INVESTIGATING THE EFFECTS OF AGE AND SEX ON MUSCULOTENDINOUS STIFFNESS AT THE VASTUS LATERALIS MUSCLE

by

HYE LYNN CHOI

A THESIS

Presented to the Department of Human Physiology
and the Robert D. Clark Honors College
in partial fulfillment of the requirements for the degree of
Bachelor of Science

May 2024

An Abstract of the Thesis of

Hye Lynn Choi for the degree of Bachelor of Science
in the Department of Human Physiology to be taken June 2024

Title: Investigating the Effects of Age and Sex on Musculotendinous Stiffness at the Vastus Lateralis Muscle

Approved: *Damien Callahan, Ph.D.*
Primary Thesis Advisor

The purpose of this study was to investigate how biological sex and chronological age influence the mechanical stiffness in muscle tissue and the response of that tissue to an acute bout of fatiguing exercise. Stiffness in the vastus lateralis (VL) muscle was measured in young (18-35 years) and older (65 to 80 years) males and females. This project tested the hypothesis that older individuals will have lower stiffness than younger individuals, and the female subjects will have lower stiffness values than male subjects. Participants were young females (n=6) and males (n=4), and older females (n=5), and males (3). Dynamometry was used to measure rate of torque development and other measures of voluntary contractile performance. Shear wave elastography (SWE) ultrasonography was used to measure stiffness in VL muscle tissue. These were complimented by more traditional B-mode ultrasound measures of stiffness and morphology (echogenicity). SWE measures were applied during passive and active contraction of the VL. B-mode ultrasound was applied during VL contractions of progressively greater intensity. Age-related reductions in VL stiffness were limited to males measured with SWE. The female group did not report a similar result, suggesting that the age-interaction effect may be specific to a certain sex. Echogenicity was significantly correlated with peak power, absolute and relative rate of torque development ($p<0.05$). The finding suggests that the age-effect on the

stiffness at the vastus lateralis muscle may be sex-specific to males. Muscle composition as defined by echogenicity may be a more highly relevant clinical measure.

Acknowledgements

I would like to express my sincere gratitude and appreciation to my primary thesis advisor, Professor Damien Callahan, for his invaluable guidance, encouragement, and support throughout this research project. His expertise, mentorship, and insightful feedback have been instrumental in shaping this thesis and enhancing my research skills. I would also like to extend my heartfelt thanks to my secondary thesis advisor, Austin Ricci, for his valuable contributions, feedback, and support during the course of this study. Additionally, I would like to thank my Clarks Honors College (CHC) advisor and committee member, Professor Courtney Thorsson, for her kindness and support throughout the thesis project process. Their expertise and guidance have significantly enriched the quality and depth of my research work and inspired me to pursue continued growth and excellence in my scholarly endeavors.

Finally, I would like to express my gratitude to all my friends and family members who have supported me through every step of my life. Their unwavering support and love have guided me, even during the most confusing and frustrating moments, and have given me the confidence to persevere. A special thank you goes to my parents, Audry Yoo and Sungwook Choi, for their unconditional love and support. I am deeply indebted to them for their unwavering support, understanding, and the sacrifices they have made to ensure my success. Their boundless love and encouragement have inspired me to strive for excellence and to never give up on my dreams.

Table of Contents

List of Figures

List of Tables

Introduction

In common English, stiffness is an "inability to move easily and without pain," or "the quality of being severe or strong" ("Oxford Languages"). However, stiffness in terms of muscles has a narrower definition. In muscular physiology, stiffness specifically refers to the mechanical characteristics of muscles concerning the "rate of force development" (Bravo-Sanchez et al., 2021). It more accurately refers to the inherent mechanical characteristics of the musculotendinous unit in terms of its ability to resist deformation in response to a load. Stiffness is critical in its impact on muscle performance because it influences how tension, generated by muscle contraction, is transmitted throughout the body (Morel et al., 2019). In the literature, higher stiffness has been correlated with an increased susceptibility to muscle strains and tendon injuries during sports and physical activities. However, decreased stiffness has been suggested to result in impaired balance recovery and diminished joint stability in older individuals (Mongold et al., 2023).

As one ages over time, muscles generally undergo sarcopenia. Sarcopenia refers to the age-related involuntary reduction in skeletal muscle mass and strength (Walston, 2012). It is usually accompanied by various alterations in muscular components, such as an increase in collagen content within the extracellular matrix (Ramaswamy, 2011). The accumulation of these changes may play a role in altering skeletal muscle stiffness among older populations. In past literature, it has been suggested that age could be a significant factor in determining one's muscle stiffness. For instance, research conducted by Alfuraih et al. (2019) found that the elderly group, consisting of individuals with an age range of 77 to 94 years old, showed lower stiffness in the vastus lateralis, rectus femoris, and other hamstring muscles, as indicated by shear wave velocity values (Alfuraih et al., 2019). A similar result was found in another study: when comparing young and old female subjects, the younger subjects showed significantly higher muscle stiffness during voluntary contraction in the right quadriceps femoris muscle (Ikezoe et al., 2011).

Assessing stiffness within the musculotendinous unit has various clinical relevance. Knowing muscle stiffness helps estimate torque and the rate of torque development, which can be further used to measure and monitor one's strength and track progress in recovery and rehabilitation (Mongold et al., 2023). Additionally, findings from other literature suggest that evaluating slight changes in muscle stiffness could be helpful in detecting the initial signs of muscle fatigue, injury, or disease (Chalchat et al., 2020). For instance, older individuals who frequently fall were found to have lower muscle stiffness in the gastrocnemius muscle (Bas et al., 2023). As much as fall injury is critical to the older population, the early onset detection of potential fall risk through musculotendinous stiffness assessment will be invaluable.

Another perspective that considers the role of age in altering muscle stiffness involves examining limited joint mobility. Joint mobility decreases with age for various reasons, including the thickening of joint capsules, stiffening of ligaments, and alterations in the structural and matrix composition of articular cartilage due to the aging process (Marcucci, Lorenzo, and Reggiani, 2020). Furthermore, age-related measures have shown that muscle stiffness is influenced by muscle length, which depends on joint angles in vivo (Xu et al., 2021). Thus, reduced joint mobility can have an overall impact on muscle performance. A restricted range of motion combined with stiff joints can hinder one's ability to fully exert force, leading to a decrease in overall muscle strength performance.

As a result, it has been argued that age-induced stiffness may serve as a compensatory mechanism to cope with reduced joint mobility and lower force production. Stiffer muscles may help transmit power throughout the muscle, preserving eccentric strength in older individuals.

This aspect may hold clinical significance for training and rehabilitation, as it can guide the regulation of resistance training intensities for the elderly with “limited muscle capacity” (Marcucci, Lorenzo, and Reggiani, 2020).

Although age-related muscle stiffness plays a critical role in overall muscle power performance, various other factors may also contribute to a general loss of power. In Clark’s study, where they attempted to quantify neuromuscular activation using the maximal voluntary rate of EMG rise, they discovered that the EMG rate decreased among older individuals, along with a decrease in leg power. This shows a correlation between neuromuscular activation and power loss. This finding suggests a possibility that factors such as the number of motor unit recruitments, motor unit discharge rate, or motor unit discharge synchronization could also affect the overall rate of torque development concerning aging (Clark, 2013). Additionally, various muscular components undergo alteration as one ages. Older individuals are known to have increased collagen content in muscles, which could lead to a stiffer extracellular matrix, but lacking important proteins such as dystrophin could inhibit older individuals from effectively transducing force (Ramaswamy et al., 2011). Thus, it is important to consider not only musculotendinous stiffness when assessing individuals in terms of age but also factors such as the rate of torque development, which reflects muscle performance, to encapsulate other potential variables that age could affect.

Another important factor that plays a role in determining skeletal muscle stiffness is sex. However, there is no set notion regarding whether females have higher or lower muscular stiffness than males. When comparing stiffness values at the biceps brachii using shear wave elastography, researchers found that females showed higher stiffness values than males within different age groups (Eby et al., 2014). A different study found that muscle stiffness was generally lower among females than in males at the vastus lateralis using myometry (Wu et al., 2016). The variation could be due to differences in the muscular area in which stiffness was observed, variations in estrogen levels among female participants in each study, or methodological differences.

The sex difference among older individuals may have a critical impact on varying muscle stiffness levels as well. Females undergo various physiological changes, especially during the menopausal period. After menopause, most sex hormones, such as progesterone or estrogen, drop to a “negligible level” (Hansen, 2018). Studies have shown that these hormones are positively related to muscle mass, and the reduction of sex hormones after menopause contributes to the loss of muscle mass and muscle strength among older females (Maltais et al., 2009). Additionally, these hormonal shifts may also affect musculotendinous stiffness. In fact, it was discovered that knee joint laxity was the highest in the blood sample collected during the late follicular phase of the menstrual cycle, where estradiol is high, and progesterone is low (Lee et al., 2013).

Currently, the “gold standard” for measuring muscle stiffness is by using the Shear Wave Elastography (SWE) method. SWE is an ultrasound method that provides a quantitative measurement of tissue stiffness based on the shear wave propagation velocity that travels through the muscle while taking the ultrasound (Brandenburg et al., 2014; Siracusa et al., 2019). SWE employs a shear-wave equation that provides the stiffness measurement in units of Pascals to help track tissue modulus (Brandenburg et al., 2014). It is advantageous to use SWE over other methods because SWE doesn’t require manual compression to measure stiffness like other strain elastography methods, thus providing more accurate results (Zardi et al., 2019). Along with SWE, B-mode ultrasonography is also often used to assess tissue structure and components

(Hullfish and Baxter, 2018). B-mode ultrasonography produces a two-dimensional image created when a linear arrangement of transducers scans through the body plane (Carovac et al., 2011).

This research project focuses heavily on investigating the vastus lateralis muscle region. The vastus lateralis, abbreviated as VL, is a unipennate muscle that constitutes one of the four major muscles in the human thigh, forming the quadriceps femoris muscle group (Biondi and Varacallo, 2023). It is situated on the lateral side of the thigh, originating around the proximal femur and attaching to the greater trochanter, the lateral side of the linea aspera, the gluteal tuberosity, and the lateral intermuscular septum (Biondi and Varacallo, 2023; Moore et al., 2015).

The vastus lateralis primarily functions as a knee extensor muscle, aiding in standing up, kicking, or maintaining positions such as squatting (Moore et al., 2015). It contracts at the end of the swing phase of walking to prepare the knee for bearing weight and, collectively with the quadriceps femoris, absorbs much of the force generated during heel strikes when walking. The vastus lateralis also undergoes eccentric contractions during activities like walking downhill or stepping downward (Biondi and Varacallo, 2023). As the largest and strongest muscle within the quadriceps femoris group, the vastus lateralis plays critical roles in a wide range of activities, including even the simplest tasks like maintaining balance.

Significance

This research is significant because it has the potential to address how age and sex may affect stiffness in relation to the rate of torque development, which plays essential roles in maintaining balance and preventing falls. This becomes even more significant when considering clinical applications among older adults, who are more prone to fall risks and subsequent injuries. In today's world, where the older population is increasing at an exponential rate but without a full understanding of their physiologies, our findings have direct implications. What sets my research apart from other projects is the incorporation of both sex and age when studying musculotendinous unit stiffness, which is more similar to the real world. Thus, our research closely resembles real life and aims to produce realistic results that could be applied in the real world. This research project aims to further consider these age and sex factors in assessing one's muscle stiffness and strength through measuring the rate of torque development. Ultimately, our research aims to contribute to understanding and reducing the risk of injury among older adults.

Hypothesis

In this project, I hypothesize that older individuals will have lower stiffness values in the vastus lateralis muscle than younger individuals, and that females will have lower stiffness values in the same muscle than male individuals.

Methods

Participants

The subjects were selected to participate in the study after going through a screening process. The participants were selected based on their age and sex. There were a total of 17 participants, with 10 females and 7 males. The sex groups were subdivided into younger subjects and older subjects for age comparison. There were 6 young females and 5 old females, and there were 4 young males and 3 old males. The younger subjects' ages ranged from 18 to 35 years old, and the older subjects' ages ranged from 65 to 80 years old.

To reduce the chance of fluctuation in circulating estradiol levels due to the menstrual cycle, all female volunteers either confirmed their use of hormonal contraceptives or were tested during the pre-follicular phase of their menstrual cycle (within 5 days of the start of menstruation). Participants did not report any orthopedic limitations such as severe osteoarthritis or prior joint replacement, nor did they have any endocrine diseases like hypo/hyperthyroidism, Addison's Disease, or Cushing's syndrome, uncontrolled hypertension (>140/90 mmHg), neuromuscular disorders, significant heart, liver, kidney, or respiratory diseases, or diabetes. Additionally, participants were non-smokers. Finally, individuals taking medications that could affect muscle contractility or beta-adrenergic signaling for neuromuscular activation, such as beta blockers, calcium channel blockers, and muscle relaxers, were excluded from the study.

Study Design

For this part of the research, the participants underwent non-invasive fatiguing protocols. We included young males, young females, old males, and old females as participants to measure stiffness in the vastus lateralis muscle during both active and passive states. For the study, we incorporated both B-Mode and Shear Wave Elastography (SWE) ultrasonography to assess musculotendinous stiffness in the muscle. The participants sat on a dynamometer during the protocol. While seated, they performed 3 MVIC kicks for maximal strength and rate of torque development measurement, and proceeded with 25%, 50%, and 100% isometric loadings for kicks. Ultrasonography was used during the passive state and at 25%, 50%, and 100% active MVIC states for stiffness measurement. Prior to the study, the research team went through the consent form with the potential subjects and obtained their consent for the study. For female subjects particularly, we tried to minimize the effect that the estrogen cycle and consequent hormonal shifts can have on female muscle by selecting female participants who are either taking oral contraception or have different hormone-regulating controls, such as IUD implantation. We also asked them about their stage in the menstrual cycle when collecting the data.

Dynamometry

For the experiment, we employed dynamometry to set up for ultrasonography data collection and other related measures from the participants using the dynamometer. We had the subjects sit and positioned their dominant leg in the Biodex dynamometer to perform a knee extension. The subjects' positions were carefully adjusted so that their axis of rotation point on their knees would match that of the dynamometer. A sponge cuff was adjusted to tightly wrap around the subject's ankle at a level that was snug but not uncomfortably so. The strength of their kick was displayed in real-time on a graph within the software used for data collection on the TV screen. After the subjects were seated, they performed 3 maximum voluntary isometric contraction (MVIC) kicks for maximal strength measurement, and we used that value to adjust the dynamometer at 25%, 50%, and 100% of maximum load for B-Mode and SWE assessment of the musculotendinous stiffness under certain submaximal loading. The dynamometer collected torque data while the subjects were undergoing 3 MVIC and submaximal loading kicks, which was later processed through custom code in MATLAB to obtain peak torque and rate of torque development during active contractions.

The variables, such as absolute rate of torque development or relative rate of torque development, were obtained using raw data digitally imported from the dynamometer. We analyzed the average of the first five contractions of the kicks they were performing at the 30% submaximal load contractions to obtain peak power and related torque values. The absolute rate of torque development was derived by taking the first derivative of the torque-versus-time function and selecting the largest positive value. The relative rate of torque development was calculated by representing the torque as a fraction of the maximum torque and then selecting the maximum value of its first derivative, defined as MVIC per second.

Ultrasound

In this project, we used shear wave elastography (SWE) mode within the Philips EPIQ Elite Diagnostic Ultrasound System device to obtain B-mode ultrasound and SWE data measurements during the passive and active contraction of the vastus lateralis. The ultrasound was used before the 3 MVIC kicks and during 3 sets of submaximal load kicks. The eL18-4 probe was placed on the upper vastus lateralis region facing vertically. B-Mode ultrasound creates a 2D, gray-scale image of anatomical structures under the skin. The images taken from the B-Mode were later used to assess intermuscular components, such as muscle thickness, subcutaneous adipose tissue (SAT), and echogenicity using software called “ImageJ.” Muscle thickness was defined as the dark area between the superficial and lower aponeurosis, and subcutaneous adipose tissue area was defined as the area between the probe and the superficial aponeurosis within the B-Mode pictures. Both muscle thickness and SAT were measured by drawing a vertical line within the muscle or SAT area in the image and measuring the length given in centimeters by the software.

Echogenicity was obtained by outlining only the vastus lateralis muscle area and assessing the average pixel intensity from the histogram. In the histogram, zero intensity is defined as pure black, and 255 or maximal intensity is defined as pure white. The closer the number is to maximal intensity, the more likely the muscle area is assumed to have more of the intermuscular components other than skeletal muscle, such as water-based adipose tissue. We used this value to calculate the corrected echogenicity with this equation:

$$Corrected\ Echogenicity\ (AU) = Echogenicity + (SAT - VL \times 40.5278)$$

We also employed B-Mode ultrasound to take a video of active contractions during the procedure. A program called “UltraTrack” was used to further analyze the ultrasound videos. Within the program, we used the fascicle tracking feature to monitor the shift in aponeurosis under different MVC loads throughout the active contractions. The fascicle displacement values were taken into account to calculate the overall stiffness values at the given submaximal load in a later analysis, using this equation:

$$Stiffness = \frac{Torque}{0.0447} \times Fascicle\ displacement$$

Shear wave elastography was used to assess tissue stiffness during passive and active contractions. SWE works by transmitting shear waves through tissues and recording how rapidly the ultrasonic waves travel through the fibers of the tissue. Unlike B-Mode, where we incorporated various software tools to analyze the data after extracting it from the ultrasound device, the SWE data was analyzed on the ultrasound device itself. A circle, typically 9 or 10mm in diameter (usually 10mm if the box was large enough), was placed in the center of the vastus lateralis muscle region to measure the shear-wave propagation velocity in the passive and active contraction footage.

Protocol

The subjects were seated on the Biodex isokinetic dynamometer, and the lever arm was attached to the ankle of the subject's dominant leg. The dynamometer was connected to the laptop running CED Spike 2 to sample the subjects' contractions. We obtained ultrasound images and videos of the vastus lateralis muscle in a passive state. Afterward, the subjects performed three kicks at maximum force. The dynamometer was set to the isometric mode during this procedure, and the participants had a one-minute gap between each maximal contraction. We used these kicks to average the maximal contraction force the subjects could perform, utilizing MATLAB, and applied this value to determine the proper amount of submaximal load.

Thereafter, they were asked to perform a series of submaximal contractions during which they would achieve submaximal target forces. During these contractions, we measured tissue displacement and inherent stiffness via B-mode ultrasound and SWE, respectively, at the vastus lateralis. We used the maximal contraction value to set the submaximal voluntary contraction thresholds at 25%, 50%, and 100%. We then adjusted the dynamometer’s loading amount to

25%, 50%, and 100% of the subjects' maximal contraction strength in the isotonic mode and had the subjects kick as hard as they could to reach the specified threshold. For each submaximal threshold, we adjusted the threshold line, which was live-broadcasted on the TV screen, for subjects to "reach" during each contraction. Once they had initially reached the required submaximal contraction level, they were told to hold the contraction for approximately 3 seconds and then release it. Throughout the entire submaximal contraction phase, we kept the ultrasound probe on the vastus lateralis region to capture footage of active contraction.

Statistical Analysis

A two-way repeated measures ANOVA was used to assess the within-subjects effect of contraction intensity (MVIC levels) and the between-subjects effects of age and sex. A two-way repeated measures ANOVA was also used to assess interaction effects within the sex variable. An independent samples t-test was conducted within the sex effect varied by intensity. A Pearson correlation analysis was used to run correlational analyses.

Results

Age Group	BMI (kg•m^{-2})	MT (cm)	SAT (cm)	Corrected Echogenicity (AU)
Young	24.0±4.5	2.11±0.4	0.63±0.3	78.4±34.7
Male	24.5±2.2	2.28±0.4	0.49±0.3	62.1±40.6
Female	23.6±5.8	1.97±0.3	0.74±0.3	91.1±24.7
Old	24.8±3.8	1.66±0.6	0.69±0.5	140.7±31.8
Male	23.8±4.4	1.64±0.7	0.24±0.4	118±36.3
Female	25.6±3.8	1.68±0.6	1.03±0.3	157.7±15.7
Total	24.3±4.2	1.97±0.5	0.65±0.3	97.4±44.3
Male	24.3±2.8	2.09±0.6	0.42±0.3	78.9±46.1
Female	24.2±5.2	1.88±0.4	0.83±0.3	111.6±38.7

Table 1. Subject Characteristics

The participants generally shared similar BMI values, but younger subjects generally showed higher muscle thickness than older subjects. The female subjects had higher subcutaneous adipose tissue (SAT) than males, and younger males showed higher SAT than older males within the same sex group. The older subjects had higher echogenicity than younger subjects, with the values almost doubled.

Age Group	Peak Torque (Nm)	Peak Power (W)	RTDabs ($Nm \bullet s^{-1}$)	RTDrel ($MVIC \bullet s^{-1}$)
Young	186.3±86.6	375.2±153.1	1213.2±782.8	6.40±2.16
Male	236.5±99.7	480.6±172.5	1474.7±950.7	5.94±2.11
Female	147.3±52.0	296.2±75.0	1009.8±604.0	6.75±2.26
Old	125.2±40.0	194.0±80.8	683.9±352.8	5.37±1.62
Male	160.2±29.7	274.4±39.5	883.7±459.8	5.35±2.0
Female	98.9±21.4	133.7±26.6	534.0±195.2	5.39±1.62
Total	167.7±79.9	314.8±157.7	1052.1±716.8	6.08±2.03
Male	213.6±90.4	411.9±172.0	1297.4±855.0	5.77±1.99
Female	132.4±49.6	242.0±100.9	863.4±552.2	6.33±2.11

Table 2. Muscular Function

In both old and young age groups, male subjects showed higher peak torque, peak power, and absolute rate of torque development (RTDabs). However, the relative rate of torque development (RTDrel) was similar among older female and male subjects, while differences were observed in younger subjects between the two sexes. Apart from that, younger subjects tended to show greater values in muscular function.

A B

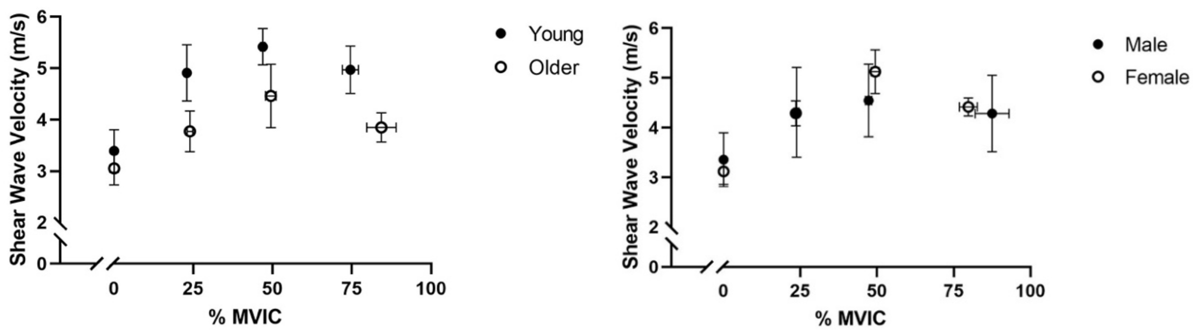

Figure 1. Tissue stiffness as measured with SWE in terms of SWV at the VL. Figure 1A describes the SWV by passive and varying contraction intensities by age effect. Figure 1B describes the SWV by passive and varying contraction intensities by sex effect.

Contraction intensity effect was observed with both age and sex variables, wherein the shear wave velocity increased as the MVIC increased (p=0.001). The older and younger subjects had very similar passive stiffness values, but the younger subjects showed slightly higher stiffness values when actively contracting. However, no differences in stiffness were observed between different sexes. There was no statistically significant interaction effect measured with contraction intensity and age or sex, nonetheless (p>0.05).

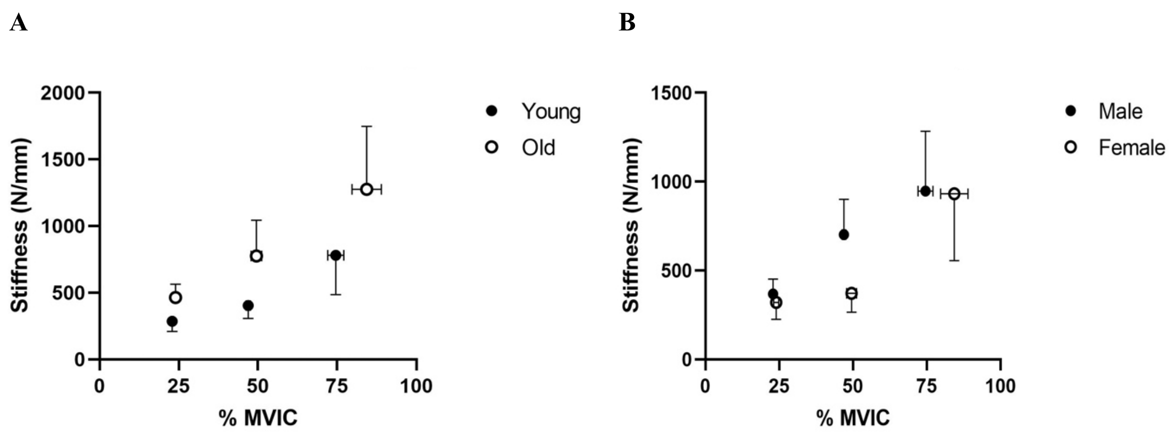

Figure 2. The relationship between tissue stiffness, calculated with values obtained from B-Mode ultrasound, and contraction intensities at the VL. Figure 2A describes the stiffness by varying contraction intensities by age effect. Figure 2B describes the stiffness by varying contraction intensities by sex effect.

Similar to what was observed with SWE, the stiffness values increased with increasing MVIC for both age and sex variables. However, unlike in SWE, B-Mode shows that older subjects have higher stiffness values, and this difference becomes even greater as the submaximal load increases during active contraction. Males showed higher stiffness at 50% MVIC than females, but otherwise, no differences were detected between different sexes.

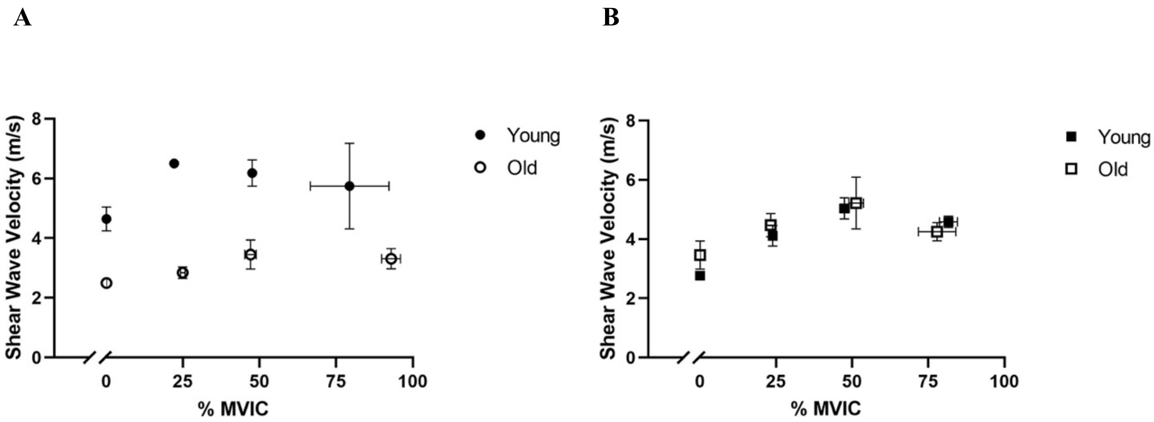

Figure 3. Tissue stiffness as measured with SWE in terms of SWV at the VL by each sex groups to assess the effect of aging. Figure 3A describes the SWV by passive and varying contraction intensities by age effect witnessed in male subjects only. Figure 3B describes the SWV by passive and varying contraction intensities by sex effect witnessed in female subjects only.

The subjects were divided into each sex group to assess the effect of age using SWE and B-Mode methodology. There was no significant between-subjects effect for sex ($p=0.524$), but a significant between-subjects effect for age was observed ($p < 0.001$). The SWE values in male subjects showed the greatest impact of aging on stiffness when compared within the same sex group. The younger male subjects generally showed higher stiffness than the older male subjects throughout the passive and active contractions. However, the female subjects did not show a significant difference in stiffness due to aging.

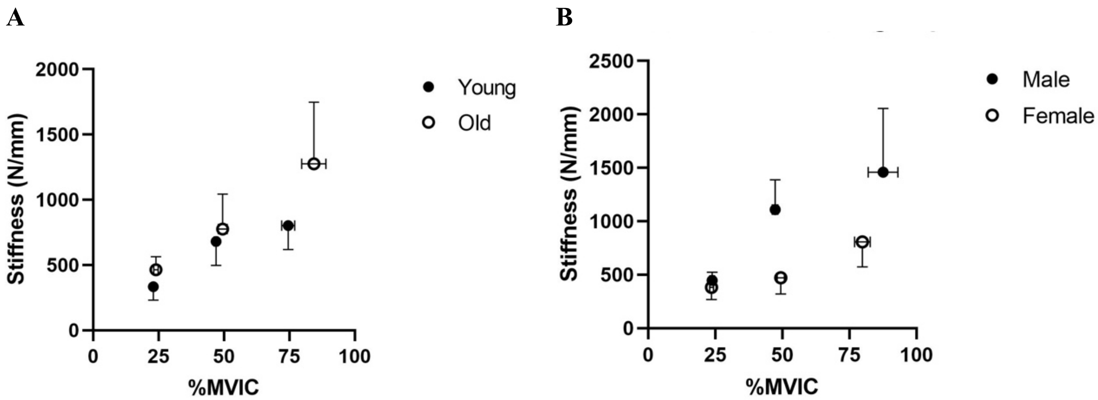

Figure 4. The relationship between tissue stiffness, calculated with values obtained from B-Mode ultrasound, and contraction intensities at the VL in the B-Mode ultrasound subgroup. Figure 4A describes the stiffness by varying contraction intensities by age effect. Figure 4B describes the stiffness by varying contraction intensities by sex effect.

Due to the relatively later implementation of SWE, the B-Mode ultrasonography included more participants in the data pool. Thus, we created a separate subgroup that was measured with both B-Mode and SWE ultrasonography to draw comparisons for age and sex. Although both the young and old subjects' stiffness increased with increasing MVIC, the stiffness difference was not significant until reaching 100% MVIC, where younger subjects had a greater stiffness value than older subjects. In the subgroup for sex comparison, both males and females showed similar stiffness values at 25% MVIC, but males showed a dramatic increase in stiffness with increasing MVIC compared to females. However, the trend generally follows the full data set for B-Mode ultrasound.

Correlation

A correlational analysis was conducted to assess any association between different intermuscular components and muscular performance variables. Muscle thickness showed a significant positive correlation with peak power (p=0.024) and peak torque (p=0.022), indicating that an increase in muscle thickness was correlated with increases in these two values.

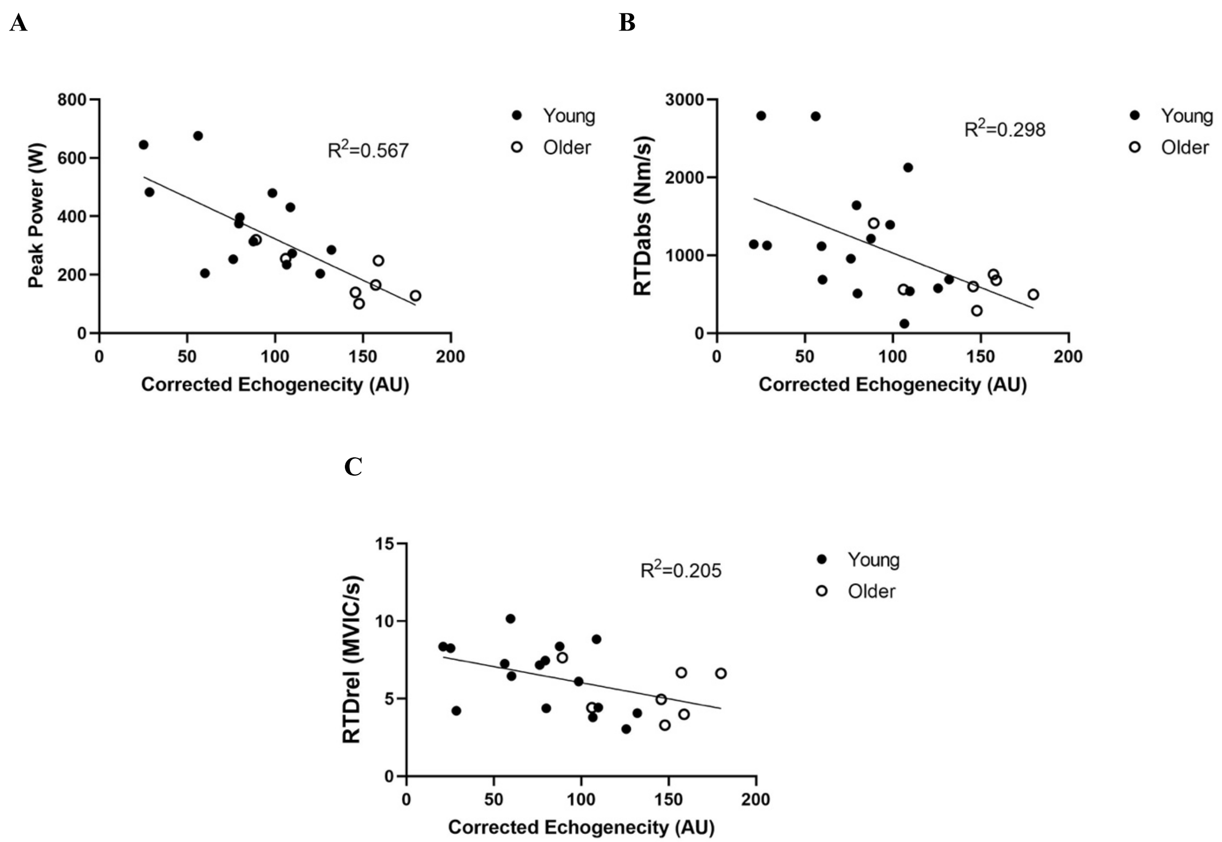

Figure 5. Relationships between corrected echogenicity (AU) and different muscular performance measurements collected through dynamometer. Figure 5A shows a negative correlation between corrected echogenicity and peak power ($p<0.05$). Figure 5B shows a negative correlation between corrected echogenicity and absolute rate of torque development ($p<0.05$). Figure 5C shows a negative correlation between corrected echogenicity and relative rate of torque development ($p<0.05$).

Corrected echogenicity showed significant negative correlations with different measures of muscle performance in our data. As echogenicity increased, peak power, absolute and relative rate of torque development decreased simultaneously. Thus, the correlations suggest that the more the muscle has other components than muscle, such as water-based adipose tissue that increases the corrected echogenicity values, the lower the muscle performance one will have. Corrected echogenicity did not correlate with SWE measurements, however ($p>0.05$), suggesting that SWE may be insensitive to variations within muscle and instead represents inherent muscle properties.

Discussion

The risk of fall-related injuries is one of the most dangerous risks associated with older populations in the modern world. Yet, the exact physiology of why and how older individuals are more prone to fall risk is not fully explored. This research project aimed to answer part of this question by incorporating both age and sex factors to assess their effect on musculotendinous stiffness in the vastus lateralis muscle, one of the most important thigh muscles aiding in ambulatory tasks. The initial hypothesis was that older subjects would show lower stiffness values than younger subjects, and females would have lower stiffness values than male individuals in the vastus lateralis.

Contrary to the hypothesis, no significant interaction effect was detected between age or sex and musculotendinous stiffness. The younger subjects had slightly higher stiffness values than older subjects in the SWE approach, but the interaction was not significant enough to suggest an effect of aging on stiffness. Similarly, male and female subjects showed almost no difference in their stiffness in the SWE approach. The stiffness calculated through B-Mode ultrasound had an opposite result to the SWE: the younger subjects showed lower stiffness than the older individuals by a slight degree. However, the difference was not significant enough to suggest an age effect. Additionally, there was no sex effect detected with the B-Mode as well. The male subjects had increased stiffness compared to females during 50% and 100% active contractions in the B-Mode subgroup analysis, but the statistic was not significant enough to suggest a sex effect on stiffness.

However, when we analyzed the data by each sex group to assess potential aging effects, the male group showed a significant difference in their stiffness. As shown in Figure 4A, the younger male subjects exhibited dramatically higher stiffness values than the older male subjects. The same trend was not observed in the female group, as the young and old female subjects had almost the same stiffness values. Thus, the result suggests that the age effect on the vastus lateralis muscle may be sex-specific to males. This may be potentially due to the younger males starting with higher stiffness values than other subject groups, thereby making the age effect within the male group more substantial. In fact, younger males show significantly higher muscular performance in every aspect, as shown in Table 2.

Initially, we expected to see lower stiffness in females compared with males, but likely enhanced stiffness in older female groups when compared with younger females, due to the effect of menopause on tissue compliance. This is contrast to the effects of estrogen on loss of muscle mass and strength (Maltais et al., 2009). The older female subjects generally exhibited lower muscle mass, higher subcutaneous adipose tissue, and higher corrected echogenicity values than the younger female subjects, as shown in Table 1. Surprisingly, stiffness was not different between young and older females, in spite of presumed effects of estrogen deficiency in older female adults. The muscular performance measures also decreased almost by half between the younger and older female subjects, as displayed in Table 2. However, when analyzed to assess the aging effect within the female group using SWE, older and younger females did not show significant differences in their stiffness at the vastus lateralis. This result may suggest that although females have lower muscle mass and strength than males initially, their overall musculotendinous stiffness may remain relatively constant throughout life, and this effect may be specific to females. The components contributing to maintaining relatively similar stiffness need further investigation in future studies.

One of the significant outcomes of this research project is the potential use of corrected echogenicity as a predictor for muscle performance measures. Echogenicity reflects the muscular tissue composition, which may include other components such as fat within the muscle. In the data analysis, corrected echogenicity showed a strong negative correlation with most power measurements, including peak power, and relative and absolute rate of torque development. This correlation is understandable because higher echogenicity values are more likely to indicate non-muscle components within the VL tissue area, which may hinder the proper transmission of force through the muscle. These results may support the use of echogenicity in a clinical setting to predict efficient muscle performance.

Limitations

One of the biggest limitations of this study is the different pattern observed in each B-Mode Ultrasound and SWE Ultrasound. While B-Mode ultrasound shows slightly higher stiffness in older subjects when analyzed to assess the effect of age, younger subjects showed higher stiffness than older subjects in SWE data. While there was almost no difference between males and females in terms of SWE stiffness, males showed higher stiffness than females in 50% and 100% MVIC in B-Mode ultrasound data analyzed for the sex effect on stiffness. Such a pattern may have arisen from the difference in the way we obtain stiffness measures from each method. Unlike SWE, where we analyze stiffness by calculating the mean shear wave velocity from the ultrasonographic video on the device itself, the B-Mode measure requires extraction of the data to be further analyzed on separate computer software. These values are then processed with the torque data obtained from the dynamometer. Thus, the stiffness calculated through B-Mode undergoes a more complicated process and may have more room for potential errors.

The other reason could be attributed to the inherent difference between the way the two methods collect data. While B-Mode is more of a 2D method, tracking the aponeurosis elongation in response to given loads, SWE is more three-dimensional as it takes into account how fast the shear wave travels along the tissue fiber. Thus, SWE may be able to account for factors such as different rate coding of the muscle fibers at certain loads. For instance, when subjects are aiming for 100% MVIC, they may have already recruited all the muscle fibers. They may have altered the rate coding frequency to accommodate more power output, in which SWE may have detected and reflected a higher shear wave velocity. However, such factors are unable to be considered in B-Mode because B-Mode stiffness calculation only relies on the fascicle elongation shown in the ultrasound videos.

Another limitation of the study is the confined data pool. We had a limited number of subjects included in the project, with the smallest number being 3 older males. Even within the subject pool, not all of them had SWE data due to the relatively later implementation of SWE. Thus, the small sample size may potentially not accurately reflect the overall population effect.

Conclusion

In conclusion, the age effect on stiffness at the vastus lateralis was only observed in male subjects, suggesting that the age effect may be specific to males only. Females did not exhibit an age effect on stiffness at the vastus lateralis, indicating that the overall lower muscle performance may be due to factors other than musculotendinous tissue stiffness itself as our measures rely on assessing tissue displacement that is in series with other, non-contractile elements and will therefore, be affected by the compliance of those structures (ex. Patellar tendon). For future studies, it will be important to take into account other anatomical structures that contribute to overall moment arm, power, or torque production, such as the patellar tendon, to assess the degree of impact that muscle stiffness has. It will also be important to consider whether certain components specific to each sex make a difference in the overall result.

The study shows the potential to use corrected echogenicity in various clinical settings as a predictor for muscle performance. Early detection through intermuscular composition measurement may aid in prescribing therapeutic exercises that could help prevent stiffness from progressing. With these findings, my thesis project aims to contribute to the understanding of age-related physiology and apply this knowledge to reduce the overall risk of injuries among older adults.

References

Alfuraih, Abdulrahman M., et al. "The Effect of Ageing on Shear Wave Elastography Muscle Stiffness in Adults." *Aging Clinical and Experimental Research*, vol. 31, no. 12, 2019, pp. 1755–63, https://doi.org/10.1007/s40520-019-01139-0.

Baş, Hakan, et al. "Lower Gastrocnemius Muscle Stiffness, Derived from Elastography, Is an Independent Factor for Falls in Older Adults." *Aging Clinical and Experimental Research*, vol. 35, no. 12, 2023, pp. 2979–86, https://doi.org/10.1007/s40520-023-02605-6.

Biondi, N. L., & Varacallo, M. (2023, August 8). *Anatomy, bony pelvis and lower limb: Vastus lateralis muscle*. National Library of Medicine. https://www.ncbi.nlm.nih.gov/books/NBK532309/

Brandenburg, Joline E., et al. "Ultrasound Elastography: The New Frontier in Direct Measurement of Muscle Stiffness." *Archives of Physical Medicine and Rehabilitation*, vol. 95, no. 11, 2014, pp. 2207–19, https://doi.org/10.1016/j.apmr.2014.07.007.

Bravo-Sánchez, Alfredo, et al. "Objective Assessment of Regional Stiffness in Vastus Lateralis with Different Measurement Methods: A Reliability Study." Sensors (Basel, Switzerland), vol. 21, no. 9, 2021, p. 3213–, https://doi.org/10.3390/s21093213.

Carovac, Aladin et al. "Application of ultrasound in medicine." *Acta informatica medica : AIM : journal of the Society for Medical Informatics of Bosnia & Herzegovina : casopis Drustva za medicinsku informatiku BiH* vol. 19,3 (2011): 168-71. doi:10.5455/aim.2011.19.168-171

Chalchat, Emeric, et al. "Changes in the Viscoelastic Properties of the Vastus Lateralis Muscle With Fatigue." Frontiers in Physiology, vol. 11, 2020, pp. 307–307, https://doi.org/10.3389/fphys.2020.00307.

Clark, David J., et al. "Longitudinal Decline of Neuromuscular Activation and Power in Healthy Older Adults." *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, vol. 68, no. 11, 2013, pp. 1419–25, https://doi.org/10.1093/gerona/glt036.

Eby, Sarah F., et al. "Shear Wave Elastography of Passive Skeletal Muscle Stiffness: Influences of Sex and Age throughout Adulthood." *Clinical Biomechanics (Bristol)*, vol. 30, no. 1, 2015, pp. 22–27, https://doi.org/10.1016/j.clinbiomech.2014.11.011.

Hansen, Mette. "Female Hormones: Do They Influence Muscle and Tendon Protein Metabolism?" Proceedings of the Nutrition Society, vol. 77, no. 1, 2018, pp. 32–41, https://doi.org/10.1017/S0029665117001951.

Hullfish, Todd J., and Josh R. Baxter. "A Reliable Method to Quantify Tendon Structure Using B-Mode Ultrasonography." *Journal of Ultrasound in Medicine*, vol. 37, no. 10, 2018, pp. 2419–24, https://doi.org/10.1002/jum.14592.

Ikezoe, Tome, et al. "Associations of Muscle Stiffness and Thickness with Muscle Strength and Muscle Power in Elderly Women." *Geriatrics & Gerontology International*, vol. 12, no. 1, 2012, pp. 86–92, https://doi.org/10.1111/j.1447-0594.2011.00735.x.

Lee H, Petrofsky JS, Daher N et al. (2013) Anterior cruciate ligament elasticity and force for flexion during the men- strual cycle. Med Sci Monit 19, 1080–1088.

Maltais, M. L., et al. "Changes in Muscle Mass and Strength after Menopause." Journal of Musculoskeletal & Neuronal Interactions, vol. 9, no. 4, 2009, pp. 186–97.

Marcucci, Lorenzo, and Carlo Reggiani. "Increase of Resting Muscle Stiffness, a Less Considered Component of Age-Related Skeletal Muscle Impairment." *European Journal of Translational Myology*, vol. 30, no. 2, 2020, pp. 223–33, https://doi.org/10.4081/ejtm.2020.8982.

Moore, Keith L., et al. *Essential Clinical Anatomy*. 5th Edition, Lippincott Williams & Wilkins, 2015.

Mongold, Scott J.; Ricci, Austin W.; Hahn, Michael E.; Callahan, Damien M.. Skeletal Muscle Compliance and Echogenicity in Resistance-Trained and Nontrained Women. The Journal of Strength & Conditioning Research ():10.1519/JSC.0000000000004669, October 16, 2023. | DOI: 10.1519/JSC.0000000000004669

Morel, Baptiste, et al. "Reduced Active Muscle Stiffness after Intermittent Submaximal Isometric Contractions." Medicine and Science in Sports and Exercise, vol. 51, no. 12, 2019, pp. 2603–09, https://doi.org/10.1249/MSS.0000000000002080.

"Oxford Languages and Google - English." *Oxford Languages*, languages.oup.com/google-dictionary-en/. Accessed 14 Feb. 2024.

Ramaswamy, Krishnan S., et al. "Lateral Transmission of Force Is Impaired in Skeletal Muscles of Dystrophic Mice and Very Old Rats." *The Journal of Physiology*, vol. 589, no. 5, 2011, pp. 1195–208, https://doi.org/10.1113/jphysiol.2010.201921.

Siracusa, Julien, et al. "Resting Muscle Shear Modulus Measured With Ultrasound Shear-Wave Elastography as an Alternative Tool to Assess Muscle Fatigue in Humans." Frontiers in Physiology, vol. 10, 2019, pp. 626–626, https://doi.org/10.3389/fphys.2019.00626.

Walston, Jeremy D. "Sarcopenia in older adults." *Current opinion in rheumatology* vol. 24,6 (2012): 623-7. doi:10.1097/BOR.0b013e328358d59b

Wu, Rui, et al. "Effects of Age and Sex on Neuromuscular-Mechanical Determinants of Muscle Strength." *AGE*, vol. 38, no. 3, 2016, pp. 57–57, https://doi.org/10.1007/s11357-016-9921-2.

Xu, Jingfei, et al. "Age-Related Increase in Muscle Stiffness Is Muscle Length Dependent and Associated with Muscle Force in Senior Females." *BMC Musculoskeletal Disorders.*, vol. 22, no. 1, 2021, https://doi.org/10.1186/s12891-021-04519-8.

Zardi, Enrico Maria, et al. "Reliability of Quantitative Point Shear-Wave Ultrasound Elastography on Vastus Medialis Muscle and Quadriceps and Patellar Tendons." Medical Ultrasonography, vol. 21, no. 1, 2019, pp. 50–55, https://doi.org/10.11152/mu-1712.