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Comparing Neuromuscular System Function in Healthy and Mobility-Impaired Older Females

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ABSTRACT

Objectives: The objective of this thesis was to evaluate and compare neuromuscular system function in a group of young, healthy old and mobility-impaired older females (gait speed less than 1m/s) in an attempt to identify which neuromuscular factors contribute most to the loss of mobility and functional performance with age.

Methods: The ankle dorsiflexor and knee extensor muscles were used as a model in these studies. First, in Chapter 2 quantitative electromyography (EMG) was used to evaluate motor unit (MU) numbers, properties and the fidelity of the neuromuscular junction. Next, Chapter 3 conducted magnetic resonance imaging (MRI) to examine contractile and non-contractile muscle components, as well as a novel technique known as magnetization transfers (MT) to assess muscle protein quality. Finally, to determine how changes in the neuromuscular system result in impaired mobility and a decline in functional performance, Chapter 4 explored isometric muscle strength, power and velocity of muscular contraction in the lower extremity muscle groups.

Results: Mobility impaired older adults had significantly lower motor unit number estimates (MUNE) in the tibialis anterior (TA) than young adults (Chapter 2). In the ankle dorsiflexor and knee extensor muscles the proportion of non-contractile muscle tissue in both healthy and mobility-impaired older adults was approximately twice that of young adults (Chapter 3). Additionally, muscle protein quality, as determined via the magnetization transfer ratio (MTR) was significantly reduced in both groups of older adults compared to young, and further reduced in healthy old

compared to mobility impaired adults (Chapter 3). While isometric strength was maintained in the ankle dorsiflexor muscles of older adults, muscular power was significantly reduced in mobility-impaired, but not healthy older adults (Chapter 4). Muscle contraction velocity was determined to be the critical component in power production, and was significantly reduced in the knee extensor muscles of mobility impaired older adults compared to young and healthy older adults (Chapter 4).

Significance: This thesis provides novel information regarding the neuromuscular factors that contribute to the loss of mobility with age. It highlights the importance of power and muscle contraction velocity for sustaining lower extremity functional performance with advanced age. These studies provide insight into important outcomes that can be used for further research to help maintain mobility into old age.

Keywords: Aging, mobility, motor unit, power, magnetic resonance imaging (MRI), electromyography (EMG)

SUMMARY FOR LAY AUDIENCE

The purpose of this research was to compare nerve and muscle health in young adults, healthy older adults and older adults with impaired mobility. We identified mobility impairment as older adults who walked slower than 1 m/s. The aim of this thesis was to identify factors that may contribute to the loss of mobility with age. To accomplish this we conducted three experiments to: 1) record the electrical activity that comes from muscle during normal muscle contraction, 2) use magnetic resonance imaging (MRI) to image muscles and compare their composition and 3) record measures of strength, power and performance in the lower extremity. We found that older adults with mobility impairment had reduced nerves supplying a muscle in the lower leg compared to young adults. Using MRI we also discovered that both groups of older adults had a greater proportion of fat within their muscles and poorer quality of muscle protein compared to young adults. Finally, we found that muscle power, which is calculated by multiplying force by angular velocity of contraction, declined more than strength with age. Specifically, we revealed that the velocity of muscle contraction seems to be the critical factor leading to the loss of power in older adult's muscles, and that those with mobility impairment had the greatest loss of muscle contraction velocity. These findings are important because they highlight some of the adaptations to nerve and muscle that occur with aging and lead to impairment in older adult's mobility. The results of this thesis provide important outcome measures that can be used to design programs aimed to maintain mobility in older adults.

Co-Authorship

Manuscripts for Chapters 2, 3 and 4 are in preparation to be submitted for publication. Neal McKinnon was first author for all manuscripts. Dr. Timothy Doherty, Dr. Susan Hunter, Dr. Charles Rice and Dr. Manuel MonteroOdasso were co-authors on all manuscripts. For the manuscript for Chapter 3, Dr. Terry Thompson, Dr. Jonathan Thiessen, John Butler and Heather Biernaski were co-authors.

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List of Abbreviations

3D: three-dimensional

2D: two-dimensional

ADLs: activities of daily living

ALS: amyotrophic lateral sclerosis

ANOVA: Analysis of variance

CCAA: Canadian Center for Activity and Aging

CMAP: compound muscle action potential

CoV: coefficient of variation

CSA: cross-sectional area

CT: computed topography

DE-STA: decomposition enhanced spike-triggered averaging

DEXA: dual-energy X-ray absorptiometry

DQEMG: decomposition-based quantitative electromyography

EMG: electromyography

IDI: inter-discharge interval

IMAT: intramuscular adipose tissue

ITT: interpolated twitch technique

MG: myasthenia gravis

MHC: myosin heavy chain

MI: mobility impaired

MN: motor neuron

MPS: multiple point stimulation

MRI: magnetic resonance imaging

MT: magnetization transfer

MTR: magnetization transfer ratio

MU: motor unit

MUNE: motor unit number estimation

MUP: motor unit potential

MVC: maximal voluntary contraction

NF: near fiber

NMJ: neuromuscular junction

pps: pulses per second

p.u.: percentage units

RF: radio frequency

ROI: region of interest

ROM: range of motion

RRA: retirement researchers association

SAT: subcutaneous adipose tissue

SD: standard deviation

SE: standard error

S-MUP: surface-detected motor unit potential

SPPB: short physical performance battery

STA: spike-triggered averaging

TA: tibialis anterior

TE: echo time

TR: repetition time

Vmax: maximal contraction velocity

VM: vastus medialis

VMO: vastus medialis oblique

Chapter 1

GENERAL INTRODUCTION AND OVERVIEW

1.0 General Introduction

1.0.1 The aging population

It has been well documented that the Canadian population has been rapidly aging. Based on the 2019 census from Statistics Canada, 1 in 6 (17.5%) of Canadians are over the age of 65 [1]. Statistics Canada also reports that the rate of growth of persons over the age of 65 (3.7%) is more than double the growth of the overall population (1.4%). Based on their projections, the number of adults over 65 will continue to increase in the coming years, reaching as high as 20% by 2025 and 25% by 2059. Additionally, due to advancements in technology and healthcare, people are living longer than ever [1]. Research has consistently shown that the very old (80+ years) exhibit even greater losses of strength [2, 3], power [4], gait velocity [3, 4] and functional performance [5] compared to the old (65-79 years), leading to a greater prevalence of mobility impairment with advancing age. With similar trends being projected globally [6], research focused on the maintenance of health and mobility with advanced age is paramount.

1.0.2 Aging and mobility

As a person ages their mobility tends to be reduced. Mobility is important for our health not only from a physical perspective, but also psychologically, as reduced mobility may lead to lack of engagement, social isolation and overall loss of independence [7]. Reduced gait velocity is one of the most commonly reported

mobility impairments in the literature [8-10]. Most researchers agree that a gait velocity of less than 1 m/s represents a critical threshold for mobility, below which subjects are categorized as having mobility impairment or "mobility disability"[11-13] and are at increased risk of complication related to their mobility impairment. It has been reported that self-selected walking speed demonstrates a precipitous decline in the 6th decade [14], and research has shown older adults with reduced mobility have an increased risk of mortality [15]. For the purposes of this dissertation, individuals with a gait velocity of less than 1 m/s will be referred to as mobility-impaired.

Some other alterations to gait and mobility with age include decreased step length [16], increased time spent in double support [9, 17] and co-contraction of agonist/antagonist muscles during locomotion [10, 18]. Although some of these variations in gait may be explained by a loss of muscular strength with aging [16, 17], it is feasible that all of these alterations to aged gait aim to increase the stability of walking [9, 10, 16]. Gait is an innately unsteady activity, and it is reasonable to suggest that as a person ages they may alter their walking pattern to improve stability and mitigate risk of falls. Falling is a significant health concern for older adults and it has been estimated that over $2 billion in annual healthcare costs are associated with falls and fall-related injuries [7]. Studies have shown that older adults with mobility impairments are twice as likely to fall as similarly aged adults [19], thus a better understanding of mobility impairment with age, and the factors that contribute to it, may help to improve physical well-being in older adults and reduce healthcare costs associated with falls.

1.0.3 Sarcopenia

It is well established that the natural process of aging leads to the loss of muscle mass and an accompanying loss of strength. This process, which is known as sarcopenia, was first described in 1989 by Irwin Rosenberg [20]. There are several factors which contribute to the development of sarcopenia with age including disruption of excitation-contraction coupling, inadequate protein intake, altered endocrine function, physical inactivity, genetic predisposition and degeneration of the neuromuscular system [21, 22]. Together, these contribute to the loss of muscle mass and concomitant loss of strength in older adults with sarcopenia. However, in the years following the publication of Rosenberg's seminal paper, researchers discovered that although there is a loss of muscle strength with age, there is an even greater loss of muscular power. Muscle power is the product of torque and velocity of muscle contraction. As both torque production and velocity of muscle contraction are independently attenuated as a person ages, their combined effect on power production may be greater than the individual contribution of either component [23]. A study by Skelton and colleagues [5] found that strength in the knee extensors declined at a rate of 1.5% per year in adults over 65 years, whereas knee extensor power declined twice as quickly (3.5% per year). This accelerated loss of muscle power has been theorized to be the result of the selective loss of type II fibers in the skeletal muscle. Due to their larger innervation ratio and relative lack of oxidative enzymes, type II motor neurons may be more susceptible to oxidative stress and chronic inflammation associated with typical aging [24-27]. If indeed the largest, fastest contracting type II muscle fibers are preferentially lost with age, it would

cause a slowing of whole muscle contractile velocity, and explain the accelerated loss of muscle power demonstrated in the study by Skelton et al. [5] and many others [28-30]. It would also indicate that muscle power, rather than muscle mass or strength, may be a more sensitive measure of degeneration and subsequent neuromuscular remodeling with age.

Strictly speaking, sarcopenia refers to the loss of muscle mass with age. [20] However, evidence suggests that even preserved muscle mass does not eliminate the weakness observed in older adults [31]. This has led some to investigate new ways to characterize aged muscle. Clark and Manini [32] have asserted that the loss of muscle mass (i.e., sarcopenia) is inadequate to describe the complexity of the aging process in muscle and have suggested a new term, dynapenia, to describe the alteration in contractile properties and neurological function that occurs with age, leading to the loss of strength and power. Muscle quality has also been described as a more robust measure of skeletal muscle aging. Muscle quality refers to the strength per cross-sectional area (CSA) of muscle tissues and represents the intrinsic force-generating capacity of a muscle [21]. A study by McNeil and colleagues [33] used magnetic resonance imaging (MRI) to determine muscle CSA and found that there was no relationship between strength and amount of muscle tissue in the dorsiflexors of young compared to older adults. This supports the assumption that there is an age-related decrease in the intrinsic force generation of individual muscle fibers (i.e., muscle quality) that leads to reduced strength and function with age, rather than being the product of the quantity of muscle mass alone. Although there are numerous factors that may affect muscle quality (for a

review see Doherty[21]), there is ample evidence to suggest that changes in the neuromuscular system play a pivotal role in reduced muscle quality with age and will be the focus of this dissertation.

1.0.4 Magnetic resonance imaging

MRI is a noninvasive imaging technique that uses strong magnetic fields paired with radio frequency (RF) waves to provide contrast and visualize soft tissues within the human body [34-36]. MRIs are based on the principle of nuclear magnetic resonance, where protons within the nuclei of atoms spin, or precess, in a particular alignment when exposed to a magnetic field [34]. These protons behave like tiny magnets that are randomly oriented at rest, but align themselves in the direction of an external magnetic field, such as that created inside an MRI scanner. There are two positions in which protons can align within a magnetic field: parallel and anti-parallel. Since parallel is a lower energy state, slightly more protons will tend to align in the parallel position creating a net magnetization in the longitudinal direction [34]. When a RF pulse with the same frequency as the precessing protons is delivered, some of the energy is absorbed by the protons and they move from the parallel position into an orthogonal position, reducing net longitudinal magnetization [34]. At the same time, the RF pulse causes the precessing protons to synchronize, creating a new transverse magnetization. When the RF pulse is turned off, longitudinal magnetization returns as protons drop back from the high-energy state to the low-energy state. This is known as longitudinal relaxation time, and is represented by the time constant T_1 [34]. Additionally, protons lose their precession coherence and transversal magnetization disappears, which is known as transversal

relaxation and is represented by time constant T_2 [34]. T_1 and T_2 are inherent properties of biological tissues and vary with the type of tissue. Using the innate difference in T_1 and T_2 in different tissues, MRI is able to create contrast to visualize structures within the body. Fluids and water tend to have a longer T_1 time (>1000 ms), whereas fats have a shorter T_1 (<200 ms) [36].

Using this technology, researchers are able to use the inherent variability in T_1/T_2 between fluids and fats to image muscle in vivo and determine CSA and volume of muscle tissue. Following motor neuron loss and subsequent muscle atrophy with age, there is a decrease in contractile muscle mass as fat and connective tissue replace atrophied muscle fibers [37-39]. To improve upon traditional measurement of muscle CSA, some studies began to separate non-contractile tissue from contractile tissue to determine total contractile muscle mass [37, 40, 41]. This allowed researchers to better account for the infiltration of fats and connective tissue into the muscle. Although this technique helps to normalize strength measurements (strength/contractile muscle mass), it is inadequate to determine the changes to intrinsic muscle force generation. Though it is possible to determine intrinsic muscle composition through muscle biopsies [42, 43], it is invasive for participants and results from muscle biopsy studies remain inconclusive [44-46]. Thus, researchers continued to pursue a noninvasive measure to assess the contents and quality of muscle protein.

1.0.5 Magnetization transfer

As discussed above, MRI uses strong magnetic fields to align protons within human tissues. Conventional MRI only affects “free” water protons; it is unable to

detect protons attached to proteins and other macromolecules because their T_2 is too short (<1ms) [47, 48]. Protons in these macromolecules can, however, affect the spin state of the "free" water protons. Therefore, by applying an off-resonance RF pulse, the MR technician can saturate protons in large macromolecules to create additional contrast on MRI known as magnetization transfer (MT) (for a review see Henkelman et al. [47]). A magnetization transfer ratio (MTR) can then be determined by subtracting the MR images with off-resonance pre-saturation RF pulse (M_1) by images without (M_0) then dividing that number by M_0 (Equation 1)[49]

Equation 1:

$$MTR = \frac{(M_0 - M_1)}{M_0}$$

MT was first used clinically to track white matter myelination primarily in multiple sclerosis [50, 51], but has since been adopted in many other clinical settings including being used to assess skeletal muscle quality. In skeletal muscle with higher protein content, MTR will be larger as proteins will be more greatly affected by the pre-saturation MT pulse. A lower MTR indicates decreased exchange of magnetization between "free" protons and protons bound to macromolecules (such as protein), and therefore, indicates a reduction in the quantity and quality of muscle protein [41, 49]. Using this technique, a recent paper by Power et al. [41] was able to identify reductions in MTR in the tibialis anterior (TA) of older adults compared to healthy young controls.

1.0.6 The motor unit and motor unit loss

A motor unit consists of a single motor neuron (located in the anterior or ventral horn of the spinal cord grey matter) and all of the muscle fibers innervated by its peripheral axon. The motor unit (MU) is regarded as the fundamental component of the motor system and the "final common pathway" for muscle activation and motor control [52]. It is responsible for all voluntary and autonomic muscle contraction. There is abundant research demonstrating that motor units are lost with age [2, 21, 28, 53-56], leading to reduced muscle mass, strength, power, and an overall decline in mobility in older adults. However, although there is evidence of significant MU loss after the age of about 60, strength is usually well maintained until much later in life [2]. This is, in part, due to the process of collateral reinnervation. As a motor neuron dies, its peripheral axon "dies back" toward the soma in the spinal cord [26]. This leaves muscle fibers previously innervated by that axon dennervated and unable to contribute to muscle contractions. Without trophic influences from the motor neuron, these muscle fibers will atrophy and die. Under normal circumstances, a neighboring motor axon will sense that these muscle fibers have been orphaned and sprout a collateral axonal branch to re-innervate these muscle fibers (Figure 1.1) [57]. Thus, much of the early MU loss in a muscle is masked through the process of collateral reinnervation. Importantly, as muscle fibers become innervated by a new axonal branch, they will begin to take on the characteristics of their new parent motor neuron. This is supported in the literature by evidence of increased co-expression of myosin heavy chain (MHC) isoforms MHC I and MHC IIA [58] as well as type I fiber grouping in aged muscle [21]. Therefore, as the larger, faster conducting type II motor neurons die off, the MU pool begins to

shift towards more type I slow twitch muscle fibers and muscles begin to lose the ability to produce rapid, high force contractions. This is why muscle peak power generating capabilities may be a more sensitive measure of neuromuscular degeneration and subsequent remodeling than muscle mass or strength. However, there is a limited capacity for collateral reinnervation, and eventually continued motor unit loss outpaces the reinnervation of muscle fibers leading to the loss of muscle mass observed in sarcopenia [59].

Conventional measures to detect sarcopenia in older adults, such as circumferential measurement of muscle mass or reduced strength, are unable to detect the reorganization of the neuromuscular system by collateral reinnervation. Thus, they are unable to detect changes in muscle until a critical threshold of MU loss has occurred [2]. This led to the demand to develop new techniques to measure MU loss and sarcopenia before it had manifested in reduced muscle mass and strength.

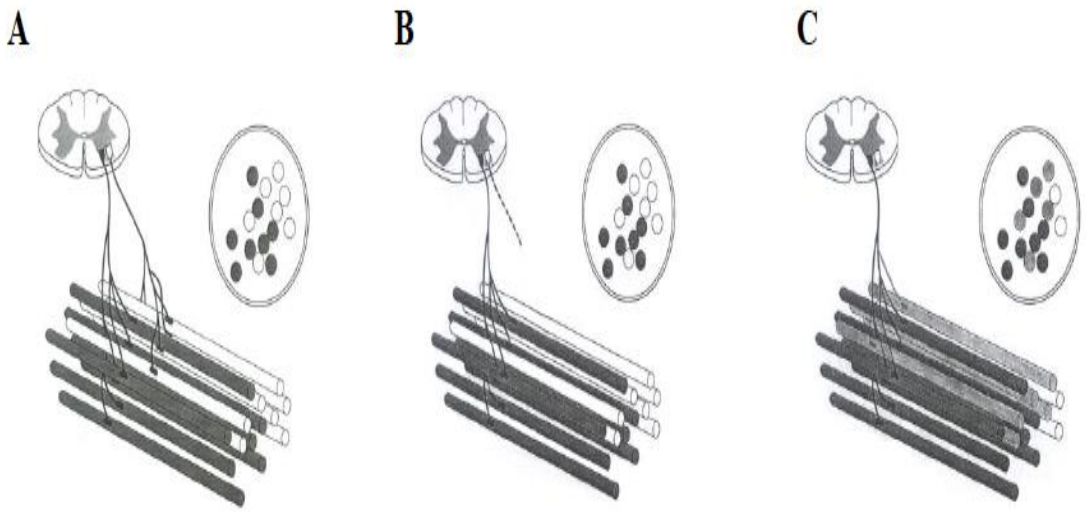


Figure 1.1. Collateral Reinnervation
(A) Depicts a cross-section of two motor neurons (MN) in the anterior horn cell of the spinal cord, and the muscle fibers they innervate comprising a motor unit (MU). (B) With age, axonal damage causes axons of some MNs to "die-back" towards the soma. This leaves muscle fibers associated with that axon dennervated and unable to contribute to muscle contractions. (C) Under normal circumstances, the surviving MN will sense that these muscle fibers have been dennervated, and sprout a collateral axon to reinnervate most of the orphaned muscle fibers. Importantly, muscle fibers reinnervated by a collateral axon will begin to demonstrate characteristics similar to their new parent neuron. Thus, through this compensatory process there is an increase in the average size of MUs and a shift towards a more homogenous MU pool. (Modified from Stalberg, E, Falck, B. The role of electromyography in neurology. *Electroencephalogr Clin Neurophysiol.* 1997; 103: 579-98) [57]

1.0.7 Motor unit number estimation

Nerve conduction studies and needle electromyography (EMG) are traditional neurophysiological diagnostic techniques, which are the foundation of clinical neurophysiology. Although these techniques are able to provide evidence of MU loss (in the form of reduced nerve conduction velocity, and increased MU action potential duration), they are unable to quantitatively determine the amount of motor axon loss in a muscle. In 1971, McComas and colleagues [60] described an innovative technique to estimate the number of motor units within a muscle. This technique was later named motor unit number estimation (MUNE) and has provided an avenue for intensive research into many different neuromuscular pathologies including post-polio syndrome [61], amyotrophic lateral sclerosis (ALS) [62, 63], diabetic polyneuropathy [64], peripheral neuropathy [65] as well as changes in the neuromuscular system with normal aging [2, 28, 56].

MUNE is a quantitative electrophysiological technique that uses surface or needle EMG to estimate the number of functioning MUs in a muscle. In the years following the publication of the groundbreaking paper by McComas and colleagues [60], many different MUNE techniques have been developed; however, they are all based around the same underlying principle. The maximal compound muscle action potential (CMAP) of the muscle under study is divided by the average surface-detected motor unit potential (S-MUP) size metric to determine the MUNE (Equation 2) [66, 67].

Equation 2:

$$\text{MUNE} = \frac{\text{maximal CMAP amplitude}}{\text{mean S-MUP amplitude}}$$

The CMAP is an evoked response by a supramaximal stimulation to the motor nerve supplying the muscle (or muscle group) and represents the combined activation of all the MUs in that muscle. The S-MUP, on the other hand, represents the electrical representation of the size of a single motor neuron and all the muscle fibers it innervates. Therefore, by dividing the size of the entire motor unit pool by the average size of a sample of individual MUs an estimate of the number of MUs in that muscle or muscle group is derived [66, 67].

1.0.8 Motor unit number estimation techniques

Different MUNE techniques vary in how the average S-MUP size is determined, each with their own inherent advantages and disadvantages. Among the most commonly used MUNE techniques are incremental stimulation, multiple point stimulation (MPS), the statistical method and spike-triggered averaging (STA) (For a review see Gooch et al. [67]). Incremental stimulation, MPS and the statistical method all rely on delivering an electrical stimulus to the motor nerve of the muscle under study. This, therefore, confines these techniques to muscle with large sections of their motor nerve available superficially for external stimulation, which tends to be smaller more distal extremity muscles [67]. STA, on the other hand, uses a combination of both needle and surface EMG to collect a sample of S-MUPs from a muscle during a moderate intensity muscle contraction [68, 69]. This allows STA to be applied to any muscle from which a valid CMAP can be measured.

1.0.9 Spike-triggered averaging

Traditional STA uses both needle and surface EMG to determine a quantitative EMG measurement. During a moderate intensity voluntary contraction, the

concentric needle electrode detects action potential impulses within the muscle. These impulses are measured and summated to produce a motor unit potential (MUP) [70]. While the needle EMG is assessing MUPs from within the muscle fibers, surface electrodes are concurrently recording EMG signals from the surface of the muscle (S-MUPs). A window-based discriminator is then used to isolate one of the MUPs from the EMG interference pattern. The individual MUP is used as a trigger and time locked to the surface EMG signal, allowing for the extraction of the surface EMG signal that is representative of the needled-detected MUP [69, 71, 72]. By measuring multiple surface detected potentials from MUPs at different depths and/or orientations of needle electrode insertion into the muscle, a representative sample of S-MUPs can be determined. The average S-MUP size is then calculated from this sample and inserted into the equation (Equation 2) to determine a MUNE [69, 71].

The main advantage of STA over some of the other conventional MUNE measurements is that STA does not rely on large sections of peripheral nerve being available for stimulation. This allows STA to be applied to larger more proximal muscles (i.e. biceps brachii, vastus medialis/lateralis, upper trapezius). Additionally, STA records electrical activity coming from the muscles during a voluntary contraction (as opposed to externally stimulated via the motor axon), which is a more practical approach and better represents how the neuromuscular system performs during voluntary muscle activation. Although research has shown STA to be a valid and reliable technique to estimate MU numbers [66, 67, 71, 73], there are some inherent limitations to this approach. Due to increasing complexity of the

EMG interference pattern with increased intensity of muscle contractions, STA is limited to lower intensity contractions. It is well established, by Henneman's size principle [74], that smaller MUs are recruited first and larger MUs are recruited later, when there is increased demand to overcome a load. Based on this principle, STA may be artificially overestimating MUNEs by collecting a biased sample of relatively small MUs that can be easily differentiated from a less complex interference pattern at lower intensity contractions. Additionally, as STA requires insertion of a needle electrode into the muscle, it is more invasive than some of the other MUNE methods. Finally, it can be an extensively time consuming process, as STA is only able to extract one MUP and S-MUP combination from each contraction, and requires substantial cooperation from the participant to maintain a low intensity muscle contraction.

1.0.10 Decomposition-enhanced spike-triggered averaging

In 1999, Stashuk [70] introduced a series of computer-based algorithms capable of decomposing a complex intramuscular EMG interference pattern into multiple discrete MUPs. Using the program decomposition-based quantitative EMG (DQEMG) allowed multiple MUPs to be extracted from an interference pattern during a single muscle contraction (Figure 1.2). The program was able to track repetitive firings of multiple MUPs during the course of a 30 second contraction and isolate characteristics of the MUP waveforms such as amplitude, duration, number of turns, area etc. All of these characteristics were then averaged to create a MUP template [70]. In 2004, Boe and colleagues [69] used these computer algorithms and applied them to the conventional STA MUNE technique. This new technique, known

as decomposition-enhance spike triggered averaging (DE-STA), was capable of taking more complex, higher force needle EMG interference patterns and decomposing them into their constituent MUPs. Therefore, by using this computer based model, DE-STA is able to decrease the sampling bias of conventional STA by allowed computer algorithms to decompose higher intensity contractions with more complex interference patters, and reduced the time and patient cooperation needed to calculate a MUNE.

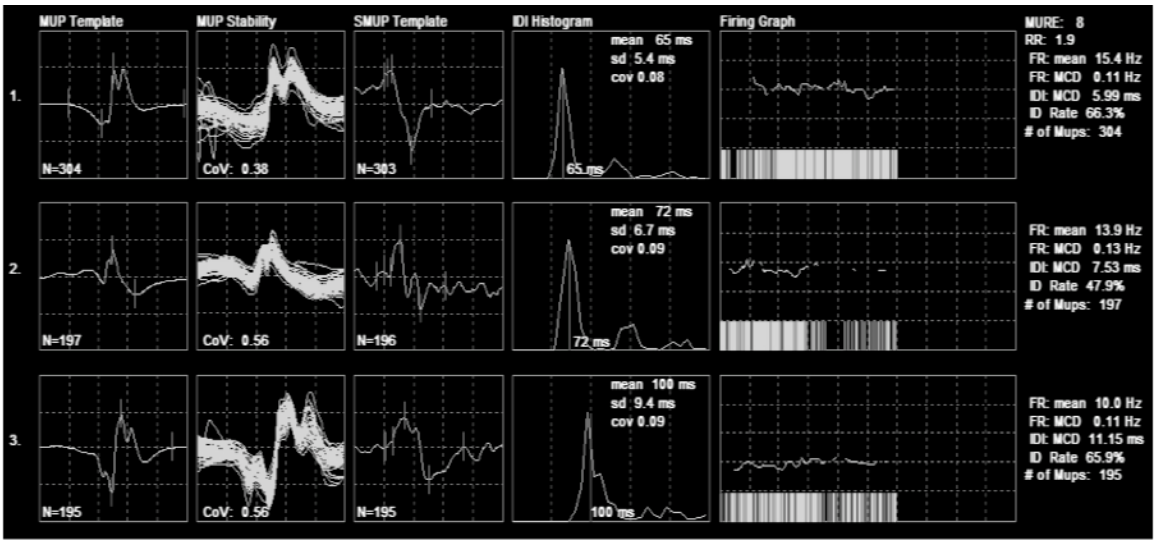


Figure 1.2. DQEMG

Example of a decomposition summary as depicted in DQEMG taken from a young participant in this study. Each row represents one motor unit collected over the span of a 30 second contraction. The first column depicts the motor unit potential (MUP) template, which is the MUP waveform averaged across all firings of the MUP (the number of MUP firings is represented in the bottom left corner of the MUP template window). The second column is known as the shimmer plot, and shows all of the MUP firings superimposed on top of each other. This gives an indication of the stability of the MU (Section 1.0.11). Column three illustrates the surface-detected MUP (S-MUP), which is a representation of the surface EMG signal that is time-locked to the discharge of the needle detected MUP and represents the size of the MU. The fourth column is the inter-discharge interval (IDI) histogram. This histogram depicts the interval between consecutive discharges of the MUP, and is used during data analysis to ensure the interval is normally distributed with a coefficient of variation (CoV) of less than 0.3. Finally, the fifth column contains the firing rate graph. The vertical lines along the bottom represent the individual MN firings, whereas the line across the top shows the instantaneous firing rate.

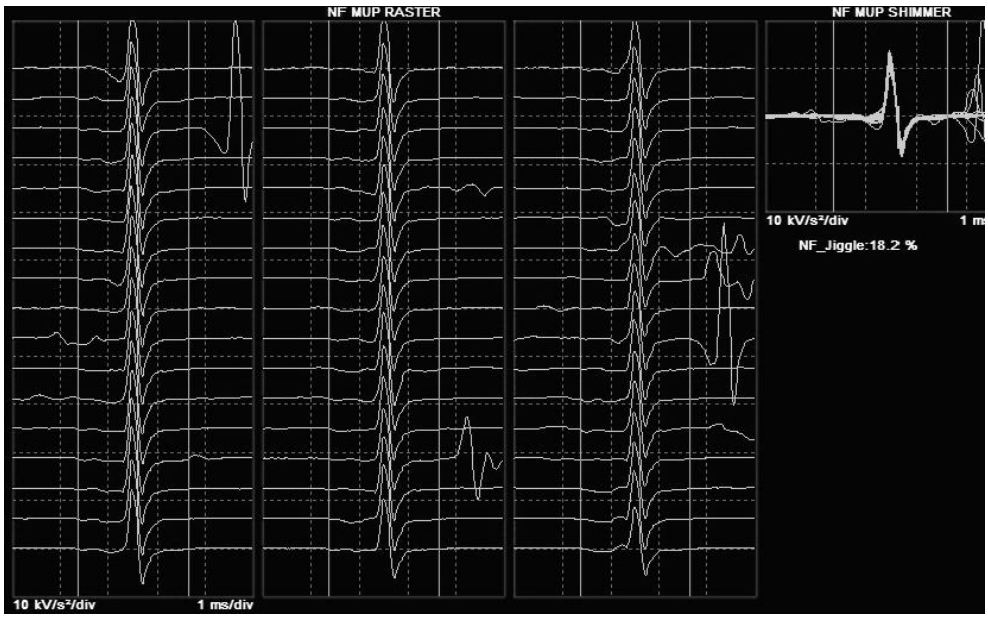
1.0.11 Neuromuscular junction instability

Although the precise underlying mechanism has not been delineated, there is evidence to suggest that aging leads to neuromuscular junction (NMJ) remodeling, which can compromise neuromuscular transmission [26, 75, 76]. Changes in neuromuscular transmission are thought to precede MU loss [77-80] and therefore may represent a clinically relevant precursor to sarcopenia. The stability of NMJ transmission can be determined electrophysiologically by measuring the variability in shape of MUPs across successive discharges of an individual MU. There are two main electrophysiological measures of MUP variability known as jitter and jiggle. Jitter represents the variability in timing of individual muscle fiber contributions to the MUP from one discharge to another [77]. Thus, if there is a disruption in NMJ transmission, the fidelity of the muscle fiber's contribution to the MUP will be reduced, resulting in an increase in the jitter measurement. Jitter has been extensively studied and shown to be a valid and reliable measure of NMJ stability [77, 81, 82], but will not be the focus of this dissertation. Jiggle, on the other hand, represents the variability in the overall shape of the MUP across repeated MU firings. Contrary to jitter, jiggle has been relatively minimally studied in the literature, although it has been shown to be increased in patients with ALS [77] and myasthenia gravis (MG)[78].

More recently, jiggle has garnered increased interest by researchers in studies of the aging neuromuscular system [79, 80]. Stashuk [83] described a novel approach to determining jiggle known as near fiber (NF) jiggle. NF jiggle uses the same general concept as traditional jiggle (measuring MUP shape deviation at

consecutive discharges), but uses a high pass filter to enhance the contribution from muscle fibers closest to the concentric needle electrode (within ~350 μm) (Figure 1.3). This allows for a more robust quantification of muscle fibers and MUs in close proximity to the needle electrode and prevents contamination from activity of other MUs farther away from the needle detection site. Using this technique, Allen et al. [79] were able to establish that patients with diabetic polyneuropathy had increased NF jiggle when compared to age-matched healthy controls. Additionally, a recent investigation by Hourigan and colleagues [80] found that healthy older adults had increased NF jiggle when compared to younger adults. These findings support the assertion that NF jiggle has utility clinically to determine instability in NMJ transmission. Importantly, it has been reported that NMJ transmission instability precedes MU loss [77, 78], thus, NF jiggle may be a more sensitive clinical measurement of impending MU loss and consequent sarcopenia.

(A)



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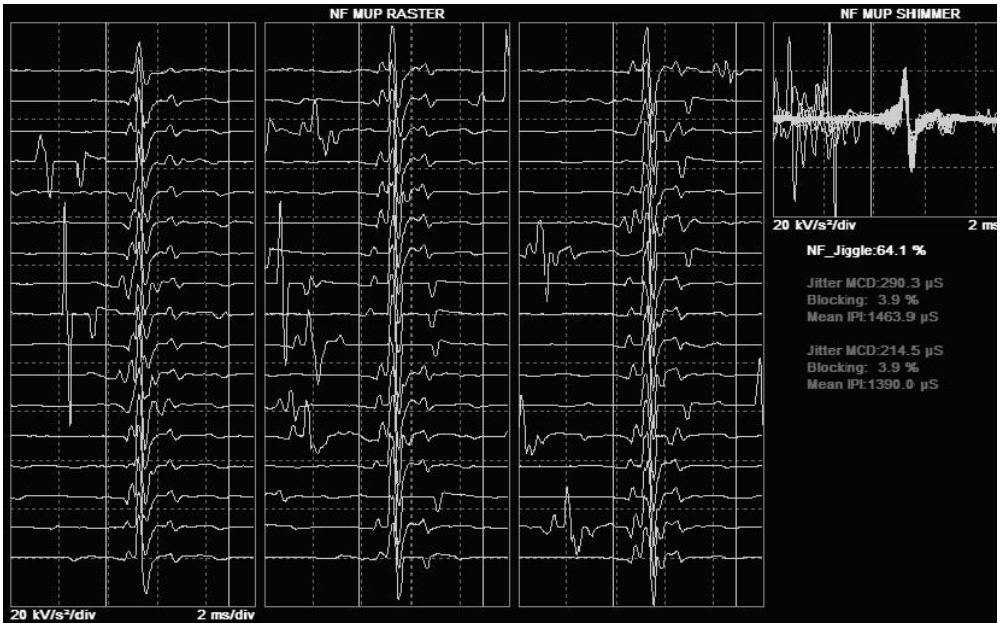

Figure 1.3 NF Jiggle

Example of NF jiggle as depicted in DQEMG. Each waveform in the MUP raster represents a different firing of the MU over the course of a 30 second contraction, and are used to create the MUP template. The shimmer plot on the right side shows all the MUP waveforms superimposed on top of each other. NF jiggle is the variability in MUP shape across consecutive discharges. (A) – MUP with a low NF jiggle value (18.2%) representing less variability in MUP shape and a more stable NMJ. (B) – MUP with a higher NF jiggle (64.1%) representing more variability in MUP shape and a less stable NMJ. Both images were taken from participants in this study

1.0.12 Tibialis anterior and vastus medialis

It is important to consider the physiology and function of the muscle under study when evaluating the health of the neuromuscular system with age. For the current investigation, muscles of the lower extremity that contribute to gait and overall functional mobility were of interest. Additionally, we aimed to include both proximal and distal muscles in our analysis to help delineate any potential length-dependent axonal changes in neuromuscular degeneration. Thus, the two muscles chosen for examination in this study were the tibialis anterior (TA) and the vastus medialis (VM).

The tibialis anterior is located below the knee and along with the extensor digitorum longus and extensor hallucis longus it makes up the anterior compartment of the lower leg [84]. Attached proximally to the lateral condyle of the tibia and to the superior portion of the lateral tibia and interosseous membrane, the TA runs parallel to the tibia and inserts distally into the medial cuneiform and base of the 1st metatarsal. Innervated by the deep fibular nerve, the TA functions primarily to dorsiflex the foot at the ankle, but it also contributes to inversion of the foot [84]. The TA is an ideal model for studies of mobility and aging as it has a principle role during the gait cycle to eccentrically control the descent of the foot at heel strike, as well as concentrically to ensure adequate toe clearance during swing phase [85, 86]. Additionally, there are numerous studies that have investigated electrophysiological and neuromuscular measurements in the TA from which a historical comparison can be made [2, 28, 56, 80, 87, 88].

The VM is one of the four quadriceps muscles that make up the bulk of the anterior thigh. Its proximal origin is along the intertrochantic line as well as the medial portion of the linea aspera of the femur [84]. It inserts distally into the common quadriceps tendon, which runs over the patella and inserts into the tibial tuberosity via the patellar tendon. The VM, like the rest of the quadriceps muscles, is innervated by the femoral nerve and functions as a unit to extend the leg at the knee joint [84]. The VM is also particularly important for patellar tracking, helping the patella to stay centered in the intercondylar groove of the femur. Although the VM undoubtedly plays an important role in gait (to help accept weight during heel strike and maintain stability during stance) [85, 86], it also serves as an ideal model for the purpose of this study as a larger, more proximal muscle. Additionally, the VM is a key contributor to large powerful movements such as climbing stairs or sit-to-stand, making it a prime candidate to investigate potential loss of muscular power with age.

1.1 Overview of thesis chapters

The objectives of this thesis were completed through a series of three studies examining nerves, muscles, strength, power and functional performance in the lower extremity of young, healthy old and mobility-impaired older adults. The aim of these investigations was to determine the differences in neuromuscular function between young, healthy older adults and those with mobility impairment. First, electrophysiological evidence of neuromuscular remodeling with age was examined using quantitative EMG analysis (Chapter 2). Next, the difference in contractile and non-contractile tissues components was studied using MRI (Chapter 3). Finally, in

Chapter 4 how changes in the neuromuscular system and composition of muscle with age affect strength and the ability to produce power was explored, with specific emphasis on the velocity of muscle contraction.

1.1.1 Chapter 2 study objectives

- To use DE-STA to evaluate quantitative MU properties of the TA and VM muscles in young, mobility-impaired and healthy older adults
- To assess NF jiggle in the TA and VM of young, mobility-impaired and healthy older adults

1.1.2 Chapter 3 study objectives

- To compare the total contractile and non-contractile muscle tissue volume in the lower extremity of young, healthy and mobility-impaired older adults
- To use MTR to assess the TA muscle quality and quantity

1.1.3 Chapter 4 study objectives

- To establish how strength and power influence functional performance in older females
- To examine the differences between specific strength and specific power in the ankle dorsiflexors and knee extensors of healthy and mobility-impaired older adults
- To determine the extent of muscle contraction velocity loss in healthy and mobility-impaired older adults

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Chapter 2

Electrophysiological evidence of motor unit remodeling with age: A comparison of healthy and mobility-impaired older adults

2.0 Introduction

The association between advanced age and a loss of total muscle mass was first described by Irwin Rosenberg [1]. This observation, which became known as sarcopenia, has been adapted over the years to incorporate the inherent complexity of the aging process and the definition now includes the loss of muscle strength and even power [2, 3]. This loss of strength and power is critical from the perspective of functional mobility. As a person's mobility is reduced they become more isolated, lose the ability to complete tasks independently and their quality of life decreases, while their mortality rate rises [4]. Researchers have identified several factors that likely contribute to the development of sarcopenia with age, which include poor diet, inactivity, hormonal variations, and degeneration of the neuromuscular system [5, 6]. While all of these factors likely contribute in isolation, as well as cumulatively, to the development of sarcopenia with age, degeneration of the neuromuscular system has been extensively studied [5-12], and is known to be a primary contributor to the loss of muscle mass and strength with age. Thus, this chapter will focus on electrophysiologically identified neuromuscular function and how it contributes to reduced mobility with age.

The MU is the fundamental component of the peripheral nervous system and there are numerous studies to support the fact that MUs are lost with advanced age

[5, 7, 9, 11, 13-16]. Most researchers agree that MU loss is relatively modest during the first 6 decades of life, but demonstrate a steep reduction thereafter [5, 7, 8, 17, 18]. Interestingly, despite evidence of significant MU loss and neuromuscular remodeling in adults over the age of 65, strength can be well maintained until much later in life (70+ years) [7]. It has been theorized that this is the result of collateral reinnervation, whereby a surviving motor neuron will sprout a collateral axonal branch to reinnervate muscle fibers that have been left behind by a dying or dysfunctional motor neuron. This would explain the relative preservation of strength, despite electrophysiological evidence of MU loss. With continued MU loss, eventually the compensatory mechanism is overtaken resulting in the observed loss of strength in very old adults. Therefore, isometric strength alone may be insufficient to capture the progression of sarcopenia until a critical threshold is achieved, wherein significant numbers of MUs have been lost and neuromuscular remodeling can no longer keep pace with such losses.

Motor unit number estimation (MUNE) is an electrophysiological technique developed to estimate the number of functioning MUs within a given muscle. MUNE provides insight into the health and number of motor neurons supplying a muscle and therefore may offer more information about the development and progression of sarcopenia during the early stages of the degenerative process. Although many different MUNE techniques have been developed and described in detail (for a review see Gooch et al. [19]), decomposition enhanced spike-triggered averaging (DE-STA) is one technique that has been shown to be valid and reliable in both healthy participants and participants with neuromuscular disease [20-25].

More recent research has focused on neuromuscular junction (NMJ) transmission stability as a potential precursor to MU loss and subsequent sarcopenia [14, 26-28]. Evidence suggests that the fidelity of NMJ transmission is compromised with age [29-31]. Although the exact mechanism is yet to be determined, this NMJ instability results in greater variability in the amplitude and shape of motor unit potentials throughout the duration of a sustained muscle contraction. This type of NMJ stability is known as "jiggle" and was first described by Stalberg & Sonoo [26]. Stashuk [32] subsequently outlined a novel approach to this measure, termed near fiber (NF) jiggle, which uses a high pass filter to amplify the contributions of muscle fibers closest to the concentric needle electrode. In effect, this allows for a more robust analysis of muscle fibers closest to the electrode, while filtering out contamination from muscle fibers further away. NF jiggle has been shown to be increased in people with diabetic polyneuropathy [28], as well as in normal healthy older adults [14]

In recent years it has been suggested that sarcopenia can be categorized into different stages of disease progression based on some key physical and physiological measures. A European working group consensus outlined grip strength, walking speed and muscle mass as three primary variables when defining sarcopenia [33]. This is supported by previous literature, which identifies gait speed as one of the most commonly reported alterations to mobility that occurs with aging [34-36] with reduced mobility being linked to increased mortality rate [4]. A gait speed of 1 m/s appears to represent a critical threshold for mobility, where people below this threshold are at the highest risk for development of secondary

complications related to their reduced mobility, and are defined as mobility-impaired [33, 37, 38].

Thus, the purpose of this study was to use DE-STA to examine MU properties, including NF jiggle, of the tibialis anterior (TA) and vastus medialis (VM) muscles in a group of young, healthy old and mobility-impaired older adults. Our aim was to categorize participants based on their mobility, rather than by age, to determine if mobility-impaired older adults would exhibit greater indices of MU loss and neuromuscular remodeling than similarly aged healthy adults. We hypothesized that both groups of older adults would demonstrate electrophysiological evidence of MU loss and neuromuscular remodeling, and further that these changes would be even more apparent in mobility-impaired older adults.

2.1 Methods

2.1.1 Participants

A total of 30 individuals took part in this study. Participants were divided into three groups: (1) healthy young adults (2) healthy older adults (3) mobility-impaired older adults. To help maintain a more homogeneous sample, all study participants were female. Female participants were selected because despite representing a significantly larger proportion of the older population [39], they tend to be less frequently studied. For inclusion in this study all participants were free from any known neuromuscular or musculoskeletal disorders. Individuals with diabetes were excluded from participation due to the well-documented impact of diabetic polyneuropathy on the neuromuscular system. Healthy young adults were recruited from the Western University graduate and undergraduate student

population and though they were recreationally active, they were not involved in any varsity or competitive sport/activity. Adults 65 years or older were eligible for the healthy (gait speed greater than 1 m/s) or mobility-impaired (gait speed less than 1 m/s) study groups. Healthy older adults were recruited from the Retirement Researchers Association (RRA) run by the Canadian Center for Activity and Aging (CCAA). The RRA is a group exercise program for adults 65 years or older. Mobility-impaired older adults were recruited from an existing database of research participants in the Gait and Brain Lab, led by Dr. Manual Montero-Odasso. Older adults were excluded if they had a recent fracture affecting their mobility, implanted metal device (such as hip or knee replacement), were unable to hold a moderate intensity dorsiflexion or knee extension contraction, or were on blood thinners. Older adults took part in a gait analysis screening session to categorize them into the healthy or mobility-impaired study group.

2.1.2 Gait analysis

Participant's walking was recorded and analyzed using the GAITRite system (CIR System Inc., Franklin, NJ). Participants were positioned in front of a 10m mat and asked to walk along the length of the mat. Participants set up ~2 feet from the start of the mat to allow for acceleration so recording would begin at a steady state gait, and were instructed to "walk right through" the far end of the mat to avoid deceleration. They were specifically instructed to walk at their regular pace. Following the first walk, they were asked to return to the starting position and repeat the test two additional times (a total of three walks). The GAITRite system provided real-time feedback to the experimenter on participant foot placement, step

length, step width and gait speed. The data were stored on the GAITRite system and later exported to Microsoft Excel for further analysis. The average gait velocity for the participant across the three trials was recorded as that individual's walking speed, and was used to categorize older adults as healthy or mobility-impaired.

2.1.3 Grip Strength

Grip strength was assessed using a handheld hydraulic dynamometer (JAMAR, Performance Health, Warrenville, IL). Participants were seated comfortably holding the dynamometer in their dominant hand with their arm tucked in against their side and elbow held at 90°. With verbal encouragement from the experimenter, participants were asked to squeeze the handle as tightly as possible for 3-4 seconds. The peak strength was recorded (in kilograms) and the dynamometer was reset. This process was repeated two more times, with one minute of timed rest between attempts, and the highest value achieved was recorded as that participant's grip strength.

2.1.4 Electromyography data collection

Electromyography (EMG) data were collected using the Viking EMG System (Natus Medical Incorporated, San Carlos CA). Self-adhering Silver Mactrode® electrodes (GE Medical Systems, Milwaukee, WI) were used to record surface potentials, while intramuscular potentials were detected using 25 mm x 30 gauge TECA™ elite Disposable Concentric Needle Electrodes (CareFusion, Middleton, WI). A bandpass filter was used at 5Hz to 5kHz and 10Hz to 10kHz for surface and needle recordings respectively.

Informal screening asking "which hand do you write with" and "with which foot would you feel most comfortable kicking a soccer ball" determined that all participants were right hand/foot dominant, and thus all testing was performed on the right limb. Participants were seated on a plinth with their back against a backrest and both legs flat across the bed. Their skin was cleansed with 70% isopropyl alcohol wipes in areas where surface electrodes would be affixed. For testing on the TA, the active electrode was placed over the motor point of the muscle (~7cm distal to the tibial tuberosity, 2cm lateral to the border of the tibia). The experimenter confirmed and repositioned this electrode, if necessary, based on the signal obtained from the excitation of the motor nerve (see below). The reference electrode for the TA was placed over the distal tendon of the TA, which was palpated by asking the participant to dorsiflex their foot against resistance provided by the experimenter. Finally, the ground electrode was positioned over the patella. Similarly for the VM, the active electrode was positioned over the muscle belly of the VM, approximately 2cm superior to the vastus medialis oblique (VMO), whereas the reference electrode was positioned over the patellar tendon and the ground electrode once again on the patella. For both muscles, active and reference electrodes were 1cm X 3.5cm and ground electrodes were 2.5cm X 3.5cm.

For the TA, a compound muscle action potential (CMAP) was determined by delivering a series of 0.1 ms electrical impulses to the common fibular nerve. A bipolar stimulator was positioned over the common fibular nerve, just posterior to the head of the fibula, and the intensity of the stimulus was increased until the amplitude of the CMAP plateaued. If necessary, the active electrode was then

repositioned to decrease the rise time and maximize the negative peak amplitude of the CMAP, ensuring the electrode was directly over the motor point of the muscle. After a plateau was reached, the stimulus intensity was increased another 20% to ensure activation of all motor axons. Once a suitable CMAP had been recorded and saved, the experimenter then affixed all electrodes in position using surgical tape to prevent displacement during further testing. A CMAP was not collected for the VM due to limitations in the validity and reliability of maximally stimulating the much larger femoral nerve, as well as EMG interference and contributions from large nearby muscles innervated by the femoral nerve [9, 40]. Thus, a MUNE was not calculated for the VM muscle in this study.

For the collection of intramuscular EMG recordings, the concentric needle electrode was inserted into the muscle under study, either slightly proximal or distal to the active surface electrode. The participant was then asked to contract against resistance provided by the experimenter and aimed to maintain a contraction intensity between 40-60 pulses per second (pps) for 30 seconds. The contraction intensity in pps represents the aggregate average of motor unit potential (MUP) discharge rate during one second of the EMG interference pattern. To help maintain adequate contraction intensity, participants received verbal feedback from the experimenter, visual feedback from a computer screen showing pps, as well as auditory feedback from the EMG signal. After each contraction the experimenter adjusted the location of the intramuscular electrode by either altering the depth of the electrode insertion into the muscle, or changing the orientation of the electrode tip. This allows for the collection of different MUs throughout the muscle. This

process was repeated until a sample of at least 20 isolated MUPs and respective S-MUPs was collected.

2.1.5 EMG signal decomposition and analysis

Once EMG signals were acquired using the Viking EMG System (Natus Medical Incorporated, San Carlos CA), they were automatically exported into DQEMG for further analysis. DQEMG uses a series of computer algorithms to decompose and isolate individual MUPs from a complex EMG interference pattern. For a detailed review of the process and algorithms used to decompose the EMG pattern see [24, 41]. Briefly, the program is able to decompose the complex EMG interference pattern into constituent MUPs and track their firing over the course of the 30-second contraction to extract multiple MUP trains. The MUPs are used as a trigger and time locked to the surface EMG signal. Thus, every time the computer detects a MUP firing, the concomitant surface EMG signal is extracted and held for processing. The S-MUP is then calculated based on the ensemble average of the surface EMG signal associated which each individual firing of the MUP.

Using DQEMG, the same experimenter inspected all data to ensure they met inclusion/exclusion criteria. First, a MUP train must have consisted of at least 51 MUP firings. Next, MU firing rates were examined to ensure they were within physiological range. This was accomplished with the assistance of the inter-discharge interval (IDI) histogram as well as the instantaneous firing rate plot. The experimenter reviewed the IDI histogram to ensure it followed a normal distribution and had a coefficient of variation (CoV) of less than 0.3. The experimenter then inspected the instantaneously firing rate plot, which should

create a reasonably straight horizontal line. If the IDI histogram displayed a non-normal distribution (CoV > 0.3) or the instantaneous firing rate plot was not a flat horizontal trace, the MUP was excluded from further analysis. A visual inspection of the MUP waveform was then conducted to rule out cannula potentials. Cannula potentials are inverted sharp positive waveforms that result from the recording of of the MUP by the cannula of the needle electrode. Any cannula MUPs were excluded from analysis; however, the S-MUP associated with the cannula potential was retained as the cannula potential still served as an appropriate trigger for surface EMG signal recordings. Finally, DQEMG would occasionally flag two MUP trains as "disparate", meaning those two MUP never fired at the same time during the 30 second contraction and it is possible that they originated from the same motor axon. In this scenario the experimenter visually reviewed the two MUPs, if they were deemed to look visually similar (similar shape, amplitude, number of turns etc.) then the MUP with the fewest number of discharges was excluded. Otherwise, both MUPs were included in the analysis.

Once all MUP and S-MUPs were deemed acceptable by the above criteria, the experimenter then visually inspected each individual waveform and adjusted markers (when applicable). MUPs had four markers (onset, negative peak, positive peak, offset) and S-MUPs had three markers (negative onset, negative peak, positive peak) (Figure 1). The same experimenter performed DQEMG analysis for all participants in this study. While adjusting these markers, two finals checks to the S-MUP were performed. First, the experimenter confirmed that the signal to noise ratio of the S-MUP was greater than 10. Any S-MUPs with a signal to noise ratio of

less than 10 were excluded from analysis. Second, the experimenter confirmed that the S-MUP occurred within 10ms of its trigger MUP. Once analysis was complete, DQEMG automatically calculated descriptive statistics based on all the included MUPs and S-MUPs. A data point-by-data point average was used to create a template S-MUP from which a MUNE could be calculated. For the purposes of this study the negative peak amplitude was used to determine MUNEs, as it has been shown to be a valid and reliable method for calculating MUNE [20-22]

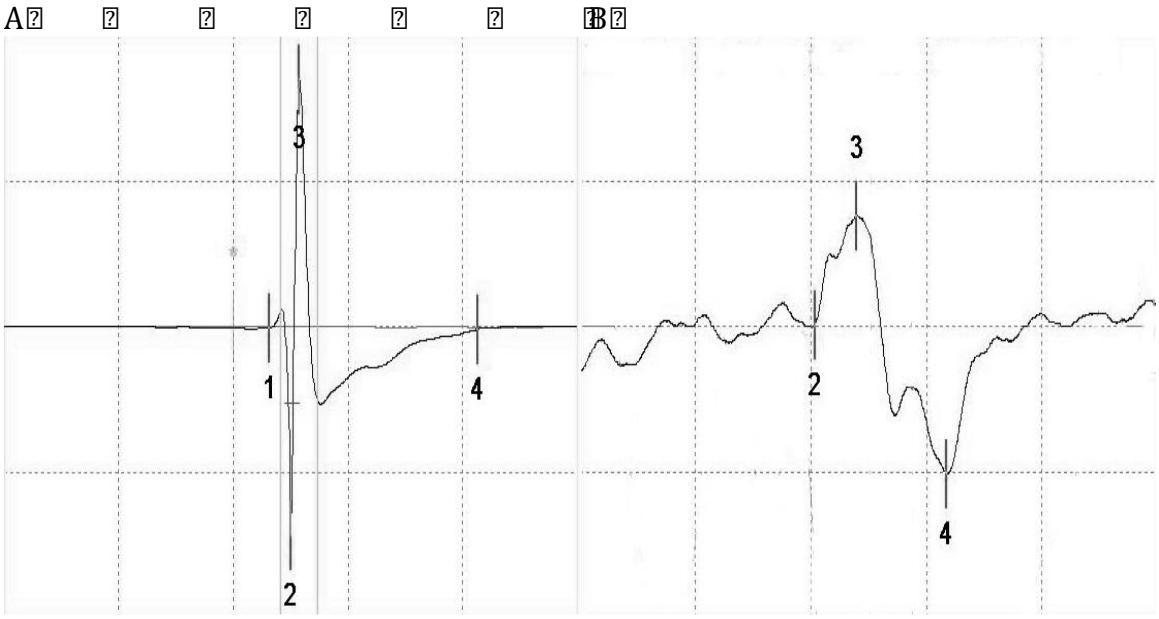

Figure 1. MUP and S-MUP
A – An example of a typical MUP as detected by the concentric needle electrode. The MUP contains markers for (1) – onset (2) – positive peak (3) – negative peak (4) – offset. B – An example of a typical S-MUP detected by the surface electrode and represented in DQEMG. S-MUP markers denote (2) – negative onset (3) negative peak (4) – positive peak. Markers are automatically set by DQEMG software. The same experimenter visually inspected and adjusted marker location if applicable. (Modified from: McKinnon, NB, Monterro-Odasso, M, Doherty, TJ. Motor unit loss is accompanied by decreased peak muscle power in the lower limb of older adults. *Experimental Gerontology.* 2015; 70: 111-8) [9]

2.1.6 NF Jiggle

Neuromuscular stability was assessed by NF jiggle, which was calculated using DQEMG software. This process has been described in detail previously [28]. In short, using a second order low-pass differentiator, the MUP template was high-pass filtered to create a NF MUP waveform. The NF MUP represents the contributions of only those muscle fibers closest to the recording surface of the needle electrode (~350 μm). A sample of 51 NF MUPs are then randomly selected and represented in a raster plot on screen. The experimenter completed a visual inspection of the NF MUPs to ensure they were appropriate for inclusion in the stability measurement. NF MUPs were excluded and replaced if they were deemed to have signal contamination from activity of nearby MUs. This process was repeated for every MUP included in the study and DQEMG automatically calculated the overall NF jiggle for each participant.

2.1.7 Statistics

All data is presented as mean ± standard deviation (SD). Statistics were analyzed using Statistical Package for the Social Sciences (Version 21; IBM SPSS Inc., Chicago, IL). A Kruskal Wallis H test was used to identify any difference between groups on MU properties. If a significant difference was detected, post-hoc analysis consisted of individual Mann Whitney U tests between groups with a modified Bonferroni correction to account for inflated alpha error. A significance level of $p \leq 0.05$ was used for all statistical tests.

2.2 Results

2.2.1 Participants

A total of twelve young adults (mean age: 25 ± 2 years), eleven healthy older adults (mean age: 74 ± 6 years) and seven mobility-impaired old adults (mean age: 72 ± 5 years) were tested. There was no difference in height (chi-square= 3.38, p=0.185) or weight (chi-square= 6.19, p=0.056) between the three experimental groups, and no difference in age between healthy old (74 years) and mobility-impaired older adults (72 years) (p=0.536). Both older adult groups had significantly reduced grip strength (chi-square= 12.12, p= 0.002) when compared to young adults (z= -3.15, p=0.01, z= -2.54, p=0.01 for healthy older and mobility-impaired older adults respectively). Mobility-impaired older adults had an average gait speed of 0.89 ± 0.10 m/s, which was significantly slower than the 1.24 ± 0.17 m/s gait speed of healthy old adults (z= -3.49, p<0.001). Participant information is summarized in Table 1. Although gait speed was not calculated for young control subjects in this study, normative data from previous research shows healthy young adults to have a gait speed between 1.2-1.6 m/s [42, 43, 44, 45].

Table 1. Study Participant Demographics

	Young	Old	MI
Sample size	12	11	7
Age (years)	25 (2)	74 (6)*	72 (5)*
Height (cm)	165 (5)	164 (5)	161 (4)
Weight (kg)	63.4 (13)	70.0 (9)	79.5 (21)
Gait speed (m/s)	-	1.24 (0.17)	0.89 (0.10)*
Grip Strength (kg)	32 (6)	24 (4)*	23 (8)*

Values presented as mean (standard deviation). * indicates significantly different from young. MI = mobility-impaired

2.2.2 TA MU properties

An average of 24 ± 2, 25 ± 3, and 24 ± 3 MUPs were collected from the TA muscle in young, old and mobility-impaired participants, respectively. From a mean of 5 total contractions, 25 ± 2 valid S-MUPs were obtained in young subjects resulting in an average of 5 ± 1 S-MUPs collected per contraction. For healthy old adults, 25 ± 3 S-MUPs were collected from an average of 4 contractions resulting in 6 ± 1 S-MUPs/contraction. Mobility-impaired older adults had on average 5 ± 1 contractions to collect 24 ± 2 valid S-MUPs, or 5 ± 1 S-MUPs per contraction. Contraction intensity, based on needled detected pps, did not differ between young, old and mobility-impaired adults (chi-square=0.32, p=0.85), indicating that MU properties were sampled at similar contraction intensity between experimental groups. A significant difference in CMAP negative peak amplitude was detected between experimental groups (chi-square=9.56, p=0.008). CMAP negative peak amplitude was 7.9 ± 2.0 mV in young subjects, which was significantly larger than the CMAP of 6.5 ± 0.9 mV in healthy old (z= -2.03, p= 0.04) and 5.2 ± 1.4 mV in mobility-impaired older adults (z= -2.53, p= 0.01). Although S-MUP negative peak amplitude did increase in both groups of older adults (30 ± 12 μV, 51 ± 44 μV, 42 ± 23 μV for young, old, and mobility-impaired respectively), this difference was not statistically significant (chi-square= 1.76, p= 0.415). For MUNE, a significant difference between groups was detected (chi-square= 6.33, p= 0.04). Post-hoc analysis determined there was no statistical difference (z=-1.9, p=0.06) in MUNE in the healthy older adults (MUNE: 193 ± 113) compared to young adults (MUNE: 287 ± 110); however, MUNEs in mobility-impaired older adults (MUNE: 160 ± 87) was

significantly reduced compared to young adults (z= -2.28, p= 0.02). A significant difference was also detected for NMJ stability (chi-square= 6.23, p= 0.04). Healthy older adults had significantly greater NF jiggle (31.8 ± 6.5%) when compared to young subjects (25.3 ± 3.7%) (z= -2.59, p= 0.009). Interestingly, although mobility-impaired older adults also had a larger NF jiggle value (30.3 ± 8.4%), it was not significantly larger than the young controls (z= -1.35, p= 0.196) (Table 2).

2.2.3 VM MU properties

For the VM muscle, a total of 24 ± 3, 24 ± 2, and 27 ± 2 MUPs were sampled for young, old and mobility-impaired respectively. For young participants, an average of 5 total contractions were used to obtain 26 ± 3 valid S-MUPs, or 5 ± 1 S-MUP's collected per contraction. Older adults required 5 contractions to collect 24 ± 2 S-MUPs resulting in 5 ± 1 S-MUPs/contraction. 26 ± 2 valid S-MUPs were obtained from 4 ± 1 contractions in mobility-impaired old adults, resulting in 6 ± 1 S-MUPs per contraction. There was no difference between groups in needle-detected contraction intensity during the acquisition of MUP data (chi-square= 0.81, p= 0.67). In the VM, the S-MUP negative peak amplitude decreased from young (54 ±23 μV) to old (45 ± 24 μV) to mobility-impaired older adults (29 ± 13 μV), although this difference was not statistically significant (chi-square= 5.71, p=0.06). Additionally, there was no difference (chi-square= 5.14, p=0.08) in NF jiggle values between groups (18.6 ± 3.8%, 21.4 ± 4.3%, 22.1 ± 4.6% for young, old and mobility-impaired old respectively) (Table 2).

Table 2. Motor unit properties of young, old and mobility-impaired older adults in the tibialis anterior and vastus medialis

	Tibialis Anterior			Vastus Medialis		
	Young	Old	MI	Young	Old	MI
CMAP Neg Peak Amp (mV)	7.9 (2.0)	6.5 (0.9)*	5.2 (1.4)*	-	-	-
S-MUP Neg Peak Amp (μV)	30 (12)	51 (44)	42 (23)	54 (23)	45 (24)	29 (13)
MUNE (#)	287 (110)	193 (113)	160 (87)*	-	-	-
NF Jiggle (%)	25.2 (3.7)	31.6 (6.5)*	30.3 (8.4)	18.6 (3.8)	21.4 (4.3)	22.1 (4.6)
Intensity (pps)	53 (5)	55 (12)	51 (10)	51 (9)	47 (9)	51 (7)

Both groups of older adults had significantly reduced CMAP amplitude in the TA muscle compared to young adults (p= 0.04, p= 0.01 for healthy and mobility-impaired old respectively). MUNEs were significantly reduced in mobility-impaired older adults (p= 0.02), but not healthy older adults (p=0.06). Only healthy older adults had significantly larger NF jiggle compared to young (p= 0.009). For the VM, S-MUP amplitude decreased from young to healthy old to mobility-impaired older adults, although this difference was not statistically significant (p= 0.06). There was no difference in NF jiggle between groups in the VM muscle (p= 0.08). There was no difference in contraction intensity during EMG recording between groups in either muscle tested (p= 0.85, p= 0.67 for TA and VM respectively). Values are presented as mean (standard deviation). * Indicates a significant difference from young. MI: mobility-impaired

2.3 Discussion

This study was designed to determine if older females with mobility impairment, defined by a gait speed of less than 1 m/s, would demonstrate electrophysiological evidence of greater MU loss and neuromuscular remodeling when compared to healthy age-matched and young controls. We observed indications of MU loss in in the TA of both groups of older adults, which appear to be more substantial based on the calculated MUNE in the mobility-impaired population. Our findings are in line with previous research indicating sarcopenia is not a function of aging alone, but rather a result of a complex interaction of multiple variables affecting a person's muscle mass, strength and overall mobility [8, 12, 46]. Thus, classification of persons with sarcopenia should not be defined based on age; instead, sarcopenia should be categorized based on a combination of defined normative values for muscle mass, strength and function [33].

Due to its functional significance during locomotion and the ease of access to the superficial muscle belly and peripheral nerve supply, the TA muscle is an ideal model for studies of neuromuscular function with age. Several studies have previously examined MU properties of the TA muscle in populations of healthy young adults, older adults and individuals with neuromuscular disorders [7, 9, 18, 47-49]. A previous study from our lab [9] investigated the relationship between MUNEs and muscular power in the TA and VM muscles of young and older adults. It was observed that older participants had significantly reduced MUNEs in the TA compared to young adults, and that this appeared to be the result of significantly larger S-MUPs in the older adults. This observation is in line with the pattern of

neuromuscular remodeling with age; as MUs are lost, through the process of collateral reinnervation, the average size of individual MUs (i.e. S-MUP) increases. The values reported for MUNE (young: 233 ± 109; old: 102 ± 76) and S-MUP negative peak amplitude (young: 27 ± 14 μV; old 63 ± 29μV) in that paper are in accordance with the values reported in the current study.

Several other studies have examined MUNEs in the TA muscle. McNeil and colleagues [7] compared MUNE and strength in young, old (65-79 years) and very old (80+ years) adults. They reported a decrease in MUNE between groups (young: 150; old: 91; very old: 59), but intriguingly strength was maintained compared to young in the old group, but reduced compared to young in the very old. The authors suggested that through collateral reinnervation, isometric strength is maintained until a critical threshold of MU loss is reached. More recent research however suggests that power may be more sensitive to MU loss associated with sarcopenia [9, 12]. As the larger fast twitch muscle fibers are lost at a greater proportion than the smaller, habitually active slow twitch fibers, there is a systemic slowing of muscle contraction velocity. Thus, even though some of the lost muscle fibers are reinnervated resulting in the maintenance of isometric strength during the early stages of dennervation, whole muscle contraction velocity is reduced, which manifests in a deficit in muscle power.

Another study by Power et al. [18] examined the effects of high-level lifelong physical activity on MU loss in the TA by comparing normal healthy older adults with “master runners” who regularly trained and competed in long distance races throughout their life. They found MU loss was attenuated in the master runners,

concluding that MU loss with age may be activity dependent. While the study by Power et al. [18] was able to establish that life-long physically active individuals may attenuate the development of sarcopenia, the current study observed the opposite end of the spectrum. Investigating older adults without any underlying neuromuscular or musculoskeletal disorders, we were able to demonstrate that those with reduced mobility showed greater indications of sarcopenia based on their estimated number of functioning MUs.

Interestingly, for the present study, although S-MUP negative peak amplitude in the TA was not significantly larger compared to young participants, MUNE was significantly reduced in the mobility-impaired older adults. Also of note, the S-MUP amplitude increased from 30 μV to 51 μV from young to healthy old adults, but then decreased slightly to 42 μV in mobility-impaired adults. Although this may seem contrary to the established model of neuromuscular remodeling with advancing age whereby MU loss leads to the expansion of surviving motor neurons [7], it may be explained as an accelerated progression of sarcopenia in the mobility-impaired older adults. It is feasible that the mobility-impaired older adults, who have presumably experienced greater MU loss (based on derived MUNE values), may have outpaced the compensatory reinnervation process. Thus, the observed decrease in S-MUP amplitude in these participants may be the result of continued MU loss, as some of the larger reinnervated parent motor neurons are lost. This notion is supported by significantly lower CMAP observed in mobility-impaired older adults, indicating that the overall MU pool has been reduced.

In comparing MUNEs in the current study with those of previously published values for the TA [7, 18, 48, 49], it is apparent that our results are slightly higher across all 3 groups. It is important to distinguish that most of these previous studies had a sample consisting of all (or mostly) male participants, whereas the present study contained all female participants. It is well documented in the literature that estrogen has neuroprotective effects [59, 60]. A study by Nakamizo et al. demonstrated that 17α-estradiol prevented nitric oxide induced motor neuron death in the spinal cord of rats [61]. Additionally, estrogen has been found to reduce oxygen free radicals, providing an antioxidant effect [59, 60]. Granted the older females in this study would be post-menopausal and have significantly reduced circulating estrogen levels; however, it is possible that the exposure to the neuroprotective effects of estrogen throughout the course of their adult life may have attenuated the progression of inflammation and oxidative stress normally experienced by motor neurons [62, 63]. Relatively few studies have examined differences in sex on MUNE, and those that have found that there was no difference in MUNE between males and females using traditional STA [11] and multiple point stimulation [64, 65]. Interestingly, all of these studies assessed muscles of the upper extremity (biceps brachii, brachialis, abductor pollicis brevis, abductor digiti minimi), to our knowledge no study to date has investigated sex differences in MUNE in the lower extremity muscles, or using DE-STA. Considering the importance of lower extremity muscles to mobility and function with age, and given that females tend to live longer and represent a significantly larger proportion of the older adult population than males [39], this may be an important area for future research.

The VM, on the other hand, has had comparatively fewer studies conducted of MUNE. Berger and colleagues [50] used DE-STA to investigate MU properties in the VM of healthy older adults (62 ± 6 years) and reported an average S-MUP negative peak amplitude of 41 ± 29 μV, which is similar to the values reported here. As well, a study by Conwit et al. [20] investigated the effects of contraction level on S-MUP negative peak amplitude in the VM using DE-STA in a sample of adults with an age ranging from 21-70 years. At contraction levels of 20% and 30% MVC (which most closely resemble the intensity of muscle contraction used in the current study) the authors reported S-MUP amplitudes of 41.3 μV and 53.5 μV respectively. These values are remarkably similar to those presented in the current study for young (54 μV) and healthy old adults (45 μV).

Results from a previously published study out of our lab show an increase in S-MUP amplitude from 48 μV in young to 60 μV in older adults [9]. In the current study however, we observed a decrease in S-MUP amplitude from young (54 μV) to healthy old (45 μV) to mobility-impaired old (29 μV). Hourigan and colleagues [14] observed a similar trend in a previous study of neuromuscular function in the VM. The authors recorded S-MUP negative peak amplitudes of 76 μV in young and 66 μV in healthy older males. Additionally, a recent study investigating age-related neuromuscular changes in the vastus lateralis found a similar decrease in S-MUP negative peak amplitude from young (96.7 μV) to older adults (87 μV) [40]. It has been suggested that due to the larger more disperse MU territories and increased depth and distance of contributing muscle fibers to the surface electrode, that larger more proximal muscle, such as the VM, may not provide an accurate reflection of the

actual MU size in these muscle [9, 51]. Previous studies have estimated the recording radius of a surface electrode to be less than 20 mm [52], which in a large muscle such as the VM, is unlikely to pick up the contributions from all of the muscle fibers of a MU. Additionally, it has been shown that older adults have increased subcutaneous and intramuscular adipose tissue [5, 53, 54], and that these tissues overlying muscle can attenuate EMG signals [55]. Data presented in Chapter 3 show that both groups of older females in this study have significantly greater subcutaneous adipose tissue compared to healthy young adults. Therefore, it is reasonable to speculate that the decrease in S-MUP amplitude observed in the VM muscle of the current study may be related to the attenuation of EMG signal activity rather than a presumed decrease in MU size. As large proximal lower extremity muscles are critically important for gait and mobility, it is important for future research to investigate different ways to accurately record and track neuromuscular changes in these muscles.

NF jiggle represents the variability in the shape of the MUP across successive discharges. It has been suggested that sarcopenia compromises the fidelity of the NMJ leading to increased variability in neuromuscular transmission [29], and that this may occur prior to actual MU loss [26, 27]. In the present study, NF jiggle in the TA increased from 25.2% in young participants to 31.6% and 30.3 % in healthy older adults and mobility-impaired older adults respectively. These values are similar to previous studies investigating NF jiggle of the TA in older adults [14, 28]. Interestingly, NF jiggle was significantly increased in healthy older adults, while MUNEs were unchanged, providing further support that NMJ instability may be a

clinically useful measure of neuromuscular damage and impending MU loss [14, 26, 28, 56].

NF jiggle values obtained for the VM in the present study were not significantly different between study groups. This is in contrast to a previous study investigating NF jiggle in young and healthy older males [14]. Using the same technique used here, Hourigan et al. [14] reported an increase in NF jiggle values in the VM from 23.9 ± 4.2% in the young to 31.3 ± 5.5% in older adults. There is some evidence in the literature to suggest that more proximal muscles may be relatively preserved from the effects of age-related neuromuscular degeneration compared to distal muscles [9, 57, 58]. However, the conflicting results between these two studies highlight the necessity for further research into sarcopenia and neuromuscular changes in large proximal muscle groups.

A recent investigation by Gilmore and colleagues [56] compared MUNEs and NF jiggle of the TA muscle in older adults categorized based on 3 levels of sarcopenia (pre-sarcopenic, sarcopenic, and severely sarcopenic). The authors reported no difference in CMAP, S-MUP amplitude or MUNE between the three groups. However, they did find that NF jiggle was significantly greater in the severely sarcopenic group compared to both the pre-sarcopenic and sarcopenic groups (31% and 26% greater respectively). Contrary to Gilmore et al. [56], the present study demonstrated a significant decrease in CMAP in both groups of older adults, and a significant decrease in MUNE in mobility-impaired adults. The current study also shows a 25% and 20% increase in NF jiggle compared to young for old and mobility-impaired adults respectively. In the study by Gilmore et al. [56]

participants were categorized based on three key variables associated with the progression of sarcopenia (grip strength, muscle mass and gait speed). Although age was not considered in their classification, there was a tendency towards adults with greater degrees of sarcopenia being older (mean age pre-sarcopenic: 75 years; sarcopenic: 78 years, severely sarcopenic: 82 years). In contrast, the present study categorized participants based on gait speed and controlled for mean age between healthy and mobility-impaired groups. It should be noted that the mean age in the present study is on the lower end of the range of participants in the study by Gilmore et al. [56]. Nonetheless, both studies underscore the importance of categorizing sarcopenia based on physical and functional performance rather than age. The present study is not without limitations. First, the sample size, especially in the mobility-impaired population, is relatively small, which limits the power of our analysis and therefore our results are conservative estimates of effect. However, it should be noted that previous studies of MU properties and EMG measures have reported significant results with similar sample sizes [7, 14, 18, 56]. Additionally, for the VM we did not record a CMAP from which we would have been able to normalize obtained S-MUP values to. This decision was made intentionally, as the reliability and validity of CMAP's obtained from this muscle have previously been called into question due to difficulty maximally stimulating the femoral nerve and because of EMG interference from large muscles nearby that are also activated with stimulation of the femoral nerve [9, 40]. Finally, the cross-sectional design of this study limits the generalizability and conclusions that can be drawn from this study.

As only females were studied this does not necessarily represent all people within these age groups, therefore we cannot generalize these results to all older adults.

In summary, the purpose of this investigation was to determine if motor unit loss and neuromuscular remodeling was accelerated in older adults with mobility impairment compared to healthy age-matched and young controls. In accordance with recently published work [33, 56], the current study provides electrophysiological evidence that sarcopenia is not defined by age alone, but rather the combination of multiple factors that contribute to the loss of strength, muscle mass and mobility as we age. Future research into sarcopenia and aging should focus on categorizing participants based on their level of impairment rather than their age, with emphasis on proximal muscles of the lower extremity, which are critically important to mobility. As well, further longitudinal studies into EMG evidence of neuromuscular remodeling at different stages of sarcopenia with aging are needed.

2.4 Reference

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Chapter 3

Investigating contractile and non-contractile muscle tissue components in healthy and mobility-impaired older adults

3.0 Introduction

Sarcopenia is the term used to describe the natural aging process whereby muscle mass and, subsequently, strength are lost [1, 2]. The progression of sarcopenia contributes to impaired mobility, and has been linked to increased disability in older adults [3]. The degradation of contractile muscle mass is the hallmark symptom of sarcopenia, and it is therefore important for studies of sarcopenia and aging to accurately document and record the atrophy of muscle tissue in vivo. Early studies used limb circumference measurement in an attempt to track muscle atrophy with advanced age; however, availability of new technologies has demonstrated the inadequacy of these measurements to accurately reflect changes in contractile muscle tissue [2-7].

First, although skin fold caliper testing can help to partially control for increases in subcutaneous adipose tissue (SAT), it is difficult to precisely control when measuring circumferential limb girth. Additionally, results from muscle biopsies [8] as well as imaging studies [3-6] consistently demonstrate that older adults have a larger proportion of intramuscular adipose tissue (IMAT) when compared to healthy young adults. Evidence suggests the accumulation of fat within muscle has a negative impact on mobility and lower extremity functional performance [9]; thus, a valid and reliable method to quantify the amount of muscle

mass separated from adipose tissue is required for the accurate tracking of sarcopenia in older adults.

Many techniques have been used to determine the size of limb muscles including ultrasound, dual-energy X-ray absorptiometry (DEXA), computed tomography (CT) and magnetic resonance imaging (MRI) [2, 4-6, 10, 11]. Due to its ease of use, high quality images and lack of exposure to radiation (such as that required for CT scanning), MRI has become one of the most predominantly used and gold standard methods for calculating muscle mass. Additionally, MRI has shown to be valid and reliable in the quantification of muscle tissue components [12, 13], whereas DEXA has been reported to potentially underestimate the loss of muscle mass associated with aging [10].

MRI is a non-invasive technique that uses extremely strong magnetic fields to image soft tissue structures. Using the principles of magnetic resonance, technologists can take advantage of the inherent differences in how fluids and fats behave when exposed to these strong magnetic fields. This allows for the production of high quality images of soft tissue in vivo. Due to advancements in technology, MRI has become faster and more practical for use in research. Furthermore, due to improvement in image quality, MRI is also able to segregate IMAT and other non-contractile tissue from within the muscle itself, allowing for the calculation of total contractile muscle mass. As it has been well established that MU loss leads to muscle fiber atrophy, which is supplanted by fat and connective tissues [2, 14], quantification of IMAT and total contractile muscle mass are important markers of sarcopenia in older adults.

Recently, researchers have described a novel MRI technique known as magnetization transfer (MT) (for a review see Henkelman et al. [15]). Although this technique was first used in studies of multiple sclerosis to record lesions in white matter of the brain [16, 17], it has increasingly been adapted for use in many other areas, including in skeletal muscle to assess muscle protein quality and quantity. MT uses an off-resonance radio frequency (RF) pulse to saturate water-bound proteins within the muscle, causing images with higher protein content to appear darker. A magnetization transfer ratio (MTR) can then be calculated by comparing images with the off-resonance RF pulse to those without [18, 19]. The ratio of change between these two images represents the quality of protein within the selected muscle. Previous studies have used MT to assess muscle protein quantity in the TA muscle and have reported reduced MTR in old adults [20], very old adults [21] and in individuals with diabetic polyneuropathy [22].

Thus, the purpose of this study was to use MRI to determine the total contractile muscle mass and amount of non-contractile tissue in the ankle dorsiflexors and knee extensor muscles in young, healthy old, and mobility-impaired older adults. Additionally, we aimed to investigate muscle protein quality and quantity in these muscle groups using this novel MT approach. Previous research has reported that older adults have increased IMAT in the ankle dorsiflexors [3, 20, 21] and knee extensor muscles [6, 7], and that increased IMAT has been linked to decreased mobility and poor functional performance in lower extremity muscles [9]. However, to our knowledge, no study to date has investigated these measures in a group of mobility-impaired older adults. Therefore, we hypothesized that both

groups of older adults would demonstrate reduced muscle mass and increased IMAT, with mobility-impaired older adults expressing even greater deficits than healthy old adults.

3.1 Methods

3.1.1 Participants

Participants were divided into one of three experimental groups based on their age and mobility status. Young participants were between the ages of 22-28 years old and recruited from the Western University student population. For inclusion to the older adult population participants must have been over the age of 65 years and have no signs of underlying health conditions which may affect muscle or nerve health. All older adults took part in a walking test to determine gait speed. Participants with a gait speed of greater than 1 m/s were classified as healthy older adults, and those with a gait speed of less than 1 m/s were classified as mobility-impaired. A gait speed of less than 1 m/s has previously been described as a critical threshold for mobility impairment in older adults [23-25]. All participants in this study were female.

3.1.2 Magnetic resonance imaging

MRIs were acquired using a Dixon technique with fat suppression via a 3T magnet (Magnetom Verio, Siemens Healthcare, Erlangen, Germany). Participants were positioned supine on the MRI table and straps were positioned around their feet and legs to prevent excessive movement during imaging. For the TA, a RF coil was positioned over the area being imaged (from the tibial plateau to the malleoli) and the participant was inserted into the bore of the magnet feet first. Sixty-six

images were acquired with a slice thickness of 5mm and no slice separation. A repetition time (TR) of 3000ms, echo time (TE) of 11ms and flip angle of 180° was used.

Due to the increased length of muscle mass being imaged for the anterior thigh muscles, two RF coils were used for these images. Each RF coil collected 58 images with a thickness of 5mm and no separation with parameters the same as outlined above. This provided some overlap of images, which were excluded during analysis.

3.1.3 Determination of muscle CSA, volume, and contractile/non-contractile tissue

All MRIs were analyzed using OsiriX DICOM viewer (Version 11.0.2, Prixmeo SARL, Geneva, Switzerland). Images were analyzed using a semi-automated process whereby the experimenter manually outlined a region of interest (ROI) on every third image starting distally and working proximally until reaching the last slice containing identifiable muscle tissue. For the ankle dorsiflexors, analysis began on the first image proximal to the malleoli containing identifiable muscle tissue and continued proximally to the tibial tuberosity. Similarly for the knee extensors, ROIs began distally with the first identified muscle fibers of the VM and continued proximally until the last slice containing muscle fibers of the rectus femoris. Due to the use of two RF coils in the knee extensors, there was some overlap of images in the middle of the thigh. For these images the experimenter visualized the area of overlap side-by-side in the DICOM viewer and outlined the images with better contrast while excluding the other images from analysis. The software was then

used to interpolate missing ROIs based on the manually outlined areas. Next, the experimenter visually inspected the computer-generated ROIs to ensure their accuracy and manual corrections were made to the outlined area if necessary. Once satisfied with the outlined muscle tissue, all pixels outside the ROI were set to zero and the experimenter used the computer software to render a 3-dimensional (3D) model of the muscle. Since there was no separation between slices in the image capture process, this 3D model of the muscle represented the entire volume of the muscle under study. The volume of muscle tissue was then determined automatically by the software and recorded as the participant's "total" muscle volume. The experimenter then used the brush tool to manually subtract any non-muscle tissue from the images (i.e., fat, connective tissue, septal space, neurovascular tissue etc.)(Figure 1). The same experimenter analyzed all images for all participants in the study. The muscle volume was then computed again and recorded as the participant's "contractile" volume allowing for the quantification of fat, connective tissue and other non-contractile tissue in the muscle. The single image slice with the largest area was recorded as the participant's CSA for each muscle group tested. The "contractile" CSA was subtracted from the "total" area to determine non-contractile CSA. Previous research has used a similar technique for MRI analysis of muscle composition [21, 26] and it has been shown to have a high degree of inter- and intra-rater reliability [26].

The amount of SAT was also determined in the thigh. SAT was measured from the single image with the largest total thigh muscle area, which was determined by manually outlining a ROI that included the entire thigh musculature.

Once the largest cross-section of muscle in the thigh was determined, the experimenter then outlined the entire mass of the thigh using a second ROI. Within this new ROI, the experimenter was then able to set a pixel value equal to an area in the image containing only adipose tissue and render a 2D model using the computer software. Finally, the experimenter used the brush tool to manually remove any IMAT that was included in the 2D modeling. The remaining area consisted of only the SAT and was recorded.

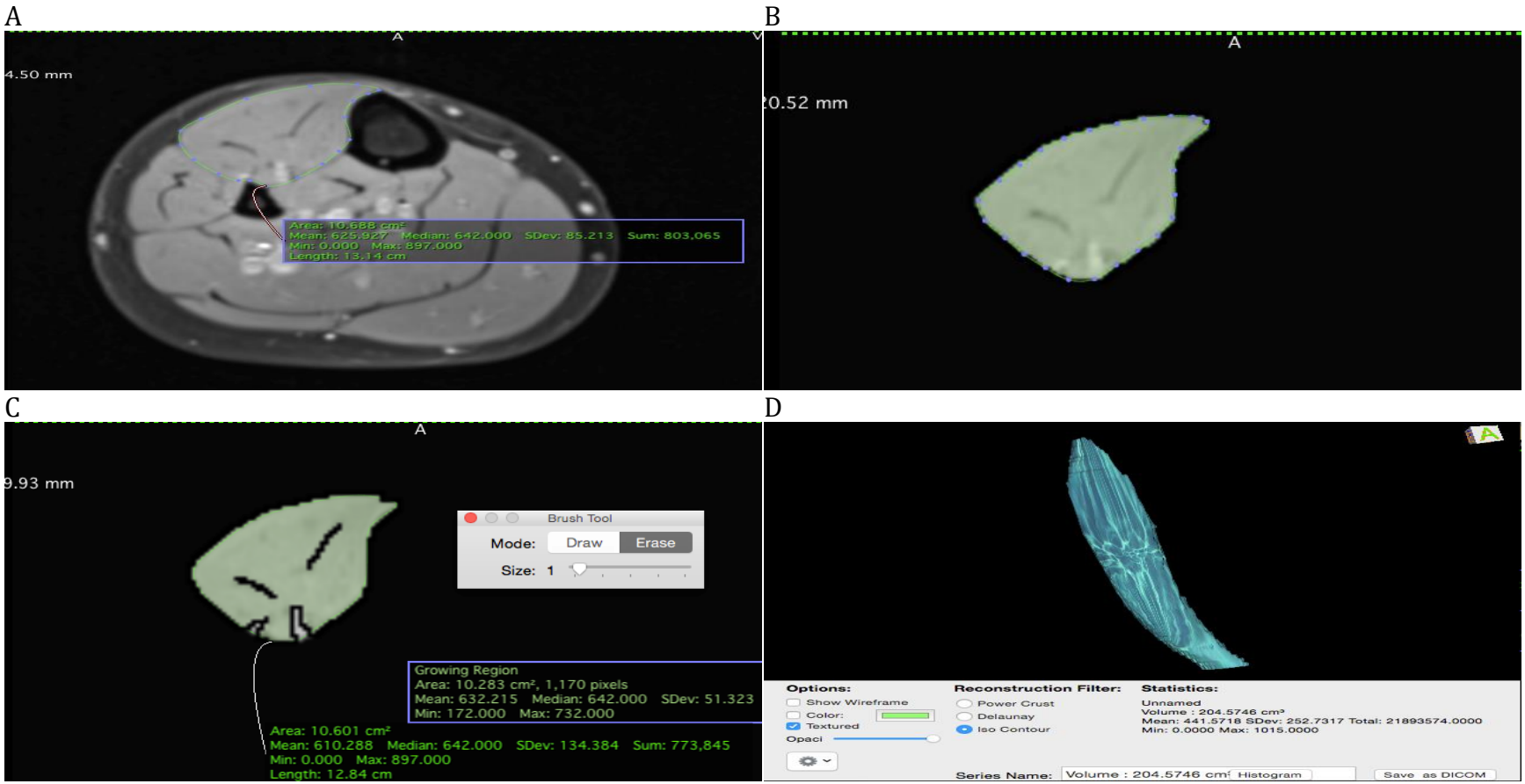


Figure 1. MRI analysis and determination of muscle CSA and volume

Representative images of the process used to determine muscle CSA and volume in the anterior compartment. A – ROIs were manually outlined on every third image starting distally. OsiriX software was then used to interpolate missing ROIs, which were manually inspected and adjusted if necessary. B – Once a ROI was determined for each image, pixel values outside the ROI were set to zero and a 3D region was determined by OsiriX software. The single image with the largest area was recorded as the participants CSA. C – Using the brush tool function, an experimenter manually subtracted any non-muscle tissue or septal spaces within the muscle from each MRI scan. The same experimenter examined all MRIs in the study. D – OsiriX software could then render a 3D model of the entire muscle to determine muscle volume.

3.1.4 Magnetization transfer

Muscle protein quality was assessed using the unique MRI protocol known as MT. MT uses an off-resonance RF pulse to saturate protons in large macromolecules (such as protein) to create additional contrast on images. These images can then be subtracted by images without the off-resonance pre-saturation pulse to determine a MTR and give an indication of the quality and quantity of protein in the muscle. A total of 32 images were taken centered around the muscle belly, with a thickness of 5mm and no separation between slices. A TR of 1120ms, TE 5ms, and flip angle of 15° was used. The MT pulse used was similar to that described previously [27]: gaussian, 10.2 ms long, 500° flip angle and 1200 Hz offset. Similar MT pulse parameters have been used previously to assess skeletal muscle quality [21, 22]. Images with off-resonance pre-saturation were acquired first and images without were obtained from the same anatomical location immediately following the first scan.

A MTR was calculated from the image with the largest CSA, which was determined in the ankle dorsiflexors by manually outlining the muscle to determine the slice with the largest area. The examiner then selected an area of muscle tissue without significant contamination of fat, connective tissue or other non-contractile tissues and recorded the mean pixel value from this area as M_1. The selected area was then copied and pasted into the same location of the images without pre-saturation and the pixel value was recorded as M_0. A MTR was then calculated using the equation $MTR = [M_0-M_1]/M_0$ (Equation 1). MTR is expressed as a ratio of relative change from baseline (i.e. images without pre-saturation).

3.1.5 Statistics

All statistical testing was completed using Statistical Package for the Social Sciences (Version 21; IBM SPSS Inc., Chicago, IL). A Kruskal-Wallis H test was used to identify any statistically significant differences between experimental groups. If a statistical difference was detected, post-hoc analysis consisted of individual Mann-Whitney U tests between groups, with a modified Bonferroni correction. A significance level of $p \leq 0.05$ was used for all statistical tests. Values are presented as means ± standard deviation.

3.2 Results

3.2.1 Participants

Twelve healthy young participants (mean age: 25 ± 2 years), ten healthy older adults (mean age: 74 ± 6 years) and six mobility-impaired older adults (mean age: 72 ± 6 years) took part in this study (Table 1). There was no difference in height (chi-square= 3.38, $p=0.185$) or weight (chi-square= 6.19, $p=0.056$) between groups. Gait speed was significantly reduced in mobility-impaired older adults compared to healthy older adults ($z = -3.49$, $p<0.001$). Participant information is summarized in Table 1.

Table 1. Study Participant Information

	Young	Old	MI
Sample size	12	10	6
Age (years)	25 (2)	74 (6)*	72 (5)*
Height (cm)	165 (5)	165 (5)	161 (5)
Weight (kg)	63.4 (13)	71.5 (8)	79.8 (23)
Gait speed (m/s)	-	1.26 (0.17)	0.87 (0.10)*

There was no difference between height ($p= 0.185$) or weight ($p= 0.056$) between participants. Mobility-impaired adults had significantly slower gait speed than healthy older adults ($p<0.001$). Values presented as mean (standard deviation). * indicates a statistically significant difference. MI = mobility-impaired

3.2.2 Ankle dorsiflexor muscle composition, CSA and volume

There was no difference in the total (chi-square= 1.7, p= 0.43) or contractile CSA (chi-square= 2.56, p= 0.28) of the muscles in the anterior compartment between young, old and mobility-impaired older adults. There was however a significantly larger amount of non-contractile tissue in both groups of older adults compared to young adults (chi-square= 9.35, p= 0.009). Older adults had 55% more non-contractile tissue (z= -2.64, p= 0.007), while mobility-impaired older adults had 58% more non-contractile tissue (z= -2.42, p= 0.01) in their dorsiflexor muscle groups compared to young adults. Similarly, there was no difference in total muscle volume between the three groups in the ankle dorsiflexors (chi-square=4.22, p= 0.12). There was a significant difference between groups in total contractile muscle volume (chi-square=8.43, p= 0.02). Post-hoc testing determined there was no difference in contractile muscle tissue between young and healthy old adults (z=-1.88, p= 0.06), while mobility-impaired older adults (162.2 ± 28.3 cm^3) had significantly reduced contractile muscle tissue volume compared to young participants (206.6 ± 35.9cm^3) (z= -2.62, p= 0.007). As well, both groups of older adults had a greater volume of non-contractile muscle tissue compared to young adults (chi-square=12.9, p= 0.002, z= -3.43, p<0.001, z= -2.34, p= 0.02 for healthy and mobility-impaired adults respectively) (Figure 2), but were not different to one another (z= -0.16, p= 0.86). When non-contractile tissue within the muscle was expressed as a percentage of total muscle volume a significant difference was detected between groups (chi-square= 18.25, p<0.001). Further analysis determined healthy old adults (10.1 ±1.6%, z= -3.89, p<0.001) and mobility-

impaired older adults (10.1 ±2.5%, z= -3.09, p=0.001) had twice as much non-contractile tissue volume as young adults (5%). Results for CSA and volume of the ankle dorsiflexor muscles are summarized in Table 2.

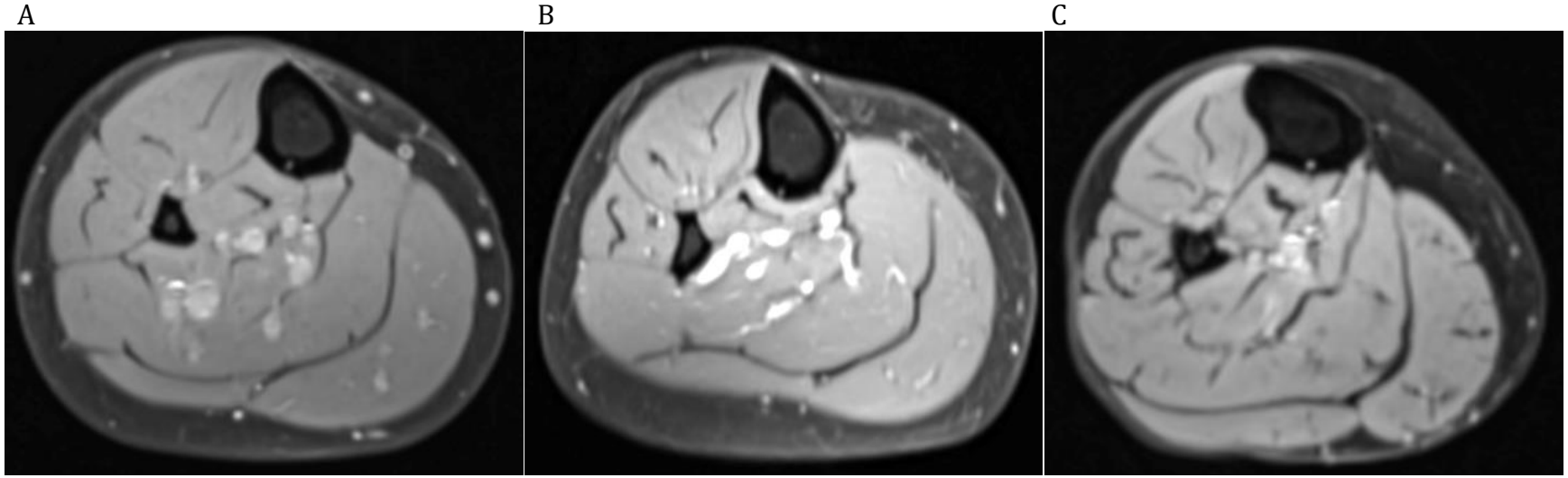


Figure 2. Representative sample of magnetic resonance images (MRI) of the ankle dorsiflexor muscles. A – young (25 years old), B – healthy old (72 years old, walking speed: 1.5 m/s), C – mobility-impaired (72 years old, walking speed: 0.96 m/s)

Table 2. Muscle CSA and Volume in the ankle dorsiflexors

	Young	Old	MI
CSA (cm^2)			
Total	11.0 (2.1)	10.56 (1.1)	9.5 (2.1)
Contractile	10.8 (2.0)	10.12 (1.3)	9.03 (2.1)
Non-contractile	0.2 (0.2)	0.44(0.23)*	0.47 (0.19)*
Volume (cm^3)			
Total	217.7 (38)	201.8 (24)	180.7 (33)
Contractile	206.6 (35.9)	181.6 (22.4)	162.2 (28.2)*
Non-contractile	11.1 (4.1)	20.2 (3.3)*	18.5 (6.0)*
% Non-contractile volume	5.0 (1.4)	10.1 (1.6)*	10.1 (2.5)*

Both groups of older adults had approximately twice as much non-contractile CSA as young adults ($p=0.007$, $p=0.01$ for healthy and mobility-impaired old respectively). Mobility-impaired older adults had significantly less contractile muscle volume compared to young adults ($p= 0.007$). Both healthy ($p<0.001$) and mobility-impaired older adults ($p=0.02$) had significantly more non-contractile muscle volume, and a two-fold increase in % non-contractile volume compared to young adults ($p<0.001$, $p=0.001$ respectively). * indicates significantly different from young. Values presented as means (standard deviation).

3.2.3 Knee extensor muscle composition, CSA and volume

A statistically significant difference was detected between groups in total (chi-square= 13.94, p= 0.001) and contractile (chi-square= 14.27, p=0.001) CSA. Post-hoc tests confirmed significant reductions in the old (z= -3.17,p= 0.001 z= -3.23, p= 0.001 for total and contractile area respectively) and mobility-impaired older adults (z= -3.0, p= 0.001 z= -3.0, p= 0.001 for total and contractile area respectively) compared to the young adults (Figure 3). There was no difference, however, in the amount of non-contractile CSA between young (2.1 ± 0.7 cm^2), old (2.5 ± 0.7 cm^2), and mobility-impaired older adults (2.6 ± 0.7 cm^2) (chi-square= 3.09, p= 0.21). A significant difference was detected for total knee extensor muscle volume (chi-square= 15.64, p<0.001). The total knee extensor muscle volume was reduced from 1564.6 ± 324cm^3 in young to 1110.9 ± 139 cm^3 in old (z= -3.56, p<0.001) and 1063.8 ± 242 cm^3 in mobility-impaired older adults (z= -2.9, p=0.002) (Table 3). Similarly, contractile muscle volume was found to be significantly lower (chi-square = 15.99, p<0.001) in both old (z= -3.63, p<0.001) and mobility-impaired (z= -2.9, p=0.002) adults compared to young adults. Although the amount of non-contractile muscle tissue did increase from 90.8 ± 17cm^3 in young to 103.6 ± 18 cm^3 in old and 107.6 ± 19 cm^3 in mobility-impaired old adults, this difference was not statistically significant (chi-square= 3.96, p= 0.14). However, when this volume was expressed as a proportion of total muscle volume, a significant difference between groups was detected (chi-square= 18.96, p<0.001). Both healthy old (9.4 ±1.8%) and mobility-impaired old adults (10.3 ± 0.8%) had a significantly higher percentage of non-contractile muscle volume than young adults (5.9 ±1.3%) (z= -3.56, p<0.001, z=

-2.9, p=0.002 for healthy and mobility-impaired older adults respectively). Finally, a significant difference was identified in subcutaneous adipose tissue area in the thigh (chi-square= 8.04, p= 0.02), which was significantly larger in both groups of older adults compared to young adults (z= -2.18, p= 0.03, z= -2.53, p= 0.01 for healthy and mobility-impaired older adults respectively) (Table 3).

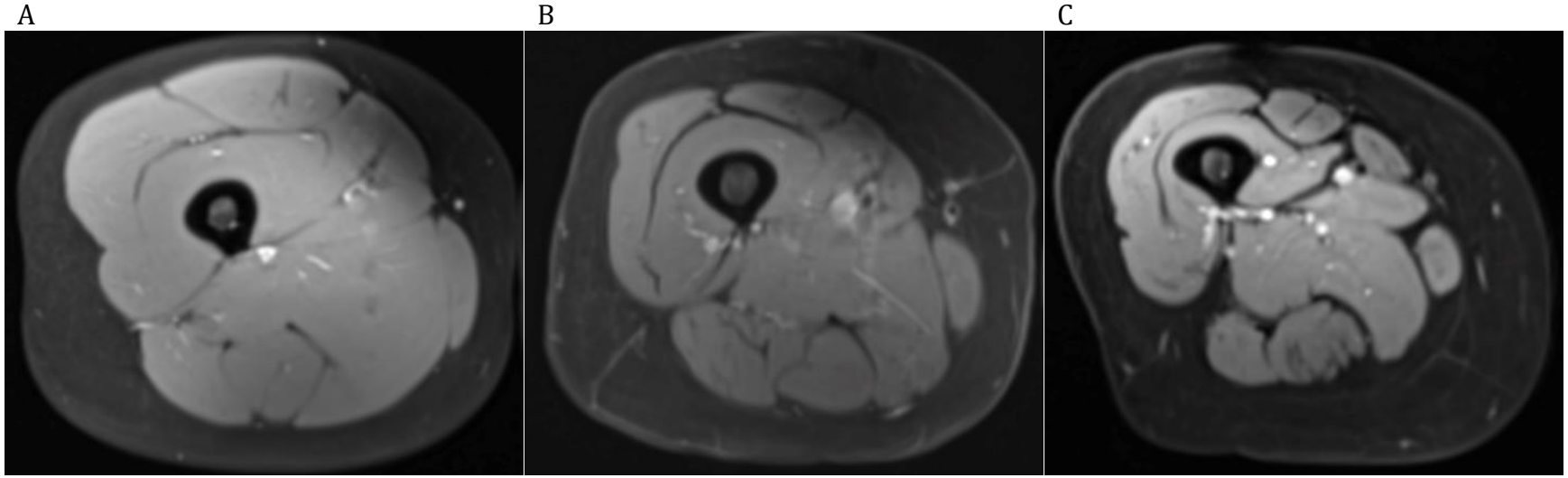


Figure 3. Representative sample of magnetic resonance images (MRI) of the mid-thigh. A – young (22 years old), B – healthy old (82 years old, walking speed: 1.3 m/s), C – mobility-impaired (72 years old, walking speed: 0.69 m/s).

Table 3. Muscle CSA and Volume in the knee extensor muscles

	Young	Old	MI
CSA (cm^2)			
Total	64.9 (13.4)	45.9 (6.9)*	43.7 (10)*
Contractile	62.8 (13.4)	43.4 (6.8)*	41.1 (9.4)*
Non-contractile	2.1 (0.7)	2.5 (0.7)	2.6 (0.7)
Volume (cm^3)			
Total	1564.6 (324)	1110.9(139)*	1063.8 (242)*
Contractile	1473.8 (315)	1007.3(135)*	956.0 (224)*
Non-contractile	90.8 (17)	103.6 (18)	107.8 (19)
% Non-contractile volume	5.9 (1.2)	9.4 (1.8)*	10.3 (0.8)*
Subcutaneous adipose tissue (cm^2)	114.2 (50)	141.2 (32)*	162.0 (52)*

Total CSA was reduced in healthy and mobility impaired older adults (p=0.001, p=0.001 respectively). Contractile CSA was significantly reduced in both older adult groups compared to young (p= 0.001, p=0.001 respectively). Young adults had greater knee extensor volume than healthy (p<0.001) and mobility impaired older adutls (p=0.002). Although absolute non-contractile volume did not differ between groups (p= 0.14), when represented as a percentage of total muscle volume, both healthy (p<0.001) and mobility-impaired older adults (p=0.002) had a significantly greater proportion of non-contractile tissue than young adults. Both older adult groups also had a greater amount of subcutaneous adipose tissue compared to young adults (p= 0.03, p= 0.01 respectively). * indicates significantly different from young. Values presented as means (standard deviation).

3.2.4 Magnetization transfer ratio in the ankle dorsiflexors

MTR represents the change in contrast of images with off-resonance RF pulse from those without (Figure 4) and is expressed as a ratio change from baseline per unit (p.u.). There was a significant difference in MTR (chi-square= 17.39, $p<0.001$), which was larger in young adults (0.43 p.u.) compared to healthy old (0.38 p.u., $z= -3.96$, $p<0.001$) and mobility-impaired older adults (0.41 p.u., $z= -1.78$, $p=0.04$). Further, MTR in healthy older adults was also significantly reduced compared to mobility-impaired adults ($z= -2.28$, $p=0.02$) (Figure 5).

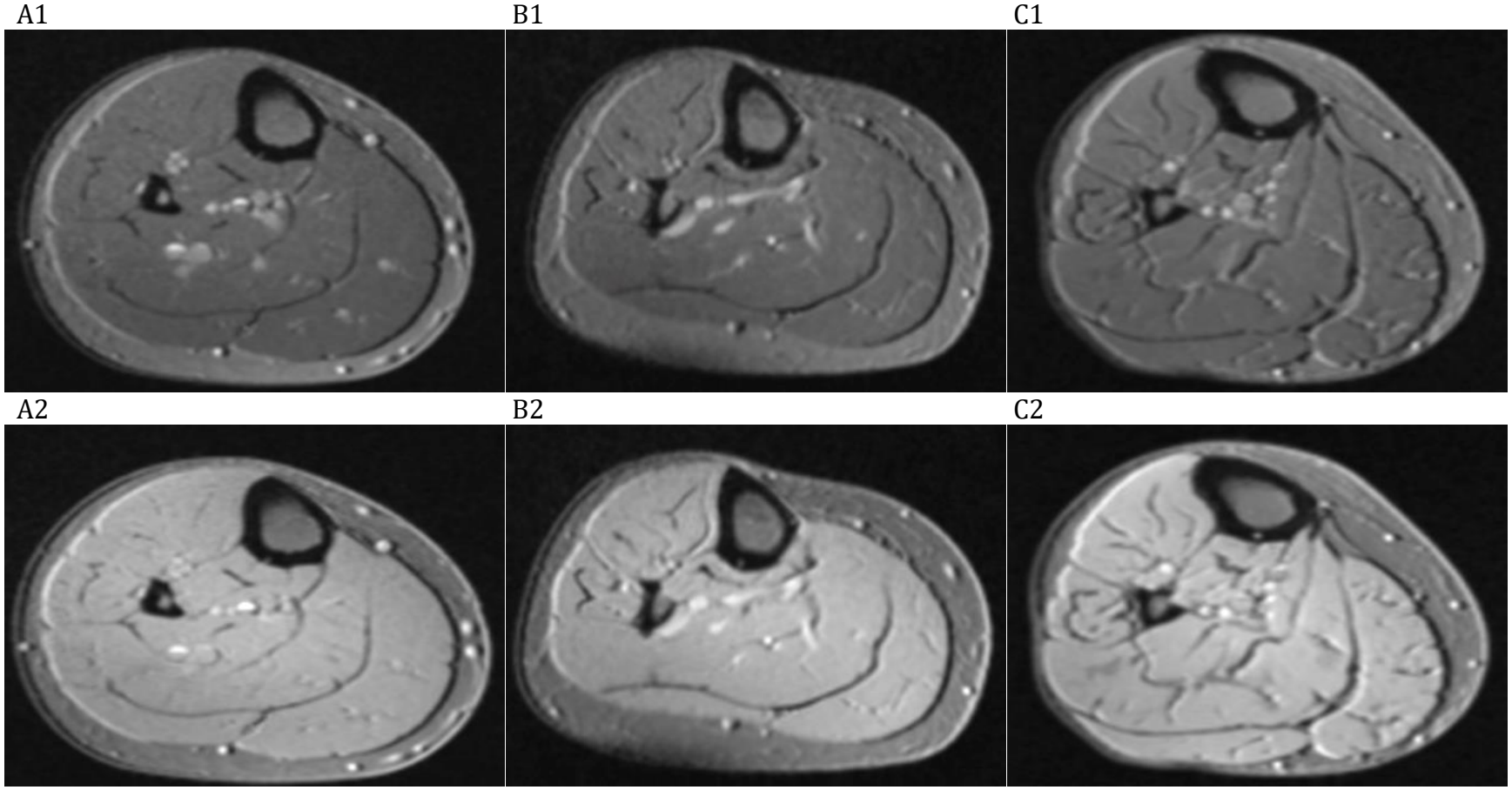
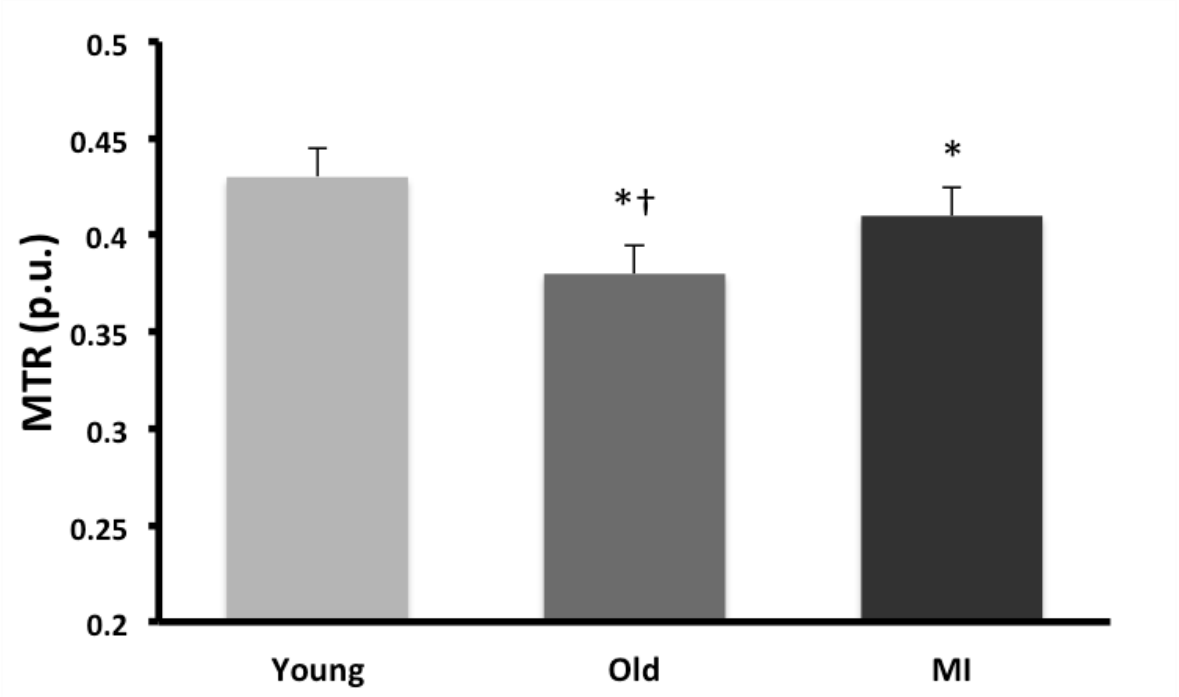


Figure 4. Sample of magnetic resonance images (MRI) of the lower leg with off-resonance radio frequency pulse (A1, B1, C1) and without (A2, B2, C2). Images were taken in the same individual at the same location in the muscle. Images with off-resonance pre-saturation (A1, B1, C1) appear darker as the radio frequency pulse excites protein molecules in the muscle. MTR is calculated based on the difference in signal contrast between these two images. A – young (25 years old, MTR: 0.43), B – healthy old (72 years old, walking speed: 1.5 m/s, MTR: 0.35), C – mobility-impaired (72 years old, walking speed: 0.96 m/s, MTR: 0.42)

Figure 5. MTR in young, healthy old and mobility-impaired older adults



MTR represented in percentage units of young (light grey) healthy old (dark grey) and mobility-impaired older adults (black). Both groups of older adults had significantly lower MTR compared to young adults ($p<0.001$, $p=0.04$ for healthy and mobility impaired old respectively). Healthy old adults were also significantly reduced compared to mobility-impaired older adults ($p=0.02$). * indicates significant difference from young adults. † Indicates significant difference from mobility-impaired old adults. Values presented as mean ± standard deviation

3.3 Discussion

The objective of this study was to determine the total muscle mass, as well as contractile and non-contractile components of the ankle dorsiflexors and knee extensors in young, healthy older and mobility-impaired older adults. Additionally, we explored the quality and quantity of muscle tissue in the ankle dorsiflexor muscles using MT. Previous research has examined differences in muscle mass [3] and MTR in healthy old [20, 21] and very old adults [21]; however, to our knowledge no study has investigated contractile and non-contractile muscle mass as well as MT in a group of older adults with mobility impairment compared to healthy similarly aged older adults. This study provides evidence that older adults have less contractile muscle mass and more non-contractile tissue within their muscles, and that these changes may be accentuated in older adults with impaired mobility.

In the present study we found that contractile sectional muscle area in the ankle dorsiflexors was not significantly different between groups. Yet, contractile volume was significantly reduced in mobility-impaired older adults, but not in healthy older adults, when compared to the young adults. As well, we observed a two-fold increase in the amount of non-contractile tissue within the muscles of both groups of older adults. While the actual composition of this non-contractile tissue could not be directly measured in the present study, previous research using muscle biopsies suggest that is it most likely comprised of fat and connective tissue [28]. Our results are in accordance with previously published studies of the ankle dorsiflexors. Kent-Braun et al. [3] used MRI to determine the proportion of contractile and non-contractile muscle area of the anterior compartment in young

(26-44 years) and old (65-83 years) men and women. Similar to the present study, the authors found that there was no difference in the total (contractile + non-contractile) area of the anterior compartment, but older adults had significantly reduced contractile area compared to young adults. As well, the authors reported ~14% of the total muscle area was non-contractile, which was over twice as much as the ~6% recorded in young adults. These data are remarkably similar to the ~5% and ~10% of non-contractile tissue volume reported in the current study for young and older adults respectively. Similarly, Moore et al [22] investigated the effects of diabetic polyneuropathy on muscle mass in the TA muscle. They reported non-contractile muscle volume to be 15% of the total TA muscle volume in a healthy control population (mean age: 59 years). Finally, Power et al [21] examined the influences of aging on muscle quality in young (22-30 years), old (60-73 years), and very old (76-85 years) males. They reported the CSA of the ankle dorsiflexors to be 15.2 cm^2 in young, 14.7 cm^2 in old, and 14.8 cm^2 in very old adults, which is slightly higher than the 11.0 cm^2, 10.6 cm^2 and 9.5 cm^2 reported here for young, old and mobility-impaired adults respectively. Similarly, the values reported for total contractile volume in that study (235.2 cm^3, 194.4 cm^3, and 191.5 cm^3 for young, old and very old) was larger than those reported in the current study (Table 2.). However, Power et al. [21] studied exclusively older males, and it has consistently been demonstrated that males have more muscle mass than females [2, 3, 7, 10, 29, 30]. While the absolute values reported for non-contractile area in that study are larger than those reported in this study, a similar trend was observed whereby old (3.0 cm^2) and older (3.6 cm^2) adults had nearly twice as much non-contractile tissue

as young adults (1.8 cm^2). Thus, based on these results it would appear that the loss of muscle mass and accumulation of intramuscular fats with age occurs at a similar relative proportion in the anterior compartment of males and females.

Numerous previous studies have examined the contractile area and volume of the knee extensor muscles. An early investigation by Trappe and colleagues [31] used MRI to investigate the age-related atrophy of muscle mass in the four quadriceps muscles in males and females. When separated based on sex, the authors reported a mean CSA of 53.4 cm^2 in young females, and 45.2 cm^2 in old females (mean age: 77 ± 6 years). Similarly, Melnyk et al. [32] reported a contractile area of 57.9 cm^2 for young females and 45.2 cm^2 for healthy older females. A longitudinal study by Delmonico et al. [33] examined muscle strength and quality in adults between 70 and 79 years old and reported an average quadriceps muscle area of 42.8 cm^2 in a sample of 865 older females. In a group of females aged 74 ± 5 years, Maden-Wilkinson et al. [30] reported a knee extensor contractile muscle volume of 993 cm^3, which was significantly less than the 1368cm^3 observed in young females. These values are similar to those presented here (Table 3) for young, old and mobility-impaired older females. A previous review of the literature for the knee extensors shows an ~29% decline in muscle mass with age [34]. Indeed, when considering total muscle mass in the current study, we observed a 29% and 32% decline in muscle mass for the healthy old and mobility-impaired older adults respectively. Thus, results from the present study are in agreement with previously published data.

Several studies have also assessed the contractile and non-contractile components of the knee extensor muscles. Hogrel et al. [7] calculated total muscle volume and intramuscular fat content of young (20-30 years) and older adults (70-80 years). The authors found young females to have a total quadriceps muscle volume of 1294 cm^3 compared to 949cm^3 in older adults. Moreover, the authors reported intramuscular fat content to be 2.8% in young adults and 4.5% in the old. This 1.5x increase in intramuscular fat content in older adults is similar to what was observed in the current study. Further, another study by Chambers et al. [34] reported an increase in IMAT percentage from 4.7% in young females to 12.3% in older females, which is comparable to the 5.9% in young, 9.4% in old and 10.3% in mobility-impaired older females reported in this study.

Recently, the effects of high levels of physical activity on skeletal muscle mass and function were investigated by comparing lifelong exercisers with a group of sedentary, but healthy, older adults [34]. The authors reported that lifelong exercise reduced the accumulation of IMAT by 50% in males. Since it is known from previous reports that greater amounts of IMAT leads to decline in lower extremity function performance [9], maintaining a healthy active lifestyle may prove critical to the prevention of mobility impairment with age. Furthermore, in 2012 Buford et al. [6] examined lower extremity tissue components in older adults categorized by physical performance. They separated older adults into either high functioning or low functioning based on their score on the Short Physical Performance Battery (SPPB). The high functioning older adults had a mean age of 76 ± 5 years of age and a gait speed of 1.10 m/s, whereas the low functioning adults were 81 ± 4 years of

age with a gait speed of 0.67 m/s. Similar to the present study, the authors found a slightly higher, although not statistically different, percentage of non-contractile muscle tissue in low functioning older adults compared to high functioning, and an approximately two-fold increase in both older groups compared to young. In the present study, although we observed a trend towards lower contractile muscle mass in the knee extensors of mobility-impaired older adults (956.0 ± 224 cm^3) compared to healthy old adults (1007.3 ± 135 cm^3), this difference was not statistically significant. Buford et al. [6] on the other hand, recorded a significant decline in contractile muscle mass in low functioning compared to high functioning older adults. There were some key differences between these two studies, which may explain the conflicting results. First, the study by Buford et al. [6] included both males and females in their sample, where the current study consisted of only females. It has been documented that age-related muscle atrophy and intramuscular fat infiltration may affect males and females differently [2, 7, 34]. Additionally, in the current study, we controlled for age between healthy old and mobility-impaired older adults, whereas in their study the low functioning older adults were on average 5 years older than the high functioning adults. A previous longitudinal study by Frontera et al [35] found a 16% decrease in knee extensor area over a 12-year period, indicating an approximate 1.3% loss of knee extensor area per year. That would imply a difference in muscle mass of 6.5% over a 5-year period. Finally, the gait speed of the individuals in the low functioning group (0.67 m/s) was significantly slower than the gait speed reported for mobility-impaired participants in this study (0.87 m/s). Thus, it is possible that individuals in the Buford et al. [6]

study were more impaired than our mobility-impaired group and consequently had greater loss of muscle mass. Together, these studies emphasize the role that mobility plays in the quantification and classification of sarcopenia with age.

Although there is an abundance of research to support the role of muscle atrophy and accumulation of IMAT in the development of mobility impairment with age, it is becoming increasingly evident that SAT also significantly contributes to this process [36]. We reported a significant increase in SAT in both groups of older adults, with a tendency towards even greater SAT in mobility-impaired older adults (Table 3). This increase in SAT was apparent despite no difference in height or weight between experimental groups. Some previous research has shown no difference in SAT area in older adults compared to young controls [6, 33]. In a longitudinal study of muscle quality Delmonico et al. [33] found that SAT increased only in participants who gained weight, and that individuals who lost weight also lost SAT area. However, despite the common misconception that adipose tissue is simply a vessel for the storage of fat, it is actually a highly active tissue responsible for the secretion of many endocrine molecules [36]. Recent work by O'Leary and colleagues [37] implicates greater amounts of SAT with an augmented secretion of resistin. In turn, resistin inhibits myogenesis while also promoting intramuscular lipid accumulation in older individuals. Adipose tissue has also been associated with the release of pro-inflammatory cytokines [36], which may amplify the oxidative stress and chronic inflammation experienced by motor axons leading to axonal death and concomitant muscle fiber atrophy and loss. Additionally, it has been shown that higher levels of total body fat predicts future incidence of mobility

disability, even in currently high functioning individuals [38]. Thus, it is conceivable that the significant increase in thigh SAT area observed in the present study may have contributed to hormonal dysregulation in older adults and resultant mobility impairment. However, further research into the effects of SAT on mobility in older adults is needed.

MTR has been increasingly utilized in studies of skeletal muscle to assess the quality and quantity of muscle protein. Using individuals with limb girdle muscular dystrophy as a model, McDaniel et al. [18] were able to demonstrate that MT can quantify pathological myogenic changes within skeletal muscle. Additionally, a more recent investigation by Sinclair and colleagues [39] examined MTR in participants with chronic demyelinating polyneuropathy and Charcot-Marie-Tooth disease. The authors found that MTRs were reduced in both these groups of patients with peripheral neuropathy compared to healthy control subjects. Finally, Moore et al. [22] examined MT in the TA muscle of participants with diabetic polyneuropathy compared to healthy age-matched controls and reported significant reductions in MTR in participants with diabetic polyneuropathy. Together, these studies highlight the efficacy of MT imaging to reflect myogenic and neurogenic changes within skeletal muscle.

Previous studies have investigated changes in MT in the TA in older adults. In a group of a 55-79 year old men and women, Schwenzer et al. [20] observed a non-significant trend towards reduced MTR in old adults (0.44 p.u.) compared to young adults (0.47 p.u.) in the TA muscle. Power and colleagues [21] used MT to examine muscle quantity and quality in the TA of young, old (60-73 years) and very old (76-

85 years) men. The authors found no difference in MTR between young and old adults; however, they reported that MTR in very old men was significantly reduced compared to both young and old participants. They concluded that the quantity of muscle protein may be preserved in the old population due to the compensatory process of reinnervation in the muscle, which is eventually outpaced in the very old population resulting in a reduction in MTR. The ~8% decrease in MTR in very old adults compared to young reported in that study is similar to the 12% and 5% reduction reported for healthy old and mobility-impaired older adults respectively in the current study.

Interestingly, in the current study, although both groups of older females had significantly lower MTR compared to young adults, our healthy older adult's MTR were also significantly lower than mobility-impaired older adults. This is contrary to our hypothesis, stating that mobility-impaired adults would likely have reduced muscle protein quality and quantity compared to healthy older adults. One possible explanation for this observation is that the quality of the muscle fibers in the healthy older adults may be reduced resulting in a lower MTR. As reported previously, the total contractile volume of the ankle dorsiflexor muscles was significantly reduced in the mobility-impaired older adults, but not in the healthy older adults, indicating that the resultant MTR was unlikely due to a decrease in muscle quantity per se. The reduced contractile volume observed in mobility-impaired older adults indicates that these individuals had likely already experienced significant denervation and muscle fiber atrophy associated with sarcopenia. The healthy older adults, on the other hand, may be actively experiencing cycles of denervation and reinnervation

resulting in a relative preservation of contractile muscle tissue, but a decrease in the quality of that tissue. This theory is corroborated by results from an unpublished study from our group denoting an increase in NF jiggle in healthy older adults, but not mobility-impaired older adults, compared to young. As motor neurons are lost and surviving motor axons work to recapture orphaned muscle fibers, the characteristics of the muscle fibers are changed and the stability of the NMJ is affected. It is possible that this may manifest as a reduction in muscle quality on MT images, however further research investigating MTRs in sarcopenic skeletal muscle is warranted.

Although there are many strengths to this study, it is not without some limitations that should be considered when interpreting the results. Namely, there is a relatively small sample size, especially in our mobility-impaired older adult group. However, it should be noted that previous studies have been able to yield significant results with a similar sample size [21, 22]. Still, the smaller sample size may impact the ability for this study to find differences between groups, leading to a potential type II error. Thus, these results of age and mobility on muscle tissue composition should be considered a conservatively.

In conclusion, this study aimed to identify the contractile and non-contractile components of ankle dorsiflexor and knee extensor muscle groups in young, healthy old and mobility-impaired older adults. Further, we endeavored to investigate the quality and quantity of muscle protein in the TA using the novel MT technique. The results of this study show that total contractile muscle mass is lost with age, which is accompanied by an increase in non-contractile tissue within the muscle. Moreover,

we provide evidence that changes within skeletal muscle may be more prominent in adults with mobility impairment. Future research into the ability of MT to identify muscle protein quality at different stages of mobility impairment and sarcopenia is required.

3.4 References

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Chapter 4

Examining specific strength and power in the lower extremity of healthy and mobility-impaired older adults: the influence of contraction velocity

4.0 Introduction

The loss of muscle mass and subsequent strength deficits associated with aging has been well defined [1, 2]. However, more recently it has been identified that, with aging, the loss of strength and function exceeds the amount of reduced muscle mass[2-4]. This has led some to suggest that aging results in changes to the neural and contractile properties of muscle fibers, leading to a decrease in the amount of force produced per unit of muscle mass [2-5]. Clark and Manini [3] have since coined the term dynapenia to refer to the loss of strength and power associated with age, to better encompass the complexity of changes that occur within muscle during senescence.

Power is the product of torque and velocity, and the reduction of muscular power with age has gained increased interest from researchers over the past two decades [4, 6-15]. Although strength is known to decrease with advanced age, it is often well maintained until beyond 70 years of age [1, 2, 6, 16, 17]. Contractile velocity during voluntary muscle contraction, on the other hand, has been shown to decrease much earlier in life than strength [6, 9, 18, 19]. This reduced contraction velocity has been linked to a preponderance of type I muscle fibers in aged muscle as muscle biopsy studies have shown a decrease in type II fiber distribution in older muscle [1, 20-22]. As well, previous works suggests that oxidative stress and

chronic inflammation can damage motor neurons [5, 23], and that type II motor neurons may be more susceptible to this process than type I motor neurons [24, 25]. An increase in the ratio of type I:II fiber area in the muscle results in a slowing of whole muscle contractile velocity. Since power consists of the product of torque and velocity of contraction, age-related differences in one or both of these measures can have a cumulative effect on power production. Therefore, power may be a more sensitive measure than the individual influence of either of its constituents.

In an experimental set up, power can be measured in one of two ways; either the velocity is held constant and the torque produced is measured (isokinetic), or the torque is held constant while velocity is measured (isotonic). Early work measuring muscular power tended to focus on isokinetic power production; however, it has been suggested that isotonic power may be more functionally relevant to how we move, as activities of daily living (ADLs) require the movement of a constant load (i.e. body mass) at varying velocities throughout differing ranges of motion (ROM) to accomplish various tasks (e.g. sit-to-stand, gait, stair climbing etc.)[6, 7]. This has led to an increase in the number of studies investigating velocity-dependent (isotonic) muscular power [6, 7, 13, 26-28]. Many studies have shown that velocity-dependent muscular power declines with age [6, 13, 26, 27], and that deficits in power appear before, or to a greater extent, than the loss of isometric strength [4, 6-8, 11]. Skelton et al. [11] have reported that leg extensor power is lost at twice the rate of isometric strength in older adults. Additionally, reductions in lower extremity muscle power have been associated with an increased incidence of mobility impairment [18, 29, 30] and increased fall risk in older adults

[31]. This has led some to suggest that exercise training programs for older adults should aim to increase velocity-dependent torque production, rather than traditional strength training [4, 14] (for a review of velocity-dependent power training see McKinnon et al. [4]).

In an attempt to control for the loss of muscle mass with age, researchers have increasingly used muscle cross-sectional area (CSA) or volume to normalize strength measurement. This normalized value, known as specific strength (or muscle quality), represents the strength per unit of muscle mass and conveys a more robust quantification of strength loss with age, taking into account the intrinsic force generation capacity of the muscle. Although not all studies agree [32-34], it has been reported that specific strength declines with age [6, 10, 35-37]. While less extensively studied, specific power has consistently been shown to decrease with age [6, 12, 15, 38-41]. A previous examination of specific strength and power production in the ankle dorsiflexor muscles shows that while specific strength was maintained in adults ~65 years of age, specific power was significantly reduced [6]. As well, a previous investigation out of our lab indicates that peak isotonic power production of the ankle dorsiflexors and knee extensors is reduced in older adults compared to young [7], although we did not control for muscle volume in that study. Indeed, it has been consistently shown that contraction velocity [6, 9] and power [7, 10-13, 15, 41] are reduced in the lower extremity with age. These studies highlight the emerging concept that muscular power is a more sensitive and possibly meaningful measure of the changes that occur in the neuromuscular system with age and further validates the use of power rather than

isometric strength for assessment of sarcopenia and its impact on function in older men and women.

Thus, the purpose of this study was to determine contraction velocity, specific strength and power in the ankle dorsiflexor and knee extensor muscles in a group of young, healthy old and mobility-impaired older adults to determine if adults with impaired mobility would exhibit reduced muscle contractile velocity and power production. Previous research suggests that reduced muscle power and contraction velocity is associated with impaired mobility in older adults [18, 29, 30, 42] putting them at increased risk for falls, as they are less able to produce force rapidly to counteract postural perturbations [4, 31]. Therefore, we hypothesized that both groups of older adults would display reductions in muscle contraction velocity compared to healthy older adults, with greater deficits recorded in mobility-impaired older adults. Moreover, we anticipated that mobility-impaired older adults would have a further reduction in specific power production.

4.1 Methods

4.1.1 Participants

The study sample consisted of females divided into three groups: 1) young 2) healthy older 3) mobility-impaired older. Females 65 years or older were eligible for one of the two older adult experimental groups. After consenting to participation in the study, all older adults took part in a walking test using the GAITRite system (CIR System Inc., Franklin, NJ). Participants with a gait speed of greater than 1 m/s were classified as healthy older adults, while those with a gait speed less than 1 m/s were categorized as mobility-impaired [43, 44]. Young participants were recruited from

the Western University student population and were recreationally active. All participants signed written informed consent before participating in the study, approved by the Western University Research Ethics Board and in accordance with the Declaration of Helsinki. All participants were free from any known neuromuscular or musculoskeletal disorders that would significantly impact their performance. Participants with diabetes, a recent fracture or injury, or implanted metal device were excluded from participating in this study.

4.1.2 Repeated chair stands

Participants were asked to perform a repeated chair stand task as a measure of functional performance. The repeated chair stands task measures the participant's ability to repetitively and rapidly move their body weight from a seated to standing position and is used as part of the Short Physical Performance Battery (SPPB). Starting from a seated position with hips and knees bent to 90° and feet firmly on the floor, participants were asked to quickly, but safely, stand and sit back down a total of 5 times. The experimenter counted the participants down and started a stop watch as soon as they began their first stand and stopped the timer once they returned to a seated position after 5 total stands.

4.1.3 Voluntary activation

The interpolated twitch technique (ITT) was used to measure voluntary activation in the ankle dorsiflexors in this study. The ITT involves delivering a supramaximal electrical stimulus to the motor nerve supplying the anterior compartment during a maximal voluntary contraction (MVC) and is used to determine the participant's percentage of voluntary activation of the motor neuron

pool [16, 45]. Participants were positioned in a custom-built ankle dynamometer with their ankle fastened to a footplate at an angle of 30° plantar flexion. Adjustable cuffs were positioned over the anterior tibia and superior surface of the knee across the distal thigh to restrict excessive lower extremity movement during testing. The footplate was attached to a load cell (LCDA-100, Omega Engineering Inc., Connecticut, USA) and amplified (1902, Cambridge Electronic Design, Cambridge, UK) for processing in Spike 2 (Version 6, Cambridge Electronic Design, Cambridge, UK). A bipolar stimulator was positioned over the common fibular nerve and a series of evoked twitch responses were recorded. The experimenter increased the intensity of the stimulus until a plateau in the twitch response was reached at which point the stimulus was increased by an additional 15% to ensure a supramaximal stimulation. Participants were then instructed to perform a maximal dorsiflexion contraction during which a supramaximal stimulus was delivered during the plateau of the MVC (interpolated twitch) and approximately one second after completion of the MVC (resting twitch). This process was repeated twice and percentage of voluntary activation was then calculated using Equation 1. The higher value of the two trials was considered that participant's percentage of voluntary activation.

Equation 1:

Voluntary activation (%) = [1 – (interpolated twitch/resting twitch)] x 100

4.1.4 Strength data collection

Strength was measured in the participant's dominant limb using the Biodex System 3 Dynamometer (Biodex Medical Systems, Shirley, NY) by performing an isometric MVC. For the ankle dorsiflexors, participants were seated in the Biodex

with their right ankle fixed to the dynamometer footplate via Velcro straps. The ankle was positioned in 30° of plantar flexion while the knee and hip were both kept at 90° angles. The participants were securely fastened in the Biodex with seatbelts across the hips and both shoulders to help mitigate any excess movement during contractions. Additionally, to help isolate torque production to the muscle under study, participants were instructed to cross their arms over their chest during testing to prevent additional force generation from muscles farther up the kinetic chain. Before testing began, participants performed some light dynamic contractions to warm up and to become familiarized with the task. Once adequately warmed up, participants were instructed to perform a maximal isometric dorsiflexion contraction. Participants were asked to perform a dorsiflexion contraction by "pulling your foot/ankle up" or "bringing your toes towards your nose" and holding that contraction for four seconds. During the contraction the participants received verbal encouragement from the experimenter as well as visual feedback of their force production on a computer screen. The torque produced was recorded (in newton meters (Nm)) and this process was repeated a total of three times, with the largest force achieved being considered the participant's MVC. Each contraction was separated by at least one minute of rest to reduce the effects of fatigue. If a participant displayed large inconsistencies between consecutive MVC attempts, additional contractions were performed, separated by one minute of rest, until the difference between attempts was less than 5%. The same protocol was followed to determine maximal knee extensor strength using a different attachment to the dynamometer which strapped around the lower leg approximately 2cm

superior to the lateral malleolus. The knee and hip joints were again positioned at 90° angles and participants were instructed to “kick out” or “straighten your knee” with as much torque as possible.

4.1.5 Power data collection

Peak muscle power was calculated using the Biodex System 3 Dynamometer (Biodex Medical Systems, Shirley, NY). Participants were seated in the dynamometer as described for strength testing. As power is the product of torque and velocity, the dynamometer was set to isotonic mode and the experimenter input mechanical resistance equal to 1 Nm and then 10, 20, 30, 40 and 50% of the participant’s MVC recorded during strength testing. 1 Nm was chosen because it is the minimum load possible when selecting isotonic resistance on the Biodex. Participants were instructed to perform two rapid dynamic contractions for each load. Instructions were given to contract as quickly as possible concentrically and then passively return to the starting position before again contracting as quickly as possible against the resistance. This process was repeated for each of the six submaximal MVC loads, and the higher of the two contraction attempts was used as the peak power produced at that load. To help reduce any potential practice effects, the order in which the participants performed each contraction was randomly selected before commencement of the study. The experimenter provided verbal encouragement during power testing, as well as visual feedback on a computer screen. Previous research has confirmed this procedure to be a reliable measure for determining isotonic velocity-dependent power [46, 47].

4.1.6 Strength & power analysis

During both strength and power testing torque and velocity data from the Biodex were amplified (Power1401-3A, Cambridge Electronic Design, Cambridge UK) and collected by Spike 2 (Version 6, Cambridge Electronic Design, Cambridge, UK). Torque and velocity of muscle contractions were displayed in real-time on a computer monitor. Muscular strength (MVC) was calculated during the data collection process, as it was required to determine submaximal loads for power testing. Maximal strength was determined by placing a marker over the plateau of the participant's isometric MVC. The highest value achieved by the participant was recorded as their MVC (provided it did not differ by more than 5% of subsequent MVCs as described above). During offline analysis, the experimenter used markers to determine the peak velocity achieved for each submaximal isotonic contraction. The values were inputted into Microsoft Excel and multiplied by the respective isotonic resistance to determine muscular power. The highest power value achieved across the six isotonic loads was considered the participant's peak power. Specific strength and specific power were determined by dividing the participants MVC and peak power by the volume of contractile muscle mass as determined by magnetic resonance imaging (MRI)

4.1.7 MRI and analysis

Using a Dixon technique with fat suppression, a 3T MRI scanner (Magnetom Verio, Siemens Healthcare, Erlangen, Germany) acquired images of the ankle dorsiflexor and knee extensor muscles. Positioned supine on the MRI table, participants were moved into the bore of the magnet feet first. For the ankle dorsiflexors, a radio frequency (RF) coil was positioned over the lower leg from the

tibial plateau to the malleoli. The entire length of the anterior compartment was imaged with a repetition time (TR) of 3000ms, echo time (TE) of 11ms and flip angle of 180° and a slice thickness of 5mm with no slice separation. The same protocol was followed for the knee extensor muscles except two RF coils were used to span the entire length of the thigh from the anterior superior iliac spine to the joint line of the knee.

OsiriX DICOM viewer (Version 11.0.2, Prixmeo SARL, Geneva, Switzerland) was used for all MRI analysis. The semi-automated process for the calculation of muscle volume was described in detail in Chapter 3. Briefly, a region of interest (ROI) containing only the muscles under study was manually outlined on every third image beginning distally and working proximally. Computer software was then used to create ROIs on the rest of the images based on the manually outlined areas. These ROIs were then used to render a three-dimensional (3-D) model of the muscle and the "total" muscle volume. Using the brush tool, the experimenter then subtracted any fat, connective tissue, neurovascular tissue or septal spaces from each image and calculated the volume as the muscle "contractile" volume. The same experimenter analyzed all the images for this study. For the knee extensors, there was some overlap of images due to the use of two RF coils. For these images, the experimenter viewed the area of overlap side-by-side in a viewing window and eliminated the duplicates with poorer signal quality. Participant's contractile muscle volume (Table 1) was used to calculate specific strength and power.

4.1.8 Statistics

Differences between groups were determined using a Kruskal Wallis H test. Post-hoc testing consisted of individual Mann-Whitney U tests with a modified Bonferonni correction to account for inflated alpha error. A two-way repeated measures analysis of variance (ANOVA) was used to investigate potential differences in power production at different loads. Experimental group (young, healthy old, mobility-impaired old) was set as between-groups factors, while submaximal isotonic load (1Nm, 10, 20, 30, 40, 50% MVC) was considered the within-group variable. A similar analysis was conducted for contraction velocity with factors of experimental group (between groups variable) and isotonic load (within-group variable). If a significant difference was detected, post hoc analysis consisted of Tukey's test, with a Bonferroni correction for multiple comparisons. All values are presented as means ± standard deviation. Statistical Package for the Social Sciences (Version 21; IBM SPSS Inc., Chicago, IL) was used for all statistical testing with a significance level of $p \leq 0.05$.

4.2 Results

4.2.1 Participants

A total of 30 females took part in this study. There was no difference in height (chi-square= 3.38, p=0.185) or weight (chi-square= 6.19, p=0.056) between young (n=12, mean age: 25 ± 2 years), healthy old (n=11, mean age: 74 ± 6 years) or mobility-impaired older adults (n=7, mean age: 72 ± 5 years). Healthy older females had a significantly faster gait speed (1.26 m/s) than mobility-impaired adults (0.89 m/s) (z = -3.49, p<0.001). A significant difference in contractile muscle mass was detected between groups (chi-square= 8.43, p= 0.015, chi-square= 16.0, p<0.001 for

ankle dorsiflexors and knee extensors respectively). Mobility-impaired older adults had significantly less ankle dorsiflexor contractile muscle volume compared to young adults ($z= -2.62$, $p=0.007$). Both groups of older adults had significantly less knee extensor muscle volume compared to young adults ($z= -3.63$, $p<0.001$, $z= -2.9$, $p= 0.002$ for healthy and mobility-impaired older adults respectively). Participant demographics are presented in Table 1

Table 1. Participant Demographics

	Young	Old	MI
Sample size	12	11	7
Age (years)	25 (2)	74 (6)*	72 (5)*
Height (cm)	165 (5)	164 (5)	161 (4)
Weight (kg)	63.4 (13)	71.5 (8)	79.5 (21)
Gait Speed (m/s)	-	1.26 (0.17)	0.89 (0.10)*
Repeated Chair Stands (seconds)	6.8 (0.9)	10.7 (2.3)*	13.9 (4.6)*†
AD contractile muscle volume (cm^3)	206.6 (35.9)	181.6 (22.4)	162.2 (28.2)*
KE contractile muscle volume (cm^3)	1473.8 (315)	1007.3 (135)*	956.0 (224)*

AD: ankle dorsiflexor, KE: knee extensor. *indicates significantly different from young. † indicates significantly different from healthy old.

4.2.2 Repeated chair stands

All participants were able to complete all 5 sit-to-stands, but there was a statistically significant difference between time taken to complete the task (chi-square= 22.1, p=0.02). Young females completed the repeated stands task significantly quicker than healthy (z= -4.05, p<0.001) and mobility-impaired older females (z= -3.47, p=0.001). Further, mobility-impaired older females were also significantly slower at completing the 5 sit-to-stands than healthy older adults (z= -2.16, p=0.031) (Table 1).

4.2.3 Voluntary activation

There was no difference in percentage of voluntary activation as recorded by the ITT (chi-square= 1.33, p=0.513). All participants were able to achieve greater than 99% activation in the ankle dorsiflexor muscles.

4.2.4 Isometric strength

Kruskal Wallis testing indicated there was a significant difference in strength between groups (chi-square= 6.57, p=0.037). Post hoc analysis determined there was no difference in isometric strength between young and healthy old participants in the ankle dorsiflexor muscles (z= -1.85, p=0.069); however, mobility-impaired older adults were significantly weaker than young adults (z= -2.2, p=0.028). In the knee extensor muscles isometric strength was significantly reduced (chi-square= 10.27, p=0.006) in both groups of older adults compared to young adults (z= -2.55, p=0.009, z= -2.58, p= 0.007 for healthy and mobility-impaired old respectively), but was not different (p=0.16) between mobility-impaired (72 ± 24 Nm) and healthy older adults (85 ± 14 Nm)(Table 2).

4.2.5 Velocity of contraction and power

There was no interaction effect detected for contraction velocity in the ankle dorsiflexors ($F(10,130) = 1.11$, $p = 0.365$). However there was a significant main effect of velocity ($F(5,130) = 312.1$, $p < 0.001$) and group ($F(2,26) = 10.7$, $p = < 0.001$). Post hoc testing determined there was a significant difference between young and healthy old ($p = 0.01$), young and mobility-impaired old ($p = < 0.001$) but not between healthy old and mobility impaired older adults ($p = 0.063$). Although mobility-impaired older adults were on average slower than healthy older adults under all six-test conditions, this difference was not statistically significant ($p > 0.05$) (Figure 1).

There was a significant interaction effect between groups on knee extension contraction velocity ($F(5,10) = 2.44$, $p = 0.028$). Knee extensor contraction velocity was significantly reduced in mobility-impaired older adults compared to young adults at each submaximal load ($p = 0.01$, < 0.001, < 0.001, < 0.001, < 0.001, < 0.001, for 1Nm-50% MVC respectively). Additionally, mobility-impaired older adults knee extensor contraction velocity was also significantly slower than healthy older adults at 1 Nm ($p = 0.04$), 10 ($p = 0.023$), 30 ($p = 0.008$) and 40% MVC ($p = 0.015$). Healthy older adults were significantly slower than young adults in the 20 ($p = 0.02$), 30 ($p = 0.03$), 40 ($p = 0.01$) and 50% MVC ($p = 0.03$) test conditions (Figure 2).

A significant difference was detected between groups for peak isotonic power in the ankle dorsiflexors (chi-square= 16.0, $p < 0.001$) and knee extensors (chi-square= 16.6, $p < 0.001$). Peak isotonic power was reduced in mobility-impaired older adults compared to young and healthy older adults in the ankle dorsiflexors

(z= -3.5, p= 0.001 z= -3.3, p= 0.001 for young and healthy old respectively). There was no difference in peak power between young and healthy older adults (z- 1.6, p=0.1). (Table 2). In the knee extensors both healthy (z= -2.5, p=0.014) and mobility-impaired older adults (z= -3.5, p=0.001) produced significantly less power than young adults, with mobility-impaired adults also producing significantly less power than healthy older adults (z= -2.8, p=0.006). Results for isometric strength and peak power are summarized in Table 2.

When peak power in the ankle dorsiflexors was assessed at each of the six submaximal isotonic loads a significant interaction effect was detected (F (10,125)= 8.9, p<0.001). Post-hoc testing determined that young were significantly more powerful than mobility impaired older adults at all test conditions (p= <0.001, 0.02, 0.02, 0.01, 0.01 <0.001 for 1Nm-50% MVC respectively). Healthy older adults also produced more power than mobility-impaired older adults at all test conditions (p= 0.003, 0.047, 0.02, 0.04, 0.008 for 1Nm, 20, 30, 40 and 50% MVC respectively) except for 10% MVC (p=0.11) (Figure 3). There was no statistically significant difference between young and healthy older adults at any of the 6 submaximal test conditions (p= 0.33, 1.0, 0.4, 0.53, 0.25, 0.23 for 1 Nm-50% respectively).

A significant interaction effect was detected in the knee extensors (F (10,125)= 13.2, p= <0.001). Mobility impaired older adults produced significantly less power than young adults at all submaximal loads (p = 0.01, p= 0.02, <0.001, <0.001, <0.001, <0.001, for 1Nm-50% respectively). Similarly, healthy older adults had significantly reduced knee extensor power at 30 (p= 0.02), 40 (p= 0.03) and 50% (p= 0.03) MVC compared to young adults. Mobility-impaired older adults also

produced significantly less power than healthy older adults at all test conditions (p= 0.03, 0.02, 0.01, 0.08, 0.02 for 1Nm, 20, 30, 40, 50% MVC respectively) except 10% MVC (p=0.052)(Figure 4).

Table 2. Isometric muscle strength and peak power in young, healthy old and mobility-impaired older adults

	Young	Old	MI
Ankle Dorsiflexors			
MVC (Nm)	30 (7)	25 (4)	21 (8)*
Power (W)	23 (7)	19 (6)	8 (4)*†
Knee Extensors			
MVC (Nm)	107 (24)	85 (14)*	72 (24)*
Power (W)	298 (75)	210 (81)*	93 (56)*†

* Indicates statistically significant difference from young. † indicates significantly different from healthy older adults. Values presented as mean (standard deviation).

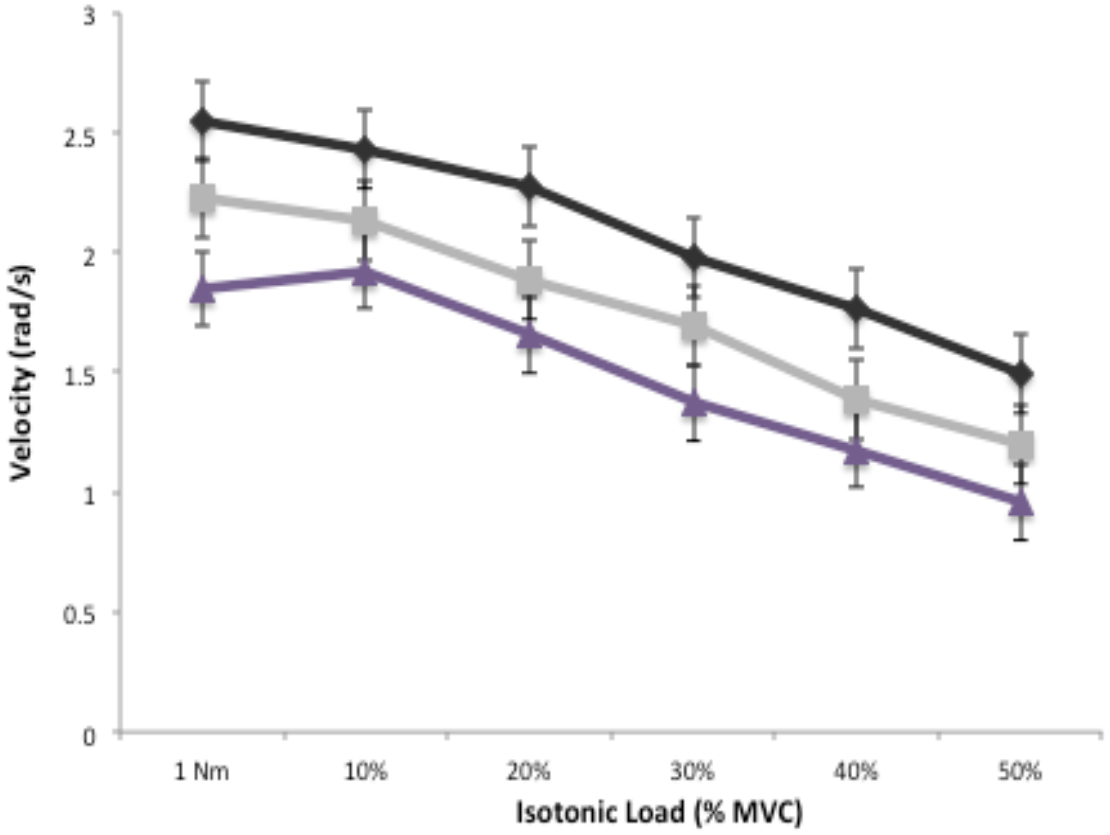

Figure 1. Ankle dorsiflexor contraction velocity
No interaction effect was detected for contraction velocity in the ankle dorsiflexors ($p= 0.365$). A significant main effect of group ($p<0.001$) showed that young adults (diamonds) were significantly faster than healthy old (squares)($p=0.01$) and mobility-impaired older adults (triangles)($p<0.001$). There was no difference between healthy and mobility-impaired older adults ($p=0.063$). Values presented as mean ± standard error.

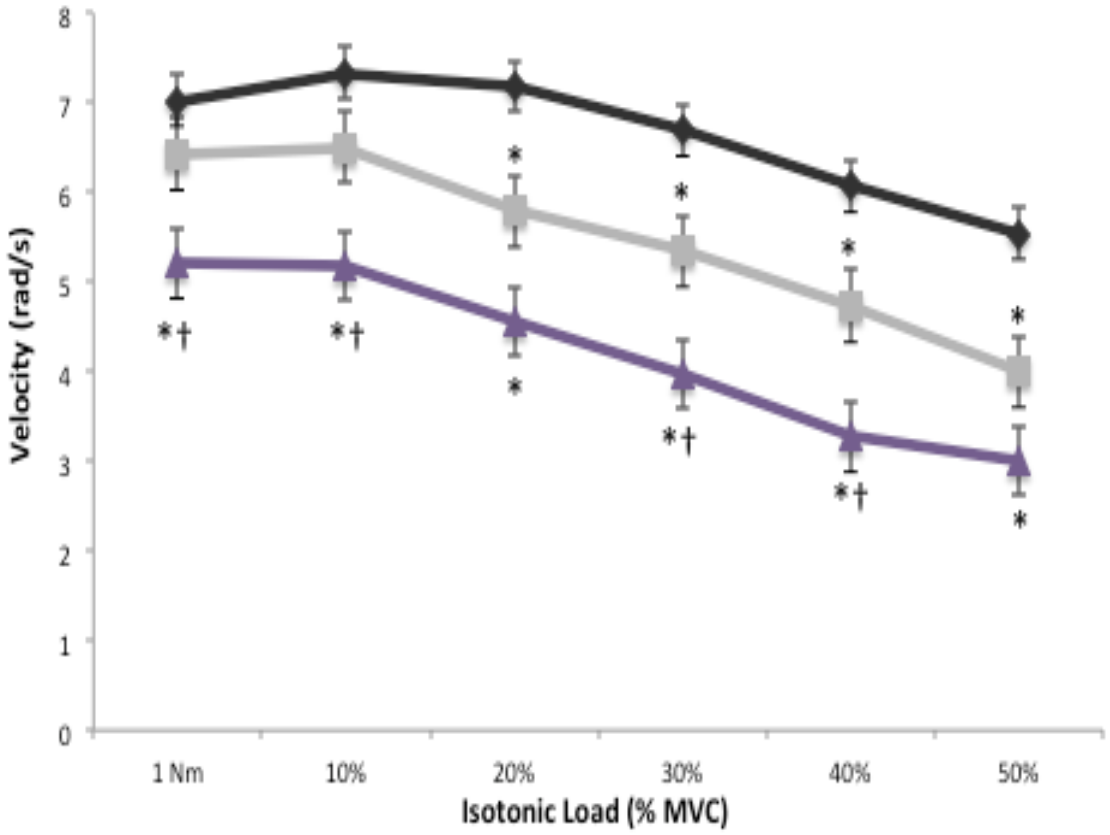

Figure 2. Knee extensor contraction velocity
A significant interaction was detected between group and isotonic load (p=0.028). Mobility-impaired (triangles) older adults had a significantly reduced contraction velocity compared to young adults (diamonds) at every submaximal load (p= 0.01, <0.001, <0.001, <0.001, <0.001, <0.001, for 1Nm-50% MVC respectively). Mobility impaired older adults were also significantly slower than healthy older adults at the 1 Nm (p= 0.04), 10 (p= 0.023), 30 (p= 0.008) and 40% MVC (p=0.015) test conditions. Healthy older adults (squares) had a slower contraction velocity than young adults at 20 (p= 0.02), 30 (p= 0.03), 40 (p= 0.01) and 50% MVC (p= 0.03). * indicates significant difference from young. † indicates significantly different from healthy old. Values presented as mean ± standard error.

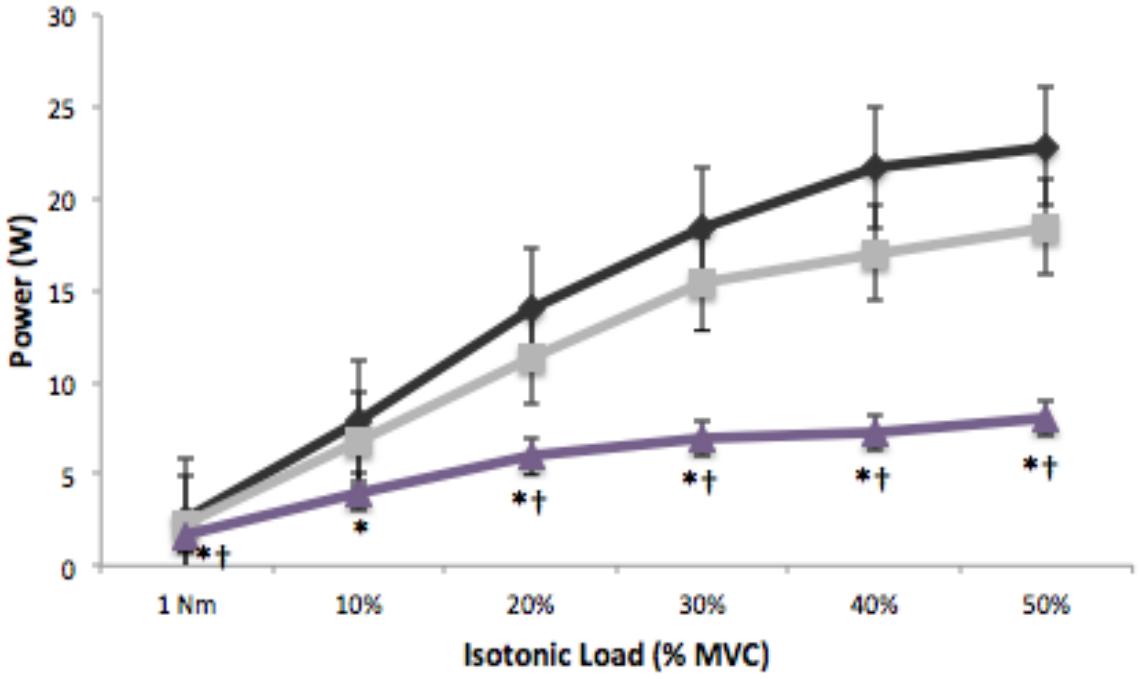

Figure 3. Ankle dorsiflexor power at submaximal isotonic loads
A significant interaction effect was detected ($p<0.001$). Young adults (diamonds) produced significantly more power than mobility impaired older adults (triangles) at al test conditions ($p= 0.003, 0.047, 0.02, 0.04, 0.008$ for 1Nm, 20, 30, 40 and 50% MVC respectively). Mobility-impaired older adults also produced less power than healthy older adults (squares) at all test conditions ($p= 0.003, 0.047, 0.02, 0.04, 0.008$ for 1Nm, 20, 30, 40 and 50% MVC respectively) except for 10% MVC ($p=0.11$). There was no difference between young and healthy older adults,($p= 0.33, 1.0, 0.4, 0.53, 0.25, 0.23$ for 1Nm-50% MVC respectively). * indicates significant difference from young. † indicates significantly different from healthy old. Values presented as mean ± standard error.

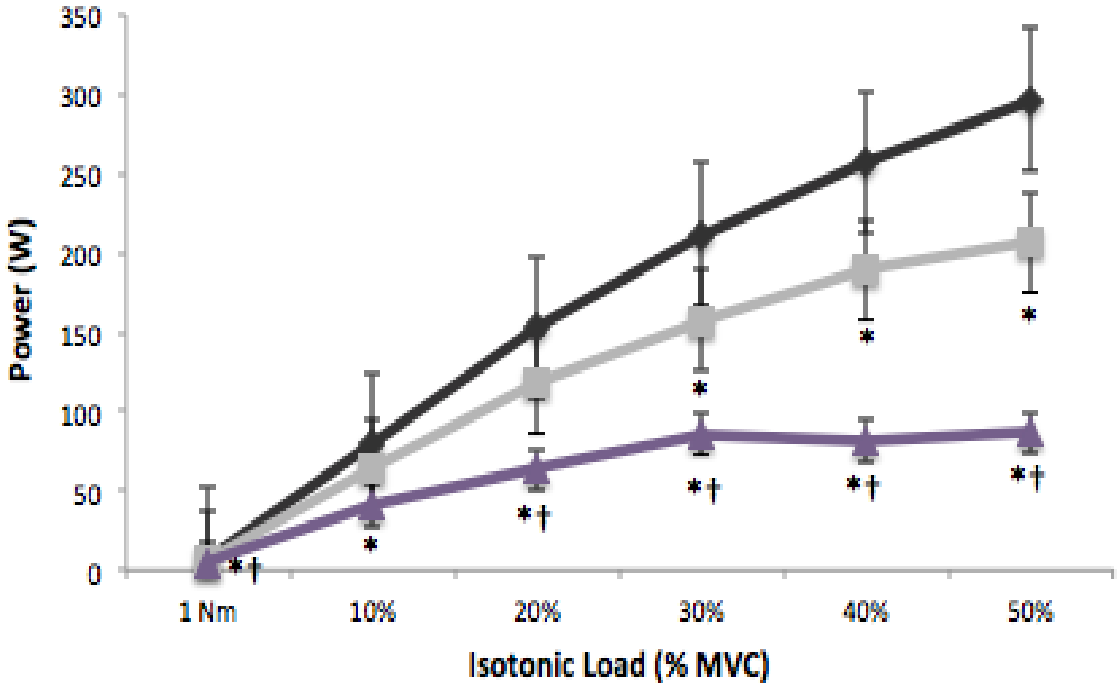

Figure 4. Knee extensor power at submaximal isotonic loads
There was a significant interaction effect between group and isotonic load ($p<0.001$). Mobility-impaired older adults (triangles) produced significantly less power than young adults (diamonds) under all test conditions ($p= 0.01, 0.02, <0.001, <0.001, <0.001, <0.001$, for 1Nm-50% respectively). Mobility-impaired older adults also produced less power than healthy older adults (squares) at 1Nm ($p=0.03$), 20 ($p=0.02$), 30 ($p=0.01$), 40 ($p= 0.008$) and 50% MVC ($p= 0.02$). Healthy older adults produced less power than young adults at 30, 40 and 50% MVC ($p= 0.02, 0.03, 0.03$, respectively) * indicates significant difference from young. † indicates significantly different from healthy old Values presented as mean ± standard error.

4.2.6 Specific strength and power

Specific strength was calculated by dividing the participants MVC by their total contractile muscle volume and is represented as Nm/cm^3. Similarly, specific power was determined from the participant's peak power output relative to their total contractile muscle volume in units of W/cm^3. There was no difference in specific strength between young, healthy old or mobility-impaired older adults in the ankle dorsiflexors (chi-square= 2.47, p=0.291) (Figure 5) or knee extensor (chi-square = 4.67, p=0.097) (Figure 6) muscle groups. Specific power, however, demonstrated a significant difference between groups in both muscles tested (chi-square= 11.6, p= 0.003 chi-square= 11.4, p= 0.002 ankle dorsiflexors and knee extensors respectively). Specific power was significantly reduced in the ankle dorsiflexors in mobility-impaired older adults compared to young and healthy older adults (z= -3.2, p= 0.002, z= -2.3 p= 0.02 respectively), but there was no difference between young and healthy older adults (z= -1.7, p= 0.09). A similar trend was observed in knee extension specific power, where both young and healthy older adults produced more power than mobility-impaired older adults (z= -3.1, p=0.002, z= -2.7, p= 0.007) but did not differ from each other (z= -099, p=0.3)

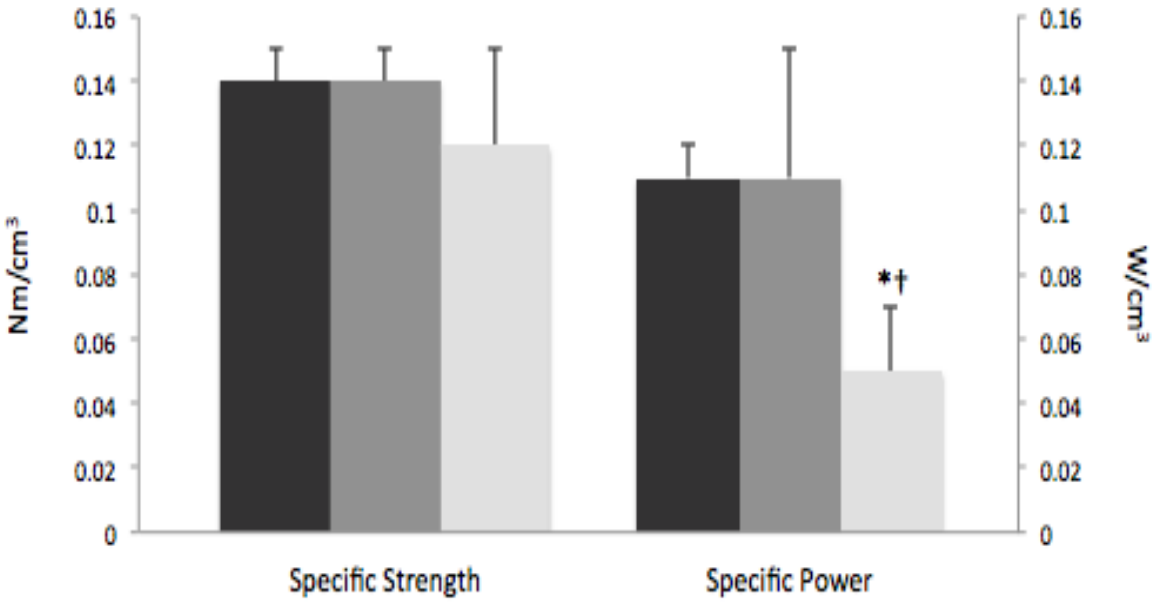

Figure 5. Specific strength and power of the ankle dorsiflexors
There was no difference between young (black), healthy old (dark grey) and mobility-impaired older adults (light grey) in specific strength (p=0.291). Specific power was reduced by 55% in mobility-impaired older adults compared to young and healthy older adults. There was no difference in specific power (p= 0.09) between young and healthy older adults. * indicates significantly different from young. † indicates significant difference from healthy old. Values are presented as means ± standard deviation.

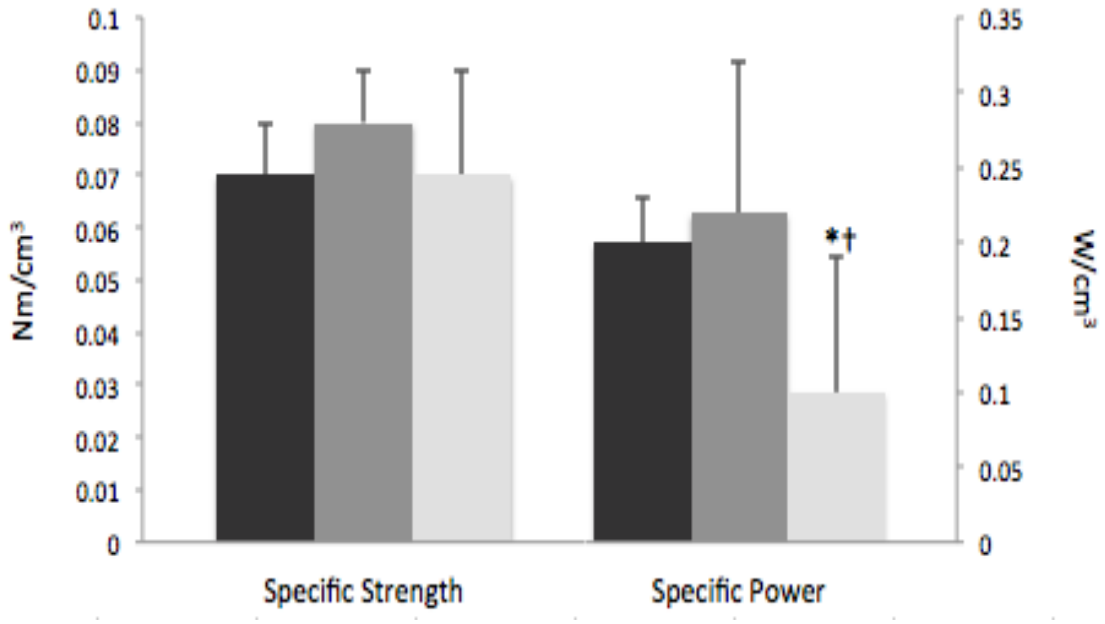

Figure 6. Specific strength and power of the knee extensors
There was no difference in specific strength between young (black), healthy old (dark grey) and mobility-impaired older adults (light grey) (p=0.097). Specific power was reduced in mobility-impaired older adults by 55% compared healthy old and 50% compared to young adults. * indicates significantly different from young. † indicates significant difference from healthy old. Values are presented as means ± standard deviation.

4.3 Discussion

This study aimed to examine the differences in the loss of isometric strength and velocity-dependent isotonic power in the lower extremity of young, healthy old and mobility-impaired older adults. Moreover, we sought to determine muscle quality by calculating specific strength and power in these three groups. We found that while strength was maintained in the ankle dorsiflexors of healthy older adults, it was significantly reduced compared to young in mobility-impaired older adults. As well, isometric knee extensor strength was reduced in all older adults compared to young adults. Additionally, we observed a decrease in contraction velocity with age, which was further attenuated in the knee extensors of mobility-impaired older adults. Further, we found that peak power production was significantly lower in mobility-impaired older adults for the ankle dorsiflexors, and both groups of older adults for the knee extensor muscles. Mobility-impaired older adults produced significantly less power than healthy older adults in both muscle groups tested. Finally, similar to previous research we observed no difference in specific strength between experimental groups [33, 34, 48], but significant reductions in specific power [6, 12, 15, 38-41] in mobility-impaired older adults compared to young and healthy old adults. The results of this study further validate the use of power, rather than isometric strength, as a more sensitive measure of neuromuscular changes with aging. As well, it underscores the importance of muscle contraction velocity in the determination of functional mobility and muscular power production.

In the present study older adults were categorized into different groups based on their functional mobility. While mobility-impaired older adults were the

same age as healthy older adults, they had significantly slower average walking speed (0.89 ± 0.10 m/s) compared to healthy old adults (1.26 ± 0.17 m/s). Previous research has defined a gait speed of less than 1 m/s to represent mobility disability, with individuals below this cutoff having increased risk of negative health consequences related to their impaired mobility [43, 44, 49, 50]. Moreover, while both groups of older females in this study were slower in performing the repeated chair stand task compared to young adults (6.8 ± 0.9 seconds), mobility-impaired older females (13.9 ± 4.6 seconds) were also significantly slower compared to healthy older females (10.7 ± 2.3). These results provide validation for our classification of participants in this study; while there was no difference in age between the two older adult groups, mobility-impaired older females performed significantly worse on measurements of functional mobility compared to healthy older females.

We found no difference in voluntary activation of the ankle dorsiflexor muscles between young, healthy old or mobility-impaired older adults. Our results are in agreement with previously published data of central activation in the tibialis anterior muscle with age [6, 16, 51]. Our results indicate that all the participants in this study were able to fully recruit their available motor unit pool, and that it is unlikely that central activation played a role in the observed loss of strength. Therefore, deficits in strength and power most likely reside in some combination of peripheral impairments.

When peak isometric strength was normalized to total contractile muscle volume, there was no difference between groups in either the ankle dorsiflexors or

knee extensor muscles. Conversely, specific power in both the ankle dorsiflexors and knee extensors was significantly reduced in mobility-impaired older adults when compared to young and healthy older adults. These observations is in line with previous work, which has shown equivocal results in the measurement of specific strength with age [10, 32-37], but unanimously reports reductions in specific power [6, 12, 15, 38-41]. A previous study by McNeil et al. [6] examined specific strength and power production in a group of young, old (mean age: 65 years), and very old men (mean age: 84 years) and found that while absolute and specific strength was maintained in old men compared to young, very old men had significant deficits in both strength measures. Further, the authors found that absolute and specific power was reduced in both groups of older adults compared to young, with very old adults also producing significantly less power compared to old adults. The results of that study are in accordance with those presented here, whereby there was no difference in specific strength between groups, but significant reduction in specific power, at least in mobility-impaired older adults. In this study we observed a 55% decrease in specific power in the ankle dorsiflexors, and 50% in knee extensor specific power in mobility-impaired older adults compared to young adults.. These values are higher than previous works that have reported reductions in specific power of 25% in the ankle dorsiflexors and 25-41% in the knee extensors of healthy older adults [6, 9].

Similar to previous work, we found that isometric strength was preserved in the ankle dorsiflexors of healthy older adults. Many studies have reported that isometric strength is well maintained into the eighth decade [6, 16, 51, 52], with

significant deficits in strength beginning to manifest in the late 70s and into the early 80 years of age [6, 16]. However, contrary to these studies we observed a significant loss of isometric strength in our mobility-impaired older adult group who had a mean age of just 72 years. Notably, the mobility-impaired older adults also had significantly less contractile muscle mass, so when isometric strength was normalized to total contractile muscle volume, although mobility-impaired adults were 19% weaker than healthy older adults, the difference was not statistically significant. Still, the reduced isometric strength and decline in muscle volume observed in the mobility-impaired older adults in this study is interesting considering previous studies show no decline in strength in similarly aged adults [16, 51]. Clearly, despite a lack of any known neuromuscular or musculoskeletal dysfunction that may affect their strength and mobility, the mobility-impaired older adults in this study differ from the older adults typically used in studies of aging and sarcopenia.

Contrary to isometric strength, peak power was reduced in the knee extensor muscle group of mobility-impaired older adults. Based on our results it appears that peak power production was more impaired in the knee extensor muscles than it was in the ankle dorsiflexors. McNeil and colleagues [6] previously reported a ~25% decline in peak power in the ankle dorsiflexors, which increased to ~60% in very old participants. Despite being similar in age to the old subjects in the McNeil et al. study, our mobility-impaired older adults displayed a 47% decline in peak power compared to young. Similarly, in a study investigating peak power production in the knee extensors, Van Driessche et al. [13] reported a decline in peak power of 24-

37% in healthy older adults with a mean age of 68 years. This is in line with peak knee extension power observed in healthy older adults in this study (30% decrease compared to young), but substantially less than the 69% reduction in mobility-impaired older adults. Thus, it appears that muscle peak power production is severely compromised in mobility-impaired older adults and may more closely resemble that of adults over the age of 80. Further, larger more proximal muscles such as the knee extensors, which are responsible for tasks that have a greater demand for power, may be more affected by the adverse adaptations to muscle with age.

When looking at peak power produced at different submaximal isotonic loads some interesting differences can be noted. While young and healthy older adults continue to increase in power production up to the 50% MVC test condition, it appears that for both the ankle dorsiflexors and knee extensors mobility-impaired older adults begin to level off in power production at around 30% MVC. Although no previous study has examined mobility-impaired older adults with this testing protocol, the trends observed in this study for young and healthy older adults are similar to those previously published for the ankle dorsiflexors [6, 7] and knee extensors [7, 13]. Mobility-impaired older adults also produced significantly less power than healthy older adults at all test conditions except for 10% MVC for both muscle groups tested. As well, it is particularly noteworthy that for both muscle groups tested there was little change in the amount of power produced by mobility-impaired adults between isotonic loads of 30-50% MVC. As power was determined isotonically, velocity was the varying factor. Therefore, it appears that mobility-

impaired older adults were unable to maintain relative contraction velocity as isotonic load increased. This has significant functional consequences for mobility, as a person's body weight, which represents an isotonic load during activities of daily living, tends to remain the same or even increase with age [53]. The inability of these older adults to maintain contraction velocity at higher isotonic loads may explain their impaired mobility. These findings underscore the growing paradigm that exercise programs for older adults should specifically emphasize velocity-dependent muscle contractions and focus on functional tasks that require strength and power [4, 14]

It has been well documented in the literature that muscle contractile velocity decreases with age [2, 6, 9, 13, 15, 54, 55]. Indeed, we observed a significant reduction in contraction velocity in both healthy and mobility-impaired older adults. Contraction velocity tended to be more impaired in older adults at higher isotonic loads. Further, mobility-impaired older adult knee extension velocity was also significantly slower than healthy older adults at loads of 1 Nm, 10, 30 and 40% MVC. The pattern towards slower contraction velocity at increased isotonic loads is similar to those presented previously in the ankle dorsiflexors [6] and knee extensors [9, 13, 54]. A previous review examining velocity-dependent power in older adults reports contraction velocity is reduced between 15-40% in older adults [2]. When pooling our results from both groups of older adults, we observed a decrease in maximal contraction velocity (Vmax) of 19% in the ankle dorsiflexors and 20% in the knee extensors compared to young adults, which is in accordance with the range reported previously. Sayers et al. [55] have shown that lower

extremity contraction velocity is significantly associated with reduced gait speed and performance on the SPPB and Van Roie et al. [28] have previously reported that contraction velocity is the single best predictor of functional performance in older adults. The results presented here support this supposition, as knee extensor contraction velocity was significantly slower in mobility-impaired older adults compared to healthy older adults. Although it is a fundamental property of muscle that contraction velocity decreases as the force that must be overcome increases [56], young adults dorsiflexion contraction velocity was reduced from 2.5 rad/s at Vmax to 1.5 rad/s at 50% MVC, a difference of 40%. Dorsiflexion contraction velocity declined by 45% in healthy older adults and 51% in mobility-impaired adults. The knee extensors had a total loss of contraction velocity of 25, 38 and 43% for young, old and mobility-impaired older adults respectively. Although many have pointed towards a change in muscle fiber composition with age and an increase in the type I:II ratio as a primary factor in reduced contraction velocity [2, 14, 20], this does not necessarily explain the significant decline observed in the ankle dorsiflexors, as the tibialis anterior is composed primarily of slow twitch muscle fibers (~80%) [57, 58]. Furthermore, studies of single muscle fibers have also reported a slowed contractile velocity in old muscle fibers [2]. This suggests that there are intrinsic changes within the muscle fiber itself which influence the loss of velocity and power observed with age. Indeed, molecular studies of aged myofibers have revealed a reduction in the concentration of myosin [59] as well as a decrease in the filament sliding speed [60]. As well, even when controlling for activity levels, older adults have been shown to have decreased fascicle length and pennation

angles compared to young adults [61, 62]. These changes to muscle architecture may negatively affect mechanical properties of older muscle such as the force-velocity relationship [4]. Additionally, the influence of antagonist co-activation [49, 63, 64], as well as slowed conduction velocity in peripheral nerves [65-67] on muscle contraction velocity and power generation cannot be ruled out. Previously, declines in lower extremity power have been associated with increased falls risk [31], and Kemoun et al. [68] have reported that delayed response of dorsiflexion contraction can predict falls. Thus, it appears that reduced muscle strength and power with age is not simply the result of lost muscle mass, but is a more complex interaction of changes that occur within individual muscles fibers and the neuromuscular system as a whole with age and this has significant consequences on an individual's functional performance.

Many previous studies have associated declines in lower extremity power with poor functional performance [14, 18, 28-30, 42, 55]. For the present study we chose the ankle dorsiflexors and knee extensors as a model for aging and mobility impairment due to their specific roles during gait and functional mobility tasks as well as their extensive use in previous works from which a comparative analysis could be drawn [6, 7, 13, 15, 41]. Additionally, although both muscles have been used to model neuromuscular change, they differ in both their function [69, 70] and relative fiber composition [57, 58, 71, 72], allowing for a more holistic view of power and mobility loss with age. Velocity is the critical determinant in muscle power loss and our results show that the knee extensor muscles seem to have greater deficits in contraction velocity, and subsequently power, than the ankle

dorsiflexors. The greater decline observed in the knee extensor muscle may be explained by muscle fiber composition. As mentioned previously, the dorsiflexor muscles are primarily slow twitch muscle fibers [57, 58]. The knee extensors on the other hand have a much more heterogenous muscle fiber composition [71, 72]. Since type IIA and IIX muscle fibers are between 3-9x stronger and faster contracting than type I fibers [2], and type II fibers represent a greater proportion of the knee extension muscles, deficits in contraction velocity may be amplified in the knee extensors. This has significant functional consequences considering the role of the knee extensors for large powerful movement tasks.

To our knowledge only two previous studies have investigated similar measures of strength and power in a group of mobility-impaired older adults [15, 41]. Similar to the results presented here, Clark et al. [15] reported a significant decrease in peak power and specific power in mobility-impaired older adults compared to middle-aged adults. The authors also reported a further decline in peak and specific power in mobility-impaired adults compared to healthy old adults. Another study lead by the same group [41] reported a decrease in muscle area as well as peak power in mobility-impaired older adults. As well, they reported no difference in specific strength, but significant reduction in specific power in both groups of older adults. Similar to our results, Reid et al. [41] observed a significant reduction in peak power in mobility-impaired older adults compared to healthy older adults. In the present study, mobility-impaired older adults were matched in age to healthy older adults. While all older adults were aged between 70-85 years in the Clark et al. [15] and Reid et al. [41] studies, healthy older adults had a mean age

of 74 ± 4 years, whereas mobility-impaired older adults were significantly older at 78 ± 5 years. It is possible that a significantly older mobility-impaired group may have contributed to their findings since previous research shows that lower extremity power is lost at a rate of 3% per year in males and 1.7% per year in females [11]. However, we controlled for age in the current study, provided further evidence that impaired mobility is associated with reduced muscular power regardless of age. To our knowledge this is the first study to investigate velocity-dependent power production in mobility-impaired older adults who were age-matched to similar healthy older individuals. Further longitudinal studies are required to delineate how these adults with early onset mobility limitation progress with age.

In conclusion, the results of this study demonstrate that contraction velocity seems to be the regulatory factor in determining muscle power, and it appears that older adults with mobility impairment may be less able to maintain contraction velocity than healthy age matched older adults. Moreover, this study provides further evidence that muscular power is reduced in older adults even when controlling for contractile muscle volume. Further research into mobility-impaired older adults is necessary to delineate the exact mechanisms that lead to deficits in contraction velocity and power.

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Chapter 5

General Discussion and Summary

5.0 General Discussion

The objective of this thesis was to examine nerve and muscle function as well as muscle mass and quality of young, healthy old and age-matched mobility-impaired older adults in an attempt to identify which neuromuscular factors may contribute to mobility loss with age. By investigating two muscle groups in the lower extremity with different roles during gait and mobility [1, 2], length of peripheral nerve supply and muscle fiber composition [3-6], we were able to outline a comprehensive analysis of how muscles in the lower extremity vary with age and mobility impairment. The objectives were accomplished through a series of three studies investigating different aspects of the nerves (Chapter 2), muscles (Chapter 3) and muscular performance (Chapter 4) in the ankle dorsiflexors and knee extensors in healthy older females, and in females with mobility impairment.

In Chapter 2 we used quantitative electromyography (EMG) analysis to assess motor unit (MU) properties in the tibialis anterior (TA) and vastus medialis (VM) muscles. Based on our derived motor unit number estimate (MUNE) from the TA muscle, it appears that mobility-impaired older adults had a greater loss of MUs compared to healthy young adults. Additionally, measures of near-fiber (NF) jiggle, which represent neuromuscular junction stability [7, 8], showed that the TA muscle was more affected with age than the VM muscle. This is in accordance with previous research, which has suggested that distal muscles may be more greatly affected by

neuromuscular changes with age than proximal muscle groups [9-11]. This study revealed differences in MU properties present between mobility-impaired older adults and young adults, that are not present in similarly aged healthy adults.. This difference may be the result of mobility-impaired older adults having an augmented loss of MUs in their TA muscle. This has significant implications for functional performance as previous research has shown impaired dorsiflexor function to be associated with increased fall risk [12].

Chapter 3 explored differences in muscle mass of the ankle dorsiflexor and knee extensor muscle groups using magnetic resonance imaging (MRI). We were also able to determine the contractile and non-contractile muscle tissue components using state of the art technology in MRI resolution capabilities. Our results demonstrate that while contractile dorsiflexor muscle volume was maintained in healthy older adults compared to young adults, it was significantly reduced in mobility-impaired adults. As well, we observed a two-fold increase in the amount of non-contractile tissue within the ankle dorsiflexors of both older adult groups compared to young adults. For the knee extensors, contractile muscle volume was significantly reduced and the percentage of non-contractile tissue volume increased in both groups of older adults compared to young adults. The results of this study provide further evidence of the loss of muscle tissue with age, and indicate, at least in the ankle dorsiflexors, that mobility-impaired older adults have a greater loss of muscle mass than young adults, which was not present in healthy similarly aged old individuals.

In Chapter 3 we also examined the quantity and quality of muscle protein in the TA muscle using the novel magnetization transfer (MT) technique. The off-resonance radio frequency pulse saturates water-bound protein molecules, allowing for an analysis of the quality of contractile tissue in muscle [13, 14]. Our results were in accordance with previously published data on MT and aging skeletal muscle, showing a decrease in the magnetization transfer ratio (MTR) in both groups of older adults compared to young adults. However, contrary to our hypothesis, healthy older adults had a significantly lower MTR than mobility-impaired older adults. We postulated that the reduced MTR in healthy older adults may be associated with an ongoing process of denervation and reinnervation of muscle fibers, leading to a reduction in the quality of nascent MUPs. In contrast, mobility-impaired older adults who have already experienced significant MU loss in the TA (as evidenced in Chapter 2), may have eliminated some of their poor quality MUs leaving more relatively stable, higher quality units remaining. However, there is a paucity of research on MT applied to skeletal muscle health, and further research into how MTR is affected by alterations in nerve and muscle health is warranted.

Chapter 4 focused on how all of the above noted changes to the neuromuscular system manifest in deficits in strength, power and functional performance. Specifically, we examined the differences between loss of strength and deficits in muscle power, while controlling for total contractile muscle volume. The results of this study showed that peak muscle power was more sensitive to changes in the neuromuscular system than isometric strength. As well, while specific strength was maintained in both groups of older adults, specific power was reduced

in both the dorsiflexors and knee extensors of mobility-impaired older adults compared to young and healthy similarly aged females. Also of note, contraction velocity appears to be the critical factor in the determination of muscular power, and older adults with mobility impairment had significantly slower knee extension contraction velocity than similarly aged healthy older adults. This result is significant, as previous work has suggested that a slowed or delayed muscle response increases the risk of falls [12, 15]. Thus, not only are mobility-impaired older adults able to produce less power, but they may also be at a higher risk for falls and fall-related injury due to a slowed rate of force generation.

Together these studies highlight the changes within the neuromuscular system with age and how those changes are associated with a person's mobility status. Our results demonstrate that despite being similar in age and having no obvious neuromuscular or musculoskeletal disorders, some older adults undergo more maladaptive changes in their nervous system and muscles than others. As well, we have shown that even when controlling for muscle mass, these changes still exist, indicating that they must arise from intrinsic changes to the contractile properties of individual muscle fibers. Our results are in line with previous research indicating sarcopenia is not as simple as a linear loss of muscle mass and subsequent strength [16-18]. Additionally, this work supports the growing paradigm that sarcopenia exists on a continuum of impairment, and for the purposes of research, participants should be classified into different categories of sarcopenia based on strength, muscle mass, and functional impairment [19].

5.1 Limitations

All of the studies in this dissertation had a relatively small sample size, especially for mobility-impaired older adults. Therefore, the consequence is a reduction in power to find statistically significant effects and the results should be considered conservatively. The mobility-impaired participants were particularly difficult to recruit as they had to be impaired enough to meet the inclusion criteria of the study, but still be able to perform maximal effort high velocity muscle contractions. In our contact with participants for recruitment, adults with impaired mobility tended to be less motivated to participate in research, and travel to/from the hospital for testing was a barrier for many participants as their mobility was compromised. Therefore, the people who are included in the mobility-impaired group do not represent all older adults with mobility deficits and likely are at the higher end of functioning of the mobility-impaired population. Therefore, finding differences between healthy older adults and the mobility-impaired older adults will be biased towards the null due to a reduction in the magnitude of difference between the groups. As well, the healthy older adults recruited for this study were highly active for their age, and were associated with a group exercise program. Consequently, they may better represent a sample of "ideal aging" rather than a typical aged person. Therefore, our mobility-impaired older adults may better represent typical aging, while our healthy older adults might be representative of very healthy and active aged women. Finally, due to the COVID-19 pandemic, the recruitment process for mobility-impaired older adults had to be cut short as all non-essential research activities were prohibited. Although a relatively small sample size does affect the power of our analysis, previous works have been able to

demonstrate significant results with a similar sample size using DE-STA [9, 20-22], MRI [23, 24] and strength/power analysis [9, 25].

The present studies are also limited by their cross-sectional design, which limits the ability to assign temporal order to the findings. Further longitudinal studies, which have the advantage of showing true change in an individual over time, into the neuromuscular properties of mobility-impaired older adults are warranted.

As all of the subjects in the present studies were females, the results can only be generalized to females and may not necessarily represent older males with mobility impairment. Previous research has shown that males and females differ in muscle mass and strength loss with age, and although males tend to be stronger than females, they also tend to lose a greater proportion of strength during senescence [26, 27]. The decision to include only females in this study was intentional in order to maintain a homogenous sample and maintain a high level of internal validity. As well, most previous studies of neuromuscular properties in older adults have focused on older men. By using similar techniques and study design as these previous studies, we were able to draw a comparison to the previously published data and present findings for a novel sample of older females.

Finally, including some additional standardized functional performance measures (such as the SPPB) may have allowed for a more comprehensive evaluation of our participants' mobility and allowed for a better comparison to previous studies [28, 29]. However, we did include many of the individual components of the SPPB (i.e. gait speed, repeated chair stands) to categorize our

older adults. As well, we evaluated all of the domains for the diagnosis of sarcopenia (grip strength, gait speed, muscle mass) as specified by the European working group consensus [19].

5.2 Future Directions

The results of these studies highlight some future directions for continued research on aging and mobility. First, in Chapter 2 we found that the S-MUP negative peak amplitude decreased from young, to older and mobility-impaired older adults in the VM muscle. Similar results have been reported in previous studies with quantitative EMG recordings of large proximal muscle such as the VM [30, 31], despite this being contrary to the established model of denervation and reinnervation with aging where surviving motor neurons sprout collateral axons to reinnervate lost fibers, thereby growing their MU size and associated S-MUP. It has been reported that surface electrodes may be unable to accurately reflect MUs in larger proximal muscles [9, 32] with an estimated recoding radius of only 20mm [33]. As well, the accumulation of IMAT and subcutaneous fats in older adults may attenuate the EMG signal [26, 34, 35]. Therefore, future research should investigate novel techniques to accurately measure MU loss and neuromuscular remodeling in large proximal muscles, especially considering their significance to functional mobility tasks. In theory NF jiggle is one technique that may be able to accomplish this task, as the high-pass filter emphasizes the contribution of only muscle fibers closest to the concentric needle electrode, eliminating the potential error of the more spatially distributed MUs in larger muscles. However, there is limited data available on NF jiggle, and the results are not always in agreement with the data

presented here [30]. More research is required to collect normative data on NF jiggle across different age groups and adults with different levels of functional performance.

Additionally, when comparing our results in Chapter 2 to previously published data, our MUNEs were higher across all groups tested. While our sample consisted of entirely females, most previous studies contained only male participants. Given the documented neuroprotective effects of estrogen [36, 37], and the fact that females characterize a larger proportion of the aged population [38], further research comparing sex differences in neuromuscular properties with aging is warranted.

Results from Chapter 3 indicate a further need for studies investigating MTR in aged muscle. Although we observed a decline in MTR in both older groups compared to young, healthy older adults actually had a further decline in MTR compared to mobility-impaired adults, which we suggested may be related to poor quality MUs in healthy adults who are undergoing cycles of denervation and reinnervation. Thus, future studies should focus on muscle quality at difference stages of sarcopenia such as those put forth by the European working group [19] to help delineate the changes in MTR in muscle at different ages and levels of function and mobility.

In Chapter 4 we observed that mobility-impaired older adults were unable to maintain relative contraction velocity as the isotonic load increased, leading to a greater reduction in muscular power at higher loads. Recent work has emphasized high velocity power training as a potential substitute for conventional strength

training to better improve overall functional performance in older adults [15, 39]. Results from this study further validate the need for studies of high velocity power training, specifically longitudinal studies investigating older adults with different levels of functional mobility. As well, considering most previous studies of mobility-impaired older adults consisted of a much older population [28, 29], further longitudinal investigations into how the older adults in this study with early onset mobility impairment progress with advanced age is warranted. Finally, though it has been identified that reduced contraction velocity is the critical component leading to muscular power loss with age, the precise intrinsic mechanisms by which contraction velocity is lost have not been identified and require further investigation.

5.3 Summary

In summary, this series of studies was designed to identify the changes to the neuromuscular system in a group of older adults with mobility impairment compared to healthy age-matched and young adults. To our knowledge, these are the first studies to investigate these measures in a group of older adults with poor functional performance who were similar in age to a group of healthy older adults. The results of this investigation demonstrate that mobility-impaired older adults had greater MU loss, as well as significantly reduced contraction velocity in their lower extremity muscles when compared to similarly aged healthy adults. These studies provide novel insights into the aging neuromuscular system, and how it translates into functional performance in older adults and further validates the use of mobility and functional performance outcome measures in the description and

classification of sarcopenia. Moreover, it emphasizes that mobility and performance are not simply a matter of preserved muscle mass, but are related to altered intrinsic force generating capacity of individual muscle fibers with advanced age.

5.4 References

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Appendix A

Western Research

Research Ethics

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Tim Doherty
File Number:103980
Review Level: Full Board
Approved Local Adult Participants:60
Approved Local Minor Participants:0
Protocol Title: Comparing Neuromuscular Function in Mobility Impaired and Healthy Older Adults
Department & Institution: Schulich School of Medicine and Dentistry\Clinical Neurological Sciences,St. Joseph's Health Care London
Sponsor:
Ethics Approval Date: July 19, 2013
Ethics Expiry Date: August 01, 2014

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Western University Protocol		
Letter of Information & Consent		2013/07/09

This is to notify you that the University of Western Ontario Health Sciences Research Ethics Board (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the University of Western Ontario Updated Approval Request form.

Member of the HSREB that are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor vote on, such studies when they are presented to the HSREB.

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

Signature

Ethics Officer to Contact for Further Information

Erika Basile (ebasile@uwo.ca)	Grace Kelly (grace.kelly@uwo.ca)	Shantel Walcott (swalcott@uwo.ca)

This is an official document. Please retain the original in your files.

Western University, Research, Support Services Bldg., Ste. 5150
London, ON, Canada N6A 3K7 t. 519.661.3036 f. 519.850.2466 www.uwo.ca/research/services/ethics

Western Research

Date: 14 June 2020

To: Tim Doherty

Project ID: 103980

Study Title: Comparing Neuromuscular Function in the Mobility Impaired and Healthy Older and Younger Adults

Application Type: Continuing Ethics Review (CER) Form

Review Type: Delegated

REB Meeting Date: 16/Jun/2020

Date Approval Issued: 14/Jun/2020

REB Approval Expiry Date: 02/Jul/2021

Dear Tim Doherty,

The Western University Research Ethics Board has reviewed the application. This study, including all currently approved documents, has been re-approved until the expiry date noted above.

REB members involved in the research project do not participate in the review, discussion or decision.

Western University REB operates in compliance with, and is constituted in accordance with, the requirements of the TriCouncil Policy Statement: Ethical Conduct for Research Involving Humans (TCPS 2); the International Conference on Harmonisation Good Clinical Practice Consolidated Guideline (ICH GCP); Part C, Division 5 of the Food and Drug Regulations; Part 4 of the Natural Health Products Regulations; Part 3 of the Medical Devices Regulations and the provisions of the Ontario Personal Health Information Protection Act (PHIPA 2004) and its applicable regulations. The REB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Please do not hesitate to contact us if you have any questions.

Sincerely,

Daniel Wyzynski, Research Ethics Coordinator, on behalf of Dr. Joseph Gilbert, HSREB Chair

Note: This correspondence includes an electronic signature (validation and approval via an online system that is compliant with all regulations).

Appendix B

ELSEVIER LICENSE
TERMS AND CONDITIONS

Aug 11, 2020

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Curriculum Vitae

Neal McKinnon

Post-Secondary Education and Degrees

09/2014 – Present	**Western University** - London, Ontario PhD Candidate Health and Rehabilitation Sciences
09/2016 – 08/2018	**Western University** - London, Ontario Masters of Physical Therapy
09/2012 – 08/2014	**Western University** - London, Ontario MSc Kinesiology
09/2008 – 04/2012	**Wilfrid Laurier University** - Waterloo, Ontario Honours BSc Kinesiology

Honours and Awards

2018-2019	Ontario Graduate Scholarship
2017-2018	Ontario Graduate Scholarship
2016-2017	Joseph A. Scott Studentship in Mobility and Aging
2015-2016	Joseph A. Scott Studentship in Mobility and Aging
2014	Southern Ontario Neuroscience Association Poster Award
2013-2014	Ontario Graduate Scholarship
2012	Dean's List Wilfrid Laurier University

Related Work Experience

11/2018 – Present	**Physiotherapist, Regional Rehabilitation** St. Joseph's Health Care Parkwood Institute 550 Wellington Road, London, Ontario
09/2018 – 11/2018	**Physiotherapist, Outpatient Orthopaedics** CBI Home Healthcare 1657 Dundas Street, London, Ontario

01/2015 – 05/2016	**Graduate Teaching Assistant** Physical Therapy 9528: Critical Appraisal and Evidence-Informed Practice Department of Health and Rehabilitation Sciences, Western University
01/2014 – 04/2014	**Graduate Teaching Assistant** Kinesiology 1080: Introduction to Psychomotor Behaviour Department of Kinesiology, Western University
09/2012 – 04/2013	**Graduate Teaching Assistant** Kinesiology 2230: Introductory Exercise Physiology Department of Kinesiology, Western University
05/2012 – 08/2012	**Research Assistant** Department of Kinesiology and Physical Education Wilfrid Laurier University
09/2011 – 02/2012	**Student Volunteer** Sun Life's Movement Disorders Research and Rehabilitation Centre (MDRC) Waterloo, Ontario
08/2010 – 11/2011	**Athletic Therapist** Men's varsity rugby Wilfrid Laurier University

Publications

McKinnon NB, Connelly, DM, Rice CL, Hunter SW, Doherty TJ. Neuromuscular contributions to the age-related reduction in muscle power: Mechanisms and potential role of high velocity power training. *Aging Research Reviews.* (2017) May;35:147-154

McKinnon NB, Montero-Odasso M, Doherty TJ. Motor unit loss is accompanied by decreased peak muscle power in the lower limb of older adults. *Experimental Gerontology.* (2015) Oct;70: 111-8

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Presentations and Conferences

McKinnon NB, Hunter SW, Rice CL, Montero Odasso M, Doherty TJ. Assessing the Neuromuscular Factors That Contribute To Mobility Impairment in Older Women. Parkwood Institute Research: Spring Update. London, Ontario. (April 21st, 2017). (Oral)

McKinnon NB, Hunter SW and Doherty TJ. Evaluating Motor Unit and Muscle Properties in Young, Old, and Mobility Impaired Older Women: A Preliminary Report. Canadian Society for Exercise Physiology (CSEP) Annual Meeting. Victoria, British Columbia. (October 12th-15th, 2016). (Poster)

McKinnon NB, Hunter SW and Doherty TJ. Motor unit loss is accompanied by decreased muscle power in healthy older adults: implications for impaired mobility in older adults. Physical Medicine and Rehabilitation Resident Research Day. London, Ontario. (January 11th, 2016). (Oral)

McKinnon NB and Doherty TJ. Comparing neuromuscular function in healthy older and young adults. Canadian Society for Exercise Physiology (CSEP) Annual Meeting. St. John's, Newfoundland. (October 22-25th, 2014). (Poster)

McKinnon NB and Doherty TJ. Comparing neuromuscular function in healthy older and young adults. Southern Ontario Neuroscience Association (SONA) Annual Meeting. London, Ontario. (May 5th, 2014). (Poster)

McKinnon NB, Graham MT, Tiidus PM. Effects of creatine supplementation on muscle damage and repair following eccentrically- induced damage to the elbow flexor muscles. Canadian Society for Exercise Physiology (CSEP) Annual Meeting, Regina, Saskatchewan. (October 10th-13th, 2012). (Oral).

McKinnon NB, Graham MT, Tiidus PM. Effects of creatine supplementation on muscle damage and repair following eccentrically- induced damage to the elbow flexor muscles. 3rd Annual Muscle Health Awareness Day, Toronto, Ontario. (May 25th, 2012). (Poster).

McKinnon NB, Graham MT, Tiidus PM. Effects of creatine supplementation on muscle damage and repair following eccentrically- induced damage to the elbow flexor muscles. Ontario Exercise Physiology Conference, Barrie, Ontario. (January 20-22nd, 2012). (Oral).