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Reductions in Muscle Quantity and Quality in Old and Very Old Men: New Insights into the Progression of Sarcopenia

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

REDUCTIONS IN MUSCLE QUANTITY AND QUALITY IN OLD AND VERY OLD MEN: NEW INSIGHTS INTO THE PROGRESSION OF SARCOPENIA

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Abstract

Healthy adult aging is associated with sarcopenia; a loss of skeletal muscle mass known as. Major contributors to this process include functional and morphological changes in the contractile tissue and within the neuromuscular system. Currently, the relationship between muscle mass, normalized strength, structural integrity, and neuromuscular properties [such as motor unit number estimates (MUNE)], in the tibialis anterior (TA) with aging is largely unknown. Therefore, to examine this relationship we recruited twelve young (25 ± 3 years old), six old (68 ± 5 years old) and six very old (79 ± 3 years old) men. Magnetic resonance (MR) images were obtained from the entire musculature of the leg from the tibial plateau to the malleoli. Muscle cross-sectional areas (CSA) were calculated using image processing software. Strength was measured as maximal isometric voluntary dorsiflexion contraction (MVC) torque; this was then normalized to CSA. Structural integrity of the muscle was evaluated by magnetization transfer ratio (MTR) using magnetic resonance imaging (MRI). Neuromuscular measures were also collected and decomposition-enhanced spike-triggered averaging was used to collect surface and intramuscular electromyography (EMG) signals. From these data, estimates of motor unit numbers were made. Muscle CSA was less only in the very old (11.2cm^2), no differences existed between the young (13.4cm^2) and old (11.7cm^2). Strength was ~26% lower in the old and ~24% in the very old than the young. When strength was normalized to CSA there were no differences between the groups. Very old men had ~8% lower MTR values than the young and old men, with no differences between young and old. Neuromuscular measures, specifically the combination of a decreased compound muscle action potential (CMAP) and increased surface motor unit potential (SMUP), resulted in a decrease in MUNE between young [~147 motor units (MU)] and old (~109 MUs) and also between young and very old (~80 MUs). In conclusion, muscular structure and function appear to be maintained in the older adult due to compensatory motor unit remodeling; however in the very old adult the structural integrity of the muscle becomes compromised as motor unit losses are greater resulting in the acceleration of sarcopenia.

Keywords: muscle, aging, normalized strength, magnetic resonance imaging magnetization transfer ratio, motor unit number estimates, neuromuscular

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List of Abbreviations and Symbols

ANOVA	analysis of variance
CMAP	compound muscle action potential
CSA	cross-sectional area
DE-STA	decomposition enhanced spike triggered averaging
DEXA	dual-energy X-ray absorptiometry
EMG	electromyography
IGF-1	insulin-like growth factor-1
MR	magnetic resonance
MRI	magnetic resonance imaging
MT	magnetization transfer
MTR	magnetization transfer ratio
MU	motor unit
MUNE	motor unit number estimates
MUP	motor unit potential
MVC	maximum voluntary contraction
ROI	region of interest
SMUP	surface motor unit potential
TA	tibialis anterior

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Chapter 1

1 Introduction

Sarcopenia is a non-pathological process characterized by the progressive loss of skeletal muscle mass, strength, and function associated with natural adult aging (Doherty, 2003; Rosenberg, 1989). In cross-sectional studies involving adults, sarcopenia has been found to be a gradual process until the seventh or eighth decade of life, at which point there is an accelerated loss of muscle mass (Doherty, 2003; Lexell, Taylor, & Sjostrom, 1988; Narici & Maffulli, 2010). Muscular strength is well-maintained until the seventh decade of life (W. F. Brown, Strong & Snow, 1988; Candow & Chilibeck, 2005; Murga Oporto, Menendez-de Leon, Bauzano Poley, & Nunez-Castain, 2003), but at a certain point in the aging process, not only does muscle mass decline, but also muscle quality. The loss of strength has been found to be accompanied by decreases in muscle mass (Frontera, Hughes, et al., 2000; Frontera et al., 2008; Jubrias, Odderson, Esselman, & Conley, 1997; Klein, Rice, & Marsh, 2001; Young, Stokes, & Crowe, 1985). However, the changes in muscle quantity alone do not always explain the decreases in strength. Reduced muscle quality with age may also be a significant contributor to the loss of strength (Newman et al., 2003).

Quantitative decreases in muscle mass can be examined using magnetic resonance imaging (MRI). Magnetic resonance imaging is a valuable non-invasive tool that has been used extensively for assessing muscle structure. Magnetic resonance imaging allows the observation of changes in muscle cross-sectional area (CSA), the ratio of contractile to non-contractile tissue, and muscle volume.

Muscle quality has previously been assessed through invasive methods such as muscle biopsy (Frontera, Hughes, et al., 2000; Frontera, Meredith, O'Reilly, Knuttgen, & Evans, 1988). Not only is muscle biopsy invasive, but it also introduces a high amount of variability as it samples just a small portion of the whole muscle (Evans & Coggan, 1995). Muscle quality using a combination of noninvasive measures has not been well studied. Muscle quality can be evaluated noninvasively in several ways. Three of these methods include normalized strength [strength per unit of muscle mass (e.g. CSA)],

magnetization transfer ratios (MTR), and neuromuscular evaluations such as electromyography (EMG) measures.

Normalized strength represents the torque per unit of muscle mass and therefore gives a measure of the ability of the muscle tissue to contract. Using normalized strength to gauge muscle quality accounts for age-related absolute loss of contractile tissue and the infiltration of noncontractile tissue within the muscle. Eliminating any noncontractile tissue from the CSA measurement allows for a relative comparison of just the contractile tissue and the strength of the muscle and therefore comparisons between age groups can be made solely on the amount of contractile tissue per se. Measuring normalized strength is a fairly simple process in which a maximum voluntary contraction (MVC) is performed to measure maximal isometric torque and a measurement of CSA is taken (either by using ultrasound, computed tomography, or MRI) and the torque value is then normalized to the CSA (MVC/CSA).

Magnetization transfer (MT) has not been widely utilized to examine healthy muscle across aged populations. Previously, MTR has been used primarily in the characterization of white matter disease in the brain (e.g. multiple sclerosis) (Henkelman, Stanisz, & Graham, 2001). Recently, Sinclair et al. (2012) examined skeletal muscle MTR and found a significant decrease in patients with clinical peripheral neuropathies. Magnetization transfer is based on longitudinal magnetization interaction between free protons and macromolecular (bound) protons. Normally, the protons associated with macromolecules such as protein are not visible in MRI due to their short T2 relaxation time. By presaturating the protons associated with macromolecules via an off-resonance pulse the excitation is transferred to the free protons decreasing the net magnetization. The magnitude of the MT is dependent on the tissue imaged; skeletal muscle normally has a prominent MT effect (Sinclair et al., 2012) because of the high concentration of protein in muscle tissue. A lower MTR indicates reduced capacity of the macromolecular protons to exchange MT with free protons and therefore reflects decreased structural integrity in muscle (McDaniel et al., 1999; Schwenzer et al., 2009).

Evaluating muscle quality in terms of the neuromuscular system examines the electrophysiologic properties of excitable muscle mass such as compound muscle action potential (CMAP) values, contraction durations, and the number of functioning motor units (MUs) (Tomlinson & Irving, 1977). The concept of age-related MU remodeling indicates that due to collateral reinnervation there is a moderate preservation of muscle mass until the eighth or ninth decades of life (W. F. Brown, 1972; W. F. Brown et al., 1988; Campbell, McComas, & Petito, 1973; Doherty, 2003; Gordon, Hegedus, & Tam, 2004; McNeil, Doherty, Stashuk, & Rice, 2005b). Collateral reinnervation is characterized by an axon sprout from a healthy MU 'recapturing' an orphaned muscle fiber when the constituent motor neuron dies thus retaining the majority of muscle fibers and therefore a minimal amount of muscle mass is lost. Typically, collateral reinnervation involves type II MUs dying and being reinnervated by slower type I MUs and results in larger MUs (more muscle fibers per single MU). With continued aging, the combination of an increased rate of MU death and larger MUs results in a higher number of muscle fibers being lost, thus explaining the accelerated loss of muscle mass in very old adults. The number of functioning MUs in a human muscle group can be estimated electrophysiologically by dividing the compound muscle action potential (CMAP) in response to maximal stimulation of its motor nerve (CMAP; maximal size of the entire MU pool within the muscle group) by the corresponding size parameter of the mean surface detected MU potentials [surface motor unit potential (SMUP); average size of the MU within a given muscle group] (Bromberg, 2007).

Overall, two major contributors to the resultant loss of strength with adult aging are changes in muscle quantity, such as CSA, CSA composition, and volume; and a decrease in muscle quality, such as normalized strength, MTRs and neuromuscular properties (e.g. MUNE).

1.1 Purpose

The purpose of this study was to explore the progression of sarcopenia and the loss of strength through both the quantitative and qualitative age-related changes in muscle in a young, old, and very old population of men.

To examine muscle quantity, CSA, CSA composition, and muscle volumes of the tibialis anterior (TA) were analyzed using MRI. To examine muscle quality, normalized strength (isometric strength relative to CSA), MTRs, and neuromuscular measures such as CMAP, contraction duration, SMUP and motor unit number estimates (MUNE), in the TA were examined.

Therefore, the following research questions were addressed in this study:

1. How does muscle quantity, including CSA, CSA composition, and muscle volume, differ between young, old, and very old populations of men?

2. How does muscle quality, such as normalized strength, MTR, and neuromuscular measures, differ between young, old, and very old populations of men?

3. Is there an ideal measure of muscle quality that reflects muscle function?

4. Does muscle quantity affect muscle quality or does muscle quality affect muscle quantity?

1.2 Hypothesis

In response to the research questions the following hypotheses were tested:

1. Muscle quantity will decrease among the three age groups. Total CSA will not change across the three groups, however in the old and very old men there will be an increase in noncontractile tissue and a decrease in contractile tissue in the TA compared to the young men. This increase in noncontractile tissue and decrease in contractile tissue will result in decreased viable muscle volume.

2. Muscle quality changes will be dependent on the evaluation technique across the three age groups. Strength will concurrently decrease with CSA resulting in normalized strength measures showing no differences. Magnetization transfer ratios will show a decrease as the structural integrity decreases in the protein with increasing age. The neuromuscular measures will reflect the decreases in the

excitable muscle mass (i.e. CMAP and SMUP) meaning a decreased number of estimated MUs in the old and very old men.

3. All three measures of muscle quality will be related to muscle function. Each measure will have advantages and disadvantages.

4. Muscle quantity will be maintained in the old men due to collateral mechanisms maintaining muscle quality, however in the very old men these compensatory mechanisms will no longer be able to keep up and muscle mass will decrease.

Chapter 2

2 Literature Review

2.1 Sarcopenia

Sarcopenia, a term of Greek origin introduced by Rosenberg in 1989, meaning poverty of flesh, refers to the loss of skeletal muscle mass associated with natural aging. However, the multidimensional nature of sarcopenia necessitates a broader definition that incorporates both the quantitative and qualitative properties of the sarcopenic process (Cesari, Ferrini, Zamboni, & Pahor, 2008). Consequently, the current definition in the research has evolved to encompass the loss of skeletal muscle mass, strength, contractile quality, and the resultant functional decline (Berger & Doherty, 2010). The causation of sarcopenia is a result of a combination of behavioral, biological, and environmental factors thus it would vary considerably among individuals. Generally, sarcopenia is a gradual process beginning after the third decade of life and accelerating after the seventh decade of life (Waters, Baumgartner, & Garry, 2000).

Although there is no specific level of muscle mass at which sarcopenia is considered to be present (Roubenoff, 2001), the relationship between muscle mass and strength makes any loss of muscle mass consequential (Roth, Ferrell, & Hurley, 2000). Currently, there is no universal diagnosis criterion for sarcopenia, but a common definition of sarcopenia was proposed by Janssen, Heymsfield, & Ross (2002) that is based on two categories of sarcopenia. Individuals with grade I sarcopenia have an appendicular skeletal muscle mass-to-body height ratio between -1 and -2 standard deviations from the gender-specific mean value of young controls. Grade II sarcopenia individuals are at least -2 standard deviations below this gender-specific value of young controls. Another definition of sarcopenia uses a percentage of skeletal muscle mass index (SMI%, total muscle mass/body mass x 100%) (Janssen, Heymsfield, Wang, & Ross, 2000).

2.2 Causes and Effects of Sarcopenia

Although the exact cause of sarcopenia is unknown; physical inactivity, alterations in the central and peripheral nervous systems, altered hormonal levels, and decreased energy

and protein intake are believed to be four main factors involved in the process (Doherty, 2003). Although there are numerous other factors involved in the progression of sarcopenia, these four are believe to be major contributing factors to the characteristic skeletal muscle atrophy and weakness due to sarcopenia contributing to the loss of functional mobility, loss of independence, and frailty present in many older adults (Roubenoff, 2000a, 2001).

Sarcopenia has been related to limited function, frailty, and an increased risk of morbidity and mortality (Bales & Ritchie, 2002; Fried et al., 2001; Janssen et al., 2002). Sarcopenia has also been linked to osteoporosis (Coin et al., 2000), insulin resistance (Boyle et al., 2001; Cefalu et al., 1995), obesity (Cesari et al., 2005 Roubenoff, 2000c), and arthritis (Toda & Kobayashi, 2000; Walsmith & Roubenoff, 2002). The sarcopenic loss of muscle mass leads to a loss of strength which leads to a loss of power and results in fragility and disability (Hairi et al., 2010; Muhlberg & Sieber, 2004).

Sarcopenia has been proposed to be associated with a reduction in both muscle fiber size and number. In the elderly, type II muscle fiber area is reduced by ~20% to ~50% whereas type I fibers are reduced ~1% to ~25% (Lexell & Downham, 1992; Lexell et al., 1988; Roos, Rice, & Vandervoort, 1997). Muscle fiber numbers were found to be more equally affected, as by the ninth decade of life there was found to be ~50% fewer type I and type II fibers when compared to a young counterpart (Lexell, 1993; Lexell et al., 1988). From this, it is believed that motor neuron loss may be a leading cause in age-related muscle atrophy (Doherty & Brown, 2002; Doherty, Vandervoort, & Brown, 1993; Doherty, Vandervoort, Taylor, & Brown, 1993). Possible supporting evidence and agreement for this conclusion are apparent in electrophysiological studies that examine the neuromuscular properties of the muscle and estimate the number of functioning MUs within the muscle (Doherty et al., 1995; McNeil et al., 2005b). Motor unit number estimation studies have found that with age it appears likely that there is a remodeling of MUs from type II to predominately type I (W. F. Brown, 1972; W. F. Brown et al., 1988; Campbell et al., 1973; Doherty, 2003; Gordon et al., 2004; McNeil et al., 2005b). This remodeling of MUs from type II to type I results in decreased strength and slower reaction times, leaving the individual with a limited ability to recover from potential falls

(Muhlberg & Sieber, 2004). As the individual continues to age and MUs continue to remodel, a greater number of muscle fibers are lost placing the individual in a condition even more susceptible to falls and fractures (Tinetti, 2003). Previous studies have found an increase in the rate of falls and injury in the elderly (Fried et al., 2001; Vellas, Wayne, Garry, & Baumgartner, 1998). Decreased reaction time may affect the ability of the individual to not only prevent falls and injury (Pai & Bhatt, 2007; Skelton, Kennedy, & Rutherford, 2002), but it may also limit their independence (Doherty, 2003; Roubenoff, 2000b, 2001). Some current research suggests that in some cases, sarcopenia is part of a cyclic process involving this loss of independence and a continual progression of sarcopenic symptoms. The cycle consists of age-related changes impairing the neuromuscular system which in turn results in a higher incidence of falls and fractures which leaves the individuals immobile. Immobilization causes a decline of nutritional status [subsequently explained by the "empty refrigerator" problem (Boumendjel, Herrmann, Girod, Sieber, & Rapin, 2000)] which results in malnutrition. Malnutrition impairs protein synthesis, therefore declining the protein reserve of the body which diminishes the capacity to meet the extra demand of protein synthesis associated with disease and injury. This results in further decreases in activity and mobility (Muhlberg & Sieber, 2004); decreases in activity and mobility then result in an even greater decline in muscle function (Bauer, Kaiser, & Sieber, 2008; Janssen et al., 2002) and consequently furthers sarcopenia processes (Muhlberg & Sieber, 2004).

2.2.1 Physical Activity

Physical inactivity is a significant contributor to sarcopenia. The relationship in older individuals between physical activity levels, disability, and skeletal muscle mass has been well established (Evans, 1997, 2002; Porter, Vandervoort, & Lexell, 1995).

Elderly individuals who are physically inactive will experience a greater decline in muscle mass than those elderly individuals who are physically active (Roubenoff, 2000b). This loss may be attributed to a reduction in intensive physical loading (Power et al., 2012; Power et al., 2010). However, not just engaging in physical activity is adequate, but the physical activity must be of sufficient intensity and duration to recruit type II muscle fibers as not recruiting these fibers results in type II fiber atrophy and the

development of sarcopenia (Roubenoff, 2000b). Others have found a dose-dependent increase in lean mass and physical function following acute resistance exercise (He, Goodpaster, & Kelley, 2004; Hillsdon, Brunner, Guralnik, & Marmot, 2005; Leveille, Guralnik, Ferrucci, & Langlois, 1999; Manini et al., 2009).

The physiological benefits of resistance exercise for the elderly are numerous. Some of these benefits include hypertrophy (Charette et al., 1991), reduced inflammation (Greiwe, Cheng, Rubin, Yarasheski, & Semenkovich, 2001), increased mitochondrial function (Melov, Tarnopolsky, Beckman, Felkey, & Hubbard, 2007), improved myogenic signaling (Kosek, Kim, Petrella, Cross, & Bamman, 2006), and increased satellite cell activity (Roth et al., 2001). Acute resistance exercise alone has been shown to be effective in increasing muscle mass and strength in sedentary individuals even in the ninth decade of life (Fiatarone et al., 1994). Following an acute bout of resistance training, the increase in muscle mass and strength has been found to be maintained with only a once a week continued resistance training program (Trappe, Williamson, & Godard, 2002).

Complementary to these resistance training results, recent studies have also found that acute aerobic exercise is assistive in the maintenance of muscle mass with age (Chomentowski et al., 2009). Aerobic exercise is beneficial through the mechanisms of improved muscle blood flow (DeSouza et al., 2000), decreased oxidative stress (Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005), and decreased glucocorticoid sensitivity (Duclos, Corcuff, Pehourcq, & Tabarin, 2001).

Chronic training effects have been studied with aging through the examination of Masters athletes. These individuals have a life-long history of physical activity and therefore provide insight into the effects of life-long training on the muscular system. As well, comparison can be made between these Masters athletes and sedentary older individuals, potentially highlighting the benefits and potential prevention of sarcopenia with a chronic physical activity regime (Hawkins, Wiswell, & Marcell, 2003). From these studies on Masters athletes, it would appear that the preservation of muscle mass, strength, and neuromuscular properties is use-dependent. Power et al. (2010) found that there was a

preservation of MUs in the active muscles in Masters runners, but not in the less active muscles. Motor unit number estimates in the TA of the Masters runners was similar to young, however the MUNE of a less active arm muscle (biceps brachii) were similar in Masters runners and their old, less active counterparts, both being less than young (Power et al., 2012). In contrast to the usage preservation theory, Pearson et al. (2002) found similar declines in Master weightlifters and sedentary controls for both peak power (1.3% vs. 1.2%) and force (0.6% vs. 0.5%). However, the declines in absolute differences allowed an ~85 year old to perform similar to an ~65 year old.

2.2.2 Nutritional Status

A decline in food intake has been found to be associated with aging (Morley, Baumgartner, Roubenoff, Mayer, & Nair, 2001). Many factors are believed to contribute to decreased food intake in the elderly including loss of appetite, gastrointestinal changes, altered taste and smell, social changes, and economic limitations (Bales & Ritchie, 2002). Inadequate nutritional intake of many elderly individuals is of concern in regards to the development of sarcopenia (Visvanathan & Chapman, 2009). In particular, insufficient protein intake may lead to the development of sarcopenia through a lack of amino acid availability and a subsequent decrease in muscle protein synthesis (V. R. Young, 1990). As a result, insufficient protein intake appears to be a critical factor for sarcopenia development in older adults and may severely compromise quality of life (Vetta, Ronzoni, Taglieri, & Bollea, 1999).

Recent data indicate that lean mass in older adults is significantly and positively associated with protein intake (Houston et al., 2008). However, it is estimated that only 15% of individuals over the age of 60 are consuming more than 75% of the 0.8g/kg/day recommend daily allowance of protein (Roubenoff & Hughes, 2000). Furthermore, it has been found that eating equal to or less than the recommend dietary allowance of protein still leads to substantial declines in muscle strength (Castaneda, Charnley, Evans, & Crim, 1995). Therefore, questions have been raised as to the adequacy of the recommendation levels of daily protein intake. In a study by Houston et al. (2008), over the course of three years, individuals who consumed more than the recommended dietary allowance for protein experienced the smallest losses of lean mass, whereas the

individuals who consumed protein quantities at or below the recommended daily allowance experienced significant muscle atrophy. This suggests that daily intake for protein in the elderly is below adequate levels to maintain optimal muscle health with aging (Evans et al., 2008; Morais, Chevalier, & Gougeon, 2006; Sood, Baker, & Coleman, 2008).

Protein synthesis may be maximized in older adults by supplementing meals with essential amino acids. Essential amino acids such as leucine, isoleucine, and valine are critical for protein synthesis in older adults (Anthony et al., 2002; Combaret et al., 2009; Dillon et al., 2009; Henderson, Irving, & Nair, 2009; Holecek, Sprongl, Tilser, & Tichy, 2001; Katsanos, Kobayashi, Sheffield-Moore, Aarsland, & Wolfe, 2006). Protein synthesis has also been found to decline in the elderly when protein is ingested in conjunction with carbohydrates (Volpi, Mittendorfer, Rasmussen, & Wolfe, 2000). Subsequently, Paddon-Jones & Rasmussen (2009) suggested that more protein be consumed with each meal in the elderly, so that there is an increase of daily overall protein intake.

The quantity and quality of protein in the body is not only affected by the decreased protein intake but is also determined through a continuous process involving a balance between protein breakdown and protein synthesis, termed protein turnover (Holloszy & Nair, 1995). Research reports that muscle protein synthesis rates are generally lower in the old when compared to young (Hasten, Pak-Loduca, Obert, & Yarasheski, 2000; Holloszy & Nair, 1995; Roth et al., 2000; Yarasheski, Zachwieja, Campbell, & Bier, 1995). Balagopal, Rooyrackers, Adey, Ades, & Nair (1997) compared protein synthesis rates in individuals in the third, sixth, and eighth decades of life and found that the rate of protein synthesis was significantly decreased by the sixth decade of life and continued to decline with age. With age, this protein turnover balance reflects a shift of a decreased synthesis rate rather than an increased catabolic rate (Combaret et al., 2009; Morley, 2012; Ryanzanov & Nefsky, 2002; Vandervoort & Symons, 2001).

Alterations of muscle protein turnover could represent an early and sensitive marker of sarcopenia (Abellan van Kan et al., 2011). The loss of muscle mass and strength with

aging is associated with the loss of skeletal muscle proteins and alterations in the balance between protein synthesis and degradation. In general, regardless of the mechanisms, muscle atrophy occurs when protein breakdown exceeds synthesis. A proper diet is critically important to slowing sarcopenia progression, as well as in maintaining overall healthy aging (Houston et al., 2008; Paddon-Jones & Rasmussen, 2009).

2.2.3 Neuromuscular Adaptations

Age-related changes in the neuromuscular system play a significant role in the onset of sarcopenia. The neuromuscular system consists of muscles and the nerves that control them. A motor neuron is an electrically excitable cell located in the central nervous system that projects axons outside the central nervous system and is responsible for sending signals to the muscles to initiate movement. A MU is a single motor neuron and all of the corresponding muscle fibers the neuron innervates. The number of fibers in a MU depends on the specific muscle and the general function of the muscle. Although muscles have mixed muscle fiber content, each motor neuron innervates the same muscle fiber type (Burke, Levine, Tsairis, & Zajac, 1973).

With age, motor neurons die and subsequently, the number of functioning MUs decline (Roth et al., 2000, Roubenoff 2001). The loss of muscle fibers begins with this loss of motor neurons; as motor neurons die, muscle fibers belonging to the MU become denervated. When a motor neuron dies, an adjacent motor neuron, usually a type I motor neuron, may reinnervate these muscle fibers, preventing atrophy. This process is called motor unit remodeling. However, not all denervated muscle fibers are reinnervated and some muscle fibers atrophy leading to a decrease in muscle mass (Roth et al., 2000). This is a continuous process throughout life and is considered irreversible (Roubenoff, 2001). When compared to type II MUs, type I MUs have slower firing rates, are slower to contract, produce less muscle force, and are smaller in size and fiber number (Henneman, 1985; De Luca & Hostage, 2010). Therefore, MU remodeling by type I motor neurons leads to less efficient MUs. The remodeled type I MU will be larger and have less precise control of movements, less force production and, the velocity of the muscle contraction will slow (Baudry, Klass, & Duchateau, 2005; Roth et al., 2000; Roubenoff, 2001; Waters et al., 2000). This helps explain the loss of balance and speed

of movement with age. In addition, with continued aging, larger MUs will die and denervation rates will exceed reinnervation rates, further explaining the increased rate of atrophy of muscle fibers and overall decrease in muscle mass in the elderly (Roth et al., 2000).

Electrophysiological studies have demonstrated the loss of whole functioning MUs in proximal and distal muscles in the upper and lower extremities (de Koning et al., 1988; McComas, 1991; Stalberg, 1980). For example, lower reported MUNE have been found in the thenar, TA, and biceps brachii muscle groups (W. F. Brown, 1972; Doherty & Brown, 1993, 1997; McNeil et al., 2005b; Power et al., 2012; Power et al., 2010) with age and are consistent with anatomic data that has demonstrated losses of anterior horn cells and ventral root fibers with aging (Mittal & Logmani, 1987; Tomlinson & Irving, 1977). Cross-sectional studies suggest that MU numbers are well maintained until the seventh decade of life and thereafter begin to decline precipitously (McComas, 1991; McNeil et al., 2005b).

Various causes for motor neuron death have been suggested, among which, on the basis of recent data in aged animals, is a crucial role of the neuromuscular junction (NMJ) (Abellan van Kan et al., 2011). Some researchers also propose that human nerve cells have a predetermined life span and the decline in quantity of these cells is dependent on the location in the body, age, and presence of disease (Vandervoort & Symons, 2001).

With regards to the neuromuscular system, the causation of sarcopenia is complex involving both central and peripheral nervous system alterations (Narici & Maffulli, 2010). Throughout the life span, muscle undergoes a continuous cycle of denervation and reinnervation, but in old age it seems that the process of reinnervation cannot keep pace with that of denervation, contributing to the loss of MUs and muscle atrophy.

2.2.4 Hormonal Changes

Aging is associated with several changes in hormonal levels, including a decrease in the concentrations of testosterone, growth hormone, and insulin-like growth factor-1 (IGF-1) (Hameed, Harridge, & Goldspink, 2002; Kamel, Maas, & Duthie, 2002). A sustained

decrease in these hormones is linked to a decrease in muscle mass and an increase in body fat (Waters et al., 2000). Testosterone, growth hormone, and IGF-1 are influential in the regulation of protein turnover; specifically, protein synthesis rates (Griggs et al., 1989), especially of myosin heavy chain and myofibrillar protein (Waters et al., 2000).

With age, there is a reduction in levels of both total testosterone and free testosterone (Morley et al., 2001; J. L. Tenover, 1997; J. S. Tenover, Matsumoto, Plymate, & Bremner, 1987). Vermeulen & Kaufman (1995) found that between the ages of 20 to 80 years old, total testosterone decreased by ~35% whereas free testosterone was reduced by ~50%. Epidemiological data support the relationship between the decline in testosterone and the decline in muscle mass (Vermeulen, Goemaere, & Kaufman, 1999), strength (Baumgartner, Waters, Gallagher, Morley, & Garry, 1999), and functional status (Perry, Miller, Patrick, & Morley, 2000). Iranmanesh, Lizarralde, & Veldhuis (1991) sampled and analyzed the blood of men between the ages of 21 and 71 for 24 hours and found growth hormone concentrations are also approximately halved between young and old individuals. Growth hormone mediates its effects on muscle by regulating the synthesis of IGF-1 (Sara & Hall, 1990) which is also decreased with age (Hennessey et al., 2001; Morley, Haren, Kim, Kevorkian, & Perry, 2005).

Some research has shown that hormone administration in pharmacological doses does increase muscle mass and strength (Gotherstrom et al., 2009; Johannsson, Grimby, Sunnerhagen, & Bengtsson, 1997; Sattler et al., 2009). Supplemental testosterone has been found to produce significant gains in total and appendicular lean mass, muscle strength, and aerobic endurance with significant reductions in whole-body and trunk fat (Sattler et al., 2009). Gotherstrom et al. (2009) found that with 10 years of growth hormone replacement therapy, there was increased muscle strength during the first five years which protected against the normal age-related decline in the subsequent five years, resulting in approximately normalized muscle strength. However, other studies have concluded that hormone replacement theory is not effective in increasing muscle mass and strength in older participants (Roubenoff, 2001; Waters et al., 2000). Due to the contradictory results in research, recommendations for hormone replacement

interventions cannot be made with regards to preventing sarcopenia (Doherty, 2003; Zachwieja & Yarasheski, 1999).

Changes in estrogen concentration in women may play a role in the development of sarcopenia during menopause. The decline in estrogen associated with menopause in women is well recognized (Messier et al., 2011). Estrogen, as a result of its conversion to testosterone, has an anabolic effect on muscle.

Altered endocrine function (Fielding et al., 2011; Nass, Johannsson, Christiansen, Kopchick, & Thorner, 2009), increased levels of proinflammatory cytokines (Jensen, 2008; Roth, Metter, Ling, & Ferrucci, 2006; Schaap, Pluijm, Deeg, & Visser, 2006), mitochondrial dysfunction (Hiona & Leeuwenburgh, 2008; Lemasters, 2005), and cellular apoptosis (Kerr, Wyllie, & Curie, 1972; Marzetti & Leeuwenburgh, 2006) are other suggested hormonal-influenced mechanisms implicated in contributing to the sarcopenic loss of muscle function.

2.3 Measuring Sarcopenia

2.3.1 Muscle Quantity

2.3.1.1 CSA

Total muscle mass peaks around the age of 24 years (Lexell et al., 1988). Some reports state that between the ages of 24 and 50, muscle mass declines slowly as only ~10% of total muscle mass is lost (Larsson, Grimby, & Karlsson, 1979). Between 50 and 80 years of age, total muscle mass decreases further by ~30% (Lexell et al., 1988). Other studies which assess muscle mass through CSA measurements have found that CSA decreases by as much as 40% between 20 and 60 years of age. Differences in reported mass and CSA vary depending on technique, skeletal site, and gender (Levine et al., 2000).

Loss of skeletal muscle mass with age has been documented by measurements using dual-energy X-ray absorptiometry (DEXA) and via muscle CSA quantified using three-dimensional imaging methods such as X-ray computer tomography or MRI (Lang et al., 2010). DEXA has been found to underestimate muscle mass by as much as 20% (Narici & Maffulli, 2010; Ryan, Dobrovolny, Smith, Silver, & Macko, 2002) whereas, use of

MRI has grown in popularity over the past number of years because of its non-invasive ability to give excellent and highly accurate insight into the human body.

When CSA of essential muscles of locomotion are evaluated, a 30% decrease in quadriceps CSA was found between young and elderly (Lexell et al., 1988; A. Young, Stokes, & Crowe, 1984). Goodpaster et al. (2006) found CSA values of the total thigh muscle and quadriceps muscles are associated with knee extensor strength. When measured at multiple muscle sites, CSAs are also associated with indices of functional capacity in the elderly, such as chair stand and leg strength measurements which have been shown to be strong predictors of falls (Visser et al., 2005; Visser et al., 2002). A major reduction in CSA and the loss of muscle strength in old age (~81 years old) has also been shown in the elbow flexor and extensor muscles by Klein et al. (2001). The decrease in CSA is a primary factor for the well documented decrease in contractile force generating potential in old age.

2.3.1.2 CSA Composition

Several computed tomography, MRI, and ultrasound studies have shown that in sarcopenia, the loss of muscle tissue is accompanied by infiltration of fat and connective tissue (Taaffe et al., 2009). As a result, the net contractile muscle mass is smaller than that measured by a simple muscle CSA methods and mistakes in the estimation of contractile muscle mass are likely to be made if this noncontractile mass is not taken into account. Musculoskeletal tissues are high in proton-containing molecules such as proteins and lipids. Magnetic resonance imaging is an inherently powerful tool at depicting these tissues, and particularly useful and accurate in quantify the adipose components of muscles (Lang et al., 2010).

Muscle quality in obese individuals is poor due to increased intramuscular adipose tissue (Villareal, Banks, Siener, Sinacore, & Klein, 2004) which contributes to muscle weakness, frailty, and disability (Blaum, Xue, Michelon, Semba, & Fried, 2005). The accumulation of intramuscular fat and connective tissue has been shown to be inversely related to the level of physical activity (Kent-Braun, Ng, & Young, 2000). However, fat infiltration of muscle causes increased difficulty of locomotion because of the added inert

mass. Fat accumulation in the muscle has been linked with decreased muscle function (Goodpaster et al., 2001; Manini et al., 2007; Visser et al., 1998).

It is also thought that excess adipose tissue disturbs the surrounding tissues through an increased inflammatory load (Lumeng, DeYoung, Bodzin, & Saltiel, 2007). The increase in adipose tissue causes a macrophage infiltration mediated-release of pro-inflammatory cytokines (such as TNF-a, IL-6, IL-1) and adipokines (leptin, adiponectin, and resistin) from adipocytes (Neels & Olefsky, 2006). Additionally, adipose tissue is known to secrete more than 50 different molecules related to inflammatory signals. These inflammatory signals may contribute to accelerating muscle atrophy in elderly obese individuals by excessively stimulating proteolysis and possibly engaging myonuclear apoptosis (Jarosz & Bellar, 2009). Fat infiltration is also a known mechanism for insulin resistance (Roubenoff, 2004). Together fat infiltration and the muscle mass loss associated with sarcopenia result in continued losses of muscle mass, increases in insulin resistance, and risk of metabolic syndrome development (Reaven, 1988). Stenholm et al. (2008) showed epidemiological evidence that obesity is associated with an accelerated functional decline and high risk of disease and mortality.

2.3.1.3 Volume

Muscle volume can account for 92% of the variance in joint torque and may be a more sensitive scaling factor of muscle strength than CSA (Fukunaga et al., 2001).

Morse, Thom, Birch, & Narici (2005) reported muscle volume assessed using MRI in the triceps surae of elderly men was overall 22% smaller in the old men than the young men. Individually, muscle volumes were 27% smaller in the lateral gastrocnemius, 29% smaller in the medial gastrocnemius, and 17% smaller in the soleus. The relative volume of the gastrocnemii is reduced to a greater extent than that of the soleus muscle. Following a 12 month resistance training program, Morse, Thom, Mian, et al. (2005) found muscle volume increased with activity with aging. Prior to training, there was no significant difference in lateral gastrocnemius, medial gastrocnemius, and soleus muscle volumes between the training and control groups. Following this training program, muscle volume increased 12% pre- to post-training within participants and was 17%

greater than age-matched controls in 70 year old men. Volume increased by 19% in the lateral gastrocnemius, 15% in the medical gastrocnemius, and 11% in the soleus. However, the relative volume occupied by each muscle within the triceps surae did not change with training.

Due to compensatory MU remodeling the higher type II fiber content of the gastrocnemii results in a greater susceptibility to atrophy whereas the high composition of type I fibers in the soleus (Enoka, 2008; Johnson, Sideri, Weightman, & Appleton, 1973) results in a greater likelihood of preservation with age (Dalton, McNeil, Doherty, & Rice, 2008).

The decrease in muscle volume (along with the decrease in CSA and fiber fascicle length) suggests that sarcopenia involves a loss of sarcomeres not only in parallel but also in series (Narici & Maffulli, 2010; Narici, Maganaris, Reeves, & Capodagilo, 2003).

2.3.2 Muscle Quality

2.3.2.1 Normalized Strength

Conflicting results have been reported regarding normalized strength changes with age. Several investigations have found significant decreases (Jubrias et al., 1997; Klein et al., 2001; McNeil, Vandervoort, & Rice, 2007; A. Young et al., 1985), whereas others have not found any differences (Frontera et al., 2008; Frontera, Suh, et al., 2000; Kent-Braun & Ng, 1999). Those reporting differences have found a greater reduction in strength than CSA and therefore reduced normalized strength.

Klein et al. (2001) examined the normalized strength of the elbow flexors and elbows extensors and found a significant decline in normalized strength in both. It is possible that the maintenance of normalized strength, like MUs, is dependent on use. McNeil et al. (2007) compared the dorsiflexors of young, old, and very old men and found that isometric strength decreased in the very old men by 33% and 25% compared to young and old men, respectively; whereas CSA was similar among the three groups. This resulted in a significant decrease in specific strength of the very old men compared to the young. However, other researchers have found opposing results. Kent-Braun & Ng (1999) found in the dorsiflexors, young men had a higher MVC compared with elderly

men, but also a decreased CSA of elderly men compared to young men. However, there was no age-related impairment of specific strength. When strength was normalized to volume, Morse, Thom, Mian, et al. (2005) found plantar flexion in elderly males is reduced compared to young males. These opposing results make a conclusion for the differences difficult. Further investigation using a combination of muscle quality evaluation techniques is necessary.

2.3.2.2 MTR

Magnetization transfer is a MRI protocol which has been used in several studies mainly to investigate the structural integrity of tissue in patients with various neurological and musculoskeletal conditions (Knight et al., 2005; Mattila et al., 1995; Schwenzer et al., 2009; Sinclair et al., 2012; Sinclair et al., 2010; Ulmer et al., 1998; Wolff & Balaban, 1994).

Normal skeletal muscle is known to have strong MT interactions, presumably related to the high concentration of hydrophilic protein macromolecules (Boss et al., 2006; Yousem, Schnall, Doughtery, Weinstein, & Hayden, 1994). It is known that MTR decreases are found in muscle edema (Boss et al., 2006), myonecrosis (Mattila et al., 1995), thyroid-related ophthalmopathy (Ulmer et al., 1998), and is also induced by exercise (Yoshioka et al., 1994; Zhu, Zhao, & Isherwood, 1992). Changes in MTR seem to depend not only on these factors but also on changes in the muscular structure itself (Schwenzer et al., 2009). It is suggested that this decrease in MTR results from increased free water content, decrease in macromolecules, and dynamic changes of water-macromolecule interaction in skeletal muscle (Grossman, Gomori, Ramer, Lexa, & Schnall, 1994; Henkelman et al., 2001).

Schwenzer et al. (2009) found a decrease in MTR in the TA with aging when comparing healthy young (~31 years old) to healthy old (~66 years old) groups. Magnetization transfer ratio, in this study decreased from 0.47 percentage units (p.u.) in young to 0.44 p.u. in old.

2.3.2.3 Neuromuscular Measures

Neuromuscular measures including CMAP, motor unit discharge rate (Bellemare, Woods, Johansson, & Bigland-Ritchie, 1983; Connelly, Rice, Roos, & Vandervoort, 1999; Dalton, Harwood, Davidson, & Rice, 2009), neural activation (Narici, Roi, Landoni, Minetti, & Cerretelli, 1989) contraction duration (Vandervoort & Symons, 2001), and MUNE (McNeil et al., 2005b; Power et al., 2010) can used to evaluate the neuromuscular health of muscle with aging.

In a review by Vandervoort (2002), examples of contraction durations between young and old were shown to have a slowing in the lower limb from ~180ms to ~255ms respectively, in the dorsiflexors and from ~260ms to ~350ms, respectively, in the plantar flexors (Vandervoort & McComas, 1986). However, in the upper limb, contraction duration was consistent across age groups; ~130ms in the elbow extensors and ~139ms in the elbow flexors (Jakobi & Rice, 2001).

As stated previously, MUNE, which is a calculation based on the SMUP and the CMAP, declines in older age groups. In a study of the TA by McNeil et al. (2005b) young men were found to have ~150 MUs, old men ~91 MUs, and very old men experiencing a significant precipitous decline with ~59 MUs.

Ideally, a comprehensive recording of all these factors, as well as the measures these factors are derived from (e.g. contraction duration is the sum of half relaxation time and time to peak torque), would provide the most insight into the neuromuscular adaptations across different age groups. These recording would allow for a complete interpretation of the excitability of the muscle. Neuromuscular excitability is influential on muscle mass and therefore, muscle function (Vandervoort, 1992).

2.4 Prevalence of Sarcopenia

In those populations over 60 years old, the prevalence of sarcopenia may be as high as 30% and may approach 50% in those populations over 75 years old; this prevalence will continue to grow as the population ages (Doherty, 2003). The strong association between sarcopenia and disability among the elderly populations bring to attention the need to

further examine the time course and causes of sarcopenia, as well as a valid way to measure the process and progress.

In the United States of America, the costs associated with caring for an individual with sarcopenia have been linked to increased overall health care costs (Buford et al., 2010; Janssen, Shepard, Katzmarzyk, & Roubenoff, 2004). The absolute costs of sarcopenia linked functional disability are expected to double over the next 25 years (Buford et al., 2010).

In the New Mexico Elder Survey, Baumgartner et al. (1998) measured muscle mass by DEXA, in 883 elderly men and women. The prevalence of sarcopenia ranged from 13% to 24% in persons between 65 and 70 years old and was over 50% for those older than 80 years old. In this study, the prevalence was higher for men (58%) than for women (45%) over 75 years old. Sarcopenic men and women had 3.6 and 4.1 times higher rates of disability, respectively, compared with those with greater muscle mass. Another study by Janssen et al. (2002) found that with sarcopenia, functional impairment and disability were two times greater in older men and three times greater in older women.

Iannuzzi-Sucich, Prestwood, & Kenny (2002) also used DEXA to measure appendicular skeletal muscle mass in women and men between the ages of 64 and 93 years old. Overall prevalence of sarcopenia was found to be 27% in men and 23% in women. However in men and women over the age of 80, these values climbed to 31% and 45%, respectively.

In the next 20 years, the number of people and proportion of the population over the age of 65 is predicted to increase (Abellan van Kan et al., 2011). The concurrent multifactorial nature of sarcopenia brings attention to the importance of intervention strategies that target multiple modifiable risk factors to help improve muscle function.

2.5 Preventing Sarcopenia

Currently, the extent to which countermeasures including exercise, nutritional, and pharmacological interventions are able to help retard sarcopenia, improve function, and decrease disability in the elderly are not well established (Hurley, Hanson, & Sheaff,

2011). A few studies have examined sarcopenia prevention and generally it has been concluded that long-term training is necessary to prevent sarcopenia. However, multiple, well-designed intervention studies, including the use of functional measures, directed towards elderly populations are still necessary (Doherty, 2003).

Short-term training studies have consistently resulted in significant strength gains in elderly men and women (A. B. Brown, McCartney, & Sale, 1990; Charette et al., 1991; Frontera et al., 1988; McCartney, McKelvie, Martin, Sale, & MacDougall, 1993). Hakkinen et al. (1998) examined the effects of a 24-week strength training and exercise regime in middle aged and elderly men. It was concluded that the adaptation to strength training among the elderly men was similar to that detected among the middle aged men, when training protocols were matched for intensity, volume, and duration.

The extent to which life-long activity and training can prevent age-related declines in strength has not been thoroughly studied. Klitgaard et al. (1990), in a cross-sectional study, compared elderly men who had either trained with running, swimming, or strength training regularly for between 12 and 17 years with young and elderly sedentary controls. In comparison to the young controls, they reported strength declines in the sedentary elderly group.

Multiple factors must be considered when designing sarcopenia prevention studies including the population, training intensity, and duration of the training, and the outcome measured.

As our population ages, it is clear that we require greater understanding of the underlying mechanisms leading to sarcopenia. Only then can we begin to develop effective targeted interventions to prevent disability and optimize independence in older men and women. An optimal exercise program for the prevention of sarcopenia will include considerations such as continuous neuromuscular training, mobilization, prevention of falls, training of nutritional skills and improvement of nutrition, and improvement of the impaired protein synthesis (Muhlberg & Sieber, 2004).

Chapter 3

3 Methods

3.1 Participants

Twelve young men (25 ± 3 years old) were recruited from the university population, and six old (68 ± 5 years old) and six very old (79 ± 3 years old) men were recruited from a local, regular exercise program designed to maintain cardiorespiratory endurance and flexibility. All participants were active with no known neurological or cardiovascular diseases, and groups were matched approximately for height and mass. Prior to the MRI, participants were given a MRI screening questionnaire to ensure safety (see Appendix A). The study protocol was approved by The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (see Appendix B) and conformed to the Declaration of Helsinki. Informed oral and written consent was obtained prior to testing.

3.2 MRI

Magnetic resonance (MR) images were acquired via serial axial plane in a 3.0 Telsa magnet. Proton density 2D FLASH images were acquired using the following parameters: 1500ms repetition time, 14ms echo time, 256x192 matrix, 243x325mm field of view, 5mm slice thickness with slice separation of 2mm. Participants were inserted into the magnet bore feet first, in the supine position, with the motor point on their right leg [7cm distal to the tibial tuberosity and 2cm lateral to the tibial anterior border (Botter et al., 2011)] isocentred to the bore of the magnet and a flex coil placed over the TA. To ensure minimal movement between scans, the feet and knees of participants were strapped together using inelastic, Velcro straps. Four series of 30 slices each were obtained imaging the entire musculature of the leg from the tibial plateau to the malleoli. Scans with the MT presaturation, off-resonance frequency were taken in series first, followed by a series of scans taken without presaturation. Presaturation scans had a higher specific absorption ratio and therefore any necessary adjustments made to the presautrated scan could be replicated for the scans without presaturation.

3.3 Muscle Quantity

3.3.1 CSA, CSA Composition & Volume

Total muscle CSAs, CSA composition, and volumes were calculated pixel-wise using a combination of manual and semi-automated techniques with open-source OsiriX image processing software (version 3.7, Geneva, Switzerland). Muscle CSAs were calculated at the slice with the largest CSA. Analysis began proximally from the first slice, in which the TA appeared to the most distal slice containing the TA. A region of interest (ROI) was manually outlined on the most proximal slice that the TA appears in, with the brush tool and repeated every five slices; missing ROIs on the skipped slices were automatically generated. With the TA outlined, all pixels outside the ROIs were set to zero. To quantify the contractile only tissue, a three-dimensional threshold-growing tool was used to ensure only muscular tissue was included in the ROIs (excluding non-contractile tissue and septal spaces). Any errors produced by the automatic generation were corrected manually. The software calculated muscle CSA and volume for the ROIs. Previous research has shown a high degree of intra- (ICC=0.997) and inter-rater reliability (ICC=0.997) with this analysis technique (Berger, 2011). In this study, images were reanalyzed at random and similar retest values were found.

3.4 Muscle Quality

3.4.1 Normalized Strength

To determine maximal dorsiflexion torque, participants were seated in a custom built isometric dynamometer with the hip and knee angles positioned at 90° and the ankle at 30° plantar flexion. To minimize confounding hip and knee joint movement during dorsiflexion contractions, an adjustable C-shaped brace was secured firmly on the distal portion of the thigh. Velcro strapping across the toes and dorsum secured the foot to the dynamometer (Figure 1).

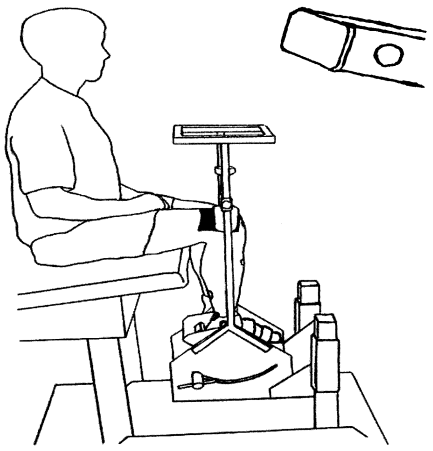

Figure 1: Isometric strength and neuromuscular properties experimental setup.

All testing was performed on the right (dominant) leg. Participants performed three MVCs, with at least 2min rest between attempts. Participants were provided with real time visual feedback of their torque and verbally exhorted. The peak torque of the three attempts was taken as the maximal torque for the participant. This torque value was then normalized to the previously found CSA, so that normalized strength equalled MVC/CSA (Nm/cm^2).

3.4.2 MTR

Using the acquired MR images, M_0 values and M_1 values (where M_0 denotes images without off-resonance presaturation and M_1 denotes images with off-resonance presaturation) were found for the same ROI as CSA. Single slice analysis was found to be not significantly different than whole muscle volume MTR, so to make relative comparison to muscle CSA the same slice ROI was used for MTR and CSA.

The interaction of macromolecular protons and free protons of a specific tissue is quantitatively described by the magnetization transfer ratio (Schwenzer et al., 2009; Sinclair et al., 2012):

$$MTR = [(M_0 - M_1) / M_0]$$

Magnetization transfer ratio was calculated in percentage units (p.u.) as change from baseline scan. It should be noted that the MTR-derived values depend on the parameters

of the sequence, and the MT prepulse and cannot be compared with values from other studies (Schwenzer et al., 2009).

3.4.3 Neuromuscular Properties

Using the same setup used during the MVC, surface EMG data were collected from the TA using self-adhering Ag-AgCl electrodes (1.5cm x 1.0cm; Marquette Medical Systems, Jupiter, Florida). The skin over the muscle was cleaned with alcohol (70% isopropyl alcohol solution) prior to application of the electrodes. An active electrode was positioned on the proximal portion of the TA over the motor point [7cm distal to the tibial tuberosity and 2cm lateral to the tibial anterior border (Botter et al., 2011)], a reference electrode was placed over the distal tendon at the malleoli, and a ground electrode was placed on the patella. Intramuscular EMG signals were recorded via a disposable concentric needle electrode with a recording surface of $0.03mm^2$ (Model N53153; Teca, Hawthorne, New York) inserted into the TA, 5-10mm proximal to the active surface electrode.

Electromyography data were acquired using decomposition enhanced spike triggered averaging (DE-STA) software on a Neuroscan Comperio system (Neurosoft, El Paso, Texas). The DE-STA and its associated algorithms have been previously described (Doherty & Stashuk, 2003; Stashuk, 1999; Stashuk, Doherty, & Brown, 2003). The surface and intramuscular EMG signals were bandpass filtered at 5Hz to 5kHz and 10Hz to 10kHz, respectively. Data collection began by determining the maximum twitch torque and CMAP responses. The CMAP was evoked via supramaximal stimulation of the common fibular nerve distal to the fibular head using a Digitimer stimulator (Model DS7AH, Digitimer Welwyn Garden City, Hertfordshire, UK) with a pulse width of 100μs and 400V. The electrical current intensity was progressively increased until a plateau in CMAP amplitude was reached. At this point, the stimulation intensity was increased 20% to ensure complete activation of all motor axons. During CMAP determination, the active electrode was repositioned to maximize the negative-peak amplitude and minimize negative-peak rise time.

Voluntary activation was assessed using the interpolated twitch technique (Belanger & McComas, 1981). The amplitude of the interpolated torque evoked during the plateau of the MVC was compared with a single 100μs resting twitch torque evoked ~1s following the MVC. Percent voluntary activation was calculated as voluntary activation (%) = [1-(interpolated twitch /resting twitch)] x 100.

Following the MVCs, participants were given 5min rest to ensure no residual fatigue. The investigator then inserted and manipulated the concentric needle to minimize rise times of the negative-peak amplitudes of the first 2-3 detected motor unit potentials (MUPs). Once the investigator was satisfied with the needle position, the participants were asked to slowly increase their dorsiflexion torque to match a target line of 25% MVC within 1-2s and hold the contraction steady for ~30s, during which both the intramuscular and surface EMG were sampled and stored for future analysis. This contraction intensity was found to be the most effective for obtaining a representative MUNE in the TA (McNeil, Doherty, Stashuk, & Rice, 2005a). Participants were given at least 1min of rest between these contractions. Between contractions, repositioning of the needle was completed by either adjusting the depth of insertion or sampling from a new area. These procedures were repeated until at least 20 suitable trains of MUPs and their respective SMUPs were collected.

Decomposed EMG signals were reviewed off-line to determine the acceptability of the needle-detected MUP trains and their corresponding SMUPs. First, an acceptable MUP train required greater than 50 detected discharges which acted as triggers for spike-triggered averaging. Then, the MU discharge pattern was inspected visually for a constant rate (i.e. coefficient of variation $\leq$ 30%) and a physiological mean discharge rate. Lastly, the interdischarge interval histogram was examined to confirm that it followed a Gaussian distribution. The SMUP trains which did not meet these criteria were excluded from further analysis (Boe, Stashuk, & Doherty, 2004). The SMUPs were inspected visually to identify a distinct waveform which was temporally linked to the needle potential. The computer generated negative-peak onset and negative-peak amplitude markers of the acceptable SMUPs were inspected to ensure they were accurate. Any markers not correctly set were repositioned manually. A computer algorithm then

aligned the negative onset markers for all accepted SMUPs and created a mean SMUP template based upon their data-point by data-point average (Doherty & Stashuk, 2003; Doherty, Stashuk, & Brown, 1993). Finally, a MUNE was derived by dividing the negative-peak amplitude of the CMAP by the negative peak amplitude of the mean SMUP. These methods have been previously used (Dalton et al., 2008; McNeil et al., 2005b; Power et al., 2012; Power et al., 2010).

3.5 Statistics

All data were analyzed using SPSS software (version 18, SPSS Inc. Chicago, Illinois). Univariate analyses of variance (ANOVA) were performed to identify the differences among groups. The level of significance was set at $P \leq 0.05$. When a significant main effect was present, a Tukey HSD post hoc test was performed to identify where significant differences existed. Since voluntary activation values are not normally distributed, a Mann-Whitney U-test was employed to test for statistical significance of this variable. A power calculation was performed on the results to ensure sufficient power ($1 - \beta > 0.80$) was achieved. All data are presented as mean ± SD.

Chapter 4

4 Results

4.1 Participant Characteristics

Table 1 shows the mean values for age, height, and mass for the participants for the three age groups. There were no significant differences among the groups for height or mass ($P > 0.05$). The young ranged in age from 22 to 30 years old, the old ranged in age from 60 to 73 years old, and the very old ranged in age from 76 to 85 years old.

Table 1: Participant characteristics.

Group	**Age (years)**	**Height (cm)**	**Mass (kg)**
Young (n = 12)	25 ± 3	179.0 ± 5.3	82.8 ± 9.5
Old (n = 6)	68 ± 5*	175.0 ± 3.9	95.1 ± 15.1
Very Old (n = 6)	79 ± 3*†	174.2 ± 3.8	80.3 ± 10.8

Values are means ± standard deviations. *significantly different than young, †significantly different than old ($P < 0.05$).

4.2 MR Sample Images

Figure 2 shows representative MR images of young, old, and very old participants. Row A contains images taken without off-resonance presaturation of the young, old, and very old men. Row B contains images taken with off-resonance presaturation of the young, old, and very old men. Sample ROIs are outlined in white.

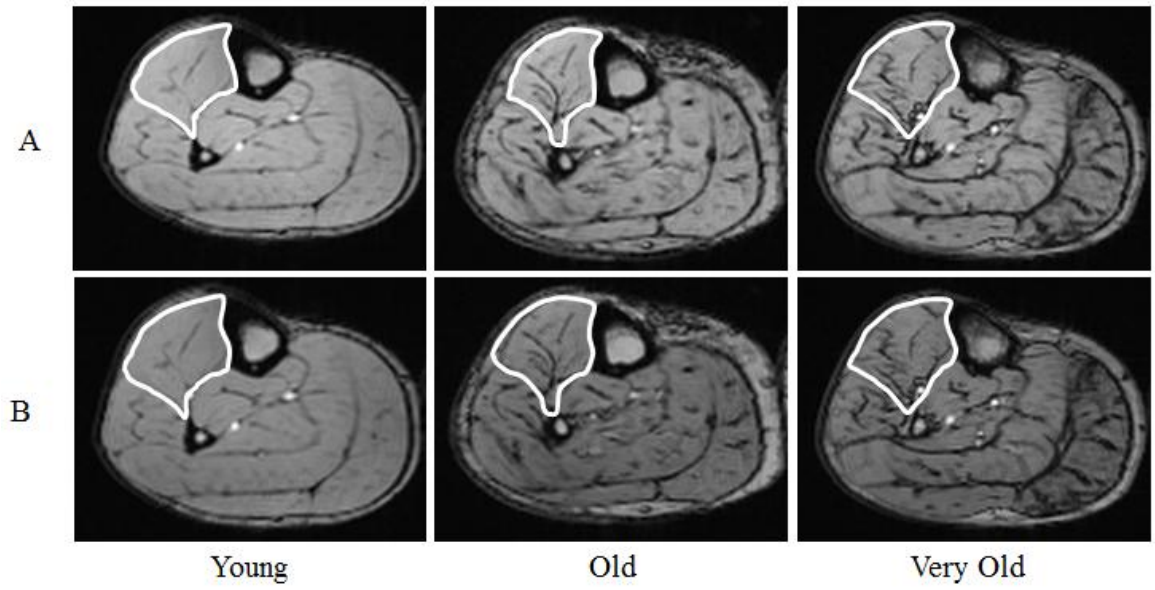

Figure 2: Representative MR images of the right leg of young, old, and very old participants including a sample outline of a ROI in white.

4.3 Muscle Quantity

4.3.1 CSA, CSA Composition, & Volume

As shown in Table 2, total CSA was not significantly different across all three groups ($P > 0.05$); however, contractile CSA was significantly lower in the very old ($11.2 \pm 1.0 cm^2$) men versus the young ($12.7 \pm 1.6 cm^2$) ($P = 0.042$) men, but not between the young and old ($P = 0.129$). Absolute noncontractile CSA was higher in the very old ($3.6 \pm 1.8\ cm^2$) than the young ($1.8 \pm 0.5 cm^2$) ($P = 0.010$) but not the old ($3.0 \pm 1.1 cm^2$) ($P = 0.105$).

Table 2: CSA & CSA composition.

Group	Contractile (cm^2)	Noncontractile (cm^2)	Total CSA (cm^2)
Young	13.4 ± 1.9	1.8 ± 0.5	15.2 ± 2.2
Old	11.7 ± 1.4	3.0 ± 1.1	14.7 ± 1.3
Very Old	11.2 ± 1.5*	3.6 ± 1.8*	14.8 ± 1.0

*significantly different than young ($P < 0.05$).

As a percentage of total muscle CSA, the noncontractile tissue accounted for a significantly higher percentage in the old (20.5 ± 7.2%) ($P = 0.048$) and very old (24.2 ± 10.6%) ($P = 0.004$) compared to the young (12.0 ± 3.3%) (Figure 3).

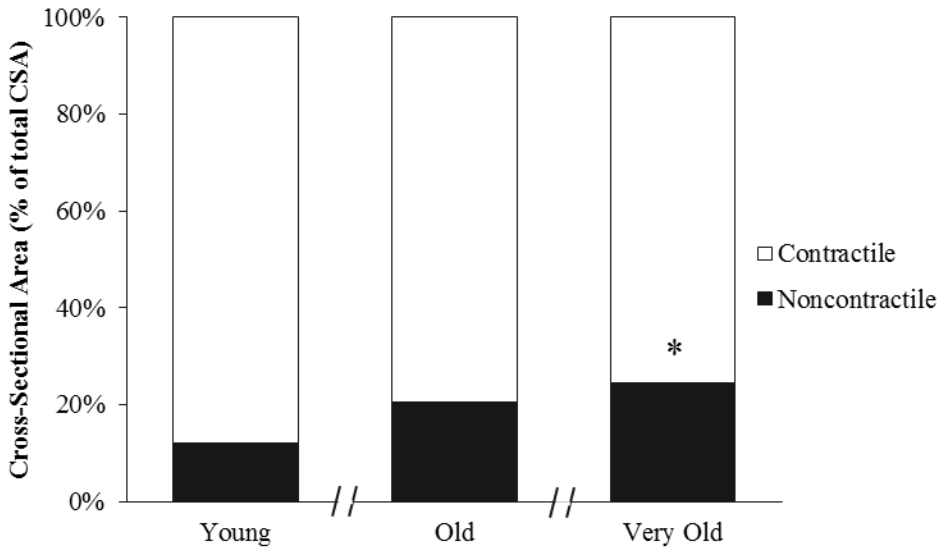

Figure 3: CSA composition as a percent of total CSA in the young, old, and very old men. *significantly different than young ($P < 0.05$).

Muscle volume showed a significant decrease among the three age groups (Figure 4). Muscle volume was ~17% lower in the old and ~19% lower in the very old men than the young men.

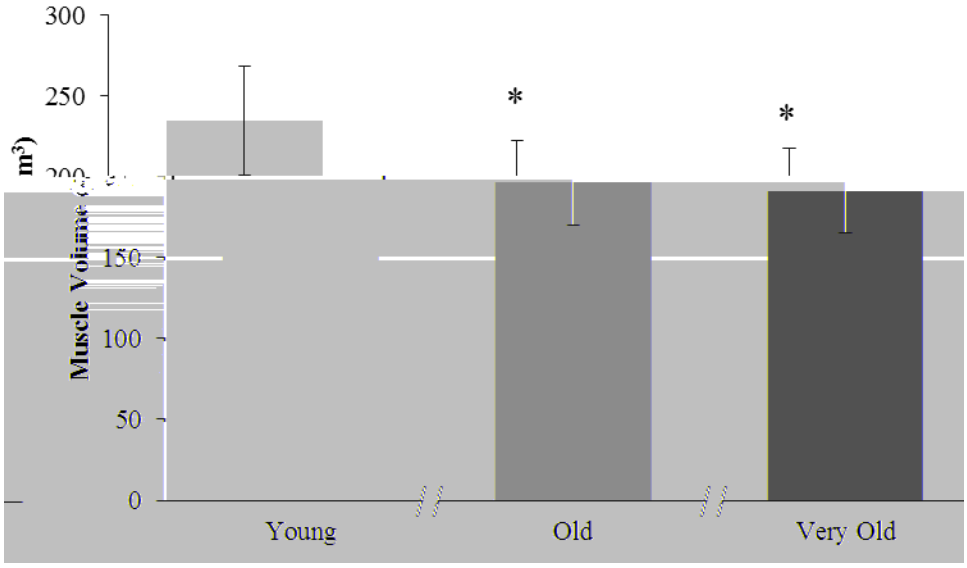

Figure 4: Muscle volume in young, old, and very old men. *significantly different than young ($P < 0.05$).

4.4 Muscle Quality

4.4.1 Normalized Strength

Maximal voluntary isometric torque was ~26% lower in the old (P = 0.002) and ~24% lower in very old men (P = 0.005) compared to the young men (Table 3).

Table 3: Strength and normalized strength values for the TA.

Group	MVC (Nm)	Strength Normalized to Total CSA (Nm/cm^2)	Strength Normalized to Contractile CSA (Nm/cm^2)	Strength Normalized to Volume (Nm/cm^3)
Young	53.9 ± 7.8	3.6 ± 0.4	4.0 ± 0.4	0.23 ± 0.02
Old	39.8 ± 6.5*	2.7 ± 0.6*	3.4 ± 0.6	0.20 ± 0.03
Very Old	40.9 ± 6.4*	2.8 ± 0.3*	3.7 ± 0.6	0.21 ± 0.03

*significantly different than young (P < 0.05).

When strength was normalized to total CSA, both the old (2.8 ± 0.3Nm/cm^2) (P = 0.002) and very old (2.7 ± 0.6Nm/cm^2) (P = 0.003) men showed significantly lower normalized strength than the young men (3.6 ± 0.4Nm/cm^2) (Figure 5 – hatched bars).

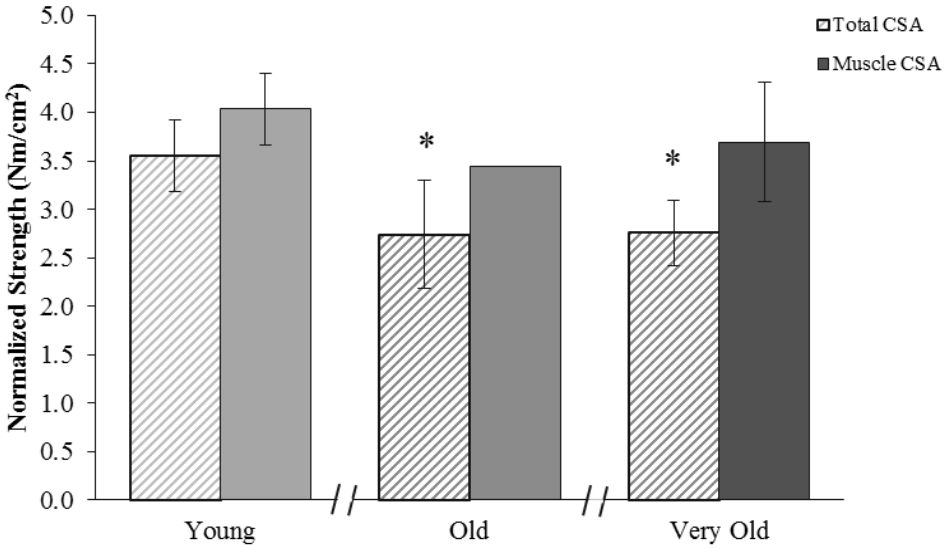

Figure 5: Strength normalized to total CSA and muscle CSA. *significantly different than young (P < 0.05).

However, when strength was normalized to only contractile CSA, the differences were no longer present between the young (4.0 ± 0.4Nm/cm^2), old (3.4 ± 0.6Nm/cm^2), and very old (3.7 ± 0.6 Nm/cm^2) ($P > 0.05$) (Figure 5 – solid bars).

Similarly to CSA, although significant differences existed among the groups in volume measures when strength was normalized to volume, no significant difference existed between the young (0.23 ± 0.02Nm/cm^3), old (0.20 ± 0.03Nm/cm^3), and very old (0.21 ± 0.03Nm/cm^3) ($P > 0.05$) (Figure 6). This is due to a concurrent decrease of strength and CSA or strength and volume.

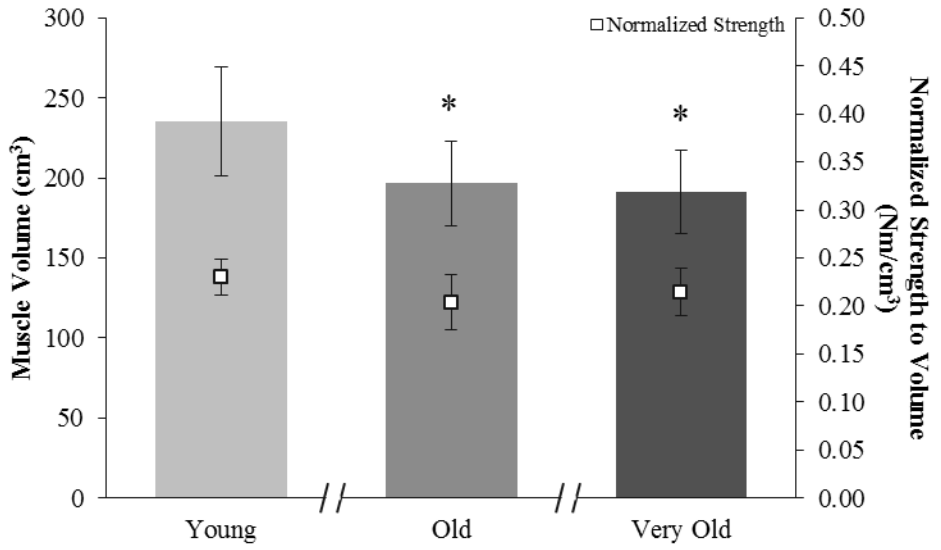

Figure 6: Absolute muscle volume to strength normalized to volume. *significantly different volume than young ($P < 0.05$).

4.4.2 MTR

As shown in Figure 7, MTRs were significantly greater between the young (0.262 ± 0.01p.u.) and very old (0.242 ± 0.02p.u.) ($P = 0.049$) as well as the old (0.266 ± 0.01p.u.) and very old ($P = 0.036$) but not significantly different between the young and old ($P = 0.844$).

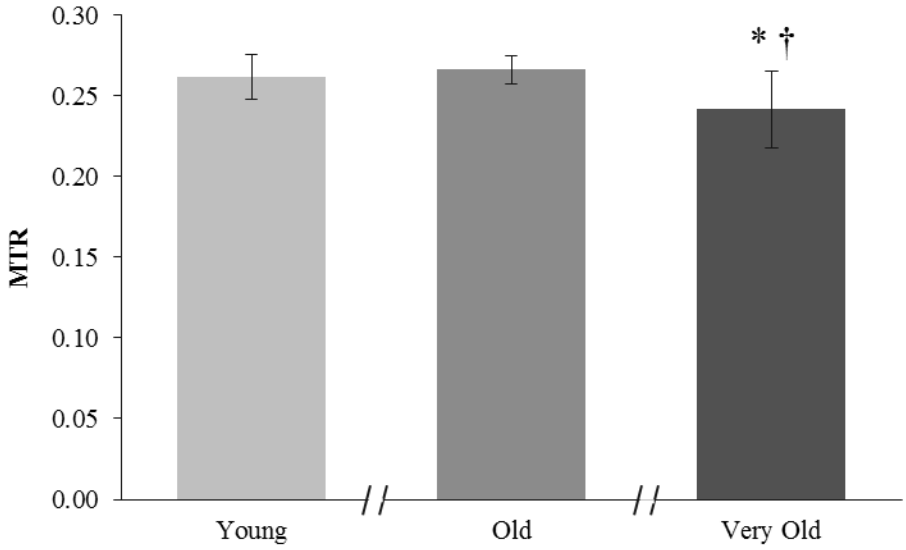

Figure 7: Magnetization transfer ratios young, old, and very old men. *significantly different than young, †significantly different than old ($P < 0.05$).

4.4.3 Neuromuscular Properties

Although there was no significant differences among groups for twitch torque ($P > 0.05$); time-to-peak torque and half relaxation times were significantly longer in the old [24.6% ($P = 0.015$) and 26.9% ($P < 0.001$), respectively] and very old [30.7% ($P = 0.003$) and 29.1% ($P < 0.001$), respectively] men compared to the young men. As a consequence, contraction duration (time-to-peak torque + half relaxation time) was ~26% longer in the old men and ~30% longer in the very old men. Voluntary activation, as assessed by the interpolated twitch technique, was near maximal (>94%) in all three groups during MVCs (Table 4).

Table 4: Neuromuscular properties of the TA.

Group	VA (%)	Pt (Nm)	TPT (ms)	HRT (ms)	CD (ms)
Young	96.2 ± 2.4	6.6 ± 2.2	78.5 ± 14.5	94.2 ± 8.5	172.7 ± 19.1
Old	95.8 ± 2.1	5.8 ± 1.3	97.8 ± 12.9*	119.5 ± 10.8*	217.3 ± 10.9*
Very Old	94.6 ± 1.9	6.2 ± 1.4	102.6 ± 5.7*	121.6 ± 13.7*	223.7 ± 15.0*

MVC = absolute maximal isometric contraction torque, VA = voluntary activation, Pt = twitch torque, TPT = time-to-peak tension, HRT = half-relaxation time, CD = contraction duration. Values are means ± standard deviations. * significantly different than young ($P < 0.05$).

The maximum negative-peak amplitude of the CMAP was not significantly different between the young (6.8 ± 1.1mV), old (5.6 ± 1.4mV), and very old (5.5 ± 1.0mV) men ($P > 0.05$) (Figure 8A). The negative-peak amplitude of the mean SMUP was only significantly higher in the very old men (72.8 ± 22.8μV) compared to the young (47.5 ± 8.3μV) ($P = 0.015$) men. The SMUP of the old men (54.3 ± 21.5μV) was not significantly different from the young or very old men ($P > 0.05$) (Figure 8B).

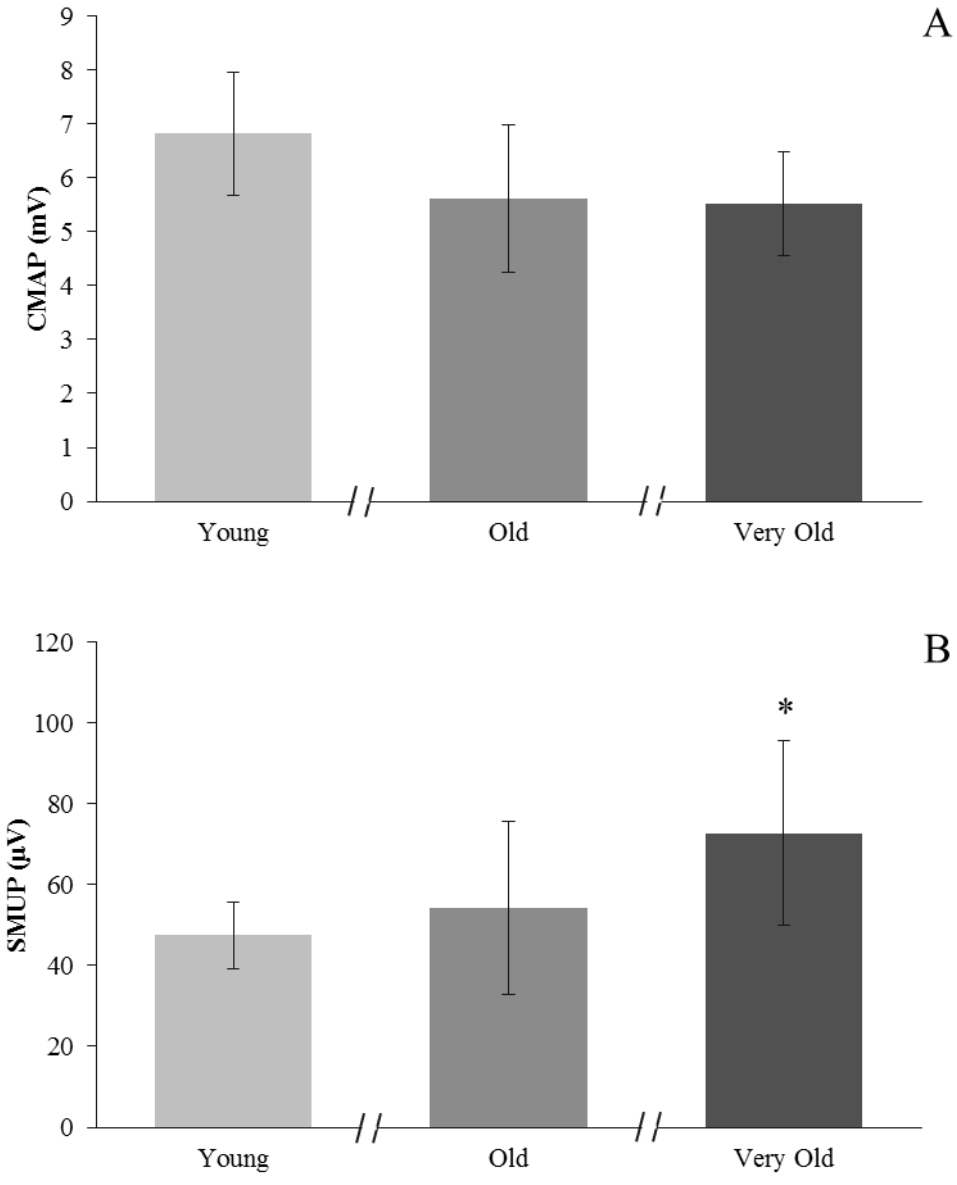

Figure 8: Compound muscle action potentials (A) and surface motor unit potentials (B) of the young, old, and very old men. *significantly different than old ($P < 0.05$).

A decrease in mean CMAPs with not significantly different SMUPs in the old men resulted in significantly lower MUNE compared to the young men ($P = 0.033$). The combination of a smaller mean CMAP with significantly lower SMUP of the very old men resulted in significantly lower MUNE than both the young ($P < 0.001$) and old ($P = 0.03$) men. It was estimated that the young men had 146 ± 31 MUs, the old men had 109 ± 25 MUs, and the very old men had 80 ± 21 MUs (Figure 9).

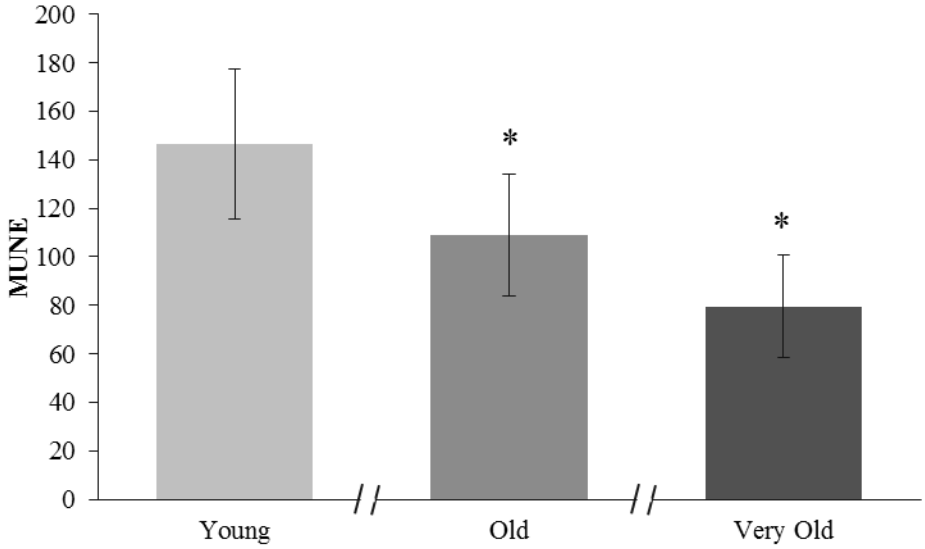

Figure 9: Motor unit number estimates for young, old, and very old men. *significantly different than young ($P < 0.05$).

Chapter 5

5 Discussion

The multidimensional nature of sarcopenia highlights the importance of examining both quantitative and qualitative changes occurring in the muscles with aging. The progression of sarcopenia is a gradual process marked by a loss of neuromuscular function up to the sixth and seventh decade of life. Following this, in the eighth decade of life and beyond, there is a precipitous loss of muscle mass and function (Berger & Doherty, 2010). Our results suggest that muscle mass is maintained in the old, likely due to collateral reinnervation, whereas a slowing of contractile properties becomes evident suggesting other intrinsic factors are affecting sarcopenia. The subsequent precipitous decline of muscular function in very old men is at least partly due to MU death and structural integrity decreases. With very old age, MU death out paces remodeling resulting in muscle fiber atrophy, and muscle quality decreases, leading to an accelerated loss of muscular function. We confirmed this via a combination of CSA, volume, normalized strength, a novel MRI technique (MTR), and electrophysiological/neuromuscular measures across three age categories of men. This combination of MRI and neuromuscular measures gave us exciting new insights into the progression of sarcopenia. However, it is important to note that in accordance with this study and previous research, sarcopenia cannot be explained by a single process but is caused by a number of contributing factors that converge to accelerate a gradual decrease into a rapid decline in muscular function.

Although total CSA was not different among the three groups, muscle atrophy did occur in the very old men. In the very old men, contractile tissue decreased and noncontractile increased. Decreased contractile CSA has been shown to be related to the strength deficits and functional limitations of sarcopenia (Newman et al., 2003; Visser et al., 2005). Contractile CSA is the more appropriate measure than total CSA for evaluating muscle quality through normalized strength. It could be speculated the quality of the contractile tissue (force per unit of muscle) is maintained in the old and very old. Therefore, the contractile tissue of the old and very old men is still capable of performing

similarly to the young men. These findings are similar to what Kent-Braun & Ng (1999) found for CSA using MR images of the TA in men with aging. With age, as muscle fibers are abandoned, muscle mass is lost and noncontractile tissue infiltrates the muscle. However, the remaining muscle in the old and very old appears to have similar contractile torque properties as young counterparts. Although absolute strength was lower in the old and very old, when this strength was normalized to muscle quantity the differences no longer existed. This coincides with previous research which found that the contractile properties of single muscle fibers are preserved with aging (Frontera et al., 2008; Frontera, Suh, et al., 2000). In contrast, previously McNeil et al. (2007) found a decrease in normalized strength in a very old age group of men. In that study, the very old men were found to have a decrease MVC, but not significantly different CSA which accounted for the difference in normalized strength. In the current study, the very old men had a concurrent loss of strength and muscle mass resulting in no differences in normalized strength. The very old group in the study by McNeil et al. (2007) was in the ninth decade of life, which may account for the differences. Muscle contractile force as a reflection of muscle quality may be more affected than CSA in the ninth decade of life. The functional effects of the muscular structure decline may not become apparent until later in the aging process (Sinclair et al., 2012). Magnetization transfer ratios may be a more sensitive measure of muscle quality and the functional declines to follow, as MTR depicts a decrease in muscle quality in the eighth decade of life, but normalized strength does not depict a decrease in muscle quality until the ninth decade of life (McNeil et al., 2007). Although the current study was cross-sectional in design, Figure 10 is a depictive possible outline of the progression of sarcopenia showing that first MUNE declines, followed by MTR then normalized strength.

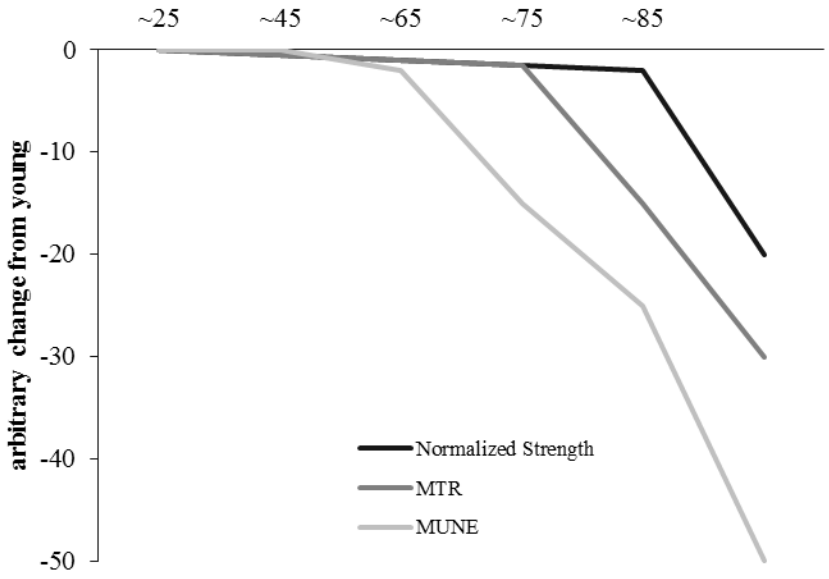

Figure 10: Predictive timeline of sarcopenia. MTR declines may precede normalized strength declines.

Magnetization transfer ratio has previously been used to measure muscular structure and therefore, muscle quality. Sinclair et al. (2012) found a significant decrease of MTR in lower limb muscle of clinical neuropathy patients. In the middle-aged neuropathy patients, ~17% lower MTR values were found compared to young. Magnetization transfer ratio is reflective of muscle quality in neuropathic cases, and therefore also a useful measure of muscle quality in healthy cases. In support, Schwenzer et al. (2009) found ~6% lower MTR values for healthy old versus young participants. Including a group in the eighth decade of life is a novel and worthwhile approach to examining muscle quality using MTR. Similarly, MTR in this study were ~8% lower in the very old than the young and old groups.

In this study, MUNE results were similar to previous research on the TA with aging in young, old, and very old men by McNeil et al (2005b). An age difference of 45 years between the young and old group showed a decrease of ~25% in mean MUNE, whereas in the 20 years difference between the old and very old groups, another ~27% decrease in mean MUNE was shown. The compensatory strategy of collateral reinnervation maintains most of the muscle mass from atrophy by dying MUs. Intact MUs sprout axon branches and acquire any orphaned muscle fibers resulting in larger MUs (increasing the

SMUP) with slower contractile speeds (TPT + HRT) due to tendency of Type II fibers to die first (Morley et al., 2001) and Type I MUs to innervate those fibers giving them Type I fiber properties. Without this compensatory mechanism, there would be a much greater loss of muscle mass. With continued aging, this compensatory strategy cannot keep up with motor neuron death, as demonstrated by the negative-peak amplitude of the SMUP being significantly higher in only the very old men. When MUs with a greater number of muscle fibers die (decreased MUNE), a larger amount of excitable muscle mass (decreased CMAP and CSA) is lost, resulting in further reduced mass and strength.

Together, the results from the current study suggest that both muscle quantity and muscle quality are declining with age. In the old men, MTR was maintained due to the ability of the compensatory mechanisms of MU remodelling maintaining muscle mass and function. In the very old men, MTR declined due to the inability of the compensatory mechanisms of MU remodeling to maintain muscle mass as a precursor to a decline in muscle function (Figure 11).

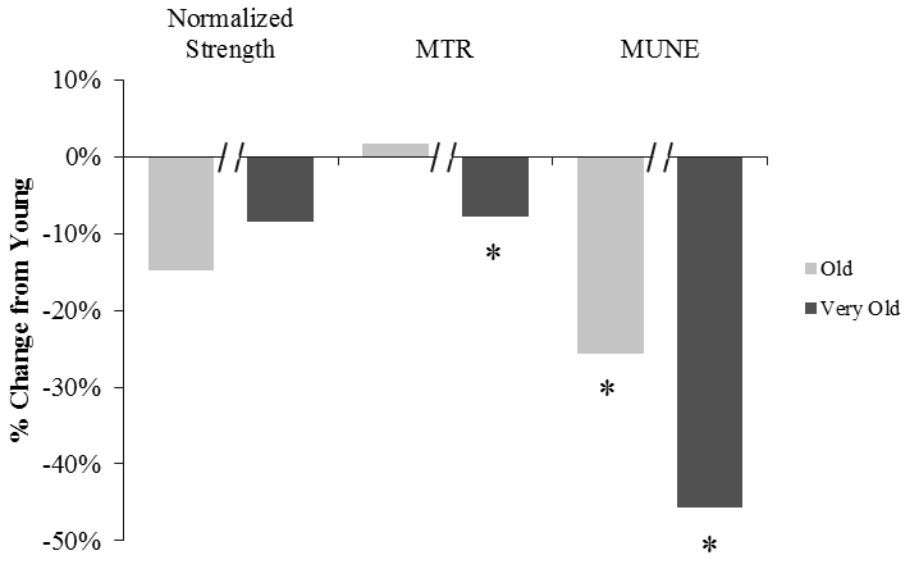

Figure 11: Percent changes from young compared to old and very old men in the measures of muscle quality. *significantly different than young ($P < 0.05$).

5.1 Summary

In response to the research questions posed at the beginning of the study, the following conclusions were made:

1. **How does muscle quantity, including CSA, CSA composition, and muscle volume differ between young, old, and very old populations of men?** Muscle quantity was decreased between the young, old, and very old men. Although total CSA was not significantly different between the three groups, the very old men had a significantly lower muscle CSA than both the young and old men. This coincided with an increase in noncontractile tissue CSA in the very old men. Muscle volume was significantly lower in both the old and very old men compared to the young men.

2. **How does muscle quality, such as normalized strength, MTR, and neuromuscular measures differ between young, old, and very old populations of men?** Muscle quality results varied among the age groups depending on the evaluation method. Normalized strength did not decline among any of the three groups (Figure 4). Magnetization transfer ratios were only significantly lower in the very old men. Neuromuscular properties, in particular MUNE, showed significant decreases between the young and both the old and very old men.

3. **Is there an ideal measure of muscle quality that reflects muscle function?** Normalized strength, MTR, and neuromuscular techniques of evaluating muscle quality each have their advantages and disadvantages. Normalized strength gives insight into the contractile properties of the muscles, but not the functional declines; MTR may be a sensitive and early detector of muscle quality changes, but does not reflect current functional properties; MUNE is able to demonstrate that MU remodeling occurs, but it is not able to show the amount of muscle mass lost.

4. **Does muscle quantity affect muscle quality or does muscle quality affect muscle quantity?** The causality dilemma of whether muscle quality affects

muscle quantity or whether muscle quality affects muscle quantity cannot be definitively answered. Muscle quantity affects muscle quality and muscle quality affects muscle quantity; it is an intricate interrelated relationship. Mechanisms that maintain muscle quantity also preserve muscle quality.

5.2 Conclusions

In conclusion, sarcopenia has two stages of progression: a gradual decline in muscle function in an old population, followed by an accelerated decline in muscle function in a very old population. During the gradual decline, muscle volume is less in the old than the young. Motor unit number estimates are less in the old than the young, but normalized strength and MTR are not different among the groups. Around the eighth decade of life, the previously gradual decline in muscle function accelerates. In this accelerated decline, contractile CSA and muscle volume are decreased in the young versus very old. Motor unit number estimates are lower in the very old than the young. Although normalized strength has yet to experience a decline, MTRs are significantly lower in the very old than both the young and old.

5.3 Strengths of the Study

A strength of this study was the use of MRI to analyze CSA and muscle volume. This non-invasive tool is very accurate and allows for the exclusion of noncontractile tissue.

Studies on human aging which compare only two groups, young and old, do not provide insight on the degree or rate of change of sarcopenia especially in the later years (i.e. >70 years old) in which faster declines are typically experienced. Another strength of this study is the implementation of a third very old group of men.

Using a muscle such as the TA was an appropriate model for examining MUNE and MTR for both qualitative and quantitative reasons. Qualitatively, participants were more comfortable having just their legs in the MR scanner. The TA is an accessible muscle for performing the MUNE technique, due to the accessibility of the fibular nerve, and unlike the soleus, the lesser volume eliminates conduction issues allowing for reliable CMAPs. Quantitatively, the TA is a high use, high impact muscle during activities such as

walking, which was the majority of activity for participants. Previous reports have been made on the TA for normalized strength (Kent-Braun & Ng, 1999; McNeil et al., 2007), MUNE (Boe, Dalton, Harwood, Doherty, & Rice, 2009; McNeil et al., 2005b), and MTR (Schwenzer et al., 2009). Typically, the muscle fiber composition of the TA is more Type I fibers than Type II (Enoka, 2008). This is an advantage because Type II muscle fiber appear to selectively die first (Morley et al., 2001), so MU death with aging would be shown earlier in muscles with this type of composition. Additionally, muscle composed of an even higher percentage Type I fibers, such as the soleus, have been shown to maintain a large majority of MUs with age (Dalton et al., 2008).

The comprehensive combination of evaluations which include measurements of both muscle quantity and muscle quality is a major strength of this study. Having physiological, morphological, and neuromuscular measures allow for an in depth and more comprehensive evaluation of the muscle properties and the development of sarcopenia.

5.4 Limitations of the Study

Limitations of this study are a small samples size in the old and very old groups, the lack of muscle biopsies, and histochemical analysis. Muscle biopsies would complement and confirm inferences made on the fiber type composition changes from type II to predominately type I. Histochemical analysis would have allowed for observations of the hormonal changes affecting the old men with sarcopenia.

Longitudinal aging studies are difficult to complete, so this study has employed a cross-sectional study design. Cross-sectional studies, however, do not allow for detail into the progression of sarcopenia along a continuous timeline but instead, a glimpse into several stages of aging. Another limitation of a cross-sectional design is in the selection of participants. Cross-sectional studies underestimate age-related change in muscle function (Frontera, Hughes, et al., 2000) and represent a comparison only with those that have survived advanced age. Persons with stronger muscles may have a better chance of being included in cross-sectional studies because they have survived to old age (Frontera et al., 2008).

5.5 Future Directions

Future studies should address the muscle biopsy and histochemical limitations and further examine muscle changes with dynamic contractions, force steadiness, and balance. Dynamic measures such as contraction velocity and power would give valuable information about the contractile and functional properties of the muscle. Force steadiness and balance are both functional measures that could be matched to the muscle measures to validate conclusions about the effects of changing muscle on function with aging. Loss of muscular strength is more subtle in eccentric testing conditions (Hortobagyi et al., 1995; Vandervoort, Kramer, & Wharram, 1990) and therefore, when examining the dynamic properties of the muscle both concentric and eccentric testing conditions should be compared.

Other MR techniques such as short tau inversion recovery imaging and T2 weighted images (Kamath, Venkatanarasimha, Walsh, Hughes, 2008; Sinclair et al., 2012) can be used to look at the denervation within the muscle and be correlated with MUNE. Ultrasound to determine fascicle length and angle of pennation would also be a potentially useful technique to combine with these other measures. Muscle strength is dependent on fascicle length and angle of pennation by which the tendon inserts into the muscle, both of which may alter with age (Binzoni et al., 2001; Narici, Maffulli, & Maganaris, 2008).

It is unknown whether our results are selective to the TA or whether a similar pattern is followed in other muscles. Muscle composition of type I and type II fibers varies among muscles depending on the requirements and function of the muscle. Examining several muscles with various muscle compositions would also be important when examining sarcopenia (Vella & Kravitz, 2002).

In order to continue furthering our insights into sarcopenia, we must also examine all these measures across several different populations. Continuing this study by including a middle-aged group and an older population of men in the ninth and tenth decades of life would give insight into the continued progress of sarcopenia, following the precipitous decline experienced beyond the eighth decade of life. Considering all of these measures

in groups of women would be a valuable next step in sarcopenia research, as women may experience sarcopenia differently than men. Examining sex differences may bring further understanding of sarcopenia and may help devise inclusive prevention techniques. Clinical populations would also be useful to compare healthy aging muscle to diseased aging muscle.

Although longitudinal studies in humans from adult to old age are challenging to accomplish, a longitudinal study would give a higher degree of specificity of age-related changes, especially during the years of faster declines.

With a thorough understanding, a valid and reliable standardized definition and clinical evaluation, that includes severity and preventions, can be created (Abellan van Kan et al., 2011; Pahor, Manini, & Cesari, 2009) for sarcopenia.

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Appendices

Appendix A: MRI Subject Screening Questionnaire.

LAWSON HEALTH RESEARCH INSTITUTE	3 TESLA MRI FACILITY	LAWSON IMAGING
SECTION: ADMINISTRATION		
SUBJECT: MR Screening Questionnaire		**NUMBER:1-07**

NAME: _____ HEIGHT: _____
DATE OF BIRTH: _____ WEIGHT: _____

1. Have you had a previous MRI? ____(circle response)_____Yes___No

2. Have you ever had a metallic object in your eye? _____Yes___No

3. Is there any chance you might be pregnant? _____Yes___No

4. Do you have any of the following?
   - HEART PACEMAKER/WIRES/STENT/DEFIBRILLATOR___Yes___No
   - BRAIN ANEURYSM CLIPS_____ Yes___No
   - SHUNT/SURGICAL CLIPS_____ Yes___No
   - SHRAPNEL/BULLETS._____ Yes___No
   - DENTURES_____ Yes___No
   - INTRA-UTERINE DEVICE (IUD)_____Yes___No
   - OTHER IMPLANTED DEVICES (HEART VALVES, EAR IMPLANTS, PROSTHESES, EYE SPRINGS) _____ Yes___No
   - MEDICATION PATCHES _____Yes___No
   - BODY PIERCING_____ Yes___No
   - PERMANENT TATTOO/EYELINER _____ Yes___No

5. Please list surgeries on the following:
   - Head _____
   - Neck _____
   - Spine _____
   - Chest _____
   - Abdomen _____
   - Extremities _____
   - Other _____

6. Are you claustrophobic? _____ _____Yes___No

Participant's Signature: _____

Date: _____

MR operator's Signature: _____

THIS FORM MUST BE COMPLETE AND ALL METAL REMOVED BEFORE YOU CAN UNDERGO YOUR MRI SCAN. ALL SUBJECTS MUST CHANGE INTO CLOTHING THAT HAS NO METAL FASTENERS OR UNDERWIRES. HOSPITAL GOWNS, PANTS, AND A LOCKER FOR VALUABLES ARE PROVIDED.

Appendix B**:** UWO Ethics Approval for MRI Research on Humans.

Office of Research Ethics

The University of Western Ontario
Room 5150 Support Services Building, London, ON, Canada N6A 3K7
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. G. Marsh
Review Number: 17762 **Review Level:** Full Board
Review Date: January 25, 2011 **Approved Local # of Participants:** 90
Protocol Title: Comparison of bone geometry and muscle volume in the legs of young, old and very old females versus males using magnetic resonance imaging (MRI).
Department and Institution: Kinesiology, University of Western Ontario
Sponsor:
Ethics Approval Date: March 09, 2011 **Expiry Date:** August 31, 2015
Documents Reviewed and Approved: UWO protocol (including instruments noted in Section 8.1), Letter of Information and Consent Form and Advertisement
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (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 REB 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 UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

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

Chair of HSREB: Dr. Joseph Gilbert
FDA Ref. #: IRB 00000940

Ethics Officer to Contact for Further Information			
☐ Janice Sutherland (jsutherl@uwo.ca)	☐ Elizabeth Wamboldt (ewamboldt@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)	

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

cc: ORE File

Appendix C: UWO Ethics Approval for Neuromuscular Research on Humans.

Use of Human Participants - Ethics Approval Notice

Principal Investigator: Dr. Tim Doherty
Review Number: 18141
Review Level: Full Board
Approved Local Adult Participants: 60
Approved Local Minor Participants: 0
Protocol Title: Impacts of Diabetes on the Neuromuscular System in Humans
Department & Institution: Clinical Neurological Sciences,London Health Sciences Centre
Sponsor:
Ethics Approval Date: August 17, 2011 **Expiry Date:** May 31, 2015

Documents Reviewed & Approved & Documents Received for Information:

Document Name	Comments	Version Date
Letter of Information		2011/07/28
UWO Protocol	Including all instruments listed in section 8.1	
Letter of Information & Consent	Healthy volunteers	2011/07/28

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 UWO 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 UWO 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

Janice Sutherland (jsuther1@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.

Curriculum Vitae

Name: William J. Booth

Post-secondary Education and Degrees:

Master of Science (*Supervisor: Dr. Greg D Marsh*)
The University of Western Ontario
London, Ontario, Canada
2010 – 2012

Bachelor of Arts in Human Kinetics
St. Francis Xavier University
Antigonish, Nova Scotia, Canada
2005 – 2010

Honours and Awards:

Western Graduate Research Scholarship
2010 – 2012

St. Francis Xavier University Dean's List
2009 – 2010

Related Work Experience

Teaching Assistant
Introductory Exercise Physiology
The University of Western Ontario
2011 – 2012

Publications:

Harwood, B., Power, G.A., Allen, M.D., Booth, W.J. (2011). Tendon vibration does not alter decreased responsiveness of motoneurones in the absence of motor cortical input during fatigue. *Journal of Physiology*, 589(23):5559-5560.

Booth, W.J., Allen, M.D., Power, G.A., Marsh, G.D., Rice, C.L. (2012). Motor unit loss precedes reductions in muscle viability in old and very old men: new insights into the progression of sarcopenia.

Conference Presentations:

Booth, W.J., Upshaw, A.N., Wilkinson, S.B., Lemon, P.W.R., Rice, C.L., Marsh, G.D. Resistance training induced similar hypertrophy in the arm and thigh musculature of young women. ACSM Annual Meeting, San Francisco, CA. 2012. Accepted.

Booth, W.J., Barnett, M., Dalton, B.H., McNeil, C.J., Rice, C.L., Marsh, G.D. Leg muscle strength, area, and contraction velocity in young, old and very old women. *Medicine & Science in Sports & Exercise*, 43:S298. ACSM Annual Meeting, Denver, CO. 2011.