MUSCLE STIFFNESS AS A CAUSE OF REDUCED MUSCLE VOLUME – A RELIABILITY STUDY

by

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STUDY.
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A leading cause of impairment in elderly individuals is sarcopenia, the progressive

deterioration of muscle size and strength, which ultimately limits muscle functionality. The loss

of muscular strength is directly correlated with increased mortality, hypertension, metabolic

syndrome, and diabetes. This corresponds with limitations to activities of daily living, therefore

further restricting the individual's independence. The purpose of our study is to test the

reliability of our methods for measuring muscle volume, stiffness, and strength for future

sarcopenia research. We have evidence proving the validity of ultrasound and elastography

techniques to measure muscle volume and stiffness. We have targeted the medial and lateral

gastrocnemius muscles in 6 young adults aged 20-30 years old. Measurements will be taken

along the longitudinal axis via ultrasound to determine muscular length. Muscular area will be

calculated by taking 5 cross-sectional ultrasound measurements across the muscle. These

measurements together will be used to calculate muscular volume, while muscular stiffness will

be measured using ultrasound elastography. Isokinetic and Isometric strength tests will be

performed on the Humac dynamometer and will represent the maximum muscular strength of

each subject. A Bland-Altman plot and regression analysis will be performed for each method

comparing data from the first testing session against the second to verify reliability. A regression

analysis will also be performed to determine the relationship between muscle volume, strength

and stiffness to determine if there is a correlation in a young population.

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Chapter 1:
Introduction

With age, people may develop sarcopenia, the gradual deterioration of muscle volume

and muscular strength associated with increasing age. Although this condition is not present in

all individuals, it affects about 50% of people over the age of 80 (Baumgartner, 1998). Muscle

loss can begin as early as age 30, and muscle volume continues to decline throughout the

individual's life span (Waters, 2010). The loss of muscle size coincides with a decline in

muscular strength, which ultimately impacts muscle functionality and limits an individual's

independence and mobility. Research has shown that strength in the quadriceps of older adults is

reduced by 48% compared to younger individuals, which results in a 45-80% increase in the time

it takes for the individual to walk, climb stairs, and rise from a chair (Haus, 2007).

Weakness in the lower extremities can reduce an individuals' independence and impact

his or her successful participation in activities of daily living. Reduced muscle functionality can

affect stability and increase the risk for older adults to fall and acquire injuries that may be

disabling or fatal (Go, 2013). Over time, the progression of sarcopenia can continue to increase

the risk for the individual to develop other serious conditions, such as metabolic syndrome and

diabetes (Moon, 2013).   These conditions alone increase the individual's risk for mortality;

however, research has shown an additional mortality risk factor observed in low handgrip

strength. Studies have shown that handgrip strength is a valid representation of an individual's

muscular strength (Rantanen, 1999). Overall, there is a negative relationship between handgrip

strength and all-cause mortality, therefore low muscular strength could increase an individual's

risk for premature death (Rantanen, 1999).

The health concerns with the progression of sarcopenia have provided significant motivation for research to determine a cause of the disease. Previous studies have already established a potential relationship between sarcopenia and changes in nutrition and physical activity typically seen in the elderly population. With old age there is often a diminution in the intake of essential nutrients and regular physical activity, which reduces the protein synthesis required to maintain muscle volume (Chapman, 2002); (Park, 2013); (Fry, 2011). Additional research studies have focused on the involuntary effects of aging that could play a role in the induction of sarcopenia. This includes the loss of motor neurons and the depletion of anabolic hormones with age. This reduction restricts muscle function and has been expected to play a role in muscle hypotrophy (Huh, 2013).

After reviewing the literature, we consider the previous evidence to be valid in determining potential causes for muscle loss and strength decline in old age. However, we do not think this evidence explains the entire cause of sarcopenia. We believe that a reduced response to exercise corresponding with old age is a relatively unexplored area that might also contribute to sarcopenia.

Several studies show that elderly subjects experienced a reduced muscular response to the designated exercise programs when compared to a younger population (Raue, 1999);(Moritani, 1980). Muscular hypertrophy and significant strength gains were observed predominantly in the younger populations in both studies (Raue, 1999); (Moritani, 1980). The research shows that the elderly subjects experienced some degree of strength improvements, but limited muscular hypertrophy in response to the exercise programs (Raue, 1999);(Moritani, 1980). The increase in muscular strength may result from increased neural stimulation following the training especially when considering the lack of muscular hypertrophy (Moritani, 1980).

Studies have linked the strength and hypertrophic restrictions to differences in gene expression and cell signaling between young and old populations. IGF1 and CNTF are both significant muscle mass regulators that have shown to be drastically reduced as a result of aging (Dennis, 2007). On the other hand, higher levels of the negative regulator GDF8 were found in older adults compared to younger adults following an exercise program (Dennis, 2007). Research also shows an increase in REDD1 mRNA in older women due to the bodies' failure to downregulate the gene after resistance training (Grieg, et al, 2011). Downregulation of REDD1 mRNA is essential to ensure the upregulation of mTOR signaling which signals protein synthesis (Grieg, et al, 2011). Researchers have concluded that the disruption of protein synthesis due to certain gene alterations can affect an elderly individual's hypertrophic response following an exercise program (Dennis, 2007);(Grieg, et al, 2011).

Statement of the Problem

The limited hypertrophic response and altered gene expression in older adults may result from changes in the mechanotransduction process due to increased muscular stiffness. Stiffening of the muscle cell can result from an accumulation of advanced glycation end products (AGE's) that are found in elderly skeletal muscle (Haus, et al, 2007). This buildup disrupts the breakdown of proteins, leading to an increase in collagen crosslinks and deformed collagen fibers - which further contributes to muscle stiffness. Muscle stiffness causes the cell membrane to become inflexible and limits stretching in response to an external stimulus. We believe this interferes with the mechanotransduction process, as the strain receptors are unable to detect the force and stimulate protein synthesis. Over time, the muscle will undergo hypotrophy due to a lack of regular forces acting upon it.

Hypothesis

Our hypothesis is that muscular atrophy and attenuated muscular strength is related to increased muscle stiffness.

Significance of the Study

The elderly population is expected to rise significantly in the next decade and with this will come an increase in sarcopenia cases. Due to the health risks associated with muscular atrophy, there are significant benefits to understanding the derivation of the disease. Our long term research goal is to evaluate muscle stiffness as a possible explanation for the development of sarcopenia. With an understanding of another potential cause of sarcopenia, future efforts can be made towards designing an intervention that may reduce the prevalence of sarcopenia in older individuals.

Our current study will provide the foundation for future sarcopenia research. We targeted a younger population to test the accuracy and practicality of our methods of measuring muscle volume, stiffness, and strength. If our methods are reliable, we can test for evidence of any correlation between the three factors in a younger population for future comparison. After we ensure the reliability of the testing procedures we will expand the research by including elderly subjects to research the affect of age-related muscle stiffness on muscle physiology.

Delimitations

This study is limited to a population between the age of 20-30 in order to refine the testing procedures before involving older subjects. To avoid differences in sex hormones and the possible affect on muscle composition and the muscular response to exercise, we have limited the study to females only.

Limitations

Our subjects are all aged between 20-30 years old and have yet to experience signs of sarcopenia. We have restricted ourselves by not collecting data from an older population. By studying the relationship between muscle size and muscle stiffness, we have limited ourselves to be able to determine only a correlation, not causation between the three factors. In addition, we are not directly studying the reduced response to exercise following a particular resistance-training program, therefore there could be several other factors contributing to sarcopenia.

Definitions

i. **Sarcopenia** - the gradual deterioration of muscle volume and muscular strength

ii. **IGF1** – a growth factor that controls muscle volume due to a mechanical or hormonal stimulus (Dennis, 2007).

iii. **CNTF** – a cytokine that regulates protein synthesis and promotes muscle excitation by preventing the death of motor neurons (Dennis, 2007).

iv. **GDF8** – a negative regulator of muscle hypertrophy – lower levels result in higher muscle mass. GDF8 decreases following an exercise program, however the duration in which this depletion occurs is individual specific (Dennis, 2007).

v. **Mechanotransduction** – the transformation of physical forces into chemical signals that stimulate muscle movements (Shwartz, et al, 2013). Stretch receptors on the cell membrane sense an external stimulus and release chemicals that signal protein synthesis (Wu, et al, 2011)

vi. **AGE's** – Advanced glycation end products – glycated proteins that result from the prolonged contace of monosaccarides with protein residue (Haus, et al, 2007)

Chapter II:
Review of Literature

This literature review will include background information on the prevalence and negative effects of sarcopenia on health and independence. Furthermore, it will include potential causes of muscular atrophy studied in previous research followed by evidence supporting our hypothesis involving muscle stiffness. In addition, the review will cover the prospective causes of muscle stiffness with age, along with a summary, which briefly covers implications for further research.

Sarcopenia

A leading cause of impairment in elderly individuals is sarcopenia, the progressive deterioration of muscle size and strength, which ultimately limits muscle functionality. The onset of muscle atrophy can occur around age 30, as individuals tend to lose about .5 pounds of muscle a year, combined with a gain of 1 pound of fat (Waters, 2010). By age 60, about 40% of the muscle cross sectional area is lost (Waters, 2010). The rate of muscle loss is not consistent with all individuals; a variety of factors also play a role, such as nutrition, physical activity level, and ethnic and environmental factors (Huh, et al, 2013). Sarcopenia exists in about 15% of individuals greater than 65 years of age and increases to about 50% around 80 years of age (Baumgartner, 1998).

In sarcopenia, Type II muscle fibers are cannibalized, accounting for the majority of the total loss in muscular strength (Lexell & Taylor, 1991). This is due to the fact that Type II muscle fibers are fast twitch fibers and promote 5-6 times the power exerted by the Type I muscle fibers (Haus, Carrithers, Trappe, & Trappe, 2007). Research shows that with age, maximum power of the quadriceps muscle decreases by 48% (Haus, 2007).

Effect on Quality of Life

The loss of muscular power and function in an elderly individual can affect activities of daily living and reduce independence. It was determined that the time it takes for individuals to climb stairs, rise from a chair, and walk is increased by 45-80% with old age (Haus, 2007). The mobility restriction and limited ability for a person to rise from a chair or a bed may require modified accommodations to satisfy the individual's changing needs (Rosenberg, 1997). Reduced mobility oftentimes forces the patient to use a cane or a walker to promote better balance during walking (Go, et al, 2013). Balance issues associated with sarcopenia make affected individuals prone to injuries due to the high prevalence of falling (Go, et al, 2013). There is a positive relationship between the amount of injuries to the hip and femur relative to the amount of falls in older adults (Jorgenson, 2014). There is a positive association between mortality and falls as well for older individuals especially due to injuries resulting in hospitalization and decreased functionality (Jorgenson, 2014). A common injury resulting from falls is a hip fracture, which affects both the individuals' mental and physical state. Following a hip fracture, most individuals don't return to their previous physical state and therefore become more dependant on others due to their prolonged disability (Karni, 2014).

The consequences of sarcopenia are not only physical, but mental as well. Sarcopenia can affect an individual's emotional state by causing anxiety and depression both of which are fairly common amongst the patients (Go, et al 2013). Falls and the resulting injuries reduce the individual's self-efficacy in their ability to move and participate in regular activities of daily living (Karni, 2014). The associated consequences of sarcopenia can result in negative attitudes toward physical activity, sociality, and the individual's quality of life (Karni, 2014).

Effect on Health and Mortality

Over time, the mental and physical consequences to sarcopenia described above can lead to other serious health concerns. Studies have shown that sarcopenic individuals are at an increased risk for hypertension, obesity, and elevated triglyceride and glucose levels –all of which are symptoms of metabolic syndrome (Moon, 2013). The risk for insulin sensitivity and, as a result, diabetes is significantly increased in sarcopenic individuals as well (Moon, 2013). The prevalence of these diseases amongst the sarcopenic population occurs mostly in adults greater than 60 years of age – there is no correlation between muscle strength and these metabolic diseases in the younger population between 20-39 years of age (Moon, 2013). These diseases occurred independently from BMI, meaning low muscle volume has a greater influence on health and mortality than body weight alone (Rantanen, 2000). However, the risk for disease was further increased in the sarcopenic population if an individual was obese (Moon, 2013).

These health concerns can ultimately predict increased mortality due to muscular strength loss. Overall, those with lower handgrip strength experienced two times the greater risk for mortality and disablement in old age (Rantanen, 1999). The risk of death may result from damages obtained through the development of the sarcopenia related diseases above. Studies show that low muscular strength impairs the body's ability to repair tissue, which leads to lower survival rates from chronic diseases and hospitalizations (Rantanen, 2000). Reduced tissue repair can result from and promote further injuries, which along with reduced muscular strength, can limit an individual's functionality (Rantanen, 2000).

Adverse health conditions are not only a result of sarcopenia, but may also contribute to the problem. Diseases and abnormal health problems may promote discomfort and unsteadiness in an individual, which lowers physical activity levels. This process ultimately comes full circle

as lower physical activity levels lead to an increased risk for future health problems and the development of sarcopenia.

Health Care Costs

The development of detrimental health conditions and the associated disabilities can put health and financial stress on the affected person. The loss of strength associated with sarcopenia results in lower health quality and an increased risk for injury. In 2000, the estimated health care costs from injuries associated with sarcopenia was about $18.5 billion and is expected to rise in the near future as the elderly population increases (Janssen, 2003). This value covered costs from hospital visits, outpatient care, and home health. The estimate didn't take into account the monetary sacrifice due to a lack of the patient's productivity (Janssen, 2003).

Causes of Sarcopenia

The increased prevalence of disabilities, health concerns, and the resulting health care costs due to sarcopenia have sparked interests in investigating the potential sources of the problem. Previous studies have researched connections between several aging factors and the onset of sarcopenia. The results have shown that neural loss, a reduction in anabolic hormones, changes in nutrition and physical activity, and a reduced response to exercise all trigger sarcopenia and result in muscular atrophy.

Neural Loss

Previous studies have predicted that sarcopenia may result from neural loss that correlates with increasing age. With decreased mobilization due to aging, some motor units become dysfunctional and deinnervate from the muscle cell, resulting in cell death (Kaya, et, al, 2013). Cell death mainly occurs in motor units connected to Type II fibers and contributes to muscular deterioration with age. With an overabundance of Type 1, slow twitch muscle fibers, the firing

rates of the remaining motor units decrease resulting in slower, less stable movements (Erim, 1999). The unattached nerve fibers can reinnervate to surrounding muscle fibers, however, this can result in the loss of fine-tune movement, due to the excessive accumulation of Type I fibers on a single muscle fiber (Erim, 1999). The deinnervation and reinnervation process that occurs with age affects the signal from the brain to the muscle fiber and the muscle loses overall power (Kaya, et al, 2013).

Changes in Anabolic Hormones

With age comes an insufficiency in anabolic hormones as well. Testosterone is a major determinant for the growth and maintenance of skeletal muscle and bone (Huh, 2013). With age, testosterone levels decrease in both men and women and eventually lower the muscle mass volume. Women have a significantly lower amount of testosterone than men, therefore the depletion of testosterone impacts male muscles to a greater degree (Huh, 2013). The more prominent reduction of testosterone in men can explain the early onset of sarcopenia in men compared to women (Huh, 2013). Estrogen has a smaller influence on the muscle, however, it is an important contributor to muscle functionality and it limits fat penetration in the muscle (Park, 2013).

Changes in Nutrition

Changes in nutrition are customary in the elderly population and these modifications are suspected to play a key role in the development of sarcopenia (Chapman, 2002). Aging results in dietary alterations due to premature satiety and adaptations to taste, changing social conditions, and monetary status that correspond with old age (Chapman, 2002); (Buford, 2010). The popular phrase "anorexia of aging" refers to the diminution of food and energy intake that occurs with age progression (Chapman, 2002). A decrease in food intake drastically lowers the

nutrients and energy available for protein synthesis and can result in a decrease in muscular volume. Protein synthesis doesn't occur as rapidly or efficiently in elderly individuals, therefore an increase in protein ingestion is expected to maintain muscle volume (Chapman, 2002). Studies show that those with sarcopenia consumed a lower amount of protein, carbohydrates, and energy than their estimated energy requirement (EER) compared to those with a healthy muscle mass (Park, 2013). Overall, 40% of elderly individuals don't consume the required amount of protein, therefore the risk for sarcopenia enhances with old age (Burton, 2010).

Elderly individuals are more susceptible to an insufficiency of Vitamin D as well due to a lack of sunlight exposure and a decrease in the efficiency of Vitamin D synthesis (Park, 2013). Vitamin D has been linked to muscle fiber maintenance and increased muscle functionality, especially in the lower extremities (Park, 2013). Muscle function is feasible through the production and availability of ATP as well, which is controlled by creatine. With age, there is a lack of obtainable creatine available for the body to produce adequate levels of ATP. Shortages of ATP during exercise and activities of daily living promote muscle failure, fatigue, and strength loss (Rom, 2012).

Changes in Physical Activity

According to the CDC, the physical activity guidelines for older adults include 150 minutes of moderate intensity aerobic exercise or 75 minutes of vigorous aerobic activity a week combined with at least 2 days of muscular strengthening activities (Center for Disease Control, 2011). Overall, a large amount of the elderly population doesn't acquire the recommended amount of physical activity (Fry, 2011). Protein synthesis depends on a mechanical stimulus and without the recommended amount of physical activity, the body is unable to maintain protein production (Fry, 2011).

Aerobic activity increases the transport and usage of nutrients in the muscle (Timmerman, 2012). A deficiency of regular aerobic exercise can lead to hypotrophy, increased fat infiltration of the muscle, and a decrease in functionality due to a lack of protein synthesis. A reduction in resistance exercise can cause a greater decline in muscular strength and results in slower gait speeds, loss of balance, and reduced physical function (Burton, 2010). Regular resistance exercise can improve joint mobility and muscle function, while decreasing pain that might limit individuals from being physically active (Shwartz, et al, 2013). Therefore, by being physically active, an individual can possibly reverse the effects of sarcopenia and increase muscular volume.

Reduced Response to Exercise

Regular physical activity may not prevent the onset of sarcopenia in all older adults, however. The progressive loss of muscle mass in older individuals can potentially be explained by a reduced muscular response to exercise. In 2009, Raue produced a study proving that following a resistance-training program, elderly individuals experienced a lower muscular hypertrophic response to exercise compared to the younger subjects. Similar results were discovered in a study by Moritani and deVries in which a significant increase in muscular cross sectional area was apparent in the younger population following an exercise program, but not in the elderly participants (Moritani, 1980). When evaluating muscular strength, both age groups improved in response to the resistance training, however strength gains were more apparent in the younger population as well (Raue, 1999);(Moritani, 1980). The observed strength improvement in the older population was deemed to be more dependant on increased neural stimulation, without a strong influence from muscle hypertrophy (Moritani, 1980).

The limited response to exercise is also demonstrated through limited gene expression with age. IGF1 is the most prevalent growth factor that responds to mechanical stimuli in order to promote and maintain muscular growth (Dennis, et al., 2007). Research comparing muscle composition between the young and old populations has shown that this growth factor is significantly diminished in the older population (Dennis, et al., 2007). Other factors, such as the CNTF growth factor, are also drastically reduced in the elderly population (Dennis, et al., 2007). This factor is responsible for neuron survival; therefore a decrease in this gene concentration will reduce muscle excitation, cell differentiation, and protein synthesis, all of which can ultimately limit muscular hypertrophy (Dennis, et al., 2007).

Protein synthesis can be halted by an overabundance of negative regulatory factors as well. The diminution of REDD1 mRNA following resistance exercise in the young population results in increased mTOR signaling and consequently the augmentation of protein synthesis (Grieg, et al, 2011). In the elderly population, REDD1 mRNA is not downregulated following the exercise program, leading to limited mTOR signaling for protein synthesis (Grieg, et al, 2011). A superfluity of the negative regulator GDF8 is also more prevalent in the elderly population following an exercise program, when compared to the younger population (Dennis, 2007). In this case, a lesser amount of the regulator results in increased muscle mass (Dennis, 2007). The reduction and overabundance of certain growth factors in the elderly population can possibly play a role in the induction of sarcopenia, even if the patient participates in regular exercise. Over time, the muscle will undergo atrophy due to the lack of a cellular response to the external forces acting upon it. As the strain sensitive receptors undergo less stimulation, the muscle cell will eventually decrease in size to compensate for the long-term decrease in perceived mechanical signaling.

We have chosen to focus our research on the reduced response to exercise as a possible cause of sarcopenia. However, if it is not a factor in muscular atrophy, the limited hypertrophic response in elderly adults still affects the treatment of sarcopenia and should undergo further research.

Mechanotransduction

To explain the reduced hypertrophic response to exercise, our overall research project focuses on alterations to the mechanotransduction process in order to target the first step in the mechanical signal transmission leading to protein synthesis. We believe that focusing on the first step of the transmission process will help us to determine the root of the signaling problem. Mechanotransduction is the transformation of physical forces into chemical signals that stimulate muscle development (Shwartz, et al, 2013). During this process, stretch receptors on the cell membrane sense an external stimulus, analyze the signal, and respond by releasing chemicals (Wu, et all, 2011). In younger individuals, the connective tissue surrounding the muscle cell stretches in response to an external force. As muscle cells age, they become inflexible and stretch less in response to the mechanical stimulus. With less muscular strain, the cell is unresponsive and doesn't release the chemicals necessary to activate certain pathways or enzymes responsible for protein production (Fry, 2011).

Chemicals released by the stretch receptors trigger signaling pathways used to phosphorylate and stimulate certain proteins necessary for a muscular hypertrophic response. These pathways are load dependant, meaning that protein synthesis occurs only when an external force acts on the muscle (Klossner, 2009). An example of a signaling pathway is the FAK/p7056k pathway in which focal adhesion kinase (FAK) reacts to the external force and activates the p7056k protein to increase muscle hypertrophy (Klossner, 2009). With age, there is

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a decrease in the FAK phosphorylation, therefore limiting pathway signaling from the muscle stretch receptors and reducing protein synthesis (Rice, 2007). In older individuals, the mTORC1 and MAPK signaling pathways are also inhibited, therefore resulting in reduced protein synthesis (Fry, 2011). This diminution results in a loss of muscular strength and mass due to a weakened cellular response to exercise. In other words, the muscle is not able to gain the benefits associated with resistance exercise (Fry, 2011).

Causes of Muscle Stiffness

There are several explanations for the decreased stretch and inflexibility of the muscle cell membrane with advancing age. Muscle fiber connective tissue in older muscle contains an increased amount of advanced glycation end products (AGEs) resulting from the prolonged contact of monosaccarides with protein residues (Haus, et al, 2007). AGEs are common in diabetics of all ages, but are also prevalent in the non-diabetic elderly population (Haus, et al. 2007). AGEs build up in immobile, elderly individuals because there is a lack of the mechanical stimulus required for normal tissue turnover (Haus, 2007).

AGEs diminish the efficiency and breakdown of proteins; therefore leading to an accumulation of inefficient proteins (Snow, et al, 2007). Studies show that aging increases AGE count by 200%, which in addition to a decrease in protein competency, results in a loss of collagen cross-linking, deformed collagen fibers, and impeded cellular communication (Haus, et al, 2007); (Reddy, 2004). Each muscle fiber is surrounded by a connective tissue supported by an arrangement of collagen fibers that provide structure and strength to the fiber (Haus, et al., 2007). Studies found that with age, the number and arrangement of collagen fibers remained the same, however, the linkage between fibers increased (Gao, 2007). Some studies have shown that with increasing age, collagen production increases as well. For this to be possible, an increase in

collagen breakdown must coincide with the increase in production in order for collagen content to appear unrelated to age (Haus, et al., 2007).   The increase in AGEs, collagen cross-linking, and a possible increase in collagen production stiffens the connective tissue surrounding the muscle fiber, therefore reducing cellular flexibility in response to an external stimuli.

Summary

Muscular atrophy and strength loss with age affects activities of daily living and individual functionality. By the year 2030, the United States population above age 65 is expected to reach 72 million, compared to the 41.4 million currently reported (Kaya, et al, 2013). Of these individuals, 15%-50% of them will develop sarcopenia and will have an increased risk for injury and mortality (Baumgartner, 1998). With sarcopenia comes strength loss, balance and mobility problems, and decreased independence (Go, et al, 2013). Furthermore, there is a positive relationship between declining muscular strength and mortality (Jorgenson, 2014). Those with sarcopenia have an increased risk for developing injuries, diabetes, metabolic syndrome, and insulin resistance, all of which could result in an early death (Moon, 2013).

Previous researchers have discovered a connection between sarcopenia and neural loss (Kaya, et, al, 2013)(Erim, 1999), changes in physical activity and nutrition, and a loss in anabolic hormones. After reviewing these theories, we have focused our research on a new hypothesis based on the reduced muscular response to exercise that is seen in the elderly population. The overall theory is that older individuals have a decreased muscular response to activity due to impairment in the mechanotransduction process. This muted cellular response ultimately will result in the loss of muscle mass. The muscle cell stiffens with age and therefore, stretch in response to a mechanical stimulus is reduced. The strain sensitive receptors will then undergo less stimulation, and the muscle cell will eventually decrease in size. The specific research I will

conduct will evaluate the methods used for further research on the relationship between muscle size, stiffness, and strength. Our overall hypothesis is that with age, the muscle may stiffen and result in reduced muscular volume.

Muscle stiffness with aging can result from several factors including an increase in the development of AGE's and the multiplication of collagen cross-links. Knowing this information, possible interventions can be established and applied to aging individuals to reduce muscle stiffness. By decreasing muscular stiffness, there is the possibility that the overall prevalence of sarcopenia could diminish in older individuals.

Chapter III:
Methods

Purpose

The purpose of the study was to test the reliability of our methods for measuring muscle volume, stiffness, and strength for future sarcopenia research.

Subjects

Six healthy female subjects were tested between the ages of 20-30 years old. The subjects were all in good health, nonsmoking, and had a BMI less than 27 kg/m2. The Institutional Review Board approved all protocol before the subjects were tested.

Data Collection

Each subject participated in two separate testing sessions. During each session, the subject underwent ultrasound, elastography, and strength testing. Ultrasound imaging was used to determine muscular volume (Infantolino, et al, 2007). Data was collected via the Aixplorer ultrasound system (Supersonic Imagine, Aix-en-Provence, France) using 2D technology. The imaging depth was set to 6 cm to account for all muscle sizes and to ensure that the entire depth of the muscle was visible in the image.

The study focused on the volume of the medial and lateral gastrocnemius muscles of the right calf. The gastrocnemius muscles were traced from the distal insertion points at the Achilles tendon to the proximal insertion points at the medial and lateral condyles. Longitudinal ultrasound scans were taken along this axis from the distal insertion point of each muscle towards the proximal insertion point to determine muscular length in centimeters.

To measure muscular cross sectional area at specific locations across the muscle, the longitudinal axes of the medial and lateral gastrocnemius muscles were divided and marked into

five equal segments. The longitudinal axis began one centimeter in from each end of the muscle. At every muscle slice, a cross-sectional ultrasound image was taken beginning at the lateral border of each muscle and moving towards the medial border. Each ultrasound scan was performed two times and an average was taken to provide the final measurement value. Separate ultrasound measurements were taken from both muscles.

To measure muscle stiffness, we used elastography - a new technology that projects sound waves into the muscle causing the tissue to vibrate. The machine measures the travel rate of the shear vibration waves to determine muscle stiffness (Eby, et. al, 2013). The machine assesses the muscle stiffness of the gastrocnemius muscles as stiff tissue transmits the waves faster than less stiff tissue. Stiff tissue was recorded as a yellow-red color and less stiff muscle appeared as a blue color.

Before testing, the machine was set for 100 kPa. The probe was placed on the center of each muscle belly and held for 10 seconds while the device recorded the shear waves transmitted through the muscle. The probe was placed at this location because this is generally the thickest part of the muscle and best represents the stiffness of the muscle as a whole. Three elastography readings were taken from the same location on each muscle for both testing days.

To determine muscular strength we performed both isometric and isokinetic strength tests on the Humac dynamometer. The isometric test was designed to test the ankle planter flexor strength at 0 degrees of flexion for a series of 5 trials. These trials were performed at the subjects' maximum strength level and held for 10 seconds after which a resting period began before the next trial. The isokinetic test targeted only concentric plantar flexion at 60 degrees per second of ankle flexion. The test consisted of 5 trials as well, in which the subjects performed at maximum capacity.

Processing the Data

The ultrasound images were uploaded into the OsiriX software program to electronically calculate the muscular length and cross-sectional area. The closed polygon feature of the program allowed the user to outline the muscle in each ultrasound image and the numerical area values were automatically presented. To determine the length, the user would select the length feature and draw a linear line from the distal to the proximal muscle boundaries and the length value was automatically presented.

To determine muscle volume, we took the total length of the muscles (minus 2 cm) and divided this value by 5 to determine the longitudinal length between each muscle slice. The separate length values were multiplied by the average of the cross sectional areas of the two bordering slices. The average was calculated to compensate for the size discrepancy of the two adjacent muscle slices. To calculate the volume of the end segments of the muscle, we estimated them as cones and multiplied the cross-sectional area of each end slice by 1/3 and by the length of the end muscle slice. After performing these calculations, we added all values together to determine the ultimate muscle volume.

The stiffness of each trial was determined by using the Q-Box function at the middle of each ten-second clip. This function placed a 2 mm circle in the center of the clip frame to designate the area where the muscle stiffness was measured. Upon the placement of the circle, the modulus mean value and the standard deviation values were automatically shown. An average was taken of the three modulus mean values recorded from the trials to represent the overall muscle stiffness.

Muscular strength data was exported from the dynamometer into separate excel spreadsheets. The strength values from the 5 trials were presented in a graph showing the peak

force values. The largest peak represented on the graph determined the subject's maximum

force. Isometric and isokinetic strength data were displayed on two separate graphs; therefore

muscular strength was denoted by two values.

Statistics

To check the accuracy of our methods for obtaining volume, stiffness, and strength data

we plotted the values from day 1 against day 2. Using this plot, we located the line of best fit

and established the coefficient of determination, which was used to verify a correlation. We also

conducted Bland-Altman assessments to demonstrate the level of intra-reliability in our methods.

The range of agreement was set at +/- 2 SD. This graph plotted the difference in data values

between the two testing sessions against the overall mean of the day 1 and day 2 values.

Through this analysis, we were able to determine the percent error in our methods and the

standard deviation of the data points.

The repeat bias in muscle size, stiffness and strength was determined by performing an

independent-sample t-test. The significance of the data variance was represented by a

corresponding p-value. The data was considered significant if the p-value was less than or equal

to 0.05.

Chapter IV:
Results

Muscle Volume. The data in Table 1 displays the calculated muscle volumes of the medial gastrocnemius (MG) and lateral gastrocnemius (LG) muscles over the two-day testing period.

Table 1: Muscle Volume Measurements (in cm³)

Subject	Medial Gastrocnemius		Lateral Gastrocnemius	
	Day 1	Day 2	Day 1	Day 2
1	201.893	229.086	124.718	126.121
2	190.164	223.041	142.109	123.455
3	169.204	172.34	93.228	122.197
4	106.829	118.525	59.033	64.864
5	147.898	142.637	98.53	115.699
6	206.549	216.22	119.673	113.513

Although there are particular discrepancies in data from day 1 compared to day 2, Figure 1 shows a positive correlation in the testing reliability of the measurements taken between the sessions. The correlation of determination is .91, which proves that the differences in volume may be insignificant when determining relative muscle volume.

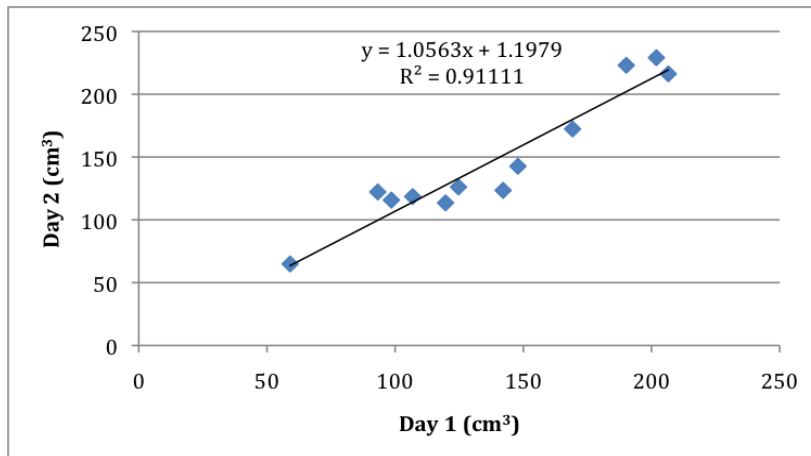*Figure 1: Volume Testing Reliability*

Figure 2 shows the Bland-Altman assessment of agreement between the two testing days. The range of agreement was set at +/- 2 SD. This is reflected in the mean bias of 5.838, which determines that these variances are likely due to systematic error. Data from the MG shows a p-value of .07, which although it is not significant, it shows there is a trend suggesting possible bias in our methods. This bias is not evident in the LG, as shown by a p-value of .52. The percent error for the data set is 15%, which signifies that our methods were satisfactory for obtaining muscle volume.

*Figure 2:Bland-Altman Analysis of Muscle Volumes Between Testing Sessions*
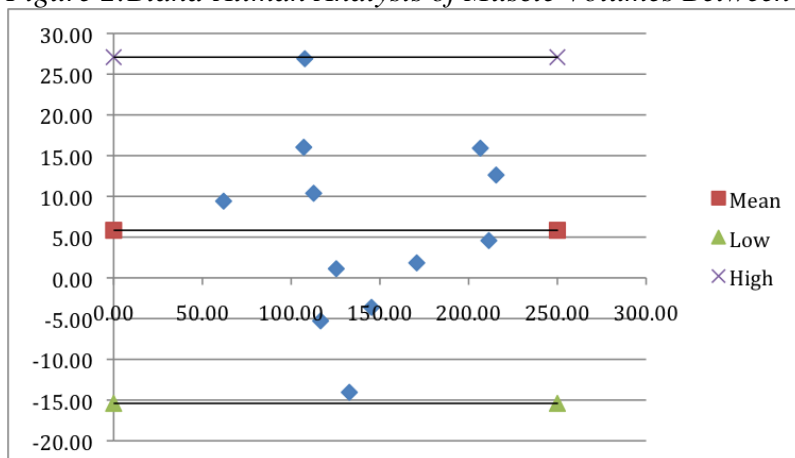
Muscle Stiffness. The muscle stiffness values in Table 2 are averages of three consecutive elastography readings taken during each testing session. Most subjects displayed a wide range of stiffness values amongst the consecutive measurements; meaning outlying data could have influenced the mean. The data shows discrepancies between day 1 and day 2 for several of the subjects, as seen with muscle volume. The stiffness values for subjects two and six varied between day 1 and 2 in the MG, while subjects one and four displayed significant stiffness variations in the LG between test days.

Table 2: Muscle Stiffness Measurements (in kPa)

| Subject	Medial Gastrocnemius		Lateral Gastrocnemius	
	Day 1	Day 2	Day 1	Day 2
1	17.5	16.6	20.7	34.03
2	16.4	27.8	18.4	20.9
3	19.8	23.6	17.6	21.4
4	26.3	24.6	20.7	34.4
5	13.2	13.7	19.2	10.7
6	21.2	13.5	22.8	18.1

The Bland-Altman analysis in Figure 3 displays a relatively equal number of subjects with negative error opposed to positive. This is reflected by a mean bias of 2.128, which is fairly close to 0. This proves that there was considerable random error in testing, opposed to a systematic error between testing sessions. Data from both the LG and MG show p-values of .41 and .74, respectively, which demonstrates a lack of evidence for bias in our testing methods.

Figure 3: Bland-Altman Analysis of Muscle Stiffness Between Testing Sessions
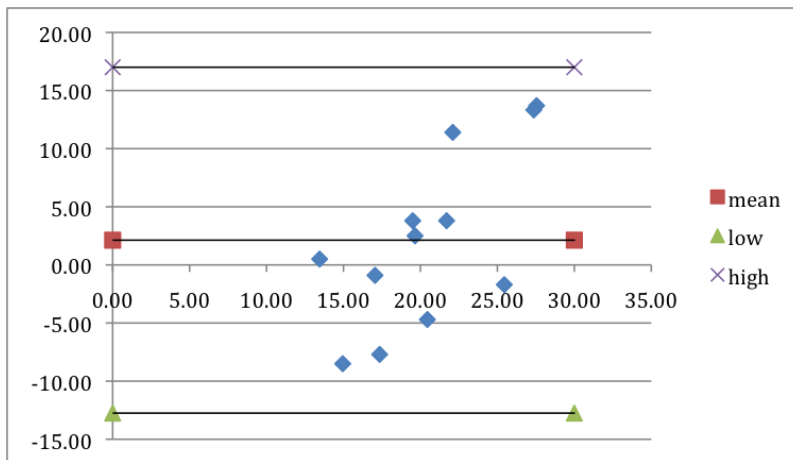
The percent error of the analysis in Figure 3 is 73.88%, therefore proving inconsistency in the methods. Figure 4 demonstrates the testing reliability when the stiffness data from day 1 was plotted against that of day 2. Based on the Figure, there is a weak correlation between testing sessions, with a coefficient of determination being 0.06343.

Figure 4: Elastography Testing Reliability
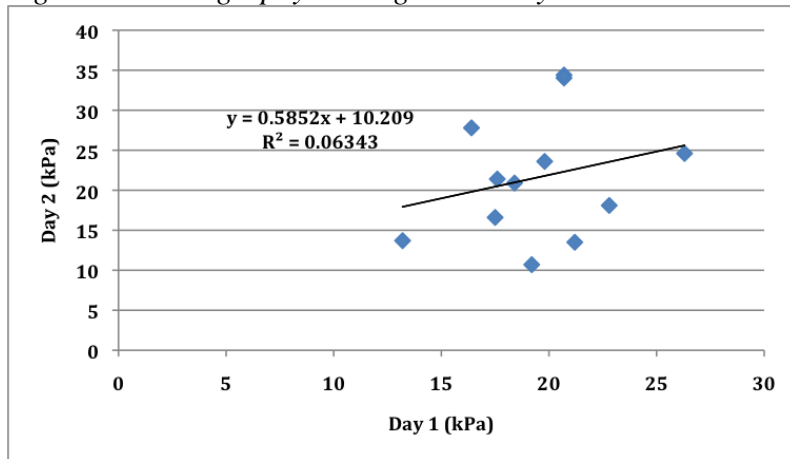
Muscular Strength. The results from the isometric and isokinetic strength tests displayed

in Table 3 represent the peak forces exerted by the subjects in a series of five trials. The peak

isokinetic torque was relatively uniform between test days for subjects 4, 5, and 6. However,

data from the remaining subjects portray strength discrepancies between days 1 and 2. This was

evident when studying the additional trials as well.

Table 3: Calf Strength Measurements (in Nm)

Subject	Isometric		Isokinetic	
	Day 2	Day 3	Day 2	Day 3
1	30.92	48.54	66.72	29.43
2	135.74	141.02	79.19	90.45
3	101.02	109.97	72.14	105.9
4	74.17	75.39	53.7	54.51
5	97.77	95.46	76.21	79.46
6	70.51	86.51	41.76	48.27

Isometric Muscular Strength. Figure 5 displays the Bland-Altman analysis of the data

comparing the two testing sessions. Five out of six subjects' strength improved during the

second testing session. This is reflected by a mean bias of 7.793, which likely means these

discrepancies were due to systematic error.   Isometric strength data shows a p-value of .06,

which is not significant, however it demonstrates that there is a trend that may provide evidence

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of a bias in our methods. The percent error was 17.91% between testing sessions. This is evident in Figure 6, which displays the testing reliability when plotting isometric strength data from day 1 against day 2. The Figure shows a strong positive correlation with a correlation coefficient of 0.9574.

Figure 5: Bland-Altman Analysis of Isometric Strength Between Testing Sessions
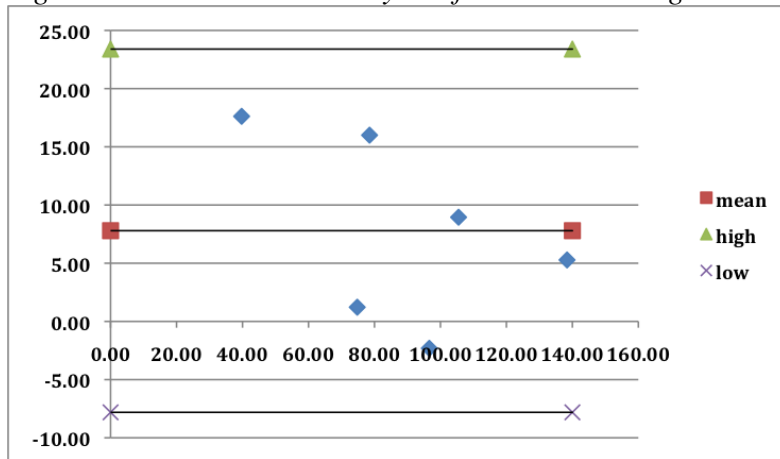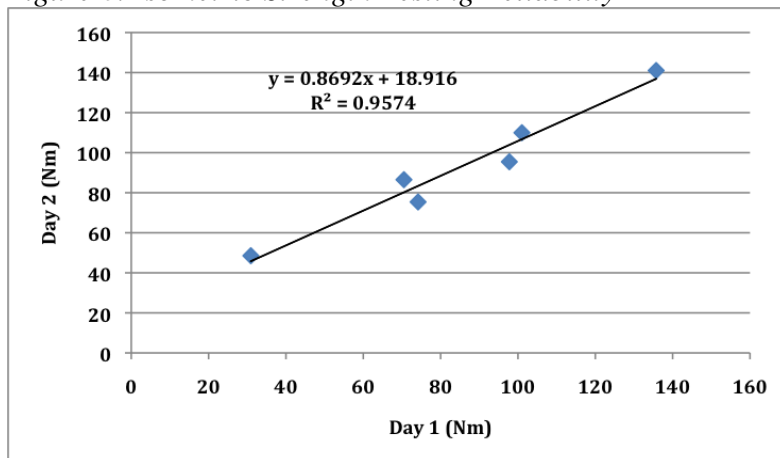

Figure 6: Isometric Strength Testing Reliability

Isokinetic Muscular Strength. Figure 7 displays the Bland-Altman analysis of agreement for the isokinetic strength values between testing sessions. It is apparent that some subjects performed better on the 2nd testing day compared to the first. This is reflected in a mean bias of

3.05, however a p-value of .76 suggests there is not evidence of significant bias in our data. The percent error for this data was relatively high at 72.7%.

Figure 7: Bland-Altman Analysis of Isokinetic Strength Between Testing Sessions
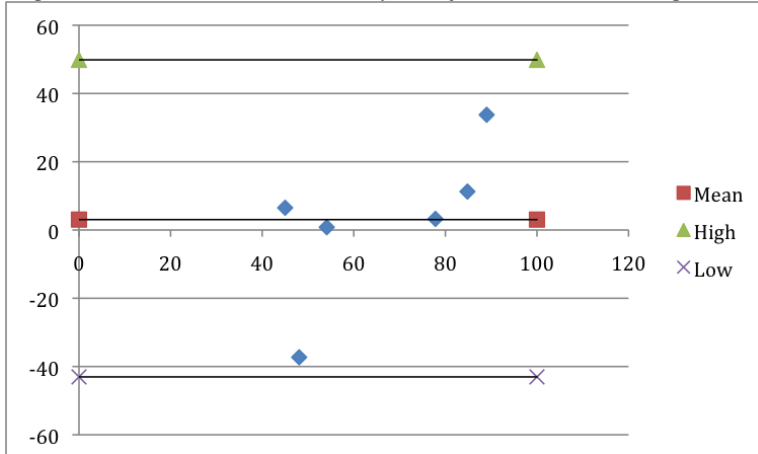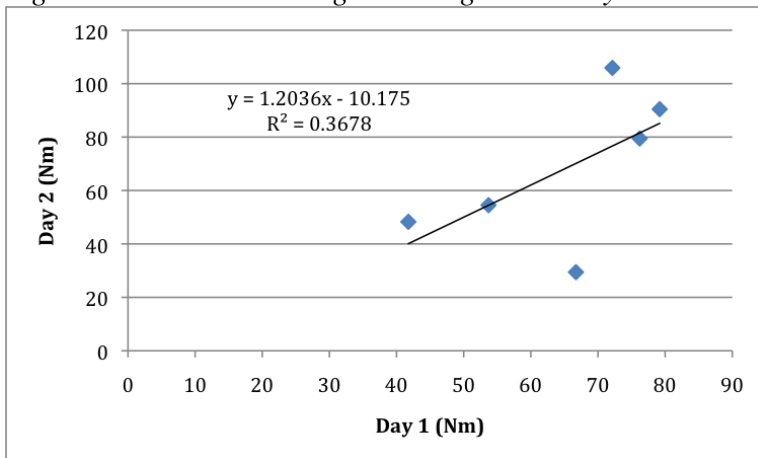

Figure 8 displays a fairly weak positive correlation between isokinetic strength values between testing days. The coefficient of determination is .3678 for this data set. This proves that there is a stronger testing reliability with isometric testing opposed to isokinetic.

Figure 8: Isokinetic Strength Testing Reliability

Chapter V:
Discussion

Based on the results, we have discovered reliability in our methods for measuring volume

and strength, but not in our stiffness methods. Even though the results were fairly reliable, they

could be improved in future studies. When comparing the calculated muscle volume and

strength values between day 1 and day 2, there are obvious discrepancies amongst the subjects.

There is a strong trend for larger volume and strength measurements to be taken on testing day 2

compared to day 1. Although our data appears statistically reliable, it is important to analyze

impending errors in order to further improve accuracy.

 A potential source of error is in our measurement of muscle length between testing

sessions. Table 4 shows that the recorded muscle lengths varied between the two days in most

subjects, showing up to a 4 cm difference. The length values used to calculate the muscle

volume were obtained manually through an external measurement across the calf. This provided

a curved surface opposed to the linear length measurement that would be more accurate.

Table 4: Measured Muscle Length (in cm)

Subject	Medial Gastrocnemius		Lateral Gastrocnemious	
	Day 1	Day 2	Day 1	Day 2
1	20	24.5	19.5	21.5
2	19.5	23.8	26.5	25
3	18.3	19.5	20.5	20.5
4	15	18.7	18.8	18.5
5	19.2	19.3	21	23.6
6	19.7	19.3	21.5	18.1

The variation in length could potentially occur from slightly different tracking paths

across the curved surface on day 1 compared to day 2. During the testing session, the operator

would angle the probe to capture the clearest ultrasound image. Slight variations in probe angle

between sessions could influence the longitudinal tracking path and skew the length measurements for the 2 days. Initially, we predicted that any length variations would be compensated for in the calculations and would have little effect on the final volume values. However, the largest volume discrepancies between the two days were evident in subjects with more significant differences in length measurement.

A second source of error could result from complications in scanning the cross-sectional slice images. Our images were scanned continuously from the lateral to the medial muscle boundary. When capturing the thickest slice of the muscle belly, the machine would occasionally distort the image due to the particular angle of the ultrasound probe. The deformed muscle slice could have provided an altered cross-sectional area measurement, which ultimately would affect the calculated muscle volume.

In a previous study, Infantolino et al proved the accuracy of the ultrasound method to determine muscle volume; however, his usage of the ultrasound differed slightly from the procedures we used in the current study. Instead of capturing a single image of each muscle slice, Infantolino focused on piecing together a series of images taken across each muscle slice. This practice could remove the distortion error seen in the current study and provide a more accurate representation of the muscle.

To improve precision, we can explore the use of ultrasound combined with motion capture to monitor the 3D location of the probe in space. This method will potentially reduce errors by ensuring that the location and the angle of the probe is consistent from day 1 to day 2. Ultrasound cross-sectional area will be computed by piecing together a series of still images using Matlab software. The motion capture equipment will process the location of the probe

during each image recording and the information will be integrated into the program to provide a more accurate volume calculation.

In our stiffness reliability analysis we discovered a weak correlation between data values taken from the opposing test days, which provides evidence for faults in our methods. Eby, et al. demonstrated the reliability of elastography in determining muscle stiffness. This suggests that our inconsistent results between sessions are due to probable human error while testing. In many subjects, there was evidence of significant variances in stiffness values recorded during the three 10 second scans. This could potentially result from changes in pressure applied by the tester while taking the elastography readings. To achieve the greatest accuracy, the ultrasound probe should be placed lightly against the muscle belly without causing stress to the muscle. Slight increases in applied pressure can alter stiffness readings and the muscle may appear stiffer.

An additional source of error could be in the placement of the probe on the muscle belly. In the current study we did not monitor the exact location of the probe between testing days, which ultimately could alter the stiffness readings. In future research, probe placement should be monitored and possibly oriented with nearby bony landmarks. For our upcoming research, the probe location will be monitored by motion capture technology in order to improve consistency between testing sessions.

The discrepancies in our stiffness data reduce the validity of the muscle volume and stiffness regression analysis. By improving our stiffness and volume methods in future research we will be able to approach a more accurate conclusion on the affect of muscle stiffness on muscle volume in a young population. When comparing muscle volume to strength, we were also unable to establish a valid correlation. Our plotted correlation showed a weak correlation

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between muscle volume and strength, which contradicts past research showing a positive correlation.

When evaluating the reliability of our methods of measuring strength, we discovered that they were inaccurate in determining the total strength of our subjects. After analyzing the strength data from both the isometric and isokinetic strength tests it was obvious that some subjects did not provide consistent effort in force production. This is evident when analyzing strength data from subject 1. In this case, the subject's isometric strength improved during the second testing session, while their isokinetic strength drastically declined. The results prove that most subjects did perform higher on the second day opposed to the first. We believe there may have been a learning effect in which the subjects' strength improved after they became more comfortable with the testing equipment and the appropriate motions.

The correlation could potentially be swayed due to the exclusion of moment arm and soleus volume data as well. Calf strength is determined by the combination of forces primarily by the MG, LG and soleus muscles. Without calculating the volume of the soleus into the combined calf muscle volume, the correlation between the muscle volume and strength is inaccurate. In addition, the moment arm data is necessary when calculating the total force exerted by each subject; without this information, our current strength data is not valid and cannot verify a correlation.

Based on these results, we have concluded that strength testing is not a good determination of the effect of a training intervention due to the increased probability of error and miscalculation of an individual's total strength. Therefore, in future study we may analyze the effect of stiffness on muscle volume, while excluding strength testing. If we do include strength training, we can apply electrical stimulation to the muscle to ensure that the participants are

exerting maximum force.   To compensate for the learning effect, researchers can allocate the first testing session for learning and collect the data on day 2.

Although we discovered potential errors in our testing methods, we found interesting results when plotting the correlation between muscle volume, stiffness and strength. We have evidence that reduced muscle volume corresponds with increased muscle stiffness and attenuated muscular strength in the elderly population; however, we were looking for evidence in the younger subjects as well. In our analysis of the relationship between muscle volume and stiffness, we discovered a slight negative correlation between the two factors in a young population, as demonstrated in Figures 9 and 10. The correlation of determination of 0.10638 in the MG and 0.13322 in the LG demonstrates that the trend in not strong, however it does exist.

. Figure 9: Muscle Volume vs. Muscle Stiffness of the Medial Gastrocnemius
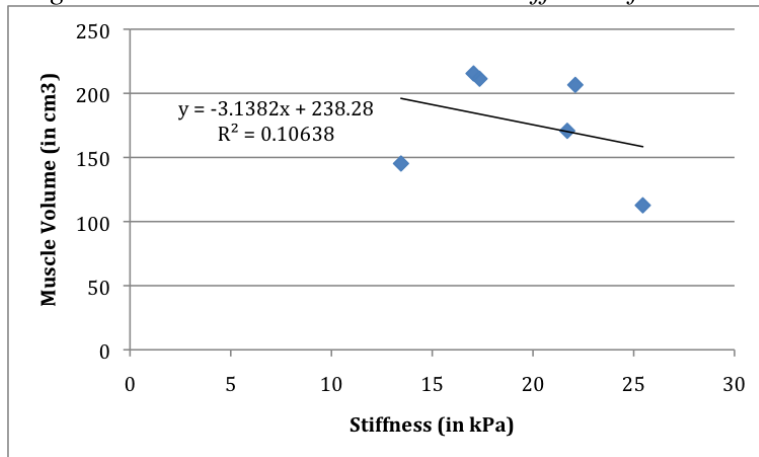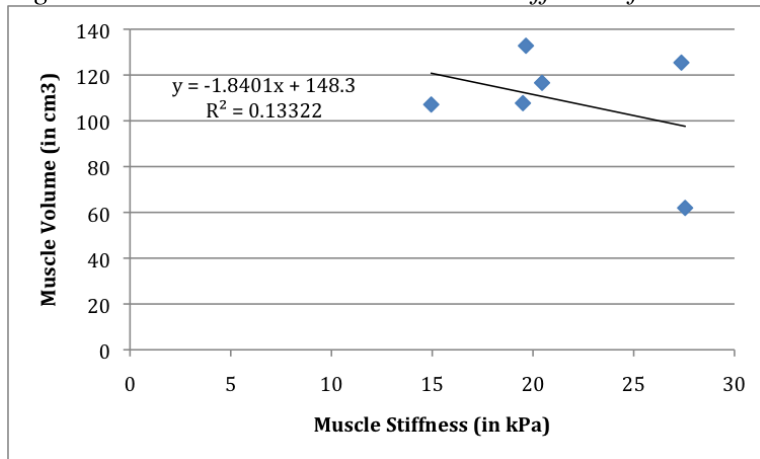
Figure 10: Muscle Volume vs. Muscle Stiffness of the Lateral Gastrocnemius

Figure 10 chart: Muscle Volume (in cm3) vs. Muscle Stiffness (in kPa). Equation: y = -1.8401x + 148.3, R^2 = 0.13322

Relationship of Muscular Strength on Stiffness. A weak negative correlation was seen when comparing both isometric and isokinetic strength with stiffness as well, as shown in Figures 11 and 12. The stiffness values are an average of the values obtained from the LG and the MG. This suggests that although the relationship is weak, after refining our methods we may be able to see a stronger correlation between the two factors in future research.

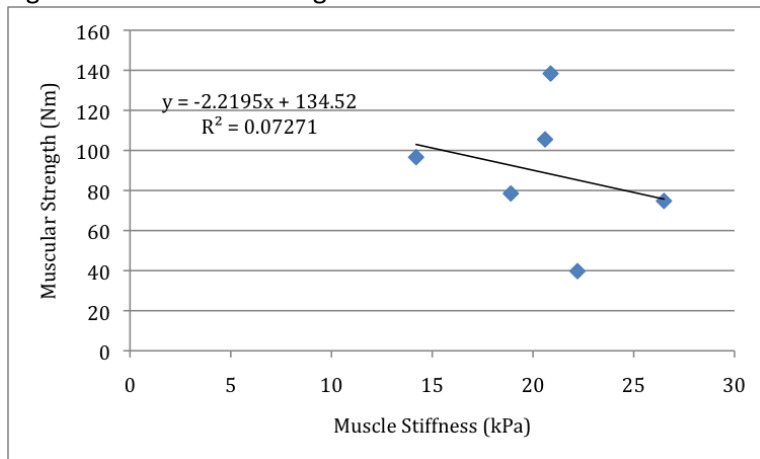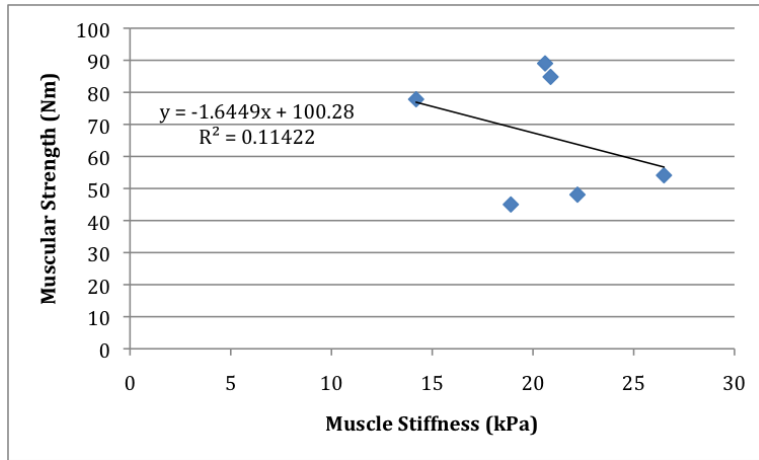Figure 11: Isometric Strength vs. Muscle Stiffness

Figure 11 chart: Muscular Strength (Nm) vs. Muscle Stiffness (kPa). Equation: y = -2.2195x + 134.52, R^2 = 0.07271

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Figure 12: Isokinetic Strength vs. Muscle Stiffness

Relationship of Muscle Volume on Strength. In our analysis of muscle volume and strength, we were surprised to discover a very weak correlation between the two factors with the coefficients of determination ranging from .00027-.04929. Figures 13 and 14 demonstrate the relationship between isometric and isokinetic strength with the LG, MG, and combined volume data. The combined values represent the sum of the MG and LG volume data. Past studies have proven a positive correlation between muscle volume and strength, therefore our misrepresentation of the relationship is likely due to errors in our methods.
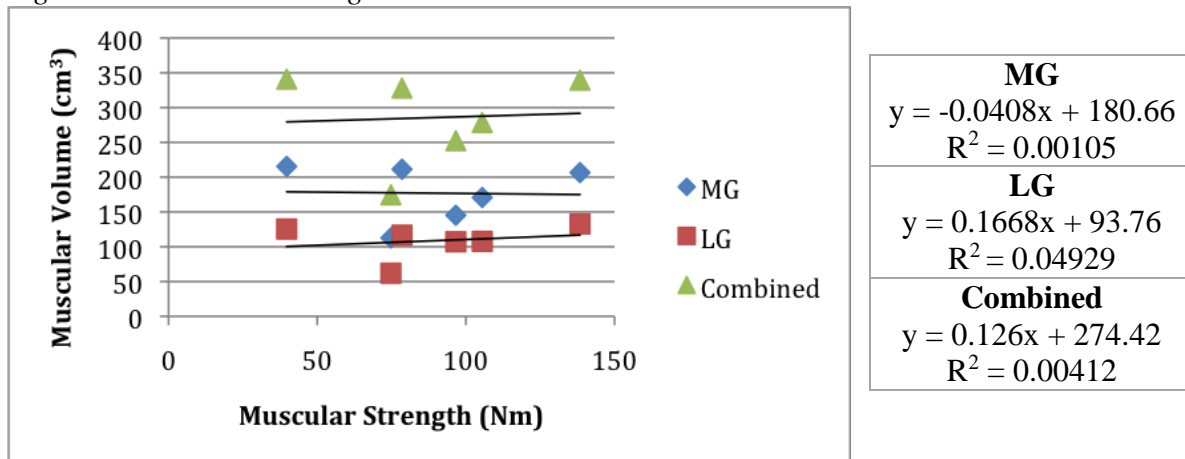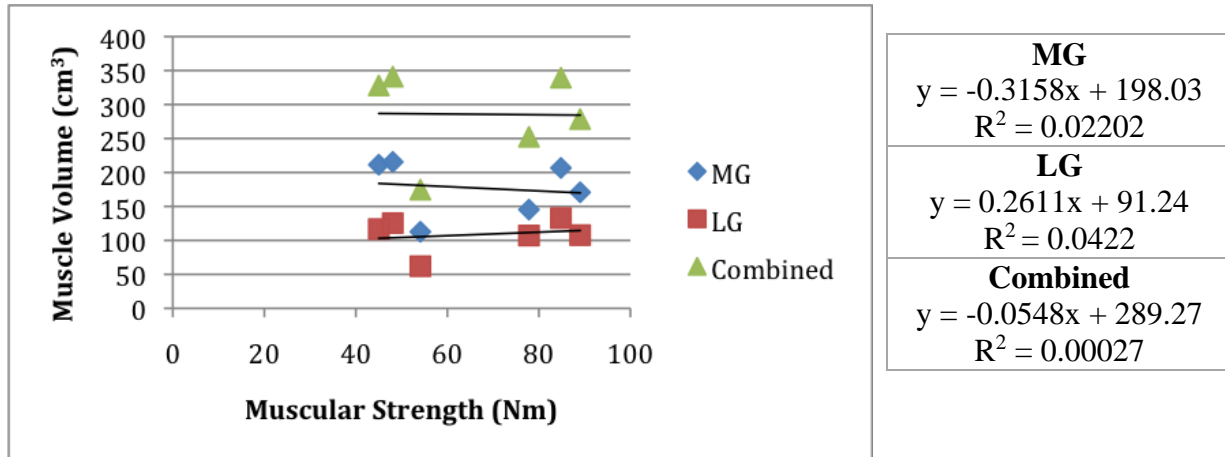
Figure 13: Isometric Strength vs. Muscle Volume

MG
$y = -0.0408x + 180.66$
$R^2 = 0.00105$
LG
$y = 0.1668x + 93.76$
$R^2 = 0.04929$
Combined
$y = 0.126x + 274.42$
$R^2 = 0.00412$

Figure 14: Isokinetic Strength vs. Muscle Volume

MG
y = -0.3158x + 198.03
$R^2 = 0.02202$
LG
y = 0.2611x + 91.24
$R^2 = 0.0422$
Combined
y = -0.0548x + 289.27
$R^2 = 0.00027$

We discovered potential errors in our methods of obtaining data, which influenced our regressions between volume, strength and stiffness. By modifying our methods we can improve our approach and determine if there is a correlation between the factors in future research. Before taking the next step in this research project, our methods should be modified and retested for reliability. The second reliability test should incorporate the use of motion capture with ultrasound and elastography technology. Also, if we plan to test muscular strength, electrical stimulation should be applied to the muscle and additional data should be collected on the soleus and moment arms.

The purpose of this study was to test the methods and provide the foundation for research that will analyze muscle stiffness as a potential cause of the reduced response to exercise. We believe that age-related muscle stiffness interferes with mechanotransduction and ultimately limits muscle hypertrophy. This could explain the reduced response to exercise seen in the elderly population and help to determine a potential cause of sarcopenia. The next step would be to expand the research to an older population. Future studies should include a larger sample size comparing data from both a younger and older population. If there is in fact a correlation

between muscle volume and stiffness, diagnosing a prospective cause of sarcopenia can allow

researchers to move forward into discovering and implementing a treatment plan.

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