.

3.5 Stability analysis

In order to calculate the NF parameters of stability, NF jiggle and NF jitter, and other NF measures of MU size (NF duration, NF dispersion, NF maximum interval, and NF count), valid MU trains were high-pass filtered to yield an acceleration template of NF MUPs whereby muscle fiber contributions between consecutive MU firings are assessed that are close to the needle electrode detection surface (Figure 7) (Boe, Stashuk, and Doherty, 2006).

The NF parameters of interest for this study included: NF duration, NF dispersion, maximum NF interval, NF count, NF jiggle, and NF jitter. These measures of features found in the NF MUP acceleration template (created with a second order low-pass differentiation of the MUP template, which high-pass filters individual MUPS) have been utilized previously in studies of aging subjects and clinically-relevant neuropathic populations (Stashuk, 1999a; Hourigan et al., 2015; Allen et al., 2014; Gilmore et al., 2017). Individual NF muscle fiber contributions are distinguished as having adequate amplitude and symmetry, with the total number of these distinctly detected contributions to an NF MUP being termed the NF count, relating to the density of a MUs constituent fibers. Where NF duration is defined as the time interval from onset to end positions of the NF MUP, NF dispersion and maximum NF interval describe the time interval from first to last detected NF contribution and the maximum between consecutive NF contributions, respectively. Increases in NF dispersion or maximum NF interval are indications of collateral reinnervation occurring (Allen et al., 2015). NF jiggle measures average percent shape variability between consecutive detected NF MUPs within a MUP

train, analogous to the traditional MUP jiggle statistic (Stålberg and Sonoo, 1994). NF jitter is a measure of average time interval differences between consecutive pairs of detected NF muscle fiber contributions within suitable set of NF MUPs as confirmed by operator analysis (Stashuk, 1999a; Allen et al., 2014).

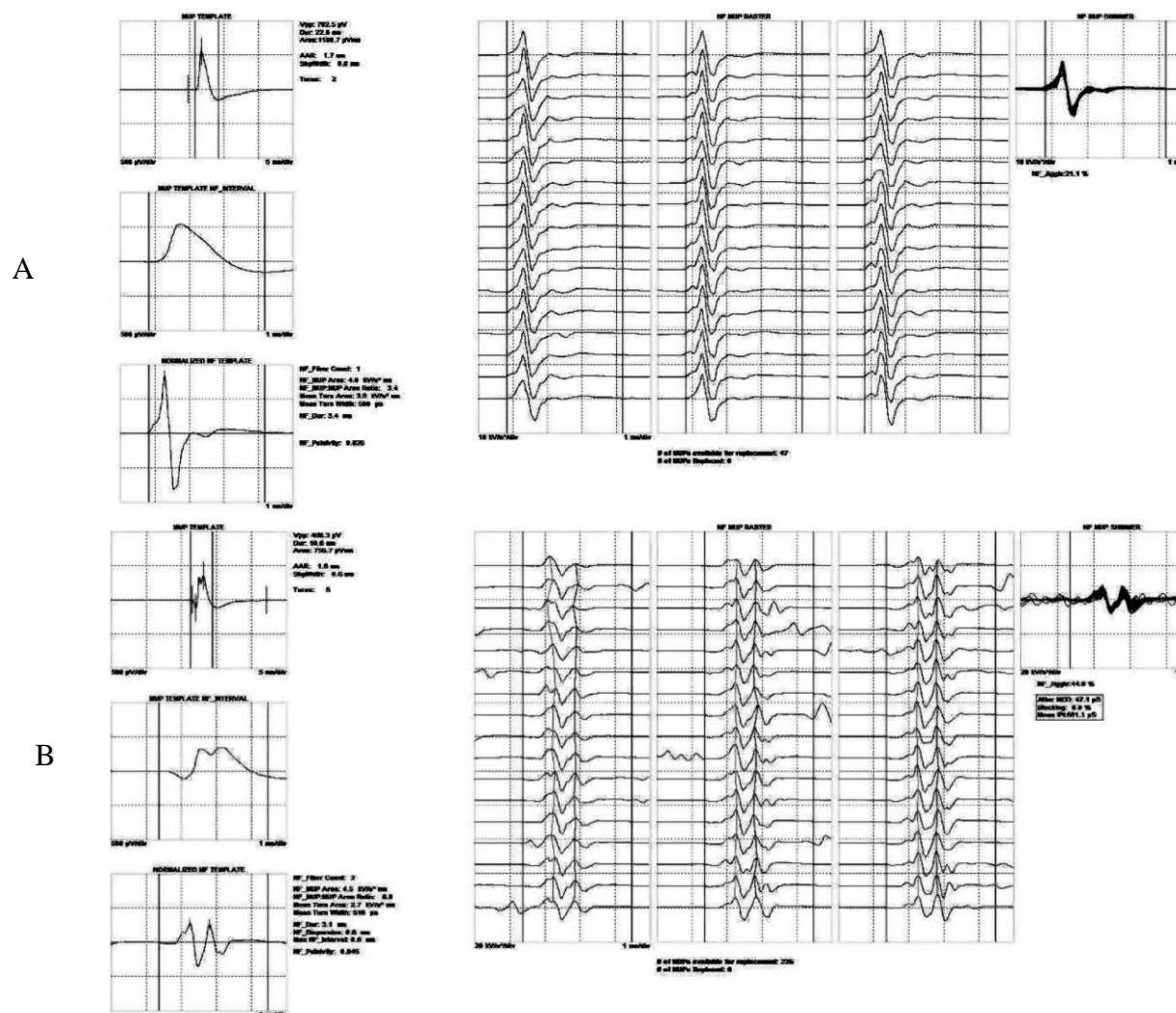


Figure 7: Near fiber (NF) MUP acceleration templates from the FDI muscle of a younger (A) and older (B) subject. A near fiber (NF) MUP acceleration template is created through high-pass filtering of valid MUP trains to accentuate muscle fiber contributions close to the needle electrode. In both the younger (A) and older (B) subject stability summaries, the left panels depict the MUP template (top), its constituent NF MUP duration (middle), and the normalized NF MUP template (bottom). The central panel displays NF MUP contributions to the NF MUP template as a raster plot. The NF MUP shimmer plot is shown on the right panel.

3.6 Statistics

Means and standard deviations were calculated for all parameters of interest. Independent two-tailed t tests were used for comparison of group means for normally distributed data. The Pearson product-moment correlation coefficient (r) was utilized to compare any correlations between near-fiber (NF) jiggle and jitter and other size related parameters or the MUP or S-MUPs. Statistical significance was represented by an alpha level of $p < 0.05$.

Chapter 4

4 Results

4.1 Muscle strength values

A summary of subject characteristics and strength values for young and older subjects is shown in Table 1. Means are calculated for each value with no significant differences observed between groups other than age (Table 1).

Table 1. Subject characteristics and muscle strength values for young and older subject groups

	Young ($n = 13$)	Older ($n = 11$)
Men/women (#)	5/8	5/6
Age (years)	31 ± 13	$74 \pm 5^\dagger$
Height (m)	1.7 ± 0	1.7 ± 0
Mass (kg)	69 ± 17	80 ± 12
FDI MRC score	5 ± 0	5 ± 0
BB MRC score	5 ± 0	5 ± 0
Grip strength (kg)	34 ± 14	30 ± 10
Pinch strength (kg)	9 ± 6	8 ± 2

Data values represented as mean \pm standard deviation. MRC, Medical Research Council.

† Significant difference ($P \geq 0.05$) vs. young group.

4.2 FDI MUP and stability values

In Tables 2 and 3, electrophysiological parameter values are expressed for both subject groups for the FDI muscle. CMAPs obtained from 13 young and 11 older subjects were used to calculate a mean value for each respective group. The older group has significantly decreased CMAP ($P = 3.8 \times 10^{-3}$) and MUNE ($P = 3.8 \times 10^{-3}$) as well as significantly increased mS-MUP ($P < 0.05$). MUP amplitudes were larger in older subjects whereas areas and durations were similar. NF jiggle ($P < 0.05$; +20%), and NF jitter ($P < 0.05$; +18%) were significantly increased when compared with the young control group, however, other NF measures were similar.

Table 2. Electrophysiological MUP values for the first dorsal interosseous (FDI) muscle in young, and older subject groups

	Young ($n = 13$)	Older ($n = 11$)
CMAP (mV)	14 ± 2	$10 \pm 3^\dagger$
mS-MUP (μV)	151 ± 42	$383 \pm 84^\dagger$
MUNE (#)	212 ± 117	$63 \pm 44^\dagger$
MUP amplitude (μV)	479 ± 154	$638 \pm 219^\dagger$
MUP duration (ms)	13 ± 3	11 ± 2
MUP Area ($\mu\text{V ms}$)	849 ± 320	877 ± 304
MUP AAR (ms)	2 ± 0	1 ± 0
MUP phases (#)	4 ± 1	4 ± 1
MUP turns (#)	2 ± 0	3 ± 1
Contractions (#)	9 ± 4	5 ± 2
MUPTS/contraction (#)	5 ± 2	5 ± 2

Data values are mean \pm standard deviation. DQEMG, decomposition-based electromyography; CMAP, compound muscle action potential; mS-MUP, mean surface motor unit action potential; MUNE, motor unit number estimate; MUP, motor unit potential; AAR, area-to-amplitude ratio; MUPT, motor unit potential train; NF, near fibre.

†Significant difference ($P \geq 0.05$) vs. young group.

Table 3. Electrophysiological near fiber (NF) stability values for the first dorsal interosseous (FDI) muscle in young and older subject groups

	Young ($n = 13$)	Older ($n = 11$)
NF duration (ms)	3 ± 1	3 ± 1
NF dispersion (ms)	1 ± 0	1 ± 0
Maximum NF interval (ms)	1 ± 0	1 ± 0
NF count (#)	2 ± 1	2 ± 1
NF jiggle (%)	28 ± 10	$35 \pm 15^\dagger (+20\%)$
NF jitter (μs)	45 ± 16	$55 \pm 19^\dagger (+18\%)$
Blocking (%)	0 ± 0	0 ± 1

Data values are mean \pm standard deviation. MUP, motor unit potential; NF, near fibre.

† Significant difference ($P \geq 0.05$) vs. young group.

4.3 BB MUP and stability values

Electrophysiological data obtained from the BB muscle across young and older subjects are represented in Tables 4 and 5. The mean SMUP, MUP and MUP durations were all significantly increased in older subjects consistent with MU loss and remodeling ($P < 0.05$). NF jiggle ($P < 0.05$; 24%), and NF jitter ($P < 0.05$; 38%) were significantly increased in the older group relative to young subjects, however, other NF measures were similar.

Table 4. Electrophysiological MUP values for the biceps brachii (BB) muscle in young and older subject groups

	Young ($n = 13$)	Older ($n = 11$)
mS-MUP (μV)	75 ± 15	$162 \pm 16^\dagger$
MUP amplitude (μV)	341 ± 89	$675 \pm 117^\dagger$
MUP duration (ms)	14 ± 3	$15 \pm 3^\dagger$
MUP area (μVms)	676 ± 171	829 ± 162
MUP AAR (ms)	2 ± 0	2 ± 0
MUP phases (#)	3 ± 0	3 ± 1
MUP turns (#)	2 ± 0	2 ± 1
Contractions (#)	7 ± 2	5 ± 3
MUPTS/contraction (#)	5 ± 1	5 ± 1

Data values are mean \pm standard deviation. mS-MUP, mean surface motor unit action potential; MUP, motor unit potential; AAR, area-to-amplitude ratio; MUPT, motor unit potential train

† Significant difference ($P \leq 0.05$) vs. young group.

Table 5. Electrophysiological near fiber (NF) stability values for the biceps brachii (BB) muscle in young and older subject groups

	Young (<i>n</i> = 13)	Older (<i>n</i> = 11)
NF duration (ms)	2 ± 1	3 ± 1
NF dispersion (ms)	1 ± 1	2 ± 1
Maximum NF interval (ms)	1 ± 0	1 ± 1
NF count (#)	2 ± 1	2 ± 1
NF jiggle (%)	22 ± 7	29 ± 10† (+24%)
NF jitter (µs)	38 ± 14	62 ± 25† (+38%)
Blocking (%)	0 ± 0	0 ± 0

Data values are mean ± standard deviation. MUP, motor unit potential; NF, near fibre.

†Significant difference ($P \geq 0.05$) vs. young group.

4.4 S-MUP distribution

The frequency distributions of acquired SMUPs are represented in Figures 8 and 9 for MUs obtained from the FDI muscle in younger and older subjects, respectively. Figures 10 and 11 show SMUP frequency distributions for the BB muscle in both younger and older subjects. Characteristic Poisson distribution is seen for each arrangement of SMUP number with greater proportion of larger SMUPs observed in older individuals compared with younger controls.

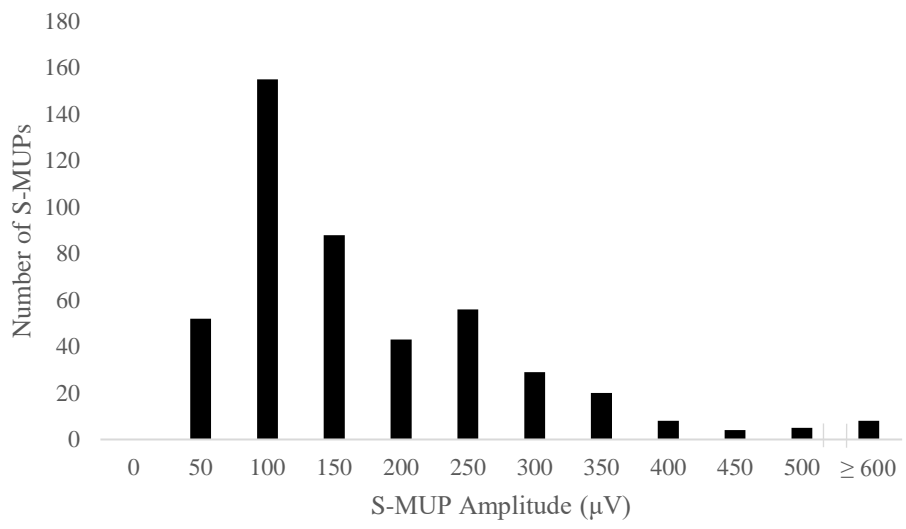


Figure 8: Frequency distribution of S-MUPs from the first dorsal interosseus (FDI) muscle of young subjects.

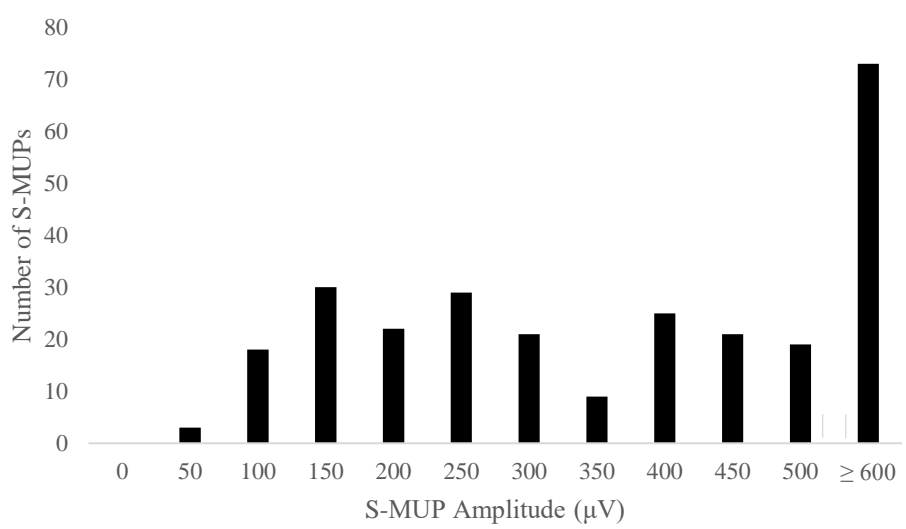


Figure 9: Frequency distribution of S-MUPs from the first dorsal interosseus (FDI) muscle of older subjects.

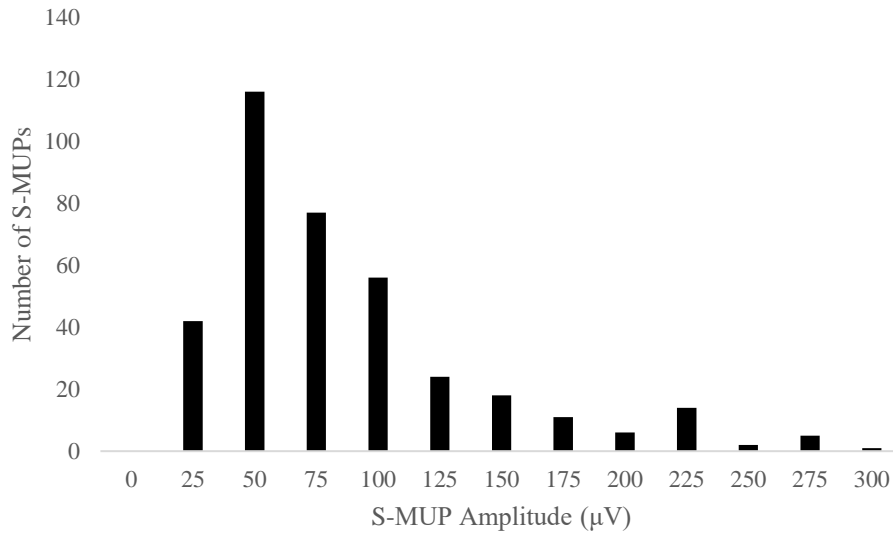


Figure 10: Frequency distribution of S-MUPs from the biceps brachii (BB) muscle of young subjects.

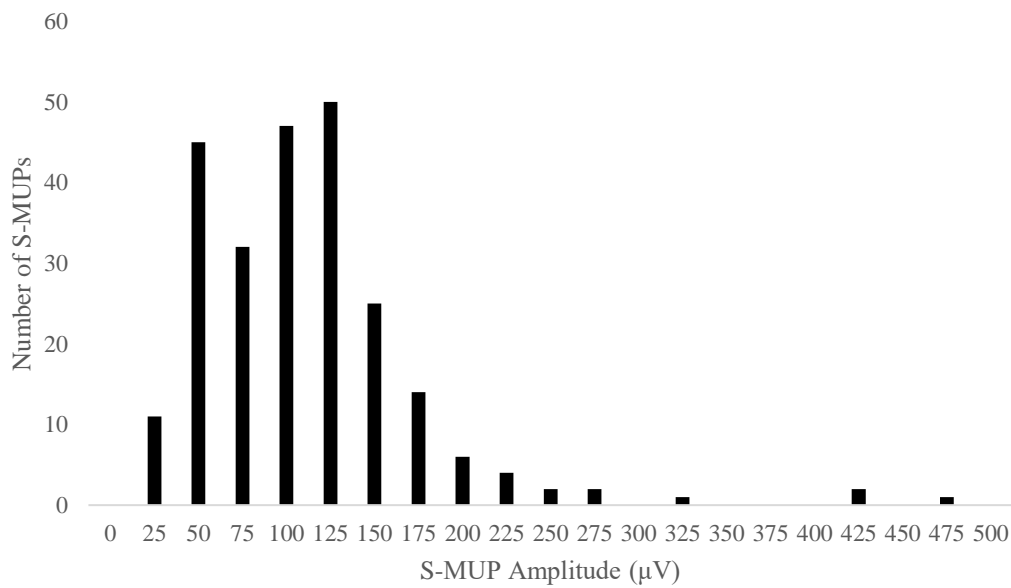


Figure 11: Frequency distribution of S-MUPs from the biceps brachii (BB) muscle of older subjects.

4.5 Correlations with stability

SMUP negative peak amplitude and MUNE are compared with NF jiggle and NF jitter values for both FDI and BB muscles across young and older subjects in Figures 12 through 17. Strong positive correlations are observed in the FDI between SMUP amplitude and NF jiggle, as well as with NF jitter, while strong negative linear correlations are observed in the FDI between MUNE and NF jiggle or jitter. The relationship between BB S-MUP size and measures of instability were not strongly correlated.

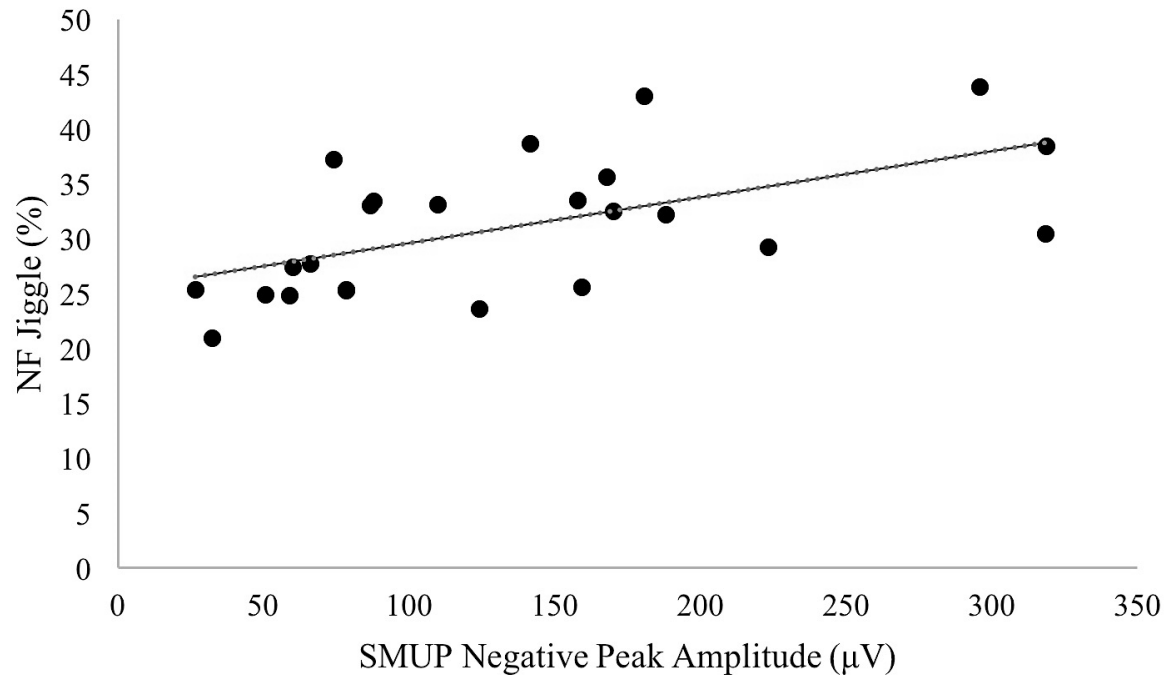


Figure 12: Relationship between NF jiggle and SMUP amplitude in the first dorsal interosseous (FDI) muscle of young and older subjects. ($r = 0.33$, $P = 6.3 \times 10^{-10}$).

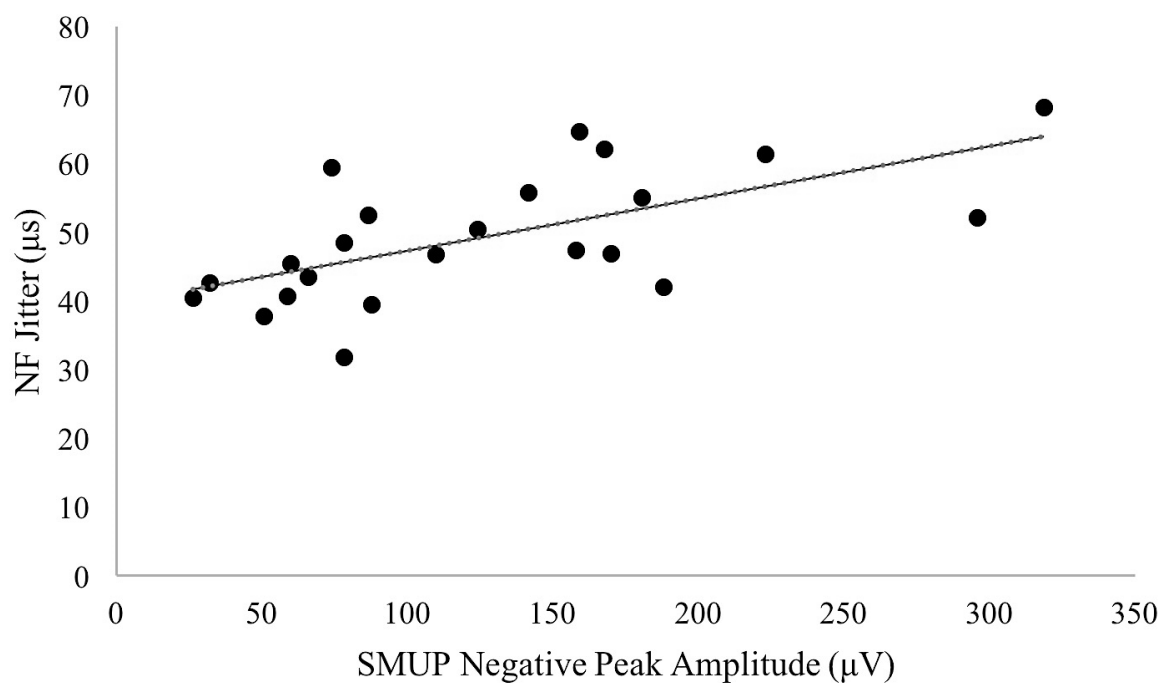


Figure 13: Relationship between NF jitter and SMUP amplitude in the first dorsal interosseous (FDI) muscle of young and older subjects ($r = 0.63^*$, $P = 1.3 \times 10^{-11}$).

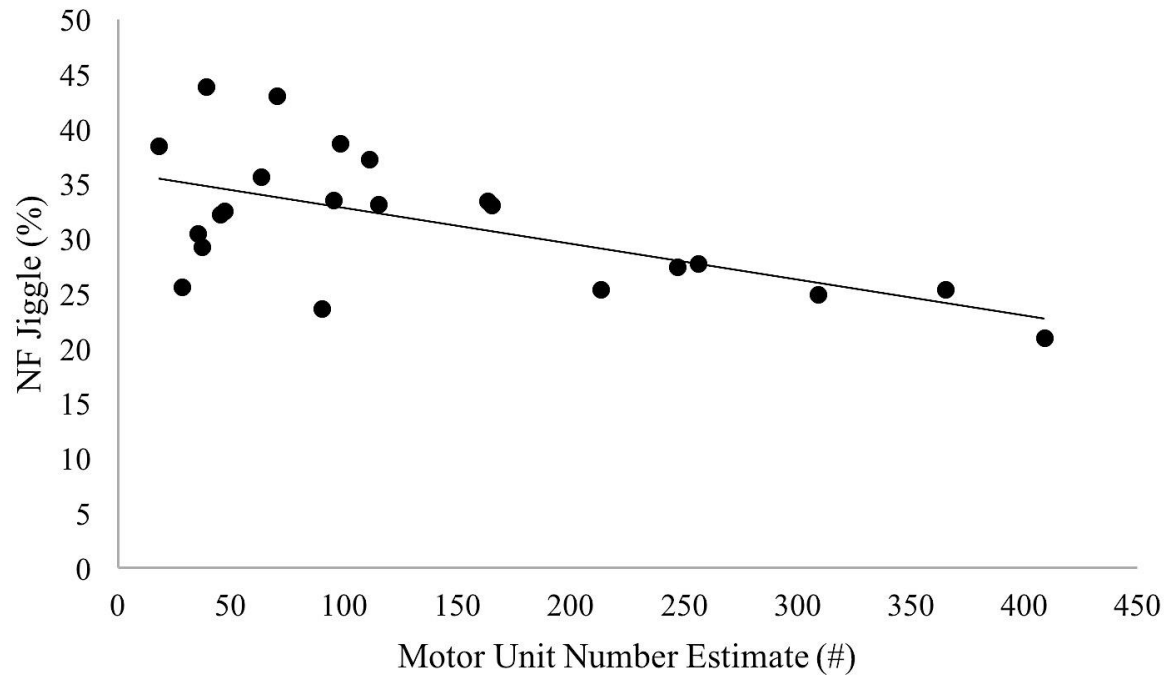


Figure 14: Relationship between NF jiggle and MUNE in the first dorsal interosseous (FDI) muscle of young and older subjects ($r = -0.61^*$, $P = 3.5 \times 10^{-15}$).

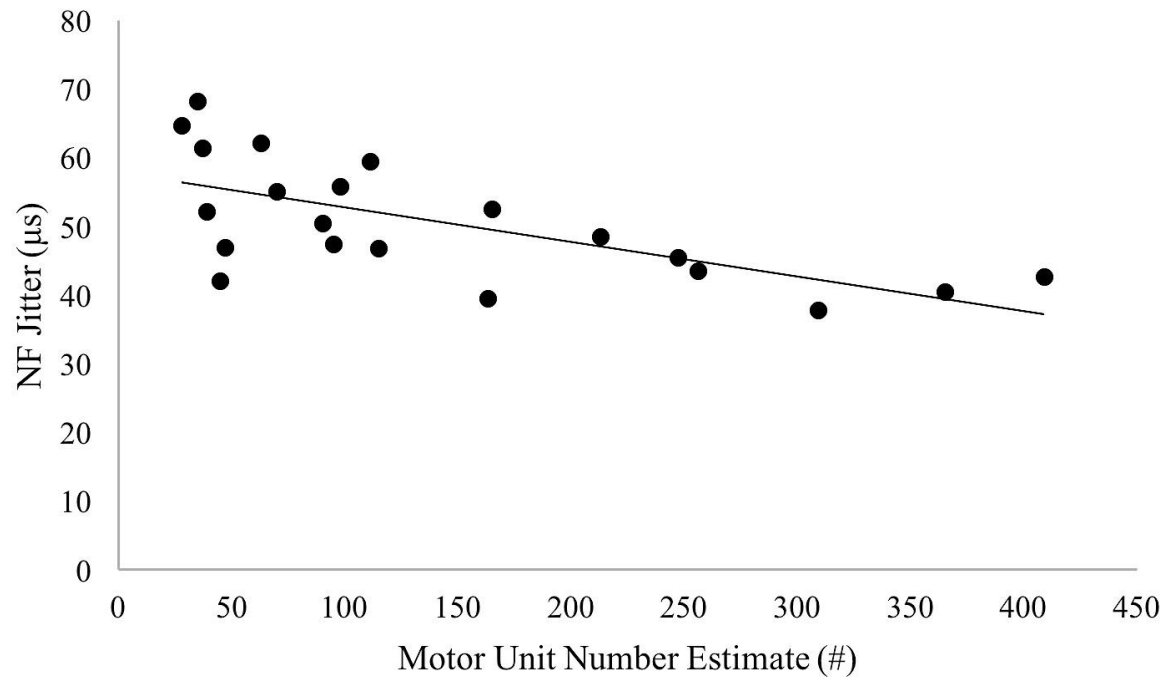


Figure 15: Relationship between NF jitter and MUNE in the first dorsal interosseus (FDI) muscle of young and older subjects ($r = -0.18$, $P = 9.5 \times 10^{-13}$).

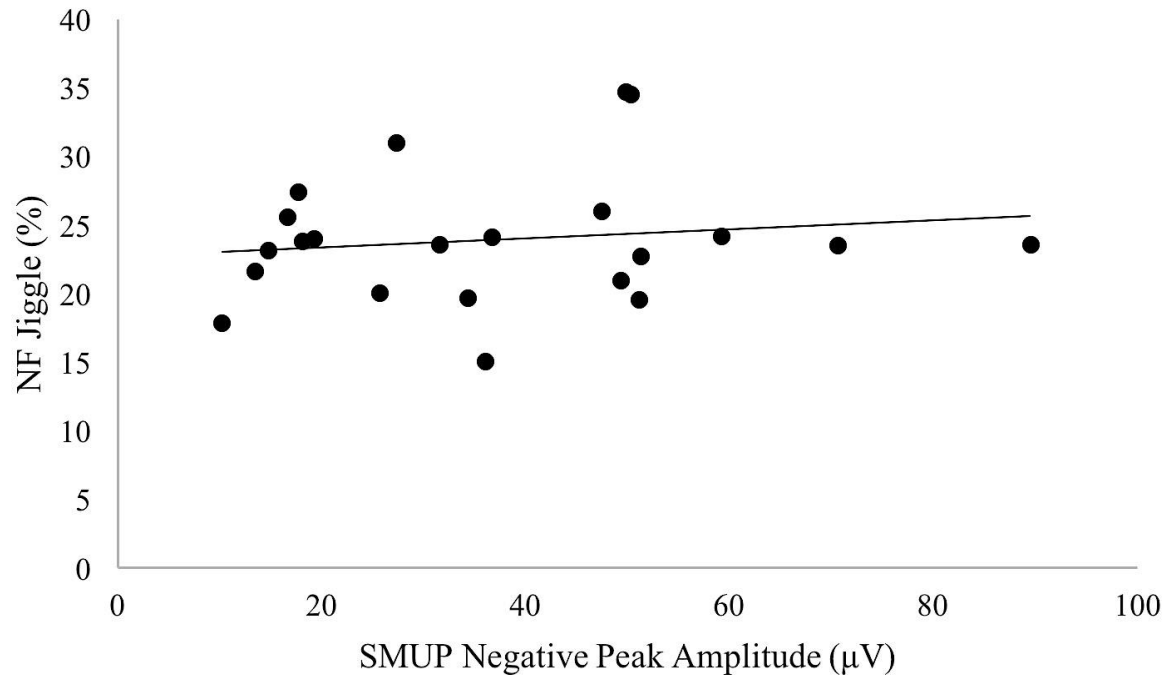


Figure 16: Relationship between NF jiggle and SMUP amplitude in the biceps brachii (BB) muscle of young and older subjects ($r = 0.099$, $P = 2.7 \times 10^{-9}$).

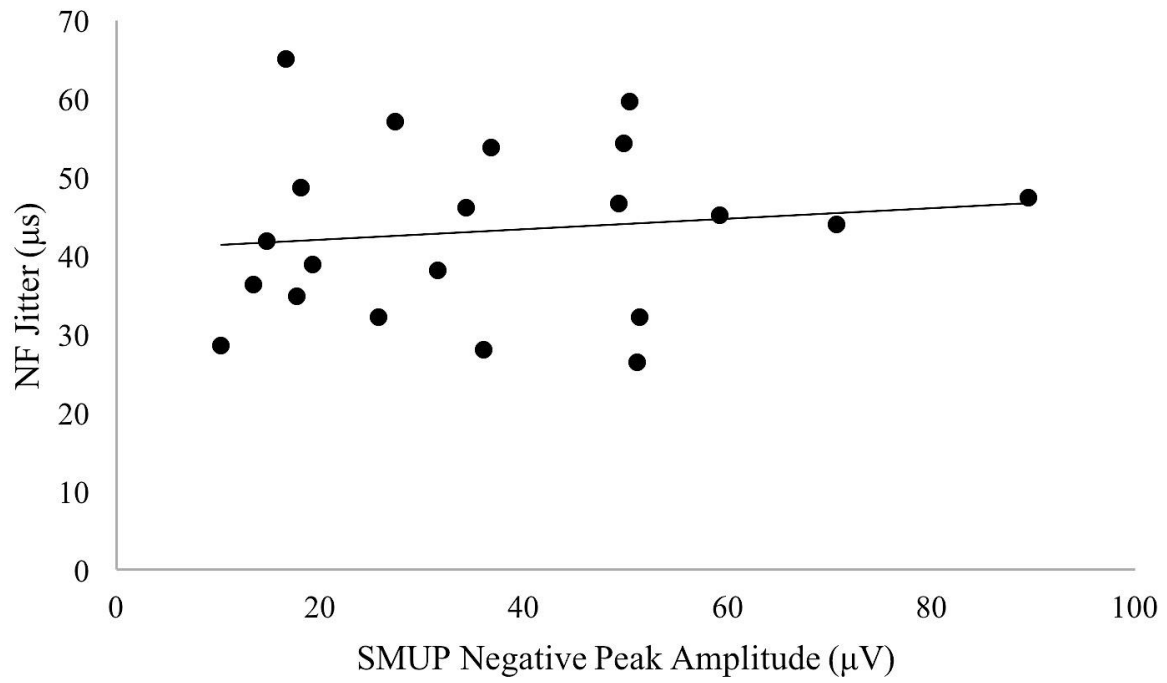


Figure 17: Relationship between NF jitter and SMUP amplitude in the biceps brachii (BB) muscle of young and older subjects ($r = 0.13$, $P = 1.3 \times 10^{-7}$).

Chapter 5

5 Discussion

This study was designed to examine age-related MU loss, MU remodeling and the impact on MU stability measured electrophysiologically using near fibre (NF) parameters from DQEMG analysis of voluntarily activated MUs in upper limb muscles of young and older healthy adults. Hourigan et. al. (2015) used DQEMG to study age-related changes in MU stability with near fiber parameters in two lower limb muscles (vastus medialis and tibialis anterior) and reported a significant positive relationship between MU size and measures of instability (NF jitter and jiggle). To expand on this work, this study examined a proximal (biceps brachii) and distal (first dorsal interosseous) upper limb muscle with regard to age-related changes in MU stability. This is the first study to utilize these methods in upper limb muscles. The contribution of MU instability to sarcopenia and the age-related decline in neuromuscular function has gained particular interest in neuromuscular research. The process of denervation and reinnervation with normal aging yields altered muscle fiber type composition in remodeled MUs with potential impact on the fidelity of new endplate connections (Hepple and Rice, 2016). To obtain further insight into impact of the ageing process of healthy individual neuromuscular systems, near fiber parameters were used to examine MU stability in two different (proximal and distal) upper limb muscles of older and younger control subjects. In general, along with evidence of substantial MU remodeling, older individuals were observed to have significantly decreased MU stability in both FDI and BB muscles compared with younger controls.

5.1 Muscle strength results

Grip and pinch strength testing revealed no significant differences for either the average pinch or grip when younger and older subjects were compared (Table 1). Mathiowetz et. al. (1985) reported normative data on the grip and pinch strength of 310 male and 328 female adults and showed similar values within approximate age ranges of the two groups compared in this study. In addition, men were found to demonstrate more force for each task in comparison to females of the same age group (Mathiowetz et. al., 1985). It is possible that the absence of any significant reduction in grip or pinch strength for older individuals may reflect the considerably smaller sample size and greater proportion of women in the young group in the current study.

5.2 CMAP results

Supramaximal stimulation of the ulnar nerve to the FDI muscle demonstrated that older subjects showed significantly reduced CMAP negative peak amplitude for this muscle (Table 2). Boe et. al. (2006) examined within-subject reliability of MUNE values for the aforementioned muscles of the upper limb using ten healthy young individuals with similar CMAP mean values (13.7 ± 3.1 mV for FDI) to those observed for the control group in the current study (14 ± 2 mV for FDI). Likewise, in the current older experimental subject group FDI mean CMAP (10 ± 3 mV) was similar to previous research on individuals (11.9 ± 2.9 mV) from 65 to 85 years of age (Kurokawa et. al., 1999). Technical limitations involved with obtaining valid maximal CMAPs from the BB muscle prevented the collection of this parameter. Therefore, the biceps MUNE was not calculated and we were reliant on increased S-MUP and MUP area measures to provide

indication of clear MU remodeling. As evidence to this the S-MUP amplitudes were 54% larger for biceps and 60% larger for FDI which provides clear indication of MU loss and remodeling in both muscles. Needle detected MUP sizes were also significantly larger in older subjects than in younger subjects for both muscles, again providing evidence of remodeling.

5.3 S-MUP results

Individual MU surface recordings extracted through spike-triggered averaging from volitional contractions of FDI and BB muscles in young and older subjects (reported as mean S-MUP amplitudes) were observed to be significantly greater ($p < 0.05$) in the older group for both muscles (Tables 2 and 4). Young control FDI mS-MUP ($151 \pm 42 \mu\text{V}$) was comparable to reported values in past research on the FDI (Boe et. al., 2004), $128.1 \pm 47.4 \mu\text{V}$ (Boe et. al., 2006), and $159 \pm 64 \mu\text{V}$ (Doherty and Stashuk, 2003). Furthermore, control group mS-MUP amplitude for the BB muscle ($75 \pm 15 \mu\text{V}$) reflected similar results from BB of healthy individuals in previous studies: $55.5 \pm 20 \mu\text{V}$ (Boe et. al., 2006) and $51 \pm 18 \mu\text{V}$ (Doherty and Stashuk, 2003). Increased mS-MUP values for older individuals indicates evidence that denervation and subsequent collateral reinnervation of muscle fibers have resulted in enlarged MUs which incorporate more muscle fibers, producing a characteristic increase in S-MUP amplitude (Doherty, Vandervoort and Brown, 1993). This significant rise in FDI and BB mS-MUP observed in older subjects of the current study would be an expected compensatory mechanism of the neuromuscular system as extensive age-related reinnervation has yielded remodeled MUs of larger size and diminished number (Hepple and Rice, 2016).

Frequency distributions of collected S-MUPs across subjects studied show a prominent shift with aging toward a greater proportion of S-MUPs with higher amplitude in each muscle tested (Figures 8 – 11). This observation is not unlike frequency distributions of twitch force values obtained from young and older subject groups (Doherty and Brown, 1997), whereby larger numbers of thenar MUs exhibit greater force in response to MU loss in ageing.

5.4 MUNE results

Calculation of average MUNE from mS-MUP and CMAP data of the FDI, revealed significantly reduced ($p < 0.05$) MU number for older subjects when compared with younger controls (Table 2). Previous studies have observed comparable number of estimated MUs for the FDI in healthy young subjects as in this study (212 ± 117): 177 ± 98 (Boe et. al., 2004) and 134 ± 44 (Boe et. al., 2006). MUNE for older adults in past studies have reported significant MU loss in lower limb muscles such as the tibialis anterior, with average MUNE for young men determined to be 187 ± 69 and for older men, 52 ± 21 (Hourigan et al., 2015). In upper limb muscles such as the BB, both lifelong master athletes (185 ± 69 MUs) and older men (133 ± 69 MUs) showed considerable MU loss in comparison to younger controls (354 ± 113 MUs) (Power et al., 2012). The extent of MU loss and associated remodeling of surviving MUs could have important functional consequences on the integrity of the neuromuscular junction, quite possibly contributing significantly to decreased MU stability as a result.

5.5 Motor unit stability results

In addition to analyzing standard electrophysiological parameters of MU size and collateral reinnervation, the main focus of the study was intramuscular data pertaining to mean MU shape variability between consecutive discharges (NF jiggle) as well as mean muscle fiber temporal variability between fiber pairs within an active MU (NF jitter). For both FDI and BB muscles, older subjects exhibited significantly greater NF jiggle and NF jitter ($p < 0.05$), representing overall increased MU instability when compared with younger controls. In a previous study, increased NF jiggle was reported in healthy older male subjects for tibialis anterior and vastus medialis muscles when compared to healthy younger males and was associated with MU loss (Hourigan et. al., 2015). NF Jiggle values for younger and older subjects (Tables 3 and 5) in the current study (FDI: 28 ± 10 %, 35 ± 15 %; BB: 22 ± 7 %, 29 ± 10 %) were comparable to reported values for distal (27 ± 0 %, 36 ± 7 %) and proximal (24 ± 4 %, 31 ± 6 %) lower limb muscles, respectively (Hourigan et. al., 2015). Significantly increased NF jitter values observed for older subjects (Tables 3 and 5) of the present study (FDI: 55 ± 19 μ s; BB: 62 ± 25 μ s) were not unlike NF jitter reported for tibialis anterior of older adults (63 ± 13 μ s; Power et. al., 2016). Positive linear correlations were found in the FDI muscle for NF instability parameters and SMUP amplitude (Figures 11, 12, 15, and 16) in addition to negative linear relationships for jiggle/jitter and MUNE (Figures 13 and 14), a trend also observed in earlier electrophysiological studies on neuromuscular aging (Power et. al., 2016; Hourigan et. al., 2015). NF MUP parameters describe temporal characteristics of muscle fiber contributions to an MU that are within 350 μ m of the needle electrode's detection surface. Spatial filtering that accounts for the NF template's distance selectivity of active

fiber contributions may reduce MU volume conduction that could obscure identification of single muscle fibers and thus might better quantify temporal relationships within MUs (Allen et. al., 2015). For this reason, NF parameters may represent neuromuscular transmission data that more accurately describes axonal sprouting associated with MU reinnervation and remodeling processes. The increased instability observed in older subjects of this present study could potentially reflect transient MU denervation-reinnervation changes, which become more pronounced with age (Hourigan et. al., 2015). As spinal motor neurons degenerate, leading to denervation at the neuromuscular junction of muscle fibers and subsequent MU loss, nerve terminal sprouting (from local unaffected motor neurons) can reinnervate these orphaned fibers through remodeling, and partially compensate for MU loss that renders larger MUs with often slower contractile properties (Power, Dalton and Rice, 2013). This denervation is thought to be a key factor in MU loss when it overwhelms normal collateral reinnervation responses, compromising neuromuscular junction integrity and furthering MU decline (Power, Dalton and Rice, 2013). It is possible that in addition to steadily declining ability for collateral reinnervation (into very old age) of affected muscle fibers, excitation-contraction uncoupling might offer a possible mechanism for early MU loss (Gonzalez-Freire et. al., 2014) and perhaps account for increased MUP variability seen in older subjects (Tables 3 and 5). As highly-specialized and energetically-demanding cells, motor neurons are particularly vulnerable to age-related accumulation of oxidation and nitrosylation products that impair mitochondrial function and could facilitate degeneration (Navarro and Boveris, 2009). Animal models of neuromuscular aging such as mice deficient in super-oxide dismutase present with many signs of sarcopenia that include increased

production of reactive oxygen species, MU loss and degradation of the neuromuscular junction (Vasilaki et. al., 2017). Recently, generation of oxidative stress in mitochondria of these mice was found to play an important role in triggering sarcopenia symptoms since localized deficiency of super-oxide dismutase to the motor neuron or muscle fiber did not produce characteristic neuromuscular junction impairment (Vasilaki et. al., 2017). Although there is no consensus on the exact mechanism of age-related NMJ remodeling, research on neuromuscular aging has shown several associated structural NMJ changes in both animals and humans. These often include increasingly complex and extensive branching of terminal axons (promoting formation of new, separate synaptic sites on muscle fibers), decreased density of pre-synaptic zones of Ach release, and decline in the proportion of the endplate that is in close association with its motor axon (Willadt, Nash and Slater, 2018). It is possible that motor neuron denervation and regeneration cycling could be an important mechanism in human NMJ aging, whereby spared axonal sprouts could target denervated muscle fibers for post-synaptic differentiation (Willadt, Nash and Slater, 2018). In studies of mice however, induced damage to muscle fibers elicited a repair process (via the corresponding nerve), which resulted in a highly fragmented NMJ, a morphology also observable in aging mice (Willadt, Nash and Slater).

It was also observed that the heart-specific component of muscular contraction known as cardiac troponin T is elevated with age in mice and has been correlated with functional deterioration of the neuromuscular junction (Xu et. al., 2017). Previous studies on old mice have revealed that axonal transport is reduced as a result of the aging process, and that this diminished capacity for motor neuron transfer of materials along the axon is associated with unsuccessful collateral reinnervation response (Delbono, 2003).

This length-dependent process of motor neuron terminal branching may account for variation in MU remodeling responses to aging when comparing distal and proximal muscles of the same limb, in addition to underlying size-dependent differences when examining severity of MU loss on different muscle groups. Though evidence of length-dependent axon loss in the present study remains unclear, it is more likely that increased remodeled MU territory due to the large size difference between the BB and FDI muscles could account for greater NF jitter and NF dispersion in the former proximal muscle. Additionally, the innervation ratio differences between the BB and FDI (whereby the former muscle exhibits greater number and size of MUs than the latter) could account for the observed increased instability of the BB (relative to the FDI, which typically has fewer motor axons), and might make it less able to compensate muscle fiber loss than the smaller FDI muscle.

5.6 Limitations

Specific limitations in this study consisted of the challenge of maintaining concentric needle electrode positioning for intramuscular recordings, which may affect the sizes of the recorded MUs across subjects tested, despite providing visual feedback and cueing. Potential bias regarding depth and location of needle electrode intramuscular insertion impacting SMUP collection was accounted for through careful manipulation so that the detection surface accessed multiple regions of each muscle tested. Since alterations in force output of a contraction can significantly impact MU estimates (Boe et al., 2005), attention on the part of the examiner was given to ensure tested subjects maintained contraction intensity within a specified consistent submaximal range defined

by pulses per minute. Inclusion of a larger number of participants would allow for sex comparisons using MUNE and NF parameters. Though this study recruited 24 old and young participants, this sample size is however comparable to that seen in related research (Allen et. al., 2015; Hourigan et. al., 2015, Boe et. al., 2006). Clearly the NF measures of MU stability are an indirect measure of integrity of the neuromuscular junction, however, the consistency of the data across multiple examiners from different laboratories would suggest that this is a robust measure (Hourigan et al., 2015, Allen et. al., 2015, Power et. al., 2016, Gilmore et. al., 2017b).

5.7 Future Directions

Investigation of MU variability in the muscles studied could be important for furthering understanding of age-related denervation mechanisms. An important protein expressed by skeletal muscle in response to denervation, glial cell-derived neurotrophic factor has been found to facilitate MU remodeling at the neuromuscular junction and its expression is elevated in response to muscle activation during short-term exercise (Gonzalez-Freire, 2014). Exercise appears to have a partial protective effect in aged rats through physiological alteration of motor neuron cell bodies, which significantly increase with more frequent physical activity (Kanda and Hashizume, 1998). In humans, similar beneficial effects of exercise are apparent with age as high performance older athletes displayed significantly improved neuromuscular transmission stability when compared with age-matched controls (Power et. al., 2016). This could highlight the relevance of examining NF measures of variability, since NMJ stability and strategies for attenuating MU loss are closely related.

5.8 Conclusion

NF jiggle and NF jitter have been successfully measured or assessed in previous studies of aging in lower limb muscles, acquired neuropathies and amyotrophic lateral sclerosis. The current study aimed to apply these techniques to comparative analysis of proximal and distal upper limb muscles in younger and older healthy adults to further the understanding of known mechanisms of neuromuscular ageing. To summarize, older subjects displayed evidence of MU remodeling with increased MUP amplitudes, S-MUP sizes and reduced MUNE in FDI. Furthermore, the older group exhibited significantly greater NF jiggle and NF jitter in the FDI and BB, as measures of MU variability, relative to the younger group. Additional investigation with larger sample sizes of aged and young healthy subjects, taking into account sex differences, may allow for greater DQEMG data reliability in interpreting causal factors in age-related neuromuscular decline for possible application in clinical diagnosis and categorizing of sarcopenic atrophy.

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Appendices

Appendix A: Research ethics board approval for current study



**Western
Research**

Western University Health Science Research Ethics Board
HSREB Annual Continuing Ethics Approval Notice

Research Ethics

Date: April 03, 2017
Principal Investigator: Dr. Tim Doherty
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,London Health Sciences Centre

Review Type: Delegated
HSREB File Number: 107854
Study Title: Examination of motor unit stability using novel near fiber MU analysis from decomposition-based quantitative electromyography (D-QEMG) in chronic ulnar nerve injury

HSREB Renewal Due Date & HSREB Expiry Date:
 Renewal Due -2018/04/30
 Expiry Date -2018/05/25

The Western University Health Science Research Ethics Board (HSREB) has reviewed the Continuing Ethics Review (CER) Form and is re-issuing approval for the above noted study.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice (ICH E6 R1), the Ontario Freedom of Information and Protection of Privacy Act (FIPPA, 1990), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

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Licensed Content Title	Assessment of variability in the shape of the motor unit action potential, the "jiggle," at consecutive discharges
Licensed Content Author	Erik V. Stålberg, Masahiro Sonoo
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Type of Use	Dissertation/Thesis
Requestor type	University/Academic
Format	Electronic
Portion	Figure/table
Number of figures/tables	1
Original Wiley figure/table number(s)	Figure 1
Will you be translating?	No
Title of your thesis / dissertation	Examining motor unit stability of first dorsal interosseous and biceps brachii muscles in healthy older adults using near fiber analysis from decomposition-based quantitative electromyography (DQEMG)
Expected completion date	Mar 2018
Expected size (number of pages)	80
Attachment	

Curriculum Vitae

EDUCATION

2015–2018 **MSc Neuroscience**, Western University
Specialization: Motor unit stability
Advisor: Dr. Timothy Doherty

2011–2015 **BSc, Physiology and Biology**, Western University

PROFESSIONAL EXPERIENCE

2015–2017 Teaching Assistant, Western University
Neuroscience 2000 Undergraduate Course

PRESENTATIONS

1. **Angelovski, I.** Motor unit variability in chronic ulnar neuropathy patients using DQEMG techniques. Presentation given at Robarts Research Institute. London, ON, 2015
2. **Angelovski, I.** Examining motor unit stability in chronic ulnar neuropathy using decomposition-based quantitative electromyography. Presentation given at Western University Hospital. London, ON, 2016