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THE EFFECTS OF AGING ON SKELETAL MUSCLE MORPHOLOGY AND NEUROMUSCULAR FUNCTION OF THE LEG EXTENSORS

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TRENT J. HERDA
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THE EFFECTS OF AGING ON SKELETAL MUSCLE MORPHOLOGY AND NEUROMUSCULAR FUNCTION OF THE LEG EXTENSORS

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF HEALTH AND EXERCISE SCIENCE

BY

_____
Dr. Joel T. Cramer, Chair

_____
Dr. Jeffrey R. Stout

_____
Dr. Chad M. Kerksick

_____
Dr. Travis W. Beck

_____
Dr. Trina L. Hope

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ABSTRACT

THE EFFECTS OF AGING ON SKELETAL MUSCLE MORPHOLOGY AND NEUROMUSCULAR FUNCTION OF THE LEG EXTENSORS

Trent J. Herda, Ph.D.

The University of Oklahoma, 2011

Supervising Professor: Joel T. Cramer, Ph.D.

The purpose of the present study was to examine the muscle cross-sectional area (CSA), thigh skinfold thickness (SF), maximal voluntary contraction (MVC) peak force (PF), and the log-transformed EMG and MMG amplitude (RMS)-force relationships (vastus lateralis [VL] and rectus femoris [RF]) of isometric step and ramp muscle actions of the right leg extensors for individuals between the ages of 20-75 years. In addition, myosin heavy chain (MHC) isoform content of the VL was analyzed to characterize individual fiber type composition. Fifty-seven healthy men volunteered for this investigation and were categorized into groups by age: 20-25 (n = 10; mean ± SD age = 22.3 ± 2.5 yrs; stature = 177.5 ±7.3 cm; mass = 82.0 ± 17.2 kg), 30-35 (n = 10; age = 32.3 ± 1.6 yrs; stature = 175.4 ± 6.8 cm; mass = 89.8 ± 13.4 kg), 40-45 (n = 10; age = 42.6 ± 2.3 yrs; stature = 180.0 ± 6.2 cm; mass = 86.7 ± 11.2 kg), 50-55 (n = 8; age = 52. 9 ± 1.9 yrs; stature = 174.8 ± 6.1 cm; mass = 93.6 ± 11.7 kg), 60-65 (n = 9; age = 62.8 ± 2.1 yrs; stature = 175.0 ± 6.3 cm; mass = 83.6

± 12.1 kg), and 70-75 (n = 10; age = 73.5 ± 2.5 yrs; stature = 171.5 ± 7.2 cm; mass = 80.2 ± 12.8 kg) years of age. Thigh SF measurements were taken from the VL and the mid-thigh muscle CSA, from a peripheral quantitative computed tomography scanner, was taken at the site of MMG electrode placement. Subjects performed two MVCs, nine submaximal voluntary contractions (10, 20, 30, 40, 50, 60, 70, 80, and 90% MVC of the highest pre-testing MVC value) in random order, and two 6-s isometric ramp muscle actions from 10 to 100% of their MVC. Linear regression models were fit to the natural log-transformed EMG_{RMS} and MMG_{RMS}-force relationships. In addition, Bergstrom muscle biopsies were taken from the VL and were analyzed for MHC isoform content. Type I MHC isoform content was higher for the 70-75 (mean ± SD 45.8 ± 5.3%) than the 20-25 age group (37.48 ± 3.94%). For MVC PF, the 20-25 (746.2 ± 305.2 N) and the 30-35 (812.9 ± 230.7 N) age groups were higher than the 70-75 (459.7 ± 223.4 N) age group. Muscle CSA was greater for the 30-35 (190.8 ± 7.9 cm^2) than the 60-65 (148.6 ± 20.4 cm^2) and 70-75 (140.0 ± 22.1 cm^2) age groups and, furthermore, the 40-45 (182.5 ± 43.1 cm^2) age group had a greater muscle CSA than the 70-75 age group. There were no age-related differences for SF thickness and *a* terms from the isometric step and ramp EMG_{RMS} and MMG_{RMS}-force relationships. However, there were muscle-related differences for the *a* terms (collapsed across age groups), such as, the *a* terms for the VL were greater than for the RF for the isometric step and ramp EMG_{RMS} and MMG_{RMS}-force relationships (except for isometric ramp MMG_{RMS}-force relationship). For the *b* terms from the isometric ramp MMG_{RMS}-force relationships,

there were differences between the 20-25 (0.60 ± 0.16) and 70-75 (0.32 ± 0.12) age groups, however, there were no other age-related differences for *b* terms from the isometric step and ramp EMG_{RMS} and MMG_{RMS}-force relationships. In addition, there were muscle-related differences for the *b* terms from the isometric step and ramp EMG_{RMS} and MMG_{RMS}-force relationships (except for isometric ramp MMG_{RMS}-force relationship). For example, the *b* terms for the RF were higher than the VL (collapsed across age groups).

The results for the present study indicated that there were no age-related changes for SF thickness and the *a* terms from the EMG_{RMS} and MMG_{RMS}-force relationships. The *a* terms (gain factors) have previously reflected differences in subcutaneous fat over the muscle. Therefore, since SF thicknesses were not different amongst the age groups, it is expected that there were no differences in the *a* terms. However, there were muscle-related differences for *a* terms, where the VL *a* terms were higher than the RF. These discrepancies between the *a* terms were likely the result of subcutaneous fat differences between the muscles with the VL having less subcutaneous fat than the RF. In addition, the results of the present study indicated that that there was an age-related increase in percent type I MHC isoform content with the 70-75 age group having a significantly greater amount of type I MHC isoform content than the 20-25 age group. The *b* terms from the log-transformed MMG_{RMS}-force relationship from the isometric ramp contractions reflected the MHC isoform content differences between the two groups (20-25 vs. 70-75 age group) since the *b* terms were lower for the 70-75 age group than the 20-25 age group.

There were age-related differences in MVC PF and muscle CSA that did not match the age-related differences in MHC isoform content or the *b* terms from the MMG_{RMS}-force relationships. Thus, the *b* terms from the MMG_{RMS}-force relationships reflected differences in motor control strategies between individuals with known type I MHC isoform content differences, but not the age-related in muscle strength or size. For EMG_{RMS}-force relationships, there were no age-related changes for the *b* terms, which suggested that the EMG_{RMS}-force relationships were unable to distinguish between different motor control strategies, between individuals with known MHC isoform content differences, or among age groups. In conclusion, the log-transformed MMG_{RMS}-force model may be an attractive model to monitor changes in fiber type composition during the aging process when type II fibers are lost. With additional research, the log-transformed MMG_{RMS}-force model may be a useful, noninvasive criterion for the diagnosis of sarcopenia.

CHAPTER 1

INTRODUCTION

The aging process in humans is associated with the progressive decline in skeletal muscle mass (35, 36, 39, 41, 42). Subsequently, this age-related loss in muscle mass is thought to contribute to a decline in muscle strength and functionality (20). This phenomenon has been defined as sarcopenia. Recently, the European Working Group on Sarcopenia in Older People was formed to determine a global consensus on a clinical and cost effective means to diagnose sarcopenia (20). It was concluded that for an individual to be diagnosed with sarcopenia they must exhibit low muscle mass accompanied by either low muscle strength and/or low physical performance (20). Although these criteria may be cost effective and easily performed in a clinical setting, the criteria for diagnoses does not attempt to measure the underlying neuromuscular mechanisms associated with the loss in muscle and function. Therefore, it may be more appropriate to examine other possible techniques to quantify muscle wasting that takes into account the underlying neuromuscular mechanisms.

Previous studies have reported age-related changes in muscle mass and muscle fiber type composition (35, 36, 39, 41, 42). For example, Lexell et al. (42) reported a 40% reduction in muscle cross-sectional area of the vastus lateralis muscle. Furthermore, there was a significant age-related reduction in type II fiber size of approximately 26% from 20 to 80 years. Larsson (39) reported that the

proportion of type I fibers increased during the aging process, which suggests that type II fibers were lost. Lexell et al. (42) and Larsson (39) both suggested that the denervation and reinnervation process resulted in a smaller cross-sectional area, which included a loss in the total number and size of the type II fibers. Consequently, there was an overall greater proportion of type I fibers. These findings have been supported by a number of other studies (35, 36, 41) and collectively indicate that the sarcopenic process may be largely related to the decrease in number and size of type II fibers.

Researchers have examined the age-related changes in neuromuscular function with invasive electromyography (EMG) (14, 15, 30, 53). For example, Brown (14) reported an age-related decline in the number of motor units in the thenar muscles with needle EMG. The authors reported that the mean motor unit potential size was enlarged for the individuals with less functioning motor units, which suggested that there was a functional compensation by the neuromuscular system that may be the result of surviving motor units increasing in size due to an overall increase in the number of fibers. Campbell et al. (15) reported similar findings in that the number of motor units decreased with age, however, the individual motor unit action potentials were greater for the elderly subjects compared to the younger subjects. The authors have concluded that the changes in the number of motor units and motor unit potential size support the hypothesis of denervation and reinnervation within the muscle during the aging process. It is possible that noninvasive measures, such as surface electromyography (EMG) and

mechanomyography (MMG), may be able to detect these age-related changes in the neuromuscular system.

Surface EMG and MMG are noninvasive techniques used to monitor motor unit activation strategies. However, the interpretation of the surface EMG and MMG is unlike indwelling EMG. Surface EMG is the linear algebraic sum of the motor units' action potentials that activate skeletal muscle fibers as detected by electrodes placed on the skin overlying the muscle (25). The amplitude of the surface EMG signal reflects muscle activation and is influenced by both the number of active motor units and their firing rates and, thus, is considered a global measure of motor unit activity (21). In contrast, MMG has been defined as the recording of low-frequency lateral oscillations of muscle fibers that occur during a contraction (6, 45, 56). Barry and Cole (6) and Orizio (45) have suggested that these oscillations are manifested through (a) the gross lateral movement of the muscle at the initiation of the contraction, (b) smaller subsequent lateral oscillations occurring at the resonant frequency of the muscle, and (c) dimensional changes in the active fibers.

Although it may initially appear that surface EMG and MMG signals provide similar information about neuromuscular function, recent studies have demonstrated that both signals provide unique information that can be used simultaneously as noninvasive measures to examine motor control issues (2, 17). There is a great amount of disparity between the EMG and MMG signals in their force-related patterns of responses. EMG amplitude-force relationships are usually characterized as linear or quadratic increases in EMG amplitude across the force spectrum (7, 50).

De Luca (21) has suggested that the EMG amplitude-force relationship reflects the concurrent increases in motor unit recruitment and motor unit firing rates that regulate muscle force output. In contrast, MMG amplitude-force relationships tend to display a cubic increase in MMG amplitude across the force spectrum, which is different from most EMG amplitude-force patterns. Previous studies have observed plateaus or decreases in MMG amplitude from 0% to 25% MVC, rapid increases from 25% to 60-80% MVC, and then plateaus or decreases to 100% MVC [1, 25]. Consequently, it has been hypothesized that the plateau or decrease in MMG amplitude from 0% to 25% MVC may reflect the initial increases in firing rates of the low-threshold motor units, the rapid increases in MMG amplitude from 25% to 60% or 80% MVC may reflect the increases in motor unit recruitment, whereas the plateau or decrease in MMG amplitude to 100% MVC may reflect the increase in active muscle stiffness due to the fusion of twitches at high force levels (2, 46). Therefore, the patterns of responses demonstrated during the MMG amplitude-force relationship may be able to distinguish between the contributions of motor unit recruitment and rate coding as the motor unit activation strategies that increase muscle force production (8, 16, 48, 50).

There have been a limited number of studies that have used surface EMG and MMG to used age-related changes in functional motor units and fiber type composition (23, 52, 57). Esposito et al. (23) reported greater mean values for EMG amplitude and frequency for the young compared to the old, but no differences in the shapes of the relationships. However, Shima et al. (52) reported differences in the

shapes of the patterns between young and old subjects for mean MMG amplitude values, but reported only greater mean values throughout the force spectrum for MMG frequency, EMG frequency, and EMG amplitude. In addition, Tian et al. (57) reported differences in the EMG and MMG amplitude-force relationships (shape of the relationship) between the young and old. The authors suggested that the EMG and MMG patterns of response at higher contraction intensities (≥ 70% MVC) may differ between younger and older individuals because of the progressive loss of type II fibers during the aging process (23, 52, 57). Therefore, limited evidence suggests that the surface EMG and MMG-force relationships may be able to detect age-related changes in skeletal muscle, such as, a loss in type II fibers during the aging process. However, these previous studies were qualitative in nature and did not attempt to quantify these relationships on a subject-by-subject basis nor were there any measures of fiber type composition (23, 52, 57).

Herda et al. (27) reported that a log-transformation applied to the surface EMG and MMG amplitude-force relationships may be an attractive, noninvasive model for statistically examining changes in motor unit activation strategies. The log-transformed procedure yields the equation $Y=a \cdot X^b$, where Y = MMG or EMG amplitude, X = force, a = gain factor, and b = exponential coefficient. The a term can be called a "gain factor," because it scales the relationship along the Y-axis variable, whereas the b term describes the nonlinearity of the relationships (27) and reflects the rate of change in the variables. Herda et al. (27) proposed that this model applied to the surface EMG and MMG-force relationships may be able to identify

differences in motor unit recruitment strategies among individuals with known differences in fiber type composition. In support of this hypothesis, Herda et al. (26) reported lower *b* terms from the log-transform MMG amplitude-force relationships for individuals with predominantly type I fiber composition compared to individuals with predominantly type II fiber composition. In contrast, the *b* term from the log-transform EMG amplitude-force relationships was unable to make this distinction. Since the log-transform model distinguished differences in the patterns of responses between individuals with known fiber type differences, it is plausible that the age-related loss of type II fibers could also be quantified with the same model. However, it is unclear if the log-transform EMG amplitude-force relationship could make this same distinction. In addition, a benefit of the log-transform model is that it quantifies the patterns on a subject-by-subject basis, which allows for comparisons to be made between individuals. It may be advantageous to use the log-transform model to noninvasively distinguish age-related differences in fiber type composition between young and old individuals to further clarify the clinical diagnoses of sarcopenia.

Therefore, the purpose of the present study was to examine the muscle cross-sectional area (CSA), thigh skinfold thickness (SF), maximal voluntary contraction (MVC) peak force (PF), and the log-transformed EMG and MMG amplitude (RMS)-force relationships (vastus lateralis [VL] and rectus femoris [RF]) of isometric step and ramp muscle actions of the right leg extensors for individuals between the ages of 20 – 75 years categorized into six cohorts (20-25, 30-35, 40-45, 50-55, 60-65, 70-

75 years of age). In addition, myosin heavy chain (MHC) isoform content of the VL was analyzed to characterize individual fiber type composition.

Hypotheses

1. It is hypothesized that there will be an age-related decrease in muscle CSA and increase in SF thickness.
2. It is hypothesized that the *b* term from the log-transformed MMG_{RMS}-force relationships (VL and RF) will indicate an age-related decrease, however, there will be no age-related differences in the *b* term from the log-transformed EMG_{RMS}-force relationships.
3. It is hypothesized that there will be an age-related decrease in the *a* term from the log-transformed EMG_{RMS} and MMG_{RMS}-force relationships (VL and RF).
4. It is hypothesized that the MHC analysis of the VL will indicate an age-related increase in type I MHC isoform content and, consequently, an age-related decrease in type II MHC isoform content.

Definition of Terms

Maximal Voluntary Contraction (MVC) – a maximal voluntary contraction that is performed for 5-s.

Isometric Peak Force (PF) – the peak force achieved during a maximal, voluntary, isometric muscle action; expressed in Newton (N).

Surface Electromyography (EMG) – a recording of the muscle action potentials that sweep across the sarcolemma and pass through the surface electrode recording areas during a skeletal muscle action. The raw signal is expressed in microvolts (μV). A bipolar EMG electrode configuration results in a differentially amplitude signal that represents the subtracted differences of the unique algebraic sums of muscle action potentials that pass within the recording areas of the two electrodes.

Surface Mechanomyography (MMG) – a recording of the lateral oscillations produced by contracting skeletal muscle fibers; contains physiological information that may represent motor unit recruitment, firing rate, and muscle stiffness; the raw signal is expressed in meters per second squared ($m \cdot s^{-2}$).

Muscle Cross-Sectional Area (CSA) – is the total muscle mass that is obtained from the peripheral quantitative computed tomography (pQCT) scanner that is calculated by subtracting the bone, skin, and subcutaneous fat CSA from the total CSA; muscle CSA is expressed in centimeters squared (cm^2).

Skinfold Thickness (SF) – is the measurement of subcutaneous fat; expressed in millimeters (mm).

Myosin Heavy Chain (MHC) – the percentage of the total myosin heavy chain isoforms that is expressed as type I, type IIa, or type IIx (or called IIb) isoforms according to their molecular masses.

Abbreviations

MVC – maximal voluntary contraction

EMG_{RMS} – electromyography amplitude

MMG_{RMS} – mechanomyography amplitude

CSA - muscle cross-sectional area

SF - skinfold thickness

MHC - myosin heavy chain

VL – vastus lateralis

RF – rectus femoris

Delimitations

Sixty men between the ages 20 and 75 years will be recruited for this study. All participants will complete a health history questionnaire and a written statement of informed consent prior to any testing and/or training. Volunteers for this study must be free from any current or ongoing neuromuscular diseases and cannot have sustained an injury or had surgery to their thigh, leg, foot, knee, or ankle within the past 6 months.

Assumptions

Theoretical Assumptions

1. Subjects will accurately answer the health history questionnaire.
2. All equipment will be calibrated and will function properly for all testing sessions.

Statistical Assumptions

1. The population from which the samples are drawn is normally distributed.
2. The variability of the samples in the experiment is equal or nearly so (homogeneity of variance).
3. The scores in all the groups are independent; that is, the scores in each group are not dependent on, not correlated with, or not taken from the same subjects as the scores in any other group.
4. The data meets the assumption of sphericity (circularity). Sphericity requires that the repeated measures data demonstrate both homogeneity of variance and homogeneity of covariance.
5. The data are based on a parametric scale, either interval or ratio.

Limitations

1. Subjects will be recruited as students from several departmental courses and responded to advertisements located within and around the Huston Huffman Center and older individuals (> 30 years of age) will be recruited around Norman, OK; therefore, the process of subject selection may not truly be random. In addition, the sample will be volunteers, therefore not meeting the underlying assumption of random selection.
2. The MHC analyses will be performed on a small tissue sample from the VL muscle and, therefore, the tissue sample may not be truly represented of the MHC isoform content of the whole VL.

CHAPTER II

REVIEW OF LITERATURE

Sarcopenia

Cruz-Jentoft, Baeyens, Bauer, Boirie, Cederholm, Landi, Martin, Michel, Rolland, Schneider, Topinkova, Vandewoude, and Zamboni (2010)

The European Working Group on Sarcopenia in Older People (EWGSOP) reported in this paper a consensus on the definition and possible diagnosis for age-related sarcopenia. The ultimate goal of the paper was to develop an "*operational definition of sarcopenia to be adopted in the mainstream of comprehensive geriatric assessment and then attempt to define the natural course of sarcopenia and to develop and define effective treatment* (p 42)". Sarcopenia has been associated with human aging as a progressive decline in skeletal muscle and, therefore, is closely associated with a decline in muscle strength. The EWGSOP group recommends that sarcopenia be thought of as a geriatric syndrome because it would help promote the identification and treatment even when the exact cause of the disorder remains unknown. The authors listed possible risk factors for sarcopenia, such as, the aging process over the life time, early life developmental influences, less-than-optimal diet, bed rest or sedentary lifestyle, chronic diseases and certain drug treatments. Sarcopenia can affect an individual in a number of ways. For example, sarcopenia can cause mobility disorders, increased risk of falls and fractures, impaired ability to

perform activities of daily living, disabilities, loss of independence and increased risk of death. The EWGSOP describes that the definition of sarcopenia should include low muscle mass (criterion 1), low muscle strength (criterion 2), and low physical performance (criterion 3) with the diagnosis requiring documentation of criterion 1 plus documentation of either criterion 2 or 3. The group did not focus on possible mechanisms for sarcopenia, but do briefly state that protein synthesis, proteolysis, neuromuscular integrity and muscle fat content may contribute to sarcopenia. However, the group did focus the majority of the paper on the stages of sarcopenia that an individual could be classified into. The EWGSOP indicated that there should be three categories: (1) presarcopenia, (2) sarcopenia, and (3) severe sarcopenia. Presarcopenia is the result of a decrease in muscle mass; sarcopenia is the result of low muscle mass and a decrease in muscle strength or physical performance; and severe sarcopenia is the result of all three criteria (decrease in muscle mass, muscle strength, and physical performance). Along with the stages, the EWGSOP reported that there are three primary types of sarcopenia: (1) primary sarcopenia (age-related sarcopenia), (2) secondary sarcopenia (activity-related sarcopenia, disease-related sarcopenia [bed rest, sedentary lifestyle, deconditioning, etc.]), (3) nutrition-related sarcopenia (results from inadequate dietary intake of energy and/or protein). Finally, the EWGSOP suggested assessments for muscle mass (CT, MRI, DXA, BIA, etc.), muscle strength (handgrip strength, knee flexion/extension, etc.), and physical performance (short physical performance battery [usual gait speed, time get-up-and-go test, and stair climb power test]) that could be used for diagnoses.

Effect of aging on muscle fiber composition

Larsson (1983)

The authors studied the effects of aging on the characteristics of skeletal muscle with a focus on eliminating differences in physical activity levels betweens subjects. The authors suggested that there is a crucial issue that arises when examining the effects of again on skeletal muscle in a cross-sectional design, such as, the differences in physical activities levels between the subjects being tested. In a cross-sectional study design that is examining the effects of aging on skeletal muscle, the environmental influence of physical activity should be held as constant as possible because of the strong influence of activity on muscle volume. Therefore, there may be differences or no differences between younger and older subjects based off their respective physical activity levels and not necessarily reflecting age-related differences in skeletal muscle (i.e. inactive young subjects versus active older subjects). In the present study, the authors selected subjects that had equal occupational and leisure physical activity levels and, specifically, reported the subjects to be male white collar workers between 22 and 65 years of age with a low physical activity level and with no prior history of cardiovascular disease or locomotor deficiency. The main findings were that an altered fiber type distribution and a decreased fiber area of the vastus lateralis during aging was characterized by a decline in the relative occurrence of type II fibers from approximately 60 to 45%

between the third and seventh decade and a fiber atrophy preferentially affecting type II fibers. Overall, authors reported a decrease in type II fiber content throughout the age spectrum and strongly recommended future researchers to account for physical activity levels between the young and old subjects.

Lexell, Henriksson-Larsen, Winblad, and Sjostrom (1983)

The authors examined the effects of aging on the total number and size of fibers, and the proportion and distribution of type I and type II fibers in cross sections of autopsied vastus lateralis muscles from two age groups (mean age 72 ± 1 years and 30 ± 6 years, respectively). The authors reported that the older subjects muscle size was 18% smaller and the total number of fibers was 25% lower than the young subjects. However, an interesting finding of the study was that there was no difference in mean fiber size. There was a reduction in type II fiber number in the aged subjects compared to the young subjects. Furthermore, the relative occurrence of the fiber types at various depths in the older individuals was more evenly distributed than the young individuals.

Lexell, Taylor, and Sjostrom (1988)

The authors examined the effects of aging on the cross-sections of autopsied whole vastus lateralis muscles from 43 healthy mean between the ages of 15 and 83. The authors reported that 25 years of age to 50 years of age, approximately 10% of the muscle area is lost and this loss of muscle area accelerates for 30 years after age

50. Specifically, the authors found that this loss in muscle area is caused by a reduction in fiber size and a reduction in fiber number. Furthermore, the majority of the fiber size reduction can be explained by the smaller type II fibers in the old muscles. Interestingly, fiber number was more closely related to the muscle area than the mean fiber size, and with no interaction with age. Therefore, the number of fibers seemed to have the greater influence on the muscle area, despite the reduction in type II fiber area also seen with aging muscle. Another unique finding of the study was that the size of type I fibers was inversely related to the total number of fibers. For example, a muscle with a few fibers had large portion of type I fibers while a muscle with many fibers had smaller portion of type I fibers. The authors speculated that the reduction in fiber number may have been caused by either irreparable damage of the fibers or a permanent loss of the contact between the nerves and the muscle fibers. The authors concluded that there were denervated fibers (flat fibers) and inactive fibers (round fibers) present in the old muscle.

Klitgaard, Zhou, Schiaffino, Betto, Salviati, and Saltin (1990)

The authors examined the myosin heavy chain composition of single fibers from biopsies of the vastus lateralis and biceps brachii in young (n = 5; 23-31 years old) and elderly (n = 5; 68-70 years old) individuals. Comparing the fiber type composition for the vastus lateralis between the young and old subjects indicated that there was a greater percentage of type I MHC content for the young (50 ± 5%) than old (33 ± 3%) and there were more type IIa MHC for the young (26 ± 3%) than old

(12 ± 2%). The MHC analyses from the biceps brachii revealed similar results to the MHC analyses of the vastus lateralis. One of the main findings of this study was it was the first to show a coexistence of three MHC isoforms within the same fiber. Furthermore, the authors reported an increased coexistence of MHC isoforms in histochemically determined fiber types with aging, however, a possible explanation for this phenomenon was not given. The authors citied previous studies that indicated an increased proportion of fibers showing coexistence of MHC isoforms in the human skeletal muscle after endurance training (Schantz & Dhott 1987, Klitgaard et al. 1990). Therefore, the higher proportion of fiber coexistence of MHC types within muscles of the elderly subjects strongly suggests that a changed activity pattern with aging might induce a transition process within the fibers of the aging human skeletal muscles. Possible explanations for this phenomenon may include: selective denervation of large fibers and a reinnervation of these by smaller motor neurons.

Klitgaard, Mantoni, Schiaffino, Ausoni, Gorza, Laurent-Winder, and Saltin (1990)

The authors examined the fiber type composition of the vastus lateralis and biceps brachii in young (28 ± 0.1 years, n = 7) and elderly sedentary subjects (68 ± 0.5 years, n = 7) and in elderly swimmers (69 ± 1.9 years, n = 6), runners (70 ± 0.7 years, n = 5) and strength-trained subjects (68 ± 0.8 years, n = 7). In addition, isometric knee extension and elbow flexion torque was reported for all groups. The maximal isometric knee extension and elbow flexion torque of the elderly sedentary,

swimming, and running subjects was less than the young individuals. Furthermore, there was a higher MHC type I fiber content of the vastus laterals and biceps brachii in the elderly sedentary, swimming, and running subjects compared to the young subjects. However, an interesting and the main finding of this study was that there were no differences between the young and elderly strength-trained subjects for MHC type II fiber content and isometric extension and elbow flexion torque. These results suggested that strength training can counteract the age-related changes in function and morphology of the aging human skeletal muscle.

Invasive electrophysiological investigations of aging on muscle

Brown (1972)

The authors examined the number of motor units in the median innervated thenar muscles in 44 subjects between the ages of 13 and 89 years. Results indicated that motor unit count decreased as the age (10 – 40 years old motor unit count was 200 – 300; >60 years of age motor unit count was 50 – 150) of the subject increased. However, twitch tensions among the different ages was not different from each other. This mechanism of functional compensation may be related to the fact that for subjects with motor unit counts < 100, the size of the remaining mean motor unit potential size was enlarged. Overall, this study reported a reduction in the number of motor units in control subjects with increasing age. Despite this decrease in motor units, motor unit twitch tension was not different among subjects, which may have

been the result of enlarged motor units. The authors hypothesized that the cause of the reduction of motor units may be the result of asymptomatic injury to the median nerve at the wrist, and primary neuronal cell death.

Campbell, McComas, and Petito (1973)

The authors examined the effects of aging on impulse propagation in the fastest and slowest conducting fibers (M-wave), amplitude, conduction velocity and configuration of muscle action potentials from an indwelling concentric needle electrode of the extensor digitorum brevis. Seventeen men and 11 women, aged between 60 and 96 years were studied. All the subjects were deemed to be in good physical condition for their age. In addition, seventy-four subjects of both sexes between the ages of 3 and 58 years served as controls. The results indicated that the mean M-wave amplitude was lower for the elderly subjects compared to the controls and, furthermore, the mean twitch tension for the elderly was lower than the controls. Regarding the twitch responses, it was reported that both phases of the twitch (contraction and half-relaxation times) were prolonged in the elderly when compared to the controls. The mean number of motor units showed no evidence of any declining between the ages of 3 and 58 years, however, subjects beyond the age of 60 years exhibited a loss of functioning units and this reduction became more apparent with advancing age. However, within the elderly population there was considerable variation in residual innervation. For the surviving motor units, the amplitudes of the individual motor unit action potentials were compared to the

controls. The amplitudes of the potentials for the elderly were greater than the controls. Thus, indicates the cross-sectional area of the surviving motor units had increased either through adoption of denervated fibers or by fiber hypertrophy or by both mechanisms. Finally, mean motor nerve impulse conduction velocity was lower in the elderly than the control. The authors concluded that the most important factor contributing to wasting and weakness of aging muscles is a reduction in the number of functional motor units. This study indicated the severity of the denervating process varies considerable among individuals but that it does not usually commence before the age of sixty years. Furthermore, the results tentatively indicated a loss of fast-twitch units, however, the fiber type composition was not assessed in the study.

Stalberg and Fawcett (1982)

The authors examined the motor unit potentials (MUP) of the biceps brachii, vastus lateralis, and tibialis anterior in 124 subjects between the ages of 12 and 75 years. For the amplitude of the MUP the authors reported medial values from all three muscles and reported that there were no changes in MUP amplitudes for the biceps brachii, however, there was a linear increase in median amplitude across the age spectrum for the vastus lateralis and tibialis anterior. In addition, the median values in both those muscles for ages above 60 were significantly higher than for the ages below. The authors did indicate that there was great variation of the data within the age groups. There was also a close correlation between amplitude and area of the

recorded signal and the amplitude/area ratio was similar for the different muscles. Furthermore, the fiber density showed a tendency to increase after the age of 50 (tibialis anterior) or 60 (biceps and vastus lateralis), and there was a slight but significant correlation between median amplitude and fiber density in all three muscles. The authors concluded that MUP amplitude and fiber density increasing across the age spectrum, especially after age 60, is probably the result of reinnervation of muscle fibers into the remaining motor units. Therefore, the increase in the number of fibers in the motor unit will increase in the amplitude of the EMG signal and this phenomenon is thought to occur in attempt to postpone development of weakness and muscle wasting.

Howard, McGill and Dorfman (1984)

The authors examined motor unit action potentials from the brachial biceps, brachial triceps, and anterior tibial muscles in 10 young (20-40 years), 10 middle-aged (40-60 years), and 10 elderly (60-80 years) healthy subjects. Decemberomposition of the electromyographic signal was recorded during isometric contractions at 10% and 30% of maximum voluntary contraction using standard concentric needle electrodes. Mean amplitudes and durations increased with age in both low-threshold and high-threshold motor unit action potentials in all muscles. Mean firing rates decreased with age when force was measured proportionately, but not when measured absolutely. These data suggested an ongoing process of

progressive denervation and reinnervation and the larger mean amplitudes, rise rates, and number of turns with age probably reflected larger muscle fiber diameters.

Stalberg, Borges, Ericsson, Essen-Gustavsson, Fawcett, Nordesjo, Nordgren, and Uhlin (1988)

The authors examined isometric and isokinetic torque, indwelling needle electromyography (EMG), and muscle fiber characteristics in the vastus lateralis muscle of both legs in healthy subjects aged between 20 and 70 years. Peak torque was greater in males and gradually decreased with age in both males and females. EMG amplitude and fiber density revealed evidence of reinnervation, indicating proceeding denervation and, therefore, loss of motor units. In addition, there were positive correlation between peak torque, body surface area, and mean fiber area. The authors concluded that a loss of motor units also contributed to the decrease in torque, but other factors may also play a role in age-related reduction in torque (reduction in muscle fiber contractility, metabolic factors, and central factors).

Noninvasive electrophysiological investigations of aging

Frontera, Hughes, Lutz, and Evans (1991)

The authors studied the isokinetic strength of the elbow and knee extensors and flexors in 200 healthy middle age and elderly men and women to examine the relationship between muscle strength, age, and body composition. Men and women

were placed into three groups determined by age (group 1 age – 50.2 years; group 2 age – 60.1 years; group 3 age – 68 years). For body composition, the older groups had lower fat-free mass (FFM) and muscle (MM). At 60 degrees per second the oldest subjects had a lower absolute strength in all muscles and after correction for FFM in the knee extension and elbow flexion in both sexes and the knee flexion of male subjects there was still a slight difference between groups. Strength per kilogram of FFM did not differ between age groups in the knee flexion of female subjects and in the elbow extension of both sexes. At 60 degrees per second, the correction of strength for MM eliminated the significant differences between age groups in all muscles. At the faster speed, older subjects had a lower absolute muscle strength in all muscle groups. However, correction for FFM and MM eliminated these differences in all muscle groups except knee extension. In summary, the results indicated difference in muscle strength with advancing age, however, when corrected for MM those differences were eliminated for the most part. Therefore, authors suggested that the decreases in strength were the result of decreases in MM and not by altered muscle function.

Doherty, Vandervoort, Taylor, and Brown (1993)

The authors examined the influence of age-associated motor unit loss on contractile strength in 24 active young and 20 older men and women. Spiked-triggered averaging was employed to extract a sample of surface-recorded single motor unit action potentials (S-MUAP) of the biceps brachii and brachialis muscles.

The amplitude of the maximum compound muscle action potential of the biceps brachii and brachialis muscles was divided by the mean S-MUAP amplitude to estimate the numbers of motor units present. In addition, maximum isometric twitch contraction, twitch contraction times and maximum voluntary contraction (MVC) of the elbow flexors were also recorded. The estimated numbers of motor units were significantly reduced in older subjects with a mean value of 189 compared with a mean of 357 in younger subjects. The sizes of the S-MUAPs were larger in the older subjects compared to the younger subjects. There was also smaller twitch force and MVC force for the old compared to the young. However, the differences between the young and old on twitch and MVC force were not as large as the differences in the number of motor units and size of S-MUAPs between groups. Overall, the large S-MUAPs and the increasing mean S-MUAP amplitude suggest that reinnervation may be able to compensate partially for the losses of motor neurons in older subjects. Furthermore, the surviving motor units in this studied exhibited substantial enlargement with respect to S-MUAP size and motor unit twitch tensions. Another finding of the present study was that there was no difference in twitch contraction times in the older subjects compared to the young subjects, which the authors contributed to the lack of loss or atrophy of type II fibers in the biceps brachii in older subjects.

Doherty, Komori, Stashuk, Kassam, and Brown (1994)

The authors examined the properties of single thenar motor units in 15 healthy younger (age = 33 years) and 15 older (age = 68 years) subjects. Evoked M-wave potentials at 10%, 20%, and 30% of the peak-to-peak amplitude of the maximum M-wave was delivered to the median nerve to examine surface detected motor unit action potentials (S-MUAPs). The S-MUAP sizes were significantly larger in older subjects, and of the range of distribution of motor unit conduction velocities were markedly shifted to reflect a slower population of motor fibers. The authors concluded that these findings suggested that age-related axonal slowing may uniformly affect all median motor fibers.

Doherty and Brown (1997)

The authors studied the effects of aging on twitch contractile properties of the thenar muscles. Seventeen younger subjects (aged 25 – 53 years) and 9 older subjects (aged 64 – 77 years) participated in the study. Electrical stimulation of the median nerve was performed to activate single motor axons of the thenar muscles to examine twitch tensions, contractile speeds, and surface-detected motor unit action potential (S-MUAP). The older subjects MU twitch force was shifted to the right and the mean value was greater compared to the younger subjects. The contraction time and relaxation time from the twitch tensions in the older subjects were prolonged compared to the younger subjects. The S-MUAP, when normalized to maximum M-wave, mean value was larger for the older subjects when compared to the younger subjects.

Kent-Bruan and Ng (1999)

In this study the researchers examined isometric maximum voluntary contraction (MVC), cross-sectional area (CSA), specific strength (MVC/CSA), and voluntary activation in the ankle dorsiflexion muscles of 24 young (mean age 32 years) and 24 elderly (mean age 72 years) healthy men and women of similar physical activity level. Voluntary muscle activation was measured with the central activation ratio, tetanic force, maximum voluntary contraction (MVC), and the maximal rate of voluntary isometric force development were also recorded. The young subjects had a greater MVC than the elderly mean and women and the young women, however, there were no differences between the young and elderly women. There were no age-related differences in titanic force, however, the rate of force development was slower in the elderly compared with the young subjects. For fat-free CSA, the men had a greater CSA than the women and young had a greater CSA than the elderly subjects. For the M-wave and specific strength (MVC/CSA), there were no age-related or gender differences between the groups. In addition, there were no differences in muscle activation (measured by CAR) between young and old or men and women. In summary, the results of this study suggested that when muscle size was accounted for, isometric specific strength was unaffected by aging. In addition, muscle activation was unaffected by aging, however, the rate of force developed was affected by aging. The rate of force development may have been affected by the possible loss of type II fibers in the elderly subjects.

Akataki, Mita, Watakabe, and Kunihiko (2002)

The authors examined the effect of age on the mechanomyogram (MMG) amplitude and mean power frequency versus force relationship of the biceps brachii muscle during a ramp contraction in 10 elderly males (age = 69.8 ± 4.7 years, mean ± SD) and 10 younger male (age = 22.7 ± 1.8 years) subjects. The authors reported that the MMG amplitude-force relationship was linear for the elderly group. In contrast, the MMG amplitude-force relationship for the young group demonstrated a linear trend up to 60% MVC followed by a gradual decrease in amplitude to 80% MVC. The MMG mean power frequency-force relationships for both groups increased in a linear fashion across the force spectrum however, the mean power frequency values were greater for the young group for the majority of the force spectrum (> 20% MVC). The authors concluded that the elderly individuals relied predominantly on slow twitch motor units to reach maximal force due to the loss of fast twitch motor units and, thus, the plateau in the MMG amplitude-force relationships may have reflected the fusion of twitches from the slow twitch motor units in the elderly subjects, which was unseen in the younger individuals.

Clark, Patten, Reid, Carabello, Phillips, and Fielding (2010)

The authors studied the effects of aging on torque, power, and electromyography in 28 older healthy adults (OH), 32 older mobility-limited adults (OML) and 29 middle-aged healthy adults (MH). Subjects were categorized into the

OH and OML groups based of the Short Physical Performance Battery (SPPB), with individuals who scored less than 9 out of 12 were placed into the OML group and the remaining subjects who scored greater than 9 were placed into the OH group. Isokinetic knee extensions were performed at 60, 90, 180, and 240 degrees per second. In addition to the performance testing, CT scans were taken of the mid-thigh to assess CSA. The CSA was largest for the MH followed by OH and OML groups. For the isokinetic testing, the MH group produced more absolute torque than OH, but the two groups did not differ for specific torque or normalized torque. The MH and OH groups produced significantly more absolute, specific, and normalized torque than OML. In addition, normalized torque in OML was significantly lower than MH and OH groups at 90, 180, and 240 degrees per second. For the slower velocities, there was a small differentiation between groups, which reached statistical significance at 240 degrees per second, with OML producing just 63% and 67% of MH and OH, respectively. For power, there were group differences with MH producing the greatest power followed by OH and OML. In addition, OML produced less absolute power than MH and OH at each velocity and significantly less specific power at each velocity except 60 degrees per second. The MH and OH groups showed differences in absolute and specific power between velocities, however, this was unseen in the OML group. For EMG amplitude of the VM, VL, and RF, mean values were higher for the MH and OH groups compared with the OML group. For EMG amplitude for the antagonist hamstring muscles (SM and BF), SM and BF activation in the MH group was less than that in OH and OML

groups. In summary, this study indicated impaired power and muscle activation in the older individuals who have an increased risk on mobility and disabilities according to the Short Physical Performance Battery when compared to healthier middle age and older individuals.

Esposito, Malgrati, Veicsteinas, and Orizio (1996)

The authors studied the effects of aging on the time and frequency properties of surface electromyography (EMG) and mechanomyography (MMG) of the elbow flexors during isometric contractions from 20% - 100% MVC. Twenty elderly subjects (65 – 78 years old) and 20 younger controls (20 – 34 years old) participated in the study. The authors reported that the maximal voluntary contraction (MVC) was lower for the elderly than the younger controls. In addition, at the %MVC the EMG and MMG amplitude and mean frequency was lower for the elderly than the younger subjects. The patterns of response plateaued or decreased from 80% to 100% for EMG frequency and MMG amplitude in the elderly, however, this was unseen in the younger controls. The authors concluded that the MVC and %MVC EMG and MMG values may be related to the reduction in the number of muscle fibers during the aging process and the decreases or plateaus in the MMG amplitude and EMG mean frequency signals may have been related to the end of the recruitment of larger motor units with high conduction velocity, which resulted in further increment of motor unit firing rate in the biceps brachii muscle.

Shima, McNeil, and Rice (2007)

The authors examined the effect of aging on mechanomyography (MMG) during evoked twitches and electromyography (EMG) and MMG during voluntary contractions at 20%, 40%, 80%, and 100% MVC of the dorsiflexors (tibialis anterior). Ten young men (21 – 33 years old) and 10 old men (75 – 83 years old) participated in this study. Electrical stimulation was delivered before and after a 10-s maximal voluntary contraction (MVC) to assess potentiation of contractile, M-wave amplitude, and MMG amplitude. The authors reported that twitch potentiation was greater in young than old subjects, however, MMG amplitude was unaffected. The EMG and MMG patterns of response were similar between groups, except for greater MMG at MVC in young subjects. The shape of the EMG amplitude versus force relationships was similar between age groups. However, MMG amplitude was different between the young and old groups, with the old group demonstrating a slight decrease from 80% - 100%. The authors concluded that MMG versus force relationships may have indicated age-related changes in motor unit recruitment.

Tian, Liu, Li, Fu, and Peng (2010)

The authors examined the time and frequency domain of electromyography (EMG) and mechanomyography (MMG) of the vastus lateralis during concentric leg extensions at 45%, 60%, and 75% of 1 repetition maximum (RM) in 10 healthy elderly (mean age 64 years) and 10 healthy young (mean age 22 years) subjects. Compared to the young, the elderly had less lean thigh volume. Absolute and

relative maximal force and absolute and relative maximal power was greater for the young group compared to the elderly group. EMG amplitude and frequency for the young group increased linearly across the three intensities, however, EMG amplitude and frequency increased to 60% and then decreased to 75% MVC. In addition, EMG amplitude and frequency was greater at 75% MVC for the young when compared to the elderly. MMG amplitude and frequency increased linearly across the force spectrum for the young, however, MMG amplitude and frequency increased up to 60% and then decreased to 75% MVC. Furthermore, MMG amplitude was greater for the young group than the elderly across the force spectrum. The authors concluded that although EMG and MMG amplitude and frequency were different between the groups, MMG amplitude was more sensitive to the possible muscle wasting condition of the elderly subjects because of the differences at all measured force intensities and not just at 75% MVC (i.e., MMG frequency and EMG amplitude and frequency).

The use of surface mechanomyography and electromyography for distinguishing between muscle fiber type compositions

Komi and Tesch (1979)

This study examined the effects of fatigue of the vastus lateralis in individuals with differences in muscle fiber type distribution. Eleven subjects performed repeated maximum voluntary knee extensions at a constant angular

velocity (180 degrees per second) with electromyographic (EMG) amplitude and frequency recorded from the vastus lateralis. In addition, muscle biopsies were collected from the vastus lateralis and fiber type analysis was performed on the muscle samples. Results indicated that for individuals that possessed a higher proportion of fast twitch muscle fibers demonstrated higher peak knee extension torque, and a greater susceptibility to fatigue than did individuals with muscles mainly composed of slow twitch muscle fibers. EMG amplitude and frequency declined during the 100 contractions in individuals with a proportion of fast twitch fibers, however, there was only a slight non significant decline in EMG amplitude and frequency for individuals with predominantly slow twitch fibers. The authors concluded that the muscles with predominantly fast twitch fibers demonstrate a greater susceptibility to fatigue and this was reflected by a rapid decrease in force output as well as by a pronounce change in the EMG signal.

Orizio and Veicsteinas (1992)

The authors studied the soundmyogram (SMG) time and frequency domain characteristics of the vastus lateralis muscle of 7 sprinters and 7 long distance runners. There were also 7 sedentary males who performed the experimental trial. The subjects performed an exhausting maximal voluntary contraction of the leg extensors. The authors reported that the sprinters had greater MVCs but shorter effort time compared to the long distance runners and sedentary subjects. In addition the SMG root mean square (RMS) and the SMG frequency content, at the onset of

contraction where higher for the sprinters than the sedentary and long distance runners. During the fatiguing MVC trial, the SMG RMS values decreased for the sprinters and sedentary individuals only and the SMG power spectra presented a compression towards the lower frequencies more so for the sprinters than sedentary individuals and long distance runners. The authors concluded these results can be explained by the percentage of fast twitch fiber area differences between the sprinters, long distance runners, and sedentary individuals.

Mealing, Long, and McCarthy (1996)

The authors examined the relationship between the frequency characteristics of vibromyographic (VMG) and the fiber composition found in postural and non-postural human muscle undergoing a standardized voluntary contraction. Eighteen healthy males performed a maximum voluntary contraction or one repetition maximum and 50% MVC was used during acquisition of the signal. The researchers examined VMG frequency at 50% of MVC on the biceps brachii and soleus muscles. The results indicated that the frequency of that VMG signal at 50% MVC distinguished between the biceps brachii (considered predominately a mixed fiber type muscle) and soleus (considered predominately type I fiber composition) muscles. The authors concluded that a large proportion of slow fibers (soleus) generated VMG signals that contained a greater percentage of low frequencies compared with muscles with a mixed population of fast and low fibers (biceps brachii). Therefore, these results supported the hypothesis that VMG, which is

generated by the mechanical twitching of motor units, may be able to distinguish between fiber types.

Beck, Housh, Fry, Cramer, Weir, Schilling, Falvo, and Moore (2009)

The authors examined the patterns of responses for electromyographic (EMG) and mechanomyographic (MMG) amplitude and mean power frequency during a fatiguing submaximal isometric muscle action. Five resistance-trained (age = 23.6 ± 3.7 years) and five aerobically-trained (age = 32.6 ± 5.2 years) subjects had muscle biopsies of their vastus lateralis muscles taken to analyze myosin heavy chain (MHC) composition. MHC of the resistance-trained subjects indicated that the fiber composition of their vastus lateralis was 59.0 ± 4.2% Type IIa, 0.1 ± 0.1% Type IIx, and 40.9 ± 4.3% Type I. The aerobically-trained subjects had 27.4 ± 7.8% Type IIa, 0.0 ± 0.0% Type IIx, and 72.6 ± 7.8% Type I MHC. The EMG amplitude versus time relationships were best fit with quadratic models for the resistance-trained subjects, however, these same relationships was best fit with a linear model for the aerobically-trained subjects. The EMG mean power frequency decreased linearly across time for both the resistance-trained and aerobically-trained subjects. There were no differences reported by the ANOVA model for these mean values from the EMG amplitude and frequency versus time relationships between the resistance- and aerobic-trained subjects. The MMG amplitude versus time relationships were fest fit with quadratic models for the resistance-trained, however, the relationships were best fit with linear models for the aerobically-trained. The MMG mean power frequency

versus time relationships for the resistance-trained models did not demonstrate any significant relationships, but for the aerobically-trained these relationships were best fit with a linear model (decreasing). An ANOVA model applied to the means of these relationships indicated that the resistance-trained MMG amplitudes were greater across the time spectrum than the aerobically-trained. The authors suggested that this mean difference was likely due to the MHC fiber composition of the subjects and not the result of possible subcutaneous fat differences, which would have resulted in the aerobically-trained subjects to have greater mean MMG amplitude values and not vise versa. The authors concluded that MMG may be a useful noninvasive tool for examining fatigue-related differences in muscle fiber type compositions.

Beck, Housh, Fry, Cramer, Weir, Schilling, Falvo, and Moore (2009)

The authors examined the mechanomyographic versus force relationships (20% to 100% MVC) of the leg extensors in five resistance-trained (age = 23.6 ± 3.7 years), five aerobically-trained (age = 32.6 ± 5.2 years), and five sedentary subjects (age = 23.4 ± 4.1 years). In addition, biopsies were taken from the vastus lateralis and MHC analyses was performed. The MMG signal from the vastus lateralis was processed with a wavelet analysis. The MHC analyses indicated that there were differences in fiber type compositions between the groups (Type I – RT = 40.9 ± 4.3%, AT = 72.6 ± 7.8%, SED = 40.1 ± 10.9%; Type IIa – RT = 59.0 ± 4.2%, AT = 27.4 ± 7.8%, SED = 42.1 ± 7.8%; Type IIx – RT = 0.1 ± 0.1%, AT = 0.0 ± 0.0%,

SED = 17.8 ± 6.4%). The wavelet analyses indicated that there were no differences between the three groups, but there were force-related differences between the intensity values in each wavelet band. Thus, the shape of the MMG frequency spectrum changed with increases in isometric force and indicated that the MMG frequency spectrum was compressed toward lower frequencies at 100% MVC. The authors concluded that the MMG frequency spectrum was not influenced by the MHC fiber composition of the vastus lateralis.

Beck, Housh, Fry, Cramer, Weir, Schilling, Falvo, and Moore (2009)

The purpose of the study was to see if isometric knee extension strength and mechanomyographic median frequency could predict myosin heavy chain (MHC) isoform content. Five resistance-trained (age = 23.6 ± 3.7 years), five aerobically-trained (age = 32.6 ± 5.2 years), and five sedentary subjects (age = 23.4 ± 4.1 years) had muscle biopsies of the vastus lateralis to determine MHC content and performed a maximum voluntary contraction (MVC) of the leg extensors. The MHC analyses indicated that there were differences in fiber type compositions between the groups (Type I – RT = 40.9 ± 4.3%, AT = 72.6 ± 7.8%, SED = 40.1 ± 10.9%; Type IIa – RT = 59.0 ± 4.2%, AT = 27.4 ± 7.8%, SED = 42.1 ± 7.8%; Type IIx – RT = 0.1 ± 0.1%, AT = 0.0 ± 0.0%, SED = 17.8 ± 6.4%). The authors reported that neither MVC and MMG median frequency could predict a significant amount of the variance of %MHC Type II content on their own, however, the MVC and MMG

median frequency together predicted a significant portion of the variance (59.8%) in %MHC Type II isoform content.

Herda, Housh, Fry, Weir, Schilling, Ryan, and Cramer (2010)

This study examined the mechanomyographic (MMG) and electromyographic (EMG) amplitude versus force relationships (isometric ramp contraction from 5% to 90% of MVC) of the vastus lateralis of five resistance-trained (age = 23.6 ± 3.7 years), five aerobically-trained (age = 32.6 ± 5.2 years), and five sedentary subjects (age = 23.4 ± 4.1 years). Simple linear regression was applied to the natural log-transform MMG and EMG versus force relationships and myosin heavy chain (MHC) isoform content was analyzed from muscle biopsies from the vastus lateralis. The MHC analyses indicated that there were differences in fiber type compositions between the groups (Type I – RT = 40.9 ± 4.3%, AT = 72.6 ± 7.8%, SED = 40.1 ± 10.9%; Type IIa – RT = 59.0 ± 4.2%, AT = 27.4 ± 7.8%, SED = 42.1 ± 7.8%; Type IIx – RT = 0.1 ± 0.1%, AT = 0.0 ± 0.0%, SED = 17.8 ± 6.4%). The slope (*b* term) from the simple linear regression indicated that there was significant differences in the MMG versus force relationships between the RT, SED, and AT, with RT and SED having lower *b* terms than the AT group. However, this was unseen with the EMG versus force relationships. The authors concluded that the *b* term from the MMG versus force relationships detected the earlier rate coding of the individuals with predominantly Type I fiber type composition. The anti-log of the y-intercept (*a* term) was higher for the AT compared to the RT and SED groups,

which may have reflected the skinfold thicknesses between those groups. Thus, the *a* term was reduced or lowered because of the greater amount of subcutaneous fat (RT and SED). The authors concluded that the log-transform MMG versus force relationships may offer an attractive, noninvasive model for statistically examining differences in the motor unit activation strategies.

Clinical applications of surface mechanomyography

Rhatigan, Mylrea, Lonsdale, and Stern (1986)

The authors examined muscle sounds with a caroid phonoangiography microphone of the biceps brachii during a muscle contraction. Thirty-seven subjects (age 22 – 86 years) volunteered for the study, with 20 subjects having some type of known neuromuscular disease (Myotonic, Friedreich's Ataxia, etc.). The authors reported average peak frequency from the caroid phonoangiography. There was a significant difference between normal subjects and subjects with neuromuscular disorders. The average peak frequency was less for subjects with a neuromuscular disease. In contrast, there was no difference between healthy older (> 60 years of age) and healthy young individuals. The authors concluded that future studies on the effects of neuromuscular diseases on muscle sounds were warranted.

Akataki, Mita, Itoh, Suzuki, and Watakabe (1996)

The authors studied electromyogram (EMG) amplitude and acoustic myogram (AMG) amplitude of the biceps brachii of 6 individuals with cerebral palsy (CP) and 8 healthy matched controls. Isometric contractions were performed at 10% to 50% (10% increments) MVC. The maximal voluntary contraction in the CP group was less than that of the normal group and, furthermore, this difference was maintained following adjustments for muscle cross-sectional area (CSA). EMG and AMG amplitude was greater at all force levels for the normal group compared to the CP group. For EMG amplitude, the force-related patterns were linear for both groups. In contrast, the AMG-force relationship demonstrated a plateau from 40% to 50% and EMG amplitude increase linearly throughout the force spectrum. The authors concluded that the alterations in the contractile properties of the CP patients were manifested in the EMG and AMG amplitude-force relationships.

Orizio, Esposito, Sansone, Parrinello, Meola, and Veicsteinas (1997)

The authors examined the mechanomyogram (MMG) and electromyographic (EMG) amplitude versus force relationships (20%, 40%, and 60% of the maximal voluntary contraction [MVC]) of the elbow flexors and finger flexors in 10 patients with myotinc dystrophy and 10 age matched controls. The MVC was lower for the patients with myotonic dystrophy for the elbow flexors and for the finger flexors. The MMG and EMG amplitude-force relationships were lower for the myotonic dystrophy patients than the controls. The authors concluded that changes in the

electromechanical coupling efficiency in patients with myotonic dystrophy can be monitored with MMG.

Yoshitake, Ue, Miyazaki, and Moritani (2001)

The authors studied the effects of fatigue of the lower-back muscles on electromyography (EMG), mechanomyography (MMG), and near-infrared spectroscopy (NIRS). Eight male subjects performed isometric back extensions for period of 60 s at 15°. EMG, MMG, and NIRS were recorded form the center of the erector spinae at the level of L3. EMG amplitude and MMG amplitude increased at the initial phase of contraction and then decreased throughout the contraction. Whereas, EMG mean power frequency decreased significantly and progressively throughout the contraction. In contrast, there was no change in MMG mean power frequency during the 60 s fatiguing contraction. Muscle blood volume and oxygenation decreased dramatically at the onset of the contraction and then remained constant throughout the rest of the contraction. The authors concluded that intramuscular mechanical pressure an important factor in lower-back muscle fatigue and, furthermore, EMG and MMG may be useful for examining mechanism of lower-back fatigue.

CHAPTER III

METHODS

Participants

Fifty-seven healthy men volunteered for this investigation. Participants were recruited between the ages of 20 and 75 and were segregated into groups by age (20-25, 30-35, 40-45, 50-55, 60-65, and 70-75 years of age). Table 1 contains the descriptive values, such as, mean ± standard deviation (SD) values for age, height, and weight for all age groups. None of the participants reported any current or ongoing neuromuscular diseases or musculoskeletal injuries specific to the ankle, knee, or hip joints. This study was approved by the University of Oklahoma Institutional Review Boards for Human Subjects, and all participants completed a written informed consent form (Appendix C) and a Pre-Exercise Testing Health & Exercise Status Questionnaire (Appendix D). Using the Procedures described by Howell (31) for estimating sample sizes for between subjects designs, a minimum sample size of n = 4 was required for each group to reach statistical power (1-β) of 0.80 based on the findings of Kiltgaard et al. (36). Sample size was based on the percentage of type I fiber area (MHC analyses) differences between young and old groups in Kiltgaard et al. (36).

Research Design

A between subjects design [age (20-25 vs. 30-35 vs. 40-45 vs. 50-55 vs. 60-65 vs. 70-75)] was used to examine peak force (PF) form the isometric maximal voluntary contractions (MVC) of the right leg extensors, the *a* and *b* terms from the natural log-transform electromyographic (EMG) and mechanomyographic (MMG) amplitude (RMS) versus force relationships (VL and RF) from the isometric step and ramp isometric contractions, thigh muscle cross-sectional area (CSA), thigh skinfold thickness (SF), and myosin heavy chain (MHC) isoform content of the VL. Each participant visited the laboratory on two occasions. The first visit included a thigh scan, skinfolds, isometric MVCs, isometric step muscle actions, and isometric ramp muscle action, while the second visit included a muscle biopsy of the VL.

Variables

The independent variable included age [20-25 vs. 30-35 vs. 40-45 vs. 50-55 vs. 60-65 vs. 70-75]. The dependent variables that were measured included: (a) MVC PF, (b) *a* term from the VL EMG_{RMS}-force step muscle actions, (c) *b* term from the VL EMG_{RMS}-force step muscle actions, (d) *a* term from the VL EMG_{RMS}-force ramp muscle actions, (e) *b* term from the VL EMG_{RMS}-force ramp muscle actions, (f) *a* term from the RF EMG_{RMS}-force step muscle actions, (g) *b* term from the RF EMG_{RMS}-force step muscle actions, (h) *a* term from the RF EMG_{RMS}-force ramp muscle actions, (i) *b* term from the RF EMG_{RMS}-force ramp muscle actions, (j) *a* term from the VL MMG_{RMS}-force step muscle actions, (k) *b* term from the VL MMG_{RMS}-force step muscle actions, (l) *a* term from the VL MMG_{RMS}-force ramp

muscle actions, (m) *b* term from the VL MMG_{RMS}-force ramp muscle actions, (n) *a* term from the RF MMG_{RMS}-force step muscle actions, (p) *b* term from the RF MMG_{RMS}-force step muscle actions, (q) *a* term from the RF MMG_{RMS}-force ramp muscle actions, (r) *b* term from the RF MMG_{RMS}-force ramp muscle actions, (s) thigh CSA, (t) SF, and (u) MHC isoform content of the VL.

Instrumentation

- Biodex Systems 3 isokinetic dynamometer (Biodex Medical Systems; Shirley, NY) fitted with a high-accuracy low-profile load cell (Omegadyne LC402, Stamford, CT) was used to measure MVC and submaximal contraction force.
- Pre-amplified (gain: x 350) active EMG electrodes (TSD150B, Biopac systems, Inc.; Santa Barbara, CA) with a 20-mm inter-electrode distance were placed over the VL and RF muscles to record surface EMG signals.
- Active miniature accelerometer MMG electrodes (EGAS-FS-10/VO5, Entran Inc., Fairfeld, NJ) were placed over the VL and RF muscle to record surface MMG signals.
- All analog signals from the Biopac acquisition system were sampled with an external analog-to-digital converter (DAQCard 6036E, National Instruments, Austin, TX).
- A personal computer (Dell Inspiron 8200, Dell, Inc., Round Rock, TX) was used to store all the digitized signals for off-line analysis.

- Custom-written software (LabVIEW 8.5, National Instruments, Austin, TX) was used to process the surface EMG, MMG, and force signals.
- A calibrated (± 1 mm) Lange Skinfold Caliper (Santa Cruz, CA) was used to obtain a thigh skinfold measurement.
- A peripheral quantitative computed topography (pQCT) scanner (XCT 3000, Orthometrix, Inc., White Plains, NY) was used to measure CSA of the mid-thigh.

Isometric Force Assessments

The isometric MVC, submaximal step, and ramp muscle actions were performed with each participant seating with restraining straps over the pelvis, trunk, and thigh, with a leg flexion angle of 90° below the horizontal plane (full extension) on a calibrated Biodex System 3 dynamometer (Biodex Medical Systems, Inc. Shirley, NY). The lateral condyle of the femur was aligned with the input axis of the dynamometer in accordance with the Biodex User's Guide (Biodex Pro Manual, Applications/Operations. Biodex Medical Systems, Inc., Shirley, NY. 1998). Submaximal warm-up trials preceded two 4-s isometric MVCs of the right leg extensors with 2-min rest between trials. Participants were asked to produce as much force as possible for 4-s, and strong verbal encouragement was provided. The higher force output between the two trials was selected as the representative MVC value. After the MVC trials, each subject performed nine submaximal isometric step muscle actions, and two or three 6-s isometric ramp muscle actions. In addition, the

order of the submaximal isometric step muscle actions was randomly administered during each experimental trial.

During the isometric step and ramp muscle actions, participants were required to track their force production on a computer monitor placed in front of them that displays the real-time, digitized force signals overlaid onto a programmed template. For the isometric step contraction, horizontal lines were programmed as templates on the computer monitor that serves as the target force lines for each submaximal step muscle action. The ramp template consisted of a 5-s horizontal baseline at 5% MVC followed by a linearly increasing ramp line lasting 6-s and ending with a 2-s horizontal plateau at 100% MVC. The isometric step and ramp muscle action templates and real-time force overlay were programmed and displayed using LabVIEW 7.1 software (National Instruments, Austin, TX).

During the experimental trial, participants performed a series of randomly ordered submaximal isometric step muscle actions at 10, 20, 30, 40, 50, 60, 70, 80, and 90% of the highest pre-testing MVC value. Each isometric step muscle action was held steady for approximately 4-s. A 2-min rest was allowed between each muscle action.

During the experimental trial, participants were asked to perform 2-3 ramp muscle actions (5-100% MVC) and verbal encouragement was provided. A 2-min rest period was allowed between each ramp muscle action. The ramp trial that best satisfies the following criteria was used for later analysis: (a) maximal force reaching

at least 90% of MVC and (b) a maximum tracking error of ± 3% MVC around the template.

Muscle Cross-Sectional Area and Subcutaneous Fat Assessments

Two-dimensional images of the right thigh were obtained using a peripheral quantitative computed tomography (pQCT) scanner (XCT 3000, Orthometrix, White Plains, New York). The subjects were seated upright in the chair of the pQCT with the right thigh flexed at 90° and leg extended. The right leg was supported by a custom-built plastic support device (Bone Diagnostics, Fort Atkinson, Wisconsin) between the chair and gantry of the pQCT. The foot was secured on the opposite side of the gantry with a Velcro® strap placed over the metatarsals. The pQCT gantry was manually positioned at the site of the muscle biopsy (OrthoMetrix, Inc., Naples, FL). The cross-sectional image obtained from the pQCT was calculated by subtracting the bone, skin, and subcutaneous fat cross-sectional areas (CSA) from the total CSA, which only left the muscle CSA (cm^2). This calculation was performed by the pQCT software (Stratec XCT 3000 software v. 6.00, Pforzheim, Germany). Previously published intraclass correlation coefficients (ICCs) for test–retest reliability for muscle CSA measured with the pQCT ranged from 0.996 to 0.998 with a standard error of the measurement (SEM) of 1.660 to 1.101 cm^2 and no significant differences among the day-to-day mean CSA values ($p \leq 0.05$) (5).

Skinfold measurements were taken at the site of MMG sensor placement on the vastus lateralis before the isometric force assessments. Measurements were

taken according to the recommendations of Jackson and Pollock (32) for thigh skinfold assessment and were performed by an experienced investigator using a calibrated Lange caliper (Cambridge Scientific Industries, Cambridge, MD). Two initial skinfold measurements were taken, and if the first two measures differed by more than 2.0 mm, a third measurement was performed. The average of the two or three measurements were used as the representative skinfold thickness for each subject.

Surface Electromyography

Pre-amplified, bipolar surface EMG electrodes (EL254S, Biopac Systems Inc.; Santa Barbara, CA, USA, gain = 350) with a fixed center-to-center inter-electrode distance of 20 mm, built-in differential amplifier with a gain of 350 (nominal), input impedance of 100 MΩ, and common mode rejection ratio of 95 dB (nominal) were taped over the VL and RF muscles of the right leg. The electrodes were placed over the VL and RF of the muscle at 50% of the distance between the greater trochanter and lateral condyle of the femur. A single pre-gelled, disposable electrode (Ag-AgCl, Quinton Quick Prep, Quintion Instruments Co., Bothell, WA, USA) was placed on the spinous process of the 7^{th} cervical vertebrae to serve as a reference electrode. To reduce inter-electrode impedance and increase the signal-to-noise ratio, local areas of the skin were shaved and cleaned with isopropyl alcohol prior to placement of the electrodes.

Surface Mechanomyography

The MMG signal was detected using an active miniature accelerometer (EGAS-FS-10-/VO5, Intran Inc., Fairfield, NJ) that was preampilfed with a gain of 200, frequency responses of 0-200 Hz, sensitivity of 70 mV/m s^{-2}, and range of ± 98 m s^{-2}. The accelerometer was placed over the VL and RF of the muscle at 50% of the distance between the greater trochanter and lateral condyle of the femur just proximal the EMG electrodes.

Signal Processing

The EMG, MMG, and force signals were recorded simultaneously with a Biopac data acquisition system (MP150WSW, Biopac Systems, Inc.; Santa Barbara, CA, USA) during each strength assessment. The force (*N*) signal from the load cell and the MMG ($m \cdot s^{-2}$) and EMG (μV) signals from the VL and RF muscles were sampled at 2 kHz during the strength assessments using a 16-big analog-to-digital converter (DHQCard-6036E, National Instruments, Austing, TX, USA) interfaced with a laptop computer (Inspiron 8200, Dell Inc., Round Rock, TX, USA). All signals were recorded, stored, and processed off-line with custom-written software (LabVIEW 8.5, National Instruments, Austin, TX, USA). EMG and MMG signals were analog filtered with a bandpass of 10-500 Hz and 5-100 Hz, respectively. The amplitudes of the EMG (EMG_{RMS}) and MMG (MMG_{RMS}) signals were quantified by calculating the root-mean-square (RMS) values for each signal epoch.

Muscle Biopsies

A muscle biopsy was taken from the vastus lateralis using the percutaneous needle biopsy methods of Bergstrom (11). After careful cleaning of the sample site, a local anesthetic was injected cutaneously, and a small incision was made through the skin and deep fascia with a scalpel. The biopsy sample was taken with a 5 mm needle (Pelomi Medical, Denmark) using the double-chop method and suction. After the biopsy was taken, the biopsied muscle sample was removed from the leg, it was rinsed in chilled phosphate-buffered saline, blotted dry and frozen in liquid nitrogen. The incision was closed with sterile strips, and a pressure bandage was placed over the incision site. Approximately 100 mg muscle samples were obtained from the right vastus lateralis of each participant. Of this sample, approximately 10 mg of wet skeletal muscle was weighed prior to being homogenized using 500 μL of cell lysis buffer (Tris-HCl, pH 6.8, 5% 2-mercaptoethanol, 10% glycerol, 2.3% SDS) using a tight-fitting pestle. Following muscle homogenization, samples were heated for 10 min at 60 C and 200 μL of 100% glycerol was added to the samples prior to storage at –80°C for subsequent protein analysis. The protein content of each sample was determined spectrophotometrically at a wavelength of 595 nm using a Bradford reagent (Bio-Rad Laboratories; catalog #: 500, Hercules, CA) in order to standardize the amount of protein loaded per well. MHC isoform content was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Homogenized muscle samples were obtained from the freezer and allowed to thaw at room temperature. One empty 1.2 ml microcentrifuge tube per sample was used to

place enough sample and Laemmli reducing buffer spiked with 5% 2-mercaptoethanol into each tube whereby 1.5 µl of protein per 30 µl of reducing buffer will be loaded per lane. Samples were heated for 2 min at 100 C and 30 µl of each sample was subsequently loaded into each well using gel loading tips. To determine MHC expression, samples were loaded on 8% gradient SDS polyacrylamide gels with 4% stacking gels, run for 11 hours at 190 V, and stained with Comassie blue. The MHC isoforms [Type I, IIa, and IIx] were identified according to their molecular masses.

Statistical Analyses

Simple linear regression models were fit to the natural log-transformed EMG_{RMS} vs. force and MMG_{RMS} vs. force relationships for the isometric step and ramp muscle actions. The equations are represented as:

$$\ln[Y] = b(\ln[X]) + \ln[a] \quad \text{(Eq. 1)}$$

Where $\ln[Y]$ = the natural log of the EMG_{RMS} or MMG_{RMS} values, $\ln[X]$ = the natural log of the force values, b = slope, and $\ln[a]$ = the natural log of the y-intercept. This can also be expressed as an exponential equation after antilog transformation of both sides of the equation:

$$Y = aX^{b} \quad \text{(Eq. 2)}$$

Where Y = the predicted EMG_{RMS} or MMG_{RMS} values, X = force, b = slope of Eq. 1, and a = the antilog of the y-intercept from Eq. 1. The slopes (b) and y-intercepts (a)

was calculated using Microsoft Excel® version 2003 (Microsoft, Inc., Redmond, WA).

A two-way mixed factorial ANOVA (age [20-25, 30-35, 40-45, 50-55, 60-65, and 70-75] x fiber type [Type I vs. Type IIa vs. Type IIx]) was used to examine the percent MHC isoform values. Three one-way between-group ANOVAs (age [20-25, 30-35, 40-45, 50-55, 60-65, vs. 70-75]) were used to analyze MVC PF, muscle cross-sectional area (CSA), and thigh skinfold thickness (SF). In addition, eight two-way mixed factorial ANOVAs (age [20-25, 30-35, 40-45, 50-55, 60-65, vs. 70-75] x muscle [VL vs. RF]) were used to analyze the isometric step and ramp muscle action EMG_{RMS} *a* and *b* values and isometric step and ramp muscle action MMG_{RMS} *a* and *b* values. When appropriate, follow-up analyses included additional ANOVAs, independent samples *t*-tests, and paired samples *t*-tests with Bonferroni corrections. An alpha level of $P \leq 0.05$ was considered statistically significant for all comparisons. All ANOVA models were conducted using SPSS v. 12.0 (SPSS Inc., Chicago, IL).

CHAPTER IV

RESULTS

Table 1 contains the mean ± standard deviation (SD) values for age, height, and weight for all age groups. The maximal voluntary contraction peak force (MVC PF), muscle cross sectional area (CSA), skinfold thickness (SF), and percent myosin heavy chain (MHC) isoform content values are presented in Table 2. Table 3 contains the *a* and *b* terms from the log-transformed mechanomyographic (MMG_{RMS}) and electromyographic (EMG_{RMS}) amplitude-force relationships from the isometric step and ramp muscle contractions for the vastus lateralis (VL) and rectus femoris (RF) muscles.

MHC

There was a significant two-way (group x fiber type) interaction ($P = 0.042$) and a main effect for fiber type ($P < 0.001$). One-way ANOVAs indicated that there were significant differences among groups for type I MHC isoform content ($P = 0.012$), however, there were no significant differences among groups for type IIx or type IIa MHC isoform content ($P > 0.05$). For type I MHC isoform content, the 70-75 age group had a higher expression of type I MHC (45.8 ± 5.28%) than the 20-25 age group (37.48 ± 3.24%) (Figure 1). In addition, paired samples *t*-tests indicated that expression of type IIx MHC isoform content (19.18 ± 7.24%) was less than type

IIa ($P < 0.001$, 39.78 ± 3.94%) and type I ($P < 0.001$, 41.04 ± 5.41%) MHC isoform content when collapsed across groups.

MVC PF

There was a significant one-way between-group interaction ($P = 0.003$). The post-hoc analyses indicated that the 20-25 ($P = 0.030$, 746.2 ± 305.2 N) and the 30-35 ($P = 0.003$, 812.9 ± 230.7 N) age groups had higher MVC PF than the 70-75 age group (459.7 ± 223.4 N) (Figure 2).

Muscle CSA and SF Thickness

There was a significant one-way between-group interaction ($P = 0.001$) for CSA. The post-hoc analyses indicated that the 30-35 age group (190.8 ± 7.9 cm^2) had a higher muscle CSA than the 60-65 ($P = 0.030$, 148.6 ± 20.4 cm^2) and 70-75 age groups ($P = 0.003$, 140.0 ± 22.1 cm^2). In addition, the 40-45 age group (182.5 ± 43.1 cm^2) had a higher CSA than the 70-75 age group ($P = 0.021$) (Figure 3). However, there was no significant one-way between-group interaction for SF thickness ($P = 0.850$) (Figure 4).

b Terms from the Isometric Step MMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.798$), no significant main effect for group ($P = 0.104$), but there was a significant main effect for muscle ($P < 0.001$). Post-hoc analyses

indicated that the *b* terms for the RF (0.76 ± 0.19) were greater than for the VL (0.62 ± 0.17) (Figure 5).

a Terms from the Isometric Step MMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.136$), no significant main effect for group ($P = 0.293$), but there was a significant main effect for muscle ($P = 0.014$). Post-hoc analyses indicated that the *a* terms for the VL (2.24 ± 2.08) where greater than for the RF (1.63 ± 1.78) (Figure 6).

b Terms from the Isometric Step EMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.800$), no significant main effect for group ($P = 0.765$), but there was a significant main effect for muscle ($P < 0.001$). Post-hoc analyses indicated that the *b* terms for the RF (1.12 ± 0.31) where greater than for the VL (0.93 ± 0.16) (Figure 7).

a Terms from the Isometric Step EMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.961$), no significant main effect for group ($P = 0.497$), but there was a significant main effect for muscle ($P = 0.029$). Post-hoc analyses

indicated that the *a* terms for the VL (0.83 ± 0.78) where greater than for the RF (0.43 ± 1.21) (Figure 8).

b Terms from the Isometric Ramp MMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.628$), no significant main effect for muscle ($P = 0.061$), but there was a significant main effect for group ($P = 0.032$). Post-hoc analyses indicated that the 20-25 age group ($P = 0.011$, 0.60 ± 0.16) had higher *b* terms than the 70-75 age group (0.32 ± 0.12) (Figure 9).

a Terms from the Isometric Ramp MMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.595$), no significant main effect for group ($P = 0.438$), and no main effect for muscle ($P = 0.454$) (Figure 10).

b Terms from the Isometric Ramp EMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.973$), no significant main effect for group ($P = 0.06$), but there was a significant main effect for muscle ($P < 0.001$). Post-hoc analyses indicated that the *b* terms for the RF (1.07 ± 0.26) where greater than for the VL (0.87 ± 0.17) (Figure 11).

a Terms from the Isometric Ramp EMG_{RMS}-Force Relationships for the VL and RF

The analyses indicated that there was no significant two-way interaction (group x muscle, $P = 0.505$), no significant main effect for group ($P = 0.067$), but there was a significant main effect for muscle ($P < 0.001$). Post-hoc analyses indicated that the *a* terms for the VL (1.54 ± 0.267) where greater than for the RF (0.47 ± 0.62) (Figure 12).

CHAPTER V

DISCUSSION

Age-related Quadriceps Muscle Strength and Morphology

Previous studies have reported age-related changes in muscle mass (39, 42). Lexell et al. (42) reported a 40% reduction in muscle CSA of the VL in previously healthy post mortem men aging from 20-80 years old. In the present study, there was an approximate 16% difference in muscle CSA between the 20-25 and 70-75 age groups. In addition, Lexell et al. (42) reported a gradual decrease in muscle CSA of the VL through the entire age spectrum. Interestingly, in the present study, muscle CSA was highest for the 30-35 and 40-45 age groups and then gradually decreased with age (Figure 3). When examining the age and muscle CSA relationship on a subject-by-subject basis in the present study (Figure 13) in conjunction with Lexell et al. (42), there is a large variation in muscle CSA within the younger age groups (20-45 years) and a noticeable decline in muscle CSA after 60 years. However, in contrast to the statistical differences reported for muscle CSA, type I MHC isoform expression was only different between the 20-25 and 70-75 age groups. Thus, muscle CSA is not solely dependent on the MHC isoform content of the muscle, which supports previous literature suggesting that the reduction in number and size of the fibers along with the shift in MHC isoform expression resulted in a decrease in muscle CSA throughout the aging spectrum (35, 36, 39, 42).

Klitgaard et al. (35) reported a 39% difference in type I MHC isoform content of the VL between sedentary young (mean age = 28 yrs) and sedentary old (mean age = 68) individuals. In addition, Larsson (38, 39) reported gradual decreases in the type II/type I fiber ratio from the age of 30 to 90 years and, thus, suggested that the percentage of type I fibers were gradually increasing. When examining the age and MHC isoform content relationships on an individual basis in the present study (Figure 14), there was a gradual increase in percent type I MHC isoform content starting after the 5th decade, whereas percent type IIx MHC isoform gradually decreased after the 5th decade. However, percent type IIa isoform content remained stable across age. The shift in percent MHC isoform content in the present study is possibly the result of the decrease in type IIx fibers. Lexell et al. (42) and Larsson (39) have suggested that the age-related denervation process results in a smaller muscle CSA because of the reduction in number and size of the type II fibers. In addition, Lexell et al. (41, 42) reported increases in the occurrence of fiber type grouping in old muscle, which suggested that the reinnervation process was also present in old muscle. Furthermore, Brown (14) and Campbell et al. (15) reported an age-related decline in the number of motor units in the thenar muscles with needle EMG. The authors reported that the mean motor unit potential size was enlarged for the individuals with less functioning motor units, which is believed to be the result of the reinnervation of inactive fibers. Therefore, the electrophysiological measurements (i.e. EMG) suggested that the changes in the number of motor units and motor unit potential size supported the hypothesis of denervation and

reinnervation within the muscle during the aging process. In summary, the MHC values from the present study support previous findings in that there was an overall increase in percent type I MHC isoform expression in aging muscle, particularly starting during the 6^{th} decade of life.

Vandervoort and McComas (58) measured plantar flexor strength in 111 healthy men and women and reported differences in isometric peak torque between young and old subjects. In the present study, MVC PF was significantly higher for the 20-25 and 30-35 age groups compared to the 70-75 age group. Examining the age and peak force/torque relationships on a subject-by-subject basis in the present study (Figure 15) in conjunction with Vandervoort and McComas (58), there was a gradual decrease in peak force/torque starting at 60 years. However, the age-related decrease in MVC PF did not match the age-related decreases in muscle CSA. MVC PF was highest for the 20-25 and 30-35 age groups, however, the 30-35 and 40-45 age groups had the largest muscle CSA. In addition, there was an approximate16% and 38% difference in muscle CSA and MVC PF between the 20-25 and 70-75 age groups in the present study. Previous studies have also reported this phenomenon and have concluded that the discrepancy between muscle CSA and MVC PF may be due to the presence of increased amounts of connective tissue in the muscles of the elderly subjects (33, 41, 60). This discrepancy between muscle CSA and MVC PF between young and old individuals has been attributed to differences in muscle quality (58). Essentially, muscle quality is the ratio of strength/force per amount of muscle mass (Figure 16). The ratio of MVC PF/CSA (N/cm^2) in the present study

(Figure 16), not statistically tested, indicated that the younger subjects were able to produce more force per muscle CSA than the older subjects. The MVC PF/CSA ratio supported the hypothesis that muscle quality is higher in the younger subjects compared to the older subjects, which may be the result of greater amounts of connective tissues in the older subjects (58). In summary, the age-related changes in peak force cannot be fully explained by the age-related muscle CSA changes in the present study.

Force-related MMG_{RMS} Patterns of Response

Surface MMG has been defined as the recording of low-frequency lateral oscillations of muscle fibers that occur during a muscle contraction and may provide unique information regarding the primary mechanism for increasing force production of a muscle (i.e., motor unit recruitment versus rate coding) (6, 47, 56). Previous studies have reported that MMG_{RMS} across the force spectrum either increases because additional motor units are recruited or plateaus and/or decreases when a higher firing rate is achieved and causes a fusion of the motor unit mechanical activity (4, 45, 54, 55). Researchers have suggested that the MMG_{RMS}-force relationships may be different between muscles that use different neuromuscular control strategies (i.e., motor unit recruitment vs. rate coding) to achieve peak force. For example, Akataki et al. (4) reported that the MMG_{RMS}-force relationship for the first dorsal interosseous (FDI) started decreasing at 45-50% MVC, whereas the MMG_{RMS}-force relationships for the biceps brachii (BB) started decreasing around

60-70% MVC. The authors concluded that because the FDI consisted of predominantly type I motor units, it relied primarily on rate coding at the higher contraction intensities to increase force. In contrast, the BB is considered a mixed fiber type muscle and relied primarily on the recruitment of addition motor units to increase force. Therefore, it is believed that the MMG_{RMS} signal may be able to distinguish between motor unit recruitment and rate coding as the primary neuromuscular control strategies to increase force. In theory, the MMG_{RMS}-force relationship may be able to distinguish between individuals with known fiber type differences, which may apply to older individuals with less type II motor units as a result of aging.

Previous studies have compared the MMG_{RMS}-force relationships between young and elderly men and have observed differences in the patterns of response (5, 57). Akataki et al. (5) reported that MMG_{RMS} during isometric ramp contractions did not demonstrate a rapid increase from 20-25 to 60-70% MVC, which is unlike younger individuals (9, 10, 18, 19). In contrast, MMG_{RMS} gradually increased from lower contraction intensities (0-25% MVC) to 60% MVC and then plateaued to 100% MVC. Akataki et al. (5) concluded that the age-related reduction of type II motor units may have explained the differences in the MMG_{RMS} patterns of response between the young and old individuals. Tian et al. (57) examined the isotonic leg extension MMG_{RMS}-force relationships in young and old individuals and reported lower MMG_{RMS} at 45%, 60%, and 75% MVC for the older compared to the young individuals. The authors speculated that the older individuals had fewer fast-twitch

motor units compared to the younger individuals, which may have explained the more rapid increases in MMG_{RMS} in the younger versus older subjects. In summary, Akataki et al. (5) and Tain et al. (57) reported age-related differences in the MMG_{RMS}-force relationship, which was believed to be due to the possible age-related loss of type II motor units.

Herda et al. (27) proposed a novel technique to examine the force-related patterns for MMG_{RMS}, which involved applying a simple linear regression model to the natural logs of the absolute force and MMG_{RMS} values. The *b* term from the log-transformed model indicates the rate of change or the linearity in the variable (MMG_{RMS}). For example, the relationship is linear if the *b* term is equal to 1, if the *b* term is not equal to 1, the relationship is nonlinear and exponential. When the *b* term >1 there is an upward acceleration, while a *b* term <1 reflects a downward deceleration (or plateau) in the relationships. Previous research studies have indicated that MMG_{RMS} increases from 20-25% to 60-70% MVC and then plateaus or decreases from 60-80% to 100% MVC (2, 46). Herda et al. (27) reported that the 95% CI intervals for the *b* terms from the MMG_{RMS}-force relationships were <1 (95% CI = 0.41 – 0.65), which reflected the plateaus at the higher contraction intensities in the MMG_{RMS}-force relationships. In theory, the *b* term from the log-transformed MMG_{RMS}–force relationships may be able to distinguish fiber type-related differences in neuromuscular control strategies (motor unit recruitment vs. firing rate). For example, a muscle that relies primarily on firing rate to reach peak force would have a lower *b* term compared to a muscle that relies primarily on

recruiting additional motor units to reach peak force. In support of this hypothesis, Herda et al. (26) reported that the *b* terms from the log-transformed MMG_{RMS}-force relationships (i.e., isometric ramp contractions) quantified on a subject-by-subject basis differences in the neuromuscular control strategies between individuals with known MHC isoform content differences. We reported that the *b* terms for the group with predominantly type I MHC isoform content were lower (95% CI = 0.26 – 0.38) compare to the group with predominantly type II MHC isoform content (collapsed across IIx and IIa subunits) (95% CI = 0.61 – 1.10). The lower *b* terms for the individuals with predominantly type I MHC isoform content suggested that there was an earlier plateau in the MMG_{RMS}-force relationship when compared to the individuals with predominantly type II MHC isoform content.

In the present study, there were differences in the *b* terms from the isometric ramp MMG_{RMS}-force relationships between the 20-25 and 70-75 age groups (collapsed across the VL and RF muscles). The *b* terms for the 20-25 age group (95% CI = 0.51 – 0.68) were higher than for the 70-75 age group (95% CI = 0.27 – 0.40), and reflected the percent type I MHC isoform content differences between the age groups. There were no other differences among the *b* terms from the isometric ramp MMG_{RMS}-force relationships, which coincided with the MHC isoform content values. Herda et al. (26) reported a 43% difference in type I MHC isoform content and a 62% difference in the *b* terms between two groups (Figure 17). In the present study, the difference in percent type I MHC isoform content between the 20-25 and 70-75 age groups was considerable lower at 18%, thus, the magnitude of difference

was far less than Herda et al. (26). Despite the smaller difference in type I MHC isoform content between groups in the present study, the *b* terms were still able to distinguished differences in the MMG_{RMS}-force relationships. In contrast, the *b* terms from the isometric step MMG_{RMS}-force relationships did not indicate a significant difference between the 20-25 and 70-75 age groups (26% difference in *b* terms). Even though there was not a significant difference between the isometric step MMG_{RMS}-force relationships, the age-related changes in *b* terms from the step and ramp muscle contractions were similar (Figure 18). The *b* terms under both conditions start noticeably decreasing during the 6th decade of life, which mimics the increase in age-related changes in type I MHC isoform.

The age-related comparison of MVC PF, muscle CSA, and *b* terms from the isometric ramp MMG_{RMS}-force relationships adds further support to the hypothesis that the log-transform MMG_{RMS} model may accurately reflect fiber type composition. In the present study, the *b* terms from the isometric ramp MMG_{RMS}-force relationships did not match the age-related changes for MVC PF and muscle CSA. For example, MVC PF was higher for the 20-25 and 30-35 age groups compared to the 70-75 age group, with the 40-45 age group having the highest MVC PF. Furthermore, muscle CSA was higher for the 30-35 and 40-45 age groups compared to the 70-75 groups, with the 30-35 age group having the highest muscle CSA. Thus, the *b* terms from the MMG_{RMS}-force relationships reflected the age-related differences in type I MHC isoform content and not the age-related changes in MVC PF or muscle CSA.

Force-related EMG_{RMS} Patterns of Response

Surface EMG is the recording of the motor units' action potentials that activate skeletal muscle fibers. The amplitude of the EMG signal reflects muscle activation and is influenced by both the number of active motor units and their firing rates (21). Researchers have reported linear and nonlinear relationships for the EMG_{RMS}-force patterns of response with the nonlinear relationships demonstrating an increase in EMG_{RMS} at a rate that is greater than the increase in force (40, 59). Woods and Bigland-Ritchie (59) reported linear relationships for the adductor policies and soleus and nonlinear relationships for the biceps brachii and triceps brachii. The authors reported that the muscles of predominantly uniform fiber composition (adductor pollicis and soleus) had linear relationships, whereas, muscles that are reported to be mix fiber type provided nonlinear responses with the EMG_{RMS} accelerating upwards across the force spectrum. Lawrence and De Luca (40) reported similar results between the EMG_{RMS}-force relationships of the biceps brachii and first dorsal interosseons with the biceps brachii demonstrating a nonlinear pattern that accelerated upwards across the force spectrum and linear relationships for the first dorsal interosseons. The authors concluded that differences in motor unit firing rate and recruitment properties between the muscles with known fiber type composition differences may partially affect EMG_{RMS} across the force spectrum. In contrast, Herda et al. (26), reported that the *b* terms from the EMG_{RMS}-force relationships were unable to distinguish differences in the patterns of response

between groups with known MHC isoform content differences. However, when examining the *b* terms on a subject-by-subject basis (Figure 1, pg. 90) in Herda et al. (26), it appears that the EMG_{RMS}-force relationships tended to be nonlinear (accelerated upwards across the force spectrum) for the individuals with predominantly type II MHC isoform content and the majority of the relationships were closer to linear for the individuals with predominantly type I MHC isoform content. Currently, it is unclear whether the EMG_{RMS}-force relationship may be able to distinguish between individuals with known fiber type differences, such as older individuals with less type II motor units as a result of aging.

There have been a limited number of studies have examined the effects of aging on the EMG_{RMS}-force relationships (23, 52, 57). Esposito et al. (23) reported that the amplitudes of the EMG signals were greater across the entire force spectrum for younger individuals when compared to the older individuals. Figure 1. (pg. 505) in Esposito et al. (23) indicated that the EMG_{RMS}-force relationship for the young individuals may have been nonlinear with an acceleration upwards across the force spectrum, while the patterns of response for the older individuals indicated a more linear relationship. The authors concluded that the possible loss of type II fibers in the elderly subjects may have contributed to the lower EMG_{RMS} values. In contrast, Shima et al. (52) reported that there were no differences between the EMG_{RMS}-force relationships between young and old individuals. In the present study, the results were very similar to Herda et al. (26) in that there was a non-significant age-related decrease in the *b* terms from the isometric ramp EMG_{RMS}-force relationships (Figure

11). The *b* terms from the EMG_{RMS}-force relationships were linear for the 20-25 age group and were nonlinear for the 70-75 age group. The 95% CI for the *b* terms from the 70-75 age group was <1 (Figure 11), which suggests that the patterns decelerated across the force spectrum and are considered to be nonlinear. Previous studies have reported that EMG_{RMS} increases either linearly or quadratically across the force spectrum and it has been hypothesized that the EMG_{RMS}-force relationships reflect the increases in both motor unit recruitment and the firing rates of the active motor units (7, 46, 47). However, these two motor control strategies (recruitment vs. rate coding) may not be as clearly distinguishable from traditional bipolar surface EMG (40) as reported for surface MMG. Considering the findings of Herda et al. (26), it was not completely unanticipated that the log-transformed EMG_{RMS}-force relationships were unable to differentiate at a significant level (alpha at 0.05) the MHC isoform content-related differences in motor control strategies between the 20-25 and 70-75 age groups.

Muscle-related Differences in the MMG_{RMS} and EMG_{RMS} Patterns of Response

Previous studies have suggested that there may be fiber type differences between the RF and VL muscles (28, 29). For example, Housh et al. (29) examined the EMG fatigue threshold during cycle ergometer on the RF, VL, and vastus medialis (VM) muscles. The authors reported that the fatigue response was different for the RF compared to the VL and VM. The results suggested that the RF may have fatigued faster than the other two muscles. The authors concluded that the RF may

have a greater percentage of fast-twitch fibers and/or because the RF is a biarticular muscle (unlike the VL and VM) may have contributed to the RF fatiguing faster than the other muscles. In support of this hypothesis, Johnson et al. (34) reported that there was a higher percentage of type I fibers for the RF compared to the VL in five of the six post-mortem individuals (age = 17-30 yrs). In theory, if there was a fiber type difference between the VL and RF, it may be reflected in the MMG_{RMS} and EMG_{RMS}-force relationships

The present study is the first effort to quantify the log-transformed EMG_{RMS} and MMG_{RMS}-force relationships in multiple muscles (VL and RF). The results indicated that the *b* terms from the EMG_{RMS} and MMG_{RMS}-force relationships for the step and ramp muscle actions were larger for the RF than the VL. The MMG_{RMS}-force relationships for the RF were closer to linear than the VL and the EMG_{RMS}-force relationships for the RF were nonlinear and accelerated upward across the force spectrum (*b* term >1) while the relationships for the VL were linear (*b* term = 1). Ryan et al. (50) examined the EMG_{RMS} and MMG_{RMS}-force relationships of the leg extensors (VL and RF) from an isometric ramp contraction. The authors normalized EMG_{RMS} and MMG_{RMS} to the highest value and applied polynomial regression to the relationships. Even though the methodologies were different between Ryan et al. (50) and the present study, the authors did graph the composite means for the relationships for which a comparison can be made. After closely examining Figure 4 (pg. 167), the MMG_{RMS} values for the RF are greater at the higher contraction intensities than for the VL (despite similar values at the lower

contraction intensities, <30% MVC) and it also appears that the plateau or decrease in MMG_{RMS} values happens slightly later in the force spectrum for the RF than for the VL. In addition, Ryan et al. (50) Figure 7 (pg. 168) contains the normalized composite patterns of response for the EMG_{RMS}-force relationships. After closely examining Figure 7, there is not a great amount of a disparity between the RF and VL compared to the MMG_{RMS}-force relationships. However, the normalized composite means for the RF does appear to be slightly greater at the higher contraction intensities than the VL, which indicates that there may have been a greater acceleration in the EMG_{RMS} across the force spectrum for the RF compared to the VL. The normalized composite patterns of response from Ryan et al. (50) tentatively supports the results in the present study in that the *b* terms from the EMG_{RMS} and MMG_{RMS}-force relationships were higher for the RF than the VL. Therefore, the results from Ryan et al. (50) and the present study suggests that there may be differences in the shape of the EMG_{RMS} and MMG_{RMS}-force relationships between the RF and VL. In addition, when considering Housh et al. (28, 29) and Johnson et al. (34), the differences in the patterns of response between the RF and VL may be the result of the RF having a greater percentage of type II MHC isoform content. However, future research is needed to further clarify these possible differences in the shapes of the EMG_{RMS} and MMG_{RMS}-force relationships.

Relationships Between Skinfold Thickness and the Amplitudes of the EMG and MMG Signals

The *a* terms from the log-transformed EMG_{RMS} or MMG_{RMS}-force relationships were recently (27) described as "gain factors" that represent upward or downward shifts in the exponential relationship without changing the shape of the curve. Therefore, if the EMG_{RMS} or MMG_{RMS} values are greater or lesser across the entire force spectrum, the *a* term would reflect this difference accordingly. In theory, subcutaneous fat acts as a low pass filter that may reduce EMG_{RMS} and MMG_{RMS} at all force levels compared to someone with less subcutaneous fat (24, 26, 44). Herda et al. (26) reported that the *a* terms from the EMG_{RMS} and MMG_{RMS}-force relationships for the group with significantly less subcutaneous fat (SF thickness = 8.7 mm) were greater than compared to the group with more subcutaneous fat (SF thickness = 25.3 mm). Therefore, the larger amount of subcutaneous fat may have been enough to act as a low pass filter that reduced the EMG_{RMS} and MMG_{RMS} values across the force spectrum. In the present study, there were no age-related differences in SF thickness and the *a* terms from the EMG_{RMS} and MMG_{RMS}-force relationships. The mean SF thickness did slightly decrease throughout the age spectrum (Figure 4) and it corresponded with a slight increase in the *a* terms from the relationships (Figures 6 and 8), however, these differences were not significant. Therefore, since there were no age-related changes in SF thickness, it is expected that there would be no age-related changes in the *a* terms from the EMG_{RMS} and MMG_{RMS}-force relationships. In contrast, there were differences in the *a* terms between the VL and RF for the isometric step and ramp EMG_{RMS} and MMG_{RMS}-force relationships (except isometric ramp MMG_{RMS}-force relationships).

In the present study, SF thicknesses were taken from the VL and not the RF. Previous unpublished data in our laboratory as indicated that for sixteen healthy college-aged men and women, there was a mean difference for SF thickness between the VL and RF. SF thicknesses for the VL were lower (mean ± SD; 22.8 ± 8.3 mm) than for the RF (29.31 ± 8.3 mm) and, furthermore, 15 of 16 subjects had a lower SF thickness for the VL than the RF. Therefore, the greater *a* terms for the VL in the present study are likely the result of the smaller amount of subcutaneous fat compared to the RF.

Isometric Ramp Versus Step Characterizations of the Force-related MMG_{RMS} and EMG_{RMS} Patterns of Response

Previous studies have examined the MMG_{RMS}-force relationships using either isometric ramp or (3, 46, 48, 49) or step (8, 16, 22, 47, 48) muscle actions. An isometric ramp muscle action is a single, nonstationary linear increase in force over an approximate 6-s period. Isometric step muscle actions are performed with discrete, stationary contractions held for 4-6 s at targeted percentages of the MVC. Aktaki et al. (3) and Orizio et al. (46) have suggested that ramp muscle actions may provide higher resolution throughout the force spectrum, require less time for data acquisition, and reduce the susceptibility to fatigue. However, ramp muscle actions are nonstationary, and may be difficult to analyze with traditional signal process techniques, whereas step muscle actions are assumed to be stationary (13). It has been well documented that there may be differences in the EMG frequency patterns

of response between isometric step and ramp muscle actions (1, 12, 13, 37, 43, 51), however, there have only been a limited number of studies that have examined the differences between isometric step and ramp muscle actions on the amplitudes of the EMG (1) and MMG (48) signals. For example, Akasaka et al. (1) examined integrated EMG_{RMS} of the plantar flexors during stepwise and ramp contractions and reported that integrated EMG_{RMS} increased in a linear fashion with force in both conditions. In contrast, Ryan et al. (48) reported differences in the MMG_{RMS} responses between step and ramp muscle actions. Ryan et al. (48) indicated that less than half the subjects exhibited the same responses during the ramp and step muscle actions for MMG_{RMS} and the authors concluded that differences existed between isometric ramp and step MMG_{RMS}-force patterns of response. In the present study, there was a significant difference (44% difference) in *b* terms between the 20-25 and 70-75 age groups. Whereas, the *b* terms from the step muscle actions were marginally different (26% difference) between the groups but not at a significant level.

Conclusion

The aging process is associated with the progressive decline in skeletal muscle mass that is accompanied by changes in muscle fiber type composition (35, 36, 39, 41, 42). Previous studies have reported the loss of type II fibers during the aging process with an invasive muscle biopsy. In addition, invasive electrophysiological measurements (i.e., indwelling EMG) have also demonstrated

motor unit remodeling during the aging process that supported the hypothesis that there is an age-related loss of type II fibers and, consequently, a greater percentage of type I fibers is reported. The age-relate loss of muscle mass with the subsequent loss of type II fibers has been termed sarcopenia (20). For the diagnoses of sarcopenia, an individual must exhibit low muscle mass accompanied by either low muscle strength and/or low physical performance (20). Currently, the diagnosis criterion for sarcopenia does not attempt to measure the underlying cause of sarcopenia, motor unit remodeling. In the present study, the *b* terms from the log-transformed MMG_{RMS}-force relationships from the ramp muscle actions reflected the difference in the MHC isoform expression between the 20-25 and 70-75 age groups. The *b* terms from the log-transformed MMG_{RMS}-force relationships from the step muscle actions also appeared to reflect the difference in MHC isoform content between the groups, however, was not a significant interaction. Furthermore, the *b* terms from the MMG_{RMS}-force relationships did not reflect the differences throughout the age spectrum for CSA or MVC PF reported in this study. In contrast, the *b* terms from the EMG_{RMS}-force relationships (step and ramp muscle actions) did not distinguish differences in the patterns of response between individuals with known MHC isoform content differences. This supports the findings of Herda et al. (26), which reported that the *b* terms from the EMG_{RMS}-force relationships did not distinguish differences in the patterns of responses between individuals with known MHC isoform content differences. The *a* terms (gain factor) from the EMG_{RMS} and MMG_{RMS}-force relationships were greater for the VL compared to the RF, which is

likely the result of the differences in overlying subcutaneous fat between the muscles. SF thickness was only taken of the VL in the present study, however, unpublished data from our laboratory suggests that SF thicknesses were greater for the RF than the VL. Therefore, the *a* terms (gain factor) shifted upwards (i.e., higher values) for the VL because of the lower amount of subcutaneous fat compared to the RF. In summary, the log-transformed MMG_{RMS}-force relationship may be an attractive model to include in the overall criterion to diagnose sarcopenia. This model is a noninvasive method that quantifies differences in fiber type composition on a subject-by-subject basis and, thus, is able to detect the loss of type II fibers throughout the age spectrum. Future research is needed to examine the effectiveness on including the MMG_{RMS}-force relationship in the overall diagnosis process of sarcopenia.

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APPENDIX A

FIGURES

Figure 1. Mean ± SD values for type IIx, type IIa, and type I percent myosin heavy chain isoform content for all age groups. * Represents a greater ($P < 0.05$) type I MHC isoform content for the 70-75 than the 20-25 age group.

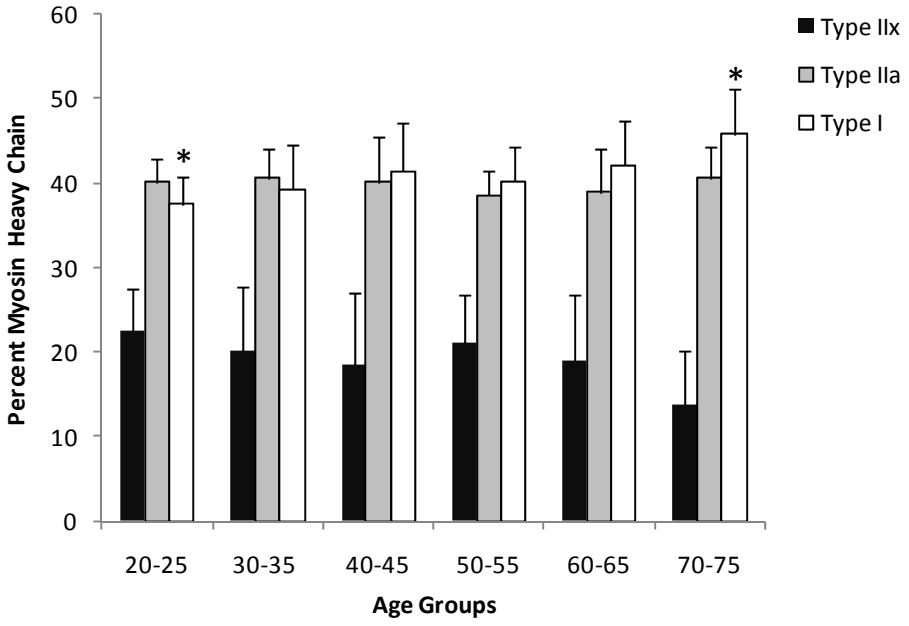


Figure 2. Mean ± SD values for maximal voluntary contraction peak force (N) for all age groups. * Represents a greater ($P < 0.05$) peak force for the 20-25 and 30-35 age groups than the 70-75 age group.

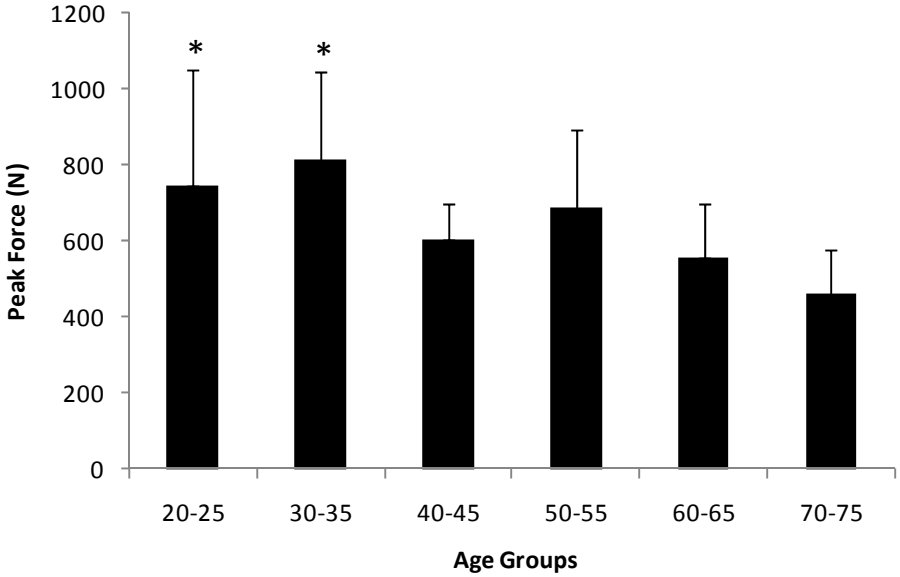


Figure 3. Mean ± SD values for muscle cross-sectional area (cm^2) for all age groups. * Represents a greater ($P < 0.05$) muscle CSA for the 30-35 than the 60-65 and 70-75 age groups. † Represents a greater ($P < 0.05$) muscle CSA for the 40-45 than the 70-75 age group.

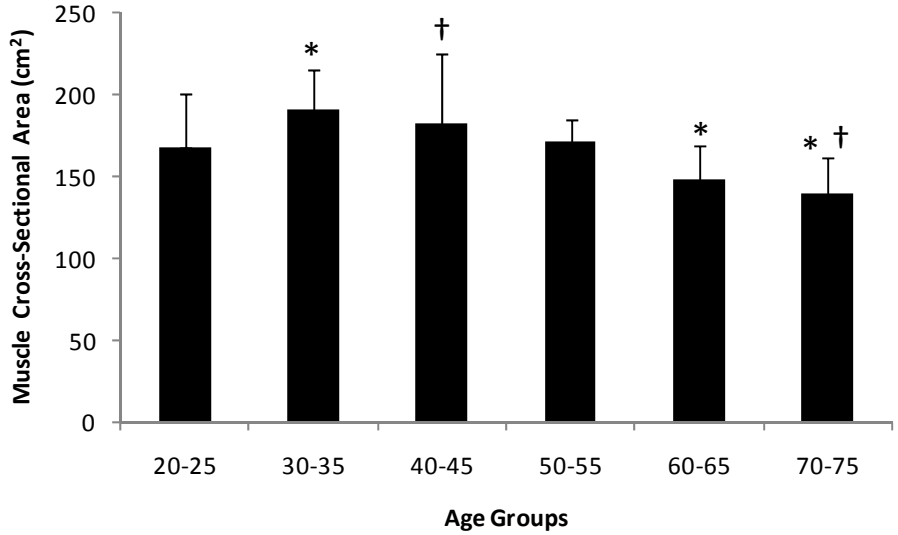


Figure 4. Mean ± SD values for thigh skinfold thickness (mm) for all age groups.

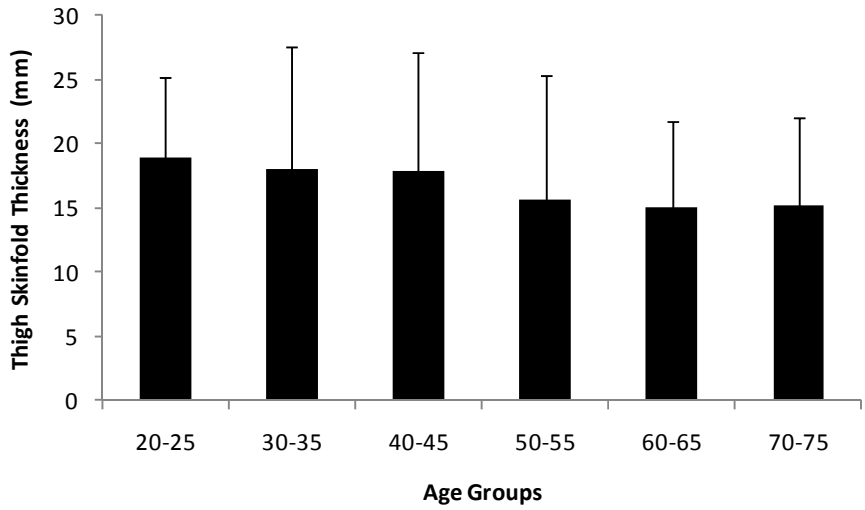


Figure 5. Mean ± 95% CI values for the *b* terms from the isometric step mechanomyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *b* terms for the RF than the VL (collapsed across groups).

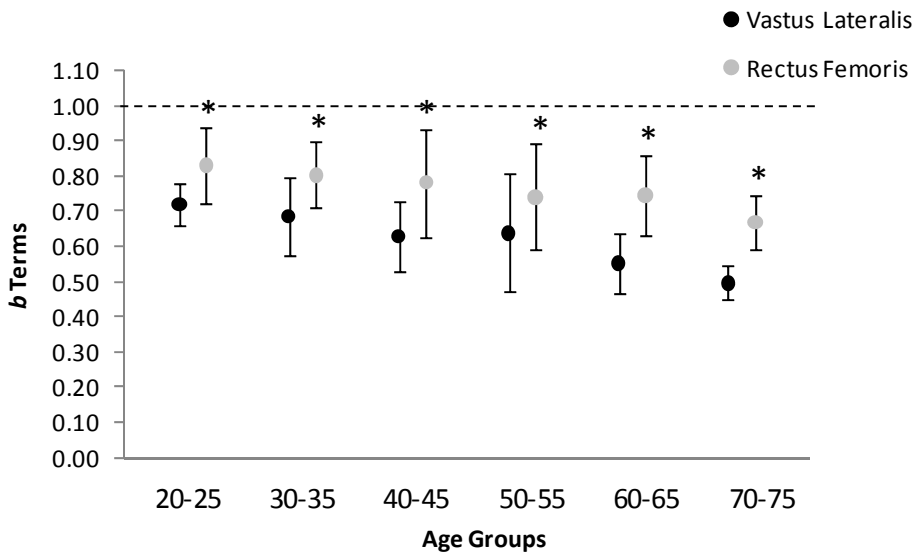


Figure 6. Mean ± 95% CI values for the *a* terms from the isometric step mechanomyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *a* terms for the VL than the RF (collapsed across groups).

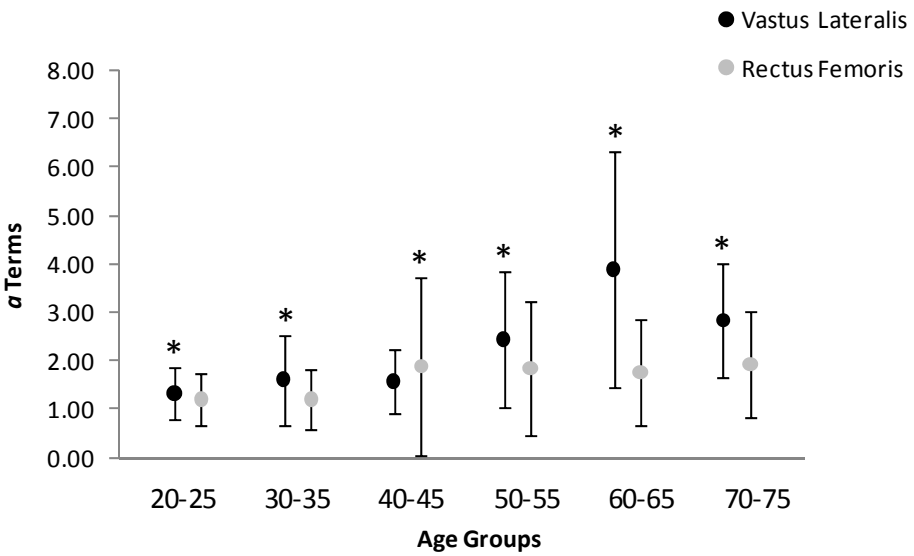


Figure 7. Mean ± 95% CI values for the *b* terms from the isometric step electromyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *b* terms for the RF than the VL (collapsed across groups).

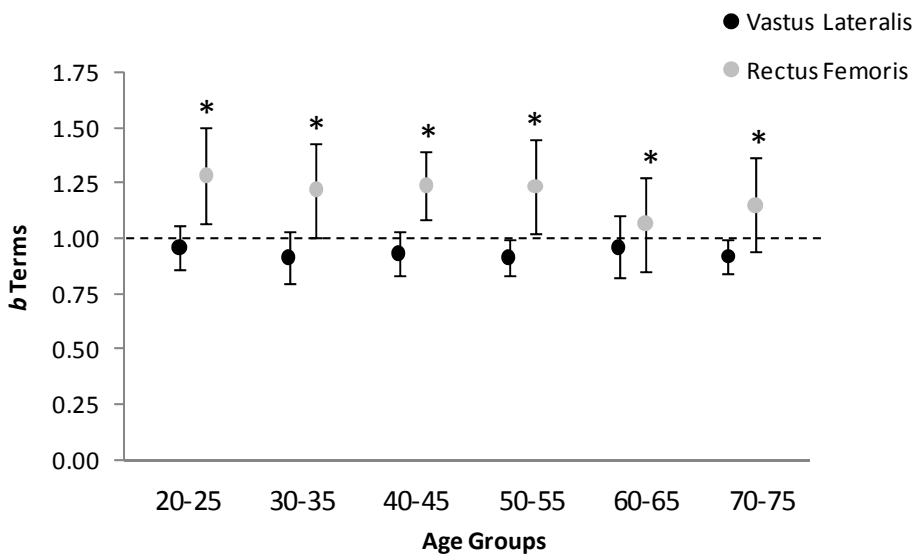


Figure 8. Mean ± 95% CI values for the *a* terms from the isometric step electromyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *a* terms for the VL than the RF (collapsed across groups).

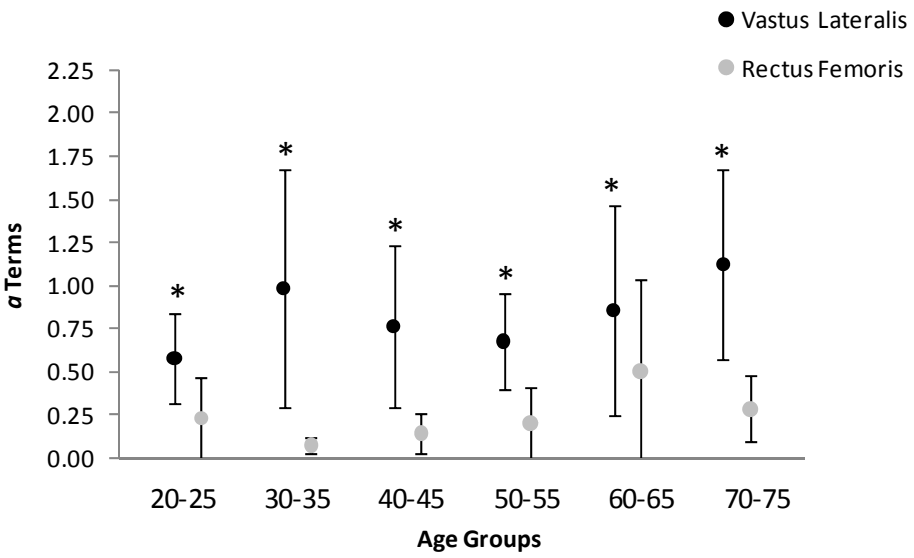


Figure 9. Mean ± 95% CI values for the *b* terms from the isometric ramp mechanomyographic amplitude versus force relationships for the vastus lateralis and rectus femoris muscles for all groups. * Represents greater ($P < 0.05$) *b* terms for the 20-25 than the 70-75 age group (collapsed across muscles).

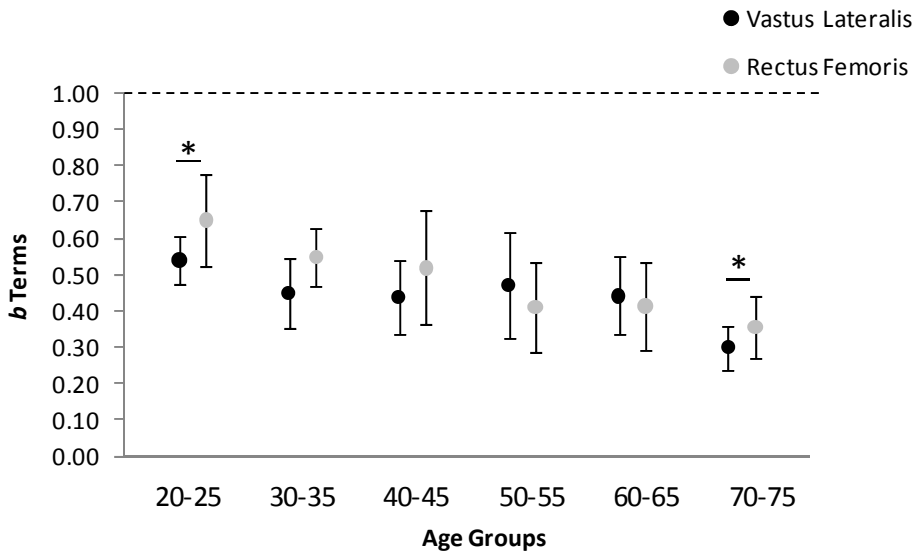


Figure 10. Mean ± 95% CI values for the *a* terms from the isometric ramp mechanomyographic amplitude versus force relationships for the vastus lateralis and rectus femoris muscles for all groups.

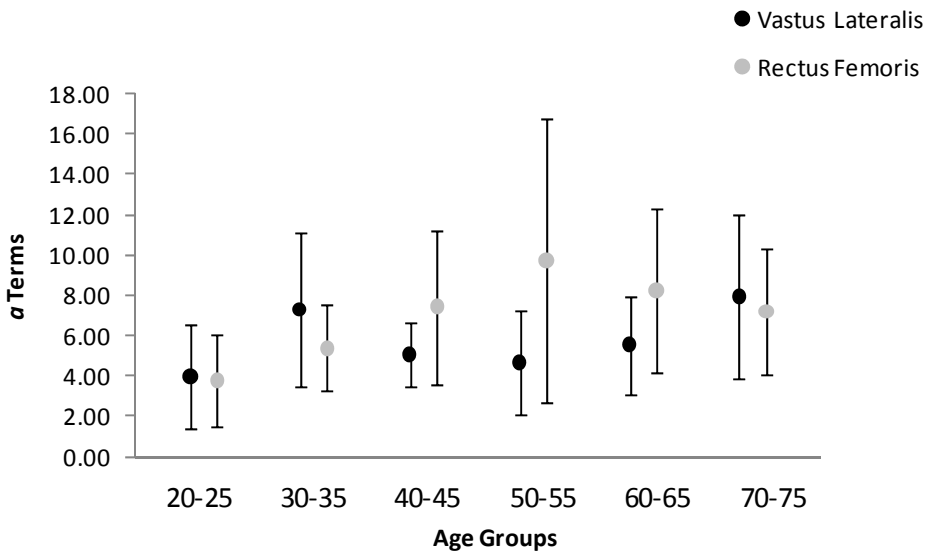


Figure 11. Mean ± 95% CI values for the *b* terms from the isometric ramp electromyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *b* terms for the RF than the VL (collapsed across groups).

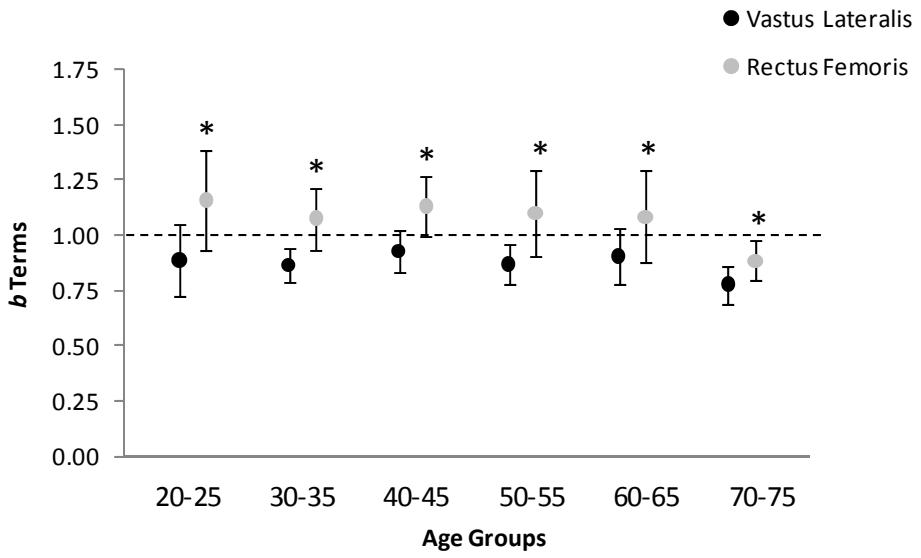


Figure 12. Mean ± SD values for the *a* terms from the isometric ramp electromyographic amplitude versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) muscles for all groups. * Represents greater ($P < 0.05$) *a* terms for the VL than the RF (collapsed across groups).

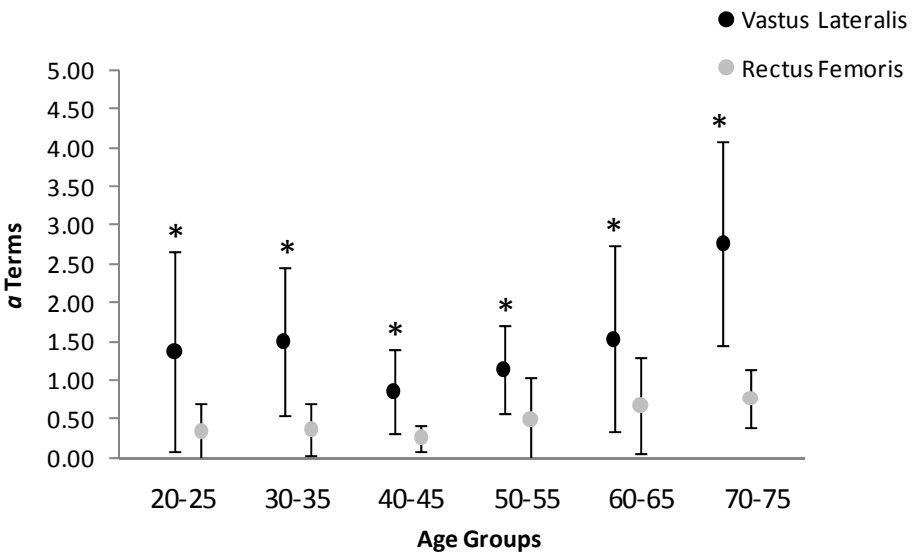


Figure 13. Plotted individual relationships for age (years) and muscle cross-sectional area (cm^2).

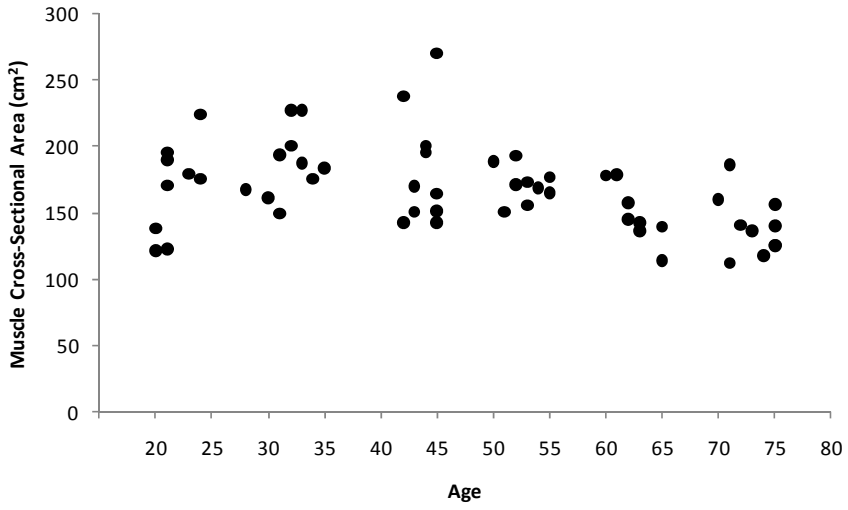


Figure 14. Plotted individual relationships for age (years) and percent myosin heavy chain isoform content for type IIx, type IIa, and type I isoforms.

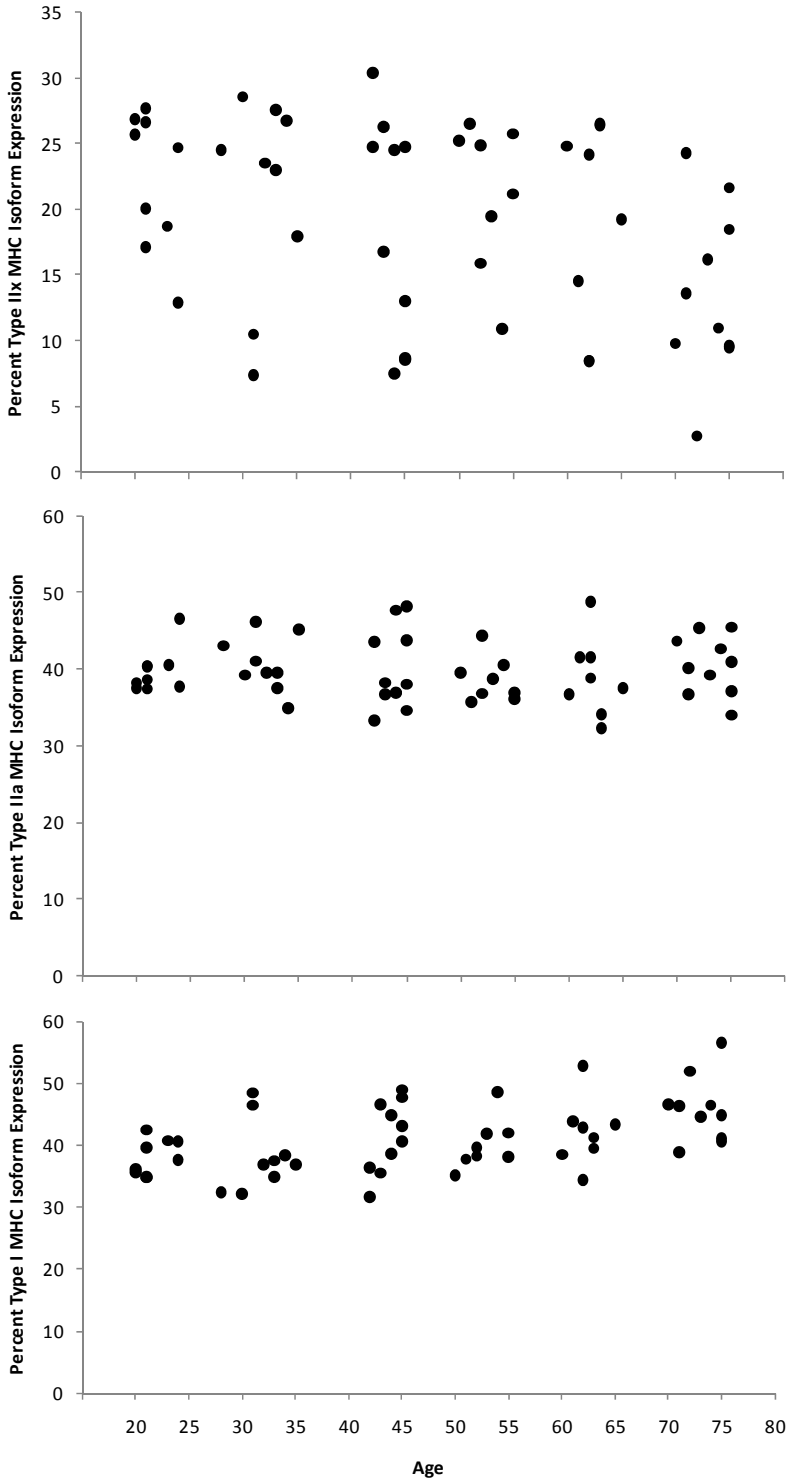


Figure 15. Plotted individual relationships for age (years) and maximal voluntary contraction force (N).

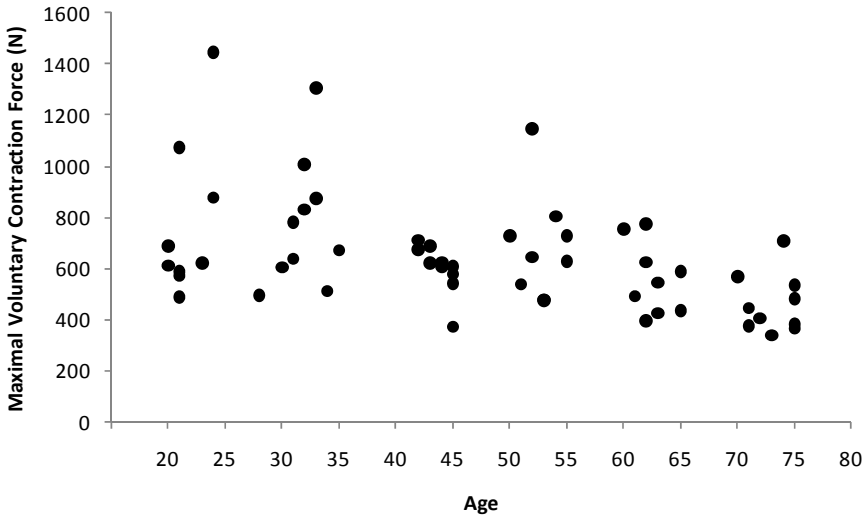


Figure 16. Mean ± SD values for the maximal voluntary contraction peak force (MVC PF) to muscle cross-sectional area (CSA) ratios for the 20-25, 30-35, 60-65, and 70-75 age groups.

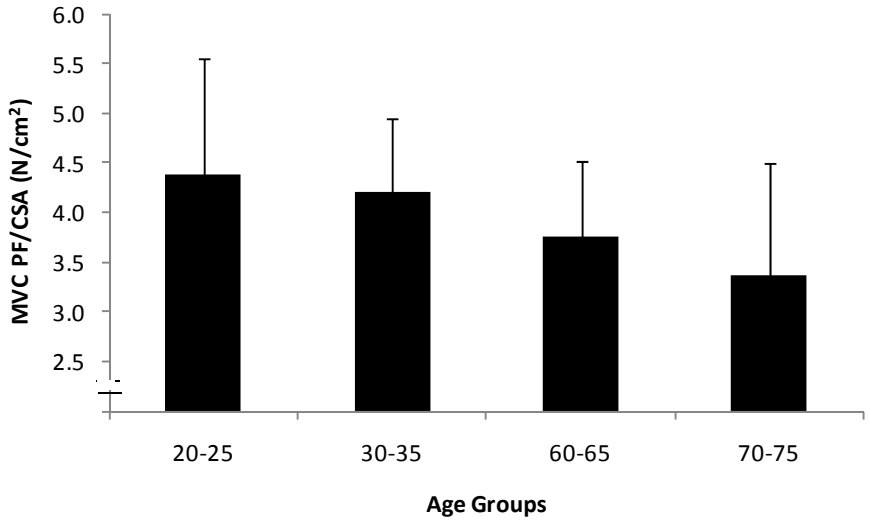


Figure 17. Percent difference between type I MHC isoform content and the *b* terms from the log-transformed mechanomyographic amplitude versus force relationships in Herda et al. (2010) *J Electromyogr Kinesiol*, 20:787-94 and the present study (20-25 vs. 70-75 age group). For Herda et al. (2010), the *b* terms were obtained from the vastus lateralis muscle and for the present study, the *b* terms are collapsed across the vastus lateralis and rectus femoris muscles.

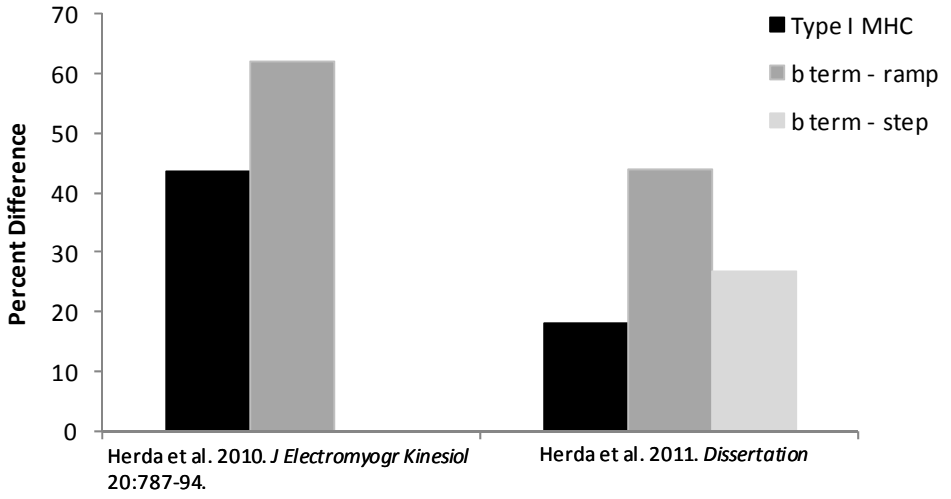
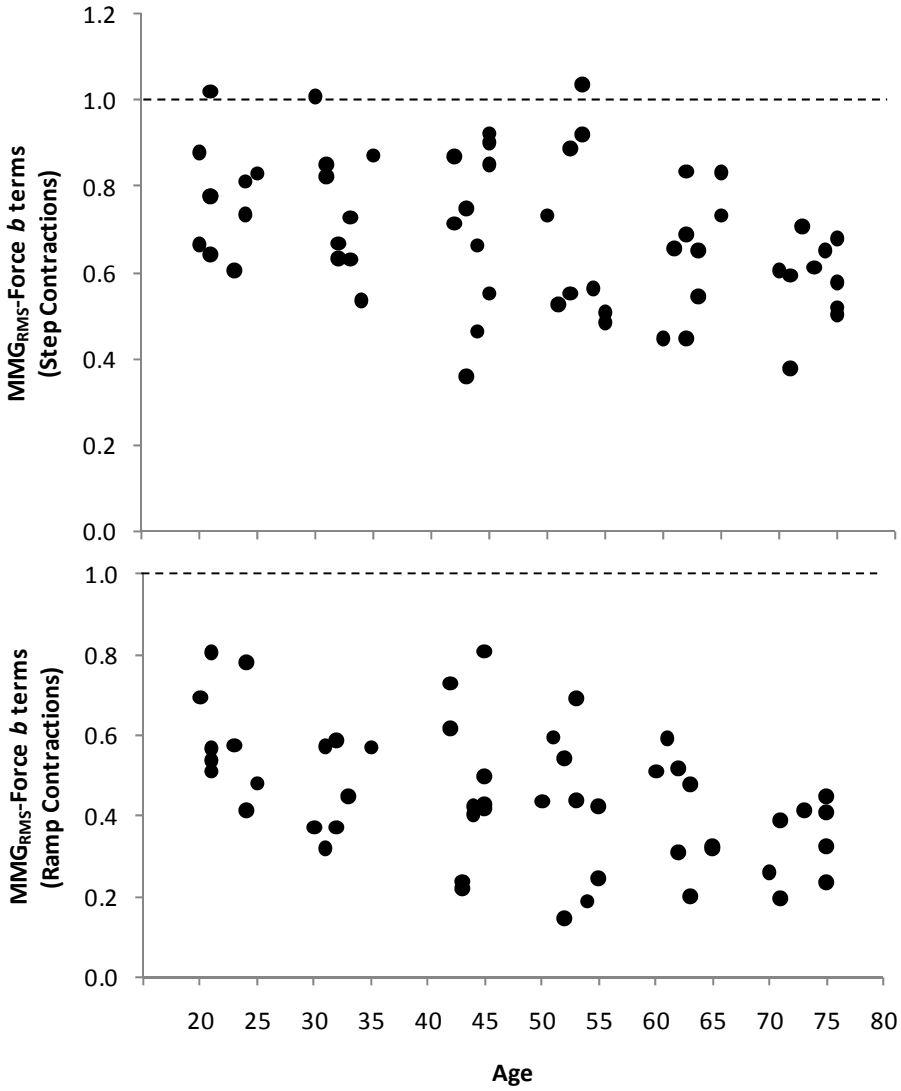


Figure 18. Plotted individual relationships for age (years) and *b* terms from the isometric ramp and step mechanomyographic amplitude (MMG_{RMS}) versus force relationships.



APPENDIX B

TABLES

Table 1. Mean ± SD values for age (yrs), weight (kg), and height (cm) for all age groups.

	Age Groups					
	20-25	**30-35**	**40-45**	**50-55**	**60-65**	**70-75**
Age (yrs)	22.3 ± 2.5	32.3 ± 1.6	42.6 ± 2.3	52.9 ± 1.9	62.8 ± 2.1	73.5 ± 2.5
Weight (kg)	82.0 ± 17.2	89.8 ± 13.4	86.7 ± 11.2	93.3 ± 11.7	83.6 ± 12.1	80.2 ± 12.8
Height (cm)	177.5 ± 7.3	175.4 ± 6.8	180.0 ± 6.2	174.8 ± 6.1	175.0 ± 6.3	171.5 ± 7.2

Table 2. Mean ± SD values for maximal voluntary contraction peak force (MVC PF), muscle cross-sectional area (CSA), skinfold thickness (SF), and the percent myosin heavy chain (%MHC) isoform content for all groups.

	Age Groups					
	20-25	**30-35**	**40-45**	**50-55**	**60-65**	**70-75**
MVC PF (N)	746.20 ± 305.24*	812.89 ± 230.67*	602.78 ± 96.06	685.80 ± 207.48	558.80 ± 137.94	459.72 ± 115.64
CSA (cm^2)	168.46 ± 32.72	190.82 ± 25.24**	182.52 ± 43.13***	171.41 ± 13.80	148.57 ± 20.43	140.00 ± 22.14
SF (mm)	18.90 ± 6.30	17.98 ± 9.57	17.90 ± 9.22	15.56 ± 9.75	15.03 ± 6.66	15.18 ± 6.87
Type IIx %MHC	22.84 ± 5.00	20.18 ± 7.61	18.50 ± 8.62	21.21 ± 5.57	19.07 ±7.69	13.66 ± 6.51
Type IIa %MHC	40.03 ± 2.92	40.56 ± 3.56	40.07 ± 5.33	38.59 ± 2.91	38.89 ± 5.14	40.53 ± 3.82
Type I %MHC	37.49 ± 3.24†	39.26 ± 5.29	41.42 ± 5.79	40.20 ± 4.05	42.04 ± 5.33	45.81 ±5.28

* Represents a significant difference between the 20-25 and 30-35 age groups compared to the 70-75 age group.

** Represents a significant difference between the 30-35 age group and the 60-65 and 70-75 age groups.

*** Represents a significant differnce between the 40-45 and 70-75 age groups.

† Represents a significant difference between the 20-25 and 70-75 age groups.

Table 3. Mean ± SD values for the *b* and *a* terms for the electromyographic (EMG_{RMS}) and mechanomyographic amplitude (MMG_{RMS}) versus force relationships for the vastus lateralis (VL) and rectus femoris (RF) during isometric step and ramp muscle contractions.

			Age Groups					
			20-25	**30-35**	**40-45**	**50-55**	**60-65**	**70-75**
Step Contraction	**MMG_{RMS} *b* term**	**VL**	0.72 ± 0.10	0.69 ± 0.18	0.63 ± 0.16	0.64 ± 0.24	0.55 ± 0.13	0.49 ± 0.09
		RF	0.83 ± 0.18*	0.80 ± 0.15*	0.78 ± 0.25*	0.74 ± 0.24*	0.74 ± 0.19*	0.67 ± 0.13*
	MMG_{RMS} *a* term	**VL**	1.34 ± 0.88**	1.60 ± 1.51**	1.57 ± 1.08**	2.44 ± 2.03**	3.89 ± 3.50**	2.84 ± 1.92**
		RF	1.21 ± 0.86	1.19 ± 1.00	1.88 ± 2.96	1.85 ± 2.02	1.76 ± 1.59	1.92 ± 1.78
	EMG_{RMS} *b* term	**VL**	0.96 ± 0.16	0.91 ± 0.19	0.93 ± 0.16	0.92 ± 0.12	0.96 ± 0.20	0.92 ± 0.13
		RF	1.28 ± 0.35*	1.22 ± 0.35*	1.24 ± 0.25*	1.23 ± 0.31*	1.06 ± 0.30*	1.15 ± 0.34*
	EMG_{RMS} *a* term	**VL**	0.58 ± 0.42**	0.98 ± 1.22**	0.76 ± 0.77**	0.68 ± 0.40**	0.85 ± 0.88**	1.12 ± 0.89**
		RF	0.23 ± 0.28	0.91 ± 2.65	0.14 ± 0.18	0.20 ± 0.30	0.50 ± 0.78	0.58 ± 0.98
Ramp Contraction	**MMG_{RMS} *b* term**	**VL**	0.54 ± 0.10†	0.46 ± 0.17	0.44 ± 0.16	0.47 ± 0.21	0.46 ± 0.16	0.30 ± 0.10†
		RF	0.65 ± 0.20†	0.55 ± 0.13	0.52 ± 0.25	0.47 ± 0.16	0.45 ± 0.16	0.35 ± 0.15†
	MMG_{RMS} *a* term	**VL**	3.97 ± 4.17	6.96 ± 6.53	5.06 ± 2.54	4.65 ± 3.76	5.96 ± 3.59	7.90 ± 6.90
		RF	3.79 ± 3.67	5.37 ± 3.43	7.41 ± 6.17	6.10 ± 3.82	8.23 ± 5.88	7.20 ± 5.01
	EMG_{RMS} *b* term	**VL**	0.89 ± 0.26	0.86 ± .12	0.92 ± 0.15	0.87 ± 0.13	0.90 ± 0.16	0.74 ± 0.11
		RF	1.16 ± 0.36*	1.02 ± 0.17*	1.13 ± 0.22*	1.10 ± 0.28*	1.08 ± 0.30*	0.88 ± 0.15*
	EMG_{RMS} *a* term	**VL**	1.37 ± 2.07**	1.50 ± 1.55**	0.86 ± 0.87**	1.14 ± 0.83**	1.53 ± 1.75**	2.77 ± 2.12**
		RF	0.33 ± 0.57	0.37 ± 0.54	0.25 ± 0.26	0.50 ± 0.78	0.67 ± 0.91	0.77 ± 0.59

* Indicates that the mean RF values are significantly greater than the mean VL values for that respected age group.

** Indicates that the mean VL values are significantly greater than the mean VL values for that respected age group.

† Represents a significant difference between the 20-25 and the 70-75 age groups.

APPENDIX C

Consent Version, Date ***IRB No: 14877***

Consent Form
University of Oklahoma Health Sciences Center (OUHSC)
University of Oklahoma-Norman

The Effects of Aging on the Neuromuscular Function of the Leg Extensors

Sponsor: Department of Health and Exercise Science, University of Oklahoma

Principle Investigator: **Chad M. Kerksick, PhD**
University of Oklahoma
405-325-9021

This is a research study. Research studies involve only individuals who choose to participate. Please take your time to make your decision. Discuss this with your family and friends.

Why Have I Been Asked To Participate In This Study?

You are being asked to take part in this trial/study because you are a healthy man who is able
to exercise and you may have completed a previous study "The Effects of Aging on the Intramuscular Markers of the Phosphocreatine System" (IRB 13637).

Why Is This Study Being Done?

Aging in men is associated with decreases in muscle mass. One contributing factor to muscle aging may included decreases in creatine levels inside your muscle. Creatine is produced by your body and helps the body to produce energy. In the previous study you participated in, muscle samples were collected in order to compare amounts of creatine in your muscle and anabolic hormone differences in men of different ages. In this study, we will be recording muscle function noninvasively, in hopes, that noninvasive methods may be able to predict age-related declines in the intramuscular phosphocreatine system.

What is the Status of the Drugs (Devices or Procedures) involved in this study?

Lidocaine is approved by the FDA as a local anesthetic.

How Many People Will Take Part In The Study?

About 60 people will take part in this study worldwide/nationwide. About 60 of these
individuals will participate at this location.

What Is Involved In The Study?

This study will consist of initial phone conversations and two testing session. During this time you will complete one visit to the lab to complete the following things:

> **Screening:** Initial screening to the study protocol will occur by phone with one of the study investigators. During this visit, the study investigator will ask you about your family and personal history in addition to various lifestyle habits, which will include your current alcohol or drug use.
>
> **Blood Collection:** (5 - 10 minutes) Approximately 15 milliliters (two tablespoons) of blood will be drawn from a vein located in the area in front of your elbow. The needle and supplies used are similar to what is used by your physician's office to draw blood. The blood will be drawn by Chad Kerksick, PhD, or graduate students trained in phlebotomy. It is important for you to follow all instructions provided to you by Dr. Kerksick and his staff to minimize any bruising and/or discomfort you may feel from the muscle collection and blood draw. To ensure your safety and provide medical care, Ryan Brown, MD will be available to provide medical consult to Dr. Kerksick and his staff if you experience and unexpected problem. This is important for you to understand since Dr. Brown will not be available on-site for emergencies but will be available for medical consultation for cases of infection, hematomas (hard bruise) etc.
>
> **Thigh Cross-sectional Area:** (20 - 30 minutes) You will have your thigh cross-sectional area assessed by the pQCT "type of xray" scanner (right leg). Furthermore, a pQCT scan is a type of x-ray procedure that will result in radiation exposure to you. This scan is not necessary for your medical care, but is being performed for research purposes only. The radiation to which you will be exposed from the pQCT scan is approximately 4% of the amount of radiation to which an x-ray technologist may be exposed in one year. You should be aware that the risk of radiation exposure is cumulative over your lifetime. Furthermore, a vertical skinfold measurement will be taken at the mid-thigh using Lange calipers (right leg). All skinfold measurements will be an average of three to ensure <10% reliability.

Passive Range of Motion: (15-25 minutes) You will perform a passive range of motion test of the right thigh muscles. You will be placed in an isokinetic dynamometer (standard physical therapy equipment) with a slight extension of the hip, then your right leg will passively move at 5º/sec from full leg extension to full leg flexion (heel to butt). Full flexion will be acknowledged by you as the point of discomfort, but not pain.

Strength Tests: (45-60 minutes) You will be re-positioned in the isokinetic dynamometer for the strength tests. After the repositioning and prior to the strength tests, electromyographic (EMG) and mechanomyographic (MMG) electrodes will be placed on the skins surface of your right thigh. Following 2-4 warm-ups, you will perform 2 maximal strength tests with 2 minutes rest between each one. Then in random order you will perform nine submaximal strength tests at 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, and 90% of your maximal strength. Then you will perform two ramp muscle actions that consists of you gradually increasing your force from 5% to 90% of your maximal strength. You will have 2 minutes rest between each strength test.

Evoked Twitch Test: (10-15 minutes) The final test will consist of five evoked twitches to your femoral nerve (thigh muscles). The electrical stimulation will be applied to you during rest.

For the second visit, you will complete the following:

Muscle Collection: (20 min) A small sample of muscle tissue will be removed from the outside portion of your thigh, halfway between your hip bone and your kneecap. The amount of muscle tissue will be equivalent to the size of a lead tip from a No. 2 pencil. During this procedure, your skin will be made numb using lidocaine, the same numbing agent that is used at the dentis. The needle used for this procedure is larger than a needle which is used for drawing blood. A small incision, approximately one-qurater of an inch will be made to more easily insert the muscle collection needle. As mentioned earlier, your skin will be made numb and as a result you will feel very little pain and likely significant pressure while the procedure is being completed. This entire process should take approximately one to two minutes. The uscle collection will be completed by Chad Kerksick, PhD, who is a professor of exercise physiology at the University of Oklahoma. Dr. Kerksick will be assisted by trained graduate students to assist him with this procedure.

How Long Will I Be In The Study?

This study should last for approximately a total of four hours. The first visit will last about 3.5 hours and the second visit will last about 30 minutes.

There may be anticipated circumstances under which your participation may be terminated by the investigator without regard to your consent, which include:

- He/She feels that it is in your medical best interest.
- Your condition worsens.
- New information becomes available.
- You fail to follow study requirements.
- The study is stopped by the sponsor.

You can stop participating in this study at any time.

What Are The Risks of The Study?

While on the study, you are at risk for these side effects. You should discuss these with the researcher and/or your regular doctor prior to providing your consent to participate.

A pQCT scan is a type of x-ray procedure that will result in radiation exposure to you. This scan is not necessary for your medical care, but is being performed for research purposes only. The radiation to which you will be exposed from the pQCT scan is approximately 4% of the amount of radiation to which an x-ray technologist may be exposed in one year. You should be aware that the risk of radiation exposure is cumulative over your lifetime.

Very Likely To Occur

- Pain, bruising, feeling faint and arm soreness from having your blood drawn and muscle collection during the 48 to 72 hours after completion.

- Muscle soreness or stiffness from completing maximal strength tests during the 48 to 72 hours after completion.

- Shortness of breath during the maximal strength testing.

Less Likely to Occur but Serious

- Chest pain, or abnormal heart rhythm during maximal strength testing.

- Bleeding from the muscle biopsy.

Less Likely to Occur

- Skin abrasions due to shaving and cleansing the skin with alcohol prior to electrode placement.

Are There Benefits to Taking Part in The Study?

There is no direct benefit to you to participate in this study, but the information from this study may increase knowledge about the effects of aging on muscle mass and muscle strength.

What Other Options Are There?

Your alternative is to not participate.

What About Confidentiality?

Efforts will be made to keep your personal information confidential. You will not be identifiable by name or description in any reports or publications about this study. We cannot guarantee absolute confidentiality. Your personal information may be disclosed if required by law. You will be asked to sign a separate authorization form for use or sharing of your protected health information.

There are organizations that may inspect and/or copy your research records for quality assurance and data analysis. These organizations include faculty members and graduate students appointed to this protocol from the Health and Exercise Science department at the University of Oklahoma and the OUHSC Institutional Review Board.

What Are the Costs?

There is no cost to you for participating in this study.

Will I Be Paid For Participating in This Study?

All individuals will be compensated for their time commitment associated with the study. Participants will be compensated $25 if they complete the one visit and $50 if they complete both visits (participants who did not complete IRB #13637). Participants who only complete the health history screening and consent process will not be compensated.

What if I am Injured or Become Ill While Participating in this Study?

In the case of injury or illness resulting from this study, emergency medical treatment is available. Payment for this treatment will be your responsibility. If injury occurs as a result of participation, you should consult with your personal physician to obtain treatment. No funds, however, have been set aside by The University of Oklahoma Health Sciences Center or University of Oklahoma to compensate you.

What Are My Rights As a Participant?

Taking part in this study is voluntary. You may choose not to participate. Refusal to participate will involve no penalty or loss of benefits to which you are otherwise entitled.
If you agree to participate and then decide against it, you can withdraw for any reason and leave the study at any time. However, at certain times during the treatment, it may be dangerous for you to withdraw, so please be sure to discuss leaving the study with the principal investigator or your regular physician. You may discontinue your participation at any time without penalty or loss of benefits, to which you are otherwise entitled.

We will provide you with any significant new findings developed during the course of the research that may affect your health, welfare or willingness to continue your participation in this study.

You have the right to access the medical information that has been collected about you as a part of this research study. However, you may not have access to this medical information until the entire research study has completely finished and you consent to this temporary restriction.

Whom Do I Call If I have Questions or Problems?

If you have questions, concerns, or complaints about the study or have a research-related injury, contact the Chad Kerksick, PhD at 405-325-9021 (office) or 405-248-8730 (cell phone 24 hours a day) or Trent Herda 405-615-8991 (cell phone 24 hours a day).

If you cannot reach the Investigator or wish to speak to someone other than the investigator, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

For questions about your rights as a research participant, contact the OUHSC Director, Office of Human Research Participant Protection at 405-271-2045.

Signature:

By signing this form, you are agreeing to participate in this research study under the conditions described. You have not given up any of your legal rights or released any individual or entity from liability for negligence. You have been given an opportunity to ask questions. You will be given a copy of this consent document.

I agree to participate in this study:

_____	_____	_____
PARTICIPANT SIGNATURE (age ≥18) (*Or Legally Authorized Representative)*	Printed Name	Date
_____	_____	_____
SIGNATURE OF PERSON OBTAINING CONSENT	Printed Name	Date

APPENDIX D

Name _____ Date_____

Home Address_____

Work Phone _____ Home Phone _____

Person to contact in case of emergency _____

Emergency Contact Phone _____ Birthday (mm/dd/yy)____/_____/_____

Personal Physician _____ Physician's Phone_____

Gender_____ Age _____(yrs) Height _____(ft)_____(in)
Weight_____(lbs)

Does the above weight indicate: a gain____ a loss____ no change____ in the past year?
If a change, how many pounds?_____(lbs)

A. JOINT-MUSCLE STATUS (✓Check areas where you currently have problems)

Joint Areas
( ) Wrists
( ) Elbows
( ) Shoulders
( ) Upper Spine & Neck
( ) Lower Spine
( ) Hips
( ) Knees
( ) Ankles
( ) Feet
( ) Other_____

Muscle Areas
( ) Arms
( ) Shoulders
( ) Chest
( ) Upper Back & Neck
( ) Abdominal Regions
( ) Lower Back
( ) Buttocks
( ) Thighs
( ) Lower Leg
( ) Feet
( )

Other_____

B. HEALTH STATUS (✓Check if you currently have any of the following conditions)

( ) High Blood Pressure
( ) Heart Disease or Dysfunction Abnormality
( ) Peripheral Circulatory Disorder
( ) Lung Disease or Dysfunction
( ) Arthritis or Gout
( ) Edema
( ) Acute Infection
( ) Diabetes or Blood Sugar Level
( ) Anemia
( ) Hernias
( ) Thyroid Dysfunction
( ) Pancreas Dysfunction

( ) Epilepsy
( ) Multiply Sclerosis
( ) High Blood Cholesterol or Triglyceride Levels
( ) Liver Dysfunction
( ) Kidney Dysfunction
( ) Phenylketonuria (PKU)
( ) Loss of Consciousness

( ) Allergic reactions to rubbing alcohol

** NOTE: If any of these conditions are checked, then a physician's health clearance will required.*

C. PHYSICAL EXAMINATION HISTORY

Approximate date of your last physical examination_____

Physical problems noted at that time_____

Has a physician ever made any recommendations relative to limiting your level of physical exertion? _____YES _____NO
If YES, what limitations were recommended?_____
_____

D. FEMALE REPRODUCTIVE HISTORY

If you are male, skip to Section E.

Did you begin menses within the past year? _____YES _____NO

Have you had consistent menstrual periods for the last 3 months?
YES____ NO____

Date of onset of last menstrual period_____

Have you used a hormonal contraceptive within the last 3 months?
YES_____ NO_____

E. CURRENT MEDICATION USAGE (List the drug name, the condition being managed, and the length of time used)

MEDICATION	CONDITION	LENGTH OF USAGE
_____	_____	_____
_____	_____	_____
_____	_____	_____

F. PHYSICAL PERCEPTIONS (Indicate any unusual sensations or perceptions. ✓Check if you have recently experienced any of the following during or soon after *physical activity* (PA); or during *sedentary periods* (SED))

PA	SED		PA	SED	
( )	( )	Chest Pain	( )	( )	Nausea
( )	( )	Heart Palpitations	( )	( )	Light Headedness
( )	( )	Unusually Rapid Breathing	( )	( )	Loss of Consciousness
( )	( )	Overheating	( )	( )	Loss of Balance
( )	( )	Muscle Cramping	( )	( )	Loss of Coordination
( )	( )	Muscle Pain	( )	( )	Extreme Weakness
( )	( )	Joint Pain	( )	( )	Numbness
( )	( )	Other_____	( )	( )	Mental Confusion

G. FAMILY HISTORY (✓Check if any of your blood relatives . . . parents, brothers, sisters, aunts, uncles, and/or grandparents . . . have or had any of the following)

( ) Heart Disease
( ) Heart Attacks or Strokes (prior to age 50)
( ) Elevated Blood Cholesterol or Triglyceride Levels
( ) High Blood Pressure
( ) Diabetes
( ) Sudden Death (other than accidental)

H. EXERCISE STATUS

Do you regularly engage in aerobic forms of exercise (i.e., jogging, cycling, walking, etc.)? YES NO

How long have you engaged in this form of exercise? ___years ___ months

How many hours per week do you spend for this type of exercise? ____hours

Do you regularly lift weights? YES NO

How long have you engaged in this form of exercise? ____ years _____months

How many hours per week do you spend for this type of exercise? _____hours

Do you regularly play recreational sports (i.e., basketball, racquetball, volleyball, etc.)? YES NO

How long have you engaged in this form of exercise? ____ years _____ months

How many hours per week do you spend for this type of exercise? ____ hours